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Breeding for Improved Flavour of Fresh Market Tomatoes: Breeders' Sensory Test and Molecular Markers

Dissertation
to attain the doctoral degree Dr. sc. agr

Submitted by
Julia Friederike Hagenguth
born in Wiesbaden

Göttingen, September 2022

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1. Referee: Prof. Dr. Heiko C. Becker

2. Referee: Prof. Dr. Elke Pawelzik

3. Examiner: Prof. Dr. Gunter Backes

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“The health of people and the planet are at critical moments. There are synergies between intensivist global food systems and phenomena such as climate change, malnutrition, and obesity. […] The alternatives to these issues go through the development of a sustainable food system, including organically grown food.”[1] “Better-tasting fruits would shift eating habits away from less healthy snack food alternatives, having a significant impact on nutrition. If we build better tasting fruits and vegetables, the consumer will come.”[2]

[1]Raigón MD, García-Martínez MD and Chiriac OP. (2022). Nutritional Characterization of a Traditional Cultivar of Tomato Grown Under Organic Conditions—cv. “Malacara”. *Frontiers in Nutrution*. 8:810812. doi: 10.3389/fnut.2021.810812; [2]Klee HJ. (2010). Improving the flavour of fresh fruits: genomics, biochemistry, and biotechnology. *New Phytologist*. 187: 44–56. doi: 10.1111/j.1469-8137.2010.03281.x

OVERVIEW OF THE STUDIES

This PhD project was conducted as part of the joint project PETRA^{q+n} (Participatory development of quality tomatoes for sustainable regional production) that was financially supported by the Ministry for Science and Culture of Lower Saxony (VWZN3255). The Section of Genetic Resources and Organic Plant Breeding (Dr. Bernd Horneburg) was supported by the Software AG Foundation. I acknowledge the Professor Werner Schulze-Stiftung zur Förderung der Pflanzenbauwissenschaften for providing me a scholarship for the completion of the thesis.

Study I: Hagenguth J, Kanski L, Kahle H, Naumann M, Pawelzik E, Becker HC, Horneburg B (2022). Breeders' Sensory Test: A new tool for early selection in breeding for tomato (*Solanum lycopersicum*) flavour. *Plant Breeding*, 141(1), 96–107. https://doi.org/10.1111/pbr.12994

Objectives were to evaluate the Breeders' Sensory Test (small team, hundreds of small samples) and the potential of selection for flavour-related traits in the first segregating generations.

Methods: F_2 plants from 32 crosses and their 12 parents were phenotyped in two contrasting cultivation systems (organic low-input, hydroponic). In both cultivation systems, a total of 910 plants were evaluated for sweetness, sourness, total and tomato aroma using the Breeders' Sensory Test, and for physicochemical measurements and volatile compounds.

Results: Corresponding physicochemical measurements and sensory attributes (sweetness and TSS, sourness and TA) were highly significantly correlated. The genetic plus environmental variance exceeded the environmental variance for most of the flavour-related traits, including sensory attributes, in most crosses.

Conclusion: The Breeders' Sensory Test is a promising tool to select for sensory attributes in early breeding generations. For most flavour-related traits, selection between single plants in the F_2 generation is expected to be successful.

Study II: Flavour Improvement in Early Generations of Fresh Market Tomatoes (*Solanum lycopersicum* L.): I. Identification of QTL for Sensory Attributes, Physicochemical Measurements and Volatile Compounds
[submitted to *Plant Breeding*]

Objectives were to map QTL for sensory attributes, physicochemical measurements, aroma volatiles and fruit weight and to identify genetic regions with co-localised QTL for multiple traits.

Methods: About 190 F_2 plants derived from a cross between two cultivars with excellent, but contrasting flavour and fruit weight, ‘Resi’ and ‘Aurigai’, were genotyped and phenotyped for sensory attributes using the Breeders' Sensory Test, physicochemical measurements, volatile compounds and fruit weight in two contrasting cultivation systems (organic low input, hydroponic).

Results: With a few exceptions, most aroma volatiles were positively correlated with tomato aroma. For the mean values of both cultivation systems, 21 sensory, 16 physicochemical, 24 volatile and 10 fruit weight QTL were mapped across all 12 chromosomes. A share of 27% of the QTL was co-located between both cultivation systems and their mean values. Nine distinct QTL clusters were identified.

Conclusion: Sensory QTL on chromosomes 5 and 10 were identified for the first time. QTL for sweetness, sourness and tomato aroma on chromosomes 2, 5, 6, 10 and 11, partly within QTL clusters, are recommended for MAS.

Study III: Flavour Improvement in Early Generations of Fresh Market Tomatoes (*Solanum lycopersicum* L.): II. Response to Breeders' Sensory and Marker-Assisted Selection
[submitted to *Plant Breeding*]

Objectives were the comparison of phenotypic selection based on the Breeders' Sensory Test (breeders' sensory selection, BS) and marker-assisted selection (MAS) with an unselected population.

Methods: Molecular markers were selected based on the QTL mapping study (part I). For two unrelated crosses, 'Resi' × 'Auriga' and 'Roterno F_1' × 'Black Cherry', BS and MAS were conducted for the sensory attributes sweetness, sourness and tomato aroma. All experimental populations were phenotyped in two contrasting cultivation systems (organic low-input, hydroponic) for sensory attributes (Breeders' Sensory Test, trained panel), physicochemical measurements, volatile compounds and fruit weight.

Results: QTL for sweetness and tomato aroma were confirmed in 'Roterno F_1' × 'Black Cherry'. Both selection methods were more efficient in the cross 'Roterno F_1' × 'Black Cherry'. A slightly higher efficiency of MAS compared to BS was observed. Increases due to BS and MAS, respectively, were observed for most sensory attributes in both crosses and cultivation systems. Selection for sensory attributes resulted in several indirect effects on physicochemical measurements and aroma volatiles, and decreased fruit weight.

Conclusion: MAS for sensory attributes is a promising method for preselection of seedlings, allowing a potentially higher selection intensity, as a very large number of plants can be analysed. The efficiency of BS is probably reduced by a genotype-by-year interaction. To capture the total genetic variation and whole flavour diversity, a combination of BS and MAS is recommended to improve tomato flavour.

FURTHER PUBLICATIONS

Published papers

Kanski L, Kahle H, Naumann M, Hagenguth J, Ulbrich A, Pawelzik E (2021). Cultivation Systems, Light Intensity, and Their Influence on Yield and Fruit Quality Parameters of Tomatoes. *Agronomy*, 11(6):1203. https://doi.org/10.3390/agronomy11061203

Talks

Hagenguth J, Kanski L, Kahle H, Persch A, Pawelzik E, Becker HC, Horneburg B (2021). Improving tomato flavour with the Breeders' Sensory Test. Pitch presentation (online) at the Organic World Congress 2021, Rennes, France (hybrid format)

Hagenguth J, Kanski L, Kahle H, Persch A, Smit I, Becker HC, Horneburg B (2018). The potential of a breeders` sensory test in the F_2 generation of tomato. Oral presentation at XIX EUCARPIA Meeting of the Tomato Working Group, Naples, Italy (Award for oral presentation)

Poster

Hagenguth J, Kanski L, Kahle H, Persch A, Pawelzik E, Becker HC, Horneburg B (2020). Breeding tomatoes with improved flavour using a breeders` sensory test. Poster presented at International Symposium of the Society for Plant Breeding e. V. (GPZ) in cooperation with Saatgut Austria, Tulln Austria (Poster award)

Hagenguth J, Kanski L, Horneburg B, Smit I, Becker HC, Naumann M, Pawelzik E (2018). Evaluation of tomato genotypes for improved taste using analytical measurements and breeders' sensory test. Poster presentation at 52. Gartenbauwissenschaftliche Jahrestagung, Geisenheim, Germany

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1 GENERAL INTRODUCTION

1.1 The tomato, the most important vegetable struggling with its flavour

1.1.1 Origin, breeding history and cultivation of tomato

The tomato (*Solanum lycopersicum* L., 2n = 24) is the most favourite and economically important vegetable globally and in Europe, consumed fresh and processed, and is an essential component of the Mediterranean diet and in cuisines around the world (Colantonio et al., 2022; Frusciante et al., 2007; Tieman et al., 2017). Since the assembly of the tomato reference genome (The Tomato Genome Consortium, 2012) and resequencing of hundreds of tomato accessions, the tomato has become a model species for molecular studies of fruit development and quality (Bauchet & Causse, 2012; Tikunov et al., 2020). Along with other agronomically important crops such as potatoes, peppers and eggplants, the tomato belongs to the large and diverse Solanaceae family (Bauchet & Causse, 2012). The *Lycopersicon* clade includes the domesticated tomato (*S. lycopersicum*, SL) and its 12 closest wild relatives, which are native to western South America (Bai & Lindhout, 2007; Bauchet & Causse, 2012; Blanca et al., 2015). The red-fruited *S. pimpinellifolium* (SP) is the closest related wild species of the cultivated tomato (Blanca et al., 2015; Lin et al., 2014). Wild tomatoes show a large genetic diversity that was used for the introgression of resistance genes into breeding material and a diversification of fruit size (Bai & Lindhout, 2007; Lin et al., 2014; Martina et al., 2021). In addition, genomic segments altering the content of primary metabolites (Capel et al., 2015) and aroma volatiles (Mathieu et al., 2009; Rambla et al., 2017; Tieman et al., 2006) were identified in wild tomatoes, but probably with a negative effect on fruit size (Capel et al., 2015). SL is divided into *S. l.* var. *cerasiforme* (SLC) and *S. l.* var. *lycopersicum* (SLL): SLL is cultivated, whereas SLC comprises a mix of wild and semi-domesticated varieties (Blanca et al., 2022). SLC and cherry tomatoes are not synonymous; cherry tomatoes are morphologically defined and include SLC, modern varieties and many admixtures between SP and SLL (Blanca et al., 2015).

During migration and domestication in the Andean region and Mesoamerica, the cultivated tomato experienced several genetic bottlenecks (Bai & Lindhout, 2007; Blanca et al., 2015). In the 16th century, tomatoes were brought to Europe by the Spanish conquistadors and were then spread around the world (Blanca et al., 2012; Lin et al., 2014). Consequently, and in combination with the high autogamy, the domesticated and cultivated tomato shows low diversity at the molecular level compared to its wild relatives (Bai & Lindhout, 2007; Gao et al., 2019; Martina et al., 2021), but shows a large range of agronomic, morphological and quality traits (Klee, 2010). Furthermore, selection for larger fruits during domestication resulted in a loss of flavour (Klee & Tieman, 2018).

In Europe, the tomato was first predominantly used as an ornamental plant (Bauchet & Causse, 2012). Starting with the end of the 18th century, tomatoes were increasingly used for human consumption. In the late 19th century, early breeding activities and the establishment of breeding programmes and the

resulting availability of diverse and numerous open-pollinated varieties (landraces or heirlooms) made this vegetable economically important (Bai & Lindhout, 2007; Bauchet & Causse, 2012). Nowadays, the majority of cultivars for the fresh market are hybrids developed by private breeding companies, and the share of hybrids for processing tomatoes is increasing (Bai & Lindhout, 2007). Fresh market tomatoes are no longer limited to the common round tomatoes, but a large range of tomatoes including truss, cocktail, cherry, long and heirloom tomatoes is available (Bai & Lindhout, 2007; Causse et al., 2010). The main breeding goals are adaption to growth constraints, disease and pest resistance, fruit productivity and quality (Bauchet & Causse, 2012), with the priority shifting from yield (1970s) to long shelf-life (1980s), then to taste (1990s) and now to nutritional quality (Bai & Lindhout, 2007). In addition, flavour has become an important goal for fresh market tomatoes (Causse et al., 2010; Colantonio et al., 2022; Klee & Tieman, 2018).

Tomato production is continuously increasing in terms of area, production volume and yield (FAO, 2022). In 2020, the global tomato production was about 186.8 million tonnes. Currently, China, India, Turkey and the USA are the main tomato producing countries with a share of almost 60%. Within Europe, Italy and Spain are the top producers. In Germany, tomato production has more than doubled in the last 20 years (FAO, 2022). In 2021, about 101,765 tonnes were produced on almost 400 ha under protected cultivation, with 18.6% of this area under organic cultivation (DESTATIS, 2022). The per capita consumption continues to increase, while the self-sufficiency rate of about 3% is the lowest among vegetables crops in Germany (BMEL, 2022).

Tomatoes are adapted to many environments and different cultivation systems (Bauchet & Causse, 2012). Nevertheless, tomatoes have a high heat and light requirement, due to their origin (Hornischer & Koller, 2015). For high yield and premium quality, dry conditions with an optimum temperature ranging from 21 to 24°C are necessary (Naika et al., 2005). Tomatoes prefer deep, well-drained, sandy loam soils (Naika et al., 2005) with low salt content and a good supply of water and nutrients (Hornischer & Koller, 2015). Tomatoes for the fresh market are grown outdoor or in protected production in cultivation systems ranging from organic outdoor production to hydroponics, while processing tomatoes are produced in open fields with mechanical harvest (Bauchet & Causse, 2012; Zörb et al., 2020). In northern and central Europe, tomatoes are mainly grown in modern, energy-intensive greenhouses due to their temperature requirements, susceptibility to frost and fungal diseases, while low energy systems are dominant in southern Europe (Hornischer & Koller, 2015; Paris et al., 2022). For conventional greenhouse production of tomatoes, hydroponic cultivation is a common method, where plants are grown in an inert substrate and fertilized along with the irrigation (Korčok et al., 2021). So far, energy use in these greenhouse systems mainly depends on fossil sources (Paris et al., 2022). However, a transformation towards more sustainable agricultural systems is currently needed (Raigón et al., 2022). Reducing energy consumption and the transition to regenerative sources is challenging for the greenhouse systems, especially while maintaining

fruit quality (Paris et al., 2022), but changes are in progress, such as the replacement of high-pressure sodium lamps by LED lamps (Kanski et al., 2021; Paris et al., 2022). In general, the high productivity of commercial tomato cultivars adapted to these greenhouse conditions negatively affects the flavour (Cebolla-Cornejo et al., 2011). At the same time, there is an increasing demand for tasty fruits that are produced with minimal negative impact on the environment (Zörb et al., 2020), leading to a growing demand of organically (Willer et al., 2022; Zörb et al., 2020) or locally produced (Adams & Salois, 2010) foods. Consequently, organic tomato production is growing (Raigón et al., 2022).

1.1.2 Tomato fruit composition and nutritional value

Tomatoes are an important source for micronutrients (vitamins and minerals), antioxidants and fibre in the human diet (Causse et al., 2003; Klee, 2010). The primary metabolites are less important for these health-promoting effects compared to secondary metabolites, while both are important for flavour (Collins et al., 2022; Klee, 2010). Water is the major compound of tomato fruits with more than 90%, (Collins et al., 2022). Depending on genotype and environmental conditions, the dry matter (DM) of tomatoes ranges between about 4.5 and 10.5% of the fresh weight (Carli et al., 2011; Chea et al., 2021) with cocktail tomatoes having a higher dry matter compared to salad tomatoes (Chea et al., 2021). The dry matter is composed approximately as follows: 50% reducing sugars, 15% organic acids, 8% minerals and 2 to 2.5% free amino acids. The remaining about 25% consists of proteins, pectin, cellulose and hemicellulose, lipids, pigments, vitamins and polyphenols. Volatile compounds account only for 0.1% of the dry matter (Yilmaz, 2001).

Fructose and Glucose are the two main sugars of tomatoes (Beckles, 2012; Klee, 2010) with fructose being present in slightly higher concentrations and perceived as sweeter (Stevens et al., 1977; Tandon et al., 2003). The most important organic acid is citric acid followed by malic acid (Beckles, 2012; Yilmaz, 2001). Glutamic acid, γ-aminobutyric acid, glutamine, and aspartic acid form the largest share of the free amino acids (Yilmaz, 2001). Among the health-promoting compounds, carotenoids have a particular importance (Causse et al., 2003; Frusciante et al., 2007). Lycopene characterised by a strong antioxidant activity and β-carotene, a provitamin A, are the main carotenoids of tomato (Capel et al., 2015; Collins et al., 2022; Frusciante et al., 2007). Tomatoes are the main source for lycopene in our diet, and this compound gives the red tomato fruits their colour (Frusciante et al., 2007). Studies have given evidence that these compounds reduce the risk for cancer and chronic degenerative diseases (Collins et al., 2022; Frusciante et al., 2007). Other important compounds with antioxidant potential are ascorbic acid, vitamin E and phenolics, in particular flavonoids (Capel et al., 2015; Frusciante et al., 2007). The accumulation of these antioxidants and micronutrients is impacted by environmental conditions (Collins et al., 2022). In terms of minerals, potassium and phosphorus are the most important in tomato fruits (Yilmaz, 2001).

The chemical composition of tomato fruits is influenced by genetics, pre- and post-harvest handling and the ripening process (Collins et al., 2022; Rambla et al., 2014; Wang et al., 2016). As a climacteric fruit, respiration and ethylene production increase at the onset of ripening (Wang et al., 2016). While ripening, tomato fruits undergo several qualitative and quantitative changes that make the fruit more attractive to seed dispersals (Rambla et al., 2014; Wang et al., 2016): while sugars increase, acids are decreasing, fruits are softening and colour changes (Gautier et al., 2008; Rambla et al., 2014). Furthermore, the volatile profile changes massively (Gautier et al., 2008; Rambla et al., 2014; Wang et al., 2016). Most of the volatile compounds increase in the later ripening stages, but some remain stable or even decrease (Wang et al., 2016).

1.1.3 Flavour of tomatoes and consumer liking

Chemically flavour is defined as the sum of primary and secondary metabolites (Klee, 2010; Piombino et al., 2013) and results from a complex interaction of taste and aroma (Beckles, 2012). In addition, flavour is influenced by texture and appearance (e.g. colour) (Baldwin et al., 2000; Klee, 2010). Taste, which is mainly determined by sugars, organic acids and their ratio, forms the foundation of flavour (Klee, 2010; Zhao et al., 2016). Free amino acids, especially glutamic acid, are important as taste-enhancers (Carli et al., 2011). However, the large flavour diversity is provided by aroma volatiles (Baldwin et al., 2000; Klee, 2010). Volatile compounds are secondary or specialized metabolites present in a large range of concentrations that may undergo different modifications (Rambla et al., 2014). The volatile compounds can be grouped according to their precursors such as apocarotenoids, fatty acids, phenolic (phenylalanine; phenylpropanoid, benzoic acid) and branched-chain amino acids (Martina et al., 2021; Rambla et al., 2014; Tikunov et al., 2020), while Zanor et al. (2009) pointed out that the rate of volatile production depends more on the transcriptional or post-transcriptional level than the precursor supply. Flavour is characterised by top and background notes (Baldwin et al., 2000). Aroma volatiles with top notes have mostly a high volatility and are predominant and very characteristic of a food. In contrast, compounds with background notes have a more subtle impact on flavour perception (Baldwin et al., 2000). Although if the characteristic tomato flavour is difficult to define (Martina et al., 2021), it is described as sweet, fruity, earthy, viney and sour (Hongsoongnern & Chambers, 2008).

Flavour perception is a complex process that involves the taste and olfactory system (Baldwin et al., 2000; Klee, 2010; Piombino et al., 2013). Humans have five taste receptors in their mouth, which measure the levels of sweet, sour, salty, bitter and umami in food (Klee, 2010; Klee & Tieman, 2018). Olfaction (smell) is another important part of flavour perception (Klee, 2010) and much more sensitive (Baldwin et al., 2000). Volatile compounds are perceived orthonasally (through the nose) or retronasally (through the mouth), with retronasal olfaction being essential for flavour and affected by temperature in the mouth, chewing and interaction with saliva (Baldwin et al., 2000; Klee & Tieman, 2013). The volatile compounds

are recognized by a large family of olfactory receptors in the nasal epithelium (Klee & Tieman, 2018). Bushdid et al. (2014) estimated that humans can differentiate between about 1 trillion different smells.

In tomatoes, more than 400 volatile compounds were detected, but only a small proportion of around 16 volatiles was identified as unique for tomato aroma based on odour thresholds and odour units (Klee, 2010; Piombino et al., 2013; Wang et al., 2016). Some of these aroma volatiles are present in the fruit at low concentrations, but are likely to influence flavour due to their comparable high odour units (Baldwin et al., 2000). Some of the aroma volatiles with negative odour units may contribute to background notes (Baldwin et al., 2000). To define the odour threshold, the lowest concentration of a compound in water solution that can be recognized by the human nose was determined (Klee & Tieman, 2013; Rambla et al., 2014). Compounds exceeding this threshold (positive odour unit) in tomato fruits were defined as important for tomato aroma and ranked according to their odour units, the concentration of a volatile compound in the tomato divided by its odour threshold, which are usually presented in their logarithmic form (Klee & Tieman, 2013; Rambla et al., 2014; Wang et al., 2016). More recent studies have shown that the approach of odour units is too simplistic: retronasal volatile perception is more important than orthonasal, a matrix effect influences the volatility of volatile compounds, aroma volatile interact with each other, odour thresholds differ among humans and change with age and experience and consumer liking remains unknown (Klee & Tieman, 2013; Rambla et al., 2014).

Therefore, several studies attempt to identify the most important compounds of tomato flavour and consumer liking using sensory panels (Baldwin et al., 2015; Piombino et al., 2013; Tandon et al., 2003; Tieman et al., 2012; Tieman et al., 2017). Perceived sweetness and flavour intensity were identified as the main drivers for liking (Causse et al., 2010; Klee & Tieman, 2018), while sourness should be intermediate (Causse et al., 2010) and the volatile profile balanced with more fruity and less green aroma notes (Baldwin et al., 2015). Tieman et al. (2012) identified 12 compounds relevant for the flavour intensity of tomatoes and pointed out that some aroma volatiles are less important than traditionally thought based on odour units. In another study, Tieman et al. (2017) identified two sugars, glutamic acid and 25 volatile compounds as important for consumer liking and flavour intensity. Among these aroma volatiles are some that enhanced perceived sweetness independent from the sugar content (Klee & Tieman, 2018; Tieman et al., 2017). These volatile compounds are interesting because they provide a way to enhance perceived sweetness and thus consumer liking without increasing the sugar content (Klee & Tieman, 2018), which is negatively correlated with fruit size and yield (Causse et al., 2003; Klee & Tieman, 2018). Other traits included, most consumers prefer medium to small, red, firm, crisp, fleshy, juicy and flavourful tomatoes with few seeds (Oltman et al., 2014).

1.1.4 Breeding for flavour

Flavour, a challenging trait neglected for a long time

Flavour was neglected as a breeding goal for several decades due to its complexity, the high cost of phenotyping and the long-standing focus on traits demanded by producers rather than consumers (Causse et al., 2010; Colantonio et al., 2022; Klee & Tieman, 2018). Especially in large breeding programmes, consumers have been left out of the development of plant varieties (Klee & Tieman, 2018). Much more attention was given to yield, shelf life, uniformity, disease resistance, and adaption to winter greenhouse conditions, traits that reduced production costs and enabled year-round production of visually perfect tomatoes (Causse et al., 2003; Folta & Klee, 2016; Klee, 2010). This resulted in consumers being satisfied with the price and availability of tomatoes, but they began to complain about the lack of flavour in modern standard tomatoes (Causse et al., 2010; Folta & Klee, 2016; Klee & Tieman, 2013). This flavour reduction was not intentional, but rather an indirect effect of the focus on producer-orientated breeding goals, as flavour and yield are negatively correlated, and missing methods to assess flavour during the breeding process (Erika et al., 2022; Folta & Klee, 2016; Klee & Tieman, 2013; Piombino et al., 2013). According to Folta & Klee (2016), breeding for flavour requires expensive analytical tools and access to consumer panels, which is beyond the capacity of most breeding programmes (Colantonio et al., 2022; Klee & Tieman, 2018). Consequently, flavour is one of the traits that is demoted to late breeding generations (Colantonio et al., 2022; Wang & Kays, 2003). In addition, flavour is influenced by environmental effects such as growing conditions, agronomic and post-harvest handling (Baldwin et al., 2015; Beckles, 2012; Klee & Tieman, 2018). Harvesting immature fruits is part of the problem, as are consumer habits when tomatoes are refrigerated at home before they are fully ripe, resulting in fewer aroma volatiles (Cebolla-Cornejo et al., 2011; Klee & Tieman, 2013).

Flavour dissatisfaction went hand in hand with the introduction of much firmer fruits and adaption to high productivity (Causse et al., 2010; Cebolla-Cornejo et al., 2011). Genes responsible for uniform ripening and firmer fruits with extended long-shelf life negatively affect flavour by decelerating the ripening process and therefore the production of sugars and volatile compounds (Baldwin et al., 2000; Folta & Klee, 2016). In northwestern Europe, a crucial point was reached in the late 1980s and early 1990s, when Dutch tomato exports to Germany dropped dramatically after it had been claimed that these tomatoes tasted “watery” (Schouten et al., 2019). As a consequence, breeding goals shifted towards quality traits demanded by consumers such as sensory quality, fruit size, shape and colour (Causse et al., 2010; Schouten et al., 2019). Results of Schouten et al. (2019) confirmed progress in tomato taste improvement by analysing 90 cultivars from the Netherlands for commercial greenhouse production released during 1950 and 2016. The sugar/acid ratio increased during the last three decades (Schouten et al., 2019). On the one hand, cherry tomatoes with high sugar content were introduced, and on the other hand medium to large-fruited tomatoes showed a reduced acidity (Schouten et al., 2019). In addition, they observed a

diversification of aroma volatiles during the last two decades. Aroma volatiles enhancing floral and sweet aroma notes increased noticeably, while volatile compounds leading to a medical, pungent or earthy aroma were reduced (Schouten et al., 2019). Despite these improvements, dissatisfaction with the flavour of tomatoes is still relevant (Baldwin et al., 2015; Colantonio et al., 2022).

Further improvement should be possible, since genetic variability of flavour-related traits was identified in wild relatives, heirlooms, breeding populations and even commercial hybrids (Rambla et al., 2014; Schouten et al., 2019). Disregarding the negative effects on fruit size, cherry tomatoes, characterised by a good flavour, high sugar and acid content, are an interesting source of flavour improvement (Causse et al., 2003). However, breeders require tools to assess this variability and clear selection criteria (Colantonio et al., 2022; Piombino et al., 2013). For flavour, objective breeding targets are difficult to define as a variety of chemical compounds and their interactions contribute to flavour (Klee & Tieman, 2013; Martina et al., 2021). Moreover, consumer preferences depend on aspects such as cultural background, age, gender and learned behaviour (Causse et al., 2010; Folta & Klee, 2016; Klee & Tieman, 2013). Folta & Klee (2016) proposed a consumer-assisted selection strategy, in which flavour compounds relevant for consumer liking are first identified, followed by the development of molecular markers for these compounds. In this approach, breeding priorities are shifted towards consumer demands and therefore are flavour, novelty, nutrition and sustainability (Folta & Klee, 2016). Nevertheless, maintaining producer-demanded traits is important, since growers are mainly paid for yield and appearance, especially in the case of large-fruited tomatoes (Folta & Klee, 2016; Klee & Tieman, 2013). This is probably part of the problem, and it is unlikely to achieve excellent flavour without scarifying yield, but a significant improvement should still be possible (Klee & Tieman, 2013). Despite these challenges, improving the flavour of tomatoes provides clear advantages (Klee, 2010). Flavourful cultivars provide the possibility to expand markets (Klee & Tieman, 2018), since eating quality is essential for subsequent purchases (Carli et al., 2011; Piombino et al., 2013) and a growing proportion of consumers is willing to pay a premium for flavour (Causse et al., 2010; Klee, 2010). Moreover, flavourful vegetables and fruits can contribute to healthier eating habits (Klee, 2010).

Flavour phenotyping

Sensory analysis is the best method to assess external (size, colour, firmness) and internal (flavour, texture) characteristics, also known as organoleptic quality, of tomatoes and other fruits and vegetables (Causse et al., 2001; Causse et al., 2003). It is the science that measures, analyses and interprets the response of people to products as perceived by their senses (Ares & Varela, 2017; Sipos et al., 2021). Sensory panels are true measuring instruments in the sense that they use human perception to quantify sensory attributes of products (Sipos et al., 2021). Sensory evaluation is grouped into analytical/descriptive tests (trained panels) and hedonic/preference tests (consumer panels) (Ares & Varela, 2017; Causse et al., 2003). For both types, standardized techniques and scaling methods are

available (Lim, 2011; Marques et al., 2022). Trained panels are used for the objective evaluation of sensory attributes of products (Ares & Varela, 2017), since they are able to provide consistent and repeatable sensory assessments of products (Sipos et al., 2021). They are generally based on 8 to 20 selected assessors that were trained prior to the assessment (Sipos et al., 2021; Vicente et al., 2014). On the other hand, consumer panels are used to evaluate the acceptance or preference of products (Ares & Varela, 2017). These panels are based on a much larger number of random, untrained people without prior selection according to defined parameters (Causse et al., 2003; Sipos et al., 2021). However, sensory analysis is expensive, time-consuming and has a low throughput (Klee & Tieman, 2018; Piombino et al., 2013; Wang & Kays, 2003). Furthermore, they require large sample sizes for replications. In addition, training of assessors is difficult if fruits, in particular specific cultivars, are only seasonally available (Vicente et al., 2014). Consequently, classical sensory panels are not suited for the application in breeding programmes. Some breeders work with small panels of one to few individuals that do not follow standard methods and are more biased by personal preferences (Colantonio et al., 2022; Vicente et al., 2014). In the organic sector in particular, some efforts have been made to develop appropriate sensory methods to assess flavour during the breeding process, as flavour is an important attribute of organic cultivars (Friedl, 2008; Wilbois & Messmer, 2017). The idea of the so-called Breeders' Sensory Test was communicated by Fleck (2009) and Horneburg et al. (2009) for carrots and parsnips. A similar method was described by Behrendt (2009) for tomatoes. The Breeders' Sensory Test was designed to organoleptically evaluate the large number of small samples typical for early segregating generations with a small team according to a scoring key. Despite the probably widespread use of such sensory tests in breeding programmes, an evaluation of this sensory method for individual plants from early generations is lacking. Furthermore, clear instrumental targets could be helpful for breeders to select for flavour-related traits (Piombino et al., 2013).

Total soluble solids (TSS) and titratable acidity (TA) are two simple and cost-efficient methods for approximate quantification of sugars and acids (Beckles, 2012) that are often used for breeding programmes (Tandon et al., 2003; Zhao et al., 2016). TSS is a refractometric measurement of the dissolved solids in a solution (about 65% sugars, 13% acids and other components of the tomato fruit pulp) that reflects DM (Beckles, 2012). Several studies showed a sufficient correlation between TSS and sugar content (Beckles, 2012) as well as TSS and perceived sweetness (Baldwin et al., 2015; Erika et al., 2022). TA measures bound and free hydrogen ions in solution (Da Conceicao Neta et al., 2007) and is correlated with perceived sourness (Baldwin et al., 2015; Erika et al., 2022; Tandon et al., 2003). In addition, the ration of these measurements (TSS/TA) is a useful indicator for tomato taste (Beckles, 2012). For these physicochemical measurements guiding values are available; a minimum TSS of 5% and a minimum TA of 0.4%, therefore a minimum level of TSS/TA of 12.5, was reported for tasty tomatoes (Beckles, 2012). However, several authors emphasized the difficulties to predict sensory attributes, particularly aroma,

from simple physicochemical measurements suitable for breeding programmes (Causse et al., 2003; Vicente et al., 2014). Aroma evaluation requires trained or consumer panels (Causse et al., 2003) that are not adapted to the large number of small samples typical of breeding programmes, especially early breeding generations as described above. To increase the throughput of objective food characterization, e-tongues and e-noses are under development (Baldwin et al., 2011; Beullens et al., 2008). Even though they are promising, correlation with sensory panels is not yet sufficient for all attributes (Beullens et al., 2008).

Genetic approaches

Since flavour phenotyping for breeding purposes is difficult using both sensory and analytical methods, marker-assisted selection (MAS) is an interesting alternative to phenotypic selection (Causse et al., 2003; Lecomte et al., 2004). However, MAS for flavour might be challenging as flavour is formed by many chemical compounds produced in different pathways (Klee & Tieman, 2018; Tikunov et al., 2020). The success of MAS depends on the number of QTL to be transferred and the distance between flanking markers and the target gene (Das et al., 2017). MAS is used to select a trait indirectly based on the genotype of molecular markers that is tightly linked to the underlying genes (Collard et al., 2005; Xu & Crouch, 2008). It is of particular interest for traits with low heritability or that are difficult to assess by phenotyping methods such as flavour (Lecomte et al., 2004). Compared to phenotypic selection, MAS provides the opportunity to increase the efficiency and effectiveness of breeding due to selection at the seedling stage, replacement of expensive or unreliable phenotyping methods, substitution of field trials that are limited to specific seasons or environments, and pyramiding of genes (Collard et al., 2005; Xu & Crouch, 2008). In the context of organic plant breeding, however, the use of molecular markers is not self-evident and frequently discussed (Lammerts van Bueren et al., 2010). This discussion is related to the basic philosophy and principles of organic farming including the concept of integrity of plants (Lammerts van Bueren et al., 2003). Since molecular markers do not directly interfere with or alter the DNA, they are not excluded by the organic standards (IFOAM, 2017; Lammerts van Bueren et al., 2003; Lammerts van Bueren et al., 2010), but their potential for organic plant breeding has first to be proven (Lammerts van Bueren et al., 2003). A major reason for the rejection of MAS are the enzymes used in the development and application of molecular markers, which are usually produced from genetically modified bacteria, but alternatives are available (IFOAM, 2017; Lammerts van Bueren et al., 2010). In general, the use of MAS in breeding programmes falls short of its expectations (Platten et al., 2019; Xu & Crouch, 2008).

In plant breeding, the application of molecular markers started in the early 1980s with the introgression of monogenic traits from exotic into breeding material (Xu & Crouch, 2008). However, most desired traits, including flavour, are quantitatively inherited, and are thus influenced by many genes, the environment and their interaction (Dekkers & Hospital, 2002; Tikunov et al., 2020). The genetic loci that contribute to the continuous distribution of a quantitative trait are known as quantitative trait loci (QTL) (Collard et al.,

2005). A breakthrough in the characterization of quantitative traits started with the development of abundant molecular markers and rapid, cost-efficient genotyping methods in the late 1980s (Collard et al., 2005; Das et al., 2017; Mackay et al., 2009). The identification of a QTL is based on the detection of associations between a phenotype and genotype by statistical methods (Collard et al., 2005; Das et al., 2017) using either traditional biparental linkage (QTL) or genome-wide association (GWAS) mapping (Mackay et al., 2009; Xu et al., 2013). Traditional linkage mapping uses recombination events in segregating populations (e.g. F_2, backcross or DH populations, recombinant inbred lines) and association mapping historical events in larger populations (Collard et al., 2005; Mackay et al., 2009). Although association mapping has some advantages (higher resolution, saving of time since no mapping population has to be developed, not limited to alleles from two parents), population substructure has to be considered, allele frequencies are unbalanced and the power to detect rare alleles and epistasis is lower compared to linkage mapping (Li et al., 2016; Ranc et al., 2012; Xu et al., 2013; Zhang et al., 2016). Linkage mapping has a high statistical power to compare pairs of alleles, although with low resolution (Li et al., 2016). Moreover, because of the relatively low genetic diversity of modern cultivated tomatoes and predominant autogamy, the linkage disequilibrium extends through long genetic distances (Ranc et al., 2012). Thus, wild tomato species were often included in studies using association mapping, but also linkage mapping (Martina et al., 2021; Ranc et al., 2012; Xu et al., 2013). However, some authors showed that linkage mapping is possible with intraspecific modern crosses, and the results of these studies are much more relevant to breeding programmes (Causse et al., 2002; Kimbara et al., 2018; Tikunov et al., 2020).

Several linkage and association studies were performed to map QTL for primary and secondary metabolites including physicochemical measurements (Martina et al., 2021; Tikunov et al., 2020), but sensory attributes (Causse et al., 2001; Tikunov et al., 2020; Zanor et al., 2009), different cultivation systems or parents with excellent flavour were only rarely included. One main perspective of mapping studies is MAS (Collard et al., 2005). In general, prior to a broader application, verification of molecular markers in diverse genetic backgrounds representing current breeding material and in different environments is essential, but mostly lacking (Chaïb et al., 2006; Collard et al., 2005; Xu & Crouch, 2008). This is probably one of the reasons, why MAS is far behind its expectations, especially in the public breeding sector (Xu & Crouch, 2008). Only the study by Lecomte et al. (2004), demonstrated the chances of marker-assisted backcrossing for flavour-related traits. Genes or enzymes underlying QTL for flavour-related traits remain largely unknown (Tikunov et al., 2020), although knowledge of the main biochemical pathways for aroma volatiles is increasing (Klee & Tieman, 2018; Martina et al., 2021). So far, only a few causative genes have been verified (Zhao et al., 2019).

Further advances in genome sequencing techniques as well as aroma characterization and quantification, made a deeper insight into the genetic variation of tomato flavour possible (Martina et al., 2021; Wang

et al., 2016). For instance, Zhao et al. (2019) conducted a meta-GWAS for flavour-related traits and detected 305 significant associations for the content of primary and secondary metabolites. Furthermore, genomic selection for tomato fruit quality traits was investigated by Duangjit et al. (2016), Hernández-Bautista et al. (2016) and Yamamoto et al. (2016). In genomic selection, a training population is phenotyped and genotyped for all available markers in order to estimate marker effects; these marker effects are used to calculate genomic estimated breeding values of individuals from a breeding population in order to conduct selection without phenotyping (Duangjit et al., 2016; Hernández-Bautista et al., 2016). Duangjit et al. (2016) and Yamamoto et al. (2016) highlight the advantages of genomic selection regarding the length of a breeding cycle and costs, although genetic gain might be equal or less compared to phenotypic selection and prediction accuracy of phenotypic values largely depends on the trait. In the study by Hernández-Bautista et al. (2016), phenotypic selection for fruit-related traits in early breeding generations was more efficient than MAS and genomic selection for most of the assessed quantitative inherited traits. Not based on molecular markers, but by quantifying the chemical profile of fruits, Colantonio et al. (2022) proposed the use of prediction models for consumer liking to increase the throughput of flavour phenotyping compared to sensory analysis and enable early selection for flavour.

1.1.5 Research gaps

According to this literature review, the following research gaps were identified. They are addressed in the three field trials conducted as part of this PhD thesis.

1a. Classical standardized sensory methods (trained and consumer panels) are not suited for breeding purposes and cannot be replaced by simple physicochemical measurements. Flavour assessment by the breeder itself or small panels has not been evaluated or standardized in terms of a guideline.

1b. Flavour assessment is relegated to advanced breeding generations, where most of the variation for this trait may be lost. Less is known about the efficiency of selection in the first segregating generations as the F_2.

2. Sensory attributes are only rarely included in QTL mapping studies. In particular, studies based on a cross between cultivars with excellent flavour are missing. Furthermore, several QTL mapping studies were conducted in more than one season or year, but very rarely in different cultivation systems.

3. Although MAS is often highlighted as a promising tool to enhance breeding for tomatoes with good flavour, verification of QTL effects in diverse backgrounds and different cultivation systems is lacking. Consequently, applied MAS programmes are rare.

1.2 From Breeders' Sensory Test to marker assisted-selection – overview of the three field trials and research questions

1.2.1 The PETRA project

This PhD project is part of PETRA^{q+n} (Participatory development of quality tomatoes for sustainable regional production, Partizipative Entwicklung von QualitätsTomaten für den nachhaltigen regionalen Anbau), an interdisciplinary project between three Divisions from the University of Goettingen (Plant Breeding Methodology, Quality of Plant Products, Marketing for Food and Agricultural Products), the University of Applied Science Osnabrueck (Department of Horticultural Production) and project partners from the whole value chain. The overall aim of this joint project was to expand the scientific basis for breeding tomato cultivars with improved quality and adaption to sustainable regional and urban production. Therefore, new selection methods were developed, the effects of more sustainable greenhouses and two household storage regimes on tomato quality were investigated, and a consumer survey was conducted. Based on previous studies (Chea et al., 2021; Erika et al., 2022), parental cultivars were selected according to their quality (sensory attributes, physicochemical measurements, volatile profile) or yield characteristics and crosses were produced. F_2 seeds from 32 of these crosses were used for the current study. As part of the project, quality breeding lines were grown and evaluated up to the F_5 generation and then handed over to a project partner for further cultivar development. The aims of the three field trial conducted were to evaluate phenotypic and molecular selection methods, to study the inheritance of flavour-related traits, in particular sensory attributes and their relationship in an organic low-input (Figure 1.1) and a hydroponic cultivation system (Figure 1.2).

Figure 1.1. Organic cultivation system

Figure 1.2. Hydroponic cultivation system

1.2.2 Overview of the three field trials

To address the research aims, three consecutive field trials were conducted from 2017 to 2019. All field trials were located in two contrasting cultivation systems, an organic low-input system at the Reinshof Research farm (University of Goettingen) and a conventional hydroponic system at the University of Applied Science Onsabrueck. At the organic site, plants were grown in a well-ventilated rainout shelter to exclude two important tomato pathogens (*Cladosporium fulvum* Cooke and *Phytophthora infestans* (Mont.) de Bary). Low-input conditions were defined as moderate irrigation and the avoidance of external products during the growing season, specifically fertiliser. The Breeders' Sensory Test, a sensory method designed to evaluate hundreds of small samples typical for early segregation generations with a small team, was used to assess sensory attributes. In addition, phenotypic selection (breeders' sensory selection, BS) was conducted based on data from the Breeders' Sensory Test. An overview of the plant material used in the three field trials is illustrated in Figure 1.3.

