Scientia Horticulturae 129 (2011) 18–26

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Utilization of correlation network analysis to identify differences in sensory attributes and organoleptic compositions of tomato cultivars grown under salt stress

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ARTICLE INFO

Article history:
Received 12 August 2010
Received in revised form
29 December 2010
Accepted 16 February 2011

Keywords:
Amino acid
Network analysis
Sensory attribute
Salt stress
Sugar
Tomato

ABSTRACT

In tomato production, salt stress is applied to improve the fruit quality. The change in sensory attributes and organoleptic compositions in salt-stressed tomato fruits have been extensively studied; however, little is known about their interaction with each other. Correlation network analysis provides a visual representation of biological systems and useful knowledge for metabolic data analysis of tomato fruits. The aim of this study is to identify the cultivar differences in sensory attributes and organoleptic compositions of tomato fruits grown under salt stress and to elucidate their interaction among different cultivars using correlation network analysis. Salt stress was applied by adding 100 mM NaCl to the nutrient solution. Fruits were harvested at red-ripe stage and used for the evaluation of sensory attributes such as sweetness, sourness, umami, and tomato like, and organoleptic compositions such as sugar, organic acid, amino acid, and sodium ion contents. Almost all of the sensory attributes and organoleptic compositions of the fruit were significantly high in salt-stressed cultivars compared to non-stressed cultivars, and significant differences were also observed among cultivars. The correlation network analysis of the control fruit showed that compared to other traits, sugar is one of the key traits for improvement of tomato fruit quality based on high connectivity and betweenness centrality. In contrast, a high degree of positive connectivity was not observed between organoleptic compositions and sensory attributes in the salt-stressed fruit network. These results indicate that the relationship between sensory attributes and organoleptic compositions in fruits were different between the control and salt-stressed cultivars, suggesting that the salt-stressed fruit may have a different circuit of relationship compared to control. Furthermore, based on the increase ratio (salt stress/control) network results, we suggest that the increased sugar, organic acid, and amino acid contents may have contributed to the salt stress-induced enhancement of sensory attributes.

1. Introduction

Recently, based on consumer and market requirements for tomato quality, increasing interest has been focused on the organoleptic aspects. Improvement of organoleptic quality is an important problem for tomato growers and breeders, and several practices have been undertaken to improve the quality. The organoleptic characteristics of tomato fruits mainly depend on a complex mixture of sugars, organic acids, and amino acids. Several studies have correlated tomato sensory traits with organoleptic compositions (Causse et al., 2007). For example, the intensity of taste of tomatoes is mainly determined by the amount of soluble solids, mostly reducing sugars and organic acids (Krumbein and Auerswald, 1998; Malundo et al., 1995). A strong positive correlation has been observed between sweetness and reducing sugars or total soluble solids content (Malundo et al., 1995; Tandon et al., 2003). The sour taste of tomatoes has been attributed mainly to citric acid (Petro-Turza, 1986). Free amino acids such as glutamic acid play an important role as umami in tomato fruit taste (Fuke and Shimizu, 1993). Furthermore, fruit texture, such as hardness, is also important to organoleptic characteristics of tomato fruits because tomato texture is one of the critical components of the consumer's perception of tomato fruit quality (Chaïb et al., 2007).

Salt stress has been applied to improve the organoleptic aspects of fruit quality in tomato production. Salt stress is known to increase the concentration of sugars, organic acid, and percentage of dry matter in fruit; however, it reduces the size and yield of the fruit (Cuartero and Fernandez-Munoz, 1998). Several studies have

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doi:10.1016/j.scienta.2011.02.011

reported about the sensory attributes and organoleptic compositions of the salt-stressed fruit. For example, in sensory analysis of tomato fruit grown under salt stress, increasing the electric conductivity (EC) in nutrient solution containing macronutrients improved the sensory attributes of the conventional round fruit but not that of the long life cultivar, indicating that cultivars did not have the same response to the changes in sensory attributes as the conventional tomato fruit when subjected to salt stress (Auerswald et al., 1999). In addition, the organoleptic parameters, such as soluble solids, fructose, glucose, titratable acid, and amino acid contents, increase with increasing salinity (Balibrea et al., 2003; Balibrea et al., 1996; Franco et al., 1999; Gao et al., 1998; Mitchell et al., 1991; Petersen et al., 1998; Zushi and Matsuzoe, 2006a, 2007). As mentioned above, the organoleptic properties of tomato fruit could be evaluated by sensory analysis or instrumental measurements only.

Tomato is represented by several hundred cultivars and hybrids in the fresh consumption market, which leads to an ever-increasing demand for fruits with varied characteristics such as fruit size, shapes, and color. However, as mentioned above, results of Auerswald et al. (1999) showed that the response to salt stress might be different among cultivars. In addition, growth, ion accumulation, and water relations in tomato plants grown under salt stress condition differ between normal-fruited and cherry tomato cultivars (Alarcón et al., 1994) and fresh market tomato cultivars (Alian et al., 2000). However, little is known about cultivar differences in sensory attributes and organoleptic compositions and their interactions with each other.

Network analysis gives a visual representation of biological systems, capturing their essential characteristics and interactions in the biological system (Barabasi and Oltvai, 2004). In recent years, network structures have been extensively characterized in an effort to elucidate design principles of metabolic or gene networks (Newman, 2003). From the agricultural viewpoint, several studies using network analysis have provided useful information for metabolic data analysis of tomato fruits (Carli et al., 2009; Causse et al., 2002; Mounet et al., 2009; Schauer et al., 2006; Ursem et al., 2008). In network analysis, the metabolic data are represented by nodes that are connected by links, with each link representing the interactions between components such as sensory attributes and organoleptic quality in tomato fruit (sugars, organic acids, and amino acids) (Carli et al., 2009). Thus, we hypothesized that the correlation network analysis could be applied to visually identify the cultivar differences in sensory attributes and organoleptic compositions of tomato fruits grown under salt stress and to elucidate their interaction among different cultivars. Furthermore, to our knowledge, no previous analyses have shown the effects of environmental factors, such as salt stress, in shaping network structures.

The aim of this study is to determine the cultivar differences in sensory attributes and organoleptic compositions of tomato fruit grown under salt stress and to elucidate their interactions using the correlation network analysis. To determine the cultivar differences in this study, we examined several commercial tomato cultivars with different fruit sizes for a period of two years.

