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Anatomical and physiological responses of two kiwifruit cultivars to bicarbonate

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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.

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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).

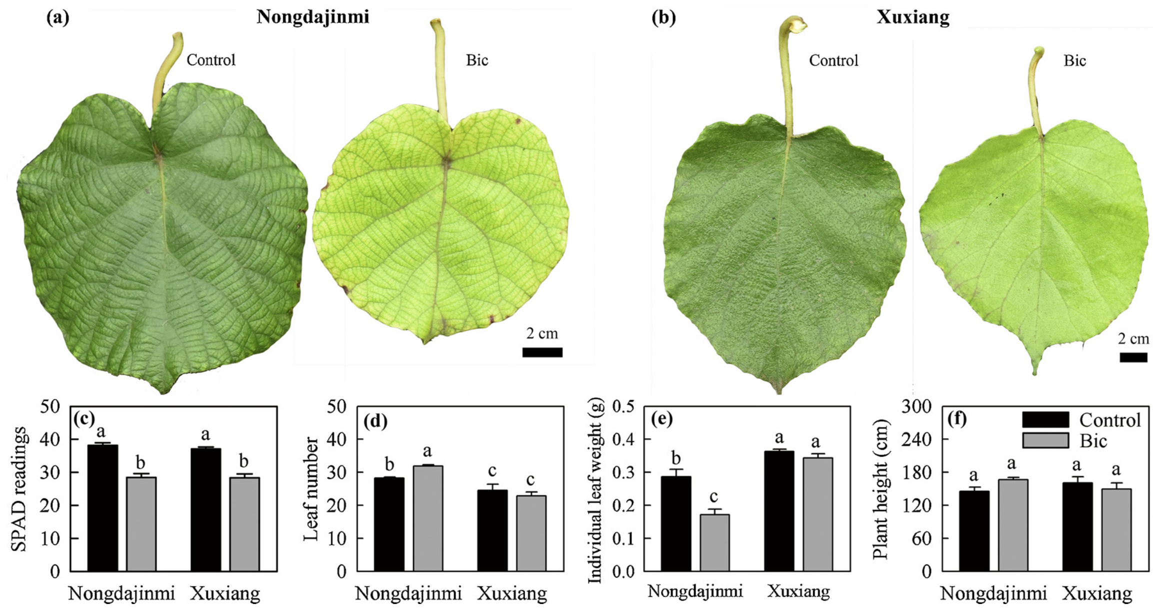

Fig. 1. Leaf symptoms (a,b), SPAD readings (c), leaf number (d), individual leaf weight (e) and plant height (f) of two kiwifruit cultivars ('Nongdajinmi' and 'Xuxiang') grafted onto the same rootstocks ('Qinmei') grown in sand culture with or without bicarbonate addition for 35 days. Values are means of four replicates ± SEs. *Bars* headed by *different letters* represent significant differences among four treatments for the same growth parameter at $P < 0.05$.

2.7. Statistical analysis

Unless otherwise stated, all reported values represent means of four replicates. Error bars indicate standard errors (SEs) of the means. Data were compared using Duncan's multiple range test in SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Visible symptoms and plant growth

Chlorosis symptoms appeared on new expanded leaves of both kiwifruit cultivars after 35 days of bicarbonate treatment (Fig. 1a, b), together with a decrease in SPAD readings (Fig. 1c). However, only 'Nongdajinmi' displayed a higher leaf number but a lower individual leaf weight under bicarbonate condition (Fig. 1d, e). Plant height of both cultivars was not modified by the addition of bicarbonate (Fig. 1f).

Bicarbonate treatment decreased the dry weight in new leaves and twigs of the two cultivars (Fig. 2a, c). However, the dry weight decreased more for 'Nongdajinmi' than for 'Xuxiang' (Fig. 2a, c). The dry weight of old leaves, stem and roots was not affected by bicarbonate stress (Fig. 2b, d, e).

3.2. Anatomical and ultrastructural changes in kiwifruit leaves

No anatomical changes were observed in 'Xuxiang' when subjected to bicarbonate treatment (Fig. 3c–j). In 'Nongdajinmi', however, bicarbonate imposition increased the palisade tissue thickness, thereby increasing leaf thickness and the ratio of the palisade to spongy tissue thickness (Fig. 3f, i, j). Irrespective of the nutrient solution treatment, 'Xuxiang' exhibited higher values of upper and lower epidermis thickness compared with 'Nongdajinmi' (Fig. 3a–e, h).

There was no apparent stomata difference in both cultivars between the control and bicarbonate treatments, except for the lower stomatal length, width and aperture size in 'Xuxiang' under bicarbonate condition (Fig. 4c–g). However, 'Xuxiang' had a greater stomatal length and width, thereby resulting in a lower stomata density when compared with 'Nongdajinmi' (Fig. 4e, f, h). Moreover, 'Xuxiang' displayed more sunken stomata than did 'Nongdajinmi' (Fig. 4a–d), consistent with the leaf anatomical observations (Fig. 3a–d).