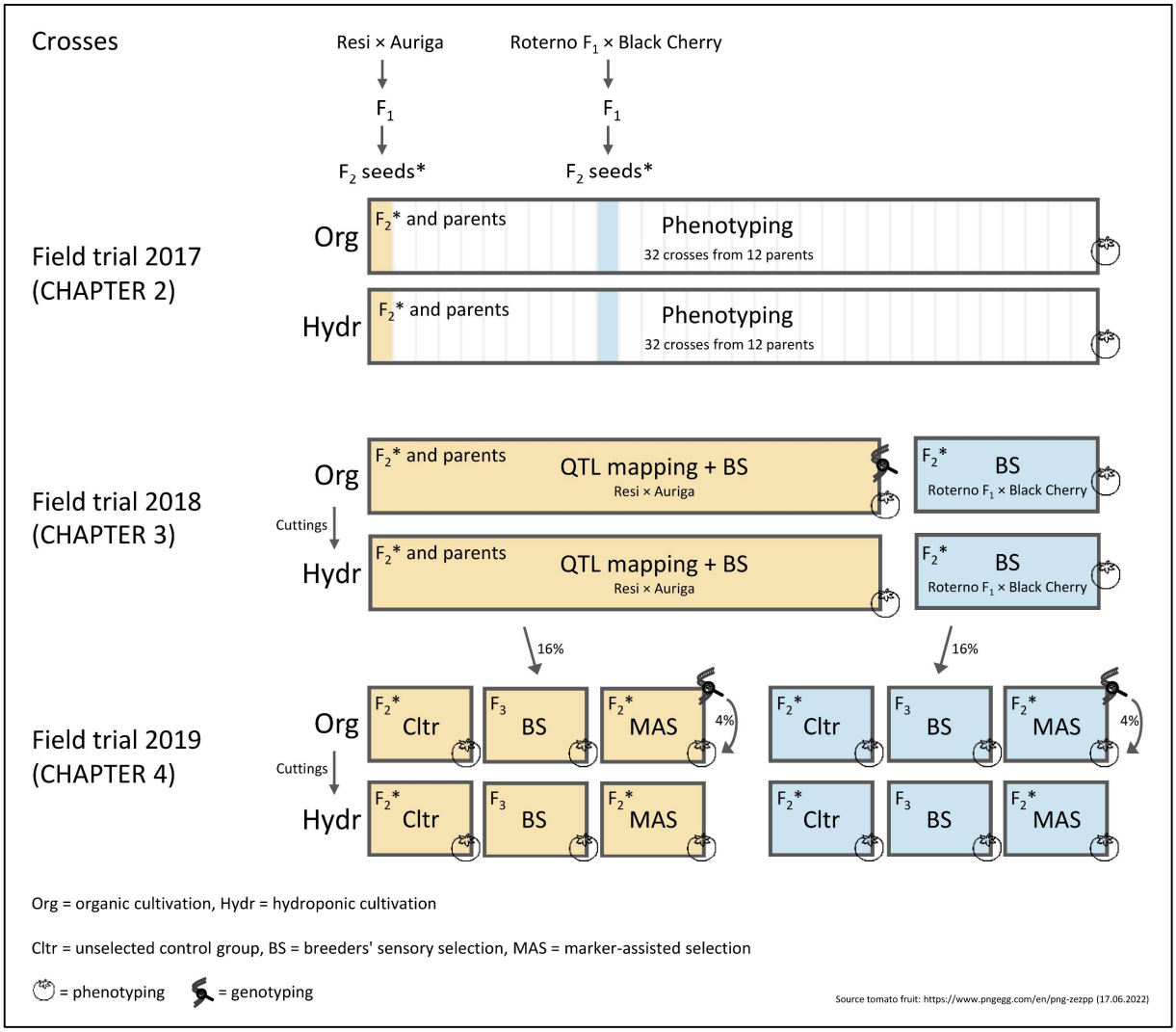

Figure 1.3. Plant material used and field trials conducted in three field seasons to address the aims of this study

1.2.3 Aims of the study and hypotheses

Based on the identified research gaps, the following research aims and hypotheses were developed, which are investigated and discussed by the three studies described in chapters 2 to 4, as well as in the general discussion.

1a. Evaluation of the Breeders' Sensory Test (CHAPTER 2, Study I).

I) Sweetness and total soluble solids (TSS) as well as sourness and titratable acidity (TA) are significantly correlated.
II) Tomato aroma and physicochemical measurements as well as tomato aroma and volatile compounds are significantly correlated.

1b. Potential of the selection for flavour-related traits in the first segregating generation (CHAPTER 2, Study I).

I) The coefficients of variation of the F_2 plants are larger than those of the parents.

2. QTL mapping for flavour-related traits and development of molecular markers (CHAPTER 3, Study II).

I) There are significant QTL for sensory attributes, physicochemical measurements, aroma volatiles and fruit weight.
II) There are co-localised QTL for related traits.

3. Comparison of phenotypic selection based on the Breeders' Sensory Test and marker-assisted selection with an unselected control (CHAPTER 4, Study III).

I) Mean values of plants selected by the Breeders' Sensory Test are significantly different from the unselected control.
II) Mean values of plants selected by molecular markers are significantly different from the unselected control.

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2 STUDY I: Breeders' Sensory Test: A new tool for early selection in breeding for tomato (*Solanum lycopersicum*) flavour

Julia Hagenguth[1, *], Larissa Kanski[2, *], Hannah Kahle[3], Marcel Naumann[2], Elke Pawelzik[2], Heiko C. Becker[1] and Bernd Horneburg[4, 5]

*Julia Hagenguth and Larissa Kanski contributed equally to this study.

[1]Division of Plant Breeding Methodology, Department of Crop Sciences, Faculty of Agriculture, University of Goettingen, Carl-Sprengel-Weg 1, 37075 Goettingen, Germany

[2]Division of Quality of Plant Products, Department of Crop Sciences, Faculty of Agriculture, University of Goettingen, Carl-Sprengel-Weg 1, 37075 Goettingen, Germany

[3]Department of Horticultural Production, University of Applied Sciences Osnabrueck, Am Krümpel 31, 49090 Osnabrueck, Germany

[4]Division of Plant Breeding, Section of Genetic Resources and Organic Plant Breeding, Department of Crop Sciences, Faculty of Agriculture, University of Goettingen, Carl-Sprengel-Weg 1, 37075 Goettingen

[5]present address: Faculty of Organic Agricultural Sciences, Section of Organic Plant Breeding and Agrobiodiversity, University of Kassel, Nordbahnhofstraße 1a, 37213 Witzenhausen, Germany

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Samples for the Breeders' Sensory Test

Standard cultivars

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BH developed the first version of the Breeders' Sensory Test, BH and HB the general approach. JH, LK, HK, BH, HB, EP, and MN planned and designed the experiment. JH, LK, and HK performed the experiment. JH and LK analysed the data and wrote the original draft of the manuscript. MN, EP, HB, and BH edited the manuscript. All authors read and approved the manuscript.

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Breeders' Sensory Test: A new tool for early selection in breeding for tomato (*Solanum lycopersicum*) flavour

Julia Hagenguth[1] | Larissa Kanski[2] | Hannah Kahle[3] | Marcel Naumann[2] | Elke Pawelzik[2] | Heiko C. Becker[1] | Bernd Horneburg[4,5]

[1]Division of Plant Breeding Methodology, Department of Crop Sciences, Faculty of Agriculture, University of Göttingen, Göttingen, Germany

[2]Division of Quality of Plant Products, Department of Crop Sciences, Faculty of Agriculture, University of Göttingen, Göttingen, Germany

[3]Department of Horticultural Production, Osnabrück University of Applied Sciences, Osnabrück, Germany

[4]Division of Plant Breeding, Section of Genetic Resources and Organic Plant Breeding, Department of Crop Sciences, Faculty of Agriculture, University of Göttingen, Göttingen, Germany

[5]Faculty of Organic Agricultural Sciences, Section of Organic Plant Breeding and Agrobiodiversity, University of Kassel, Witzenhausen, Germany

Correspondence
Julia Hagenguth, Division of Plant Breeding Methodology, Department of Crop Sciences, Faculty of Agriculture, University of Göttingen, Carl-Sprengel-Weg 1, 37075 Göttingen, Germany.
Email: julia.hagenguth@agr.uni-goettingen.de

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Abstract

For several decades, improved flavour has not been a main focus in tomato breeding because it is difficult and expensive to evaluate. Sensory panels are designed to assess flavour, but they are not able to evaluate large sample numbers typical for early breeding generations. Selection in the first segregating generation could enhance breeding for flavour by preventing the loss of favourable alleles. The efficiency of early selection is unknown due to the polygenetic inheritance of flavour. To address these issues, F_2 plants from 32 crosses and their parents (910 individuals) were evaluated for aroma, sweetness and sourness with the Breeders' Sensory Test (small team and large number of small samples from individuals), as well as for physicochemical traits (total soluble solids, titratable acidity and dry matter), and volatile compounds in low-input organic and hydroponic cultivation. Corresponding physicochemical and sensory traits were significantly correlated. For most of the studied traits, it was possible to select between single plants in the F_2 generation. Thus, the Breeders' Sensory Test can be used as a new tool in breeding for flavour.

KEYWORDS

F_2 generation, flavour, heritability, sensory evaluation, tomato (*Solanum lycopersicum*)

Julia Hagenguth and Larissa Kanski contributed equally to this study.

1 | INTRODUCTION

The tomato, a vegetable consumed worldwide, is still struggling with its reputation as far as the flavour of its fruits is concerned (Klee & Tieman, 2018). This is also a challenge for other fresh fruits and vegetables (Bruhn et al., 1991). As Folta and Klee (2016) aptly described, plant breeders have made enormous progress to meet the demand for flawless products that are available throughout the year. For decades, the main aims of producer-oriented breeding and selection have focused on production and trade-related traits, such as disease resistance, shelf life, yield and uniformity, instead of considering consumer-preferred traits (Folta & Klee, 2016; Tieman et al., 2017). Consumers prefer fruits that are intense in flavour and nutritious (Klee & Tieman, 2018), where flavour is the interaction of taste, aroma and texture (Tikunov et al., 2020). The compounds contributing to the specific tomato flavour are mainly sugars, acids and aroma volatiles (Baldwin et al., 2008). Although over 400 volatiles have been detected in tomatoes, only a small fraction contributes to tomato flavour (Baldwin et al., 2000; Causse et al., 2017). One of the reasons for neglecting flavour in tomato breeding is its complexity and the resulting difficulty in evaluating it. In tomato, a negative correlation between sugar content and fruit weight, as shown by Causse et al. (2017), makes it even more challenging to satisfy producer and consumer demands. Moreover, most flavour components are influenced by genetics, the environment, agronomic practices and postharvest handling (Causse et al., 2017; Klee & Tieman, 2013, 2018; Tieman et al., 2017). In recent years, quality traits, such as flavour, fruit shape and colour, have received increasing importance during the breeding process (Causse et al., 2017; Schouten et al., 2019) because consumers are willing to pay higher prices for products of high quality, and this is an opportunity to expand markets (Klee & Tieman, 2018). This trend could be facilitated by modern breeding techniques, such as marker-assisted selection (Klee, 2010; Klee & Tieman, 2018).

The content of total soluble solids (TSS) and titratable acidity (TA) are widely used as quality parameters in tomato (Tigist et al., 2013). TSS are the sum of sugars, acids and minor compounds in the pulp and correlate sufficiently with the sugar content in tomatoes (Beckles, 2012), as well as with the perception of sweetness (Baldwin et al., 1998; Causse et al., 2003; Kanski et al., 2020). Higher TSS values can influence the perceived sweetness and flavour of tomatoes (Chassy et al., 2006). TA determines the soluble acid concentration in the fruits and is a good indicator of perceived sourness (Baldwin et al., 1998; Causse et al., 2002, 2003; Tandon et al., 2003).

Sensory analysis is an effective way to characterize internal properties and study consumer preferences (Causse et al., 2010). Therefore, either trained sensory panels or consumer panels are used (Piombino et al., 2013). Both approaches are not suited for the initial steps of a breeding programme that starts with a large number of genetically different plants and very little available plant material. Due to these small sample sizes, the possibility of conducting analyses such as volatile measurements or sensory analysis is limited, and these analyses are still expensive and time-consuming (Klee & Tieman, 2018). In consumer panels or trained sensory panels, in particular, a large number of fruits for each sample are required. To surmount this constraint, herein, the Breeders' Sensory Test was introduced and presented as a new tool, in which a small and trained team organoleptically evaluates a large set of small samples according to a scoring key.

A similar sensory method was developed and used for the improvement of parsnip flavour (Horneburg et al., 2009). A team of two experienced persons evaluated parsnips. It was found that organoleptic selection of individual plants significantly improved sweetness and sugar content (Horneburg et al., 2009). According to Hardner et al. (2016), the application of such a small panel is common in apple breeding because large-scale testing is not possible with a trained panel. In a breeding programme described by Behrendt (2009), sensory evaluation was conducted by three to four persons on tomatoes after preselection for other traits. Breeders, especially in smaller breeding programmes, probably apply similar procedures. To our knowledge, no study investigating early generation sensory analysis of individual plants has been conducted for tomato.

An experiment was designed to evaluate the Breeders' Sensory Test, which is intended for the early phase of a tomato breeding programme. A large number of individual plants of a diverse set of 32 crosses in the F_2 generation and the corresponding 12 parental cultivars were investigated. Using the Breeders' Sensory Test, 910 individual plants were evaluated. Additionally, TSS, TA, dry matter content (DM) and volatile compounds were analysed and correlated with the sensory data. The experiments were conducted in two largely contrasting cultivation systems, that is, organic and hydroponic. With this experimental set-up, (i) the effectiveness of the Breeders' Sensory Test and (ii) the potential for the selection of flavour-related traits in the first segregating generation were studied.

2 | MATERIALS AND METHODS

2.1 | Plant material

Twelve indeterminate parental cultivars of tomato (*Solanum lycopersicum* L.), open pollinated cultivars and F_1 hybrids (Table 1), with a wide variation of quality traits (TSS, TA, sweetness and sourness) and fruit yield, were chosen on the basis of a previous study (Chea et al., 2021). In winter 2015/2016, the parental cultivars were crossed at the University of Göttingen, Section of Genetic Resources and Organic Plant Breeding, Germany. F_2 seeds were produced one year later in the greenhouse. A total of 32 crosses (Table S1) were chosen for the present study. In 2017, the F_2 plants were cultivated in two cultivation systems, an organic low-input and a hydroponic production site, together with the parental cultivars. Ten F_2 plants per cross and six to 20 parental plants, based on their frequency as a parental cultivar, were grown. The F_2 plants were planted in groups of five individuals, separated by the corresponding parents with two to three plants.

TABLE 1 Parental cultivars used to produce the 32 crosses

Cultivar	Fruit type[a]	Fruit colour	Breeder	Year of release
Auriga	Salad	Orange	Saatzucht Quedlinburg	1980[b]
Black cherry	Cocktail	Red-brown	Reinsaat KG	2009[c]
Bocati F_1	Salad	Red	Enza Zaden	2011[c]
Cappricia F_1	Salad	Red	Rijk Zwaan	2009[c]
Goldita	Cocktail	Orange	De Ruiter/Arche Noah	1997[d]
Green Zebra	Salad	Green, yellow stripes	Wagner	1972[e]
Lyterno F_1	Salad	Red	Rijk Zwaan	2010[c]
Primabella	Cocktail	Red	OOTP	2012[c]
Resi	Cocktail	Red	OOTP	2010[c]
Roterno F_1	Salad	Red	Rijk Zwaan	2007[c]
Sakura F_1	Cocktail	Red	Enza Zaden	1999[c]
Supersweet 100 F_1	Cocktail	Red	Syngenta seeds	1992[b]

Abbreviation: OOTP, Organic Outdoor Tomato Project (Zörb et al., 2020).
[a]Cocktail ≤52 g and salad >52 g (classification according to Erika et al., 2020).
[b]CPVO (2021).
[c]European Commission (2021).
[d]An inbred on-farm selection derived from the original hybrid cultivar was used (Arche Noah, personal communication, February 27, 2015).
[e]T. Wagner (personal communication, June 13, 2016).

2.2 | Organic field trial

The organic low-input trial was located at Reinshof experimental farm, Göttingen, Germany (51°30′15.4″ N, 9°55′16.3″ E). Tomato plants were grown under a well-ventilated rainout shelter to exclude major pathogens that are relevant in greenhouses and polytunnels (*Cladosporium fulvum* Cooke) or outdoors (*Phytophthora infestans* (Mont.) de Bary). Seeds were sown in 96 Quick pot trays in Bio-Traysubstrat (Klasmann-Deilmann, Geeste, Germany) in the greenhouse (23°C day/18°C night, photoperiod of 16 h) in Week 14. After 19 days, plants were transplanted into 1.1-L pots filled with Bio-Kräutererde (Klasmann-Deilmann, Geeste, Germany). In Week 21, the plants were transferred to the field. All plants were cultivated with 0.5 m between plants within the row and 1 m between rows. No fertilizer or plant protection was applied during the cultivation period. For irrigation, a drip system was used. Further details of the growing conditions and the soil analysis are summarized in Tables 2 and S2.

TABLE 2 Comparison of the two cultivation systems

	Hydroponic trial	Field trial
Location	Osnabrück, Germany	Reinshof, Göttingen, Germany
Cultivation system	Greenhouse, single glazed, conventional	Rainout shelter, greenhouse film euro 4, organic low input
Growing material	Rock wool	Soil (silty loam)
Plant distance within rows	0.25 m	0.5 m
Plant distance between rows	0.5 m between double rows; 1-m path width	1 m
Climatic conditions during harvest period	Minimum 19°C day/18°C night	See Figure S1
Fertilization	Nutrient solution with electric conductance of 3 to 5 mS	No fertilization. Soil nutrient availability according to Table S2
Maintenance	Weekly, pruning	Weekly, pruning
Irrigation	Drip system including fertilization (amount regulated according to the sum of irradiation)	Drip system (total 166 L m^{-2})

2.3 | Hydroponic trial

Plants were cultivated under conventional hydroponic conditions at the Osnabrück University of Applied Sciences, Department of Horticultural Production, Germany. In Week 19, seeds were sown in 77 Quick pot trays in Seedlingsubstrat (Klasmann-Deilmann, Geeste, Germany) in the greenhouse (20°C day/20°C night, daylight). Plants were transferred to Grodan cubes (rock wool cubes, Roermond, the Netherlands) after 14 days and, subsequently, placed on Grodan slabs in the greenhouse (19°C day/18°C night, daylight) in Week 25. The cultivation system was designed with double rows (0.5-m distance), 0.25 m between plants and 1 m between rows. Depending on solar irradiation and the state of development, a nutrient solution was applied with irrigation, prepared according to de Kreij et al. (2003). The targeted electric conductance was 3 to 5 mS.

2.4 | Harvest and sampling processing

Fully mature fruits were harvested in the organic field trial in Week 33 and in the hydroponic trial in Week 35 for analyses. Up to 99 samples per day were evaluated with the Breeders' Sensory Test and processed for physicochemical and volatile measurements. The Breeders' Sensory Test was conducted with mixed samples of up to four fresh tomatoes. On the same day, up to 10 fruits per sample were processed for aroma extraction or prepared for the physicochemical measurements of TSS, TA, DM and pH value and subsequently frozen at −20°C for the analyses.

2.5 | Breeders' Sensory Test

The Breeders' Sensory Test was conducted by a team of two to three persons. This team was trained for four weeks before the first evaluation on eight dates (16 h in total). A scale from 1 to 9 was developed for sweetness, sourness, tomato aroma (tomato typical aroma) and total aroma (Supporting Information S1). Tomato aroma reflects the perception of taste associated with tomatoes and is described as sweet, fruity, green grassy, ripe and sour (Hongsoongnern & Chambers, 2008). A special focus was given to the differentiation between tomato aroma and total aroma and between sweetness and tomato aroma. Total aroma was defined as the sum of tomato aroma and further aroma attributes like berry or smoky. The team was trained to handle a large number of samples. During this training period, a protocol for scoring was established (Supporting Information S1). Four standard cultivars with low, medium and high scores for sweetness, sourness, total aroma and tomato aroma were defined. Each evaluation started by tasting these standard cultivars, followed by tasting three to five random samples and discussing the scores. Ideally, each expert received four pieces from different fruits for the evaluation of sweetness, sourness, total aroma and tomato aroma on a scale from 1 to 9 with increments of 0.5 (1 = not detectable and 9 = highest intensity). In addition, special aroma components, firmness and juiciness, were noted if they attracted attention. Samples were randomized (double blind) and served on transparent plastic trays. The test was conducted in a calm room during the day. Tap water, herbal tea, white and crisp bread, and yoghurt were available for neutralization. Breaks were taken regularly in accordance with the needs of the team, including 1-h-long break halfway through all daily samples.

2.6 | Physicochemical and volatile measurements

All analyses were carried out at the University of Göttingen, Division Quality of Plant Products, Germany. The TSS, TA and DM analyses were performed as described in Kanski et al. (2020). The pH value was recorded at the beginning of the TA measurement with a pH electrode (pH titrator Titroline 96, SCHOTT AG, Mainz, Germany). The measurements of the volatile compounds were conducted according to Kanski et al. (2020). For evaluation, the 18 identified volatile compounds were used. The calculation was done as described in Zhang et al. (2015) with 1-octanol as the internal standard. The relative concentration in relation to 1-octanol was expressed in ng ml^{-1} sample.

2.7 | Statistical analyses

For each cross, the coefficient of variation (CV) of F_2 plants (genetic and environmental variance) was compared with the mean CV of both parents (environmental variance) to evaluate the efficiency of (early) selection with the Breeders' Sensory Test. Phenotypic correlations were estimated by Spearman's correlation coefficient. Welch's *t* test was conducted to compare the two cultivation systems. A significance test was only performed for the parental cultivars because F_2 plants were not identical in both environments. Phenotypic data for physicochemical and volatile measurements were further analysed, on the basis of the following linear model:

$$x_{ij} = \mu + C_i + E_j + \varepsilon_{ij},$$

where x_{ij} designates the observed phenotypic value, μ the intercept term, C_i the effect of the *i*th cultivar, E_j the effect of the *j*th environment (cultivation system) and ε_{ij} the residual effect. For sensory attributes, the model was extended by the factor person:

$$x_{ijk} = \mu + C_i + E_j + P_k + CE_{ij} + CP_{ik} + EP_{jk} + \varepsilon_{ijk},$$

where x_{ijk} indicates the observed phenotypic value, μ the intercept term, C_i the effect of the *i*th cultivar, E_j the effect of *j*th environment, P_k the effect of the *k*th person, CE_{ij} the *ij*th effect of the cultivar–environment interaction, CP_{ik} the *ik*th effect of the cultivar–person interaction, EP_{jk} the effect of the *jk*th environment–person interaction and ε_{ijk} the residual effect. The effect of person and all interactions with this effect was treated as random; all other effects were treated as fixed. For physicochemical measurements and volatile compounds, *p* values for each source of variation were obtained by carrying out an analysis of variance. Sensory attributes were analysed using the lmerTest R package (Kuznetsova et al., 2017). The model was fitted using restricted maximum likelihood. Fixed effects were tested using an *F* test with Kenward–Roger approximation for degrees of freedom and random effects with a likelihood ratio test. For the estimation of broad-sense heritability, variance components were determined with the restricted maximum likelihood (REML) method using a random model. Broad-sense heritability was estimated as follows:

- Physicochemical measurements and volatile compounds: $H^2 = \frac{V_C}{V_C + \frac{V_\varepsilon}{n_E}}$.
- Sensory attributes: $H^2 = \frac{V_C}{V_C + \frac{V_{CP}}{n_P} + \frac{V_{CE}}{n_E} + \frac{V_\varepsilon}{n_{PE}}}$, with variance components of the genetic (V_C), cultivar–person (V_{CP}), cultivar–environment (V_{CE}) and residual (V_ε) effects, number of persons (n_P), environments (n_E) and persons × environments (n_{PE}). All statistical analyses were carried out in the R programming environment (R Core Team, 2021).

3 | RESULTS

3.1 | Correlation of TSS, TA and volatiles with attributes from the Breeders' Sensory Test

The correlation between the physicochemical traits TSS and TA and the corresponding sensory attributes sweetness and sourness was highly significant ($p = .001$) for both the hydroponic and organic cultivation systems (Figure 1). The correlation for TSS and sweetness was higher ($r_{\text{hydroponic}} = .644$ and $r_{\text{organic}} = .699$) than that for TA and sourness ($r_{\text{hydroponic}} = .535$ and $r_{\text{organic}} = .441$).

Figure 2 shows the correlation between sensory attributes, physicochemical measurements and some key aroma volatiles for both cultivation systems. Benzaldehyde, 2-phenylethanol and phenylacetaldehyde were significantly positively correlated ($p = .001$) with sweetness ($r = .49$, $r = .38$ and $r = .12$), tomato aroma ($r = .5$, $r = .41$ and $r = .15$) and total aroma ($r = .47$, $r = .3$ and $r = .11$; phenylacetaldehyde: $p = .01$). Benzaldehyde ($p = .001$), 2-phenylethanol ($p = .01$) and phenylacetaldehyde ($p = .01$) showed significant negative correlations with TA ($r = -.13$, $r = -.10$ and $r = -.09$). 2-Isobutylthiazole had no significant correlations with any of the sensory attributes.

3.2 | Selection for flavour in the F_2 generation

The CV of F_2 plants was mostly higher than those for the parental plants for all sensory and physicochemical traits in both cultivation systems (53.8% to 96.9%), except for pH in the hydroponic cultivation system (42.3%; Figure 3 and Table S3). The CV ratios of the F_2 plants and the mean of the parental CVs were similar in the organic and hydroponic cultivation systems, except for sweetness (Figure 3a) and pH (Table S3). For sweetness, the percentage of crosses with a higher CV of the F_2 plants than of the parental mean was larger in the organic cultivation system (90.6%) than in the hydroponic system (61.5%). This ratio was lower for sourness than for the other traits in both cultivation systems (53.8% and 59.4%, Figure 3c and Table S3). For the two volatile compounds, benzaldehyde and 2-phenylethanol, which showed a positive correlation with the sensory attributes, the CV of the F_2 plants exceeded the mean of the parental CV in most cases (69.2% to 90.6%; Figure 4a,b and Table S3). Similar observations were made for methyl salicylate in both cultivation systems (69.2% to 93.8%); neral in the hydroponic cultivation system (65.4%); and hexanal, 6-methyl-5-hepten-2-one, hexanol, 2-isobutylthiazole and β-ionone in the organic cultivation system (≥75.0%). For other volatiles, the ratio between the CV of the F_2 plants and the mean CV of the parents was lower (<65%, Table S3).

3.3 | Effect of cultivar and cultivation system and heritability of quality parameters

For all sensory attributes, the effect of the cultivar was highly significant ($p = .001$, Table 3) and larger than the effect of the person. The effect of the cultivar was higher for total aroma than for sweetness, sourness and tomato aroma. Except for sourness, the environmental effect was significant ($p = .05$) and larger than the effect of the person. The effect of the person was not significant for all sensory attributes. Heritability for all sensory traits was high (≥0.75). Among the physicochemical traits, TSS and DM showed a significant effect of the cultivar ($p = .001$), whereas this effect was not or only slightly significant for pH, TA and TSS/TA (Table 4). There was a highly significant effect of the environment ($p = .001$; DM $p = .01$) on these traits. For pH and TSS/TA, the effect of the environment was considerably higher than the effect of the cultivar. Accordingly, TSS/TA and pH showed a low heritability (0.25). Heritability was high for TA, TSS and

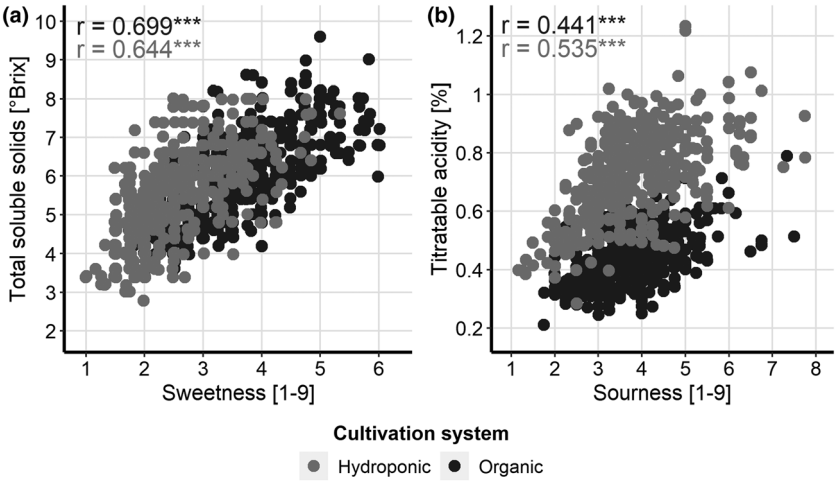

FIGURE 1 Correlation between (a) sweetness and total soluble solids and (b) sourness and titratable acidity with Spearman correlation coefficients (r) of F_2 plants and parental cultivars in two contrasting cultivation systems ($n_{\text{hydroponic}} = 428$ and $n_{\text{organic}} = 450$). ***Significant at $p = .001$

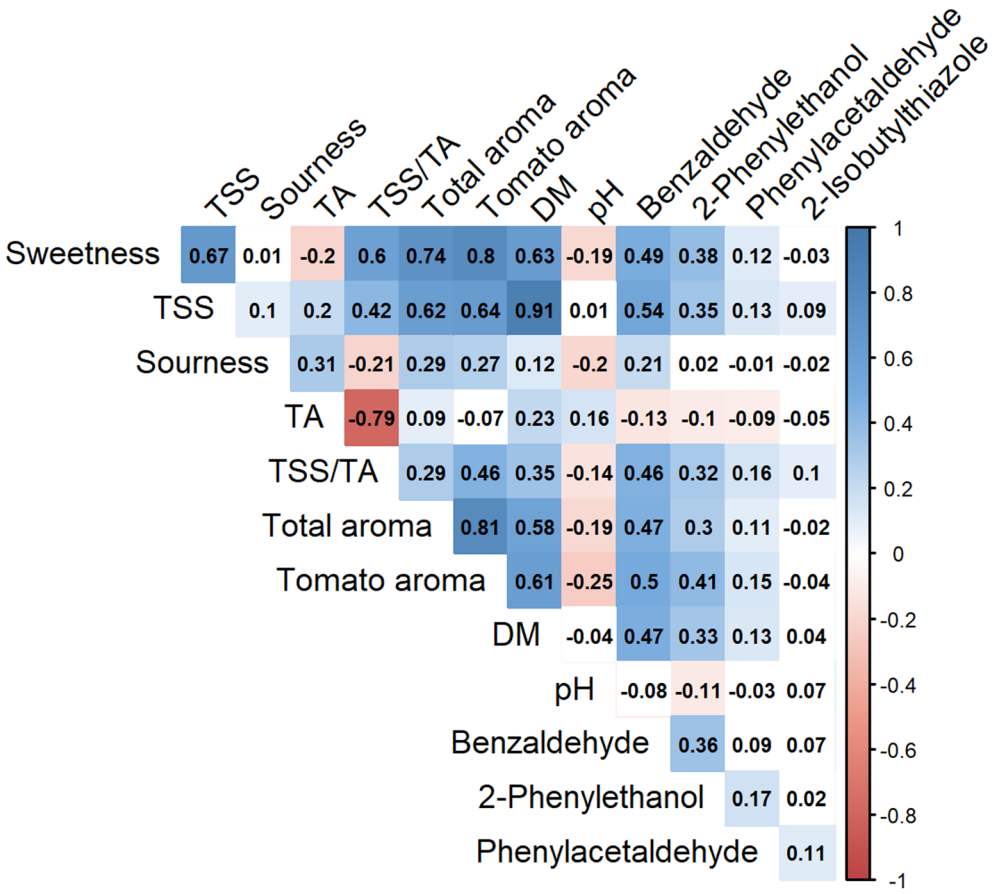

FIGURE 2 Spearman correlation coefficients for sensory attributes, physicochemical measurements and selected key aroma volatiles ($n = 851$) analysed in two cultivation systems; significant positive correlations are shown in blue and significant negative correlations in red with $p = .01$. DM, dry matter; TSS, total soluble solids; TA, titratable acidity [Color figure can be viewed at wileyonlinelibrary.com]

DM (≥0.77). Hexanal, benzaldehyde, β-damascenone, Z-geranylacetone and 2-phenylethanol showed a significant effect of the cultivar and cultivation system ($p = .05$), whereas for 2-phenylethanol, the effect of the cultivar was higher than that of the environment. Heritability for these aroma volatiles was high (0.71 to 0.89). The effect of the cultivar and the heritability was zero for 1-penten-3-one, Z-3-hexenol and phenylacetaldehyde. For all other volatile compounds, heritability ranged from 0.13 to 0.82. The comparison of the two cultivation systems showed significant differences between most parameters (Table 5). According to Welch's test, no significant difference was observed between the two cultivation systems for sourness and the volatile compounds hexanol, 2-isobutylthiazole and phenylacetaldehyde. The relative concentration of 6-methyl-5-hepten-2-one was significantly higher in the hydroponic cultivation system than in the organic cultivation system, whereas the other volatiles showed inverse results.

4 | DISCUSSION

4.1 | Correlation of TSS, TA and volatiles with attributes from the Breeders' Sensory Test

The accuracy of the Breeders' Sensory Test was examined by correlating the physicochemical traits TSS and TA with sweetness and sourness scores of the Breeders' Sensory Test. Correlations between TSS and sweetness and between TA and sourness were highly significant ($p = .001$) (Figure 1). This shows that the Breeders' Sensory Test was able to effectively describe the amount of sugars and acids, which are decisive characteristics for the flavour of tomatoes.

In several studies, sensory descriptors have been linked to physicochemical measurements and aroma volatiles to identify relationships and the contribution of individual chemicals to tomato flavour (Carli et al., 2009; Tikunov et al., 2020; Zanor et al., 2009). The TSS/TA ratio showed that a significant positive correlation with sweetness and TA was significantly correlated with sourness (Figure 2), both in accordance with Tandon et al. (2003) and Baldwin et al. (2015). In addition to sugars and acids, aroma volatiles are the most important components that greatly influence the perceived flavour of tomatoes (Zanor et al., 2009). In the review by Klee and Tieman (2018), 20 volatiles were listed that correlated positively with consumer preferences, including benzaldehyde, 2-phenylethanol, phenylacetaldehyde and 2-isobutylthiazole, which were focused on in the present study. 2-Phenylethanol and phenylacetaldehyde were positively correlated with all sensory attributes except sourness (Figure 2). Tikunov et al. (2020) also observed a positive response of 2-phenylethanol to sweet taste, aroma and aftertaste. Zanor et al. (2009) expected that 2-phenylethanol has a positive synergistic effect with sugars, which leads to the perception of an even sweeter flavour. Tandon et al. (2003) showed that 2-isobutylthiazole was positively correlated with fruity aroma and sweetness, whereas the present study could not find any significant correlation with the analysed

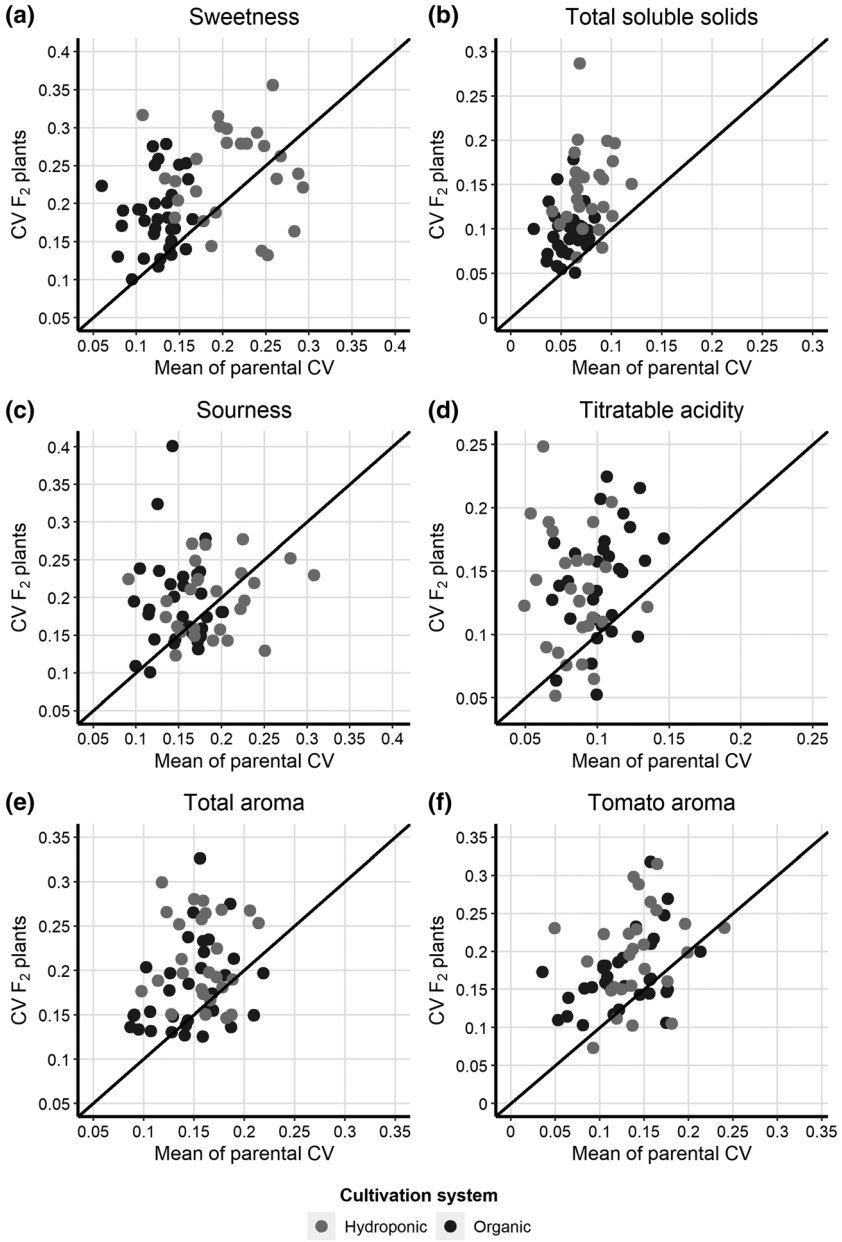

FIGURE 3 Coefficient of variance (CV) of F_2 plants ($n_{hydroponic} = 26$ crosses and $n_{organic} = 32$ crosses) and the mean CV of the respective parents for (a) sweetness, (b) total soluble solids, (c) sourness, (d) titratable acidity, (e) total aroma and (f) tomato aroma in two cultivation systems. CV F_2 plants = genetic + environmental variance; mean of parental CV = environmental variance

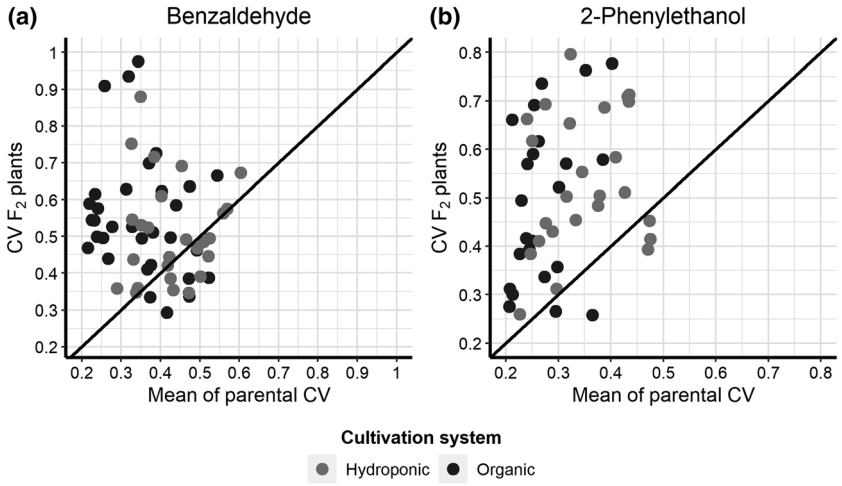

FIGURE 4 Coefficient of variance (CV) of F_2 plants ($n_{hydroponic} = 26$ crosses and $n_{organic} = 32$ crosses) and the mean CV of the respective parents for the aroma volatiles (a) benzaldehyde and (b) 2-phenylethanol in two cultivation systems. CV F_2 plants = genetic + environmental variance; mean of parental CV = environmental variance

TABLE 3 Mean values and ±standard deviation (SD) of all sensory traits and variance components for the effects of cultivar (V_C), person (V_P), environment (V_E), cultivar–person interaction (V_{CP}), cultivar–environment interaction (V_{CE}), person–environment interaction (V_{PE}), and residuals (V_ε) and broad-sense heritability (H^2) for the parents ($n = 11$) in two cultivation systems

Trait	Mean	SD	V_C	V_P	V_E	V_{CP}[a]	V_{CE}	V_{PE}	V_ε	H^2
Sweetness (1–9)	2.98	±1.41	0.579***	0.072	0.853*	0.000	0.137**	0.037	0.105	0.87
Sourness (1–9)	3.60	±1.12	0.643***	0.081	0.030	0.008	0.350***	0.050	0.206	0.75
Total aroma (1–9)	3.64	±1.27	1.149***	0.053	0.625*	0.010	0.038	0.011	0.126	0.96
Tomato aroma (1–9)	3.06	±1.01	0.507***	0.100	0.566*	0.043	0.057*	0.012	0.076	0.90

[a]Causes singularity in the analysis for sweetness but was kept in the model because it did not change the model results.
*Significant at 0.05 level.
**Significant at 0.01 level.
***Significant at 0.001 level.

TABLE 4 Mean values and ±standard deviation (SD) of physicochemical measurements and volatile compounds (ng ml^{-1} sample) and variance components for the effects of cultivar (V_C), person (V_P), environment (V_E), and residuals (V_ε) and broad-sense heritability (H^2) for the parents ($n = 11$) in two cultivation systems

Trait	Mean	SD	V_C	V_E	V_ε	H^2
TSS (°Bx)	5.55	±1.35	1.591***	0.315***	0.155	0.95
pH	4.53	±0.11	0.001	0.014***	0.004	0.25
TA (%)	0.56	±0.19	0.011*	0.035***	0.006	0.77
TSS/TA	1.83	±3.62	0.307	21.065***	1.800	0.25
DM (%)	6.94	±1.67	2.339***	0.399**	0.353	0.93
1-Penten-3-one	0.13	±0.08	0.000	0.008***	0.002	0.00
Hexanal	86.04	±66.10	2037.461***	3638.221***	523.555	0.89
Z-3-Hexenal	1.05	±2.01	0.690	0.570	3.067	0.31
E-2-Hexenal	16.24	±12.53	8.204	161.382***	64.603	0.20
6-Methyl-5-hepten-2-one	22.51	±18.71	153.857	83.994*	159.593	0.66
Hexanol	2.25	±1.18	0.538	0.000	0.873	0.55
Z-3-Hexenol	1.17	±0.94	0.000	1.161***	0.269	0.00
2-Isobutylthiazole	37.16	±23.19	24.482	0.000	308.830	0.61
Benzaldehyde	5.08	±4.28	7.791*	8.628**	6.399	0.71
Phenylacetaldehyde	1.34	±0.48	0.000	0.000	0.229	0.00
Neral	0.53	±0.41	0.100*	0.015	0.065	0.75
Geranial	4.07	±1.98	0.127	4.165***	1.630	0.13
Methyl salicylate	0.69	±0.74	0.140	0.211*	0.305	0.48
β-Damascenone	6.87	±2.79	3.046*	4.830***	2.355	0.72
Z-Geranylacetone	0.19	±0.10	0.004**	0.007***	0.002	0.82
E-Geranylacetone	32.54	±32.06	743.043**	7.344	316.067	0.82
2-Phenylethanol	3.88	±2.63	4.813**	0.812*	1.919	0.83
β-Ionone	6.90	±6.05	12.715	21.807**	13.126	0.66

Abbreviations: DM, dry matter; TSS, total soluble solids; TA, titratable acidity.
*Significant at 0.05 level.
**Significant at 0.01 level.
***Significant at 0.001 level.

sensory attributes. In a study by Zanor et al. (2009), benzaldehyde was the only metabolite that was significantly positively correlated with the typical tomato aroma ($r = .91$). This positive correlation was also observed in the present study ($r = .50$), and benzaldehyde was also significantly positively correlated with total aroma, tomato aroma, sweetness and sourness (Figure 2). In general, selection for aroma volatiles positively linked to flavour intensity and consumer liking remains challenging (Klee & Tieman, 2018). However, recent progress in genomics will lead to a better understanding of the underlying genetics and thus facilitate selection (Tikunov et al., 2020; Zhao et al., 2019).