2. Materials and methods

2.1. Plant materials and growth conditions

In an attempt to include many cultivars, the experiment was repeated over two years (2006 and 2007) using different cultivars, except 'Tio Cook'. In the 2006 experiment, the following four cultivars were used: cherry-type 'Mini Carol' (MC), medium-fruited type 'Rui 60' (RU), large-fruited type 'House Momotaro' (HM), and processing-type 'Tio Cook' (TC). In the 2007 experiment, the following five cultivars were used: cherry-type 'Chika' (CH), medium-fruited type 'Furutyka' (FU), large-fruited type 'Momotaro 8' (M8), 'Kyoryoku Beijyu' (KB), and processing-type 'Tio Cook' (TC). These cultivars were grown under greenhouse conditions at Prefectural University of Kumamoto from spring to summer. Seeds were sown at the beginning of April during both years, and seedlings with two true leaves were transplanted in a 12-cm-diameter pot filled with pumice. At the end of May, when the inflorescence appeared, plants were transplanted into a closed irrigation system in a randomized complete block design with two replications at a density of 4 plants per meter. Each plot consisted of 6 plants. The closed irrigation system consisted of a culture bed filled with pumice (particle size, approximately 5 mm), a 100 L tank for nutrient solutions, and a timer for irrigation. The plants were fertilized with a nutrient solution (half-strength Otsuka-B solution; Otsuka Chemical Co., Osaka, Japan) having an electrical conductivity of 1.6 dS m^{-1} until the start of the salt stress treatment. The nutrient solution was supplied through a drip irrigation tube at 2-h intervals during daytime (6:00–18:00) for 15 min, and the drainage was reused. Concentrations of the nutrient solution were determined using RQ flex (NO_3^-, PO_4^{3-}, Merck; Germany) and ion chromatography (K^+, Ca^{2+}, Mg^{2+}, and Na^+; Shimadzu, Japan) at 7-day intervals and readjusted accordingly by adding the concentrated fertilizers. The pH of the nutrient solutions was measured daily, and when necessary, corrected with 1.0 M H_2SO_4 to maintain the pH at 6.5–7.0. Nutrient solutions were renewed at 4-week intervals. All the plants were topped just below the fourth truss, and all lateral shoots were removed periodically.

2.2. Salt stress treatment

After two weeks of transplantation, salt treatments were applied by adding 100 mM NaCl to the nutrient solution. Nutrient solution without NaCl served as the control. The salt treatments were continued until the end of the experiment. The NaCl concentration in the nutrient solutions was readjusted as mentioned above.

2.3. Fruit harvest

Red-ripe fruits were harvested from the end of June to the beginning of July during both years. For large-fruited and cooked-type cultivars, such as HM, KB, TC, and M8, fruits were harvested from the distal second or third fruit on the second truss. For cherry-type and medium-fruited cultivars such as MC, CH, FU, and RU, all trusses with more than 10–20 fruits were harvested from the middle of the truss to improve uniformity of size and growth conditions. For each treatment and cultivar, approximately 20 fruits from different plants (2 fruits per plant) were harvested, and then, at least 6 fruits were selected based on the average size and color. The fruit surface color was measured using a colorimeter (Minolta CR-300; Minolta Corp, Japan) in CIELAB units. Measurements were taken in triplicates at the equator of the fruit (data not shown). Following the measurement of fruit weight and fruit surface color, the fruits were cut into quarter sections for all cultivars, except for cherry-type cultivar fruits that were cut in half. The sliced fruits were then stored at $-80\,^{\circ}C$ until analysis.

2.4. Sensory evaluation

The sensory panel comprised 14 panelists recruited from among the students of the Department of Food and Health Sciences at Prefectural University of Kumamoto on the basis of willingness to consume fresh tomatoes. No panelists reported negative reactions or allergies to tomato fruit. Red-ripe fruits were harvested approximately 1 h before the sensory evaluation and kept at room temperature. Fruit samples were sliced into two (cherry-type and medium-fruited cultivars) or four (other cultivars) parts. Slices were placed in white dishes labeled with a random number. Evaluations were conducted in individual booths and samples were presented in a random order. Water was provided for panelists to cleanse their palates in between tasting of samples. Panelists were instructed to open the lid from each tomato sample dish and then proceed with the sensory evaluations of the following characteristics: appearance, tomato smell, hardness, granulosity, sweetness, sourness, umami, saltiness, aftertaste, overall taste, and tomato like. Appearance is judged totally from external attributes such as color intensity, color uniformity, mechanical damage, and small cracks. The sensory panel evaluated the intensity of various attributes on a 5-point scale, where −2 represented "very weak intensity," 0 represented "fair intensity," and 2 represented "very strong intensity."

2.5. Analysis of organoleptic compositions

To evaluate sugar, organic acid, and amino acid contents, 0.2 g of the lyophilized sample dissolved in distilled water was maintained in a hot water bath at 80 °C for 10 min, and then centrifuged at 10,000 × g for 15 min. The extraction was repeated three times. Three extractions were pooled with 50 ml distilled water and then filtered through a 0.45-μm filter. The filtrate was subjected to high-performance liquid chromatography (HPLC). HPLC separation and quantification of sugar, organic acid, and amino acid contents were carried out according to Zushi and Matsuzoe (2006b). The extraction and measurement of Na^+ concentration of cell sap was carried out according to Zushi et al. (2009).

2.6. Statistical analysis

All statistical analyses were carried out using JMP version 7.0 (SAS Institute, NC, USA). Mean comparison and interaction between treatments and cultivars were calculated using a two-way analysis of variance (ANOVA) test at a significance level (P) of <0.05. Principal component analysis (PCA) was carried out to describe patterns of variation in sets of sensory data.

2.7. Correlation network analysis

To elucidate the interactions between the different sensory attributes and organoleptic compositions of salt-stressed and control fruit cultivars, their relationships were visualized using correlation networks analysis. Correlation between all trait (sensory attributes and organoleptic compositions) pairs was tested using mean values.

Networks are defined as set of nodes (N, sensory attributes and organoleptic compositions) and links (l) among them. Two nodes are considered to be connected by a link if the correlation (positive or negative) between traits is significant at $P < 0.05$. Networks were constructed using the Pajek version 1.24 software (http://vlado.fmf.uni-lj.si/pub/networks/pajek/). To measure the topological properties of the network, we calculated the average degree, average clustering coefficient, average shortest path, and betweenness centrality (BC) of each network. Degree (K) of a node is defined as the number of links of such a node. The average degree $\langle K \rangle$ is defined as $\langle K \rangle = 2l/N$. The clustering coefficient was used to evaluate the extent of interconnections among neighbors (Watts and Strogatz, 1998). The average clustering coefficient $\langle C \rangle$ is obtained by averaging all nodes of a network. The average path length (L) indicates the average number of nodes that separates each node from the other (Rodriguez-Caso et al., 2005). BC of a node is the fraction of those directed shortest paths between all pairs of vertices that pass through the node (Freeman, 1977). BC values are used to identify some important nodes in the network. We calculated $\langle C \rangle$, L, and BC using the Network Workbench (NWB) Tool Version 1.0.0 (http://nwb.slis.indiana.edu./).

3. Results

3.1. Sensory evaluation

For each cultivar and NaCl treatment, the data obtained from ANOVA of all sensory attributes measured in the two experimental years is reported in Table 1. Significant differences among cultivars were found for 10 traits at $P < 0.001$, except for tomato smell, and between NaCl treatment significant differences were found for 9 traits at $P < 0.001$, except for tomato smell and granulosity. Interaction between cultivars and treatments were found for the following 4 traits: appearance, tomato smell, hardness, and sweetness.

The increase ratio of salt-stressed to control fruit (the average of salt-stressed fruit/the average of control fruit) of all cultivars is shown in Fig. 1. In the 2006 experiment, the increase ratio for all sensory attributes of cherry-type cultivar MC was significantly low compared to that of other cultivars. Similarly, in the 2007 experiment, the increase ratio for all sensory attributes of cherry-type cultivar CH was significantly low compared to that of other cultivars. A significantly high increase ratio was observed for sweetness of cultivars M8 and KB.