Control plants showed well-organized ultrastructure independent of the genotype, as suggested by the absence of alterations in the cell wall and chloroplast (Fig. 5a, c). However, bicarbonate induced cell plasmolysis of both scions, particularly in 'Xuxiang' (thin arrows, Fig. 5b, d). Moreover, the chloroplasts showed irregular shapes and tended to degenerate, particularly in 'Nongdajinmi' (thick arrows, Fig. 5b, d).

3.3. Concentrations of organic compounds and mineral elements in kiwifruit leaves

Bicarbonate treatment decreased the concentrations of procyanidin B2, chlorogenic acid, and caffeic acid in the leaves of 'Nongdajinmi' and decreased the malic and succinic acid concentrations in 'Xuxiang' (Fig. 6a–e).

Bicarbonate treatment decreased N and Cu concentrations in new leaves and P concentration in old leaves of both cultivars (Table 1). Moreover, bicarbonate raised Ca concentration in new leaves but reduced Mg concentration in old leaves in 'Nongdajinmi' (Table 1). In 'Xuxiang', on the contrary, bicarbonate decreased Ca concentration of new leaves (even though not statistically significant) and increased Mg concentration of old leaves (Table 1).

4. Discussion

Leaf chlorosis is frequently observed under field conditions when kiwifruit vines are grown on calcareous soils (Liu et al., 2002; Song et al., 2003; Tran et al., 2012), owing to their high sensitivity to bicarbonate stress (Tagliavini and Rombolà, 2001). However, limited information is available on the responses of kiwifruit to bicarbonate. Here, we compared the anatomical and physiological responses of two kiwifruit cultivars to bicarbonate under sand culture and found that 'Nongdajinmi', an *A. chinensis* var. *chinensis* cultivar, is more sensitive to bicarbonate than 'Xuxiang', an *A. chinensis* var. *deliciosa* cultivar. This result would be beneficial to the cultivar selections for kiwifruit orchards established on calcareous soils.

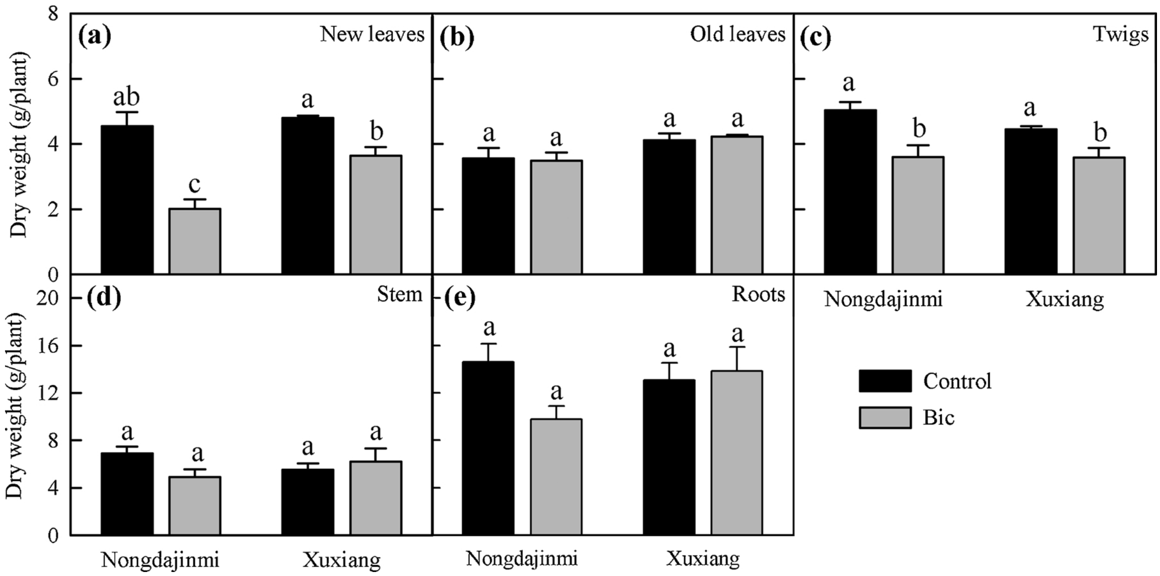

Fig. 2. Dry weight in various parts of two kiwifruit cultivars ('Nongdajinmi' and 'Xuxiang') grafted onto the same rootstocks ('Qinmei') grown in sand culture with or without bicarbonate addition for 35 days. Values are means of four replicates ± SEs. *Bars* headed by *different letters* represent significant differences among four treatments for the same plant tissue at $P < 0.05$.