4.2 | Selection in the F_2 generation by the Breeders' Sensory Test

Due to its complexity, flavour is often assessed in late stages of a breeding programme, when most of the variation is already lost

TABLE 5 Mean values and ±standard deviation (SD) of F_2 plants and parental cultivars and comparison of sensory attributes, physicochemical measurements and volatile compounds (ng ml^{-1} sample) for two cultivation systems ($n_{hydroponic} = 428$ and $n_{organic} = 450$)

	Hydroponic cultivation		Organic cultivation		
Trait	Mean	SD	Mean	SD	*p* value
Sweetness (1–9)	2.59	±0.79	3.60	±0.94	***
Sourness (1–9)	3.75	±1.10	3.73	±0.89	ns
Total aroma (1–9)	3.48	±0.92	4.13	±1.09	***
Tomato aroma (1–9)	2.80	±0.68	3.55	±0.78	***
TSS (°Bx)	5.41	±1.16	5.98	±1.12	***
pH	4.59	±0.20	4.44	±0.24	***
TA (%)	0.72	±0.15	0.44	±0.09	***
TSS/TA	7.65	±1.41	13.72	±2.37	***
DM (%)	6.78	±1.49	7.44	±1.35	***
1-Penten-3-one	0.06	±0.06	0.22	±0.10	***
Hexanal	56.28	±49.13	134.19	±71.55	***
Z-3-Hexenal	0.46	±0.42	1.36	±1.76	***
E-2-Hexenal	8.39	±5.33	27.50	±13.95	***
6-Methyl-5-hepten-2-one	33.04	±31.01	20.28	±17.28	***
Hexanol	2.41	±2.27	2.37	±1.58	ns
Z-3-Hexenol	0.58	±0.64	1.89	±0.88	***
2-Isobutylthiazole	37.99	±31.78	37.83	±25.07	ns
Benzaldehyde	3.04	±2.41	7.35	±5.81	***
Phenylacetaldehyde	1.27	±0.75	1.33	±0.42	ns
Neral	0.45	±0.52	0.63	±0.75	***
Geranial	2.62	±1.16	5.59	±3.84	***
Methyl salicylate	0.39	±0.36	1.24	±1.11	***
β-Damascenone	5.80	±3.64	9.36	±4.30	***
Z-Geranylacetone	0.15	±0.12	0.25	±0.12	***
E-Geranylacetone	23.61	±25.06	30.24	±19.90	***
2-Phenylethanol	3.41	±2.34	4.91	±3.26	***
β-Ionone	3.91	±2.90	11.28	±9.27	***

Note: ns, $p > .05$.
Abbreviations: DM, dry matter; TSS, total soluble solids; TA, titratable acidity.
*Significant at 0.05 level, Welch's test.
**Significant at 0.01 level, Welch's test.
***Significant at 0.001 level, Welch's test.

(Wang & Kays, 2003). This loss of favourable alleles might be reduced drastically by early selection, ideally starting in the F_2 generation. However, the usefulness of F_2 selection for polygenic traits is still questionable (Liu & Constable, 2017) because the genetic differences between single plants are masked by large environmental effects. In this study, the variation between F_2 plants of a cross, which is due to genetic and environmental causes, is compared with the variation between genetically identical plants of the parents, which is purely environmental. A coefficient of variation (CV) between F_2 plants that is larger than the CV between parental plants is an indicator that selection between F_2 plants would be efficient. The CV of the F_2 plants was higher than the mean of the corresponding parental CV for most crosses for sweetness, total aroma and tomato aroma in both cultivation systems (Figure 3 and Table S3). This was also observed for sourness but less notably. Early selection for total aroma and tomato aroma, both complex traits and only quantifiable with a sensory method, was possible in both cultivation systems. Similar observations were made for physicochemical traits except pH in the hydroponic cultivation system. These results indicate good chances to improve sensory characteristics of fresh tomatoes by the Breeders' Sensory Test, independent of the cultivation system. de Bruyn et al. (1971) also concluded by comparing F_2 plants with their F_3 offspring that selection for sensory traits (taste intensity, taste quality and sweet–acid ratio), which were assessed by a panel consisting of five persons, was possible at least in some selection environments. Studies on other crops (e.g., parsnip and apple) have shown that small panels can successfully improve sensory attributes (Hampson et al., 2000; Horneburg et al., 2009). Additionally, the assessment of TSS and TA in early generations might support the improvement of flavour. Nevertheless, these analytical measurements reflect the

perceived sweetness and sourness only to some extent. Moreover, aroma is an essential component of flavour, which can only be assessed with a sensory panel (Causse et al., 2003; Piombino et al., 2013). An analytical assessment of aroma volatiles in early generations is far beyond the capacity of practical breeding programmes (Klee & Tieman, 2018; Tieman et al., 2017).

4.3 | Heritability of quality parameters and effect of the cultivation system

The heritability of 0.75 for sourness and the high heritability (≥0.87) for the other sensory attributes support the high quality of the Breeders' Sensory Test (Table 3). The heritability was high for TSS, TA and DM (≥0.77) and low for pH and TSS/TA (both 0.25, Table 4). For physicochemical traits, the heritability estimated by Saliba-Colombani et al. (2001), Xu et al. (2013), Ruggieri et al. (2014) and Bauchet et al. (2017) for recombinant inbred lines or diverse material was in a similar range (0.51–0.87), except for a higher heritability for pH compared with our study. The heritability for volatile compounds ranged from 0 to 0.89 in the current work. Heritability estimated for volatile compounds ranged from 0.14 to 0.88 in a study by Saliba-Colombani et al. (2001), from 0.30 to 0.76 in Zhang et al. (2016) and from 0.29 to 0.79 in Bauchet et al. (2017). This large variation indicates that estimates of heritability largely depend on the genetic material used and the impact of the environment and agronomic practices on the profile of volatile compounds, as also indicated by Bauchet et al. (2017).

This environmental influence was also indicated by the comparison of two cultivation systems, which showed significant differences in most quality parameters (Table 5). Mean values for most traits were significantly higher in the organic cultivation system, except pH, TA and 6-methyl-5-hepten-2-one, which were higher in the hydroponic system. Quality parameters and flavour attributes in general are significantly influenced by cultivar, year and agronomic practices, whereas no general advantage was found for organic or conventional production according to several studies (Aldrich et al., 2010; Pieper & Barrett, 2009).

In summary, the Breeders' Sensory Test, conducted by a small team and with a high number of small sample sizes, showed significant correlations with TSS and TA. The method was applied in the F_2 generation on a total of 910 individual plants in two contrasting production systems. For most flavour-related traits, the genetic plus environmental variance (CV of F_2 plants) was higher than the environmental variance (mean of parental CV) for most crosses. Thus, the Breeders' Sensory Test is a promising tool to select for flavour during the early breeding stages. Selection between single plants in the F_2 generation is expected to be successful regardless of the selection environment.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

B. H. developed the first version of the Breeders' Sensory Test and B. H. and H. C. B. the general approach. J. H., L. K., H. K., B. H., H. C. B., E. P. and M. N. planned and designed the experiment. J. H., L. K. and H. K. performed the experiment. J. H. and L. K. analysed the data. L. K., J. H., M. N., E. P., H. C. B. and B. H. wrote the manuscript. All authors read and approved the manuscript.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Julia Hagenguth https://orcid.org/0000-0001-9159-9327

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SUPPORTING INFORMATION

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Supplementary Materials

For Breeders' Sensory Test: A new tool for early selection in breeding for tomato (Solanum lycopersicum) flavour

Table S1. 32 crosses analysed with the Breeders' Sensory Test, physicochemical measurements, and for volatile compounds in two contrasting (organic, hydroponic) cultivation systems

Cross
Black Cherry x Resi
Auriga x Black Cherry
Black Cherry x Green Zebra
Resi x Auriga
Auriga x Green Zebra
Black Cherry x Primabella
Resi x Primabella
Auriga x Primabella
Green Zebra x Primabella
Goldita x Primabella
Supersweet 100 F_1 x Primabella
Sakura F_1 x Primabella
Bocati F_1 x Primabella
Lyterno F_1 x Primabella
Supersweet 100 F_1 x Black Cherry
Black Cherry x Sakura F_1
Supersweet 100 F_1 x Resi
Sakura F_1 x Resi
Auriga x Supersweet 100 F_1
Green Zebra x Supersweet 100 F_1
Roterno F_1 x Black Cherry
Bocati F_1 x Resi
Resi x Roterno F_1
Auriga x Bocati F_1
Cappricia F_1 x Auriga
Bocati F_1 x Green Zebra
Green Zebra x Roterno F_1
Bocati F_1 x Goldita
Lyterno F_1 x Goldita
Supersweet 100 F_1 x Bocati F_1
Cappricia F_1 x Supersweet 100 F_1
Lyterno F_1 x Supersweet 100 F_1

Table S2. Soil analyses in the organic low-input cultivation system at a soil depth of 0–30 cm

Soil sample	Soil type[1] (Group)	Humus content[1] [%]	Calcium carbonate[1] [pH-value]	Phosphorus[1] (P) [mg/100g]	Potassium[1] (K) [mg/100g]	Magnesium[1] (Mg) [mg/100g]	Total nitrogen[1] (N_{tot}) [%]	Mineral Nitrogen[2] (N_{min}) [kg/ha]
			$CaCl_2$[§]	CAL[§]	CAL[§]	$CaCl_2$[§]	DIN EN 16168, 2012[§]	$CaCl_2$[§]
18.05.2017 start of experiment	uL[†]	2.0	7.2 D[‡]	6.2 C[‡]	9.4 B[‡]	6.6 C[‡]	0.13	57.18

[†]uL = silty loam; [‡]A = very low, B = low, C = to target, D = high, E = very high, F = extremely high; [§]standard methods according to VDLUFA method manual I (ISBN 978-3-941273-13-9); [1]results were measured and provided by Agrolab Agrarzentrum GmbH, Leinefelde-Worbis, Germany; [2]results were measured and provided by University of Goettingen, Division of Agronomy, Goettingen, Germany

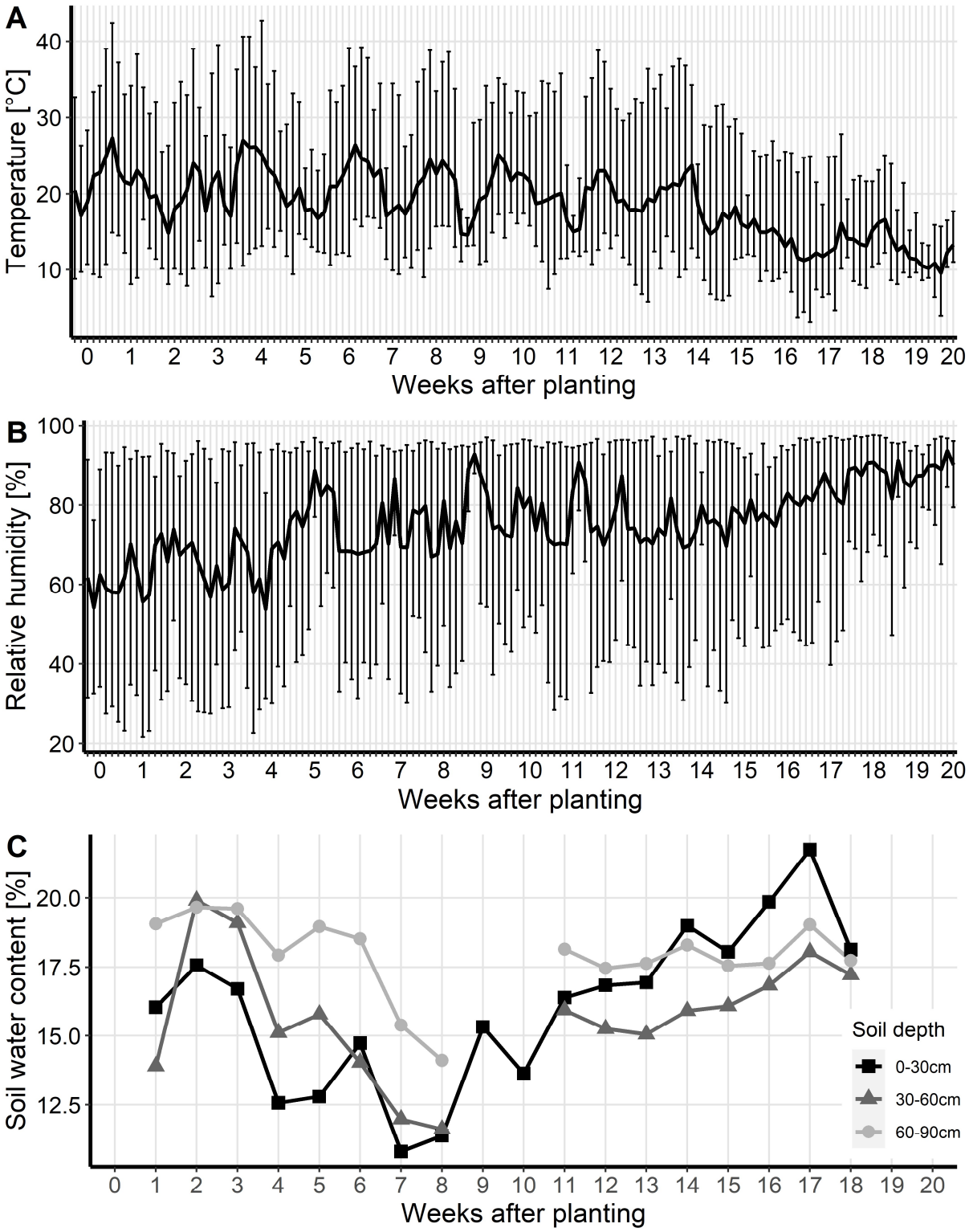

Figure S1. (A) Mean, minimum and maximum temperature per day, (B) mean relative humidity with minimum and maximum per day, and (C) soil water content in the organic cultivation system; temperature and humidity data were recorded every 30 min in about 0.5 m above soil surface using an EBI 20-TH Data Logger (ebro Electronic GmbH & Co. KG, Ingolstadt, Germany); soil water content is expressed as gravimetric moisture content

Table S3: Proportion of crosses with a coefficient of variance (CV) of the F_2 plants larger than the mean CV of the respective parents for both cultivation systems ($n_{hydroponic}$ = 26 crosses, $n_{organic}$ = 32 crosses)

	hydroponic	**organic**
Sweetness	61.5	90.6
Sourness	53.8	59.4
Total aroma	88.5	71.9
Tomato aroma	76.9	81.3
TSS[†]	96.2	96.9
pH	42.3	68.8
TA[‡]	80.8	81.3
TSS/TA	92.3	90.6
DM[§]	88.5	78.1
1-Penten-3-one	42.3	46.9
Hexanal	53.8	78.1
Z-3-Hexenal	50.0	46.7
E-2-Hexenal	30.8	59.4
6-Methyl-5-hepten-2-one	42.3	93.8
Hexanol	61.5	75.0
Z-3-Hexenol	61.5	34.4
2-Isobutylthiazole	57.7	87.5
Benzaldehyde	69.2	81.3
Phenylacetaldehyde	34.6	37.5
Neral	65.4	46.9
Geranial	53.8	46.9
Methyl salicylate	69.2	93.8
ß-Damascenone	61.5	46.9
Z-Geranylacetone	46.2	59.4
E-Geranylacetone	42.3	62.5
2-Phenylethanol	88.5	90.6
ß-Ionone	61.5	78.1
Total yield[††]	NA	78.1
Fruit weight[††]	NA	93.8

[†]TSS = Total soluble solids, [‡]TA = Titratable acidity, [§]DM = Dry matter

[††]Mature fruits were harvested every second week, starting at eight weeks after planting. Fruits from each single plant were counted and weighed to obtain average fruit weight and total yield. Data are only available for the organic cultivation system.

Supplement 1 - Guideline for the Breeders' Sensory Test

The Breeders' Sensory Test is used to evaluate a high number of small samples with a small team. In this guideline, the general procedure (1) is described, followed by additional instructions (2), a description of assessed sensory attributes (3), recommendations regarding standards (4) and an example of a scoring sheet (5).

1. Procedure

1. Training period for new team members or refreshment for experienced persons. Topics are i) scoring scale for all assed traits, ii) definition of traits, iii) differentiation of traits e.g. tomato typical aroma vs. total aroma, sweetness vs. aroma, iv) handling large numbers of samples
2. Prepare randomization (ideally double blind, at least blind) and labels (harvest boxes and samples) in advance
3. On the preceding day, preparation of the room for sensory evaluation (calm; windows for daylight, northern exposition; moderate temperature; ventilation): arrangement of tables and chairs for relaxed and efficient assessment
4. Provide all necessary material to each individual tester: trays, petri dishes to serve the samples, plates, knifes, teaspoons, non-transparent cups, material for neutralization, paper towels, scoring lists (paper and digital), guideline, paper, pencil, pencil sharpener, rubber, standard cultivars, random samples to warm up and for calibration, bucket for compost, box for used plates
5. Harvest fruits in labelled boxes: fully ripe, on each day of an assessment
6. Order samples according to randomization
7. An extra person prepares samples fresh (if possible, four pieces from four different fruits per sample); each sample is labelled. Note number of fruits per sample. The size of suitable pieces varies with fruit size: cocktail tomatoes 1/2 or 1/4, salad tomatoes 1/8 to 1/12 of a fruit
8. During preparation of the first samples, the sensory team tests the standard cultivars and three to five random samples, scorings will be discussed
9. Sensory assessment with data collection on laptop, hard copy as backup
10. Short breaks after about 20 to 30 samples. Larger blocks in the morning than in the afternoon, adapted to the abilities of the testers. Standard cultivars and random samples are always available for recalibration.
11. One large break of about one hour after a bit more than 50% of the samples
12. As needed: preparation of room and material for the next day

2. Instructions

It is important to work with alert senses. Avoid strong spices already on the day before an assessment. Do not use fragrance, aftershave, or perfumed soap. Avoid being hungry or saturated.

Samples can be spit out directly after the test is completed to avoid a sense of satiety (or other problems with the stomach) resulting in reduced awareness.

Serve samples from different fruits, so that samples with off-taste (e.g. overripe, lesions) can be recognized and discarded. This should occur only rarely because samples will be prepared with care.

The size of the slices influences the perceived intensity of the scored traits. Knifes are available to adapt the size (see above) of a sample.

For neutralization tap water and weak herbal or black tea at moderate temperature, white and crisp bread as well as plain yoghurt are available. Take short brakes according to your personal needs.

3. Assessed traits

a) Quantitative attributes

Attribute	Description	Instructions	Scale
Sweetness	Taste associated with the impression of sweetness.	Chew a mix of all fruit parts; do not crunch seeds because of their often bitter taste.	1 - 9
Sourness	Taste associated with the impression of sourness.	Chew a mix of all fruit parts; do not crunch seeds.	1 - 9
Total aroma	The sum of tomato typical aroma and additional aroma components incl. off-taste.	Chew a mix of all fruit parts; do not crunch seeds. Distinguish aroma from sweetness and sourness.	1 - 9
Tomato typical aroma	Aroma associated with tomato.	Chew a mix of all fruit parts; do not crunch seeds.	1 - 9

1 indicates the lowest level of the trait scored (e.g. no sweetness) and 9 the highest level (e.g. extremely sweet)

Often perceptions of taste and flavour develop dynamically (e.g. brief and intense, long-lasting, slow emergence during chewing). Score the overall impression.

b) Qualitative attributes

Special aspects and aftertaste including bitterness, firmness, and juiciness will be recorded in a separate column if they can be named, even though they will be included in the scoring of total aroma. Special aroma compounds that were observed during the training period might be added to quantitative attributes.

Examples

- Spicy, green-grassy, smoked
- Fruity, banana-like, sweetish
- Musty, fermented, unpleasant
- Chemical, perfumed, metallic
- Floury, watery, chewing gum like

Yellow shoulder, a physiological deficiency at the stem end of the fruit, will be removed but noted with YS in the corresponding column.

c) Other attributes

These attributes were not included in the present study, but might be relevant for some objectives or later breeding generations.

Attribute	Description	Instructions	Scale
Firmness of the pericarp	Resistance during initial chewing. Very overripe fruits are scored as 1.	See above.	1 - 9
Firmness of the epidermis	Degree to which the epidermis remains intact during chewing.	With increasing firmness of the pericarp, the less dominant appears the epidermis.	1 - 9
Juiciness	Amount of liquid expressed during initial chewing. Nearly liquid overripe fruits are scored as 9.	Crush a mix of all fruit parts. See above.	1 - 9

1 indicates the lowest level of the trait scored (very soft, very dry) and 9 the highest level (extreme firm, extreme juicy)

Hints to distinguish firmness of the pericarp and juiciness:

Soft and dry = mealy.
Soft and juicy = overripe, macerating.
Firm and dry = high dry matter, storable.

4. Standard samples

Define standard samples for sweetness, sourness, total aroma and tomato typical aroma that are available during the training period and all assessments. Adapt the scorings of the standard samples within the team due to seasonal changes. Include also a year-round available supermarket tomato with long-shelf life that typically represents the lower range of scorings. Ideally cover the entire scale from 1 to 9 with the different standard samples.
Alternatively, use a wide range of samples to train and standardize traits within the team and/or work with reference substances in specific concentrations such as sucrose solutions for sweetness or citric acid solutions for sourness[1].

5. Example of the scoring sheet

SENSORY ASSESSMENT			**Date:**	**Name:**		
Sample no.	**Sweetness [1-9]**	**Sourness [1-9]**	**Total aroma [1-9]**	**Tomato typical aroma [1-9]**	**Special characteristics incl. off-taste**	**Yellow-shoulder [YS]**
1						
2						
…						

[1]**General instructions:** Sensory analysis - General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors (ISO 8586:2012); Examples for tomato: i) Baldwin, E.A., Goodner, K., Plotto, A., Pritchett, K., & Einstein, M. (2004). Effect of Volatiles and their Concentration on Perception of Tomato Descriptors. Journal of Food Science, 69(8), S310–S318. https://doi.org/10.1111/j.1750-3841.2004.tb18023.x; ii) Hongsoongnern, P., & Chambers, E. (2008). A lexicon for texture and flavor characteristics of fresh and processed tomatoes. Journal of Sensory Studies, 23(5), 583–599. https://doi.org/10.1111/j.1745-459X.2008.00174.x

3 STUDY II: Flavour Improvement in Early Generations of Fresh Market Tomatoes (*Solanum lycopersicum* L.): I. Identification of QTL for Sensory Attributes, Physicochemical Measurements and Volatile Compounds

Julia Hagenguth[1], Larissa Kanski[2], Hannah Kahle[3], Heiko C. Becker[1] and Bernd Horneburg[4, 5]

[1]Division of Plant Breeding Methodology, Department of Crop Sciences, Faculty of Agriculture, University of Goettingen, Carl-Sprengel-Weg 1, 37075 Goettingen, Germany

[2]Division of Quality of Plant Products, Department of Crop Sciences, Faculty of Agriculture, University of Goettingen, Carl-Sprengel-Weg 1, 37075 Goettingen, Germany

[3]Department of Horticultural Production, University of Applied Sciences Osnabrueck, Am Krümpel 31, 49090 Osnabrueck, Germany

[4]Division of Plant Breeding, Section of Genetic Resources and Organic Plant Breeding, Department of Crop Sciences, Faculty of Agriculture, University of Goettingen, Carl-Sprengel-Weg 1, 37075 Goettingen

[5]present address: Faculty of Organic Agricultural Sciences, Section of Organic Plant Breeding and Agrobiodiversity, University of Kassel, Nordbahnhofstraße 1a, 37213 Witzenhausen, Germany

Submitted to *Plant Breeding*.

Production of cuttings

Tomato fruit samples of the mapping population Resi x Auriga

Author contributions:

JH, HB and BH planned and designed the experiment. JH performed the experiment in the organic and HK in the hydroponic cultivation system. LK conducted all physicochemical measurements and the aroma volatile analysis. JH analysed the data, wrote the original draft, and was supervised by HB and BH. All authors reviewed, edited and approved the manuscript.

Abstract

Human sensory analysis is the most appropriate method for assessing the flavour of fresh market tomatoes, but it is very labour and time consuming. Therefore, sensory attributes are often neglected in early generations of breeding programmes and genetic studies, although there is a demand for tomatoes with improved flavour. In this study, the recently developed Breeders' Sensory Test was applied to an F_2 mapping population derived from two parents with superior flavour. Sensory attributes, physicochemical measurements, volatiles and fruit weight were assessed in organic low-input and hydroponic cultivation. A linkage map spanning 1070 cM was developed. In total, 71 quantitative trait loci (QTL) were detected for the means of both cultivation systems, 61 for organic and 46 for hydroponic cultivation. A proportion of 27% of the loci were co-localised between both cultivation systems. Nine distinct QTL clusters for flavour-related traits were identified, including a large cluster on chromosome 6 comprising five sensory and nine volatile QTL. The sensory QTL on chromosomes 2, 5, 6, 10 and 11, partly within clusters, are recommended for marker-assisted selection.

Keywords: tomato, *Solanum*, fruit flavour, QTL mapping, sensory analysis, volatiles

3.1 Introduction

Tomatoes (*Solanum lycopersicum* L., 2n = 2x = 24) are among the most popular vegetables worldwide, consumed fresh and processed, and are an important source of micronutrients, such as antioxidants and vitamins (Klee, 2010; Piombino et al., 2013). Nevertheless, the loss of flavour in fresh market tomatoes is a major cause of consumer complaints (Causse et al., 2010; Colantonio et al., 2022; Folta & Klee, 2016). Flavour results from the interaction of primary and secondary metabolites, and flavour perception is additionally influenced by texture and external properties, such as colour and size (Causse et al., 2003; Klee, 2010; Piombino et al., 2013). Sugars (glucose and fructose) and acids (citric, malic and glutamic acid) are the most important compounds contributing to taste; both are necessary in sufficient quantities and in an appropriate balance (Klee, 2010; Stevens et al., 1977). Aroma volatiles cause the diversity of flavours (Klee, 2010; Klee & Tieman, 2018). The increasing demand for flavourful tomatoes raises the need for breeding high-yielding cultivars with outstanding flavour (Colantonio et al., 2022; Zörb et al., 2020). Nonetheless, improving flavour remains a challenge due to the difficulties in assessing this complex trait, the lack of clear selection criteria, and a negative correlation between quality characteristics and fruit size or yield (Causse et al., 2003; Klee & Tieman, 2013; Klee & Tieman, 2018).

According to Tieman et al. (2017), flavour phenotyping is expensive, limited to a small number of samples and therefore not possible in the first segregating generations of a breeding programme. Sensory analysis by a trained or consumer panel is the best method for evaluating taste and aroma attributes but is not

suitable for breeding purposes (Causse et al., 2001; Causse et al., 2010; Piombino et al., 2013). Thus, the Breeders' Sensory Test (Hagenguth et al., 2022) was introduced. Since simple physicochemical measurements are not sufficient to predict flavour, molecular markers for key aroma compounds and sensory attributes can contribute substantially to flavour improvement (Causse et al., 2003; Klee & Tieman, 2018; Tieman et al., 2017). As simultaneous selection for many molecular markers is challenging (Xu & Crouch, 2008), the number of aroma volatiles contributing to tomato flavour and consumer liking needs to be reduced to a smaller set of primary or secondary metabolites, which is possible, as many volatile compounds are metabolically linked (Klee & Tieman, 2018; Martina et al., 2021; Rambla et al., 2014). An alternative is to develop molecular markers directly for sensory attributes that reflect the perceived flavour. Such quantitative trait loci (QTL) are a promising tool for the preselection of seedlings and thus in reducing the loss of valuable genotypes. Several studies have been conducted to identify genetic regions controlling the quantitative variation of fresh tomato flavour, focusing on primary and secondary metabolites (Martina et al., 2021; Tikunov et al., 2020). Sensory attributes, however, are only considered by a few authors (Causse et al., 2001; Tikunov et al., 2020; Zanor et al., 2009). Tikunov et al. (2020)) identified several QTL for sensory properties, but fewer than for primary metabolites and volatile compounds. One reason for this might be the complexity of flavour perception; many genetically and functionally independent loci are likely to be involved in the variation in sensory attributes (Tikunov et al., 2020). Many studies have worked with genetically distant material, such as crosses between cultivated and wild tomatoes to increase genetic and phenotypic variation. However, studies using modern cultivars are needed for the direct implementation of QTL into practical breeding programmes (Kimbara et al., 2018; Tikunov et al., 2020). In particular, studies using mapping populations developed from a cross between cultivars with superior flavours are missing.

Flavour is not only influenced by genetics but also by the cultivation system and agronomic practices (Beckles, 2012; Causse et al., 2001; Klee, 2010). The majority of greenhouse tomatoes are grown in conventional hydroponic systems, where plants are grown in an inert substrate (Korčok et al., 2021), but the demand for organically produced tomatoes is constantly increasing (Raigón et al., 2022; Willer et al., 2022). QTL might not only be specific for the plant material used but also for the cultivation system. QTL studies are often conducted over several seasons or years (Bauchet et al., 2017; Capel et al., 2015; Zanor et al., 2009) but rarely in different cultivation systems, such as fields and greenhouses, as in Tieman et al. (2006, 2017) and Mathieu et al. (2009). Co-localised QTL detected in contrasting cultivation systems are of special interest for breeding programmes.

To map QTL for superior flavour in organic low-input and hydroponic cultivation, an F_2 mapping population was developed from an intraspecific cross between two high-quality cultivars. The parents are characterised by excellent but contrasting quality attributes and different fruit weights. Sensory

attributes, physicochemical measurements (total soluble solids, pH, titratable acidity, dry matter), volatile compounds and fruit weight were assessed.

3.2 Materials and methods

3.2.1 Mapping population

An F_2 population of 188 individuals, originating from a single F_1 plant, was developed from a cross between the two open-pollinated cultivars 'Resi' (Organic Outdoor Tomato Project, released in 2010, Zörb et al. (2020) and CPVO (2022)) and 'Auriga' (Saatzucht Quedlinburg, released in 1980, CPVO (2022)). Resi, a red cocktail tomato, was chosen for its sweetness, tomato and fruity (named banana-melon) aroma. Auriga, an orange salad tomato, has distinctly sour fruit and a characteristic aroma profile (named orange aroma).

3.2.2 Cultivation systems

The F_2 mapping population and three plants per parental cultivar were phenotyped in an organic low-input system at Reinshof experimental farm (51°30'17.0" N, 9°55'14.5" E), University of Goettingen, Germany, and in hydroponic cultivation at the University of Applied Sciences, Department of Horticultural Production, Osnabrueck, Germany, in 2018. In both cultivation systems, plants were grown in a randomised complete block design with two replications (blocks) surrounded by border plants.

Seeds were sown in trays in Bio-Traysubstrat (Klasmann-Deilmann, Geeste, Germany) in a greenhouse (22°C day/18°C night, 16/8 h) in week 13. Seedlings with fully developed cotyledons were transferred eight days later into QP96 trays (Hermann Meyer, Rellingen, Germany) filled with Bio-Traysubstrat. After another eight days, the seedlings were potted in 1.1 L pots with Bio-Kräutererde (Klasmann-Deilmann, Geeste, Germany). In week 19, three side shoots were taken from each individual plant. The largest one was planted into a 1.1 L pot; the two smaller ones were cut to an equal size and planted in QP96 trays filled with Bio-Traysubstrat.

In the organic cultivation system, the original plants were transferred to the field in week 21 (replication 1) and the largest cuttings one week later (replication 2). Plants were grown in silty loam (Hagenguth et al., 2022) under a well-ventilated rain-out shelter (greenhouse film Euro 4, Folien Bernhard, Dreieich, Germany) to minimise major pathogens that are relevant in greenhouses (e.g. *Cladosporium fulvum* Cooke) and the open field (*Phytophthora infestans* (Mont.) de Bary). Plants were spaced 0.5 m apart within and 1 m between rows. Low-input conditions were defined as no application of fertiliser and moderate irrigation. During the entire growing season, 239 L m^{-2} were irrigated with a drip system. Temperature, relative humidity and soil water content are given in Figure SII.1 and mineral nitrogen in Table SII.1.

In hydroponic cultivation, the two smaller cuttings were transferred to rockwool cubes (10 × 10 × 6.5 cm, Grodan®, Roermond, The Netherlands) in week 22. Three weeks later, Grodan cubes were placed on rockwool slabs (100 × 15 × 7.5 cm, Grodan®, Roermond, The Netherlands) in the greenhouse (19°C day/17°C night; single glazed) in double rows (distance 0.5 m) with 0.36 m between plants and 1 m between double rows. The plants were irrigated with a nutrient solution. This nutrient solution was prepared according to De Kreij et al. (2003). The amount and concentration of nutrients were adapted according to solar irradiation and development stage.

3.2.3 Evaluation of F_2 plants

In the organic trial, mature fruits were harvested, weighted and counted every second week from week 27 onwards. Ideally, four fruits per plant were weighed in the hydroponic trial at weeks 37 and 39 to obtain the average fruit weight. Fruits with blossom end rot were discarded. Fully mature fruits were harvested in the organic cultivation system in week 33 and in hydroponic cultivation in week 36 for sensory, physicochemical and volatile analyses. Up to 82 samples were evaluated and processed each day.

Sensory evaluation

For sensory evaluation, the Breeders' Sensory Test (Hagenguth et al., 2022) was applied by a three-person team. Depending on the range of experience, the team members were trained on two to six dates for four weeks before the evaluation (5 to 12 hours in total). Sweetness, sourness, total aroma, tomato aroma and the special aroma attributes banana, melon, orange, berry, spicy and green (Table 3.1) were evaluated on a scale from 1 to 9 (1 = not perceptible, 9 = maximum intensity).

Table 3.1. Description of sensory attributes as developed for the Breeders' Sensory Test

Attribute	Description	Scale[1]
Sweetness[†]	Taste associated with the impression of sweetness	1–9
Sourness[†]	Taste associated with the impression of sourness	1–9
Total aroma[†]	Sum of tomato aroma and additional aroma components including off-taste	1–9
Tomato aroma[†]	Aroma associated with tomato	1–9
Banana [like] aroma[‡]	Fruity aroma associated with banana; typical for the parent Resi	1–9
Melon [like] aroma[‡]	Fruity aroma associated with honeydew melon; typical for the parent Resi	1–9
Orange [like] aroma	Fruity aroma associated with citrus fruits; typical for the parent Auriga	1–9
Berry [like] aroma	Fruity aroma associated with berry fruits such as gooseberry	1–9
Spicy aroma	Spicy, tangy aroma	1–9
Green aroma	Aroma associated with freshly cut tomato stems, vines or grass and green vegetables	1–9

[1]1 indicates the lowest level of the trait scored (e.g. no sweetness) and 9 the highest level (e.g. extremely sweet)
[†]Hagenguth et al. (2022)
[‡]For the final analysis, the sum of banana and melon aroma was used [2–18]

The following four standard cultivars were used to define the scale of the assessed attributes: mini plum (origin and cultivar unknown, high score for sweetness), a standard salad tomato purchased at a supermarket (low scores) and both parental cultivars. Each evaluation started by tasting these standard cultivars, followed by tasting three to five random samples to calibrate the team on each evaluation day. Samples were double-blind randomised and served on transparent plastic trays (Petri dishes). For neutralisation, tap water, herb tea, brown bread and yoghurt were served. Breaks were regularly taken as required by the team, including a one-hour break after about 50% of the daily samples.

Physicochemical measurements and volatile analysis

The physicochemical measurement of total soluble solids (TSS), titratable acidity (TA), and dry matter (DM) and the analysis of volatiles were performed according to Kanski et al. (2020) at the University of Goettingen, Division Quality of Plant Products, Germany. The pH value was recorded at the beginning of the TA measurement with a pH electrode (pH titrator Titroline 96, SCHOTT AG, Mainz, Germany). The 18 identified volatile compounds were used for evaluation. The relative concentration was expressed in relation to the internal standard 1-octanol in ng mL^{-1} sample according to Zhang et al. (2015).

3.2.4 Phenotypic data analysis

The following linear mixed model was applied to physicochemical measurements, agronomic traits and volatile compounds:

$$x_{ijk} = \mu + G_i + R_j{:}E_k + E_k + GE_{ik} + \varepsilon_{ijk}$$

where x_{ijk} represents the observed phenotypic value, μ the general mean, G_i the effect of the ith genotype, $R_j{:}E_k$ the effect of the jth replication within the kth environment (cultivation system), E_k the effect of the kth environment, GE_{ik} the effect of the genotype-by-environment interaction, and ε_{ijk} the residual effect. For sensory attributes, the model was extended by the factor person:

$$x_{ijkl} = \mu + G_i + R_j{:}E_k + E_k + P_l + GE_{ik} + GP_{il} + EP_{kl} + GEP_{ikl} + \varepsilon_{ijkl}$$

where x_{ijkl} represents the observed phenotypic value, μ the general mean, G_i the effect of the ith genotype, $R_j{:}E_k$ the effect of the jth replication within the kth environment (cultivation system), E_k the effect of the kth environment, P_l the effect of the lth person, GE_{ik} the effect of the genotype-by-environment interaction, GP_{il} the effect of the genotype-by-person interaction, EP_{kl} the effect of the environment-by-person interaction, GEP_{ikl} the effect of the genotype-by-environment-by-person interaction and ε_{ijkl} the residual effect. The effect of genotype, replication and person were treated as random, and the effect of the environment as fixed. Genotypes that were completely missing in one environment were discarded from the analysis of the corresponding trait. The number of F_2 plants used for the different traits is shown in Table SII.2. Linear mixed models were used for the calculation of least square means and the analysis of variance. Least squares were also calculated for the mean values of the

individual cultivation systems based on linear models without the factor environment and corresponding interactions. These analyses and the estimation of heritability were conducted in Plabstat version 3Bp-rep (Utz, 2014). Heritability was estimated according to Knapp & Bridges (1987). Correlations between all phenotypic traits were estimated by Spearman's correlation coefficients in the R programming environment version 4.0.5 (R Core Team, 2021).

3.2.5 Linkage map construction

Leaf samples for DNA extraction were taken from young leaves of the original plants (organic cultivation system) 10 weeks after planting. DNA extraction and genotyping using the Axiom 200K SOLCUC vegetable array (Graner et al., 2017) was conducted by the SGS Institut Fresenius GmbH, TraitGenetics Section (Seeland, Germany). The linkage map was constructed using the R package ASMap version 1.0-4 (Taylor & Butler, 2017). Initially, genotypic data from 188 F_2 plants and 6113 pre-filtered (polymorphic, <10% missing allele scores) SNP markers were available. Preliminary linkage groups were constructed using a threshold of $p = 1 \times 10^{-8}$. Genetic distances were estimated based on the Kosambi mapping function. Subsequently, low-quality markers were dropped according to the following strict filtering protocol to obtain linkage groups with a typical length for tomato: <1% missing allele scores, significant segregation distortion using Bonferroni-adjusted alpha level (0.05/3391), co-located markers and ≥ 1 double crossover. In addition, genotypes were investigated for high genotyping error rates and double crossovers. Finally, 178 F_2 plants and 738 SNP markers were available for the construction of the linkage map. After fixing the linkage groups, markers were reordered using a less strict threshold of $p = 1 \times 10^{-6}$. Linkage groups were assigned to the particular tomato chromosome and oriented according to the physical map. Finally, a framework map aiming at a distance of 5 to 10 cM between markers was constructed, resulting in linkage groups with 12 to 21 markers. To fill the gaps, a few of the discarded markers were reintroduced into the framework map. The final map included 205 markers spanning a total length of 1070.26 cM. The average length of the linkage groups was 89.19 cM and the average distance between markers was 5.2 cM, with a maximum gap of 14.75 cM. A linkage map, including graphical visualisation of QTL positions and intervals, was drawn in MapChart 2.32 (Voorrips, 2002).

3.2.6 QTL mapping

QTL analysis was performed with the R package R/qtl version 1.48-1 (Broman et al., 2003) for least square means over both cultivation systems and for each system individually. Logarithm of the odds (LOD) significance thresholds for a type I error rate of $\alpha = 0.05$ were obtained by running 1000 permutations (*scanone* and *scantwo*) for the respective trait and cultivation system and their mean values. Single QTL mapping was applied using the Haley–Knott regression method (Haley & Knott, 1992), followed by two-dimensional QTL scans. Afterwards, a multiple-QTL model was fitted, including all significant QTL and QTL-by-QTL interactions. The model was further explored for the presence of additional QTL and QTL-by-QTL

interactions. If there was an indication of a second QTL on a chromosome, *addpair* was used for further investigation. Finally, QTL positions were optimised based on the final multiple-QTL model, which contained all significant QTL and QTL-by-QTL interactions. The overall fit of the full model was tested against the null model using an analysis of variance. In the drop-one analysis, the effect of each single QTL was determined by comparing the full model and the model with the respective term omitted. For each QTL, a 95% Bayesian confidence interval was calculated. Co-localised QTL between the individual cultivation systems and their mean values were defined as QTL with overlapping confidence intervals or peak positions within 15 cM. Regions harbouring QTL associated with two or more traits with overlapping confidence intervals were defined as QTL clusters.

3.3 Results

3.3.1 Phenotypic analysis and heritability

The 9 sensory attributes, 5 physicochemical traits, 18 volatile compounds and fruit weight displayed continuous distributions (Tables 3.2 and 3.3). In the mapping population, transgressive segregation was observed in both directions for most traits and was most clear in the organic cultivation system (Table SII.3). The parental cultivars differed in most traits, but the differences were only significant for some of them (Tables 3.2 and 3.3). Resi was characterised by higher values for sweetness, total, tomato and banana-melon aroma, TSS, TSS/TA, and DM compared to Auriga in both cultivation systems, spicy aroma in the organic cultivation and berry aroma in hydroponic cultivation. Most volatiles except ß-damascenone, ß-ionone, Z-3-hexenal, methyl salicylate and benzaldehyde were more abundant in Resi. For benzaldehyde, this was only true for the organic cultivation system.