In PCA, Fig. 2A shows the loadings of the used factors and relationship among the sensory attributes associated with the first two principal axes. The loading that positively correlated with principal component 1 (61.4%) included overall taste, tomato like, aftertaste, umami, and sweetness, and that with principal component 2 (18.4%) were hardness and granulosity (Fig. 2A). In all cultivars, the score of PCA moved to different points between control and salt-stressed fruits, but the movement depended on cultivars. The movement of PCA scores in cherry-type cultivars, such as MC and CH, was small compared to that in other cultivars (Fig. 2B).

Table 1
Results of two-way analysis of variance (ANOVA) of cultivar (C), salt treatments (T), and their interactions (C × T) data from the 2006 and 2007 experiments.

Traits	Cultivar (C)	Treatment (T)	C × T
Appearance	**	**	**
Tomato smell	**	ns	**
Hardness	**	**	**
Granulosity	**	ns	ns
Sweetness	**	**	ns
Sourness	ns	**	**
Umami	**	**	ns
Saltiness	**	**	ns
After taste	**	**	ns
Overall taste	**	**	ns
Tomato like	**	**	ns
Glucose	**	**	ns
Fructose	**	**	ns
Citric acid	**	**	ns
Malic acid	**	ns	ns
Aspartic acid	**	**	**
Glutamic acid	**	**	ns
Asparagine	**	**	**
Serine	**	**	**
Glutamine	**	**	**
Glycine	**	**	**
Threonine	**	**	**
Arginine	**	**	**
Alanine	**	ns	ns
γ-Aminobutyric acid	**	**	**
Na	**	**	**

ns: not significant.
** Significant at $P < 0.01$.

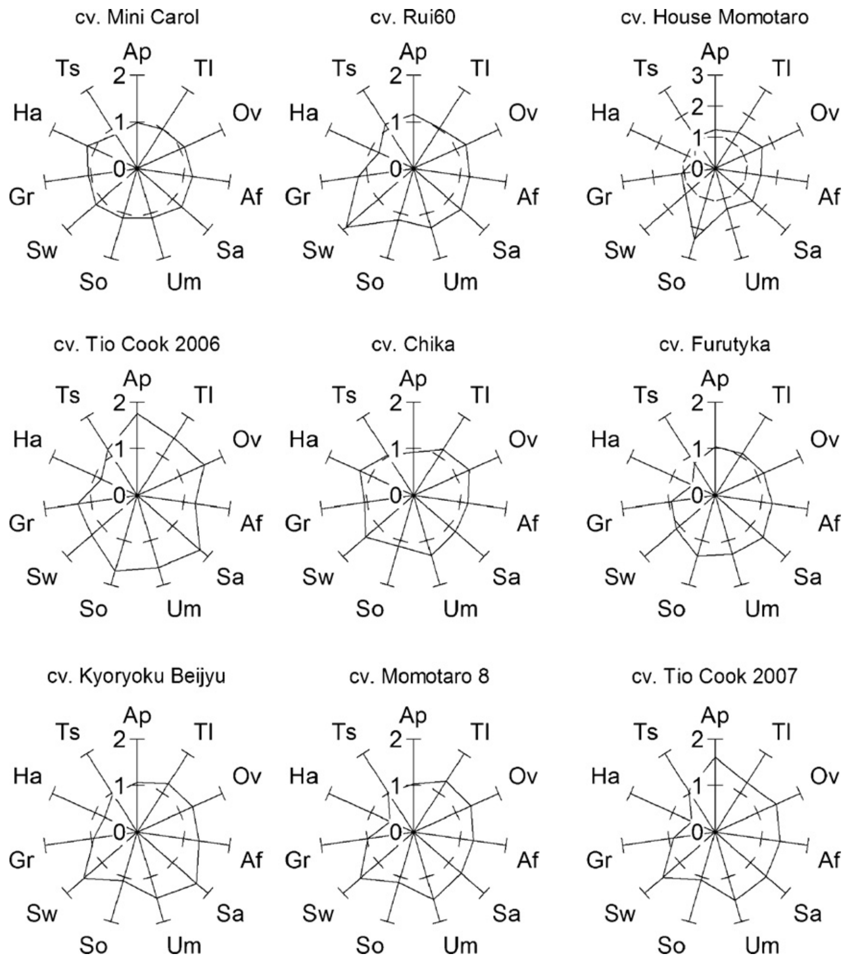

Fig. 1. Effect of salt stress on sensory attributes of fruits belonging to different tomato cultivars from the 2006 and 2007 experiments. Values represent the increase ratio of salt stress to control (mean of salt-stressed fruit/mean of control fruit) ($n = 14$). Abbreviations of sensory attributes in figure represent the following: Ap, appearance; Tl, tomato like; Ov, overall taste; Af, after taste; Sa, saltiness; Um, umami; So, sourness; Sw, sweetness; Gr, granulosity; Ha, hardness; Ts, tomato smell.

3.2. Organoleptic compositions

In both experimental years, the sugar content was significantly different among cultivars, and the effect of salt stress on sugar content was also detected in both experimental years (Table 1). Cherry-type cultivars had high glucose and fructose contents compared to normal-fruited or cooking-type varieties (Figs. 3 and 4). In addition, glucose and fructose contents were higher in salt-stressed fruits than in control fruits of all cultivars in both experimental years (Figs. 3 and 4).

Both citric acid and malic acid contents showed significant differences among cultivars and treatments (Table 1). In addition, citric acid showed the association between salt stress treatment and cultivars; citric acid content of almost all cultivars was higher in salt-stressed fruit than in control fruit in both experimental years (Figs. 3 and 4). However, this was not true in case of malic acid content in both experimental years.

In both experimental years, the effect of salt stress was significant on amino acid content among varieties (Table 1). In case of cherry-type cultivars (CH and MC), a significantly higher content of γ-aminobutylic acid was found in the salt-stressed fruit than in the control fruit, but no significant differences were observed for other amino acids (Figs. 3 and 4). In case of normal-fruited and cooking-type cultivars, such as HM and TC, in 2006 experiment and KB, M8, and TC in the 2007 experiment, almost all amino acids, especially glutamic acid, were significantly higher in salt-stressed fruits than in control fruits (Figs. 3 and 4).

With respect to sodium ion content, significant differences among varieties and the effect of salt stress were found in both experimental years ($P < 0.01$; Table 1). Sodium ion content was higher in salt-stressed fruits than in control fruits of all cultivars (Figs. 3 and 4).

3.3. Correlation network analysis

Correlation matrices in both experimental years were revealed by the calculation of pairwise correlation coefficients at $P < 0.05$ (data not shown). On the basis of these matrices, a correlation network was constructed where each trait is represented as a node possibly connected to any other node at $P < 0.05$. Of the 253 possible pairs, the network consisted of 19 nodes and 38 links in control fruits (Table 2, Fig. 5) and 19 nodes and 50 links in salt-stressed fruits (Table 2, Fig. 6).