4.1. Plant growth

After 35 days of bicarbonate treatment, new expanded leaves of both cultivars exhibited yellowing symptoms and a reduction in SPAD readings (Fig. 1a–c), and thus the dry weight in new tissues (i.e., new leaves and twigs) declined (Fig. 2a, c). These results concur with those reported in other plant species, such as citrus (Byrne and Rouse, 1994; Castle et al., 2009; Benyahia et al., 2011), plum (Cinelli and Loreti, 2004; Assimakopoulou et al., 2011), grape (Ksouri et al., 2005, 2007; Assimakopoulou et al., 2016), *Medicago ciliaris* (M'Sehli et al., 2008), pear and quince (Donnini et al., 2009; Alcántara et al., 2012), apple (Sahin et al., 2017), and evergreen azaleas (Demasi et al., 2017). These results suggest that the major effect of bicarbonate on plant growth is leaf chlorosis and biomass reduction, caused by a decrease in chlorophyll content and thus photosynthesis (Han et al., 1994; Jelali et al., 2011; Cambrollé et al., 2014, 2015).

Differences in the tolerance to high bicarbonate levels among genotypes within one species and among plant species are well documented (Tagliavini and Rombolà, 2001). Our study has shown that 'Nongdajinmi' was more susceptible to bicarbonate than 'Xuxiang', as supported by more reduction in the dry weight of new leaves and twigs in 'Nongdajinmi' under bicarbonate treatment (Fig. 2a, c). The high susceptibility of 'Nongdajinmi' to bicarbonate could be explained by its lower ploidy (as it is a hybrid of diploid and tetraploid progenitors) when compared with 'Xuxiang' (which is hexaploid; Zhong et al., 2016; Yao et al., 2017), since polyploid plants are usually better adapted to a variety of abiotic stresses (e.g., Chao et al., 2013; Tan et al., 2015). Another possible explanation is the discrepancy of two kiwifruit species: *A. chinensis* var. *chinensis* (e.g., 'Nongdajinmi') and *A. chinensis* var. *deliciosa* (e.g., 'Xuxiang'), the former species mainly originates from lower latitudes (i.e., southern China) where the soils are acidic whereas the latter originates from higher latitudes with alkaline soils (Huang, 2014; Kang et al., 2014).

Interestingly, in the presence of bicarbonate, 'Nongdajinmi' leaf number increased despite its reduction in individual leaf weight as a consequence of the decrease in new leaf dry weight (Figs. 1d, e and 2 a). These results agree with the investigations on chlorotic leaves from 'Qinmei' kiwifruit under field conditions (Yao et al., 2005). The increase in the leaf number of bicarbonate-treated plants may be a growth adaptation related to increasing the photosynthetic area, which is similar to the foraging response displayed under mild N deficiency through increasing total root length (Giehl and Von, 2014). These results suggest that the bicarbonate-treated plants in the present study may be in a mild response (i.e., foraging response) rather than a severe response (i.e., survival strategy) to bicarbonate stress.

4.2. Anatomical changes

Kiwifruit is one of the most recently domesticated fruit crops in the world, and thus, anatomical research on it is very limited (Wang et al., 1994; Sotiropoulos et al., 2002; Yin et al., 2017). Anatomical observations in bicarbonate-treated leaves showed that chloroplast degeneration occurred in both cultivars (Fig. 5b, d), consistent with leaf chlorosis and a decrease in chlorophyll content (Fig. 1a–c), indicating that bicarbonate imposition probably impairs the chloroplast structure and function of kiwifruit leaves. These results are consistent with other abiotic stresses, such as sulfur toxicity in kiwifruit (Yin et al., 2017), manganese toxicity in sugarcane (Zambrosi et al., 2016), phosphorus deficiency with phosphite spray (Zambrosi et al., 2017) and boron toxicity in citrus (Mesquita et al., 2016), and drought in apple (Wang et al., 2012). However, the stomatal and anatomical changes of the two cultivars differed. For example, the chloroplast ultrastructure of 'Nongdajinmi' was more modified by the addition of bicarbonate than that of 'Xuxiang' (Fig. 5b, d), suggesting that 'Nongdajinmi' is more sensitive to bicarbonate than 'Xuxiang'. Our results also showed that 'Nongdajinmi' exhibited an increase in the palisade tissue thickness (Fig. 3f), while 'Xuxiang' displayed lower stomata length, width and aperture size (Fig. 4e–g), along with more apparent cell plasmolysis than 'Nongdajinmi' when grown in bicarbonate treatment (Fig. 5b, d). These results are similar to the adaptation of several plants to drought stress (Du et al., 2010), indicating that bicarbonate treatment may trigger osmotic stress as a side effect besides the pH-increasing effect. It should be noted that 'Xuxiang' seems to be sensitive to osmotic stress than 'Nongdajinmi' due to its more apparent stomatal changes and cell plasmolysis, consistent with our field observation that 'Xuxiang' is one of the most sensitive kiwifruit cultivars to osmotic stress induced by drought.