The effect of the genotype was highly significant ($p = 0.01$) for all sensory attributes except green aroma, for all physicochemical measurements except pH, for fruit weight, and for most volatile compounds (Tables SII.4 and SII.5). The effect of the cultivation system (environment) was significant for most physicochemical measurements and the volatile compounds hexanol, Z-3-hexenol and hexanal (Table SII.5) but not for the sensory attributes (Table SII.4). Nevertheless, the mean values of the F_2 population were mostly higher in the organic cultivation system (Tables 3.2 and 3.3). Inverse results were found for berry aroma, pH, TA and a few volatile compounds. The genotype-by-environment interaction was significant for most sensory attributes, physicochemical measurements, fruit weight and a few volatiles (Tables SII.4 and SII.5).

Table 3.2. Phenotypic variation (Min, minimum; Mean; Max, maximum; SD, standard deviation) of parental cultivars 'Resi' (R; n = 3) and 'Auriga' (A; n = 3) and their F_2 mapping population (n ≥ 172) for sensory attributes, physicochemical measurements and fruit weight for two cultivation systems and broad-sense heritability (H^2)

Trait	Organic cultivation						Hydroponic cultivation						H^2
	R	A	Resi x Auriga (F_2)				R	A	Resi x Auriga (F_2)				
			Min	Mean	Max	SD			Min	Mean	Max	SD	
Sweetness [1–9]	4.89[a]	3.59[b]	2.67	3.99	5.67	±0.57	4.39[a]	2.92[b]	2.17	3.63	5.58	±0.68	0.73
Sourness [1–9]	4.00	5.17	2.83	4.43	6.50	±0.69	3.64[b]	5.17[a]	2.36	4.12	6.75	±0.78	0.69
Total aroma [1–9]	5.39	4.88	3.92	5.29	7.00	±0.57	5.50	4.67	3.42	5.05	6.75	±0.67	0.72
Tomato aroma [1–9]	4.19	3.79	2.83	4.00	5.42	±0.49	4.22	3.25	2.42	3.59	5.11	±0.46	0.55
Banana-melon aroma [2–18][†]	3.53[a]	2.30[b]	2.00	3.39	7.92	±1.39	4.03	2.34	2.00	3.38	8.83	±1.52	0.87
Orange aroma [1–9]	1.67	2.50	0.95	2.10	5.05	±0.77	1.00[b]	3.84[a]	0.89	1.72	5.67	±0.78	0.50
Berry aroma [1–9]	1.53	1.84	1.00	1.84	3.95	±0.59	2.67	1.34	0.95	2.08	4.17	±0.76	0.27
Spicy aroma [1–9]	2.67	1.75	1.00	1.82	4.83	±0.70	1.69	1.67	0.93	1.40	2.92	±0.42	0.36
Green aroma [1–9]	1.22	2.58	1.00	1.81	3.08	±0.51	2.11	2.34	0.99	1.77	2.99	±0.43	0.15
TSS [°Brix]	7.32[a]	6.04[b]	6.00	7.04	8.20	±0.41	6.98[a]	5.60[b]	5.40	6.54	7.65	±0.40	0.80
pH	3.94	3.99	3.60	3.96	4.40	±0.15	4.09	4.03[a]	3.59[b]	4.08	4.54	±0.16	0.11
TA [%]	0.49	0.53	0.42	0.52	0.68	±0.05	0.87	0.86	0.77	0.87	0.97	±0.04	0.27
TSS/TA	15.27[a]	11.55[a]	10.69	13.62	16.85	±1.17	10.93[a]	7.65[b]	7.54	10.47	14.36	±1.37	0.64
DM [%]	8.72[a]	7.23[b]	6.86	8.24	10.02	±0.56	8.64[a]	6.37[b]	6.02	7.72	9.42	±0.61	0.84
FW [g]	17.33[a]	74.84[b]	22.01	33.65	56.25	±6.58	15.26[b]	59.80[a]	16.05	28.65	52.69	±7.18	0.93

Small letters indicate significant differences between the parental cultivars within one cultivation system (LSD, p = 0.05), only significant differences are indicated

Abbreviation: TSS, total soluble solids; TA, titratable acidity; DM, dry matter; FW, fruit weight

[†]sum of banana and melon aroma

Table 3.3. Phenotypic variation (Min, minimum; Mean; Max, maximum; SD, standard deviation) of parental cultivars 'Resi' (R; n = 3) and 'Auriga' (A; n = 3) and their F_2 mapping population (n ≥ 163) for volatile compounds [ng mL^{-1} sample] including their precursor and flavour description for two cultivation systems and broad-sense heritability (H^2)

Trait	Precursor[1]	Flavour[2]	Organic cultivation						Hydroponic cultivation						H^2
			R	A	Resi x Auriga (F_2)				R	A	Resi x Auriga (F_2)				
					Min	Mean	Max	SD			Min	Mean	Max	SD	
6-Methyl-5-hepten-2-one	AC	green, musty	4.94[a]	1.22[b]	0.00	2.12	8.38	±1.53	6.56[a]	1.42[b]	0.70	4.11	17.75	±2.53	0.82
Neral	AC	citrus, lemon	0.41	0.16	0.04	0.24	1.11	±0.17	0.72[a]	0.17[b]	0.00	0.42	2.54	±0.29	0.71
Geranial	AC	citrus, lemon	1.49[a]	0.27[b]	0.01	0.58	2.52	±0.46	1.69[a]	0.44[b]	0.15	1.16	4.26	±0.77	0.83
E-Geranylacetone	AC	floral, fruity	6.09[a]	1.69[b]	0.38	2.48	8.28	±1.44	3.15	1.57	0.54	2.25	6.40	±1.16	0.72
β-Damascenone	AC	woody, herbal	1.87	2.35	0.33	1.19	3.74	±0.62	0.98	2.15	0.20	1.05	3.42	±0.64	0.32
β-Ionone	AC	woody, berry	0.96[b]	3.60[a]	0.24	1.99	6.14	±1.01	0.55[b]	3.56[a]	0.11	2.17	6.83	±1.12	0.79
1-Penten-3-one	FA	spicy, pungent	0.09	0.04	0.00	0.09	0.28	±0.05	0.02	0.01	0.01	0.02	0.04	±0.01	0.20
Hexanol	FA	green, fruity	2.69[a]	0.94[b]	0.46	1.73	4.08	±0.68	1.92[a]	0.29[b]	0.14	1.10	5.50	±0.78	0.66
Z-3-Hexenol	FA	green, fresh	2.35	1.57	0.84	2.42	5.38	±0.76	1.16	0.61	0.29	1.00	3.03	±0.50	0.62
Hexanal	FA	green, woody	83.35[a]	16.77[b]	11.17	31.09	99.39	±12.81	43.15[a]	6.69[b]	4.19	19.77	56.52	±10.77	0.57
E-2-Hexenal	FA	green, fruity	6.66	3.71	0.11	5.38	13.53	±2.43	2.00	2.09	0.61	1.86	4.30	±0.81	0.04
Z-3-Hexenal	FA	green, grassy	1.69	2.15	0.14	1.99	4.47	±0.72	0.67[b]	2.16[a]	0.37	1.28	3.27	±0.59	0.43
E-2-Heptenal	FA	green, sweet	0.33	0.21	0.01	0.27	0.67	±0.12	0.14	0.07	0.03	0.11	0.22	±0.04	0.13
Phenylacetaldehyde	PHA	honey, floral	0.19	0.12	0.04	0.20	0.86	±0.10	0.27[a]	0.06[b]	0.06	0.21	0.47	±0.08	0.49
2-Phenylethanol	PHA	floral, sweet	1.08[b]	0.49[a]	0.25	0.56	1.00	±0.14	0.72	0.60	0.16	0.46	0.84	±0.12	0.53
Methyl salicylate	PHP	minty, sweet	0.57[b]	3.20[a]	0.00	0.88	7.95	±1.33	0.05[b]	0.20[a]	0.02	0.07	0.40	±0.06	0.78
Benzaldehyde	BA	fruity, almond	0.28[b]	0.46[a]	0.09	0.32	0.68	±0.10	0.50	0.26	0.13	0.43	1.08	±0.17	0.58
2-Isobutylthiazole	BCA	green, tomato	1.54[a]	0.45[b]	0.00	0.68	1.72	±0.39	0.67	0.31	0.04	0.47	1.05	±0.21	0.27

Small letters indicate significant differences between the parental cultivars within cultivation systems (LSD, p = 0.05), only significant differences are indicated

[1]precursors of volatile compounds (Martina et al., 2021; Rambla et al., 2014; Tikunov et al., 2020): AC= apocarotenoids; FA = fatty acids; phenolic volatiles derived from PHA = phenylalanine, PHP = phenylpropanoid and BA = benzoic acid; BCA = branched chain amino acids

[2]flavour description obtained from The Good Scents Company Information System (http://www.thegoodscentscompany.com/search2.html, 10.01.2021)

Among the sensory attributes, heritability was high (≥ 0.69) for sweetness, sourness, and total and banana-melon aroma. For tomato, orange and spicy aroma the heritability was medium (0.36–0.55), and low (≤ 0.27) for berry and green aroma (Table 3.2). Heritability was high (≥ 0.80) for TSS, DM, and fruit weight, medium (0.64) for TSS/TA, and low (≤ 0.27) for pH and TA (Table 3.2). For most volatile compounds, the heritability was medium to high (0.32–0.83) (Table 3.3).

3.3.2 Correlations

Sweetness was significantly correlated with the physicochemical trait TSS (r = 0.57) and sourness with TA (r = 0.23) (Figure 3.1). Tomato aroma showed significant positive correlations with sweetness, sourness, total and berry aroma, TSS, TA, DM, and several aroma volatiles derived from apocarotenoids, fatty acids, and phenylalanine, while it was highly negatively correlated with orange aroma, β-ionone, Z-3-hexenal and fruit weight. Total aroma showed highly significant positive correlations with sweetness and, specifically, banana-melon aroma (r = 0.61). Among the volatiles, apocarotenoid-derived volatiles showed the strongest positive correlation with tomato aroma (r ≥ 0.43). Banana-melon aroma was positively correlated with the volatiles hexanol and Z-3-hexenol (r ≥ 0.37) as well as neral and methyl salicylate (r ≥ 0.22) (Figure SII.2). In contrast to the other sensory attributes, orange aroma showed positive correlations with β-ionone and Z-3-hexenal (r ≥ 0.33) and negative correlations with several other volatiles. The apocarotenoid-derived volatiles 6-methyl-5-hepten-2-one, neral, geranial and E-geranylacetone were highly significantly and strongly (r ≥ 0.68) correlated with each other, whereas these volatiles were negatively correlated with β-ionone. Fruit weight was negatively correlated with sweetness, tomato and berry aroma, a few volatile compounds, and DM.

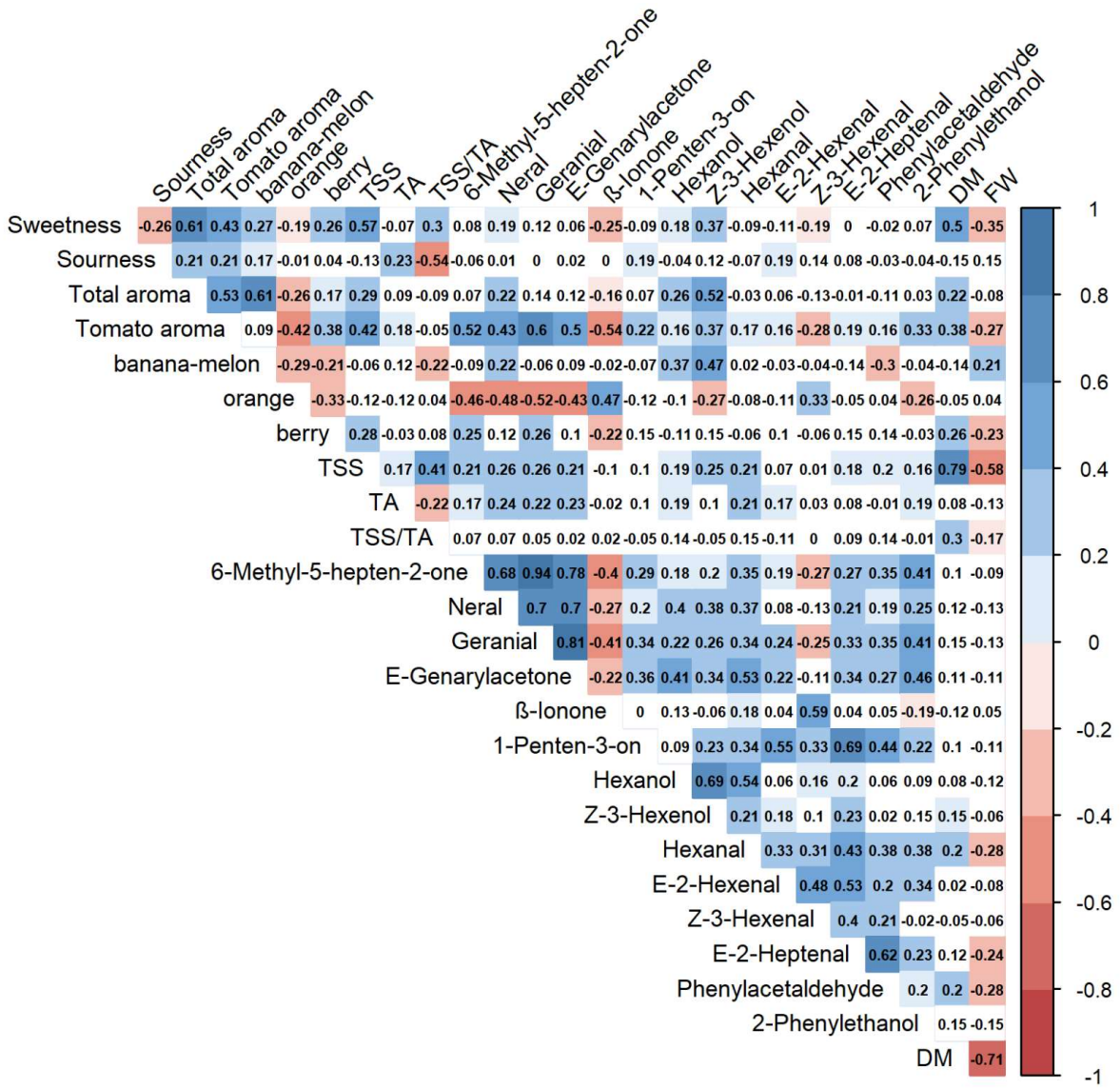

Figure 3.1. Spearman's correlation coefficients (r) for selected sensory attributes, physicochemical measurements (TSS = total soluble solids, TA = titratable acidity, DM = dry matter), aroma volatiles, and fruit weight analysed in two cultivation systems (n ≥ 163); significant positive correlations are shown in blue and significant negative correlations in red with p = 0.05

3.3.3 QTL detection

QTL were mapped on all chromosomes. For 18 of the 33 traits, QTL with relatively major effects (percentage of phenotypic variation explained by a QTL, PVE > 20%) were identified on chromosomes 1, 3, 5, 6 and 9 (Figure 3.2, Table 3.4). Of the total number of 100 significant QTL, 27 were co-localised between both cultivation systems and their mean values (Table SII.6). A total of 71 QTL (sensory attributes: 21, physicochemical traits: 16, volatiles: 24, fruit weight: 10) and two QTL-by-QTL interactions were detected for the mean values of both cultivation systems (Table 3.4, Figure 3.2). A total of 61 QTL (sensory attributes: 15, physicochemical traits: 14, volatiles: 22, fruit weight: 10) were mapped in the organic cultivation system and 46 QTL (sensory attributes: 12, physicochemical traits: 14, volatiles: 17, fruit weight: 3) were mapped in hydroponic cultivation, each with one QTL-by-QTL interaction (Tables SII.7

and SII.8, Figures SII.3 and SII.4). QTL for green aroma were only found in organic cultivation and for pH and TA only in hydroponic cultivation. Hereafter, we focus on the QTL detected for the mean values of both cultivation systems.

A minimum of one QTL (banana-melon aroma, ten volatile compounds) and a maximum of ten QTL (fruit weight) were identified per trait (Table 3.4). The individual contribution of a QTL to the phenotypic variance explained ranged from 1.9 (fruit weight) to 74.4% (banana-melon aroma). For the sensory attributes, we detected two QTL for sweetness (percentage of phenotypic variation explained by the multiple-QTL model, PVE_{full} = 33.63%), three for sourness (PVE_{full} = 35.7%), five and one QTL-by-QTL interaction for total aroma (PVE_{full} = 55.2%), and two for tomato aroma (PVE_{full} = 50.6%). Both QTL for tomato aroma overlapped with QTL for total aroma (Figure 3.2). The phenotypic variance explained ranged from 19.6 to 74.4% for the special sensory attributes and from 33.1 to 80.6% for the physicochemical measurements. QTL were detected for all aroma volatiles (PVE_{full} from 10.44 to 68.7%) except ß-damascenone, 2-isobutylthiazole and phenylacetaldehyde. Auriga carried most alleles that increased sourness, orange aroma and fruit weight. For most of the other QTL, Resi contributed to increased phenotypic values, with the exception of the QTL mapped on chromosome 7 and some volatile compounds.

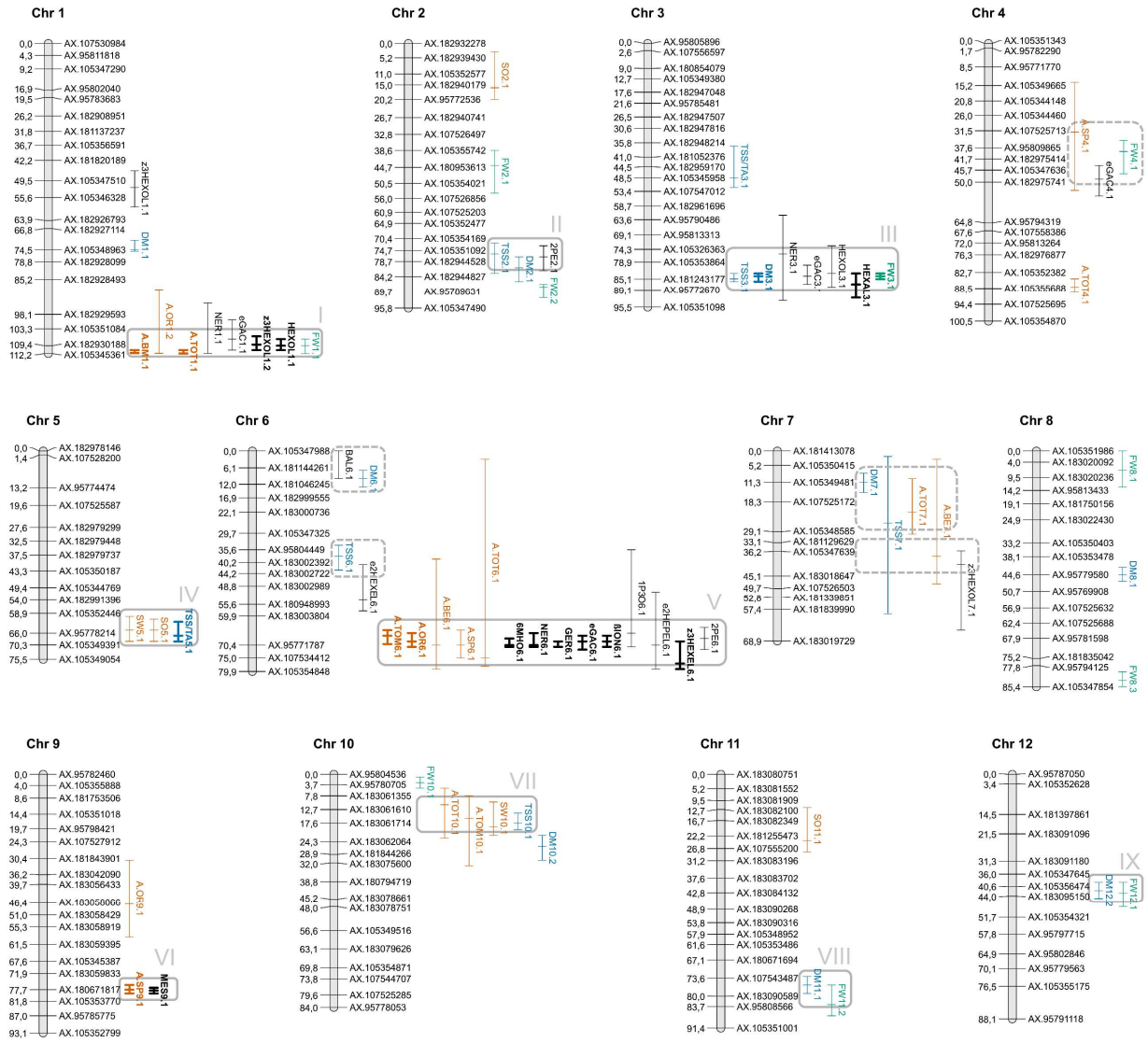

Figure 3.2. Linkage map constructed from 178 F_2 plants of the cross 'Resi' × 'Auriga' using the Axiom 200K SOLCUC vegetable array; quantitative trait loci (QTL; peak position and 95% Bayesian confidence interval) for the sensory attributes (orange), physicochemical measurements (blue), aroma volatiles (black) and fruit weight (green) detected by multiple-QTL mapping for the mean values of two cultivation systems; QTL with an phenotypic variance >20% are marked in bold; QTL enclosed in boxes indicate clusters for co-localised QTL (distinct clusters: solid line; suspected cluster: dashed line)

A total of nine clusters were identified and five more suspected (Figure 3.2). The largest cluster was located on chromosome 6, comprising QTL for five sensory attributes, five volatiles derived from apocarotenoids, three from fatty acids and one from phenylalanine. QTL for several sensory attributes were clustered together with QTL for physicochemical measurements on chromosomes 5 and 10 and for aroma volatiles on chromosomes 1, 6 and 9. On chromosomes 2 and 3, QTL for physicochemical measurements and aroma volatiles were co-localised. In addition, QTL for fruit weight were mapped within the cluster on chromosomes 1 and 3 and close to those on chromosomes 2 and 10.

Table 3.4. Location and estimates of QTL for sensory attributes, physicochemical measurements, fruit weight and volatile compounds detected by multiple-QTL mapping in an F_2 population of 'Resi' × 'Auriga' for the mean values of two cultivation systems

Trait	QTL	Chr[1]	CS[2]	Closest marker	Pos[3]	LOD[4]	PVE[5]	PVE_{full}[6]	Add[7]	Dom[8]	Allele[9]
Sweetness											
	SW5.1	5	AV, Or	AX-95778214	65.0 (60.0–69.0)	6.30	11.82	33.63	−0.26	0.05	A
	SW10.1	10	AV, Or, Hy	AX-183061714	19.0 (10.0–22.0)	8.57	16.57		−0.26	0.17	A
Sourness											
	SO2.1	2	AV	AX-182940179	16.0 (3.0–20.2)	5.24	9.39		−0.22	0.14	A
	SO5.1	5	AV, Or	AX-95778214	65.0 (61.0–69.0)	8.38	15.67	35.67	0.29	0.13	B
	SO11.1	11	AV, Or, Hy	AX-181255473	24.0 (12.0–28.0)	6.68	12.22		0.28	−0.08	B
Total aroma											
	A.TOT1.1	1	AV, Or, Hy	AX-105345361	112.2 (111.0–112.2)	15.05	21.46		−0.33	−0.14	A
	A.TOT4.1	4	AV	AX-105355688	88.0 (85.0–90.0)	6.44	8.16		−0.06	0.14	A
	A.TOT6.1	6	AV	AX-107534412	75.0 (3.0–78.0)	5.58	6.99	55.23	−0.02	−0.13	A
	A.TOT7.1	7	AV, Hy	AX-107525172	22.0 (10.0–30.0)	4.63	5.73		0.18	0.03	B
	A.TOT10.1	10	AV, Hy	AX-183061610	11.0 (5.0–23.0)	4.63	5.74		−0.18	0.03	A
	A.TOT4.1:6.1		AV			4.95	6.16				
Tomato aroma											
	A.TOM6.1	6	AV, Or, Hy	AX-95771787	67.0 (65.0–70.0)	23.25	41.08	50.57	−0.38	−0.08	A
	A.TOM10.1	10	AV	AX-183061714	16.0 (7.8–33.0)	3.84	5.19		−0.10	0.11	A
Banana-melon aroma											
	A.BM1.1	1	AV, Or, Hy	AX-105345361	111.0 (111.0–112.0)	52.37	74.40	74.40	−1.44	−0.81	A
Orange aroma											
	A.OR1.2	1	AV	AX-105345361	112.0 (89.0–112.2)	4.35	6.47		0.21	0.08	B
	A.OR6.1	6	AV, Or, Hy	AX-95771787	69.0 (66.0–71.0)	18.18	32.64	46.03	0.54	−0.07	B
	A.OR9.1	9	AV	AX-183058066	47.0 (31.0–59.0)	3.63	5.35		−0.20	−0.16	A
Berry aroma											
	A.BE6.1	6	AV	AX-95771787	70.4 (39.0–79.0)	3.74	8.22	19.64	−0.22	−0.06	A
	A.BE7.1	7	AV, Or	AX-105347639	38.0 (3.0–48.0)	3.82	8.39		0.21	0.01	B
Spicy aroma											
	A.SP4.1	4	AV	AX-107525713	32.0 (14.0–53.0)	3.66	6.11		−0.15	−0.10	A
	A.SP6.1	6	AV, Or	AX-95771787	70.4 (65.0–75.0)	6.08	10.49	38.75	−0.21	0.02	A
	A.SP9.1	9	AV, Or	AX-180671817	77.7 (76.0–79.0)	12.44	23.41		0.26	−0.19	B
Total soluble solids											
	TSS2.1	2	AV, Hy	AX-105351092	76.0 (72.0–83.0)	6.49	8.04		−0.13	−0.10	A
	TSS3.1	3	AV, Or, Hy	AX-181243177	85.0 (83.0–86.0)	12.26	16.46		−0.24	0.10	A
	TSS6.1	6	AV, Or, Hy	AX-183002392	38.0 (34.0–43.0)	8.52	10.86	57.38	−0.18	0.00	A
	TSS7.1	7	AV	AX-105348585	26.0 (2.0–59.0)	4.00	4.79		0.09	−0.10	B
	TSS10.1	10	AV, Or, Hy	AX-183061714	17.6 (14.0–20.0)	13.27	18.07		−0.19	0.10	A
TSS/TA											
	TSS/TA3.1	3	AV	AX-105345958	48.5 (37.0–52.0)	4.06	7.69	33.06	−0.39	0.30	A
	TSS/TA5.1	5	AV, Or, Hy	AX-95778214	67.0 (62.0–69.0)	12.81	27.38		−0.74	−0.05	A
Dry matter											
	DM1.1	1	AV, Or	AX-105348963	74.5 (71.0–75.0)	12.52	7.63		0.18	0.05	B
	DM2.1	2	AV, Or, Hy	AX-182944528	81.0 (77.0–86.0)	13.36	8.23		−0.22	−0.04	A
	DM3.1	3	AV, Or, Hy	AX-181243177	84.0 (83.0–86.0)	32.83	26.87	80.59	−0.44	0.05	A
	DM6.1	6	AV, Or	AX-181046245	10.0 (7.0–13.0)	8.57	4.94		−0.17	−0.06	A
	DM7.1	7	AV, Hy	AX-105349481	11.3 (8.0–15.0)	5.99	3.33		0.14	0.01	B

Continued next page.

Table 3.4. Continued.

Trait	QTL	Chr[1]	CS[2]	Closest marker	Pos[3]	LOD[4]	PVE[5]	PVE$_{full}$[6]	Add[7]	Dom[8]	Allele[9]
	DM8.1	8	AV	AX-95779580	44.6 (42.0–47.0)	9.17	5.33		−0.13	−0.09	A
	DM10.2	10	AV, Or	AX-183062064	26.0 (22.0–31.0)	18.91	12.61		−0.27	0.01	A
	DM11.1	11	AV, Or	AX-107543487	76.0 (73.0–79.0)	12.34	7.50		−0.17	0.20	A
	DM12.2	12	AV	AX-105356474	42.0 (39.0–45.0)	13.37	8.25		−0.22	0.07	A
	DM1.1:8.1		AV			4.64	2.54				
Fruit weight											
	FW1.1	1	AV	AX-182930188	109.4 (107.0–112.2)	5.82	2.55		−1.30	0.74	A
	FW2.1	2	AV, Or	AX-180953613	44.0 (38.6–54.0)	6.13	2.69		1.60	0.09	B
	FW2.2	2	AV, Or, Hy	AX-95789631	88.0 (87.0–92.0)	28.28	17.06		3.90	−2.39	B
	FW3.1	3	AV, Or, Hy	AX-181243177	84.0 (83.0–85.1)	38.20	26.80		5.39	0.72	B
	FW4.1	4	AV	AX-95809865	39.0 (35.0–47.0)	7.74	3.48	84.81	1.87	0.25	B
	FW8.1	8	AV	AX-183020236	7.0 (0.0–13.0)	4.52	1.94		1.29	0.58	B
	FW8.3	8	AV, Or	AX-105347854	83.0 (80.0–85.4)	8.42	3.82		1.37	2.04	B
	FW10.1	10	AV	AX-95780705	3.0 (1.0–5.0)	15.88	7.99		2.88	−0.82	B
	FW11.2	11	AV, Or	AX-95808566	83.0 (76.0–87.0)	12.24	5.85		2.32	−0.33	B
	FW12.1	12	AV, Or, Hy	AX-183095150	43.0 (39.0–48.0)	9.57	4.41		2.10	0.13	B
6-Methyl-5-hepten-2-one											
	6MHO6.1	6	AV, Or, Hy	AX-95771787	70.4 (68.0–71.0)	31.27	58.22	58.22	−2.03	−0.88	A
Neral											
	NER1.1	1	AV, Or	AX-105345361	112.2 (94.0–112.2)	5.50	9.46		−0.07	0.05	A
	NER3.1	3	AV	AX-105326363	76.0 (62.0–93.0)	3.81	6.39	42.98	−0.08	0.01	A
	NER6.1	6	AV, Or, Hy	AX-95771787	70.4 (66.0–71.0)	12.86	24.62		−0.14	−0.07	A
Geranial											
	GER6.1	6	AV, Or, Hy	AX-95771787	71.0 (69.0–71.0)	41.82	68.65	68.65	−0.65	−0.36	A
E-Geranylacetone											
	eGAC1.1	1	AV, Or	AX-182930188	107.0 (100.0–111.0)	6.34	7.72		−0.41	0.17	A
	eGAC3.1	3	AV, Or, Hy	AX-181243177	84.0 (80.0–87.0)	7.35	9.10	60.06	−0.53	0.00	A
	eGAC4.1	4	AV	AX-182975741	49.0 (44.0–55.0)	3.66	4.30		−0.13	−0.43	A
	eGAC6.1	6	AV, Or, Hy	AX-95771787	69.0 (67.0–72.0)	21.34	32.51		−0.96	−0.14	A
ß-Ionone											
	ßION6.1	6	AV, Or, Hy	AX-95771787	69.0 (67.0–71.0)	35.29	62.21	62.21	1.09	0.29	B
1-Penten-3-one											
	1P3O6.1	6	AV, Or	AX-95771787	66.0 (35.6–71.0)	4.46	11.64	11.64	−0.01	−0.01	A
Hexanol											
	HEXOL1.1	1	AV, Or, Hy	AX-182930188	109.4 (107.0–111.0)	11.85	25.91	34.82	−0.40	−0.10	A
	HEXOL3.1	3	AV	AX-181243177	83.0 (73.0–88.0)	5.10	10.11		−0.32	0.08	A
Z-3-Hexenol											
	z3HEXOL1.1	1	AV, Or	AX-105347510	52.0 (46.0–59.0)	4.06	7.22		0.20	0.05	B
	z3HEXOL1.2	1	AV, Or, Hy	AX-182930188	109.0 (106.0–111.0)	11.98	23.90	39.81	−0.33	0.02	A
	z3HEXOL7.1	7	AV, Or	AX-183018647	41.0 (36.0–65.0)	5.13	9.26		0.22	−0.02	B
Hexanal											
	HEXAL3.1	3	AV, Or, Hy	AX-181243177	87.0 (83.0–92.0)	10.13	24.37	24.37	−7.36	−0.15	A
E-2-Hexenal											
	e2HEXEL6.1	6	AV, Or	AX-180948993	54.0 (41.0–58.0)	4.90	12.72	12.72	−0.33	−0.82	A
Z-3-Hexenal											
	z3HEXEL6.1	6	AV, Or, Hy	AX-107534412	77.0 (69.0–79.0)	10.64	25.43	25.43	0.36	−0.10	B

Continued next page.

Table 3.4. Continued.

Trait	QTL	Chr[1]	CS[2]	Closest marker	Pos[3]	LOD[4]	PVE[5]	PVE_{full}[6]	Add[7]	Dom[8]	Allele[9]
E-2-Heptenal											
	e2HEPEL6.1	6	AV	AX-95771787	70.4 (51.0–79.0)	4.00	10.44	10.44	−0.02	−0.03	A
2-Phenylethanol											
	2PE2.1	2	AV, Or, Hy	AX-182944528	77.0 (73.0–82.0)	8.26	16.99	34.00	−0.06	0.00	A
	2PE6.1	6	AV, Hy	AX-95771787	68.0 (64.0–72.0)	8.56	17.70		−0.06	−0.01	A
Methyl salicylate											
	MES9.1	9	AV, Or, Hy	AX-180671817	78.0 (77.0–79.0)	29.21	56.18	56.18	0.58	−0.53	B
Benzaldehyde											
	BAL6.1	6	AV, Or	AX-105347988	0.0 (0.0–10.0)	4.54	11.83	11.83	0.06	−0.01	B

[1]Chr, chromosome; [2]CS, cultivation system with AV = environmental means, Or = organic cultivation system, Hy = hydroponic cultivation (details for the QTL mapped in Or and Hy are available in Tables SII.7 and SII.8); [3]Pos, peak position with 95% Bayesian confidence interval; [4]LOD, log of likelihood ratio; [5]PVE, percentage of phenotypic variation explained by the QTL; [6]PVE_{full}, percentage of phenotypic variation explained by the multiple-QTL model; [7]Add, additive effect (positive effect denote increasing effect of the B allele); [8]Dom, dominance effects; [9]Allele, allele increasing the phenotypic value (A from Resi, B from Auriga)

3.4 Discussion

3.4.1 Phenotyping of the parental cultivars and the effect of the cultivation system

In the present study, the Breeders' Sensory Test (Hagenguth et al., 2022), introduced as a sensory method for small sample sizes from a high number of individual plants, was successfully implemented for QTL mapping. Sensory differences between the parental cultivars Resi and Auriga, characterised by different fruit sizes, colours, and sensory and metabolic profiles, were detected for most attributes (Table 3.2). Resi, a cocktail tomato, had higher scorings for sweetness, tomato, total and banana-melon aroma, higher values for TSS, TSS/TA, and DM, and higher concentrations of most volatile compounds in both cultivation systems (Tables 3.2 and 3.3). Auriga, a salad tomato, had higher scores for sourness and orange aroma.

Flavour is not only influenced by genetics but also by environmental factors and agronomic handling (Baldwin et al., 2015; Erika et al., 2022; Klee & Tieman, 2018), as also shown by our results (Tables SII.4 and SII.5). For the mean values of the F_2 population, a slight trend towards higher values in organic cultivation was observed for sensory attributes and physicochemical measurements, with the exception of berry aroma and TA (Table 3.2). Additionally, most aroma volatiles were more abundant in organic cultivation (Table 3.3). The differences between the cultivation systems are probably due to higher stress levels, reduced fertilisation and a longer time for fruit development in the organic system, which favours higher production of primary and secondary metabolites in organic cultivation (Mitchell et al., 2007; Oliveira et al., 2013). However, summarising several studies, no clear trend of tomato quality was identified as favouring a specific cultivation system (Pieper & Barrett, 2009).

3.4.2 Correlation of sensory attributes with physicochemical measurements and volatiles

Tomato aroma was significantly ($p \geq 0.21$) positively correlated with the sensory attributes sweetness, sourness, and total and berry aroma (Figure 3.1). These findings are consistent with the description of tomato flavour as sweet, fruity, green-grassy, ripe and sour (Hongsoongnern & Chambers, 2008). However, tomato aroma was not correlated with banana-melon aroma and negatively correlated with orange aroma ($r = -0.42$). Apparently, these two special sensory attributes were not perceived as typical for tomatoes and might be specific to this mapping population. In agreement with Baldwin et al. (2015) and Erika et al. (2022), we found significant correlations between TSS and sweetness ($r = 0.57$) and between TA and sourness ($r = 0.23$). Although measuring TSS and TA allows breeders to select for tomato taste (Tandon et al. 2003), Colantonio et al. (2022) emphasised the importance of aroma volatiles for sensory attributes by quantifying the proportion of phenotypic variance explained by sugars, acids and volatile compounds. Volatile compounds explained 68% of flavour intensity and 62% of sweetness assessed by a consumer panel, while sourness was much less affected by volatiles (Colantonio et al., 2022).

Among the sensory attributes, tomato aroma showed the highest number of significant correlations with volatile compounds (Figure 3.1). The most important contributors to tomato aroma were aroma volatiles derived from apocarotenoids ($r \geq 0.43$) with the exception of β-damascenone (Figure SII.2). Consistent with these results, apocarotenoid-derived volatiles, characterised by fruity or floral aroma notes, have been described as important contributors to tomato aroma (Martina et al., 2021; Rambla et al., 2014). However, the importance of specific apocarotenoid-derived volatiles has been questioned by more recent studies (Tieman et al., 2012), as also seen for β-damascenone in the present study. In contrast to earlier descriptions by Baldwin et al. (2000) and Baldwin et al. (2015), β-ionone was negatively correlated with tomato aroma ($r = -0.54$), possibly due to the relatively high concentration in the mapping population. Because β-ionone has a very low odour threshold (Baldwin et al., 2000; Rambla et al., 2014), high concentrations might lead to a negative effect on tomato aroma. As described in previous studies (Klee & Tieman, 2018; Piombino et al., 2013; Tikunov et al., 2020), the fatty acid-derived volatile Z-3-hexenol ($r = 0.37$) and the phenylalanine-derived volatile 2-phenylethanol ($r = 0.33$) were also important contributors to tomato aroma. In agreement with Tandon et al. (2003), a negative effect of Z-3-hexenal on tomato aroma ($r = -0.28$) was observed, while this volatile was positively correlated with orange aroma ($r = 0.33$) in our study and with fruitiness in their study.

3.4.3 QTL for flavour-related traits, including sensory attributes

Despite the difficulty of assessing sensory attributes in large mapping populations, we detected QTL for all sensory attributes, with the exception of green aroma (only in the organic cultivation system) (Figure 3.2). For the main sensory attributes sweetness, sourness and tomato aroma, the PVE per attribute ranged from about 34 to 51% (Table 3.4). The QTL for these sensory attributes on chromosomes 2, 5, 6, 10 and

11 are of interest for marker-assisted selection (MAS), as they directly contributed to the perceived taste and aroma. The largest number of QTL per trait mapped in this study was identified for fruit weight with 10 QTL. This was expected since the two parents of the mapping population were characterised by very different fruit weights. All QTL for fruit weight were consistent with previously published genetic regions for this trait (Grandillo et al., 1999; Pereira et al., 2021; Saliba-Colombani et al., 2001). QTL for the special aroma attributes banana-melon, orange, berry and spicy provide novel information, although previous studies have worked with different aroma notes and naming is not standardised. Therefore, the QTL for the spicy aroma on chromosome 9 might overlap with the smoky QTL detected by Tikunov et al. (2020). The QTL for methyl salicylate co-localised with the QTL for spicy aroma corresponds to the SlSAMT gene identified by Tieman et al. (2010), involved in the synthesis of this aroma volatile. Working with an F_2 mapping population enabled the estimation of additive and dominance effects. For most QTL, the additive effect was larger than the dominance effect, while for some QTL the effect was similar, and for the three QTL A.TOT4.1, A.TOT6.1 and FW8.3 the dominance effect was larger (Table 3.4).

Several QTL formed clusters resulting from either physiological relationships or genetically linked genes (Bauchet et al., 2017; Zanor et al., 2009). Such clusters are of great interest because they provide the opportunity to identify genetic loci associated with large sets of metabolic changes affecting tomato flavour (Folta & Klee, 2016). Genetic regions altering the concentration of several volatiles with a common biological origin have been reported by several authors (Bauchet et al., 2017; Rambla et al., 2017; Zhang et al., 2015), but QTL studies combining physicochemical measurements or primary metabolites and volatile compounds with sensory attributes are rare (Causse et al., 2002; Tikunov et al., 2020; Zanor et al., 2009). As expected from the correlations, most of the sensory QTL were co-localised with QTL for either other sensory attributes, physicochemical measurements, aroma volatiles or fruit weight (Figure 3.2).

In the largest identified cluster on chromosome 6, the major QTL for tomato aroma was co-localised with QTL for orange, berry, spicy, and total aroma, five apocarotenoid-volatiles, three fatty acid-derived volatiles, and the phenylalanine-derived volatile 2-phenylethanol (Figure 3.2). Tikunov et al. (2020) detected QTL for aroma intensity, sour taste and TSS on the same linkage group, confirming that this genetic region may be important for improving sensory attributes in tomato. Within this cluster and the clusters on chromosomes 1 and 3, the co-localisation of QTL for volatiles derived from apocarotenoids and fatty acids is striking. Similar to this observation, the apocarotenoids 6-methyl-5-hepten-2-one and geranylacetone clustered together with C_6 volatiles (fatty acids) in the construction of a metabolic tree by Mathieu et al. (2009), and a metabolic dependency was proposed (Mathieu et al., 2009). Most of the volatile QTL from our study could be roughly classified into the QTL genomic regions summarised by Martina et al. (2021) and, in some cases, complement the volatile groups; e.g. E-geranylacetone, neral and hexanol complement the cluster of apocarotenoid- and fatty acid-derived QTL towards the end of chromosome 1.