Among the topological properties of each network, $\langle K \rangle$ and $\langle C \rangle$ of the salt-stressed fruit network were higher than those of the control fruit network (Table 2). In contrast, L of the salt-stressed fruit network was slightly lower than that of the control fruit network (Table 2). The highest BC value was for glucose in case of the control fruit network, while it was for serine in case of the salt-stressed fruit network (Figs. 5 and 6).

In each connection of the networks, the difference between control and salt-stressed fruit networks was clearly observed. For example, glucose and fructose in the control fruit network were highly interconnected between sensory attributes (e.g., tomato like

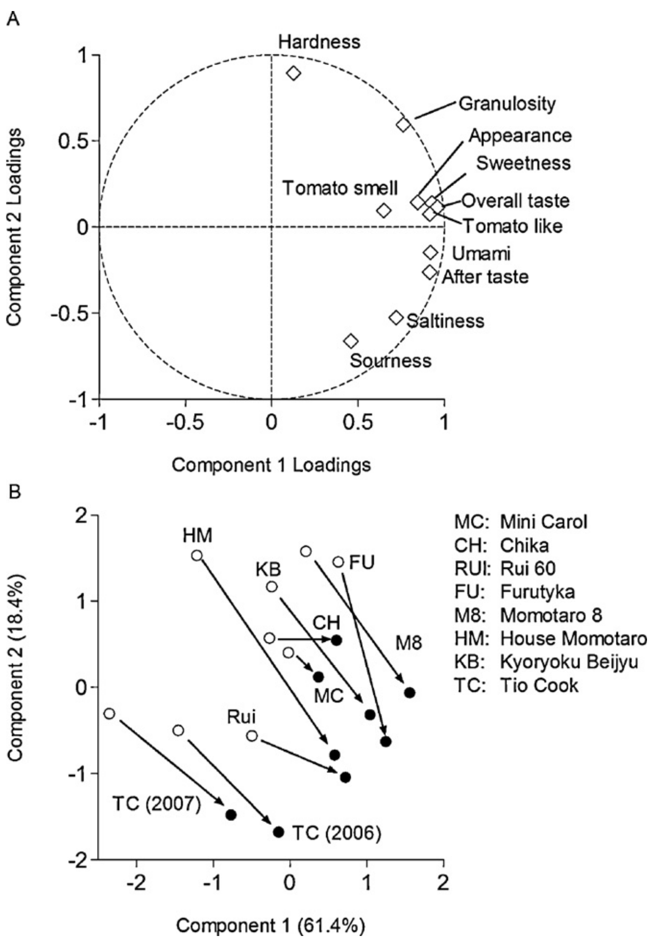

Fig. 2. Principal component analysis (PCA) of sensory evaluation of tomato fruit grown under salt stress and control conditions in the pool of 2006 and 2007experiments. (A) PCA loading plot. (B) PCA scores plot. Circles in B represent the score of PCA of salt-stressed (closed circles) and control (open circles) fruits. Arrows indicate the changes from the control fruit to salt-stressed fruit.

and overall taste) and amino acids (e.g., glutamic acid), and had the highest BC, but other compositions showed fewer links outside the other group and had lower BC than sugars (Fig. 5). In contrast, sugars in the salt-stressed fruit network showed fewer links outside the other group (Fig. 6). Consequently, BC of glucose and fructose was lower in the salt-stressed fruit network than in the control fruit network. In addition, the interconnection of amino acids was much stronger in the salt-stressed fruit network than in the control fruit network. Furthermore, in the salt-stressed network, malic acid, threonine, and serine were negatively linked to several sensory attributes.

To further evaluate this data, we examined the correlation network within the increase ratio by salt stress; the network consisted

Table 2
Topological properties of networks of control fruit, salt-stressed fruit, and the increase ratio of salt stress.

	Control	NaCl stress	Increase ratio
N	19	19	21
l	38	50	78
$\langle K \rangle$	4	5.3	7.4
$\langle C \rangle$	0.50	0.60	0.64
L	2.4	2.2	2.1

N, total number of nodes; l, total number of links; $\langle K \rangle$, average degree; $\langle C \rangle$, average clustering coefficient; L, average path length.

of 21 nodes and 78 links (Table 2, Fig. 7). In the increase ratio network, the topological properties, such as N, L, $\langle K \rangle$, and $\langle C \rangle$, were higher than those in both control and salt-stressed fruit networks (Table 2). In each connection of the increase ratio network, interconnection and centrality were different between the control and salt-stressed fruit networks (Fig. 7). For example, amino acids displayed high interconnectivity. In addition, glucose and fructose were highly connected with sensory attributes and amino acids. Umami also showed high interconnection between citric acid and amino acids such as glycine, serine, and glutamic acid. Furthermore, BC of glucose and fructose was lower in the increase ratio network than in the control fruit network (Figs. 5 and 7).

4. Discussion

Sensory properties are very important for the assessment of vegetable quality by consumers and for their purchase behavior. Tomato fruits grown under increased EC and nutrient solution with NaCl show increased sweetness, acidity, umami, and overall preference (Sato et al., 2006), but this increase is dependent on cultivars (Auerswald et al., 1999). Similarly, in our study, although all sensory attributes except for hardness were higher in salt-stressed fruit than in control fruit, the increase ratios depended on cultivars (Fig. 1). For example, in cherry-type cultivars, the increase ratio of sensory attributes by salt stress was small compared to normal-fruited or cooking-type cultivars (Fig. 1). Furthermore, changes in PCA score by salt stress were greater in normal-fruited and cooking-type cultivars than in cherry-type cultivars (Fig. 2). These results suggest that the effect of salt stress on sensory attributes of cherry-type cultivars was small compared to that on normal-fruited or cooking-type cultivars.

In addition, several studies have reported that salt stress enhanced the contents of organoleptic composition including sugars (Balibrea et al., 2003; Balibrea et al., 1996), organic acids (Mitchell et al., 1991), and amino acids (Franco et al., 1999; Zushi and Matsuzoe, 2006a). In our study as well as previous reports, organoleptic compositions were higher in the salt-stressed fruit than in the control fruit (Figs. 3 and 4). However, despite many studies showing the increase in organoleptic quality by salt stress, it is still unclear whether and how the changes in organoleptic compositions are related to sensory attributes, and how these relationships are affected by cultivar differences.

The graphic representation of network analysis has revealed various types of interactions among several traits in biological and social sciences. In our study, we explored how sensory attributes and organoleptic compositions are interconnected through the structure in control and salt-stressed fruit networks and their importance in shaping the network. In network analysis, the most elementary characteristic of a node is its degree, which indicates the number of links the node has to other nodes (Steuer and López, 2008). $\langle C \rangle$ characterizes the overall tendency of nodes to form clusters in the networks. L represents the static diameter of the network (Steuer and López, 2008). In our study, $\langle K \rangle$ and $\langle C \rangle$ of the salt-stressed fruit network were higher than those of the control fruit network (Table 2). In contrast, L of the salt-stressed fruit network was smaller than that of the control fruit network (Table 2). These results indicate that the correlation network of sensory attributes and organoleptic compositions among different cultivar varies among control and salt-stressed fruits, and the network of salt-stressed fruit is rather compact compared to that of the control fruit.

