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Metabolic profile of organoleptic and health-promoting qualities in two tomato cultivars subjected to salt stress and their interactions using correlation network analysis

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ABSTRACT

For tomato production, salt stress is applied to improve the organoleptic and health-promoting qualities of tomato fruits; however, both the qualities and its association between them remain unclear. Here, we investigated the metabolic profile of tomato fruits grown under salt stress, and their association with organoleptic and health-promoting qualities by multivariate analysis and correlation network analysis. Cherry tomato cultivar Mini Carol (MC) and normal-fruited tomato cultivar House Momotaro (HM) that have different responses to salt stress were grown under different salt intensities (25, 50, 75, and 100 mM) in a closed irrigation system. For both cultivars, although fruit weight decreased with an increase in salt intensity, several organoleptic and health-promoting qualities on a fresh weight (FW) basis increased with salt stress. When calculated on a dry weight basis, we suggest that the higher level of sugars and organic acids, except for sucrose on an FW basis, may be a result of the concentration effect, but salt-induced changes in sucrose, amino acid, and health-promoting compounds may lead to net metabolic changes. Furthermore, in the statistical comparison of metabolites, principal component and cluster analyses clearly discriminated between salt-stressed and control fruit, and grouped them into two primary clusters separated by cultivars. Correlation network analysis enabled us to visualize the association with metabolic profiles, such that networks for HM fruits comprised highly connected nodes, such as antioxidant activities, citric acid, γ-aminobutyric acid, and ascorbic acid; however, in MC fruits, most connective nodes were fruit weight, glutamic acid, and glucose. These results suggest that for the two cultivars, different connectivity and key components may lead to different metabolic changes under salt stress and that salt stress modulates the design principles of metabolic networks. Finally, we discussed the possibilities of correlation network analysis for the prediction of change in fruit quality grown under salt stress.

1. Introduction

Based on the consumer and market requirements for tomato quality, there has been an increasing focus on its organoleptic and health-promoting qualities. Organoleptic qualities of tomato fruits mainly depend on the complex mixtures of sugars, organic acids, and amino acids. Several studies have reported that sensory traits of tomato correlate with its organoleptic compositions (Carli et al., 2009, 2011; Causse et al., 2003; Sinesio et al., 2010; Zushi and Matsuzoe, 2011). In addition, a health-promoting quality of tomato fruits is that it is an important source of lycopene, which is a red-colored carotenoid associated with several health benefits with implications for inflammation, cardiovascular diseases, and cancer (Ellinger, 2010; Zhang et al., 2008). Further, in addition to several vitamins, such as ascorbic acid and β-carotene, tomato contains a number of phenolic acids that can contribute to a healthy diet (Slimestad and Verheul, 2009). Thus, improvements in both the organoleptic and health-promoting qualities are an important target for tomato growers and breeders.

In tomato fruit, the organoleptic and health-promoting qualities vary due to genetics, environmental factors, and cultural practices (Dorais et al., 2008). For example, in cultural practice, salt stress has been applied to improve the organoleptic qualities. Salt stress is known to increase the concentration of sugars, organic acids,

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and amino acids, although it reduces the size and yield of the fruit (Balibrea et al., 1996, 2003; Franco et al., 1999; Gao et al., 1998; Petersen et al., 1998; Sato et al., 2006; Zushi and Matsuzoe, 2011). Moreover, regarding the health-promoting qualities, rising salinity levels in a nutrient solution significantly increased ascorbic acid, lycopene, and β-carotene, and the phenol concentration was tendentiously enhanced; the antioxidant capacity of phenol and carotenoid increased on a fresh weight (FW) basis (Krauss et al., 2006). Although the effects of salt stress on the organoleptic and health-promoting qualities have been investigated, these studies were conducted on an individual quality, such as each organoleptic or health-promoting quality. In a comprehensive study of biochemicals in salt-stressed tomato fruit, Johnson et al. (2003) have reported differences between the control and salt-treated tomato fruits with respect to the metabolic fingerprints using chemometric techniques. However, salt-induced changes in the content of each organoleptic or health-promoting quality were not determined. Furthermore, associations between the organoleptic and health-promoting qualities are important for evaluating fruit quality because this investigation can help in interpreting complex fruit qualities by identifying key quality components in salt-stressed fruits. Therefore, a comprehensive study of both the organoleptic and health-promoting qualities under salt stress condition is necessary.

For a comprehensive analysis of tomato fruit metabolites, selecting an optimal statistical method is important. In general, well-known statistical methods [e.g., analysis of variance (ANOVA)] that investigate small data sets are not effective at displaying the effects and associations within a large data set, such as fruit metabolites. Therefore, multivariate statistical analyses, principal component analysis (PCA), and cluster analysis are used to determine the differences in biological components that show different behaviors between conditions (Toubiana et al., 2013). In addition, a network analysis can help to interpret complex data sets through the identification of key components (Toubiana et al., 2013). Correlation network analysis has been previously used to explore how sensory attributes and organoleptic compounds are interconnected in different tomato cultivars (Zushi and Matsuzoe, 2011). In other studies, network analysis of metabolic data has provided useful information about tomato fruits (Aurand et al., 2012; Carli et al., 2009; DiLeo et al., 2011; Mounet et al., 2009; Schauer et al., 2006; Ursem et al., 2008). To investigate the interaction between fruit qualities, we hypothesized that a correlation network analysis could identify the association between the organoleptic and health-promoting qualities of tomato fruits grown under salt stress.