4.3. Organic compounds and nutrient homeostasis

Bicarbonate treatment has been reported to induce a strong accumulation of phenolics and organic acids in roots of some non-

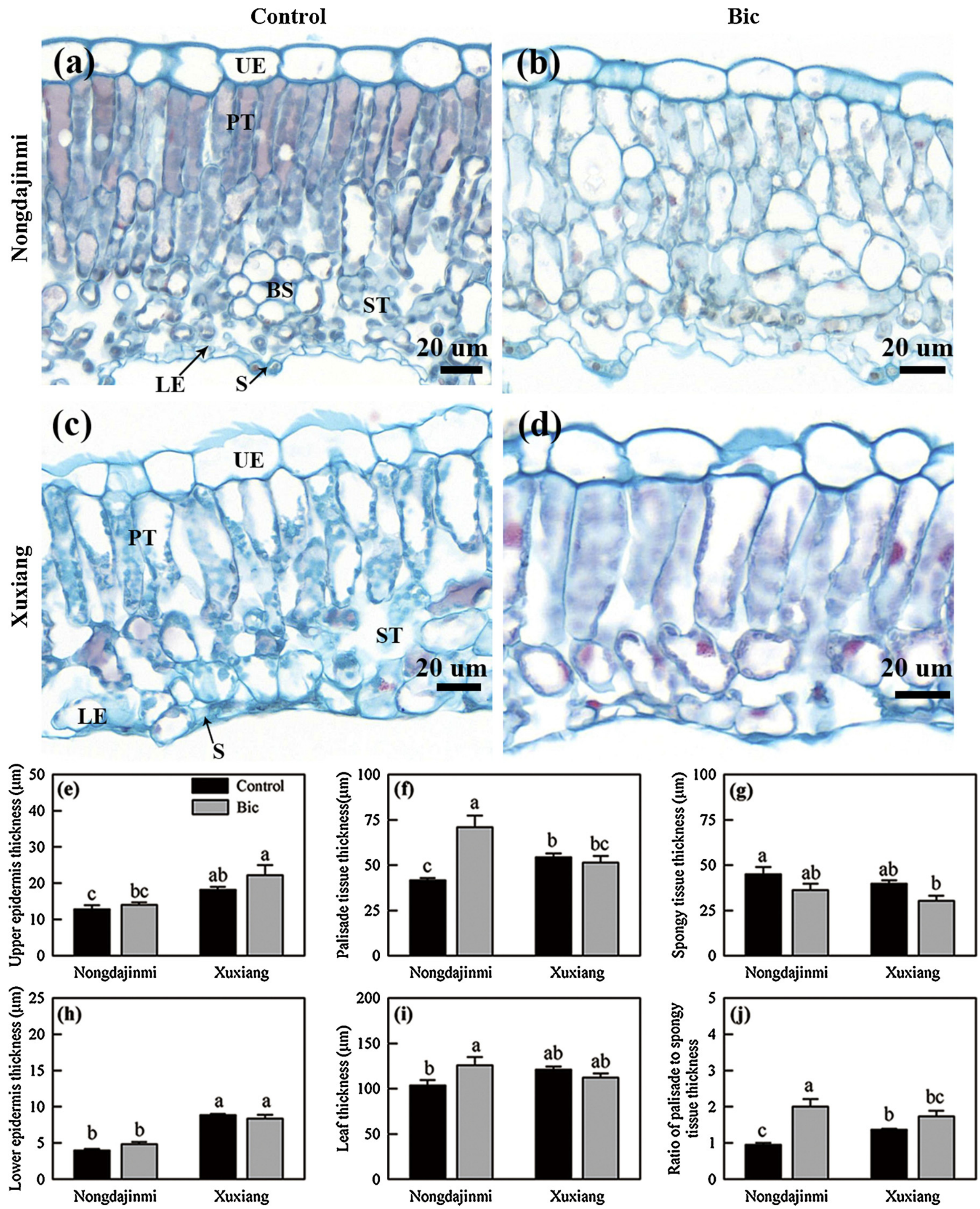

Fig. 3. Cross sections of leaves under light microscopy (a–d) and anatomical features (e–j) of two kiwifruit cultivars ('Nongdajinmi' and 'Xuxiang') grafted onto the same rootstock ('Qinmei') grown in sand culture with or without bicarbonate addition for 35 days. Values are means of four replicates ± SEs. *Bars* headed by *different letters* represent significant differences among four treatments for the same anatomical feature at $P < 0.05$.
UE, upper epidermis; LE, lower epidermis; PT, palisade tissue; ST, spongy tissue; BS, bundle sheath; and S, stomata.

graminaceous angiosperm species, such as *Pisum sativum* (Jelali et al., 2010) and *Parietaria diffusa* (Donnini et al., 2012; Tato et al., 2013), which alleviates the pH-increasing effect of the apoplasmic space in roots grown on calcareous soils. By contrast, in graminaceous plants (e.g., rice and wheat), high pH caused significant reductions in root phenolics and thus hindered nutrient transport and homeostasis in the shoot (Chen et al., 2018). These results suggest that the effect of bicarbonate on phenolics and organic acids might be species-dependent. Moreover, this effect within one plant may be tissue-dependent. In the presence of bicarbonate, organic acid concentrations in grape roots accumulated significantly to mitigate the apoplasmic alkalization but decreased significantly in pH-increasing xylem sap (Covarrubias and Rombolà, 2013). Moreover, in kiwifruit leaves, significant decreases in phenolics and organic acids were observed under bicarbonate condition (Fig. 6a–e). This may be explained by (1) more organic compound transportation from leaves to roots to alleviate the apoplasmic alkalization of kiwifruit roots induced by high bicarbonate, (2) less accumulation of organic compounds in leaves due to the weaker tissue