Several studies have reported that $\langle K \rangle$ and $\langle C \rangle$ are different among several networks, such as gene, metabolic, protein interaction, food web, and neural networks (Newman, 2003). Furthermore, in prokaryotic metabolic networks, the environmental factor (the

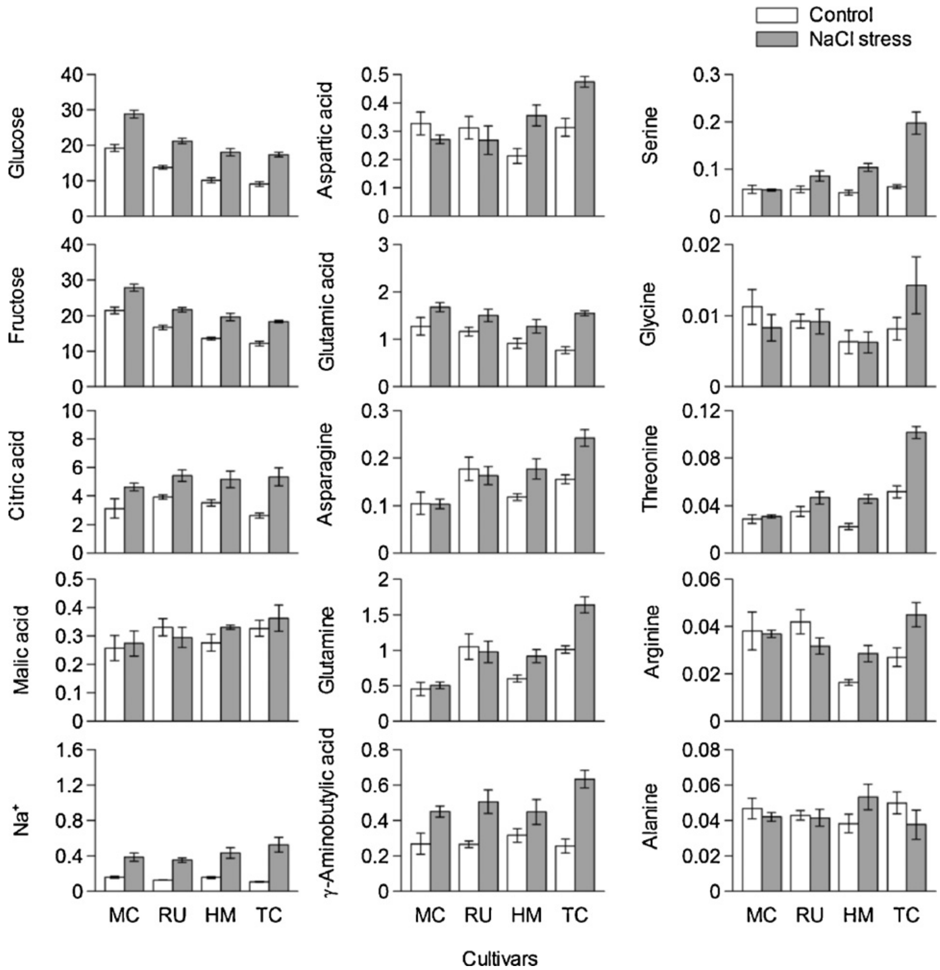

Fig. 3. Effect of salt stress on sugar (glucose, fructose; $mg\,g^{-1}$ fresh weight), organic acid (citric acid, malic acid; $mg\,g^{-1}$ fresh weight), sodium ion (Na^+; $mg\,ml^{-1}$ cell sap), and amino acid (aspartic acid, glutamic acid, asparagine, glutamine, γ-aminobutylic acid, serine, glycine, threonine, arginine, alanine; $mg\,g^{-1}$ fresh weight) contents in tomato fruit in the 2006 experiment. Bars represent the average ± standard error ($n = 8$) in control fruit (white bar) and salt-stressed fruit (gray bar).

effect of temperature) plays an important role in the design principles such as ⟨C⟩ of metabolic networks (Takemoto et al., 2007). The finding of different topology in the correlation network of control and salt-stressed fruits was somewhat unexpected; however, this finding suggests that environmental factors, such as salt stress, may play a crucial role in the design principles of sensory and organoleptic networks in tomato fruits. This finding is accordance with Takemoto et al. (2007).

In addition, in our study, the different network topology in salt-stressed fruit may have caused the cultivar differences in increase ratios of organoleptic compositions and sensory attributes by salt stress. The increase ratios of sensory attributes and organoleptic compositions by salt stress were different between cultivars (Figs. 1, 3 and 4). Therefore, the many correlations (links) were induced in salt-stressed fruit because their variances among the cultivars were higher in the salt-stressed fruit than that in the control fruit. Consequently, higher ⟨K⟩ and ⟨C⟩ were found in salt-stressed fruit than in control fruit, and salt-stressed fruit exhibited different design principles of sensory and organoleptic networks.

In general, network studies address issues of centrality (which individuals are best connected to others or have most influence) and connectivity (whether and how individuals are connected to one another through the network) (Newman, 2003). In the control fruit network, a few organoleptic compositions interacted with sensory attributes. For example, sugars interacted with sensory attributes such as tomato like, overall taste, and amino acids such as glutamic acid, but other compositions showed fewer links outside the other group and had lower BC than sugars (Fig. 5). Therefore, those contents would have resulted from cultivar differences in the control fruit. However, these results are not in agreement with the previously reported correlations between sweetness and reducing sugars (Malundo et al., 1995; Tandon et al., 2003), and umami and glutamic acid (Fuke and Shimizu, 1993). These disagreements may be due to the differences in the measurement composition; for example, in some studies, organoleptic composition has been measured only for sugars and acid (Fuke and Shimizu, 1993; Malundo et al., 1995; Tandon et al., 2003), whereas in our study many relevant attributes were measured. Thus, we believe that these relevant attributes influence sweetness and umami perceptions, and consequently, our results may not show the interactions between sweetness and sugars, and umami and glutamic acid. Similar to our study, few metabolic traits seem to have a direct influence on important sensory traits in the correlation network analysis of several tomato cultivars (Carli et al., 2009). Based on these data, Carli et al. (2009) suggested that the regulatory factors responsible for balancing several classes of metabolites act on different circuits determining the perception of tomato flavor.

