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Vegetative growth, mineral change, and fruit quality of 'Fuji' tree as affected by foliar seawater application

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ABSTRACT

Extensive researches have been conducted over the decades to investigate the effects of seawater on many crop plants either by irrigation or foliar spray in an attempt to enhance the yield and quality. The purpose of this study was to quantitatively determine the effects of different foliar seawater sprays on vegetative growth, fruit quality and yield of eight-year-old 'Fuji'/M.9 apple trees. A field experiment was conducted using two different concentrations of seawater (100 or 50-fold dilution) starting from 130 DAFB (days after full bloom) at 5-day intervals between the treatments. Foliar seawater sprays to 'Fuji' tree led to increased levels of fruit soluble solids along with higher activities of sucrose phosphate synthase (SPS) (EC 2.4.1.14), sucrose synthase (SS) (EC 2.4.1.13) and neutral invertase (EC 3.2.1.26). In addition, a significant increase in anthocyanin concentration was observed, especially when 'Fuji' trees were sprayed three times with 50-fold diluted seawater. Foliar seawater sprays also resulted in increases in Na^+ concentration and K^+/Ca^{2+} ratio in fruit. In contrast to fruit, the levels of N, Na^+, K^+, Mg^{2+} in leaves remained unchanged regardless of different seawater treatments. Moreover, foliar seawater sprays did not affect the vegetative growth since leaf area, leaf fresh weight, shoot elongation, and chlorophyll content did not differ significantly from those of control plants. The results suggest that foliar seawater treatments could be useful for improving fruit quality without affecting the yield.

1. Introduction

Plants are frequently exposed to diverse abiotic stress conditions, such as high and low temperature, water deficit, UV light, oxidative stress, heavy metal toxicity and salinity. These factors greatly influence the growth and development of plants, as well as the crop productivity. In the course of evolution, plants have developed mechanisms to cope with and adapt to various adverse environmental stresses, most often by accumulating specific defensive secondary metabolites through signaling pathways (Ramakrishna and Ravishankar, 2011). So, moderate environmental stress conditions could be beneficial to improve the quality and to control the growth of certain crops. Salinity usually leads to a marked increase in soluble sugars and protein that may act as osmolytes for lowering the osmotic potential (Chen et al., 2009).

Seawater has a salinity of nearly 3.5%, and the major components of the dissolved salts are sodium, magnesium, calcium and potassium; excessive accumulation is harmful to plant by causing osmotic injury and specific ion toxicity (Millero et al., 2008). Exogenous high salt application caused a reduction in pigment concentrations, as well as the uptake of K^+, Ca^{2+}, Mg^{2+}, and N in tissues (Cambrollé et al., 2011; Chakraborty et al., 2012). It was reported that high salinity of seawater reduces the ratio of Mg^{2+}/ Na^+; thus salinity may decrease photosynthesis by reducing concentration of Mg^{2+}, a major component of leaf chlorophyll.

Several lines of evidence have suggested that seawater irrigation affects contents of specific metabolites in higher plants (Ferrante et al., 2011; Long et al., 2009; Ventura et al., 2011; Zhang et al., 2009). Moderate seawater treatment resulted in the marked accumulation of soluble sugar, protein, polyphenol, carotene, fatty acid (Long et al., 2008; Ventura et al., 2011). However, as the salinity was further increased, levels of electrolyte leakage and proline concentration rose, and this was typically accompanied by reduced levels of chlorophyll and carotenoid and more protein degradation (Ferrante et al., 2011; Long et al., 2009; Misra and Saxena, 2009). The high salinity stress resulted in reduced growth rate and biomass, shorter stature, smaller leaves, nutritional deficiency and mineral disorders (Ferrante et al., 2011; Long et al., 2009; Sekmen et al.,

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2012; Ventura et al., 2011; Zhang et al., 2009). The activity of superoxide dismutase was transiently stimulated and then decreased gradually under high salinity stress. Song and Ryou (2008) reported that as seawater spray frequency increased in grapevines, leaf browning and leaf drop occurred along with lower concentrations of soluble solids and anthocyanin in fruits. Thus, use of appropriate levels of salinity is important in agricultural practices, especially for the fruit quality improvement. Unlike grapevines, there was no leaf toxicity or symptoms of chlorosis and necrosis observed when olive trees were irrigated with up to 21.3% diluted seawater for five months (Vigo et al., 2005). The effects of saline water irrigation on growth and fruit quality have been investigated in other crop plants including melon and tomato (Botia et al., 2005; Maggio et al., 2004).

To date a few scientific data about the effect of diluted seawater spray treatments on fruit quality of apple (*Malus domestica* Borkh.) have been reported. There is a lack of information on the proper timing and concentrations of seawater application in apple trees to improve fruit quality. In order to support agricultural extension service to fruit growers for the scientific management in seawater application, we investigated the effects of foliar seawater sprays on growth, fruit quality, and mineral nutrients of 'Fuji'/M.9 apple over two consecutive years.