The aim of this study was to determine the metabolic profile of the organoleptic and health-promoting qualities and the underlying possible mechanism associated with salt-stressed tomato fruit and to elucidate their association using multivariate analysis and network analysis. We analyzed the salt-induced changes in tomato fruit grown under different salt intensities, including organoleptic compounds (sugar, organic acid, and amino acid) and health-promoting compounds (lycopene, β-carotene, ascorbic acid, polyphenol, and several antioxidant capacities). Furthermore, we compared the cultivar differences using large-fruited and cherry cultivars because our previous reports showed that several responses to salt stress differed between these two cultivars (Zushi and Matsuzoe, 2009, 2011). For example, the effect of salt stress on sensory evaluations and organoleptic components was greater in the normal-fruited cultivar than in the cherry cultivar (Zushi and Matsuzoe, 2011), and the effect of salt stress on antioxidant systems differed between these two cultivars (Zushi and Matsuzoe, 2009). We used multivariate analysis and correlation network analysis to visualize and interpret the results and we discussed the possibilities of correlation network analysis for the prediction of changes in fruit quality grown under salt stress.

2. Materials and methods

2.1. Plant materials and growth conditions

Two tomato cultivars, normal-fruited cultivar House Momotaro (HM) and cherry cultivar Mini Carol (MC), were grown from spring to summer under greenhouse conditions at the Prefectural University of Kumamoto. Seeds were sown on March 22, 2011, and seedlings with two true leaves were transplanted into 12-cm diameter pots filled with pumice. At the end of April, when inflorescences appeared, plants were transplanted into a closed irrigation system with two replicates. Each plot comprised 10 plants at a density of 4 plants per meter. The closed irrigation system, nutrient solution, and other growth conditions were described by Zushi and Matsuzoe (2011).

2.2. Salt stress treatments

Two weeks after transplantation into the closed irrigation system, 25, 50, 75, and 100 mM NaCl were added to the nutrient solutions. To avoid osmotic shock to the plants, the 50, 75, and 100 mM NaCl treatments were applied at 25 mM per day until the target concentrations were reached. Nutrient solutions without NaCl served as the control treatment. The salt treatments were continued until the end of the experiment.

2.3. Fruit harvest

Red ripe fruits were harvested from the end of June to the beginning of July. HM fruits were harvested from the distal second or third fruit on the second truss. The harvested HM fruits were 44–47 days after flowering in all treatments. MC fruits were harvested from the middle of the truss to improve the uniformity of size and growth conditions. The harvested MC fruits were 40 (50, 75, and 100 mM NaCl), 42 (25 mM NaCl), and 48 (control) days after flowering. For each treatment and cultivar, approximately 20 fruits (2 fruits from each of the 10 plants) were harvested and then measured for weight and color. Fruit color was measured using a colorimeter (Minolta CR-300, Minolta Co. Ltd, Japan) in CIELAB units; it was measured in triplicates at the equator of the fruit.

HM fruits were cut into quarter sections and MC fruits were cut into half sections and then stored at −40 °C until analysis. Subsamples were freeze-dried for 72 h using a freeze dryer (FDU-830, EYELA Co. Ltd, Tokyo, Japan) and then ground to a fine powder. Each sample was weighed before and after freeze–drying.

The fruit sap was obtained by hand squeezing the sliced fruits. The following agricultural parameters were determined on all sap samples: total soluble solids (TSS; PAL-1, Atago, Tokyo, Japan), total acidity (TA; fruit tester FT-1+, TGK Co. Ltd, Tokyo, Japan), and Na^+ (compact ion meter C-122, HORIBA Co. Ltd, Tokyo, Japan).

2.4. Measurement of metabolite contents

To evaluate the organoleptic qualities of sugar, organic acid, and amino acid contents, 0.1–0.13 g of each freeze-dried sample was dissolved in 80% ethanol (v/v), maintained in a hot water bath at 80 °C for 10 min, and then centrifuged at 3000 × *g* for 15 min. The extraction was repeated three times, and the pooled extracts were evaporated to dryness at 50 °C and dissolved in 5 ml of distilled water. The extract was filtered through a 0.2-μm filter, and the filtrate was analyzed using high performance liquid chromatography (HPLC). Further, the sugar and organic acid contents were separated and quantified by HPLC according to Zushi and Matsuzoe (2006b). Amino acid contents were measured using the *o*-phthalaldehyde/9-fluorenylmethyl chloroformate pre-column derivatization ultra HPLC system by SIL-30AC autosampler (Shimadzu Co., Kyoto, Japan)