In contrast to the control fruit network, sugars in the salt-stressed fruit network showed fewer links outside the other group and had lower BC (Fig. 6). These results suggest that the key process of correlation among sensory attributes and organoleptic compositions were vastly different under salt stress conditions. In addition,

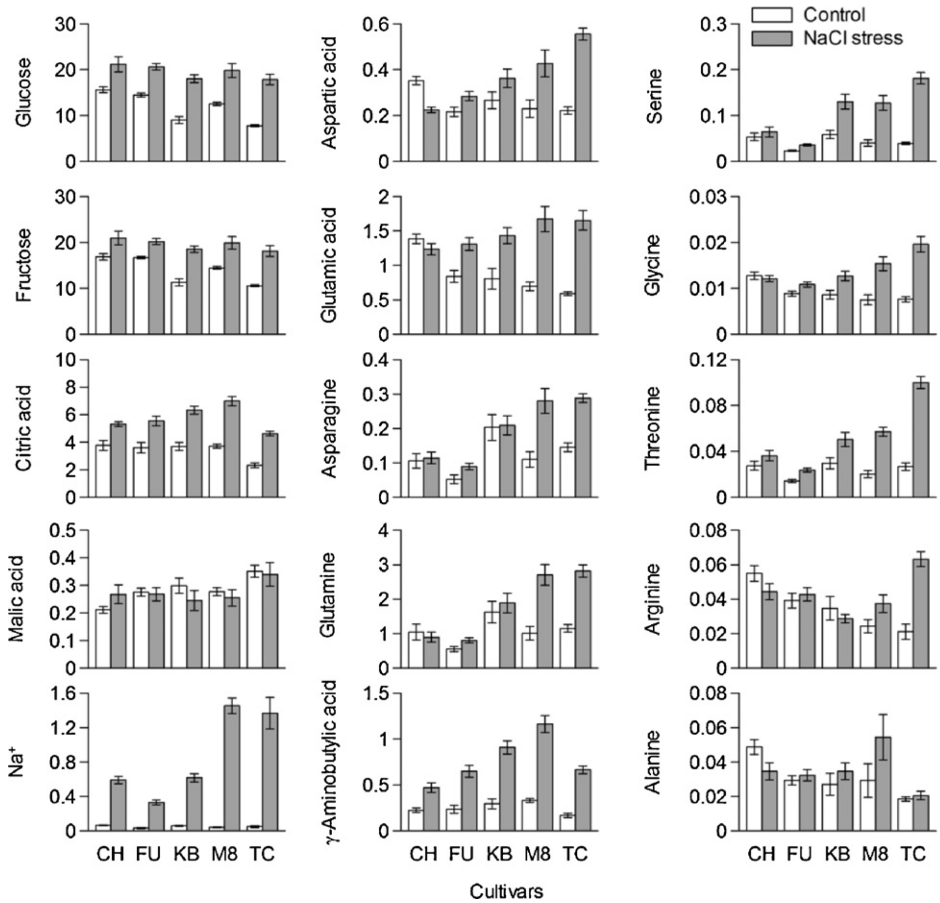

Fig. 4. Effect of salt stress on sugar (glucose, fructose; mg g^{-1} fresh weight), organic acid (citric acid, malic acid; mg g^{-1} fresh weight), sodium ion (Na^+; mg ml^{-1} cell sap), and amino acid (aspartic acid, glutamic acid, asparagine, glutamine, γ-aminobutylic acid, serine, glycine, threonine, arginine, alanine; mg g^{-1} fresh weight) contents in tomato fruit in the 2007 experiment. Bars represent the average ± standard error (n = 8) in control fruit (white bar) and salt-stressed fruit (gray bar).

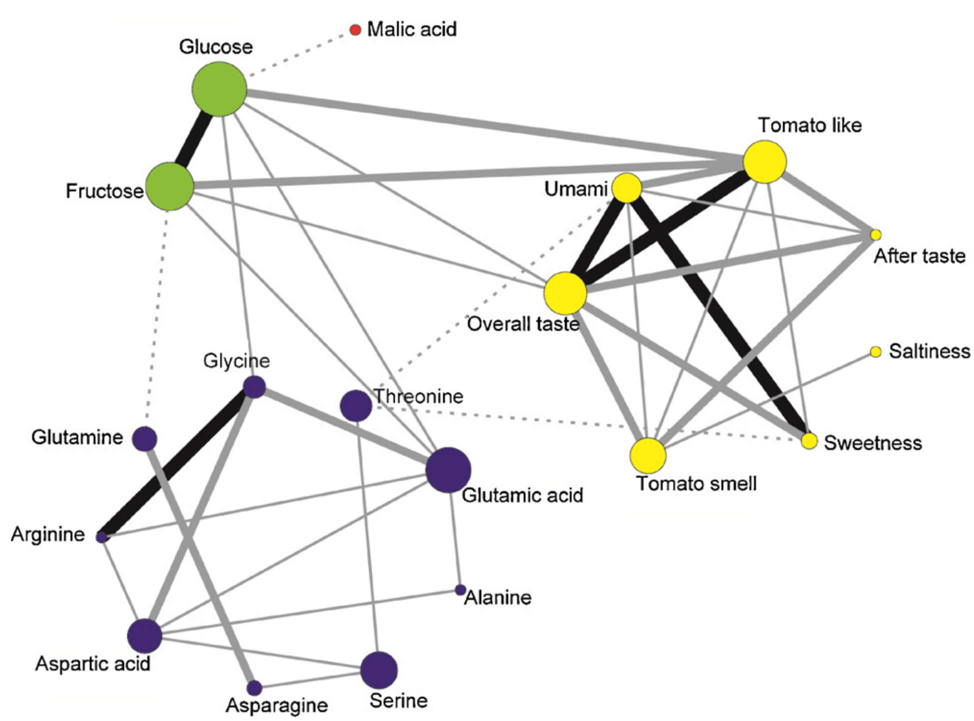

Fig. 5. Correlation network analysis of control fruits visualized using the Pajek software package. Only significant (P < 0.05) correlations are drawn. Correlations are indicated with solid lines (positive correlation) or dotted lines (negative correlation). Line thickness and color indicates correlation strength: thin gray lines represent 0.01 < P ≤ 0.05; heavy gray lines represent 0.001 < P ≤ 0.01; black lines represent P ≤ 0.001. Sizes of vertices indicate the betweenness centrality.

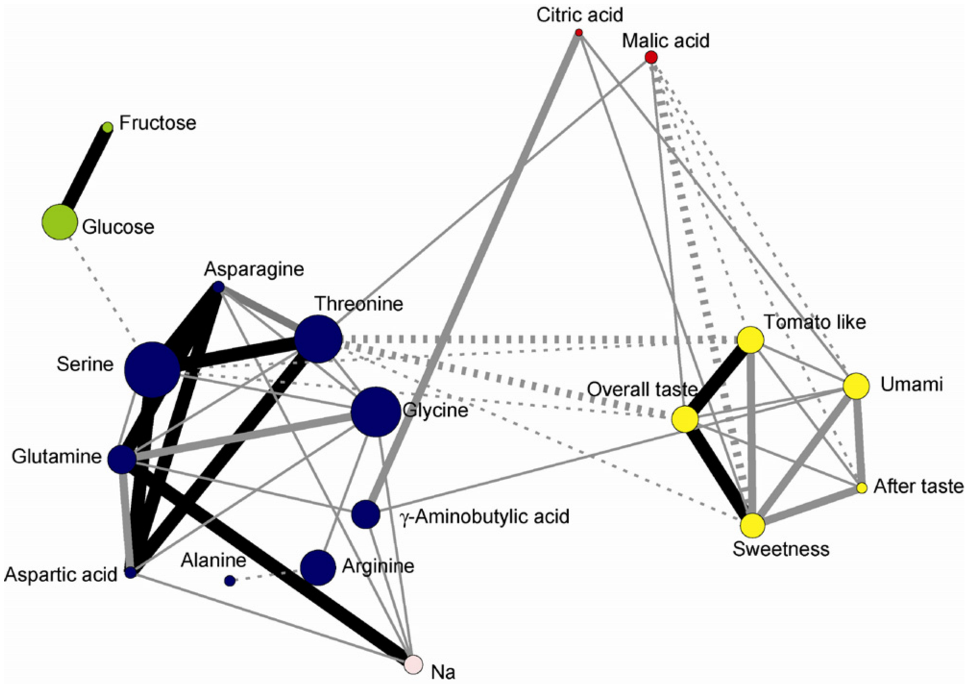

Fig. 6. Correlation network analysis of salt-stressed fruits visualized using the Pajek software package. Other details are as indicated in Fig. 5.