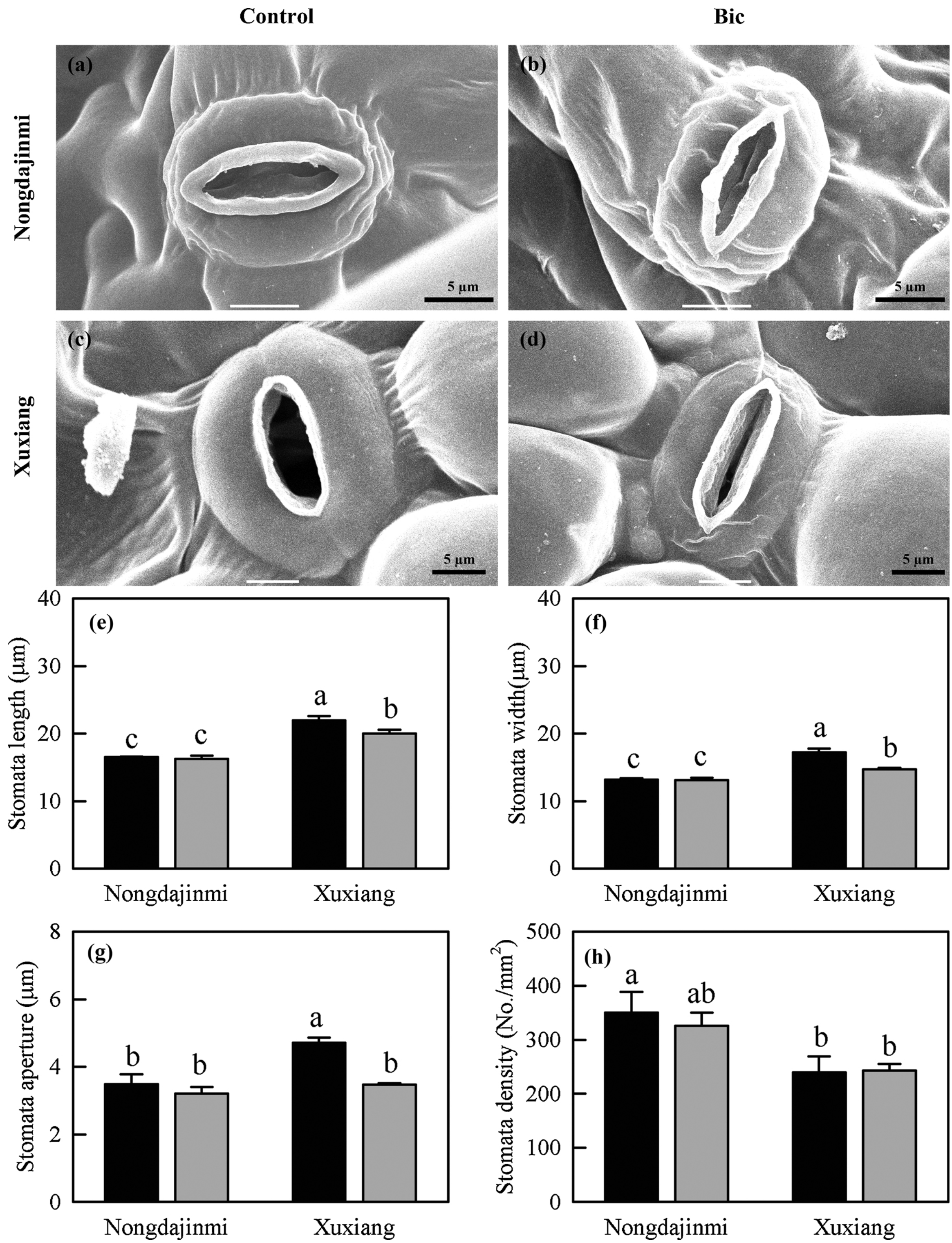

Fig. 4. Scanning electron microscopy on the abaxial surface of leaves (a–d) and stomata features (e–h) of two kiwifruit cultivars ('Nongdajinmi' and 'Xuxiang') grafted onto the same rootstock ('Qinmei') grown in sand culture with or without bicarbonate addition for 35 days. Values are means of four replicates ± SEs. *Bars* headed by *different letters* represent significant differences among four treatments for the same stomata feature at $P < 0.05$.

response to external bicarbonate when compared with roots, and (3) mild plant response to bicarbonate stress as mentioned above.