The QTL for sweetness and sourness on chromosome 5 (PVE ≥ 11.8%) and for sweetness, total and tomato aroma on chromosome 10 (PVE ≥ 5.2%) were mapped for the first time. In a similar region of chromosome 5, Causse et al. (2001) and Tikunov et al. (2020) mapped QTL for texture-related traits but reported no sensory QTL. The QTL cluster on chromosome 5 is likely to influence the perceived sweet–sour taste, as indicated by the presence of a QTL for TSS/TA (PVE = 27.4%). Both QTL clusters provide interesting candidates for MAS to improve the sweet and sour taste of tomatoes. In addition, QTL for physicochemical measurements and aroma volatiles were co-localised on chromosomes 2 and 3 in similar regions, where Causse et al. (2001), Causse et al. (2002) and Tikunov et al. (2020) also identified QTL clusters for flavour-related traits. The genetic region towards the end on chromosome 2 is well studied due to the presence of *fw2.2*, a gene largely involved in increased fruit size during domestication (Frary et al., 2000), but antagonistic effects for flavour-related traits were observed, most likely due to a dilution effect (Causse et al., 2002; Lecomte et al., 2004).

3.5 Conclusions

QTL mapping based on an F_2 population derived from two tomato cultivars with superior quality but different fruit weights revealed many insights into the relationship between sensory attributes, physicochemical measurements, aroma volatiles and fruit weight and their inheritance. Phenotyping conducted in two contrasting cultivation systems, organic low-input and hydroponic, enabled the identification of robust QTL. This study highlights QTL for sensory attributes, including novel ones on chromosomes 5 and 10, which are partially co-localised with QTL for physicochemical measurements, aroma volatiles and fruit weight. QTL for sourness on chromosomes 2 and 11 and genetic regions harbouring QTL for multiple flavour-related traits, including sweetness and tomato aroma, on chromosomes 5, 6 and 10 are recommended for MAS to improve the flavour of fresh market tomatoes. The application of molecular markers for sensory attributes that directly reflect human flavour perception to seedlings enables breeders to consider sensory attributes in the first segregating generations and reduce the risk of losing genotypes with favourable attributes. However, QTL may partly be specific to the mapping population and the environments of this experiment. Therefore, we verified the applicability of the identified QTL for MAS in the following year for both the mapping population and a second independent population and compared it with the response to phenotypic selection (Hagenguth et al., n.d.).

3.6 References

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Conflict of interest

The authors declare no conflicts of interest.

Data Availability Statement

Data are available upon request from the corresponding author.

3.7 Supplementary Materials

For Flavour Improvement in Early Generations of Fresh Market Tomatoes (Solanum lycopersicum L.): I. Identification of QTL for Sensory Attributes, Physicochemical Measurements and Volatile Compounds

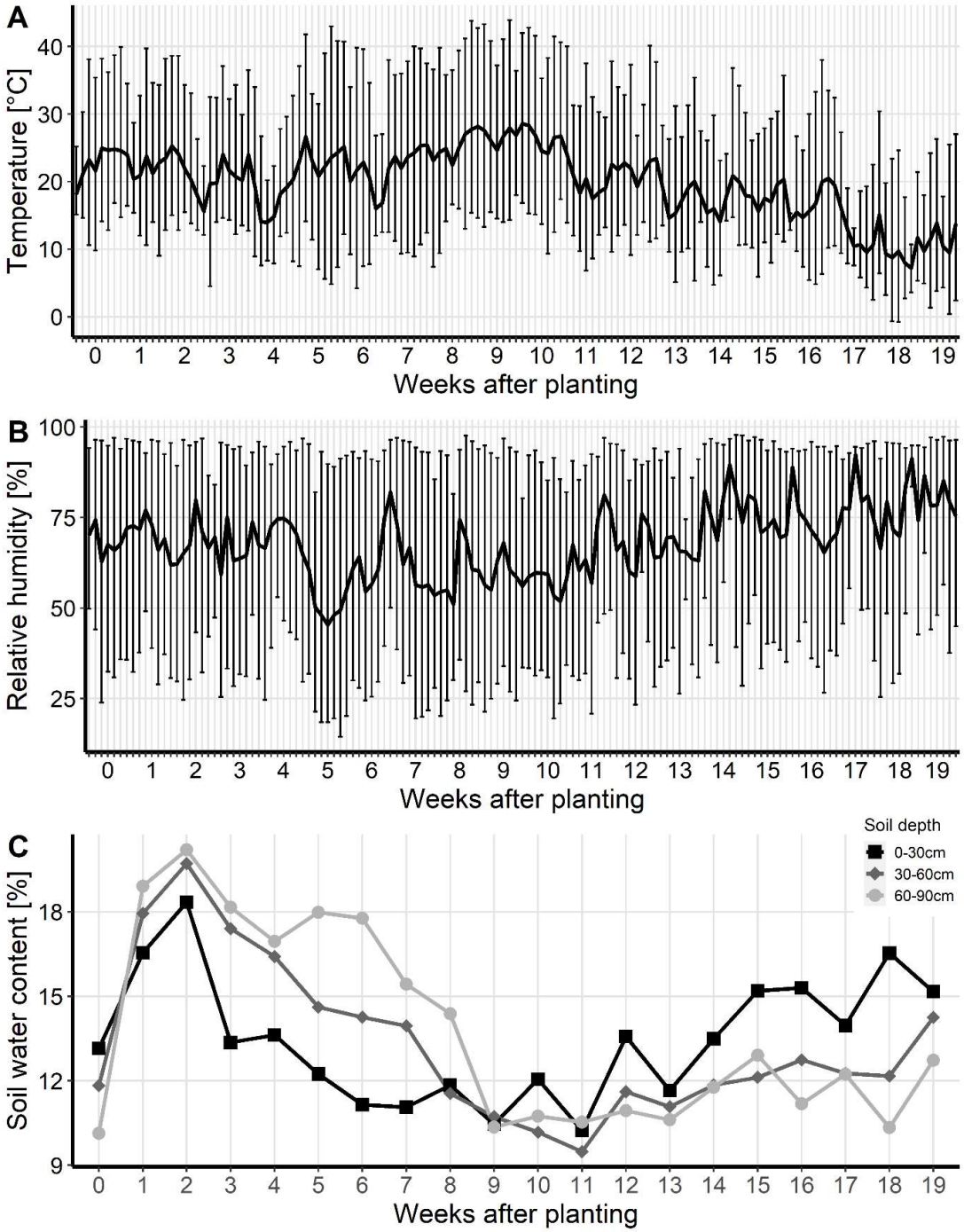

Figure SII.1. (A) Mean, minimum and maximum temperature per day, (B) mean relative humidity with minimum and maximum per day, and (C) soil water content in the organic cultivation system.
Temperature and humidity data were recorded every 15 min in about 0.5 m above soil surface using an EBI 20-TH Data Logger (ebro Electronic GmbH & Co. KG, Ingolstadt, Germany); soil water content is expressed as gravimetric moisture content

Table SII.1. Mineral nitrogen in the organic low-input cultivation system

Soil sample	Soil depth (cm)	Mineral nitrogen[1] (N_{min}) [kg/ha]
23.05.2018 start of experiment	0–30	73.57
	30–60	94.33
31.07.2018	0–30	15.23
	30–60	52.84
09.10.2018 end of experiment	0–30	10.90
	30–60	7.40

[1]analysed by University of Goettingen, Division of Agronomy, Goettingen, Germany

Table SII.2. Number of F_2 genotypes used for the estimation of least square means and QTL analysis

Trait	n
Sweetness	177
Sourness	177
Total aroma	177
Tomato aroma	177
Banana-melon aroma	177
Orange aroma	177
Berry aroma	177
Spicy aroma	177
Green aroma	177
TSS	173
pH	174
TA	173
TSS/TA	172
DM	174
FW	176
6-Methyl-5-hepten-2-one	165
Neral	165
Geranial	166
E-Geranylacetone	165
ß-Damascenone	166
ß-Ionone	167
1-Penten-3-one	166
Hexanol	163
Z-3-Hexenol	165
Hexanal	167
E-2-Hexenal	166
Z-3-Hexenal	167
E-2-Heptenal	167
Phenylacetaldehyde	166
2-Phenylethanol	166
Methyl salicylate	163
Benzaldehyde	166
2-Isobutylthiazole	166

Abbreviation: TSS, total soluble solids; TA, titratable acidity; DM, dry matter; FW, fruit weight

Table SII.3. Minimum (Min) and maximum (Max) of the parental cultivars 'Resi' (R, n = 3) and 'Auriga' (A, n = 3) and their F_2 mapping population (n ≥ 163) for sensory attributes, physicochemical measurements, fruit weight and volatile compounds [ng mL^{-1} sample] for two cultivation systems

Trait	Organic cultivation						Hydroponic cultivation					
	Resi		Auriga		F_2		Resi		Auriga		F_2	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Sweetness [1–9]	4.25	5.42	2.92	4.25	2.67	5.67	4.17	4.67	2.71	3.12	2.17	5.58
Sourness [1–9]	3.58	4.58	4.08	6.25	2.83	6.50	3.25	3.92	4.19	6.14	2.36	6.75
Total aroma [1–9]	5.17	5.67	4.67	5.08	3.92	7.00	5.33	5.83	4.25	5.08	3.42	6.75
Tomato aroma [1–9]	4.00	4.33	3.75	3.83	2.83	5.42	4.08	4.42	3.06	3.44	2.42	5.11
Banana-melon aroma [2–18] ‡	2.88	4.67	2.17	2.42	2.00	7.92	3.00	5.42	2.00	2.67	2.00	8.83
Orange aroma [1–9]	1.00	2.17	2.33	2.67	0.95	5.05	1.00	1.00	2.45	5.22	0.89	5.67
Berry aroma [1–9]	1.00	2.25	1.67	2.00	1.00	3.95	1.67	3.33	1.05	1.62	0.95	4.17
Spicy aroma [1–9]	2.17	3.17	1.17	2.33	1.00	4.83	1.50	1.83	0.93	2.40	0.93	2.92
Green aroma [1–9]	1.00	1.33	1.83	3.33	1.00	3.08	1.67	2.50	2.33	2.34	0.99	2.99
TSS [°Brix]	7.20	7.45	5.80	6.27	6.00	8.20	6.95	7.05	5.01	6.19	5.40	7.65
pH	3.75	4.03	3.95	4.03	3.60	4.40	3.84	4.24	3.74	4.32	3.59	4.54
TA [%]	0.44	0.52	0.52	0.54	0.42	0.68	0.82	0.90	0.80	0.92	0.77	0.97
TSS/TA	14.36	16.66	11.23	11.86	10.69	16.85	9.76	11.87	6.02	9.27	7.54	14.36
DM [%]	8.67	8.79	6.85	7.60	6.86	10.02	8.28	9.08	5.75	6.99	6.02	9.42
FW [g]	16.93	17.68	74.84 †		22.01	56.25	15.04	15.44	59.80 †		16.05	52.69
6-Methyl-5-hepten-2-one	3.90	6.49	1.20	1.23	0.00	8.38	2.76	10.41	0.69	2.15	0.70	17.75
Neral	0.34	0.51	0.13	0.19	0.04	1.11	0.37	1.00	0.00	0.42	0.00	2.54
Geranial	1.26	1.93	0.24	0.29	0.01	2.52	0.67	2.41	0.19	0.69	0.15	4.26
E-Geranylacetone	5.40	7.23	1.45	1.93	0.38	8.28	1.41	4.70	1.07	2.06	0.54	6.40
ß-Damascenone	1.15	2.56	1.72	2.97	0.33	3.74	0.75	1.32	0.81	3.49	0.20	3.42
ß-Ionone	0.80	1.22	3.17	4.03	0.24	6.14	0.45	0.65	2.82	4.29	0.11	6.83
1-Penten-3-one	0.07	0.11	0.04 †		0.00	0.28	0.01	0.02	0.01 †		0.01	0.04
Hexanol	1.87	4.20	0.70	1.17	0.46	4.08	1.27	3.07	0.08	0.49	0.14	5.50
Z-3-Hexenol	1.91	2.98	1.47	1.66	0.84	5.38	0.82	1.45	0.39	0.83	0.29	3.03
Hexanal	66.50	93.41	15.36	18.18	11.17	99.39	26.91	61.61	5.88	7.50	4.19	56.52
E-2-Hexenal	4.40	8.18	1.86	5.55	0.11	13.53	1.56	2.23	1.91	2.27	0.61	4.30

Continued next page.

Table SII.3. Continued.

Trait	Organic cultivation						Hydroponic cultivation					
	Resi		Auriga		F_2		Resi		Auriga		F_2	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Z-3-Hexenal	0.83	2.92	1.73	2.56	0.14	4.47	0.51	0.83	1.11	3.20	0.37	3.27
E-2-Heptenal	0.24	0.41	0.12	0.30	0.01	0.67	0.07	0.25	0.05	0.09	0.03	0.22
Phenylacetaldehyde	0.13	0.25	0.10	0.14	0.04	0.86	0.13	0.45	0.05	0.06	0.06	0.47
2-Phenylethanol	0.82	1.28	0.38	0.59	0.25	1.00	0.68	0.80	0.56	0.63	0.16	0.84
Methyl salicylate	0.42	0.81	2.95	3.44	0.00	7.95	0.04	0.06	0.12	0.28	0.02	0.40
Benzaldehyde	0.27	0.30	0.41	0.50	0.09	0.68	0.34	0.64	0.26	0.26	0.13	1.08
2-Isobutylthiazole	1.32	1.66	0.32	0.57	0.00	1.72	0.62	0.77	0.18	0.43	0.04	1.05

Abbreviation: TSS, total soluble solids; TA, titratable acidity; DM, dry matter; FW, fruit weight

† Data only available for one of the three parental plants; ‡sum of banana and melon aroma

Table SII.4. Mean values and standard deviation (SD) of sensory attributes and variance components for the effects of genotype (V_G), person (V_P), replication within environment ($V_{R:E}$), environment (V_E), genotype-by-person interaction (V_{GP}), genotype-by-environment interaction (V_{GE}), person-by-environment interaction (V_{PE}), genotype-by-person-by-environment interaction (V_{GPE}) and residuals (V_ε) of the F_2 mapping population 'Resi' × 'Auriga' (n = 177) in two cultivation systems

Trait	Mean	SD	V_G	V_P	$V_{R:E}$	V_E†	V_{GP}	V_{GE}	V_{PE}	V_{GPE}	V_ε
Sweetness [1–9]	3.81	±0.51	0.192**	0.093**	0.044**	0.001	0.000	0.136**	0.125**	0.000	0.987
Sourness [1–9]	4.28	±0.53	0.196**	0.128**	0.045**	0.022	0.000	0.350**	0.005	0.000	1.147
Total aroma [1–9]	5.17	±0.51	0.187**	0.018**	0.013**	0.018	0.000	0.114**	0.010**	0.000	0.930
Tomato aroma [1–9]	3.80	±0.40	0.086**	0.018**	0.010**	0.048	0.066**	0.038**	0.081**	0.005	0.581
Banana–melon aroma [2–18]‡	3.38	±1.30	1.482**	0.272**	0.040**	0.000	0.098*	0.404**	0.059**	0.000	2.240
Orange aroma [1–9]	1.91	±0.63	0.200**	0.002	0.012*	0.053	0.096*	0.102*	0.028**	0.000	2.021
Berry aroma [1–9]	1.96	±0.52	0.072**	0.018**	0.002	0.019	0.130**	0.044	0.018*	0.111	1.855
Spicy aroma [1–9]	1.61	±0.45	0.072**	0.139**	0.004	0.072	0.071**	0.069*	0.033**	0.000	1.257
Green aroma [1–9]	1.79	±0.36	0.019	0.844**	0.000	0.000	0.021	0.000	0.002	0.060	1.234

*, ** significant at 0.05 and 0.01 level, respectively

†cultivation system; ‡sum of banana and melon aroma

Table SII.5. Mean values and standard deviation (SD) of physicochemical measurements, fruit weight and volatile compounds [ng mL^{-1} sample] and variance components for the effects of genotype (V_G), replication within environment ($V_{R:E}$), environment (V_E), genotype-by-environment interaction (V_{GE}) and residuals (V_ε) of the mapping population 'Resi' × 'Auriga' (n ≥ 163) in two cultivation systems

Trait	Mean	SD	V_G	$V_{R:E}$	V_E[†]	V_{GE}	V_ε
TSS [°Brix]	6.79	±0.36	0.1012**	0.0000	0.1243**	0.0272**	0.1004
pH	4.02	±0.11	0.0013	0.0000	0.0062**	0.0039	0.0438
TA [%]	0.70	±0.03	0.0002**	0.0002**	0.0600**	0.0005**	0.0024
TSS/TA	12.05	±0.99	0.6246**	0.1187**	4.9102**	0.5846**	1.4054
DM [%]	7.98	±0.52	0.2240**	0.0196**	0.1216	0.0699**	0.1707
FW [g]	31.15	±6.48	39.6614**	1.2613**	11.2666*	5.2358**	12.2807
6-Methyl-5-hepten-2-one	3.11	±1.87	2.8689**	0.5318**	1.7142	0.5240**	2.4908
Neral	0.33	±0.20	0.0278**	0.0539**	0.0000	0.0130**	0.0445
Geranial	0.87	±0.56	0.2597**	0.0440**	0.1434	0.0652**	0.2106
E-Geranylacetone	2.37	±1.10	0.8752**	0.0485**	0.0000	0.2920**	1.3765
ß-Damascenone	1.12	±0.44	0.0632**	0.0273**	0.0000	0.1263**	0.5338
ß-Ionone	2.08	±0.94	0.7030**	0.0470**	0.0000	0.1352*	0.7310
1-Penten-3-one	0.06	±0.03	0.0001	0.0012**	0.0017	0.0003	0.0024
Hexanol	1.42	±0.60	0.2353**	0.0180**	0.1924*	0.1215**	0.4880
Z-3-Hexenol	1.71	±0.52	0.1704**	0.0048	1.0034**	0.0716*	0.4126
Hexanal	25.43	±9.62	52.6905**	1.8817	62.4638**	15.2686	159.4186
E-2-Hexenal	3.62	±1.27	0.0675	12.3140**	0.0251	0.2726	6.1584
Z-3-Hexenal	1.64	±0.50	0.1100**	0.5230**	0.0000	0.0715	0.5783
E-2-Heptenal	0.19	±0.06	0.0005	0.0101**	0.0076	0.0001	0.0139
Phenylacetaldehyde	0.20	±0.07	0.0025**	0.0015**	0.0000	0.0013	0.0104
2-Phenylethanol	0.51	±0.10	0.0054**	0.0157**	0.0000	0.0028*	0.0193
Methyl salicylate	0.48	±0.68	0.3582**	0.1048**	0.2653	0.6477**	0.4100
Benzaldehyde	0.37	±0.11	0.0065**	0.0030**	0.0036	0.0056**	0.0191
2-Isobutylthiazole	0.58	±0.24	0.0153*	0.1144**	0.0000	0.0000	0.1684

*, ** significant at 0.05 and 0.01 level, respectively

Abbreviation: TSS, total soluble solids; TA, titratable acidity; DM = dry matter; FW = fruit weight

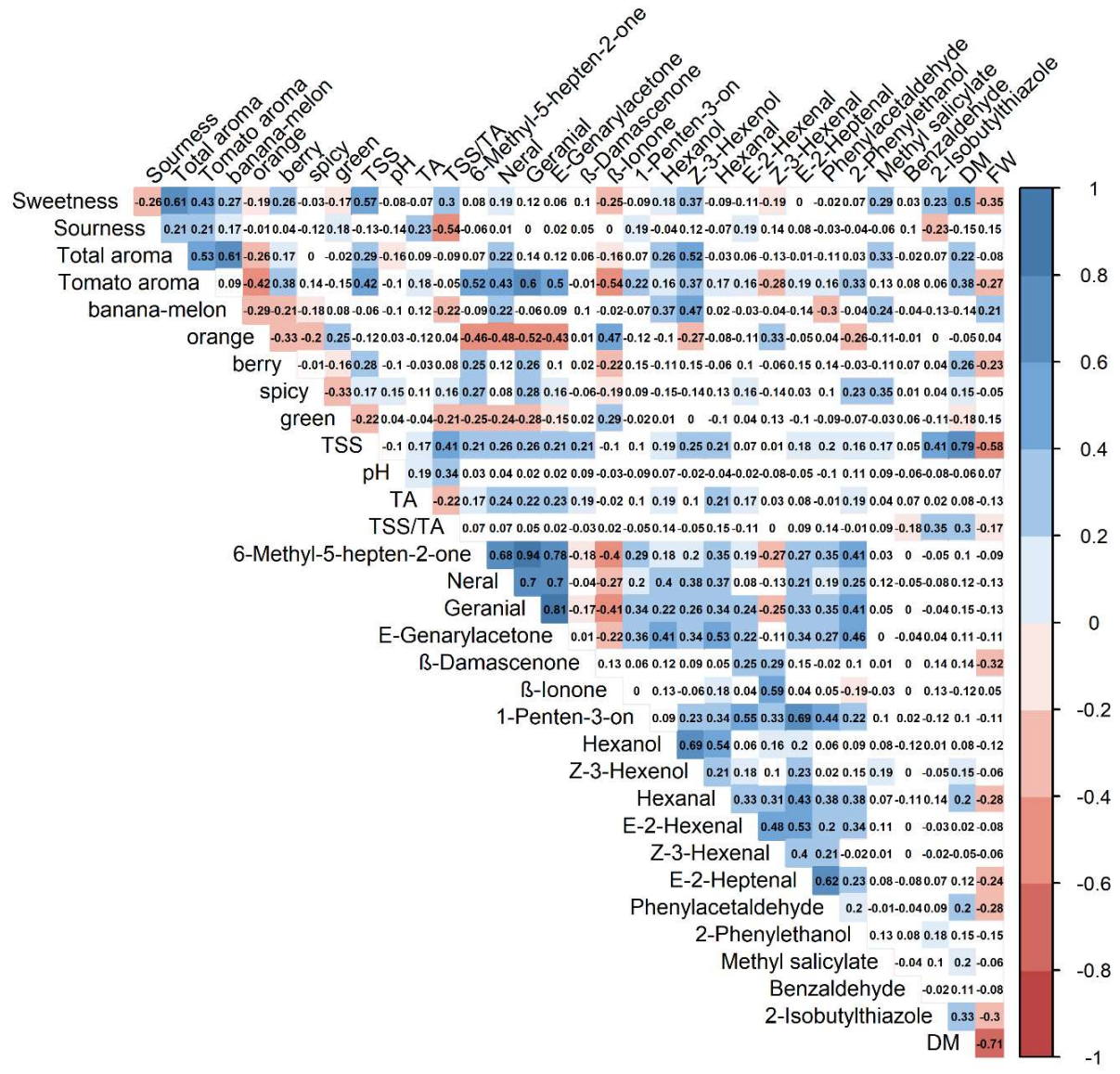

Figure SII.2. Spearman's correlation coefficients (r) for all sensory attributes, physicochemical measurements (TSS, total soluble solids; TA, titratable acidity; DM, dry matter), volatile compounds and fruit weight (FW) analysed in two cultivation systems (n ≥ 163); significant positive correlations are shown in blue and significant negative correlations in red with p = 0.05

Table SII.6. Comparison of QTL detected for the environmental means (AV), the organic cultivation system (Or) and the hydroponic cultivation systems (Hy) with their peak position and 95% Bayesian confidence interval; classification into co-localised QTL between the different environments

#	Name	AV	Or	Hy	All	AV/Or	AV/Hy	AV	Or	Hy	Note
	Frequency				27	19	5	20	15	14	
	Percentage [%]				27	19	5	20	15	14	
Sweetness											
1	SW2.1			86 (76–94)						x	
2	SW5.1	65 (60–69)	65 (58.9–72)	-		x					
3	SW10.1	19 (10–22)	21 (9–27)	11 (6–20)	x						
Sourness											
4	SO2.1	16 (3–20.2)	-	-				x			
5	SO3.1	-	-	85.1 (82–87)						x	
6	SO5.1	65 (61–69)	68 (58.9–75.5)	-		x					
7	SO11.1	24 (12–28)	12.7 (8–33)	24 (15–34)	x						
Total aroma											
8	A.TOT1.1	112.2 (111–112.2)	112 (106–112.2)	112.2 (111–112.2)	x						
9	A.TOT4.1	88 (85–90)	-	-				x			
10	A.TOT6.1	75 (3–78)	-	-				x			
11	A.TOT7.1	22 (10–30)	-	9 (4–51)			x				
12	A.TOT10.1	11 (5–23)	-	10 (5–32)			x				
Int[†]	A.TOT4.1:6.1	x	-	-							
Tomato aroma											
13	A.TOM2.1	-	-	87 (70–95.8)						x	
14	A.TOM6.1	67 (65–70)	67 (64–70)	69 (65–72)	x						
15	A.TOM10.1	16 (7.8–33)	-	-				x			
Banana-melon aroma											
16	A.BM1.1	111 (111–112)	111 (111–112)	111 (110–112)	x						
17	A.BM5.1	-	14 (8–18)	-					x		
18	A.BM6.1	-	68 (59–74)	-					x		

Continued next page.

Table SII.6. Continued.

#	Name	AV	Or	Hy	All	AV/Or	AV/Hy	AV	Or	Hy	Note
19	A.BM8.1	-	71 (48–76)	-					x		
Int[†]	A.BM5.1:8.1	-	x	-							
Orange aroma											
20	A.OR1.1	-	-	83 (77–92)						x	close
21	A.OR1.2	112 (89–112.2)	-	-				x			
22	A.OR6.1	69 (66–71)	70.4 (67–72)	67 (64–71)	x						
23	A.OR9.1	47 (31–59)	-	-				x			
Berry aroma											
24	A.BE6.1	70.4 (39–79)	-	-				x			
25	A.BE7.1	38 (3–48)	36.2 (12–43)	-		x					
Spicy aroma											
26	A.SP4.1	32 (14–53)	-	-				x			
27	A.SP6.1	70.4 (65–75)	70.4 (65–75)	-		x					
28	A.SP9.1	77.7 (76–79)	77.7 (76–79)	-		x					
Green aroma											
29	A.GR6.1	-	75 (64–78)	-					x		
Total soluble solids (TSS)											
30	TSS2.1	76 (72–83)	-	74.7 (71–77)			x				
31	TSS3.1	85 (83–86)	84 (82–86)	85.1 (82–91)	x						
32	TSS6.1	38 (34–43)	42 (38–45)	37 (32–58)	x						
33	TSS7.1	26 (2–59)	-	-				x			
34	TSS10.1	17.6 (14–20)	20 (16–23)	17 (6–22)	x						
35	TSS11.1	-	-	68 (65–77)						x	
36	TSS12.1	-	30 (19–42)	-					x		
Titratable acidity (TA)											
37	TA7.1	-	-	36.2 (24–46)						x	
pH											
38	pH7.1	-	-	36.2 (26–47)						x	

Continued next page.

Table SII.6. Continued.

#	Name	AV	Or	Hy	All	AV/Or	AV/Hy	AV	Or	Hy	Note
TSS/TA											
39	TSS/TA3.1	48.5 (37–52)	-	-				x			
40	TSS/TA3.2	-	-	85.1 (37–91)						x	
41	TSS/TA5.1	67 (62–69)	64 (51–72)	66 (61–72)	x						
42	TSS/TA6.1	-	11 (5–15)	-					x		
43	TSS/TA10.1	-	10 (5–26)	-					x		
Dry matter											
44	DM1.1	74.5 (71–75)	75 (70–91)	-		x					
45	DM2.1	81 (77–86)	81 (76–87)	80 (76–87)	x						
46	DM3.1	84 (83–86)	85 (83–86)	82 (78.9–84)	x						
47	DM6.1	10 (7–13)	12 (5–24)	-		x					
48	DM7.1	11.3 (8–15)	-	8 (4–12)			x				
49	DM8.1	44.6 (42–47)	-	-				x			
50	DM8.2	-	-	75.2 (46–81)						x	
51	DM10.1	-	-	14 (9–18)						x	close
52	DM10.2	26 (22–31)	32 (22–34)	-		x					
53	DM11.1	76 (73–79)	84 (76–87)	-		x					
54	DM12.1	-	26 (20–30)	-					x		close
55	DM12.2	42 (39–45)	-	-				x			
Int[†]	DM1.1:8.1	x	-	-							
Int[†]	DM3.1:7.1	-	-	x							
Fruit weight											
56	FW1.1	109.4 (107–112.2)	-	-				x			
57	FW2.1	44 (38.6–54)	46 (43–49)	-		x					
58	FW2.2	88 (87–92)	89 (86–92)	83 (81–86)	x						
59	FW3.1	84 (83–85.1)	84 (83–85.1)	77 (76–83)	x						

Continued next page.

Table SII.6. Continued.

#	Name	AV	Or	Hy	All	AV/Or	AV/Hy	AV	Or	Hy	Note
60	FW4.1	39 (35–47)	-	-				x			close
61	FW4.2	-	58 (47–66)	-					x		
62	FW8.1	7 (0–13)	-	-				x			
63	FW8.2	-	38.1 (36–42)	-					x		
64	FW8.3	83 (80–85.4)	83 (76–85.4)	-		x					
65	FW10.1	3 (1–5)	-	-				x			close
66	FW10.2	-	17.6 (15–20)	-					x		
67	FW11.1	-	12.7 (11–15)	-					x		
68	FW11.2	83 (76–87)	88 (85–91.4)	-		x					
69	FW12.1	43 (39–48)	56 (48–62)	43 (20–61)	x						
6-Methyl-5-hepten-2-one											
70	6MHO6.1	70.4 (68–71)	70 (68–71)	70.4 (69–72)	x						
71	6MHO9.1	-	-	0 (0–43)						x	
Neral											
72	NER1.1	112.2 (94–112.2)	111 (102–112.2)	-		x					
73	NER3.1	76 (62–93)	-	-				x			
74	NER6.1	70.4 (66–71)	67 (64–71)	70.4 (66–72)	x						
Geranial											
75	GER6.1	71 (69–71)	70 (68–71)	71 (69–72)	x						
E-Geranylacetone											
76	eGAC1.1	107 (100–111)	103 (98–111)	-		x					
77	eGAC3.1	84 (80–87)	84 (40–93)	85.1 (76–91)	x						
78	eGAC4.1	49 (44–55)	-	-				x			
79	eGAC6.1	69 (67–72)	68 (65–70.4)	72 (67–77)	x						
ß-Ionone											
80	ßION6.1	69 (67–71)	69 (67–72)	69 (67–72)	x						

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Table SII.6. Continued.

#	Name	AV	Or	Hy	All	AV/Or	AV/Hy	AV	Or	Hy	Note
1-Penten-3-one											
81	1P3O1.1	-	-	107 (105–112)						x	
82	1P3O6.1	66 (35.6–71)	39 (35–71)	-		x					
Hexanol											
83	HEXOL1.1	109.4 (107–111)	108 (105–112)	109.4 (98–112.2)	x						
84	HEXOL3.1	83 (73–88)	-	-				x			
Z-3-Hexenol											
85	z3HEXOL1.1	52 (46–59)	55.6 (15–62)	-		x					
86	z3HEXOL1.2	109 (106–111)	111 (106–112.2)	109.4 (107–112)	x						
87	z3HEXOL4.1	-	-	17 (4–25)						x	
88	z3HEXOL7.1	41 (36–65)	41 (35–65)	-		x					
89	z3HEXOL10.1	-	10 (5–21)	-					x		
Hexanal											
90	HEXAL3.1	87 (83–92)	86 (71–94)	87 (83–92)	x						
E-2-Hexenal											
91	e2HEXEL6.1	54 (41–58)	53 (39–58)	-		x					
Z-3-Hexenal											
92	z3HEXEL6.1	77 (69–79)	78 (42–79.9)	68 (65–72)	x						
E-2-Heptenal											
93	e2HEPEL6.1	70.4 (51–79)	-	-				x			
2-Phenylethanol											
94	2PE1.1	-	-	112 (106–112.2)						x	
95	2PE2.1	77 (73–82)	67 (42–87)	82 (74.7–87)	x						
96	2PE6.1	68 (64–72)	-	70.4 (66–72)			x				
Methyl salicylate											
97	MES9.1	78 (77–79)	78 (77–79)	80 (77–85)	x						

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Table SII.6. Continued.

#	Name	AV	Or	Hy	All	AV/Or	AV/Hy	AV	Or	Hy	Note
Benzaldehyde											
98	BAL4.1	-	73 (70–84)	-					x		
99	BAL6.1	0 (0–10)	18 (3–31)	-		x					
100	BAL10.1	-	12 (9–49)	-					x		

[†]QTL-by-QTL interaction

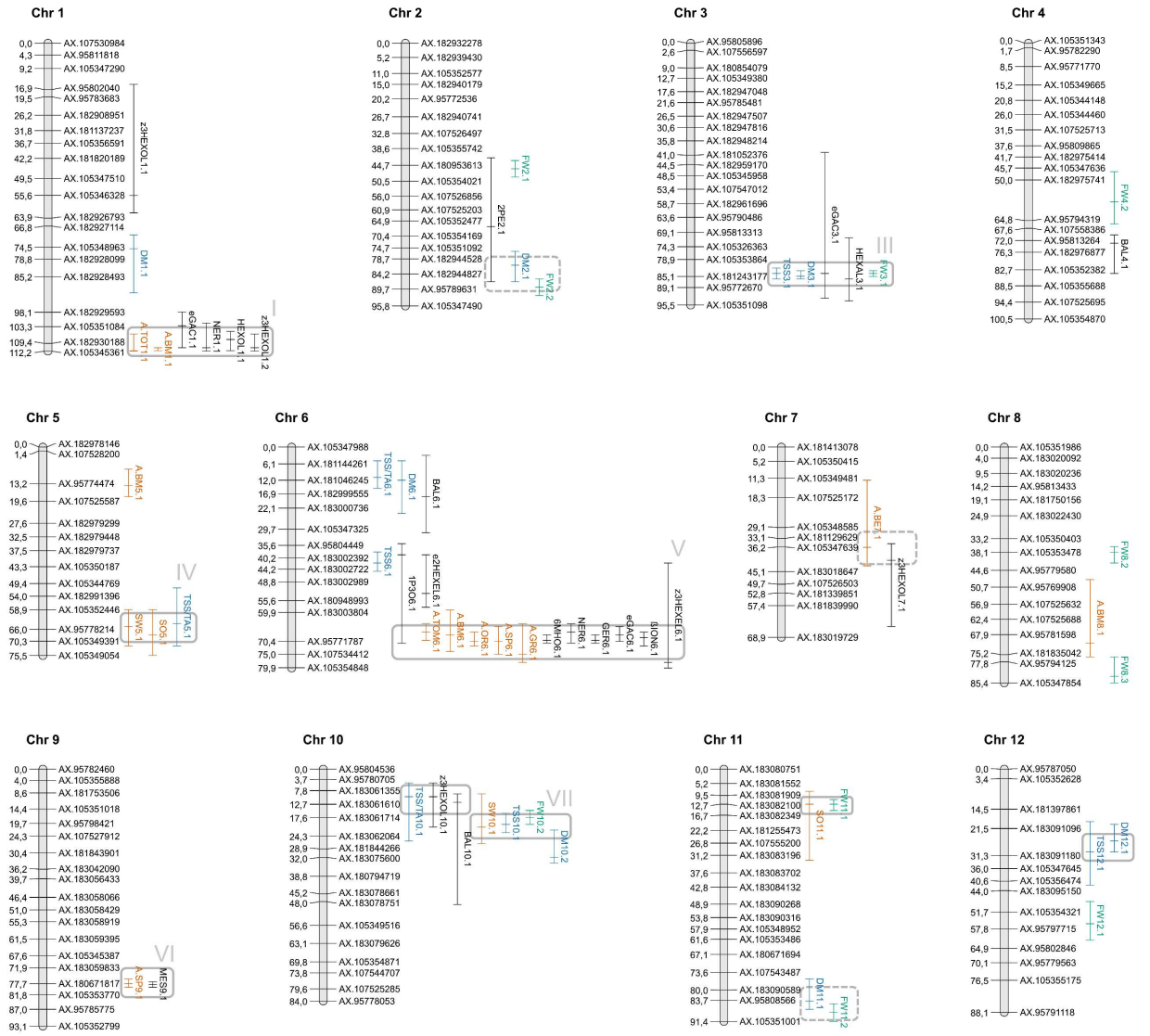

Figure SII.3. Organic cultivation: QTL (peak position and 95% Bayesian confidence interval) for the sensory attributes (orange), physicochemical measurements (blue), volatile compounds (black) and fruit weight (green) on the linkage map of 'Resi' × 'Auriga' detected by multiple-QTL mapping; QTL enclosed in boxes indicate clusters for co-localised QTL (distinct clusters: solid line; suspected clusters: dashed line), numbering according to QTL clusters identified for mean values of both cultivation systems

Figure SII.4. Hydroponic cultivation: QTL (peak position and 95% Bayesian confidence interval) for sensory attributes (orange), physicochemical measurements (blue), volatile compounds (black) and fruit weight (green) on the linkage map of 'Resi' × 'Auriga' detected by multiple-QTL mapping; QTL enclosed in boxes indicate clusters for co-localised QTL (distinct clusters: solid line; suspected clusters: dashed line), numbering according to QTL clusters identified for mean values of both cultivation systems

Table SII.7. Organic cultivation: Location and estimates of QTL for sensory attributes, physicochemical measurements, fruit weight and volatile compounds detected by multiple-QTL mapping in an F_2 population of 'Resi' × 'Auriga'

Trait	QTL	Chr[1]	Closest marker	Pos[2]	LOD[3]	PVE[4]	PVE$_{full}$[5]	Add[6]	Dom[7]	Allel[8]
Sweetness										
	SW5.1	5	AX-95778214	65.0 (58.9–72.0)	4.59	9.94	21.62	−0.26	0.04	A
	SW10.1	10	AX-183062064	21.0 (9.0–27.0)	3.82	8.19		−0.21	0.13	A
Sourness										
	SO5.1	5	AX-95778214	68.0 (58.9–75.5)	4.57	10.29	18.40	0.32	0.01	B
	SO11.1	11	AX-183082100	12.7 (8.0–33.0)	3.67	8.17		0.28	−0.17	B
Total aroma										
	A.TOT1.1	1	AX-105345361	112.0 (106.0–112.2)	8.29	19.39	19.39	−0.32	−0.13	A
Tomato aroma										
	A.TOM6.1	6	AX-95771787	67.0 (64.0–70.0)	19.66	40.04	40.04	−0.46	−0.13	A
Banana-melon aroma										
	A.BM1.1	1	AX-105345361	111.0 (111.0–112.0)	42.44	50.50		−1.38	−0.52	A
	A.BM5.1	5	AX-95774474	14.0 (8.0–18.0)	9.63	7.13		−0.001	−0.41	A
	A.BM6.1	6	AX-95771787	68.0 (59.0–74.0)	4.92	3.42	74.95	0.37	0.26	B
	A.BM8.1	8	AX-95781598	71.0 (48.0–76.0)	9.02	6.63		−0.26	−0.15	A
	A.BM5.1:8.1				7.35	5.28				
Orange aroma										
	A.OR6.1	6	AX-95771787	70.4 (67.0–72.0)	8.63	20.12	20.12	0.48	0.24	B
Berry aroma										
	A.BE7.1	7	AX-105347639	36.2 (12.0–43.0)	4.02	9.92	9.92	0.24	−0.10	B
Spicy aroma										
	A.SP6.1	6	AX-95771787	70.4 (65.0–75.0)	5.91	11.70	29.68	−0.35	−0.01	A
	A.SP9.1	9	AX-180671817	77.7 (76.0–79.0)	10.22	21.41		0.38	−0.31	B
Green aroma										
	A.GR6.1	6	AX-107534412	75.0 (64.0–78.0)	4.05	10.00	10.00	0.23	0.06	B
Total soluble solids (TSS)										
	TSS3.1	3	AX-181243177	84.0 (82.0–86.0)	10.97	15.96		−0.27	0.09	A
	TSS6.1	6	AX-183002392	42.0 (38.0–45.0)	8.55	12.02	52.97	−0.22	0.05	A
	TSS10.1	10	AX-183061714	20.0 (16.0–23.0)	11.46	16.77		−0.22	0.13	A
	TSS12.1	12	AX-183091180	30.0 (19.0–42.0)	5.07	6.80		−0.15	−0.06	A
TSS/TA										
	TSS/TA5.1	5	AX-95778214	64.0 (51.0–72.0)	5.97	11.79		−0.59	0.12	A
	TSS/TA6.1	6	AX-181046245	11.0 (5.0–15.0)	5.37	10.54	31.92	−0.54	0.40	A
	TSS/TA10.1	10	AX-183061355	10.0 (5.0–26.0)	3.93	7.56		−0.36	0.42	A
Dry matter										
	DM1.1	1	AX-105348963	75.0 (70.0–91.0)	5.25	5.10		0.18	0.14	B
	DM2.1	2	AX-182944528	81.0 (76.0–87.0)	5.59	5.46		−0.18	−0.08	A
	DM3.1	3	AX-181243177	85.0 (83.0–86.0)	22.13	27.25		−0.44	−0.08	A
	DM6.1	6	AX-181046245	12.0 (5.0–24.0)	4.72	4.56	65.77	−0.19	0.07	A
	DM10.2	10	AX-183075600	32.0 (22.0–34.0)	10.59	11.07		−0.28	−0.01	A
	DM11.1	11	AX-95808566	84.0 (76.0–87.0)	8.95	9.15		−0.23	0.14	A
	DM12.1	12	AX-183091096	26.0 (20.0–30.0)	10.82	11.35		−0.27	0.02	A
Fruit weight										
	FW2.1	2	AX-180953613	46.0 (43.0–49.0)	10.46	5.03		2.24	−0.55	B
	FW2.2	2	AX-95789631	89.0 (86.0–92.0)	14.18	7.18	84.34	2.59	−1.31	B

Continued next page.

Table SII.7. Continued.