2. Materials and methods

2.1. Field condition, plant material and seawater dilution management

This study was carried out on eight-year-old 'Fuji'/M.9 cultivar apple trees for two consecutive seasons of 2010 and 2011. All the trees were planted with a distance of 3.8 m between rows and 1.8 m within the rows. Trees of 3 m high and 1.5 m wide were trained as a slender spindle, and those with similar growth vigor were selected as test materials. Soil properties were tested as pH 6.52, 13.2% organic matter, 0.75 g kg^{-1} available phosphorus, 0.46 g kg^{-1} available nitrogen, 0.48 g kg^{-1} available potassium. The trees were placed at yearly average temperature 11.8 °C, sunshine hours 2698.4 h, average humidity 68%, precipitation 651.9 mm. The surface seawater was purified through a simple reverse osmosis system to eliminate dirt and microorganism and to reduce ion content. The desalted seawater (0.35 g kg^{-1} K^+, 0.31 g kg^{-1} Ca^{2+}, 0.94 g kg^{-1} Mg^{2+}, 0.89 g kg^{-1} Na^+) was used for treatment. The experimental design consisted of nine treatments crossing two seawater concentrations. A standard randomized block design with six replications including one tree in replicate of each treatment was used. The trees to be tested were isolated in a distance with at least one untreated guard tree. All the trees were grown under the same environmental conditions with the same doses of irrigation, fertilization and phytosanitary treatments.

All of the foliar seawater applications were performed by a hand sprayer (knapsack) at 20 L/six trees in the morning, starting from 130 DAFB (days after full bloom) at 5-day intervals between the sprays. In 2010, single, double and triple spray treatments with 100-fold diluted seawater were implemented, along with control treatment of water. In 2011, double and triple spray treatments with both 50- and 100-fold diluted seawater were implemented. The apple fruits were harvested at the time of optimum maturity which is at 175 DAFB.

2.2. Sampling and fruit characteristics

Leaf area, fresh/dry weight, shoot elongation, and chlorophyll content were determined at 45 DAFFS (days after the first foliar spray application). Meanwhile fruits were selected based on the medium size, uniform shape and color scales in the morning. To determine physical and chemical characteristics of fruit, each treated fruit sample was divided into two groups; one was used immediately and the other was stored in storage room maintaining 4 °C and 80–85% relative humidity. Coloration was determined using a Color-Reader (KONICA MINOLTA SENSING, INC. Japan). Fruit firmness was measured by compression of individual apple fruits with a fruit texture analyzer (GUSS.ZA/GS-14). Soluble solid concentration and titratable acidity in apple juice were determined by a digital refractometer (PR-101, Cat. No. 3412, ATAGO, Japan) and a digital fruit acidity analyzer (Model: GMK-708, GVK, South Korea), respectively.

Anthocyanin contents were determined as described by Rosso and Mercadante (2007). About 5 g of fresh fruit peel tissues from six randomly selected fruits in each treatment was pulverized and extracted with 1% (v/v) methanol. The homogenate was centrifuged at 19,000 × *g* for 15 min, and diluted with 1% HCl–methanol to a final volume of 500 ml. Absorbance of the diluent was measured at 530 nm.

2.3. Mineral analysis

Leaves and fruits were washed in deionized water and dried at 80 °C for 48 h, ground and stored in an oven at 60 °C until analysis. Samples were then ashed in a muffle furnace at 600 °C overnight, and dissolved in 0.1 N HCl. The N level in leaves was analyzed by Kjeldahl method (Page et al., 1982). The levels of Na^+, Ca^{2+}, Mg^{2+}, and K^+ in fruits and leaves were analyzed by ICP-OES (Optima2000DV, Perkin Elmer Instruments Inc., CT, USA). All analyses were conducted in triplicate.

2.4. Enzyme activity assays

Frozen mesocarp of pulp (0.5 g) was pulverized with pre-cooled mortar and pestle and mixed with 4 ml of extraction buffer containing 50 mM HEPES–NaOH (pH 7.5), 5 mM $MgCl_2$, 1 mM EDTA, 2.5 mM dithiothreitol (DTT), 0.2% of bovine serum albumin (BSA) and 0.05% Triton X-100. The mixture was centrifuged at 4 °C for 10 min, and supernatant was desalted immediately through a Sephadex G-25 column pre-equilibrated with 50 mM Hepes–NaOH (pH 7.5), 5 mM $MgCl_2$, 2.5 mM DTT and 0.2% BSA. All procedures were carried out at 4 °C or lower.

Sucrose phosphate synthase (SPS) activity was assayed in a reaction mixture (0.3 ml) containing 7.5 mM UDP-glucose, 7.5 mM fructose-6-P, 10 mM glucose-6-P, 1.5 mM $MgCl_2$, 100 mM Tris–HCl (pH 7.5) and 50 μl of the desalted extract at 30 °C. The reaction was terminated by adding 75 μl of 4 M NaOH after 20 min. The boiled enzyme was used as a control in each assay. Unreacted fructose-6-P was removed by placing the reaction mixture in a boiling water bath for 10 min. After cooling, 0.25 ml of 0.1% (v/v) resorcinol in 95% (v/v) ethanol and 0.75 ml of 30% (w/v) HCl were added. The mixture was then incubated at 80 °C for 8 min and allowed to cool, and its absorbance was determined at 540 nm.

The procedure for sucrose synthase (SS) assay was identical to that of SPS except that the reaction mixtures contained 10 mM fructose instead of fructose-6-P. One unit of SPS and SS is defined as amount of the enzyme producing 1 μmol sucrose per min.

Acid and neutral invertase assays were performed at 30 °C for 15 min in a total reaction volume of 300 μl containing 50 μl of desalted protein extract, 120 mM sucrose, and 100 mM citrate-phosphate (pH 5.0, for acid invertase), or 120 mM Hepes–NaOH buffer (pH 7.5, for neutral invertase). The reactions were terminated by boiling in a water bath for 5 min and the product (glucose) was assayed using dinitrosalicylic acid agent. Each assay had a boiled enzyme as control. One unit of acid and neutral invertase is defined as amount of the enzyme producing 1 μmol glucose per min.