although previous studies showed that both sugar and glutamic acid contents play an important role in tomato fruit taste (Fuke and Shimizu, 1993; Grierson and Kader, 1986), our results indicate that these contents did not influence the cultivar differences of the salt-stressed fruit network. As possible explanations for this phenomenon, we suggest that the absolute concentrations of compounds may be a contributing factor depending on the sensory intensity differences For example, at high sugar and glutamic acid concentrations, such as in salt-stressed fruit, the change in these contents may lead to lower sensory attribute variations among cultivars when compared to those at a lower concentrations, such as in control fruit. Consequently, in the salt-stressed fruit network, sugars showed fewer links outside the other group and had lower BC, and glutamic acid had no links. As another possible explanation is that a correlation can only be found when data are spread, and hence, more elaborate research is needed to clear this possibility.

As the next step, to evaluate how cultivar differences occurred in salt-stressed fruits, we examined the increase ratio network. Our result showed that the increase ratio network had a greater difference compared to both control and salt-stressed fruit networks because of the differences in the topological properties of the network and the connectivity (Fig. 7). Especially, the finding that N, L, $\langle K \rangle$, and $\langle C \rangle$ were high in the increase ratio network compared to both control and salt-stressed fruit networks may provide evi-

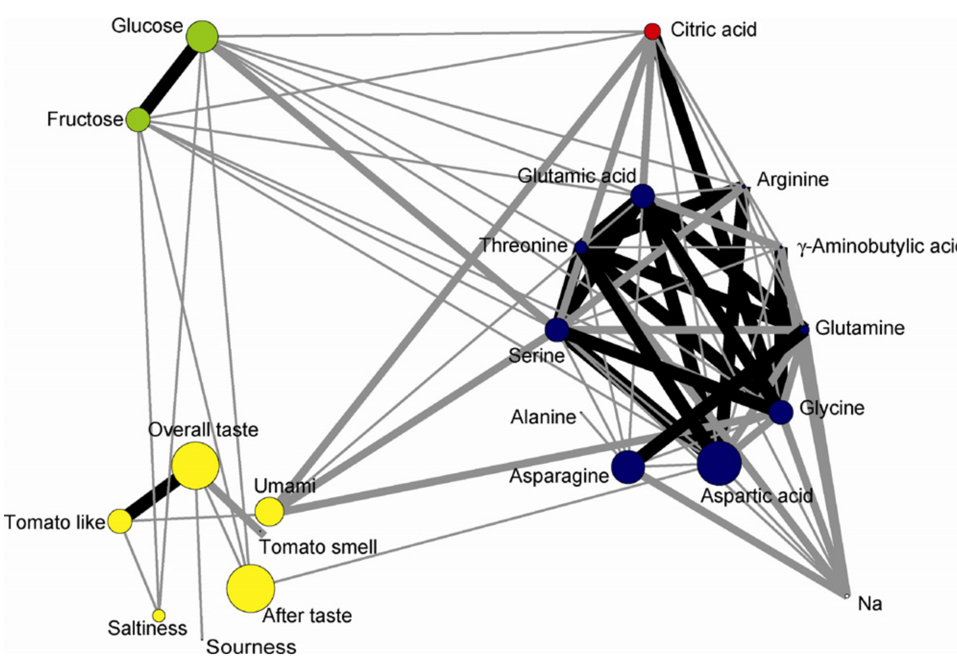

Fig. 7. Correlation network analysis of the increase ratio by salt stress visualized using the Pajek software package. Other details are as indicated in Fig. 5.

dence that cultivar differences of sensory attributes in salt-stressed fruit result from interaction between the increase ratio of sensory attributes and organoleptic compositions. In addition, the increase of glucose and fructose by salt stress may be a key factor in cultivar differences because of their high connectivity among other traits (Fig. 7). However, in the increase ratio network, glucose and fructose were highly connected with sensory attributes but not with sweet or sour. These results may have resulted from the interference of other attributes or organoleptic compositions such as amino acids. Furthermore, umami in the increase ratio network showed higher interconnection among citric acid and amino acids such as glycine, serine, and glutamic acid (Fig. 7). These results visually represent for the first time that, in addition to the increase in sugar content, the increase in citric acid and amino acid contents by salt stress may have contributed to the salt-stress-induced enhancement of sensory attributes.

5. Conclusion

In conclusion, our results show that the salt stress-induced changes in sensory attributes and organoleptic compositions of tomato fruit were different between cultivars. For example, in cherry-type cultivars, the increase ratio by salt stress was smaller compared to that in normal-fruited or cooking-type cultivars. Furthermore, the correlation network analysis showed a significant difference between the topological properties of the control and the salt-stressed fruits. These results show for the first time that the correlation network between sensory attributes and organoleptic compositions is different in growing conditions such as salt stress, and salt stress plays an important role in the topology of the correlation network in tomato fruit. Therefore, the correlation network is very useful to study changes in sensory attributes and organoleptic compositions among the cultivars grown under the salt stress conditions.

Furthermore, the correlation network analysis is valuable for identifying key factors in cultivar differences of sensory attributes and organoleptic compositions of tomato fruit grown under salt stress. Sensory evaluation is time consuming and expensive (Causse et al., 2007). Thus, the key factors in cultivar differences need to be clearly identified using instrumental measures, which could replace the sensory evaluation. In our study, a number of interesting links between glucose, fructose, citric acid, and amino acids and several sensory attributes could be identified in the increase ratio network using correlation network analysis. Thus, these links can provide direct benefits of key factors in the improvement of tomato quality by salt stress. Further research is needed to clarify the effect of other environmental factors, such as light and temperature, on the network structure of sensory and organoleptic interactions in tomato fruit.

Acknowledgment

This study was supported by a Grant-in-Aid for Young Scientists (B) (No. 21780234) from the Japan Society for the Promotion of Science.

References

Alarcón, J., Bolarín, M., Sánchez-Blanco, M.J., Torrecillas, A., 1994. Growth, yield and water relations of normal fruited and cherry tomato cultivars irrigated with saline water. J. Hort. Sci. 69, 283–288.

Alian, A., Altman, A., Heuer, B., 2000. Genotypic difference in salinity and water stress tolerance of fresh market tomato cultivars. Plant Sci. 152, 59–65.