Trait	QTL	Chr[1]	Closest marker	Pos[2]	LOD[3]	PVE[4]	PVE_{full}[5]	Add[6]	Dom[7]	Allel[8]
	FW3.1	3	AX-181243177	84.0 (83.0–85.1)	37.12	26.40		5.37	0.63	B
	FW4.2	4	AX-95794319	58.0 (47.0–66.0)	5.95	2.69		1.69	−1.18	B
	FW8.2	8	AX-105353478	38.1 (36.0–42.0)	3.91	1.72		0.67	−1.65	B
	FW8.3	8	AX-105347854	83.0 (76.0–85.4)	3.80	1.67		1.18	0.85	B
	FW10.2	10	AX-183061714	17.6 (15.0–20.0)	20.09	11.07		3.06	−0.92	B
	FW11.1	11	AX-183082100	12.7 (11.0–15.0)	7.62	3.52		1.95	−0.62	B
	FW11.2	11	AX-105351001	88.0 (85.0–91.4)	11.75	5.75		2.23	−1.25	B
	FW12.1	12	AX-95797715	56.0 (48.0–62.0)	9.82	4.68		1.90	−1.27	B
6-Methyl-5-hepten-2-one										
	6MHO6.1	6	AX-95771787	70.0 (68.0–71.0)	34.84	62.18	62.18	−1.72	−0.81	A
Neral										
	NER1.1	1	AX-105345361	111.0 (102.0–112.2)	6.11	11.78	36.62	−0.07	0.03	A
	NER6.1	6	AX-95771787	67.0 (64.0–71.0)	10.39	21.32		−0.11	−0.07	A
Geranial										
	GER6.1	6	AX-95771787	70.0 (68.0–71.0)	33.46	60.47	60.47	−0.49	−0.28	A
E-Geranylacetone										
	eGAC1.1	1	AX-105351084	103.0 (98.0–111.0)	4.95	6.84		−0.46	0.34	A
	eGAC3.1	3	AX-181243177	84.0 (40.0–93.0)	3.74	5.08	53.83	−0.48	−0.15	A
	eGAC6.1	6	AX-95771787	68.0 (65.0–70.4)	22.46	40.26		−1.37	−0.30	A
ß-Ionone										
	ßION6.1	6	AX-95771787	69.0 (67.0–72.0)	24.76	49.47	49.47	1.05	0.23	B
1-Penten-3-one										
	1P3O6.1	6	AX-183002392	39.0 (35.0–71.0)	3.89	10.24	10.24	−0.02	−0.02	A
Hexanol										
	HEXOL1.1	1	AX-182930188	108.0 (105.0–112.0)	8.00	20.24	20.24	−0.41	−0.03	A
Z-3-Hexenol										
	z3HEXOL1.1	1	AX-105346328	55.6 (15.0–62.0)	3.66	6.24		0.26	0.10	B
	z3HEXOL1.2	1	AX-105345361	111.0 (106.0–112.2)	8.65	15.84	41.98	−0.37	0.21	A
	z3HEXOL7.1	7	AX-183018647	41.0 (35.0–65.0)	6.80	12.12		0.37	−0.02	B
	z3HEXOL10.1	10	AX-183061355	10.0 (5.0–21.0)	4.82	8.35		−0.29	0.20	A
Hexanal										
	HEXAL3.1	3	AX-181243177	86.0 (71.0–94.0)	4.05	10.56	10.56	−6.28	−0.88	A
E-2-Hexenal										
	e2HEXEL6.1	6	AX-180948993	53.0 (39.0–58.0)	3.98	10.46	10.46	−0.56	−1.48	A
Z-3-Hexenal										
	z3HEXEL6.1	6	AX-105354848	78.0 (42.0–79.9)	3.72	9.75	9.75	0.29	−0.21	B
2-Phenylethanol										
	2PE2.1	2	AX-105352477	67.0 (42.0–87.0)	3.78	9.97	9.97	−0.06	0.03	A
Methyl salicylate										
	MES9.1	9	AX-180671817	78.0 (77.0–79.0)	28.44	55.23	55.23	1.13	−1.03	B
Benzaldehyde										
	BAL4.1	4	AX-95813264	73.0 (70.0–84.0)	4.31	8.72		0.04	0.02	B
	BAL6.1	6	AX-182999555	18.0 (3.0–31.0)	5.03	10.28	31.35	0.05	−0.01	B
	BAL10.1	10	AX-183061610	12.0 (9.0–49.0)	5.10	10.44		−0.03	0.04	A

[1]Chr, chromosome; [2]Pos, peak position with 95% Bayesian confidence interval; [3]LOD, log of likelihood ratio; [4]PVE, percentage of phenotypic variation explained by the QTL; [5]PVE_{full}, percentage of phenotypic variation explained by the multiple-QTL model; [6]Add, additive effect (positive effect denote increasing effect of the B allele); [7]Dom. dominance; [8]Allele, allele increasing the phenotypic value (A from Resi, B from Auriga)

Table SII.8. Hydroponic cultivation: Location and estimates of QTL for sensory attributes, physicochemical measurements, fruit weight and volatile compounds detected by multiple-QTL mapping in an F_2 population of 'Resi' × 'Auriga'

Trait	QTL	Chr[1]	Closest marker	Pos[2]	LOD[3]	PVE[4]	PVE_{full}[5]	Add[6]	Dom[7]	Allel[8]
Sweetness										
	SW2.1	2	AX-182944827	86.0 (76.0–94.0)	3.75	7.58	25.95	−0.26	0.00	A
	SW10.1	10	AX-183061610	11.0 (6.0–20.0)	8.63	18.64		−0.43	0.08	A
Sourness										
	SO3.1	3	AX-181243177	85.1 (82.0–87.0)	9.04	19.61	26.04	0.52	−0.41	B
	SO11.1	11	AX-181255473	24.0 (15.0–34.0)	4.13	8.40		0.34	−0.03	B
Total aroma										
	A.TOT1.1	1	AX-105345361	112.2 (111.0–112.2)	12.00	22.70		−0.40	−0.24	A
	A.TOT7.1	7	AX-105349481	9.0 (4.0–51.0)	3.64	6.15	38.07	0.24	−0.03	B
	A.TOT10.1	10	AX-183061355	10.0 (5.0–32.0)	5.09	8.77		−0.29	0.01	A
Tomato aroma										
	A.TOM2.1	2	AX-95789631	87.0 (70.0–95.8)	3.96	7.41	31.66	−0.17	0.09	A
	A.TOM6.1	6	AX-95771787	69.0 (65.0–72.0)	11.82	24.60		−0.34	−0.06	A
Banana-melon aroma										
	A.BM1.1	1	AX-105345361	111.0 (110.0–112.0)	34.92	59.69	59.69	−1.47	−0.98	A
Orange aroma										
	A.OR1.1	1	AX-182928493	83.0 (77.0–92.0)	4.86	8.13	39.66	0.32	0.10	B
	A.OR6.1	6	AX-95771787	67.0 (64.0–71.0)	16.67	32.77		0.63	−0.32	B
Total soluble solids (TSS)										
	TSS2.1	2	AX-105351092	74.7 (71.0–77.0)	6.76	10.67		−0.16	−0.12	A
	TSS3.1	3	AX-181243177	85.1 (82.0–91.0)	7.23	11.49		−0.22	0.15	A
	TSS6.1	6	AX-95804449	37.0 (32.0–58.0)	4.59	7.03	45.87	−0.15	−0.06	A
	TSS10.1	10	AX-183061714	17.0 (6.0–22.0)	6.00	9.38		−0.17	0.05	A
	TSS11.1	11	AX-180671694	68.0 (65.0–77.0)	5.09	7.86		−0.11	0.17	A
Titratable acidity (TA)										
	TA7.1	7	AX-105347639	36.2 (24.0–46.0)	3.65	9.25	9.25	−0.01	0.00	A
pH										
	pH7.1	7	AX-105347639	36.2 (26.0–47.0)	3.66	9.23	9.23	−0.07	0.00	A
TSS/TA										
	TSS/TA3.2	3	AX-181243177	85.1 (37.0–91.0)	5.44	11.51	26.57	−0.69	0.58	A
	TSS/TA5.1	5	AX-95778214	66.0 (61.0–72.0)	5.93	12.64		−0.69	−0.04	A
Dry matter										
	DM2.1	2	AX-182944528	80.0 (76.0–87.0)	11.87	15.53		−0.35	−0.04	A
	DM3.1	3	AX-105353864	82.0 (78.9–84.0)	16.21	22.54		−0.39	0.09	A
	DM7.1	7	AX-105350415	8.0 (4.0–12.0)	9.99	12.73	57.93	0.17	−0.01	B
	DM8.2	8	AX-181835042	75.2 (46.0–81.0)	3.76	4.41		−0.17	−0.05	A
	DM10.1	10	AX-183061610	14.0 (9.0–18.0)	9.35	11.80		−0.31	0.02	A
	DM3.1:7.1				6.26	7.58				
Fruit weight										
	FW2.2	2	AX-182944827	83.0 (81.0–86.0)	17.32	25.51		4.94	−2.99	B
	FW3.1	3	AX-105353864	77.0 (76.0–83.0)	19.86	30.33	56.46	5.74	2.19	B
	FW12.1	12	AX-183095150	43.0 (20.0–61.0)	4.36	5.35		2.48	1.03	B
6-Methyl-5-hepten-2-one										
	6MHO6.1	6	AX-95771787	70.4 (69.0–72.0)	21.21	42.20	47.75	−2.33	−1.07	A
	6MHO9.1	9	AX-95782460	0.0 (0.0–43.0)	3.65	5.60		−0.66	−0.72	A

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Table SII.8. Continued.

Trait	QTL	Chr[1]	Closest marker	Pos[2]	LOD[3]	PVE[4]	PVE_{full}[5]	Add[6]	Dom[7]	Allel[8]
Neral										
	NER6.1	6	AX-95771787	70.4 (66.0–72.0)	7.85	19.68	19.68	−0.18	−0.10	A
Geranial										
	GER6.1	6	AX-95771787	71.0 (69.0–72.0)	29.36	55.72	55.72	−0.80	−0.43	A
E-Geranylacetone										
	eGAC3.1	3	AX-181243177	85.1 (76.0–91.0)	4.79	10.45	26.95	−0.59	0.11	A
	eGAC6.1	6	AX-95771787	72.0 (67.0–77.0)	7.15	16.13		−0.69	−0.14	A
ß-Ionone										
	ßION6.1	6	AX-95771787	69.0 (67.0–72.0)	23.71	48.00	48.00	1.14	0.35	B
1-Penten-3-one										
	1P3O1.1	1	AX-182930188	107.0 (105.0–112.0)	5.95	15.23	15.23	0.001	0.01	B
Hexanol										
	HEXOL1.1	1	AX-182930188	109.4 (98.0–112.2)	5.22	13.71	13.71	−0.37	−0.14	A
Z-3-Hexenol										
	z3HEXOL1.2	1	AX-182930188	109.4 (107.0–112.0)	7.79	17.20	29.22	−0.27	−0.07	A
	z3HEXOL4.1	4	AX-105349665	17.0 (4.0–25.0)	3.70	7.69		−0.13	−0.22	A
Hexanal										
	HEXAL3.1	3	AX-181243177	87.0 (83.0–92.0)	10.42	24.98	24.98	−8.47	0.63	A
Z-3-Hexenal										
	z3HEXEL6.1	6	AX-95771787	68.0 (65.0–72.0)	14.78	33.47	33.47	0.51	−0.05	B
2-Phenylethanol										
	2PE1.1	1	AX-105345361	112.0 (106.0–112.2)	6.12	10.59		−0.05	0.03	A
	2PE2.1	2	AX-182944827	82.0 (74.7–87.0)	8.11	14.42	42.83	−0.06	−0.01	A
	2PE6.1	6	AX-95771787	70.4 (66.0–72.0)	9.22	16.67		−0.07	−0.02	A
Methyl salicylate										
	MES9.1	9	AX-105353770	80.0 (77.0–85.0)	8.60	21.58	21.58	0.04	−0.03	B

[1]Chr, chromosome; [2]Pos, peak position with 95% Bayesian confidence interval; [3]LOD, log of likelihood ratio; [4]PVE, percentage of phenotypic variation explained by the QTL; [5]PVE_{full}, percentage of phenotypic variation explained by the multiple-QTL model; [6]Add, additive effect (positive effect denote increasing effect of the B allele); [7]Dom. dominance; [8]Allele, allele increasing the phenotypic value (A from Resi, B from Auriga)

4 STUDY III: Flavour Improvement in Early Generations of Fresh Market Tomatoes (*Solanum lycopersicum* L.): II. Response to Breeders' Sensory and Marker-Assisted Selection

Julia Hagenguth[1], Larissa Kanski[2], Hannah Kahle[3], Heiko C. Becker[1] and Bernd Horneburg[4, 5]

[1]Division of Plant Breeding Methodology, Department of Crop Sciences, Faculty of Agriculture, University of Goettingen, Carl-Sprengel-Weg 1, 37075 Goettingen, Germany

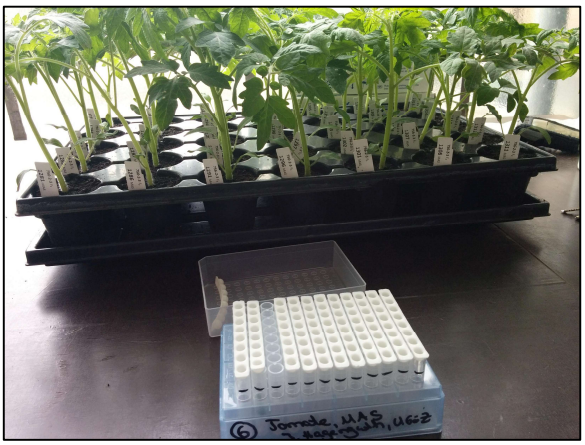
[2]Division of Quality of Plant Products, Department of Crop Sciences, Faculty of Agriculture, University of Goettingen, Carl-Sprengel-Weg 1, 37075 Goettingen, Germany

[3]Department of Horticultural Production, University of Applied Sciences Osnabrueck, Am Krümpel 31, 49090 Osnabrueck, Germany

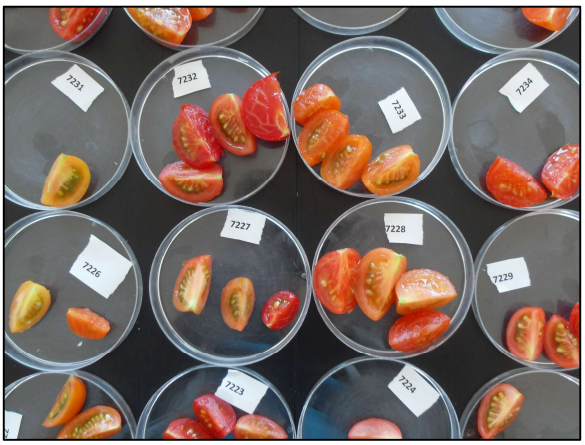
[4]Division of Plant Breeding, Section of Genetic Resources and Organic Plant Breeding, Department of Crop Sciences, Faculty of Agriculture, University of Goettingen, Carl-Sprengel-Weg 1, 37075 Goettingen

[5]present address: Faculty of Organic Agricultural Sciences, Section of Organic Plant Breeding and Agrobiodiversity, University of Kassel, Nordbahnhofstraße 1a, 37213 Witzenhausen, Germany

Submitted to *Plant Breeding*.



Leaf samples for marker-assisted selection



Samples for the Breeders' Sensory Test

Author contributions:

JH, LK, HB and BH planned and designed the experimental setup. JH, HB and BH developed the experimental populations. JH performed the experiment at the organic and HK at the hydroponic site. JH guided the Breeders' Sensory Test. LK conducted the physicochemical measurements and aroma volatile analysis and guided the trained panel. HB and BH supervised the experiment. JH analysed the data, wrote the original draft, and was supervised by HB and BH. All authors reviewed, edited and approved the manuscript.

Abstract

Fresh market tomatoes are often characterised by poor flavour. Flavour is a quantitative trait difficult to assess and often neglected in breeding. Classical sensory methods are not suitable for the evaluation of early segregating generations; simple physicochemical measurements are not sufficient to predict perceived aroma. Alternative selection methods are needed, such as the recently introduced Breeders' Sensory Test or marker-assisted selection (MAS). Two unrelated crosses were chosen to evaluate the efficiency of phenotypic selection with the Breeders' Sensory Test (breeders' sensory selection, BS) and MAS for five quantitative trait loci for sweetness, sourness and tomato aroma (mapped in 'Resi' × 'Auriga') in organic low-input and hydroponic cultivation. Selection for sensory attributes reduced fruit weight, emphasising the challenge of breeding flavourful, large-fruited tomatoes. Both selection methods were more effective in 'Roterno F_1' × 'Black Cherry' derived from more distant parents. A trend towards higher efficiency of MAS in early segregating generations was observed, most evident for tomato aroma. However, a major advantage of BS is its universal applicability. To improve flavour, combining both methods is recommended.

Keywords: flavour improvement, phenotyping, molecular marker, sensory, tomato

4.1 Introduction

Improving tomato flavour remains a major challenge in breeding (Klee & Tieman, 2018; Piombino et al., 2013; Zhao et al., 2019). Until recently, flavour was a low priority breeding goal relegated to advanced breeding generations (Causse et al., 2010; Klee & Tieman, 2018; Wang & Kays, 2003). The focus on producer-oriented breeding goals, such as yield, disease resistance, long shelf life and adaption to winter greenhouse conditions, has enabled the year-round availability of tomato fruits with excellent appearance (Causse et al., 2010; Folta & Klee, 2016). However, in the early 1990s, consumers started to complain about the poor flavour of commercial tomato cultivars (Causse et al., 2010; Schouten et al., 2019). Flavour is determined by an interaction of taste, aroma and texture (Beckles, 2012; Tikunov et al., 2020). Due to its chemical complexity and the polygenic nature of this trait, suitable methods for assessing flavour are lacking (Causse et al., 2010; Piombino et al., 2013). Since consumers demand flavourful tomatoes and are willing to pay a premium for flavour, priority has shifted towards flavour and consumer demands (Causse et al., 2010; Klee & Tieman, 2018; Schouten et al., 2019). Nevertheless, balancing producer and consumer demands is challenging since quality attributes, such as tomato flavour, are negatively correlated with fruit size and yield (Causse et al., 2003; Erika et al., 2022; Klee & Tieman, 2018).

Breeders need appropriate tools to incorporate flavour into the breeding process (Causse et al., 2010; Colantonio et al., 2022), especially suitable for early segregating generations with hundreds of small

samples. Sensory analysis is the most powerful method for studying organoleptic quality, including taste and aroma, and for analysing consumer preferences (Causse et al., 2001; Causse et al., 2010). Descriptive analysis by a trained panel is objective, accurate and standardised but time consuming and expensive (Piombino et al., 2013) and requires large sample sizes to ensure replicability. They might be suitable for late breeding generations close to cultivar release but not for early segregating generations. Physicochemical measurements, such as total soluble solids (TSS) and titratable acidity (TA), helped breeders improve the taste of tomato (Tandon et al., 2003), but aroma cannot reliably be predicted from these simple measurements (Causse et al., 2003; Lecomte et al., 2004a). Therefore, Hagenguth et al. (2022) recommended using the Breeders' Sensory Test in the early generations. Similar methods are probably used in vegetable and fruit breeding programmes (Behrendt, 2009; Horneburg et al., 2009; Vicente et al., 2014) and in field evaluations (Colantonio et al., 2022) but were evaluated for the first time in tomato (Hagenguth et al., 2022). Furthermore, phenotypic selection is particularly important in organic plant breeding, where the use of marker-assisted selection (MAS) is less self-evident due to the basic principles of organic farming (Lammerts van Bueren et al., 2003; Lammerts van Bueren et al., 2010). However, it remains unclear how efficient phenotypic selection for sensory attributes can be.

Several quantitative trait loci (QTL) have been identified for primary and secondary metabolites in tomatoes (Martina et al., 2021; Tikunov et al., 2020), and a few have been identified for sensory attributes (Causse et al., 2001; Tikunov et al., 2020; Zanor et al., 2009). These QTL can be used for MAS (Flint-Garcia et al., 2003; Lecomte et al., 2004a). Due to difficulties in flavour phenotyping, molecular markers are a promising tool to enhance tomato flavour (Causse et al., 2002; Lecomte et al., 2004a; Tieman et al., 2017). A major advantage is that molecular markers can be applied to seedlings and thus to the large number of individuals in the first segregating generations (Hernández-Bautista et al., 2016). However, verification of QTL in diverse genetic backgrounds is essential before they are used in breeding programmes, but is rarely done (Chaïb et al., 2006; Platten et al., 2019).

In a previous study, we identified QTL for the sensory attributes sweetness, sourness and tomato aroma, some of which were novel (Hagenguth et al., n.d.). For this study, five genetic regions were chosen to test molecular markers for sensory attributes in the cross used for QTL mapping and in an unrelated cross. In parallel, plants from both crosses were phenotypically selected for the same attributes based on the Breeders' Sensory Test (breeders' sensory selection, BS). Three experimental populations (unselected control, BS and MAS) were developed for each cross. These populations were phenotyped in organic low-input and hydroponic cultivation for sensory attributes, physicochemical measurements and aroma volatiles to evaluate the response to selection by *i)* phenotypic selection based on the Breeders' Sensory Test and *ii)* molecular markers for sensory attributes.

4.2 Materials and methods

4.2.1 Plant material, experimental design and selection methods

This study was carried out based on two crosses: *i)* 'Resi' × 'Auriga', a cross between two open-pollinated tomato cultivars with superior fruit quality (Chea et al., 2021; Erika et al., 2022) and *ii)* 'Roterno F_1' × 'Black Cherry', a cross between a high-yielding F_1 hybrid (Chea et al., 2021) and an open-pollinated cultivar characterised by superior flavour (Erika et al., 2022) (Table 4.1). Of these crosses, Resi × Auriga has previously been used to map QTL for flavour-related traits (Hagenguth et al., n.d.).

Table 4.1. Crosses and their parental cultivars used to develop three experimental populations

	Fruit type[1]	Fruit colour	Attribute[2]	Breeder[3]	Year of release[3]
Resi × Auriga					
Resi	Cocktail	Red	Quality	OOTP[†]	2010
Auriga	Salad	Orange	Quality	Saatzucht Quedlinburg	1980
Roterno F_1 × Black Cherry					
Roterno F1	Salad	Red	Yield	Rijk Zwaan	2007
Black Cherry	Cocktail	Red-brown	Quality	Reinsaat KG	2009

[1]Cocktail ≤ 52 g, Salad > 52g; classification according to Erika et al. (2020)
[2]according Chea et al (2021)
[3]CPVO (2022)
[†]OOTP = Organic Outdoor Tomato Project (Zörb et al., 2020)

In 2019, three experimental populations per cross were phenotyped in organic low-input and hydroponic cultivation, as described below. All experimental populations of a cross originated from a single F_1 plant. Two methods were used to identify the best F_2 plants and to compare them to an unselected control (Figure 4.1).

Breeders' sensory selection (BS): In 2018, 190 F_2 plants of Resi × Auriga and 62 plants of Roterno F_1 × Black Cherry were phenotyped for sensory attributes using the Breeders' Sensory Test (Hagenguth et al., 2022) in both cultivation systems. The sum of the mean values of both cultivation systems for sweetness, sourness and tomato aroma was used to select the top 16% of the plants. This resulted in 30 selected F_2 plants of Resi × Auriga and 10 selected F_2 plants of Roterno F_1 × Black Cherry. In the following year, two F_3 plants per selected F_2 plant of Resi × Auriga and six F_3 plants per selected F_2 plant of Roterno F_1 × Black Cherry were phenotyped.

Marker-assisted selection (MAS): A total of 15 Kompetitive allele specific PCR (KASP) markers, summarised in Table 4.2, were used to genotype 473 F_2 plants per cross. These molecular markers were selected based on the QTL mapping study by Hagenguth et al. (n.d.) and represented five loci expected to enhance the sensory attributes sweetness, sourness and tomato aroma. Leaf samples for DNA extraction were collected from young plants in the 4-leaf stage 3 weeks after sowing. Leaf samples of three plants per parental cultivar were pooled. DNA extraction and KASP marker analysis were conducted at the SGS

Institut Fresenius GmbH, TraitGenetics Section (Seeland, Germany). Homozygote loci and as few heterozygote loci as necessary were selected. In total, 20 selected plants (4%) were used per cross.

Unselected control (Ctrl): Thirty F_2 plants were randomly chosen per cross.

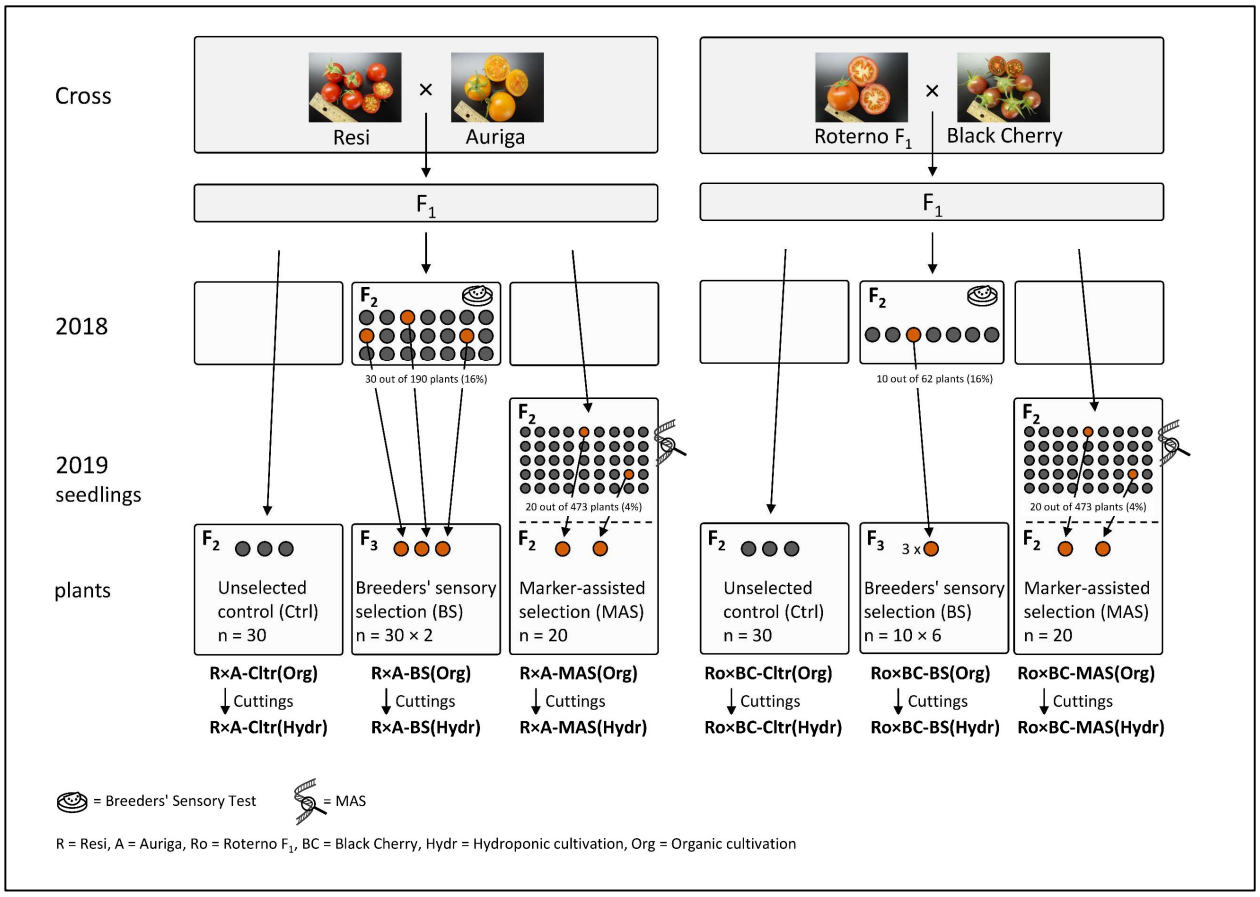


Figure 4.1. Selection scheme and selection intensity for the development of three experimental populations derived from two crosses of tomato

Table 4.2. KASP markers used to genotype two crosses to perform marker-assisted selection

Closest marker	Flanking marker top	Flanking marker bottom	Trait[1]	Chromosome[1]	QTL Peak[1,2]	PVE[1,3]	Allele[1,4]
AX-182940179	AX-105352577	AX-95772536	Sourness	2	16.0 (3.0–20.2)	9.4	B
AX-95778214	AX-105352446	AX-105349391	Sweetness	5	65.0 (60.0–69.0)	11.8	A
AX-95771787	AX-183003804	AX-107534412	Tomato aroma	6	67.0 (65.0–70.0)	41.1	A
AX-183061714	AX-183061610	AX-183062064	Sweetness	10	19.0 (10.0–22.0)	16.6	A
			Tomato aroma	10	16.0 (7.8–33.0)	5.2	A
AX-181255473	AX-183082349	AX-107555200	Sourness	11	24.0 (12.0–28.0)	12.2	B

[1]Hagenguth et al. (n.d.)
[2]Position of the QTL peak and 95% Bayesian confidence interval
[3]Percentage of phenotypic variation explained by the QTL
[4]Allele increasing the phenotypic value

For hydroponic cultivation, clones (cuttings) produced from the side shoots of plants from organic cultivation were used. Two replications were grown per cultivation system. Plants from the experimental populations were divided equally among the replications. The details of the experimental design are shown in Figure 4.2. The trials were surrounded by border plants.

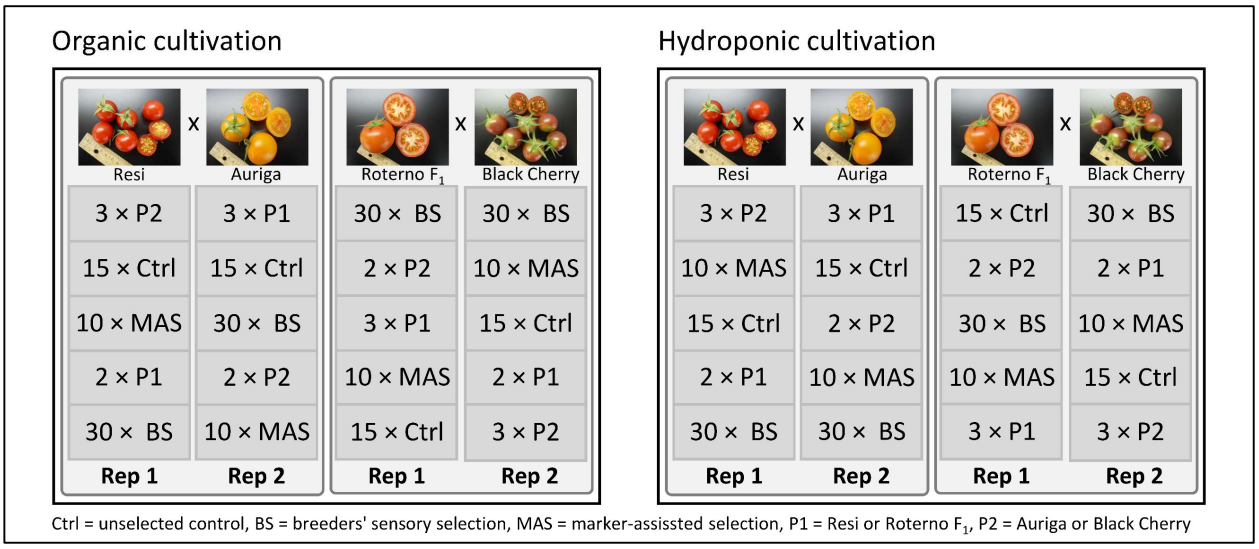


Figure 4.2. Experimental design used to assess the response to selection by breeders' sensory selection and molecular markers for the sensory attributes sweetness, sourness and tomato aroma

4.2.2 Cultivation systems

Per cross, 120 indeterminate tomato plants, including the parental cultivars, were grown in an organic low-input and a hydroponic cultivation system. In week 15, seeds were sown in trays in Bio-Traysubstrat (Klasmann-Deilmann, Geeste, Germany) in a greenhouse (20°C day/18°C night, 16/8 h). After 12 days, seedlings with fully developed cotyledons and emerging first leaves were transferred to QP 35R (Hermann Meyer, Rellingen, Germany) trays filled with Bio-Traysubstrat. Seedlings were potted in 0.69 L pots filled with Bio-Kräutererde (Klasmann-Deilmann, Geeste, Germany) 16 days later.

Planting in the organic low-input cultivation system took place seven weeks after sowing. Low-input conditions were defined as no application of fertiliser and moderate irrigation. The organic cultivation system was located at Reinshof experimental station (51°30'15.1" N, 9°55'14.7" E, 152 m above sea level), University of Goettingen, Germany. Plants were grown under a well-ventilated rain-out shelter (greenhouse film FVG Euro 4, FVG Folien-Vertriebs GmbH, Dernbach, Germany) with a distance of 0.5 m between plants and 1 m between rows. This growing system was chosen to exclude major pathogens that are relevant in greenhouses (*Cladosporium fulvum* Cooke) and in the field (*Phytophthora infestans* (Mont.) de Bary). A drip system was used for irrigation (187 L m^{-2} during the entire field season). An overview of soil properties, temperature, relative humidity and soil water content during the field season is available in Table SIII.1 and Figure SIII.1.

For the conventional hydroponic cultivation system at the University of Applied Sciences Osnabrueck, Department of Horticultural Production, Germany, one side shoot per individual plant was collected 12 days after planting. Cuttings were planted into QP 96 trays (Hermann Meyer, Rellingen, Germany) filled with Bio-Traysubstrat (Klasmann-Deilmann, Geeste, Germany). Two weeks later, they were transferred to rockwool cubes (10 × 10 × 6.5 cm, Grodan®, Roermond, The Netherlands). After another two weeks, Grodan cubes were placed on rockwool slabs (100 × 15 × 7.5 cm, Grodan®, Roermond, The Netherlands) in the greenhouse (19°C day/17°C night, daylight; single glazed) in double rows (distance 0.5 m) with 0.36 m between plants and 1 m between double rows. A standard nutrient solution, according to de Kreij et al. (2003), was applied together with irrigation. The amount and concentration of nutrients were adapted depending on solar irradiation and development stage.

4.2.3 Phenotyping of experimental populations

In the organic cultivation system, from week 31 onwards, mature fruits were harvested, counted and weighted every second week to obtain the average fruit weight for each individual plant. If available, four fruits were weighed in the hydroponic trial at weeks 36 and 38 to determine the average fruit weight. Fruits with blossom end rot were discarded.

All individual plants were evaluated with the Breeders' Sensory Test. For sensory attributes assessed by the trained panel, physicochemical measurements and aroma volatiles, ten plants per cross, experimental population and replication were randomly chosen. Parental cultivars were analysed as mixed samples. For the Breeders' Sensory Test, fully mature fruits were harvested in the hydroponic cultivation system in week 38 and one week later in the organic system. The fruits were evaluated on the day of harvest. In both cultivation systems, fruits were harvested in week 37 for physicochemical measurements, volatile analysis and sensory assessment by a trained panel. Fruits were stored at room temperature for two days prior to panel evaluation and sample preparation for subsequent analysis.

Sensory evaluation by the Breeders' Sensory Test

For sensory evaluation of all individual plants, the Breeders' Sensory Test (Hagenguth et al., 2022) was conducted by a three-person team involved in previous assessments. Depending on the range of experience, the members of this team conducted 2–4 training sessions (5–8 hours in total) in the four weeks prior to the assessment. The evaluation was conducted in a quiet room with moderate natural light. Sweetness, sourness and tomato aroma were evaluated on a scale from 1 to 9 with increments of 0.5 (1 = not perceptible, 9 = maximum intensity). Two standard cultivars and randomly selected fruits from the study were used to define the scale. All genotypes were evaluated over three days per cultivation system. Each evaluation started by tasting the two standard cultivars, followed by tasting three to five random samples to calibrate the team on each evaluation day. Samples were randomised (double blind) within each replication of a cross. For each sample, four fruits (if available) were equally sliced and served

on transparent plastic trays (Petri dishes). For neutralisation, tap water, herbal tea and brown bread were available. Breaks were taken regularly, as required by the team, including a one-hour break after about 50% of all daily samples.

Sensory evaluation by a trained panel

A trained panel rated the sweet taste, sour taste and tomato aroma of selected plants. The trained panel consisted of 10 assessors selected according to DIN EN ISO 8586 (Deutsches Institut für Normung e. V., 2014a). The assessors were trained during twelve consecutive two-hour sessions twice a week prior to the assessment, as described in Kanski et al. (2021). The sensory evaluation was conducted in the sensory lab of the University of Goettingen with separated booths set in daylight conditions designed in accordance with DIN EN ISO 8589 (Deutsches Institut für Normung e. V., 2014b). The attributes were assessed on unstructured line scales ranging from not perceptible to very strongly perceptible (0–100%). One session was conducted per cultivation system, both in the same week. Each assessor received one fruit per experimental population, replication and cross, resulting in 12 samples per assessor and assessment. Parents were not evaluated. The sessions started with tasting the standard cultivar Auriga to warm up taste and olfactory receptors. Whole fruits were served for the assessment and assessors were asked to cut the fruits in slices of equal size. Tap water, unsalted cracker (P. Heumann's Matzen, Aerzen, Germany) and peeled cucumbers were available for neutralisation. After six samples, a break of 10 minutes was taken.

Physicochemical measurements and volatile analysis

In preparation for the physicochemical measurements of total soluble solids (TSS), titratable acidity (TA) and dry matter (DM), the fruits were cut and preserved at −20°C. Samples for volatile analysis were extracted as described in Kanski et al. (2020) and frozen at −20°C until analysis. Physicochemical measurements and aroma volatile analysis were performed according to Kanski et al. (2020) at the University of Goettingen, Division of Quality of Plant Products, Germany. The relative concentration of aroma volatiles was expressed in relation to the internal standard 1-octanol in ng mL^{-1} sample according to the equation described in Zhang et al. (2015). Aroma volatiles were selected according to the QTL mapping study by Hagenguth et al. (n.d.). In addition, benzaldehyde was selected because of the highly significant correlations with sweetness and tomato aroma in Hagenguth et al. (2022).

4.2.4 Statistical analyses

Crosses and cultivation systems were analysed separately. Data from the two replications were adjusted for the difference between the mean value of the respective replication and both replications. A Student's t-test was conducted to compare both cultivation systems and to compare the selected experimental populations with the unselected control. If one of the comparisons with the control was significant at p =

0.10, a t-test was conducted comparing both selection methods. Statistical analysis was performed in the R programming environment version 4.0.5 (R Core Team, 2021).

4.3 Results

4.3.1 Usable molecular markers for selection

For the F_2 plants derived from Resi × Auriga, the cross used to map the QTL, at least one KASP marker per QTL could be used for MAS (Table 4.3). Except for the loci on chromosome 6, the marker closest to the QTL peak was usable. The F_2 plants derived from Roterno F_1 × Black Cherry were not polymorphic for the three markers on chromosome 5. At least one marker for the other genetic regions could be used for MAS in this cross (Table 4.3).

Table 4.3. Availability of KASP markers for marker-assisted selection in two crosses of tomato

Marker	Chromosome	Position[1]	Resi × Auriga	Roterno F_1 × Black Cherry
Sourness				
AX-182940179	2	peak	yes	no
AX-105352577	2	top	yes	yes
AX-95772536	2	bottom	yes	no
Sweetness				
AX-95778214	5	peak	yes	no
AX-105352446	5	top	yes	no
AX-105349391	5	bottom	yes	no
Tomato aroma				
AX-95771787	6	peak	no	yes
AX-183003804	6	top	no	no
AX-107534412	6	bottom	yes	yes
Sweetness/tomato aroma				
AX-183061714	10	peak	yes	no
AX-183061610	10	top	yes	no
AX-183062064	10	bottom	yes	yes
Sourness				
AX-181255473	11	peak	yes	no
AX-183082349	11	top	no	yes
AX-107555200	11	bottom	yes	no

[1]Marker position in relation to the QTL peak (peak, closest marker; top and bottom, flanking markers)

4.3.2 Effect of breeders' sensory and marker-assisted selection in organic and hydroponic cultivation for two crosses

For the majority of traits, the comparison between organic and hydroponic cultivation showed significant differences, with higher values for sweetness, TSS, DM, some aroma volatiles and fruit weight in organic cultivation, irrespective of the cross (Table SIII.2). Therefore, the comparison of the selected populations with the unselected control was conducted separately for both cultivation systems.

Resi × Auriga, the cross used for QTL mapping

For all selected attributes except sweetness in the organic cultivation system, increases up to 0.22 scores were observed for the population selected by BS in the cross used to map the QTL but did not reach the significance threshold (Table 4.4). In the same cross, MAS significantly (p = 0.1) enhanced tomato aroma in both cultivation systems in comparison to the unselected population by at least 0.28 scores (Figure 4.3, Table 4.4).

Indirect positive effects of phenotypic selection on TSS and DM were observed in the organic cultivation system, although they did not reach the significance threshold (Table 4.4). MAS resulted in significant increases in these traits (TSS, p = 0.01; DM, p = 0.05) (Table 4.4, Figure 4.4). The concentrations of the apocarotenoid-derived volatiles neral, E-geranylacetone and β-ionone were significantly affected by BS in the organic cultivation system; neral increased (p = 0.01), while E-geranylacetone (p = 0.1) and β-ionone (p = 0.05) decreased (Table 4.4). MAS enhanced the concentration of 6-methyl-5-hepten-2-one in the organic cultivation system and decreased Z-3-hexenal in both cultivation systems (p = 0.05). Fruit weight was significantly ($p_{organic}$ = 0.1, $p_{hydroponic}$ = 0.05) reduced by BS. Significant differences between both selected populations were found for TSS (p = 0.05) and DM (p = 0.1) in the organic cultivation system, each with higher mean values for the population selected by molecular markers and for some volatile compounds in both cultivation systems (Table 4.4, Figure 4.4).

Roterno F_1 × Black Cherry, an unrelated cross

Of the selected attributes, sweetness in the cross unrelated to the mapping population was significantly (p = 0.05) improved by 0.72 scores by BS in the organic cultivation system (Table 4.5). The other sensory attributes were not significantly affected by phenotypic selection, but increases of up to 0.16 scores were observed in organic cultivation and by 0.14 scores for sourness in hydroponic cultivation. MAS was effective (p = 0.1) for sweetness and tomato aroma in organic cultivation, with increases of 0.73 and 0.28 scores, respectively (Figure 4.3, Table 4.5). Selection progress due to MAS, as observed by the Breeders' Sensory Test, was confirmed by the trained panel for tomato aroma (p = 0.1) and for sweetness, although not significantly.

For all physicochemical measurements, with the exception of TA, indirect effects of BS were observed in both cultivation systems (Table 4.5). Of these traits, DM was significantly (p = 0.05) enhanced by BS in organic cultivation. Compared to the unselected control, the population selected by MAS showed significant increases for TSS ($p_{organic}$ = 0.05, $p_{hydroponic}$ = 0.01) in both cultivation systems (Figure 4.4) and DM (p = 0.05) in the organic system (Table 4.5). All aroma volatiles except neral were significantly influenced by either BS or MAS or both in at least one of the cultivation systems (Table 4.5). The apocarotenoid-derived volatiles neral, E-gernanylacetone and β-ionone and the volatile compounds 2-phenylethanol and benzaldehyde were increased by both selection methods in both cultivation systems.

