Scientia Horticulturae 243 (2019) 528–536

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Anatomical and physiological responses of two kiwifruit cultivars to bicarbonate

Nannan Wang, Chunchao Yao, Mingjun Li, Cuiying Li, Zhande Liu, Fengwang Ma*

State Key Laboratory of Crop Stress Biology for Arid Areas, Shaanxi Key Laboratory of Apple, College of Horticulture, Northwest A&F University, Yangling 712100, Shaanxi, China

ARTICLE INFO

Keywords:
Bicarbonate-induced chlorosis
Actinidia
Microscopy
Organic compound
Nutrient imbalance

ABSTRACT

Bicarbonate-induced chlorosis is a common agricultural problem that affects both the quantity and quality of fruit crops grown on calcareous soils. However, little is known about the responses of kiwifruit to bicarbonate stress. In the present study, we investigated the anatomical and physiological responses of two kiwifruit cultivars ('Nongdajinmi' and 'Xuxiang') grafted onto the same rootstock ('Qinmei') to bicarbonate (0.50 g/L $CaCO_3$ and 0.84 g/L $NaHCO_3$) under sand culture. After 35 days, bicarbonate-treated vines exhibited yellowing symptoms and decreased chlorophyll content in new expanded leaves. Bicarbonate imposition decreased N and Cu concentration in new leaves and P in old leaves. Moreover, bicarbonate decreased the dry weight of the new leaves and twigs and degenerated the chloroplast, particularly in 'Nongdajinmi', suggesting that 'Nongdajinmi' is more sensitive to bicarbonate than 'Xuxiang'.

However, bicarbonate treatment thickened palisade tissue and decreased the phenolic content in 'Nongdajinmi' and lowered the organic acid content and stomatal length, width and aperture size in 'Xuxiang'. Interestingly, bicarbonate raised Ca concentration in new leaves and reduced Mg in old leaves of 'Nongdajinmi', but the opposite was true for 'Xuxiang'. These results implied that the two cultivars adopted two different mechanisms in response to bicarbonate stress. Our study contributes to better understanding the responsive mechanisms of kiwifruit to bicarbonate and the cultivar selections for kiwifruit orchards established on calcareous soils.

1. Introduction

Kiwifruit (*Actinidia* Lindl.) is very susceptible to bicarbonate-induced chlorosis (Tagliavini and Rombolà, 2001). When cultivated on calcareous soils, kiwifruit vines frequently exhibit typical yellowing symptoms in new expanded leaves caused by > 50 g/kg active carbonate content in the soils (Tagliavini and Rombolà, 2001). These symptoms are more severe during fruit setting and expansion stages, thereby leading to a loss of kiwifruit yield and quality (Yao et al., 2005). Over 30% of global kiwifruit production is from Shaanxi and Sichuan provinces in China, the country's two largest kiwifruit-producing areas, which are characterized by soils with high bicarbonate content (Xiong and Li, 1987; Zhai, 2015). Consequently, bicarbonate-induced chlorosis has become one of the most important limiting factors that influence the sustainability of the kiwifruit industry worldwide.

The phenomenon of leaf chlorosis is usually accompanied by damage to the anatomical structures of leaf under some abiotic stresses, such as manganese toxicity in sugarcane (Zambrosi et al., 2016), boron toxicity in citrus (Mesquita et al., 2016), and drought in apple (Wang et al., 2012). Moreover, the apoplasmic alkalization within plant tissues induced by high bicarbonate blocks nutrient absorption, transport and utilization, thereby leading to an imbalance of ions in leaves (Bavaresco and Poni, 2003; Cambrollé et al., 2015). To adapt to this adverse environment, the plant roots secrete organic compounds that buffer the pH-increasing effect induced by bicarbonate, thereby improving nutrient use efficiency (Jelali et al., 2010; Donnini et al., 2012; Tato et al., 2013; Chen et al., 2018). The effects of bicarbonate on plant growth, organic compounds and nutrient homeostasis have been reported in a number of plant species, including citrus (Byrne and Rouse, 1994), plum (Cinelli and Loreti, 2004), *Medicago ciliaris* (M'Sehli et al., 2008), pear and quince (Donnini et al., 2009; Alcántara et al., 2012), *Pisum sativum* (Jelali et al., 2010), *Parietaria diffusa* (Donnini et al., 2012; Tato et al., 2013), grape (Covarrubias and Rombolà, 2013), apple (Sahin et al., 2017), and evergreen azalea (Demasi et al., 2017). However, the mechanism by which kiwifruit plants respond to bicarbonate is still unclear.