Nutrient imbalance is a common response to high bicarbonate in fruit crops, such as grape (Bavaresco and Poni, 2003; Cambrollé et al., 2015; Assimakopoulou et al., 2016) and plum (Assimakopoulou et al., 2011). In fact, nutrient disorders are frequently observed in kiwifruit production (e.g., Parent et al., 2015; Lu et al., 2016) and the integrated nutrient management is required (Müller et al., 2015; Zhao et al., 2017). Thus, understanding the relationship between nutrient homeostasis and bicarbonate stress is useful for the sustainability of kiwifruit orchards on calcareous soils. A field investigation showed that yellowing kiwifruit leaves had significant reductions in N, P, K, and Fe compared with green leaves (Tran et al., 2012), but another study reported a significant increase in P, K, Zn, and Mn, as well as a significant

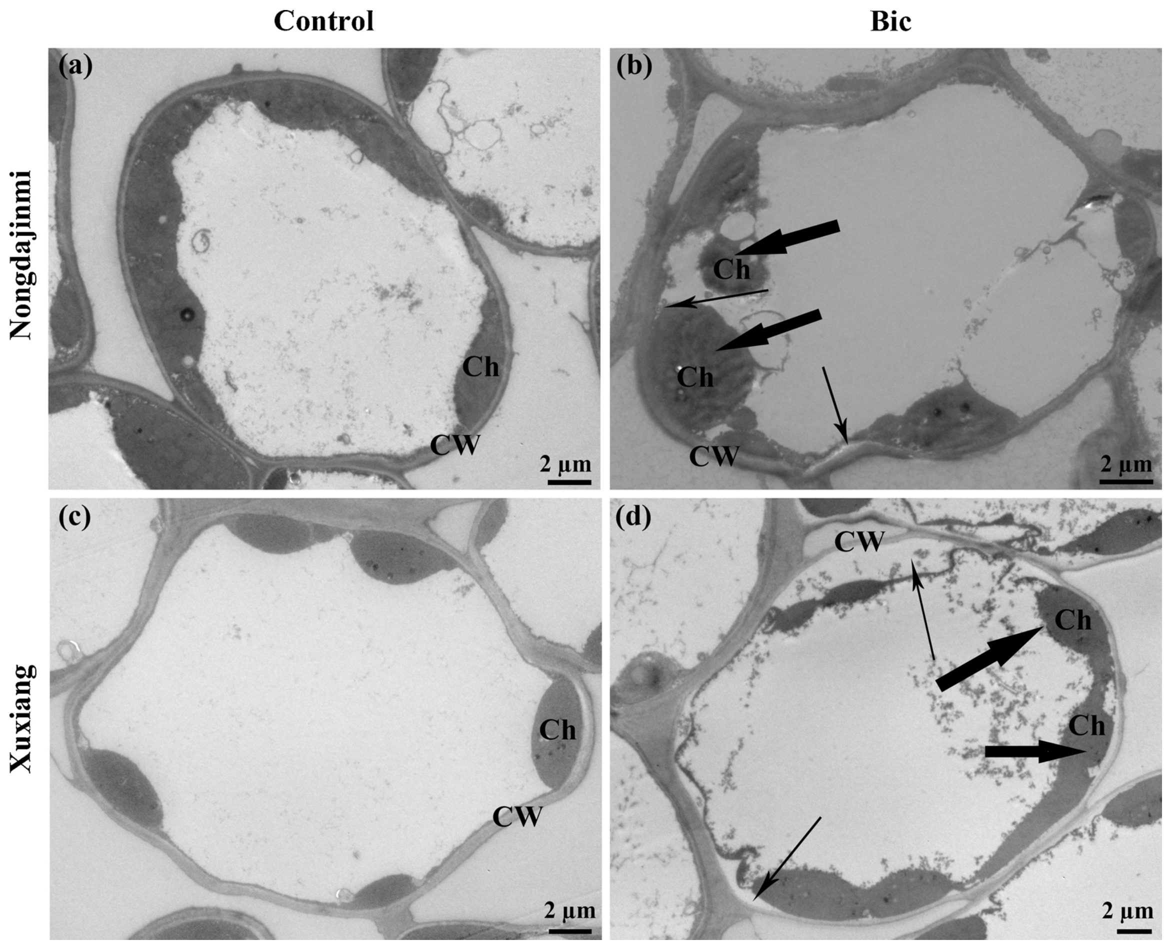

Fig. 5. Transmission electron microscopy of the leaves of two kiwifruit cultivars ('Nongdajinmi' and 'Xuxiang') grafted onto the same rootstock ('Qinmei') grown in sand culture with or without bicarbonate addition for 35 days.
Ch, chloroplast; CW, cell wall; and thin arrows indicate cell plasmolysis, while thick arrows indicate alterations of chloroplast.