For the other volatiles, both increases and decreases were observed, depending on the selection method or the cultivation system. Significant differences between the two selected populations were observed for TSS (p = 0.1) in hydroponic cultivation and the volatile compound β-ionone ($p_{organic}$ = 0.01, $p_{hydroponic}$ = 0.05), each with higher mean values for the population selected by MAS (Table 4.5, Figure 4.4). A significantly higher concentration of Z-3-hexenal ($p_{organic}$ = 0.001, $p_{hydroponic}$ = 0.05) was found in the population selected by BS compared to the population selected by molecular markers.

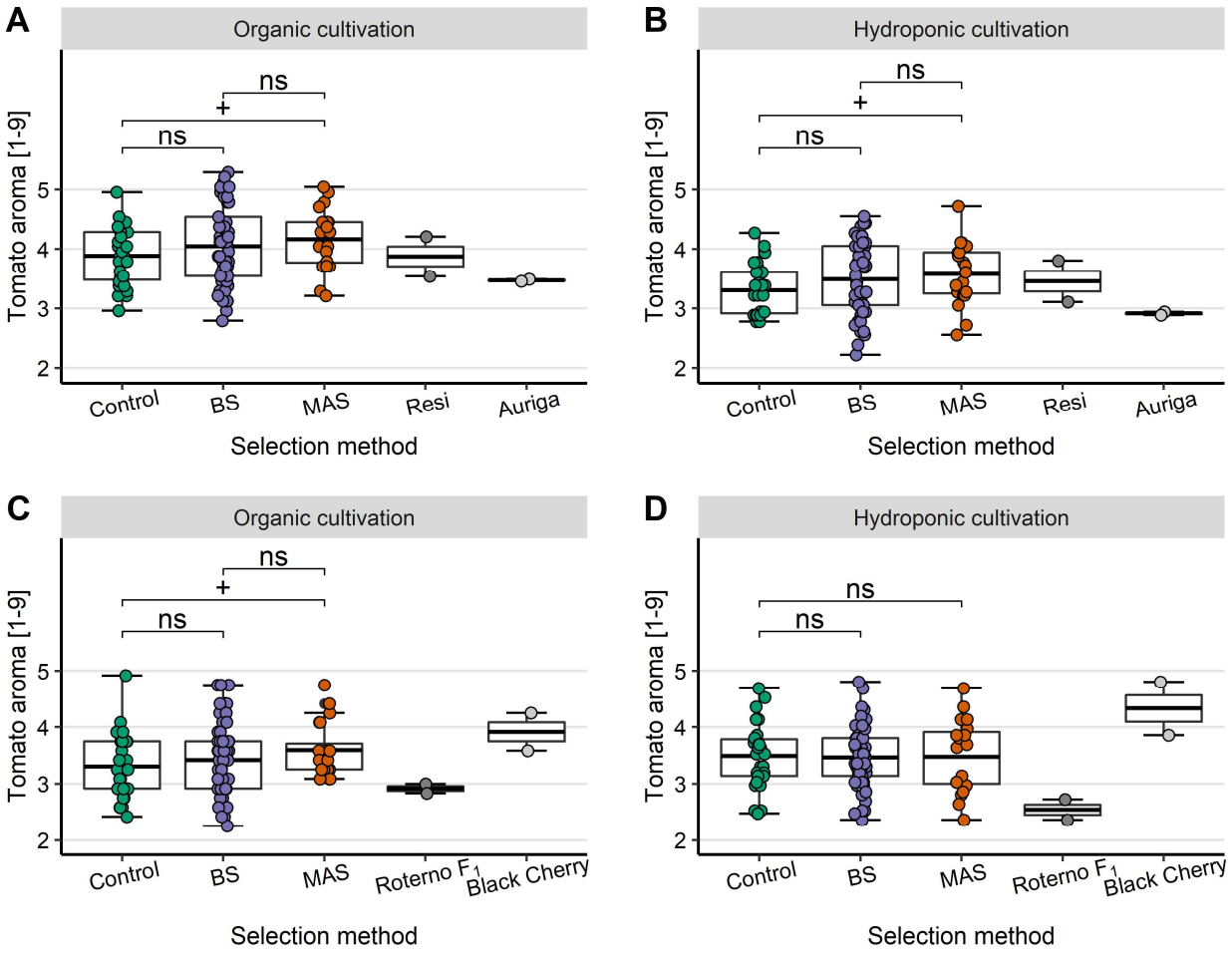


Figure 4.3. Comparison of breeders' sensory selection (BS) and marker-assisted selection (MAS) with an unselected control for tomato aroma assessed with the Breeders' Sensory Test for 'Resi' × 'Auriga' in (A) organic and (B) hydroponic cultivation and 'Roterno F_1' × 'Black Cherry' in (C) organic and (D) hydroponic cultivation.
The bold line represents the mean value; + p = 0.1

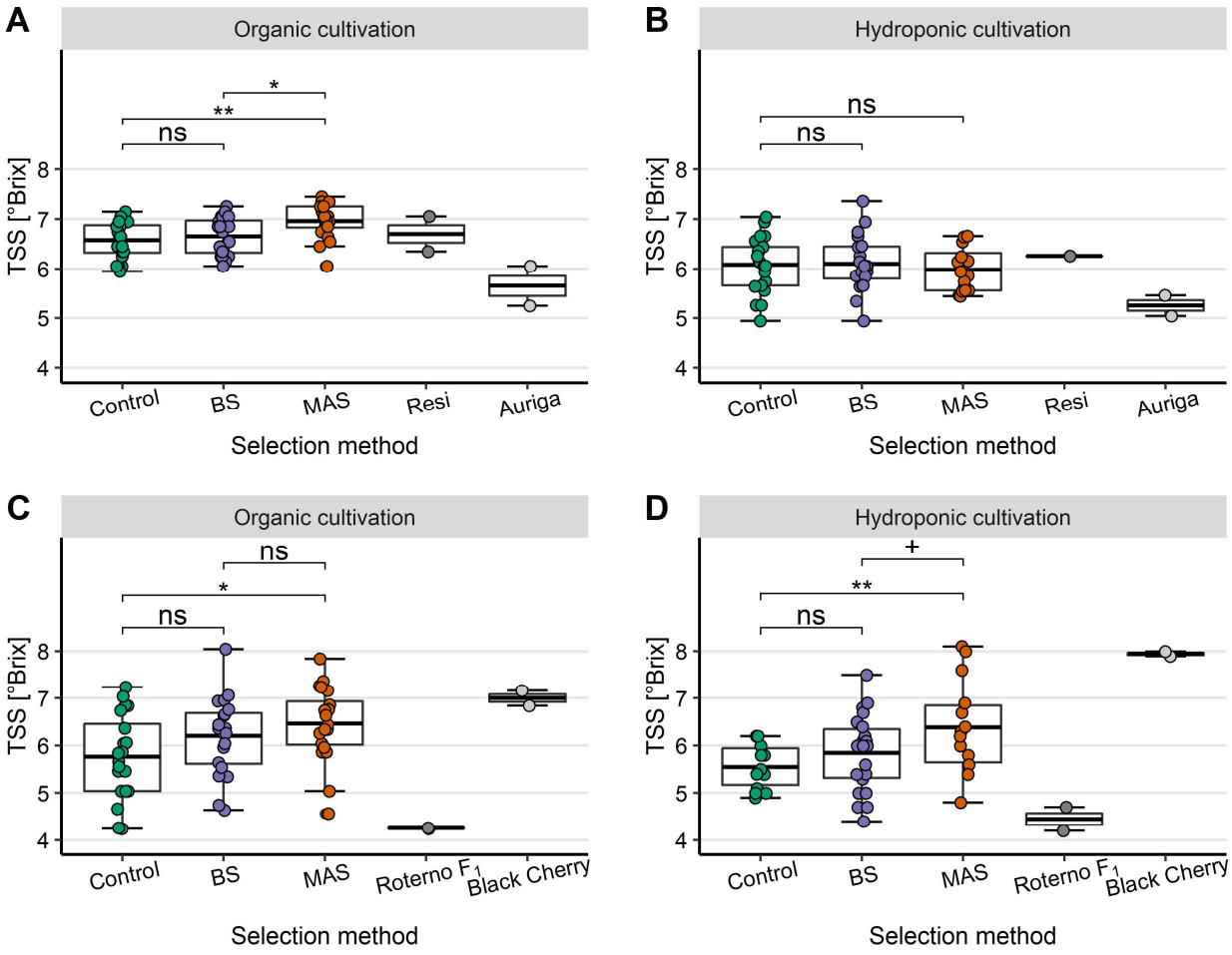


Figure 4.4. Comparison of breeders' sensory selection (BS) and marker-assisted selection (MAS) with an unselected control for total soluble solids (TSS) for 'Resi' × 'Auriga' in (A) organic and (B) hydroponic cultivation and 'Roterno F_1' × 'Black Cherry' in (C) organic and (D) hydroponic cultivation.
The bold line represents the mean value; +p = 0.1, *p = 0.05, **p = 0.01

Table 4.4. Comparison of breeders' sensory selection (BS) and marker-assisted selection (MAS) with an unselected control (Ctrl) and for the cross 'Resi' × 'Auriga' and both parents (R, 'Resi; A, 'Auriga') in two cultivation systems, p-values obtained from Student's t-test

	Resi × Auriga															
	Organic cultivation								Hydroponic cultivation							
	Mean values					t-test			Mean values					t-test		
Trait	**Ctrl**	**BS**	**MAS**	**R**	**A**	**Ctrl - BS**	**Ctrl - MAS**	**BS – MAS[1]**	**Ctrl**	**BS**	**MAS**	**R**	**A**	**Ctrl - BS**	**Ctrl - MAS**	**BS– MAS[1]**
Breeders' Sensory Test [1–9]																
Sweetness	5.12	5.07	5.27	5.25	3.76	0.840	0.615		3.65	3.87	3.60	4.25	2.65	0.339	0.814	
Sourness	3.98	4.09	3.90	3.57	3.42	0.479	0.670		3.89	3.95	3.98	3.09	3.45	0.789	0.710	
Tomato aroma	3.88	4.04	4.17	3.76	3.48	0.236	0.051+	0.451	3.30	3.49	3.58	3.57	2.92	0.156	0.056+	0.588
Trained panel [%]																
Sweetness	42.59	44.45	46.90			0.684	0.338		29.90	27.65	28.66			0.606	0.784	
Sourness	40.76	40.57	39.45			0.974	0.805		47.90	46.58	48.39			0.792	0.921	
Tomato aroma	65.55	65.26	68.70			0.937	0.380		63.94	62.98	64.57			0.762	0.857	
Physicochemical measurements																
TSS [°Brix]	6.58	6.66	6.96	6.70	5.65	0.502	0.002**	0.010*	6.08	6.10	5.98	6.26	5.25	0.909	0.566	
TA [%]	0.46	0.45	0.47	0.50	0.54	0.738	0.710		0.73	0.71	0.69	0.73	0.75	0.542	0.148	
TSS/TA	14.32	15.05	14.82	13.61	10.53	0.421	0.568		8.40	8.66	8.87	8.47	6.95	0.464	0.261	
DM [%]	7.28	7.41	7.67	7.58	6.45	0.454	0.031*	0.087+	6.99	6.79	6.95	7.35	5.76	0.298	0.854	
Aroma volatiles [ng mL^{-1} sample]																
6-Methyl-5-hepten-2-one	2.26	2.43	3.43	3.83	1.01	0.693	0.012*	0.045*	2.93	2.78	2.64	na[†]	1.69	0.780	0.543	
Neral	0.07	0.16	0.08	0.32	0.06	0.005**	0.804	0.005**	0.28	0.31	0.31	na[†]	0.13	0.472	0.433	
E-Geranylacetone	2.45	1.77	2.48	3.04	1.27	0.052+	0.923	0.013*	3.78	3.66	3.64	na[†]	2.33	0.825	0.784	
β-Ionone	1.32	0.86	1.08	0.65	2.22	0.040*	0.292	0.272	1.50	1.20	1.31	na[†]	1.91	0.297	0.518	
Z-3-Hexenol	0.37	0.33	0.42	0.45	0.54	0.411	0.358		0.24	0.26	0.23	na[†]	0.21	0.245	0.374	
Z-3-Hexenal	2.99	2.70	2.11	3.44	3.46	0.394	0.011*	0.027*	3.95	4.05	3.01	na[†]	4.43	0.808	0.044*	0.021*
2-Phenylethanol	0.25	0.28	0.22	0.37	0.26	0.403	0.110		0.57	0.53	0.51	na[†]	0.65	0.533	0.434	
Benzaldehyde	0.24	0.25	0.27	0.18	0.34	0.557	0.295		0.31	0.27	0.34	na[†]	0.32	0.239	0.284	
Agronomic trait																
Fruit weight [g]	36.17	33.00	35.56	16.81	67.66	0.056+	0.789	0.188	23.73	21.13	22.85	11.86	54.01	0.049*	0.488	0.227

+,*, **, *** significant at 0.1, 0.05, 0.01 and 0.001 level, respectively; [1]only calculated if one of the comparisons with the control group was significant; [†]not available

Table 4.5. Comparison of breeders' sensory selection (BS) and marker-assisted selection (MAS) with an unselected control (Ctrl) for the cross 'Roterno F_1' × 'Black Cherry' and both parents (Ro, 'Roterno F_1'; BC, 'Black Cherry') in two cultivation systems, p-values obtained from Student's t-test

	Roterno F_1 × Black Cherry															
	Organic cultivation								Hydroponic cultivation							
	Mean values					t-test			Mean values					t-test		
Trait	**Ctrl**	**BS**	**MAS**	**Ro**	**BC**	**Ctrl - BS**	**Ctrl - MAS**	**BS – MAS[1]**	**Ctrl**	**BS**	**MAS**	**Ro**	**BC**	**Ctrl - BS**	**Ctrl - MAS**	**BS – MAS[1]**
Breeders' Sensory Test [1–9]																
Sweetness	3.86	4.58	4.59	2.68	6.31	0.019*	0.050+	0.994	3.28	3.24	3.65	2.54	3.67	0.844	0.238	
Sourness	3.07	3.23	3.13	2.67	3.70	0.184	0.639		3.52	3.66	3.58	2.51	4.68	0.445	0.788	
Tomato aroma	3.31	3.42	3.59	2.92	3.92	0.398	0.063+	0.262	3.49	3.46	3.47	2.60	4.10	0.837	0.935	
Trained panel [%]																
Sweetness	39.72	43.90	44.99			0.358	0.221		23.05	24.25	27.99			0.790	0.312	
Sourness	34.68	36.41	35.15			0.719	0.921		41.24	42.03	47.28			0.877	0.286	
Tomato aroma	62.37	64.28	67.82			0.553	0.096+	0.214	61.98	60.07	66.48			0.591	0.138	
Physicochemical measurements																
TSS [°Brix]	5.77	6.20	6.47	4.25	7.00	0.119	0.012*	0.305	5.54	5.76	6.38	4.44	7.94	0.391	0.008**	0.055+
TA [%]	0.44	0.45	0.44	0.36	0.50	0.849	0.836		0.68	0.69	0.65	0.57	0.76	0.669	0.354	
TSS/TA	13.55	13.75	15.13	12.07	14.17	0.817	0.052+	0.069+	8.30	8.40	10.00	7.86	10.47	0.835	0.008**	0.006**
DM [%]	6.53	7.20	7.24	5.02	7.93	0.022*	0.018*	0.887	6.40	6.44	6.96	4.65	8.44	0.910	0.119	0.120
Aroma volatiles [ng mL^{-1} sample]																
6-Methyl-5-hepten-2-one	3.11	2.92	3.23	2.27	2.65	0.549	0.695		4.49	6.65	7.11	5.23	na[†]	0.007**	0.001**	0.565
Neral	0.14	0.18	0.20	0.13	0.01	0.434	0.211		0.19	0.23	0.29	0.15	0.09	0.438	0.145	
E-Geranylacetone	1.93	2.10	2.43	1.31	0.98	0.524	0.059+	0.312	3.80	5.32	5.64	4.61	6.29	0.054+	0.029*	0.703
β-Ionone	0.48	0.53	0.69	0.45	0.46	0.220	<0.001***	0.002**	0.54	0.67	0.81	0.45	0.42	0.023	<0.001***	0.013*
Z-3-Hexenol	0.56	0.51	0.41	0.47	0.26	0.328	0.013*	0.135	0.27	0.29	0.26	0.34	0.20	0.536	0.960	
Z-3-Hexenal	3.02	3.76	2.44	2.94	2.92	0.044*	0.130	<0.001***	3.68	2.91	1.89	3.71	1.84	0.150	0.001**	0.018*
2-Phenylethanol	0.40	0.61	0.59	0.15	0.31	0.007**	0.029*	0.836	0.76	1.15	1.10	1.04	0.76	0.075+	0.112	0.819
Benzaldehyde	0.13	0.22	0.27	0.05	0.21	0.017*	<0.001***	0.246	0.38	0.45	0.56	0.25	0.89	0.398	0.071+	0.237
Agronomic trait																
Fruit weight [g]	52.67	42.99	46.79	106.53	22.15	0.005**	0.150	0.333	33.51	27.33	29.10	68.55	15.53	0.003**	0.122	0.426

+,*, **, *** significant at 0.1, 0.05, 0.01 and 0.001 level, respectively; [1]only calculated if one of the comparisons with the control group was significant; [†]not available

4.4 Discussion

We used five QTL previously identified by Hagenguth et al. (n.d.) for MAS. They were expected to enhance sweetness, sourness and tomato aroma in two crosses, the mapping population Resi × Auriga and the unrelated cross Roterno F_1 × Black Cherry. The QTL effects were mapped for the means of two cultivation systems, organic and hydroponic. The molecular markers were developed based on phenotypic data from the Breeders' Sensory Test (Hagenguth et al., 2022) that was also used for BS. The molecular markers were polymorphic not only for the population derived from Resi × Auriga but also for the unrelated cross, with the exception of the markers on chromosome 5 (Table 4.3). This is a first indication that these could be of interest for broader application.

4.4.1 Effectiveness of breeders' sensory and marker-assisted selection

Effect of cultivation systems and parental cultivars

Since flavour is influenced by growing conditions, e.g. solar irradiation, fertilisation regime and irrigation (Beckles, 2012), the response to selection was investigated in two distinctly different cultivation systems, i.e. organic low-input and hydroponics. To clearly distinguish between the effect of the cultivation system, as observed for most traits (Table SIII.2), and the response to selection, both cultivation systems were treated separately. The effects of selection were similar in both cultivation systems with more pronounced effects in organic cultivation (Tables 4.4 and 4.5).

For both selection methods, sensory attributes and physicochemical measurements were more frequently enhanced in the populations derived from Roterno F_1 × Black Cherry (Tables 4.4 and 4.5). Roterno F_1 is a large-fruited high-yielding cultivar, and Black Cherry is a cocktail tomato with excellent flavour. In contrast, both parents of the mapping population are characterised by good flavour. Consequently, the differences for the sensory attributes were larger between the parental cultivars Roterno F_1 and Black Cherry (Tables 4.4 and 4.5), and the variance in the F_2 population used for BS of Roterno F_1 × Black Cherry was larger than in Resi × Auriga (Table SIII.3). This most likely explains why superior genotypes were more efficiently selected in the populations derived from Roterno F_1 × Black Cherry.

Response to direct selection

BS was conducted based on the sum of sweetness, sourness and tomato aroma for individual plants in the F_2 generation and the response to selection was investigated on their F_3 progeny. For the selected attributes, no significant differences from the unselected population were found for the phenotypically selected population derived from Resi × Auriga (Table 4.4). However, BS significantly enhanced sweetness in the population derived from Roterno F_1 × Black Cherry in the organic cultivation system by 0.72 scores (Table 4.5). In addition, some increases in the population mean up to 0.22 scores in sweetness, sourness and tomato aroma were observed independently of the cross and cultivation system, but they did not

reach the significance threshold (Tables 4.4 and 4.5). These findings are particularly relevant for organic plant breeding, where phenotypic selection is considered the most important, especially for quantitative traits (Lammerts van Bueren et al., 2010). Other studies have shown that early phenotypic selection for flavour-related traits is expected to be successful. By comparing F_3 plants with their F_2 parents, De Bruyn et al. (1971) identified the sweet/acid ratio, taste intensity and taste quality as attributes in which selection is likely possible. Working with F_2 plants from 32 crosses and their corresponding parents, Hagenguth et al. (2022) described selection for flavour-related traits in early generations as a promising tool to improve tomato flavour.

For Roterno F_1 × Black Cherry, significant selection success by MAS was found for sweetness and tomato aroma in the organic cultivation system with increases up to 0.73 scores, but not for sourness (Table 4.5). For the population derived from Resi × Auriga, a significant improvement up to 0.29 scores was observed for tomato aroma in both cultivation systems (Table 4.4). Lecomte et al. (2004) also demonstrated the odds of MAS for flavour-related traits. In their study, marker-assisted backcrossing for five genetic regions carrying QTL for quality traits was successful.

In addition to the assessment by the Breeders' Sensory Test, which was also used to map the QTL, a trained panel was included in the present study to investigate the response to selection. However, each genotype could only be tested by one assessor, likely leading to large experimental errors. This might explain why sensory attributes assessed with the trained panel indicated less significant results compared to the Breeders' Sensory Test. Nevertheless, the positive effects of selection on sweetness and tomato aroma were confirmed; they were significant for tomato aroma of Roterno F_1 × Black Cherry selected by MAS in the organic cultivation system.

Response to indirect selection

Selection for sensory attributes resulted in several indirect effects on physicochemical measurements with the exception of TA, aroma volatiles and fruit weight (Tables 4.4 and 4.5). In terms of volatiles, the concentration is critical in determining whether they enhance or reduce tomato aroma and consumer liking, as well as the overall aroma profile since volatiles interact with each other (Piombino et al., 2013; Rambla et al., 2014). In both crosses, increases in TSS by BS were observed. MAS significantly increased TSS with the exception of Resi × Auriga in the hydroponic cultivation system. This was expected since TSS is not only a trait correlated with the sensory attributes sweetness and tomato aroma, as reported by Hagenguth et al. (n.d.), but it is also co-located in the QTL clusters on chromosomes 5 (sweetness and TSS) and 10 (sweetness, tomato aroma and TSS) in the same study. Fruit weight and DM, two negatively correlated traits (Beckles, 2012), were indirectly influenced by the selection for sensory attributes (Tables 4.4 and 4.5). Fruits selected based on the Breeders' Sensory Test were, on average, smaller compared to the unselected population, while MAS did not significantly affect fruit size. Dry matter was significantly increased by both selection methods for Roterno F_1 × Black Cherry and by BS for Resi × Auriga in the

organic cultivation system. Due to a well-known negative correlation between tomato quality and fruit size, breeding large-sized tomatoes with good flavour is challenging (Causse et al., 2003; Klee & Tieman, 2018; Lecomte et al., 2004b). In our previous study (Hagenguth et al., n.d.), a QTL for fruit weight was located close to a genetic region carrying QTL for sweetness and tomato aroma (allele from Resi increasing the trait value) on chromosome 10 with the allele from the other parent increasing fruit weight; similar observations were made for other genetic regions. This is consistent with studies that identified genetic regions containing co-localised QTL for flavour-related traits and fruit weight on multiple chromosomes (Capel et al., 2015; Causse et al., 2002; Xu et al., 2013).

4.4.2 Comparison of breeders' sensory and marker-assisted selection

In the direct comparison between the selected populations, no significant difference was found in the sensory attributes sweetness and tomato aroma in either of the two crosses (Tables 4.4 and 4.5). For physicochemical measurements and volatile compounds, significant differences between the populations selected by BS and MAS were observed in some cases. In both populations selected by MAS, a tendency towards the highest mean value of sensory attributes and physicochemical measurements was observed, while BS also often increased the population mean. The most clear result was obtained for the improvement of tomato aroma by MAS, which was significant in most cases. In addition, according to the trained panel, tomato aroma was improved by MAS in both crosses in both cultivation systems, although in most cases it was not statistically significant at $p = 0.1$. These observations confirm the large part of the phenotypic variation explained (41.1%) of the QTL for tomato aroma on chromosome 6, as reported in Hagenguth et al. (n.d).

When comparing BS and MAS, differences in selection intensity must be considered. Of the plants, 16% were selected using BS, while only the best 4% of the seedlings were selected by MAS. Such a difference in selection intensity is realistic when applying these two selection methods. The Breeders' Sensory Test is designed for segregating generations with a large number of small samples, but mature fruits are required and resources to grow plants are limited. A large advantage of MAS is that it can be conducted on seedlings, and therefore, many more plants can be screened. Thus, MAS for sensory attributes in the first segregating generations is an interesting tool for the preselection of seedlings and might be more efficient than BS. Apart from these advantages of MAS, it has to be considered that molecular markers developed from QTL are not perfectly linked with the genes of interest and in most cases only a part of the genetic variability is captured. For the genetic loci we used, about 5 to 41% of the phenotypic variance was explained by a single QTL (Hagenguth et al., n.d.). Dekkers and Hospital (2002) also pointed out that selection based on the genotype alone is not sufficient to maximise the response to selection, as only the phenotype reflects the combined effect of all genes, including those unidentified. A major advantage of phenotypic selection based on the Breeders' Sensory Test is its universal applicability without any knowledge of the genetic background of the genotypes and without an elaborate development process.

This makes BS the most important tool in breeding and screening programmes, with limited resources, restricted access to laboratory facilities and other limitations in the application of molecular methods.

In conclusion, to improve the flavour of fresh market tomatoes, a combination of MAS and phenotypic selection is recommended, which also balances the costs and effort of both methods. The possible linkage of molecular markers with other agronomically important traits, mainly fruit size, needs further investigation.

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Ethics Statement

The study was approved by Ethics Committee, University of Goettingen, P.O. Box 37 44, 37027 Goettingen, Chair: Prof. Dr. Hans Michael Heinig (Date of approval was 26 July 2019). The participants provided their written informed consent to participate in this study.

Conflict of interest

The authors declare that they have no conflict of interest.

Data Availability Statement

Data are available upon request from the corresponding author.

4.6 Supplementary Materials

Flavour Improvement in Early Generations of Fresh Market Tomatoes (Solanum lycopersicum L.): II. Response to Breeders' Sensory and Marker-Assisted Selection

Table SIII.1 Soil analyses in the organic low-input cultivation system at a soil depth of 0–30 cm

soil sample	Soil type[1] (Group)	Calcium carbonate[1] [pH-value] $CaCl_2$[§]	Phosphorus[1] (P) [mg/100g] CAL[§]	Potassium[1] (K) [mg/100g] CAL[§]	Magnesium[1] (Mg) [mg/100g] $CaCl_2$[§]	Mineral Nitrogen[2] (N_{min}) [kg/ha] $CaCl_2$[§]
03.06.2019 start of experiment	(h) ttU[†]	7.0 C[‡]	4.6 C[‡]	11.7 C[‡]	13.6 D[‡]	39.0
15.08.2019	-	-	-	-	-	18.7
08.10.2019 end of experiment	(h) ttU[†]	7.0 C[‡]	4.4 B[‡]	9.1 B[‡]	13.3 D[‡]	7.4

[†](h) = low in humus (< 4%), ttU = silty loam; [‡]A = very low, B = low, C = to target, D = high, E = very high, F = extremely high; [§]standard methods according to VDLUFA method manual I (ISBN 978-3-941273-13-9)
[1]results were measured and provided by LUFA Nord-West, Institut für Boden und Umwelt, Hameln, Germany; [2]analysed by University of Goettingen, Division of Agronomy, Goettingen, Germany

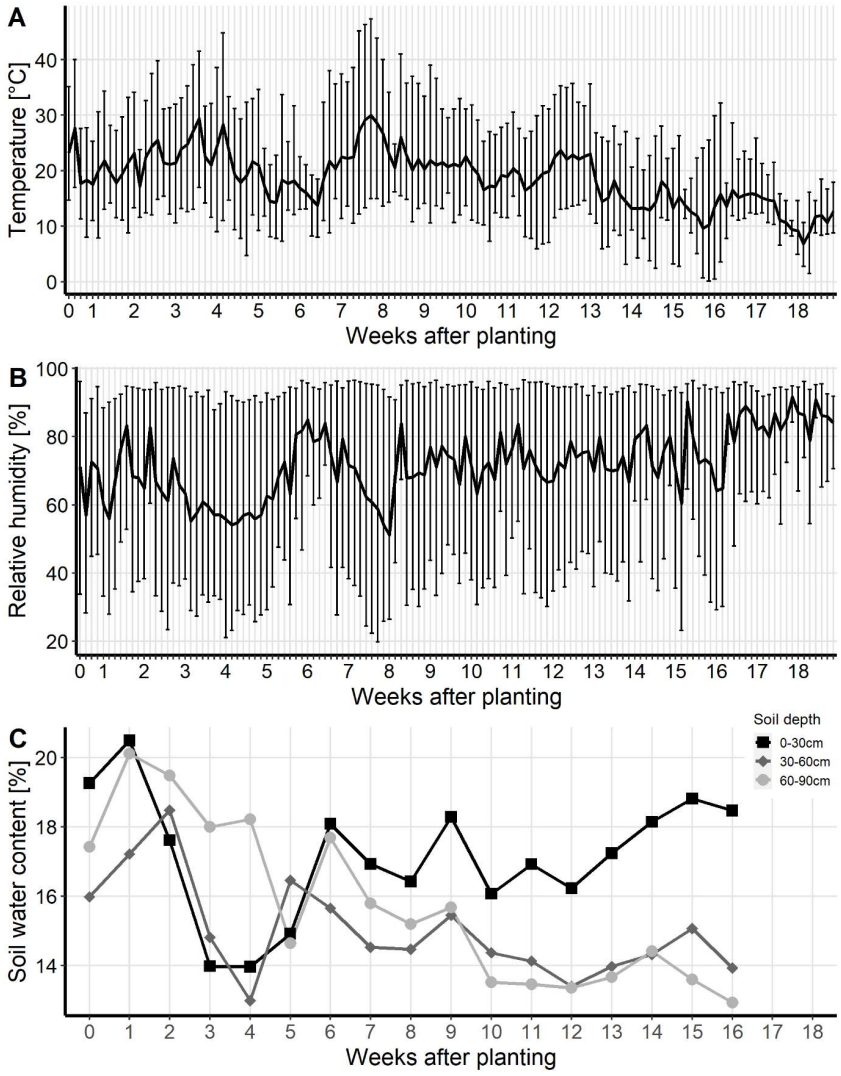

Figure SIII.1 (A) Mean, minimum and maximum temperature per day, (B) mean relative humidity with minimum and maximum per day, and (C) soil water content in the organic cultivation system. Temperature and humidity data were recorded every 15 min in about 1.0 m above soil surface in the row between plants using an EBI 20-TH Data Logger (ebro Electronic GmbH & Co. KG, Ingolstadt, Germany); soil water content is expressed as gravimetric moisture content.

Table SIII.2. Comparison of sensory attributes, physicochemical measurements, and aroma volatiles for organic low-input and hydroponic cultivation (n = 114 per cross and cultivation system), mean values and standard deviation (SD) of all experimental populations and parental cultivars

Trait	Resi × Auriga					Roterno F_1 × Black Cherry				
	Organic cultivation		Hydroponic cultivation			Organic cultivation		Hydroponic cultivation		
	Mean	SD	Mean	SD	p-value	Mean	SD	Mean	SD	p-value
Breeders' Sensory Test										
Sweetness [1–9]	5.10	±1.05	3.73	±0.92	***	4.39	±1.38	3.32	±0.86	***
Sourness [1–9]	4.00	±0.67	3.91	±0.88	ns	3.17	±0.53	3.61	±0.75	***
Tomato aroma [1–9]	4.01	±0.59	3.44	±0.55	***	3.42	±0.58	3.47	±0.58	ns
Trained Panel										
Sweetness [%]	44.65	±12.88	28.78	±13.80	***	42.91	±13.17	24.84	±13.50	***
Sourness [%]	40.27	±16.51	47.58	±15.20	*	35.44	±14.22	43.36	±14.99	**
Tomato aroma [%]	66.44	±10.81	63.81	±9.57	ns	64.81	±9.37	62.53	±9.38	ns
Physicochemical measurements										
TSS [°Brix]	6.70	±0.44	6.04	±0.53	***	6.11	±0.92	5.90	±0.96	ns
TA [%]	0.47	±0.07	0.72	±0.09	***	0.44	±0.09	0.68	±0.09	***
TSS/TA	14.56	±2.72	8.56	±1.15	***	14.08	±2.51	8.82	±1.62	***
DM [%]	7.42	±0.54	6.88	±0.67	***	6.95	±0.99	6.57	±1.03	*
Aroma volatiles [ng mL^{-1} sample]										
6-Methyl-5-hepten-2-one	2.71	±1.41	2.74	±1.27	ns	3.04	±0.94	6.20	±2.01	***
Neral	0.11	±0.09	0.29	±0.13	***	0.16	±0.15	0.23	±0.15	*
E-Geranylacetone	2.21	±0.96	3.63	±1.38	***	2.08	±0.88	5.05	±1.95	***
ß-Ionone	1.10	±0.66	1.36	±0.74	ns	0.56	±0.16	0.65	±0.17	**
Hexanol	0.85	±0.52	0.69	±0.36	ns	0.41	±0.19	0.30	±0.16	**
Z-3-Hexenol	0.38	±0.12	0.24	±0.04	***	0.49	±0.18	0.28	±0.08	***
Z-3-Hexenal	2.65	±0.94	3.74	±1.25	***	3.07	±1.15	2.80	±1.26	ns
2-Phenylethanol	0.26	±0.07	0.54	±0.18	***	0.51	±0.27	1.05	±0.52	***
Benzaldehyde	0.25	±0.09	0.31	±0.08	**	0.20	±0.13	0.45	±0.23	***
Agronomic trait										
Fruit weight [g]	34.63	±9.11	22.58	±6.97	***	46.97	±17.35	29.80	±10.76	***

ns, $p > 0.05$; *, **, *** significant at 0.05, 0.01 and 0.001 level, respectively; Student's t-test or Welch's t-test if p value from Levene's test is > 0.05

Abbreviation: TSS, total soluble solids; TA, titratable acidity; DM, dry matter

Table SIII.3. Phenotypic variation (Min, minimum; Mean; Max, maximum; σ^2, variance) of sensory attributes for the F_2 populations used for breeders' sensory selection for mean values of the organic low-input and hydroponic cultivation system in 2018

Trait	Resi × Auriga				Roterno F_1 × Black Cherry			
	Min	Mean	Max	σ^2	Min	Mean	Max	σ^2
Sweetness [1–9]	2.58	3.83	5.25	0.27	2.33	3.52	5.00	0.39
Sourness [1–9]	3.00	4.29	5.79	0.27	2.42	3.94	6.50	0.74
Tomato aroma [1–9]	2.63	3.82	5.11	0.16	2.08	3.31	5.25	0.24

5 GENERAL DISCUSSION AND CONCLUSIONS

5.1 Background and key results

Despite the general popularity of tomatoes, consumers are dissatisfied with the flavour of fresh market tomatoes (Beckles, 2012; Causse et al., 2010; Colantonio et al., 2022). Flavour is one of the most difficult traits to evaluate routinely in breeding programmes and has therefore been a low priority breeding target in tomatoes and other crops until recently (Causse et al., 2010; Ferrão et al., 2020; Klee, 2010). Plant breeders lack suitable methods for flavour assessment and objective breeding targets (Beckles, 2012; Causse et al., 2010; Piombino et al., 2013). In addition, improving flavour without compromising agronomically important traits is challenging (Causse et al., 2003; Klee & Tieman, 2018). Small sensory panels of one to a few persons are probably a common practice in vegetable and fruit breeding programmes (Behrendt, 2009; Colantonio et al., 2022; Horneburg et al., 2009; Vicente et al., 2014), but are less evaluated and not standardized. Marker-assisted selection (MAS) is an interesting alternative (Causse et al., 2003; Klee & Tieman, 2018). However, verification of QTL for flavour-related traits is rare, even though it is a prerequisite for MAS. In the course of this study, the following key results were obtained according to the hypotheses established in 1.2.3, which will be discussed below:

1a. The Breeders' Sensory Test is a promising method to select for sensory attributes in the first segregating generations (CHAPTER 2, Study I).

I) Sweetness and TSS as well as sourness and TA were highly significantly correlated.
II) TSS, TA/TSS were significantly correlated with sweetness and tomato aroma, and some volatile compounds with sweetness and most with tomato aroma (CHAPTER 2 and 3).

1b. Selection of single plants for flavour-related traits in the F_2 generation is expected to be successful (CHAPTER 2, Study I), but might be decelerated by a genotype-by-year interaction (CHAPTER 4, Study III).

I) For most flavour-related traits, including sensory attributes, the coefficients of variation of the F_2 plants exceeded those of the corresponding parental means in most crosses.

2. QTL for most flavour-related traits, including sensory attributes, were mapped (CHAPTER 3, Study II).

I) Sensory QTL on chromosomes 5 and 10 and QTL for most special aroma attributes were identified for the first time. Sensory QTL, partly within QTL clusters, are highly interesting for MAS.
II) Nine distinct QTL clusters were identified for the mean values of both cultivation systems.

3. MAS for sensory attributes is a promising method for the preselection of seedlings, particularly in the first segregating generations. At least in advanced generations, a combination of phenotypic and marker-assisted selection is necessary (CHAPTER 4, Study III).

I) The sweetness of Roterno F_1 × Black Cherry was significantly improved by phenotypic selection in the organic cultivation system; increases were observed for most sensory attributes.
II) The effect of MAS for sensory attributes was most evident for tomato aroma in both crosses and cultivation systems; increases were observed for most sensory attributes.
III) Selection for sensory attributes resulted in several indirect effects on physicochemical measurements and aroma volatiles, and reduced fruit size.

5.2 Selection for sensory attributes: Breeders' Sensory Test and molecular markers

5.2.1 Phenotypic and molecular methods for early selection

Tomato producers and consumers demand a wide range of traits from high productivity to flavour and nutritional value (Cebolla-Cornejo et al., 2011; Kimbara et al., 2018). Until the early 1990s, breeders focused on traits required by producers, including appearance, whereas more attention is now paid to flavour (Causse et al., 2010). Consumers are willing to pay a premium for flavourful tomatoes and this offers an opportunity to expand markets (Klee & Tieman, 2018). Breeders now need tools to select for flavour-related traits, ideally suitable for the first segregating generations. MAS is an interesting alternative to phenotypic selection, particularly interesting for a large number of individuals, and offers the possibility to track volatile compounds (Ferrão et al., 2020; Hernández-Bautista et al., 2016). However, due to the principles of organic agriculture, the use of MAS in organic plant breeding is less self-evident, although it is allowed (Lammerts van Bueren et al., 2010). Nevertheless, the private breeding sector requires a good balance between phenotypic and molecular selection techniques (Lammerts van Bueren et al., 2010). Not only, but in particular, organic plant breeders need improved phenotypic selection methods (Lammerts van Bueren et al., 2010).

Phenotypic selection for sensory attributes in early generations

The Breeders' Sensory Test was developed to overcome the limitations of trained and consumer panels and uses a small team (two to three persons) to evaluate hundreds of small samples, as is typical for early breeding generations. We evaluated the accuracy of this method and created a guideline for this test (CHAPTER 2). Highly significant correlations between corresponding sensory attributes and physicochemical measurements (sweetness and TSS, sourness and TA; Study I, Figure 1) indicate an adequate quality of the Breeders' Sensory Test. The same significant correlations were found by Baldwin et al. (2015), Erika et al. (2022) and for small-fruited hybrids by Causse et al. (2003) using trained or consumer panels. These physicochemical measurements are used in many breeding programmes to account for tomato taste (Tandon et al., 2000; Zhao et al., 2016). However, using these measurements alone neglects aroma, which is essential for the typical tomato flavour and consumer liking, and analytical aroma analysis is far behind the capacity of most breeding programmes (Causse et al., 2003; Causse et al., 2010; Klee & Tieman, 2018). The Breeders' Sensory Test offers the possibility to directly assess perceived sweetness, sourness and aroma. The high heritability (0.75 – 0.96) of sensory attributes estimated based on the parental cultivars supports the high quality of the Breeders' Sensory Test (Study I, Table 3). Therefore, the Breeders' Sensory Test is recommended as a promising tool to select for sensory attributes in early breeding generations starting with the F_2.

Early selection for sensory attributes, ideally starting with the F_2 generation, might prevent the loss of favourable alleles. However, the usefulness of early selection for quantitative inherited traits is still under discussion, since genetic differences might be diminished by environmental effects (Liu & Constable, 2017). The genetic plus environmental variance (coefficient of variation of the F_2 plants) exceeded the environmental variance (mean of the parental coefficient of variation) for most flavour-related traits in most crosses, thus fulfilling the prerequisite for successful selection (CHAPTER 2; Study I, Figures 3 and 4, Table S3). These traits included tomato aroma, an attribute that cannot be predicted by simple physicochemical measurements suitable for breeding programmes. In accordance with our results, De Bruyn et al. (1971) illustrated the chances of early phenotypic selection for sensory attributes (taste intensity, taste quality, and sweet-acid ratio). In the second study, the heritability for an F_2 generation was estimated to be medium to high (0.55 – 0.73) for the sensory attributes sweetness, sourness and tomato aroma (CHAPTER 3; Study II, Table 3.2), showing that both genetic and environmental effects including the cultivation system affect sensory attributes. In addition, significant genotype-by-environment interactions were observed in the second study (Study II, Table SII.4). In particular, the third study showed the challenges of phenotypic selection for flavour-related traits, including sensory attributes (CHAPTER 4). An observable but low selection progress is probably caused by a genotype-by-year interaction as described by Baldwin et al. (2015). Nevertheless, also some sensory attributes and volatile compounds less sensitive to environmental effects were identified, particularly sourness, phenylacetaldehyde and 2-isobuthylthiaziole (CHAPTER 2 and 3; Study I, Table 5; Study II, Table SII.5).

Early selection using the Breeders' Sensory Test is not limited to tomatoes, but is also suitable for other vegetables and fruits, some of which also suffer from a lack of flavour (Ferrão et al., 2020; Folta & Klee, 2016). However, sensory attributes must be adapted according to the flavour characteristic of the particular vegetable or fruit. A comparison of corresponding sensory attributes and physicochemical measurements for crops other than tomato would be interesting and could lead to a broader application of the Breeders' Sensory Test. In order to simplify selection decisions for sensory attributes, the introduction of a score for the overall impression similar to that of acceptability might be necessary as also used by Erika et al. (2022), although this score normally requires large consumer panels (Carneiro et al., 2020), otherwise combining the scores for the different attributes might complicate the selection.