* Corresponding author.
E-mail address: fwm64@sina.com (F. Ma).

https://doi.org/10.1016/j.scienta.2018.09.011
Received 17 April 2018; Received in revised form 28 July 2018; Accepted 5 September 2018

Differences in the degree of bicarbonate susceptibility between rootstock genotypes within one species have been investigated in apple (Han et al., 1994), grape (Ksouri et al., 2005), citrus (Byrne and Rouse, 1994), and plum (Cinelli and Loreti, 2004). However, limited information is available on the scion (Assimakopoulou et al., 2016; Sahin et al., 2017). For example, anatomical and organic compound changes have not yet been investigated under bicarbonate condition. As we know, the responsive mechanisms of abiotic stresses vary between the rootstock and scion (e.g., Wang et al., 2014). In recent years, the number of kiwifruit cultivars has dramatically increased, particularly for *Actinidia chinensis* var. *chinensis* and *A. chinensis* var. *deliciosa* (Huang, 2014; Yu, 2017). However, the differential sensitivity of kiwifruit genotypes to bicarbonate is mostly unknown.

The objective of the present study was to investigate the anatomical and physiological responses of two kiwifruit cultivars ('Nongdajinmi' and 'Xuxiang') grafted on the same rootstock ('Qinmei') to bicarbonate stress under sand culture.

2. Materials and methods

2.1. Plant materials

Two kiwifruit cultivars of 'Nongdajinmi' (*Actinidia chinensis* var. *chinensis* 'Nongdajinmi') and 'Xuxiang' (*A. chinensis* var. *deliciosa* 'Xuxiang') were used in this study. Both the yellow-fleshed 'Nongdajinmi' and green-fleshed 'Xuxiang' cultivars were bred in China (Huang, 2014; Yao et al., 2017). 'Qinmei' seedlings (*A. chinensis* var. *deliciosa* 'Qinmei' × 'Qinxiong401') were used as rootstock. Tongue-grafting with three buds in each cane was employed to form two scion–rootstock combinations: Ns/Qr, 'Nongdajinmi' scion grafted onto 'Qinmei' rootstock; and Xs/Qr, 'Xuxiang' scion grafted onto 'Qinmei' rootstock.

2.2. Growth conditions and treatments

The experiment was performed outdoors in late March 2017 at the Agriculture Experiment Station, Northwest Agriculture & Forestry University, Yangling, China. After fifteen days, all the grafted plants were washed with tap water to remove surface contaminants and transplanted into 7-L opaque plastic pots (one plant per pot) filled with a mixture of sand and perlite (1:3, *v:v*). Then, the plants were irrigated with a 1/4-strength nutrient solution for two weeks and then with a 1/2-strength solution for about eight weeks until another 18–24 leaves expanded for each of the scions. The nutrient composition as described by Smith et al. (1989) with a few modifications was as follows: 7.7 mM KNO_3, 5 mM $Ca(NO_3)_2$, 1.1 mM $NH_4H_2PO_4$, 1.6 mM $MgSO_4$, 23 μM H_3BO_3, 9 μM $MnCl_2$, 0.8 μM $ZnSO_4$, 0.3 μM $CuSO_4$, 0.01 μM H_2MoO_4, and 50 μM Fe-EDTA.