decrease in the active Fe of the yellowing leaves relative to green leaves (Wang et al., 2011). Our results showed that bicarbonate treatment decreased N and Cu concentrations in new leaves and P concentration in old leaves of both cultivars (Table 1), consistent with the results described by Tran et al. (2012). Our study also indicated that bicarbonate imposition increased Ca concentration in new leaves but reduced Mg concentration in old leaves in 'Nongdajinmi', but the opposite trend was observed in 'Xuxiang' (Table 1). These results indicate that, in the presence of $CaCO_3$, the sensitive cultivar 'Nongdajinmi' is unable to block Ca flooding and thus antagonize Mg absorption, but the

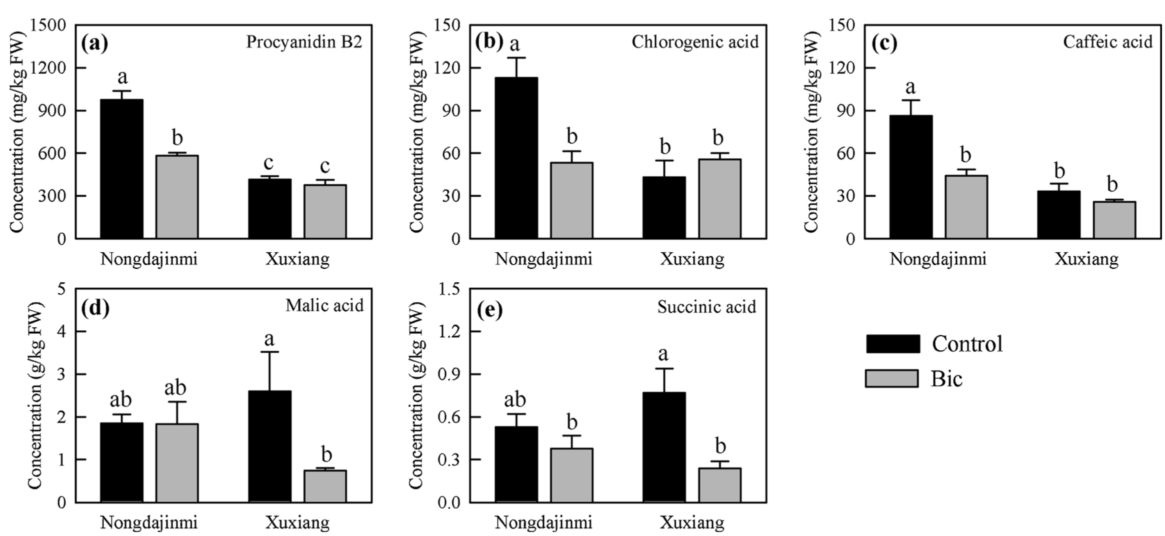

Fig. 6. Procyaniadin B2 (a), chlorogenic acid (b), caffeic acid (c), malic acid (d) and succinic acid (e) concentrations in new leaves of two kiwifruit cultivars ('Nongdajinmi' and 'Xuxiang') grafted onto the same rootstock ('Qinmei') grown in sand culture with or without bicarbonate addition for 35 days. Values are means of four replicates ± SEs. *Bars* headed by *different letters* represent significant differences among four treatments for the same organic compound at $P < 0.05$.

Table 1
Nutrient concentrations in new and old leaves of two kiwifruit cultivars ('Nongdajinmi' and 'Xuxiang') grafted onto the same rootstock ('Qinmei') grown in sand culture with or without bicarbonate addition for 35 days.

			N	P	K	Ca	Mg	Fe	Mn	Cu	Zn
NL	ND	Control	25.1bc (1.0)	2.2ab (0.2)	14.0b (1.0)	25.0b (0.7)	7.3a (0.2)	134.4a (7.5)	42.4a (4.3)	6.7a (0.3)	49.1ab (7.9)
		Bic	22.4c(0.7)	2.0b (0.1)	14.0b (1.2)	31.1a (2.5)	7.0a (0.2)	117.2a (2.0)	39.3a (1.2)	5.1b (0.2)	57.8a (17.8)
	XX	Control	29.4a (0.3)	2.5a (0.1)	39.0a (1.2)	26.2b (1.4)	6.9a (0.2)	140.2a (9.4)	23.9b (1.1)	4.9b (0.8)	22.2b (1.1)
		Bic	26.6b(1.2)	2.4ab (0.2)	38.6a (0.5)	21.6b (0.9)	7.3a (0.2)	142.0a (15.3)	28.3b (1.3)	1.8c (0.0)	27.6ab (4.7)
OL	ND	Control	20.0b (0.6)	1.5c (0.1)	11.6b (1.0)	26.3a (0.8)	6.6a (0.2)	270.5ab (13.7)	41.0ab (3.1)	7.1a (1.2)	34.6a (6.2)
		Bic	19.1b (0.4)	1.2d (0.0)	11.3b (0.6)	26.1a (1.4)	5.9bc (0.2)	235.1b (16.2)	45.0a (2.6)	8.1a (1.2)	34.8a (6.9)
	XX	Control	24.1a (0.9)	2.2a (0.1)	29.5a (1.3)	18.1b (0.5)	5.6c (0.1)	316.2a (6.0)	29.2c (3.3)	7.4a (0.9)	20.6a (1.8)
		Bic	22.5a (0.6)	1.8b (0.0)	28.4a (0.9)	19.1b (0.7)	6.4ab (0.2)	301.0a (21.3)	32.3bc (4.4)	5.8a (0.6)	23.3a (1.3)