Marker-assisted selection for sensory attributes in an early generation

MAS is a promising method to evaluate the large number of individuals in the first segregating generations and an important application of QTL studies (Hernández-Bautista et al., 2016; Lecomte et al., 2004a). However, little is known about its efficiency for flavour-related traits (Ferrão et al., 2020). Although QTL for sensory attributes are of direct relevance to breeding programmes (Amyotte et al., 2017), QTL studies that consider sensory attributes are rare (Causse et al., 2001; Tikunov et al., 2020; Zanor et al., 2009). Therefore, we focused on the identification of sensory QTL and potential co-localisation with

physicochemical measurements, volatile compounds and fruit weight in two contrasting cultivation systems (CHAPTER 3). QTL studies using both organic and conventional cultivation systems are particularly relevant to organic plant breeding, as one concern is that molecular markers developed only in a conventional context may not be suitable for selection under organic conditions (Lammerts van Bueren et al., 2010).

The use of sensory attributes enabled the discovery of previously unreported QTL for sweetness (SW5.1), sourness (SO5.1), total (A.TOT10.1) and tomato aroma (A.TOM10.1). In addition, most QTL for special aroma attributes provide novel information. The other sensory QTL confirm previously published QTL (Causse et al., 2001; Tikunov et al., 2020; Zanor et al., 2009). For the main sensory attributes sweetness, sourness and tomato aroma, two to three QTL per attribute were detected with a phenotypic variance explained (PVE) above or close to 10%, except for A.TOM10.1 (PVE = 5.5%), ranging from about 9.4 to 41.1% (Study II, Table 3.4). The PVE per QTL is similar to that of Causse et al. (2001), except for the QTL for tomato flavour on chromosome 6, which showed a much higher PVE. Texture attributes were not included in this study, but QTL for these attributes were identified by Causse et al. (200), Tikunov et al. (2020) and Zanor et al. (2009). QTL for sensory attributes were co-localised with QTL for physicochemical measurements on chromosomes 5 and 10 and with QTL for volatile compounds on chromosomes 1 and 6, with the latter forming the largest identified cluster (Study II, Figure 3.2). These clusters are probably caused by physiological relationships, as sensory attributes result from both physicochemical measurements and volatile compounds. In addition, especially for the different groups of volatile compounds, linked QTL are probably also involved. For most flavour-related QTL, but with exception of chromosome 7, the allele of the small-fruited parent increased the phenotypic value, confirming the potential of cocktail tomatoes for improving tomato flavour as described by Causse et al. (2003). Sensory QTL for sweetness, sourness and tomato aroma on chromosomes 2, 5, 6, 10 and 11 are recommended for MAS.

To verify the QTL for these sensory attributes, MAS was conducted on F_2 seedlings in two unrelated crosses, the mapping population Resi × Auriga and an alternative cross Roterno F_1 × Black Cherry, and was compared to an unselected population (CHAPTER 4). Molecular markers were polymorphic for all loci of Resi × Auriga and most of Roterno F_1 × Black Cherry (Study III, Table 4.3), a first indication that they might be interesting for a broader application. The QTL for sweetness was confirmed in Roterno F_1 × Black Cherry and the QTL for tomato aroma in both crosses, most clearly in the organic cultivation system (Study III, Tables 4.4 and 4.5). Furthermore, the usefulness of MAS for sensory attributes in the first segregation generations was underlined by an improvement of most sensory attributes, including sourness, although not statistically significant, independent of the cross and cultivation system.

Combining phenotypic and marker-assisted selection for flavour improvement

According to Dekkers & Hospital (2002), MAS has to be combined with phenotypic selection to maximize genetic gain, because the phenotypic value reflects the collective action of all genes, including those which were not identified. Therefore, we compared both, phenotypic selection based on the Breeders' Sensory Test (breeders' sensory selection, BS) and MAS with an unselected population in the third study (CHAPTER 4). MAS showed a tendency towards a higher efficiency than breeders' sensory selection, most evident for tomato aroma, which was significantly improved in most cases (Study III, Figure 4.3). This was confirmed by the direct comparison of both selected populations, especially by indirect effects on the physicochemical measurements TSS and TSS/TA and the often highest mean value for the population selected by MAS (Study III, Tables 4.4 and 4.5). One main reason for the higher response to selection by MAS is probably the much higher selection intensity. A higher number of individual plants (seedlings) could be analysed with molecular markers and thus it was possible to select the best 4% of the F_2 plants, whereas 16% were selected by breeders' sensory selection (mature fruits necessary). If replications are not necessary, as in the case of the F_2 mapping population, a higher selection intensity for breeders' sensory selection should be possible. However, space in protected cultivation for growing the plants to maturity is likely to be limited. Nevertheless, breeders' sensory selection increased the population mean for most sensory attributes, although rarely statistically significant (Study III, Tables 4.4 and 4.5). Furthermore, selection was more efficient in Roterno F_1 × Black Cherry as identification of superior plants was easier due to more diverse parents (Study III, Table SIII.3). Studies comparing phenotypic and marker-assisted selection for flavour-related traits are rare. In the study of Hernández-Bautista et al. (2016), the genetic gain was higher for phenotypic selection for most fruit-related traits (no sensory attributes were included), while MAS outperformed phenotypic selection for TSS (Hernández-Bautista et al., 2016). To account for the different advantages of phenotypic and marker-assisted selection and thus maximise the selection success, a combination of both methods is recommended.

For an efficient use of molecular markers in breeding programmes, breeders need access to efficient systems for DNA extraction, genotyping, and data management and processing (Xu & Crouch, 2008). While large breeding companies have their own facilities, smaller companies rely on service providers, in particular time-efficient selection decisions might be critical. Phenotypic methods to assess sensory attributes as the Breeders' Sensory Test might be easier to integrate into smaller breeding programmes, as this method does not require equipment that is expensive or difficult to use, but the overall throughput tends to be lower compared to MAS. Since ripe fruits are needed for the Breeders' Sensory Test, plants could be preselected for other agronomically important traits, while the negative correlation between flavour-related traits and fruit weight (Causse et al., 2003; Klee & Tieman, 2018) as well as yield (Erika et al., 2022) should be considered.

5.2.2 Relevance of aroma volatiles for breeding and key volatiles for tomato aroma

Due to a lack of methods for identification and quantification of volatile compounds in the breeding process, volatile compounds, which form the aroma, have largely been neglected (Ferrão et al., 2020; Rambla et al., 2014). MAS offers the chance to integrate volatile compounds into the breeding process (Causse et al., 2003; Ferrão et al., 2020; Klee & Tieman, 2018). However, the large number of volatile compounds contributing to tomato flavour and consumer liking poses a challenge (Klee & Tieman, 2018; Tikunov et al., 2020). For instance, Tieman et al. (2017) identified two sugars, glutamic acid and 25 volatile compounds as positively or negatively correlated with consumer liking and flavour intensity. The number of traits or target loci that can be manipulated in one cycle is limited because the population size to provide the necessary recombinants increases exponentially with the number of target loci (Xu & Crouch, 2008). Hence, it is necessary to reduce the number of key compounds contributing positively or negatively to tomato aroma and consumer liking to a manageable number, ideally based on their physiological relationships (Klee & Tieman, 2013; Klee & Tieman, 2018). An important observation was therefore that volatiles derived from the same precursors such as apocarotenoids or fatty acids (C_6 volatiles) were co-localised on multiple chromosomes (CHAPTER 3; Study II, Figure 3.2), as also indicated by Rambla et al. (2017). Moreover, the co-localisation of QTL for apocarotenoid and C_6 volatiles on chromosomes 1, 3 and 6 could be caused due to metabolic dependence (Mathieu et al., 2009). Nevertheless, improving flavour by manipulating volatile profiles is complex as volatile compounds interact with primary metabolites and each other and are influenced by various factors such as environmental conditions, maturity stage and extraction methods (Rambla et al., 2014). In addition, the concentration of volatile compounds is crucial in determining whether it enhances or reduces tomato aroma and consumer liking (Rambla et al., 2014). Consequently, and due to the complexity of flavour perception, the effect of changes in the composition of aroma volatiles is difficult to predict (Rambla et al., 2014) and thus the effect of MAS for volatile compounds. Ferrão et al. (2020) described MAS for aroma volatiles to be feasible and efficient in blueberries, while the authors also pointed out that the volatile compounds were controlled by only a few major loci. For tomatoes, further research is needed to evaluate the effect of MAS for volatile compounds on sensory attributes. MAS for sensory attributes could be an alternative to select for taste and especially tomato aroma as perceived by the human senses. Perceived sensory attributes are of direct relevance for plant breeders (Amyotte et al., 2017). Moreover, molecular markers for sensory attributes will also change volatile profiles in the desired direction. As shown by our results, improving perceived tomato aroma is possible using molecular markers (CHAPTER 4; Study III, Figure 4.3).

Although accounting for aroma volatiles in the breeding process is difficult, to better understand this complex trait, it is important to identify the volatile compounds responsible for the tomato aroma and to study their relationships with each other and primary metabolites. According to the first two studies, sweetness, TSS and sourness enhanced tomato aroma. Comparing the Spearman correlation

coefficients (r), sweetness was more important for tomato aroma than sourness (CHAPTER 2 and 3; Study I, Figures 2 and AI.1; Study II, Figure 3.1). In accordance with Colantonio et al. (2022), volatiles were less important for sourness than for sweetness. Our first study showed the tendency of volatile compounds positively correlated with sweetness and TSS to also enhance tomato aroma (Study I, Figure AI.1). In agreement with Erika et al. (2022), hexanal was the most abundant volatile compound (Study I, Table 4; Study II, Table SII.5). Other volatile compounds with high concentrations in the first two studies were 6-methyl-5-hepten-2-on and E-geranylacetone. In both studies, with a few exceptions, the analysed volatile compounds were significantly correlated with tomato aroma. 2-Isobuthylthiazole was the only volatile compound that did not significantly contribute to tomato aroma in both studies, which is in agreement with Baldwin et al. (2015), but contradictory to Klee & Tieman (2018) and Piombino et al. (2013), who investigated consumer liking. β-Ionone and Z-3-hexenal showed a positive correlation with tomato aroma for the diverse plant material, but a negative correlation for the F_2 mapping population, demonstrating the difficulties associated with manipulating the concentration of individual aroma volatiles. Apocarotenoid-derived volatiles were most important for tomato aroma with $r \geq 0.43$ in the F_2 mapping population and are described as important for tomato flavour and acceptability (Martina et al., 2021; Rambla et al., 2014), while benzaldehyde had the highest r in the previous study, a volatile compound identified as important for consumer liking by Klee & Tieman (2018). In both studies, 2-phenylethanol was relatively important for tomato aroma as in agreement with earlier studies (Rambla et al., 2014). Among the fatty acid-derived volatiles, 1-penten-3-one and Z-3-hexenol were important contributors to tomato aroma and in addition E-2-hexenal in the first study. Fatty acid-derived volatiles are the most abundant volatiles of tomatoes, while more recent studies suggest a more limited impact of these volatiles on tomato flavour and consumer liking as assumed from the odour units (Martina et al., 2021; Rambla et al., 2014; Tieman et al., 2012). As expected from these correlations, selection for sensory attributes indirectly affected physicochemical measurements and aroma volatiles (CHAPTER 4).

5.2.3 Effect of flavour improvement on fruit weight

The results from all three studies confirm the challenge of breeding large-fruited tomatoes with good flavour as emphasized by Causse et al. (2003), Klee & Tieman (2018) and Lecomte et al. (2004b). Negative correlations between all sensory attributes except sourness, all physicochemical measurements except pH and some aroma volatiles with fruit weight were observed in the first study (CHAPTER 2; Study I, Figure 2). Some of them were confirmed using an F_2 mapping population (CHAPTER 3; Study II, Figure 3.1). These negative correlations led to indirect negative effects on fruit weight due to breeders' sensory selection (CHAPTER 4; Study III, Tables 4.4 and 4.5). Likewise, although not statistically significant, MAS reduced fruit weight. DM was increased by breeders' sensory selection and MAS, especially in the organic cultivation system, additionally indicating a decreased fruit size, since DM and fruit weight are negatively correlated (Beckles, 2012; Study II, Figure 3.1). The negative relationship between flavour-related traits

and fruit weight can be explained by antagonistic allelic effects of co-localised or close QTL for these traits (Study II, Figure 3.2, Table 3.4). This has already been described by several authors, in particular for fruit weight and sugar content or TSS (Causse et al., 2002; Causse et al., 2003; Chen et al., 1999; Lecomte et al., 2004b), also for the well-characterised *fw2.2*, a QTL cloned by Frary et al. (2000) and co-localised with QTL for DM, total sugars and TA (Lecomte et al., 2004b). In our study, breeders' sensory selection might have influenced several genetic regions, whereas for MAS only the co-localised QTL for sweetness and tomato aroma on chromosome 10 were close to a QTL for fruit weight (PVE = 8.0 %). TSS was also included in this QTL cluster on chromosome 10 (Study II, Figure 3.2). Furthermore, TSS and FW were co-localised or located in similar regions on chromosomes 2 and 3. As fruit weight and DM are negatively correlated and TSS and DM positively, a higher fruit weight is mainly due to increased water content, resulting in a dilution effect. This dilution effect is likely caused by a pleiotropic effect of single QTL, while linkage of different QTL could not be completely excluded (Causse et al., 2002; Chen et al., 1999; Lecomte et al., 2004b; Prudent et al., 2009).

In addition, fruit weight was co-localised with sensory attributes on chromosome 10 (as mentioned above), with volatile compounds on chromosome 3 and both on chromosome 1 (Study II, Figure 3.2). This might be caused by a combination of pleiotropic effects and linked genes. A dilution effect probably also affects sensory attributes and volatile compounds. Furthermore, it is likely that with decreasing TSS, positively correlated traits such as sensory attributes especially sweetness and tomato aroma, as well as some volatile compounds also decrease. Nevertheless, linked QTL for FW and volatile compounds, and thus sensory attributes might be additionally involved. Genes controlling fruit weight mainly affect floral meristem enlargement and organization, cell division and cell expansion (Pereira et al., 2021), while volatile compounds are formed in different metabolic pathways (Klee & Tieman, 2018; Martina et al., 2021). Further genetic studies such as fine mapping would be necessary to differentiate between closely linked and pleiotropic genes. Still, some improvement of taste and aroma independent of fruit weight should be possible as indicated by our results and pointed out by others. According to our results, QTL for sweetness, sourness and TSS/TA on chromosome 5 are not co-localised with a QTL for fruit weight (Study II, Figure 3.2). Chen et al. (1999) also identified a few QTL only for TSS, while a significant increase in TSS is probably not possible without reducing fruit weight. The large QTL cluster on chromosome 6 covering QTL for sensory and volatile QTL did not co-segregate with QTL for fruit weight. Other studies identified volatile compounds with a positive effect on sweetness independent of sugar content, highly interesting for breeding (Klee & Tieman, 2018). As reported by others, significantly improving in the flavour of tomatoes without sacrificing yield is an additional challenge (Erika et al., 2022; Klee & Tieman, 2013).

5.3 Conclusions and perspective

The results of the three trials provide relevant information to implement phenotypic and marker-assisted selection for sensory attributes in the first segregating generations of a breeding programme and expands the knowledge on the inheritance of flavour-related traits and their relationships. Novel QTL for sensory attributes co-localised with physicochemical measurements were identified. Such genetic targets reflecting sensory attributes as perceived by human senses are of direct relevance for plant breeders. Early selection for sensory attributes using the Breeders' Sensory Test and molecular markers were both investigated as promising methods to improve the flavour of fresh market tomatoes. Marker-assisted selection is particularly interesting for preselection of seedlings and allows a potentially higher selection intensity, as a very large number of plants can be analysed. However, phenotypic selection using the Breeders' Sensory Test accounts for the whole flavour diversity and captures all loci, including the unidentified ones. To maximize the response to selection, a combination of phenotypic and marker-assisted selection is recommended. Flavour-related traits were influenced by environmental effects including the cultivation system. Most traits showed higher values in the organic cultivation system, but no general conclusions are possible since the cultivation systems differed not only in their management strategy. Significant genotype-by-environment interactions were observed for most flavour-related traits. Neverthelss, some attributes and volatile compounds less sensitive to environmental effects have also been identified. The selection progress is probably decelerated by a genotype-by-year interaction. Our results confirm the challenge of breeding large-fruited tomatoes with improved flavour, as also described by others for yield. Regional sustainable and organic production offers a potentially suitable market that could accept some loss of yield if flavour is superior. Genetic improvement starting with the F_2 generation is expected to be possible, although environmental effects make it more difficult to identify superior genotypes. Breeding is the first step in the whole value chain and provides the basis for flavourful tomatoes, while also cultivation systems, agronomic handling and post-harvest treatments have to be optimized so that the genotype can fulfil its potential.

The studies presented provide opportunities for further research, including the following topics:

- Determining the optimal number of samples per day for the Breeders' Sensory Test as a compromise between throughput and data quality. Adaption of the Breeders' Sensory Test to other crops.
- Fine mapping to differentiate between pleiotropic and linked QTL within the QTL clusters and identification of candidate genes for flavour-related traits.
- Identification of key compounds for flavour and investigation of MAS for volatile compounds and their effects on sensory attributes.
- Distinguishing between the effects of organic vs. conventional, greenhouse vs. sheltered vs. outdoor and inert vs. soil-based tomato cultivation on sensory attributes, primary and secondary metabolites.

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APPENDIX

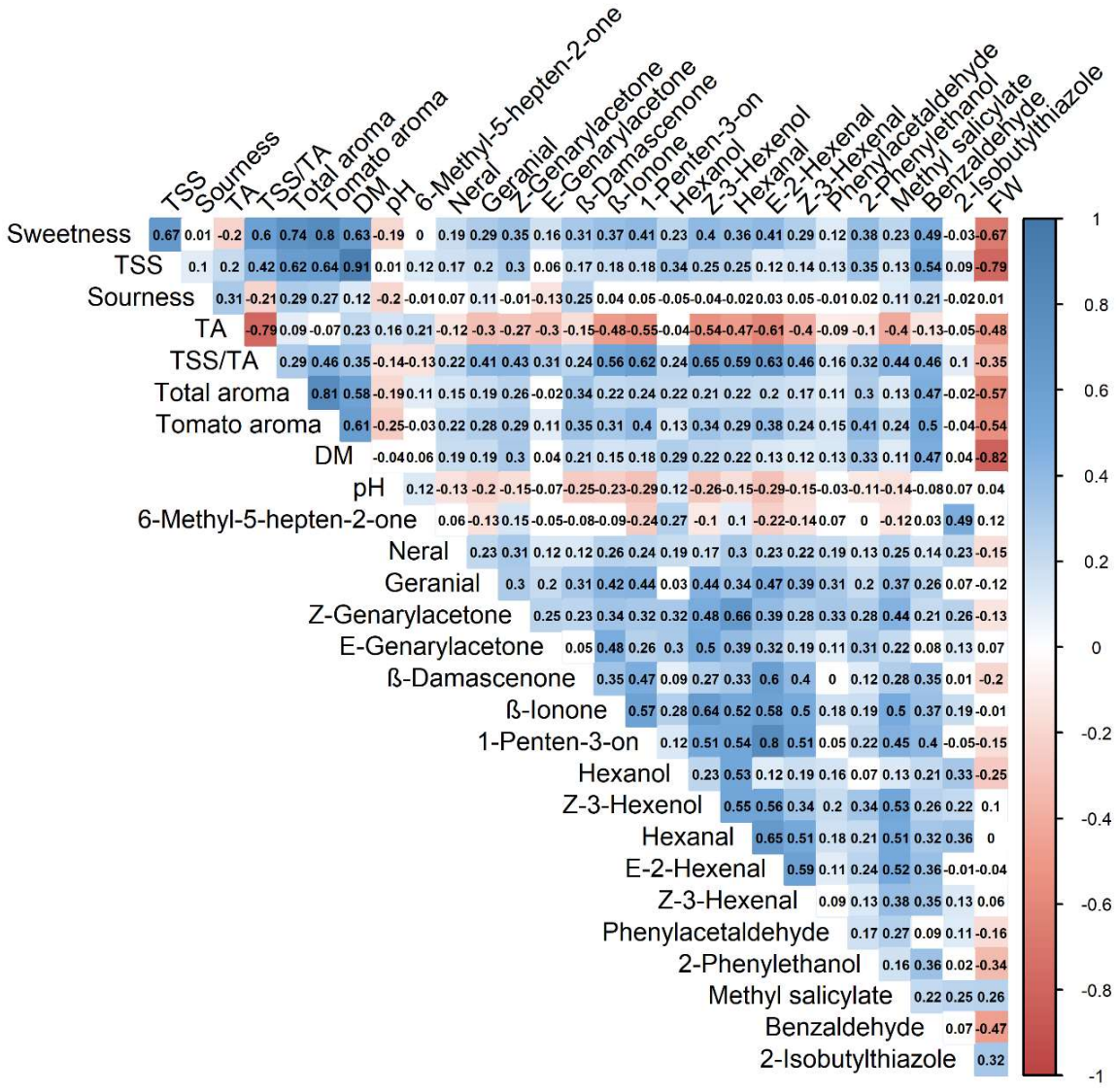

Figure AI.1. Spearman correlation coefficients for sensory attributes, physicochemical measurements (TSS, total soluble solids; TA, titratable acidity), and aroma volatiles (n= 851) analysed in two cultivation systems; significant positive correlations are shown in blue and significant negative correlations in red with p = 0.01

SUMMARY

Flavour was not a primary breeding target in tomatoes (*Solanum lycopersicum* L.) until recently. However, the poor flavour of tomatoes is a major cause of consumer complains. Nevertheless, the tomato is one of the most important and popular vegetables in Europe and worldwide. Flavour results from a complex interaction of sugars, acids (taste) and volatile compounds (aroma). This chemical complexity makes it difficult to assess flavour, particularly for hundreds of samples with a small sample size as it is typical for early breeding generations. In addition, environmental factors including the cultivation system affect tomato flavour. It is challenging to improve flavour while maintaining agronomically important traits, as superior flavour is negatively correlated with productivity. Trained and consumer panels are not suitable for large numbers of samples. Simple physicochemical measurements as total soluble solids (TSS) and titratable acidity (TA) can be used as approximations for sugars and acids (taste), but not for perceived aroma. To meet consumer demands for better flavour, plant breeders need appropriate methods for flavour assessment. The so-called Breeders' Sensory Test, in which hundreds of small samples can be assessed by a small team, has not yet been evaluated or standardized. Marker-assisted selection (MAS) is a promising alternative to phenotypic selection, but is controversially discussed in the organic sector. However, sensory attributes, crosses of parents with excellent flavour and different cultivation systems have rarely been investigated in mapping studies. Studies verifying molecular markers in multiple genetic backgrounds, as required for a broader application, are even rarer.

Our study was based on 32 crosses whose parental cultivars were selected based on their quality traits and yield from a previous study. The main research aims were *i)* to evaluate the Breeders' Sensory Test and the potential of selection for flavour-related traits, particularly sensory attributes, in early segregating generations, *ii)* to map QTL for flavour-related traits and develop molecular markers for sensory attributes, and *iii)* to compare the means of populations selected by phenotypic selection the Breeders' Sensory Test or molecular markers for sensory attributes with an unselected control.

To address these aims, three trials were conducted in two contrasting cultivation systems, namely organic low-input and hydroponic. *i)* In 2017, ten F_2 plants from each of the 32 crosses and the corresponding parents (in total 910 individuals) were characterised for sensory attributes, physicochemical measurements and aroma volatiles. Perceived sweetness, sourness, total and tomato aroma were assessed with the Breeders' Sensory Test, which was conducted by a team of two to three persons. *ii)* One of the crosses, Resi × Auriga, was chosen as F_2 mapping population for the second trial in 2018. Both open-pollinated cultivars had been characterised by superior but contrasting fruit quality and very different fruit weights. In each cultivation system, 190 individuals of the F_2 mapping population were grown with two replications. Plants were genotyped with the Axiom 200K SOLCUC vegetable array and phenotyped for the same traits as in the previous year to map QTL for flavour-related traits, including sensory

attributes. *iii)* In the same field season, phenotypic selection based on the Breeders' Sensory Test (breeders' sensory selection, BS) was performed on F_2 plants of the mapping population and the unrelated cross Roterno F_1 × Black Cherry. Roterno F_1 is a high yielding hybrid and Black Cherry an open-pollinated cultivar with excellent sensory attributes. Based on the results of the mapping study, five QTL for the sensory attributes sweetness, sourness and tomato aroma were selected for verification in two genetic backgrounds. In both crosses, Resi × Auriga and Roterno F_1 × Black Cherry, MAS was performed on F_2 seedlings in 2019. Subsequently, F_3 progenies of plants selected by BS and F_2 plants selected by MAS were phenotyped together with an unselected F_2 population for sensory attributes (Breeders' Sensory Test and trained panel), physicochemical measurements, aroma volatiles and fruit weight.

The following results were obtained: *i)* Highly significant correlations between corresponding sensory attributes and physicochemical measurements, namely sweetness and TSS as well as sourness and TA, were observed, indicating that the sensory attributes assessed with the Breeders' Sensory Test adequately described the sugar and acid content of the fruits. The genetic plus environmental variance (coefficient of variation of the F_2 plants) exceeded the environmental variance (mean of the parental coefficients of variation) for most flavour-related traits of most crosses regardless of the cultivation system. For sensory attributes, this was true for about 54 to 91% of the crosses. That means that the prerequisite for successful early selection of individual plants is fulfilled. *ii)* A total of 71 QTL was detected for the mean values of both cultivation systems, 61 QTL for organic and 46 QTL for hydroponic cultivation. Accounting for co-localised QTL between these environments, a total of 100 QTL were detected. A proportion of 27% of the QTL was co-localised between both cultivation systems and their mean values, representing robust QTL. QTL for sensory attributes on chromosomes 5 and 10 and most QTL for specific aroma attributes provide novel information. Nine QTL clusters were identified for the mean values of both cultivation systems, comprising co-localised QTL for different trait classes, e.g. sensory attributes and physicochemical measurements or volatile compounds. Co-localised QTL for fruit weight and flavour-related traits with antagonistic effects were identified. QTL for the sensory attributes sweetness, sourness and tomato aroma, partly within QTL clusters, on chromosomes 2, 5, 6, 10 and 11 are highly interesting for MAS. *iii)* BS significantly increased sweetness of Roterno F_1 × Black Cherry in the organic cultivation system. In both crosses, MAS was most efficient for tomato aroma. In addition, a significant increase in sweetness by MAS was observed in Roterno F_1 × Black Cherry in the organic cultivation system. For both selection methods, increases in the population means were observed for most sensory attributes, including sourness, in both crosses and cultivation systems. For most traits, the experimental populations selected by molecular markers showed the highest mean value for both crosses and cultivation system. Selection for sensory attributes by BS and MAS, respectively, resulted in indirect changes of the level of some physicochemical measurements and volatile compounds; fruit weight decreased.

In conclusion, the three trials suggest methods to select for flavour-related traits, particularly sensory attributes and reveal many insights into their relationship and inheritance. The Breeders' Sensory Test and MAS are both promising methods to select for sensory attributes in the first segregating generations. MAS is particularly interesting for preselection of seedlings and allows a potentially higher selection intensity, as a very large number of plants can be analysed. The Breeders' Sensory Test reflects the whole flavour diversity and is particularly interesting for organic plant breeding, where phenotypic selection is considered most important. To maximize the response to selection and capture all genetic loci including unidentified ones, a combination of breeders' and marker-assisted selection is recommended. Novel QTL for the sensory attributes sweetness, sourness, and tomato aroma were identified on the chromosomes 5 and 10. The QTL for sweetness and tomato aroma were confirmed in a non-related genetic background. The results of all three trials underline the challenge of breeding large-fruited tomatoes with improved flavour. Genetic improvement starting with selection in the F_2 generation is expected to be successful. A genotype-by-year interaction probably decelerates the selection progress. Our results provide relevant information to improve the flavour of fresh market tomatoes, a trait demanded by consumers. Breeding is the first step in the value chain and improved genetics form the basis for flavourful tomatoes.

SUMMARY *deutsch*

Geschmack war bis vor kurzem kein vorrangiges Zuchtziel bei Tomaten (*Solanum lycopersicum* L.). Allerdings ist der geringe Geschmack von Tomaten ein Hauptgrund für Verbraucherbeschwerden. Trotzdem ist die Tomate sowohl in Europa als auch weltweit eines der wichtigsten und beliebtesten Gemüse. Geschmack (engl. flavour) resultiert aus einem komplexen Zusammenspiel von Zuckern, Säuren (engl. taste) und flüchtigen Verbindungen (Aroma). Diese chemische Komplexität erschwert die Quantifizierung des Geschmacks, insbesondere bei Hunderten von Proben mit geringer Probenmenge, wie es typisch für die ersten Züchtungsgenerationen ist. Zudem wird der Tomatengeschmack von Umwelteffekten wie dem Anbausystem beeinflusst. Es ist eine Herausforderung, den Geschmack zu verbessern und gleichzeitig das aktuelle Niveau agronomisch wichtiger Eigenschaften beizubehalten, da hervorragender Geschmack und Produktivität negativ miteinander korreliert sind. Geschulte Panels und Konsumentenpanels sind für eine große Anzahl an Proben ungeeignet. Einfache physikalisch-chemische Messungen wie der Gehalt an löslichen Feststoffen (Brix) und titrierbaren Säuren (TS) können als Näherungswerte für Zucker und Säuren verwendet werden, die Analyse von Aromastoffen ist allerdings komplex und aufwendig. Um den Konsumentenforderungen nach besserem Geschmack nachkommen zu können, benötigen Pflanzenzüchter nun geeignete Methoden zur Geschmackserfassung. Die sogenannte züchterische Sensorik (Breeders' Sensory Test), bei der Hunderte von Proben durch ein kleines Team bewertet werden können, wurde bisher noch nicht evaluiert oder standardisiert. Markergestützte Selektion (MAS) ist eine vielversprechende Alternative zur phänotypischen Selektion, die in der Biobranche allerdings kontrovers diskutiert wird. Allerdings wurden bisher nur selten sensorische Merkmale, Kreuzungen von Eltern mit hervorragendem Geschmack und unterschiedliche Anbausysteme in Kartierungsstudien berücksichtigt. Noch seltener sind Studien, die molekulare Marker in mehreren genetischen Hintergründen überprüfen, wie es für eine breite Anwendung nötig ist.

Die Grundlage für unsere Studie waren 32 Kreuzungen, deren Elternsorten basierend auf ihren Qualitätsmerkmalen und Erträgen aus einer früheren Studie ausgewählt wurden. Die wichtigsten Forschungsziele waren *i)* die Evaluierung der züchterischen Sensorik und des Potenzials der Selektion auf geschmacksrelevante Merkmale, insbesondere sensorische Eigenschaften, in frühen Generationen, *ii)* die Kartierung von QTL für geschmacksrelevante Merkmale und die Entwicklung molekularer Marker für sensorische Eigenschaften und *iii)* der Vergleich von Mittelwerten für Populationen, die durch phänotypische Selektion mittels der züchterischer Sensorik oder mit molekularen Markern für sensorische Merkmale selektiert wurden, mit einer nicht selektierten Kontrollgruppe.

Um diese Ziele zu erreichen, wurden drei Versuche in zwei kontrastierenden Anbausystemen (ökologischer low-input und hydroponischer Anbau) durchgeführt. *i)* 2017 wurden zehn F_2-Pflanzen aus jeder der 32 Kreuzungen und ihre Eltern (insgesamt 910 Individuen) für sensorische Eigenschaften,

physikalisch-chemische Merkmale und Aromastoffe charakterisiert. Süße, Säure, Gesamt- und tomatentypisches Aroma wurden mit der züchterischen Sensorik erfasst, die von einem Team aus zwei bis drei Personen durchgeführt wurde. *ii)* Resi × Auriga wurde als F_2-Kartierungspopulation für das zweite Versuchsjahr in 2018 ausgewählt. Beide Liniensorten zeichnen sich vorherigen Studien nach durch eine hervorragende, aber gegensätzliche Fruchtqualität und sehr unterschiedliche Fruchtgewichte aus. Je Anbausystem wurden 190 Individuen der F_2-Kartierungspopulation in zwei Wiederholungen angebaut. Die Pflanzen wurden mit dem Axiom 200K SOLCUC vegetable array genotypisiert und für dieselben Merkmale wie im Vorjahr phänotypisiert, um QTL für geschmacksbezogene Merkmale, einschließlich sensorischer Eigenschaften, zu kartieren. *iii)* Im gleichen Versuchsjahr wurde eine phänotypische Selektion mittels der züchterischen Sensorik (züchterische Selektion, ZS) an F_2-Pflanzen der Kartierungspopulation und der nicht verwandten Kreuzung Roterno F_1 × Black Cherry durchgeführt. Roterno F_1 ist eine ertragreiche Hybride und Black Cherry eine Liniensorte mit außergewöhnlichem Geschmack. Basierend auf den Ergebnissen der Kartierungsstudie wurden fünf QTL für die sensorischen Eigenschaften Süße, Säure und Tomatenaroma zur Überprüfung in zwei genetischen Hintergründen ausgewählt. In den beiden Kreuzungen Resi × Auriga und Roterno F_1 × Black Cherry wurde 2019 eine MAS an F_2-Sämlingen durchgeführt. Anschließend wurden F_3-Nachkommen der phänotypisch selektierten Pflanzen und F_2-Pflanzen aus der MAS gemeinsam mit einer unselektierten F_2 Population für sensorische Eigenschaften (züchterische Sensorik und geschultes Panel), physikalisch-chemische Merkmale, Aromastoffe und Fruchtgewicht phänotypisiert.

Die folgenden Ergebnisse wurden erzielt: *i)* Hochsignifikante Korrelationen zwischen sich entsprechenden sensorischen Eigenschaften und physikalisch-chemischen Messungen (Süße und Brix sowie Säure und TS) wurden beobachtet, was darauf hindeutet, dass die sensorischen Eigenschaften aus der züchterischen Sensorik den Zucker- und Säuregehalt der Früchte ausreichend quantifizieren. Die genetische plus umweltbedingte Varianz (Variationskoeffizient der F_2-Pflanzen) übertraf die rein umweltbedingte Varianz (Mittelwerte der elterlichen Variationskoeffizienten) für die Mehrheit der geschmacksrelevanten Merkmale in den meisten Kreuzungen unabhängig vom Anbausystem. Bei den sensorischen Eigenschaften traft dies auf etwa 54 bis 91% der Kreuzungen zu. Dies bedeutet, dass die Voraussetzung für eine erfolgreiche frühe Selektion von Einzelpflanzen erfüllt ist. *ii)* Insgesamt wurden 71 QTL für die Mittelwerte beider Anbausysteme identifiziert, 61 QTL für den ökologischen und 46 QTL für den hydroponischen Anbau. Unter der Berücksichtigung von kolokalisierten QTL zwischen diesen verschiedenen Umwelten wurden insgesamt 100 QTL entdeckt. Ein Anteil von 27% der QTL war zwischen den beiden Anbausystemen und ihren Mittelwerten kolokalisiert und stellt damit robuste QTL dar. QTL für sensorische Eigenschaften auf den Chromosomen 5 und 10 sowie die meisten QTL für spezifische Aromaeigenschaften wurden zum ersten Mal kartiert. Für die Mittelwerte beider Anbausysteme wurden neun QTL-Cluster identifiziert, welche kolokalisierte QTL für verschiedene Merkmalsgruppen, z. B.

sensorische Eigenschaften und physikalisch-chemische Messungen oder flüchtige Verbindungen umfassen. Es wurden kolokalisierte QTL für das Fruchtgewicht und geschmacksrelevante Merkmale mit antagonistischen Effekten kartiert. QTL für die sensorische Merkmale Süße, Säure und tomatentypisches Aroma, teilweise innerhalb von QTL-Clustern, auf den Chromosomen 2, 5, 6, 10 und 11 sind für MAS von großem Interesse. *iii)* ZS erhöhte die Süße der Kreuzung Roterno F_1 × Black Cherry im ökologischen Anbau signifikant. In beiden Kreuzungen war die MAS war für das Tomatenaroma am erfolgreichsten. Darüber hinaus wurde eine signifikante Steigerung der Süße durch MAS in Roterno F1 × Black Cherry im ökologischen Anbausystem beobachtet. Für beide Selektionsmethoden und die meisten sensorischen Merkmale, einschließlich Säure, wurden Zunahmen der Populationsmittelwerte in beiden Kreuzungen und Anbausystemen beobachtet. Für die meisten Merkmale zeigten die mit molekularen Markern selektierten Populationen unabhängig von der Kreuzung und dem Anbausystem den höchsten Mittelwert. Die Selektion auf sensorische Eigenschaften mittels ZS beziehungsweise MAS hatte indirekte Auswirkungen auf das Niveau einiger physikalisch-chemische Merkmale und die Konzentration zahlreicher Aromastoffe; das Fruchtgewicht wurde reduziert.

Zusammenfassend lässt sich sagen, dass die drei durchgeführten Versuche Methoden für die Selektion auf geschmacksbezogene Merkmale, insbesondere sensorische Eigenschaften, vorschlagen und viele Erkenntnisse über deren Beziehung und Vererbung liefern. Sowohl die züchterische Sensorik als auch MAS sind vielversprechende Methoden zur Selektion auf sensorische Eigenschaften in den ersten aufspaltenden Züchtungsgenerationen. MAS ist besonders interessant für die Vorselektion von Sämlingen und ermöglicht eine potenziell höhere Selektionsintensität, da eine sehr große Anzahl von Pflanzen analysiert werden kann. Die züchterische Sensorik erfasst die gesamte Aromavielfalt und ist besonders für die ökologische Pflanzenzüchtung interessant, welche die phänotypische Selektion als am wichtigsten betrachtet. Um den Selektionserfolg zu maximieren und alle genetischen Loci, einschließlich der nicht kartierten zu berücksichtigen, wird eine Kombination aus züchterischer und markergestützter Selektion empfohlen. QTL für die sensorischen Eigenschaften Süße, Säure und Tomatenaroma wurden erstmalig auf den Chromosomen 5 und 10 kartiert. Die QTL für Süße und Tomatenaroma wurden in einer zweiten Kreuzung verifiziert. Die Ergebnisse aller drei Feldversuche bestätigen die Herausforderung großfrüchtige Tomaten mit verbessertem Geschmack zu züchten. Es wird erwartet, dass eine genetische Verbesserung, die mit der F_2-Generation beginnt, erfolgreich sein wird. Eine Wechselwirkung zwischen Genotyp und Jahr verlangsamt vermutlich den Selektionsfortschritt. Unsere Ergebnisse liefern relevante Informationen zur Verbesserung des Geschmacks von Tomaten, einer von Verbrauchern geforderten Eigenschaft. Die Züchtung ist der erste Schritt in der Wertschöpfungskette und eine verbesserte Genetik bildet die Grundlage für schmackhafte Tomaten.

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DECLARATION

I, Julia Friederike Hagenguth, born on 01.09.1987 in Wiesbaden, hereby declare that

1. this dissertation was conducted independently and without unauthorized references and assistance
2. this dissertation has not been presented to any other examining body either in its present or a similar form
3. I have not applied for a doctoral degree at any other universities.

The versions for printing and reading online are identical, except for some details of formatting.

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CURRICULUM VITAE

Personal details

Name	Julia Friederike Hagenguth
Date of Birth	01.09.1987 in Wiesbaden, Germany

Education

Since 04/2017	**PhD candidate**, PhD Programme for Agricultural Sciences in Göttingen (Division of Plant Breeding Methodology, University of Goettingen, Göttingen, Germany)
02/2013 – 11/2016	**Master of Agricultural Biology** University of Natural Resources and Life Sciences, Vienna, Austria Major: plant breeding, plant production, agroecology
10/2009 – 11/2012	**Bachelor of Agricultural Biology** University of Hohenheim, Stuttgart, Germany Major: biology of crops, plant breeding and seed science

Awards and Scholarships

07/2021 – 12/2021	Scholarship of the Professor Werner Schulze-Stiftung zur Förderung der Pflanzenbauwissenschaften
02/2020	Poster award, International Symposium of the Society for Plant Breeding e.V. (GPZ), University of Natural Resources and Life Sciences, Tulln, Austria
05/2018	Award for oral presentation, XIX EUCARPIA Meeting of the Tomato Working Group, University of Naples Federico II, Naples, Italy

Work experience and Internships

03/2017 – 12/2021	**Research assistant** in plant breeding, project PETRA^{q+n} (Participatory development of quality tomatoes for sustainable regional production), Division of Plant Breeding Methodology, University of Goettingen, Göttingen Germany
8/2016 - 10/2016	Internship, Getreidezüchtung Peter Kunz Deutschland, Darmstadt, Germany
07/2016	Internship/Student assistant, University of Utah, Department of plants, soils and climate, Prof David Hole, Logan, United States of America
5/2015 - 11/2015, 11/2013	Student assistant, University of Natural Resources and Life Sciences, Institute of Biotechnology in Plant Production, Tulln, Vienna, Austria
09/2014	Internship, Saatzucht Gleisdorf, Gleisdorf, Austria
07/2013 – 08/2013	Internship, University of Minnesota, Department of Plant Pathology, Prof Ruth Dill Macky's lab, St. Paul, United States of America
08/2011 – 09/2011	Internship with a focus on seed propagation, Biohof Bienert Hänsel GbR Taucha, Germany
08/2010	Internship, Demeter marketgarden Sonnenberg, Wiesbaden, Germany
08/2008 – 07/2009	Voluntary ecological year, Schloss Freudenberg, Wiesbaden, Germany and Demeter beekeeping Robert Friedrich, Mainz, Germany

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Conference contributions

09/2021	**Hagenguth J**, Kanski L, Kahle H, Persch A, Pawelzik E, Becker HC, Horneburg B (2021). Improving flavour with the Breeders' Sensory Test. Pitch presentation at: 20th Organic World Congress 2021, Rennes, France (hybrid)
02/2020	**Hagenguth J**, Kanski L, Kahle H, Persch A, Pawelzik E, Becker HC, Horneburg B (2020). Breeding tomatoes with improved flavour using a breeders' sensory test. Poster presented at: International Symposium of the Society for Plant Breeding e.V. (GPZ), University of Natural Resources and Life Sciences, Tulln, Austria
05/2018	**Hagenguth J**, Kanski L, Kahle H, Persch A, Smit I, Becker HC, Horneburg B (2018). The potential of a breeders' sensory test in the F_2 generation of tomato. Oral presentation at: XIX EUCARPIA Meeting of the Tomato Working Group, University of Naples Federico II, Naples, Italy
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11/2016	**Hagenguth J** (2016). Evaluation of common bunt resistance in three winter wheat populations. Poster presentation at: ELLS scientific student conference, University of Hohenheim, Stuttgart, Germany

Skills

Languages	German: native language English: fluent
Computer literacy	MS Office: Word, PowerPoint, Excel Programming language R (advanced)

References and supporting documents will be provided upon request.

[redacted]