One week before the treatments, the shoots of the plants were pruned at the tenth node above the graft union to stimulate new shoot development. Uniform plants with emerging buds were selected for two treatments: (1) control, with complete nutrient supply, and (2) bicarbonate treatment (Bic), with the addition of 0.50 g/L $CaCO_3$ and 0.84 g/L $NaHCO_3$ into the complete nutrient solution to bring the pH to approximately 7.8. $CaCO_3$ and $NaHCO_3$ were applied to mimic the effect of a calcareous soil. Each treatment was replicated four times with four plants (pots) for each replication. The pots were randomly arranged with one guarding row pot outside. The plants were irrigated every five days, and the pH of the nutrient solution was adjusted by adding NaOH or HCl to obtain a pH of 6.2 and 7.8 for control and bicarbonate treatments, respectively. To avoid salt accumulation in the medium, the pots were flushed with deionized water every ten days. This experiment was terminated when leaf yellowing occurred in the apical leaves of bicarbonate-treated vines.

2.3. Measurement of chlorophyll content, leaf number, individual leaf weight and shoot length

One day before the end of the experiment, leaf chlorophyll content was measured and expressed as soil plant analysis development (SPAD) reading, which was obtained from the average value of all the new leaves that had emerged since the beginning of treatments for each plant using a portable chlorophyll meter (Konica Minolta SPAD 502 Plus, Tokyo, Japan). Meanwhile, leaf number and shoot length for each vine were recorded. Individual leaf weight was calculated by total leaf dry weight divided by leaf number.

2.4. Analysis of dry weight and nutrient concentrations

At the end of the experiment, new leaves (emerged since the beginning of the treatments), old leaves (emerged before the treatments), twigs (emerged since the beginning of the treatments), stem, and roots were sampled separately. All the plant parts were initially washed with tap water and then with deionized water. The samples were then quickly blotted with tissue paper, oven dried at 65 °C for 72 h and weighed. Each dry sample was ground, dry ashed in a muffle furnace at 520 °C for 6 h, dissolved in 0.25 mol/L HNO_3 for nutrient determination using an inductively coupled plasma optical emission spectrometry (ICP-OES), and N concentration was measured using a continuous flowing analyzer after digestion with H_2SO_4-H_2O_2 (Bao, 2000).

2.5. Microscopy analysis

After 35 days of bicarbonate treatment, anatomical characteristics of the leaf tissue were evaluated in new leaves. Tissue samples (approximately 20 mm^2 in area) were collected from the middle of the fifth fully expanded leaf from the shoot top with four plants per treatment between 9:00 and 10:00 a.m. and fixed in Karnovsky solution (Karnovsky, 1965). Some of the blocks were then dehydrated in an increasing ethanol series (30%, 50%, 70%, 90%, and 100%, with each series three times), infiltrated with resin ethanol for polymerization, and sectioned with a microtome. Similarly, samples for scanning electron microscopy (SEM) analysis were fixed, dried to the critical point and gold-sputtered before observation. Some of the leaf samples fixed in Karnovsky solution were then post-fixed for 1 h with 1% osmium tetroxide, dehydrated, infiltrated and cut for transmission electron microscopy (TEM) observation. Details about light microscopy, SEM and TEM protocols are well described by Zambrosi et al. (2017). The parameters of foliar anatomical structures and stomata features in images were measured using Image-Pro Plus 6.0 (Media Cybernetics, Inc., Silver Spring, MD, USA). A set of 64 cells randomly selected from 16 images of leaves were examined for each treatment.

2.6. Assay of phenolics and organic acids

A 0.20-g sample of leaf tissue was ground in liquid nitrogen at 0 °C, transferred into a centrifuge tube with 2 mL of solvent containing 70% methanol and 2% formic acid. The mixture was heated at 30 °C for 30 min in a thermomixer at 1000 rpm and then centrifuged at 10 000 × *g* for 10 min. The supernatant was passed through a 0.21-μm syringe filter into a vial for phenolic analysis. Phenolic compounds were analyzed using high-performance liquid chromatography (HPLC) as described in detail by Wang et al. (2015).

Approximately 0.15 g of each sample was ground in liquid nitrogen, and extracted with 1.5 mL of deionized water. After centrifugation at 6000 × *g* for 10 min, the supernatant was passed through a 0.21-μm syringe filter into a vial for organic acid analysis. The concentrations of organic acids were measured with HPLC according to the method reported by Ma et al. (2015).