Values are means of four replicates with SEs in parentheses. *Different letters* represent significant differences among four treatments for the same plant tissue at $P < 0.05$.
The unit of N, P, K, Ca and Mg is g/kg and the unit of Fe, Mn, Cu and Zn is mg/kg based on dry weight. NL, new leaves that emerged since the beginning of bicarbonate treatment; OL, old leaves that emerged before bicarbonate treatment; ND, 'Nongdajinmi'; and XX, 'Xuxiang'.

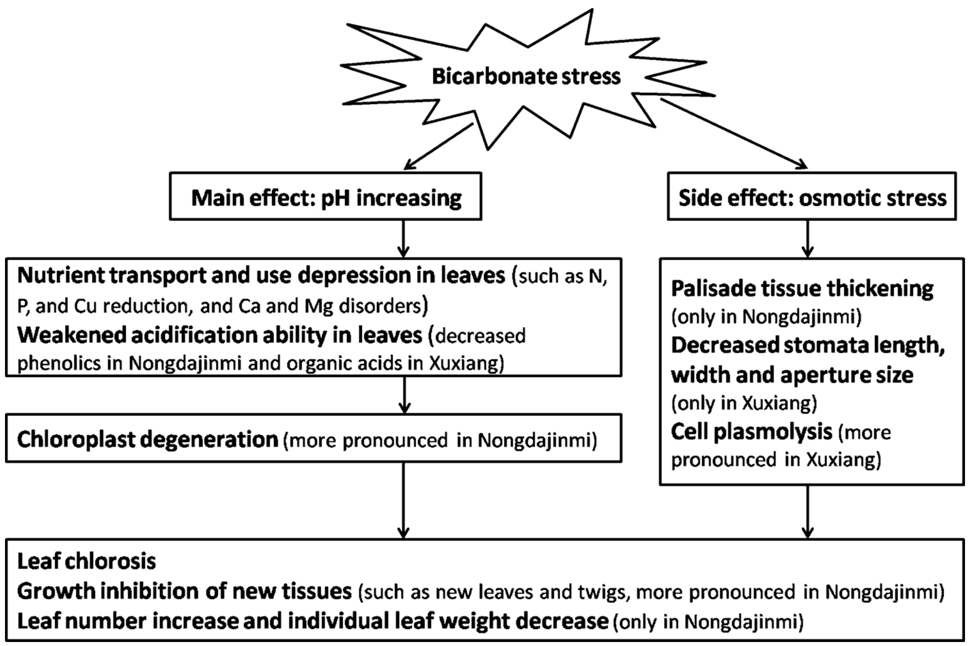

Fig. 7. A model for bicarbonate responses in the leaves of two kiwifruit cultivars ('Nongdajinmi' and 'Xuxiang') grafted on the same rootstock.

tolerant cultivar 'Xuxiang' absorbs more Mg to resist Ca flooding into leaves and thus maintain nutrient homeostasis within the whole vine.

Based on the present study, here we propose a model to describe the responsive process of two kiwifruit cultivars to bicarbonate (Fig. 7). Our results indicate that 'Nongdajinmi' is more sensitive to bicarbonate than 'Xuxiang' for the following reasons: (1) 'Nongdajinmi' was less able to restrict Ca uptake and transport in the presence of $CaCO_3$; (2) more severe chloroplast degeneration and biomass reduction occurred in 'Nongdajinmi'; and (3) thickening palisade tissue, increasing leaf number and decreasing individual leaf weight were observed only in 'Nongdajinmi' under bicarbonate condition (Fig. 7). These results would contribute to better understanding the mechanisms of anatomical and physiological responses of kiwifruit to bicarbonate, and to selecting the appropriate kiwifruit cultivars in calcareous soils.

5. Conclusion

Our present work indicates that the 'Nongdajinmi' and 'Xuxiang' kiwifruit cultivars differ in their susceptibility to bicarbonate-induced chlorosis. The sensitivity of 'Nongdajinmi' to bicarbonate may be associated with increased Ca concentrations in leaves, palisade tissue thickening, and greater chloroplast degeneration and dry weight reductions in new tissues and individual leaf. These results would help to explore the physiological mechanisms of bicarbonate stress, and provide valuable references (bicarbonate-tolerant cultivar 'Xuxiang' vs bicarbonate-sensitive cultivar 'Nongdajinmi') in breeding new tolerant cultivars and selecting the appropriate cultivars for kiwifruit orchards established on calcareous soils.

Conflict of interest

The authors declare that they have no competing interests.

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