Auerswald, H., Schwarz, D., Kornelson, C., Krumbein, A., Bruckner, B., 1999. Sensory analysis, sugar and acid content of tomato at different EC values of the nutrient solution. Sci. Hort. 82, 227–242.

Balibrea, M.E., Cuartero, J., Bolarin, M.C., Perez-Alfocea, F., 2003. Sucrolytic activities during fruit development of Lycopersicon genotypes differing in tolerance to salinity. Physiol. Plant. 118, 38–46.

Balibrea, M.E., Santa Cruz, A.M., Bolarin, M.C., Perez-Alfocea, F., 1996. Sucrolytic activities in relation to sink strength and carbohydrate composition in tomato fruit growing under salinity. Plant Sci. 118, 47–55.

Barabasi, A.L., Oltvai, Z.N., 2004. Network biology: understanding the cell's functional organization. Nat. Rev. Genet. 5, 101–113.

Carli, P., Arima, S., Fogliano, V., Tardella, L., Frusciante, L., Ercolano, M.R., 2009. Use of network analysis to capture key traits affecting tomato organoleptic quality. J. Exp. Bot. 60, 3379–3386.

Causse, M., Damidaux, R., Rousselle, P., 2007. Traditional and enhanced breeding for quality traits in tomato. In: Razden, M.K., Matto, A.K. (Eds.), Genetic Improvement of Solanaceous Crops. Vol.2: Tomato. Science Publisher, New Hampshire, pp. 153–192.

Causse, M., Saliba-Colombani, V., Lecomte, L., Duffe, P., Rousselle, P., Buret, M., 2002. QTL analysis of fruit quality in fresh market tomato: a few chromosome regions control the variation of sensory and instrumental traits. J. Exp. Bot. 53, 2089–2098.

Chaïb, J., Devaux, M.-F., Grotte, M.-G., Robini, K., Causse, M., Lahaye, M., Marty, I., 2007. Physiological relationships among physical, sensory, and morphological attributes of texture in tomato fruits. J. Exp. Bot. 58, 1915–1925.

Cuartero, J., Fernandez-Munoz, R., 1998. Tomato and salinity. Sci. Hort. 78, 83–125.

Franco, J.A., Pérez-Saura, P.J., Fernández, J.A., Parra, M., García, A.L., 1999. Effect of two irrigation rates on yield, incidence of blossom-end rot, mineral content and free amino acid levels in tomato cultivated under drip irrigation using saline water. J. Hort. Sci. Biotechnol. 74, 430–435.

Freeman, L.C., 1977. A set of measuring centrality based on betweenness. Sociometry 40, 35–41.

Fuke, S., Shimizu, T., 1993. Sensory and preference aspects of umami. Trends Food Sci. Technol. 4, 246–251.

Gao, Z., Sagi, M., Lips, S.H., 1998. Carbohydrate metabolism in leaves and assimilate partitioning in fruits of tomato (*Lycopersicon esculentum* L.) as. Plant Sci. 135, 149–159.

Grierson, D., Kader, A.A., 1986. Fruit ripening and quality. In: Atherton, J.G., Rudich, J. (Eds.), Tomato Crop. A Scientific Basis for Improvement. Chapman & Hall, London, pp. 241–280.

Krumbein, A., Auerswald, H., 1998. Characterization of aroma volatiles in tomatoes by sensory analyses. Nahrung 42, 395–399.

Malundo, T.M.M., Shewfelt, R.L., Scott, J.W., 1995. Flavor quality of fresh tomato (*Lycopersicon esculentum* Mill.) as affected by sugar and acid levels. Postharvest Biol. Technol. 6, 103–110.

Mitchell, J.P., Shennan, C., Grattan, S.R., May, D.M., 1991. Tomato fruit yields and quality under water deficit and salinity. J. Am. Soc. Hort. Sci. 116, 215–221.

Mounet, F., Moing, A., Garcia, V., Petit, J., Maucourt, M., Deborde, C., Bernillon, S., Le Gall, G., Colquhoun, I., Defernez, M., Giraudel, J.-L., Rolin, D., Rothan, C., Lemaire-Chamley, M., 2009. Gene and metabolite regulatory network analysis of early developing fruit tissues highlights new candidate genes for the control of tomato fruit composition and development. Plant Physiol. 149, 1505–1528.

Newman, M.E.J., 2003. The structure and function of complex networks. SIAM Rev. 45, 167–256.

Petersen, K.K., Willumsen, J., Kaack, K., 1998. Composition and taste of tomatoes as affected by increased salinity and different salinity sources. J. Hort. Sci. Biotechnol. 73, 205–215.

Petro-Turza, M., 1986. Flavor of tomato and tomato products. Food Rev. Int. 2, 309–351.

Rodriguez-Caso, C., Medina, M., Sole, R., 2005. Topology, tinkering and evolution of the human transcription factor network. FEBS J. 272, 6423–6434.

Sato, S., Sakaguchi, S., Furukawa, H., Ikeda, H., 2006. Effects of NaCl application to hydroponic nutrient solution on fruit characteristics of tomato (*Lycopersicon esculentum*). Sci. Hort. 109, 248–253.

Schauer, N., Semel, Y., Roessner, U., Gur, A., Balbo, I., Carrari, F., Pleban, T., Perez-Melis, A., Bruedigam, C., Kopka, J., Willmitzer, L., Zamir, D., Fernie, A.R., 2006. Comprehensive metabolic profiling and phenotyping of interspecific introgression lines for tomato improvement. Nat. Biotechnol. 24, 447–454.

Steuer, R., López, G.Z., 2008. Global network properties. In: Junker, B.H., Schreiber, F. (Eds.), Analysis of Biological Network. John Wiley & Sons, Inc, Hoboken, New Jersey, pp. 31–63.

Takemoto, K., Nacher, J., Akutsu, T., 2007. Correlation between structure and temperature in prokaryotic metabolic networks. BMC Bioinformatics 8, 303.

Tandon, K.S., Baldwin, E.A., Scott, J.W., Shewfelt, R.L., 2003. Linking sensory descriptors to volatile and nonvolatile components of fresh tomato flavor. J. Food Sci. 68, 2366–2371.

Ursem, R., Tikunov, Y., Bovy, A., van Berloo, R., van Eeuwijk, F., 2008. A correlation network approach to metabolic data analysis for tomato fruits. Euphytica 161, 181–193.

Watts, D., Strogatz, S., 1998. Collective dynamics of small-world networks. Nature 393, 440–442.

Zushi, K., Matsuzoe, N., 2006a. Free amino acid contents of tomato fruit grown under water and salinity stresses. Acta Hort. 724, 91–96.

Zushi, K., Matsuzoe, N., 2006b. Postharvest changes in sugar, organic acid, glutamic acid and antioxidant contents in tomato fruit grown under salinity stress. Environ. Cont. Biol. 44, 111–117.

Zushi, K., Matsuzoe, N., 2007. Salt stress-enhanced γ-aminobutyric acid (GABA) in tomato fruit. Acta Hort. 761, 431–435.

Zushi, K., Matsuzoe, N., Kitano, M., 2009. Developmental and tissue-specific changes in oxidative parameters and antioxidant systems in tomato fruits grown under salt stress. Sci. Hort. 122, 362–368.