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The effects of high concentration of bicarbonate applications on kiwifruit genotypes with different ploidy levels on some growth parameters of leaves

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Abstract: The high concentration of bicarbonate ion (HCO_3^-) in irrigation water or soil is a limiting factor for the growth and production of kiwifruit vineyards. Especially the high concentration of bicarbonate in irrigation water can cause negative effects on the development of many plants at different rates. In this study, the effects of different concentrations (0, 170, 350, and 550 mg L-1) of bicarbonate ion (HCO_3^-) in irrigation water on six kiwifruit seedlings (DA_1, DA_2, CK_1, CK_2, CK_3, AA) were studied in the greenhouse condition. The six kiwifruit seedlings used in the study belong to a total of 3 different species (*A. deliciosa, A. chinensis*, and *A. arguta*) and also have different ploidy levels (2n, 4n, and 6n). The highest (16.6%) leaf abscission was found in AA (*A. arguta*) seedlings when exposed to HCO_3- stress, and the lowest value (1.19%) was found in DA_1 (*A. deliciosa* cv. 'Bruno') seedlings. Depending on the cultivar or species, bicarbonate caused leaf chlorosis at different rates in kiwifruit seedlings. CK_1 (*A. chinensis*) seedlings showed the highest (38.46%) leaf chlorosis and DA_2 (*A. deliciosa*) seedlings showed the lowest (6.95%) leaf chlorosis. Bicarbonate reduced chlorophylls and carotenoid content in all kiwifruit genotypes. Furthermore, it induced leaf iron (Fe), manganese (Mn), and zinc (Zn) deficiency. Seedlings of 'Bruno' showed the lowest decline of leaf iron (Fe) and Zinc (Zn) content when exposed to HCO_3- stress. In contrast, a high level of Manganese (Mn) was found in seedlings of CK3. Overall, it was detected a close relationship between bicarbonate stress and nutrient homeostasis in different kiwifruit genotypes.

Keywords: *Actinidia* spp., bicarbonate stress, leaf abscission, nutrient homeostasis, chlorosis

1. Introduction

Kiwifruit has been known to be sensitive to bicarbonate-derived chlorosis (Wang et al., 2020). When kiwifruit vines are cultivated on soils with over 50 g kg^{-1} active carbonate, or irrigated with water of more than 200 mg L^{-1} bicarbonate, the newly expanded leaves usually display typical yellowing symptoms, which are more severe throughout fruit expansion and setting process, thereby causing a loss of quality and yield in kiwifruit (Yao et al., 2005). Safe levels for irrigation water should include chloride of less than 70 ppm, bicarbonate of less than 200 ppm, and sodium of less than 50 ppm for kiwifruit production. High bicarbonate content in the soil is a limiting factor for kiwifruit vineyard growth and productivity in some parts of the world (Zhao et al., 2017). High bicarbonate ion in irrigation water is the main cause of reduced productivity of kiwifruit in some part of Iran. As a result, HCO_3^--derived chlorosis has appeared as one of the key restricting factors that threaten the production sustainability in the kiwifruit industry worldwide.

The chlorosis is generally followed by damage to plant leaves as mineral cytotoxicity (Wang et al., 2019). Furthermore, high HCO_3^- concentration induces apoplasmic alkalization within plant tissue, which in turn, blocks nutrient utilization, transport, absorption (Cambrollé et al., 2015), and thereby causing an ion imbalance in the leaf. To adapt to this unfavourable condition, the root starts to secrete organic compounds that alleviate the impact of high pH and improve the use efficiency of nutrients (Wang et al., 2019). The effect of HCO_3^- on nutrient homeostasis, organic compounds, and plant growth have been reported in many plants, such as evergreen azalea (Demasi et al., 2017), apple (Şahin et al., 2017), grape (Covarrubias and Rombolà, 2013), *Parietaria diffusa* (Tato et al., 2013), *Pisum sativum* (Jelali et al., 2010), *Medicago ciliarys* (M'Sehli et al., 2008), and quince and pear (Donini et al., 2009). However, the modes of action by which kiwifruit responds to HCO_3^- are still unknown.

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The difference in the level of HCO_3^- sensitivity occurred between genotypes within one species has been evaluated in grapes (Ksouri et al., 2005). However, data on the influence of irrigation water HCO_3^- on nutrient uptake and physiological traits is limited. For instance, leaf mineral changes have not been studied very well under HCO_3^- stress. On the other, the mechanisms involved in response to HCO_3^- vary between different genotypes (Wang et al., 2019). In the recent decade, the release of kiwifruit cultivars derived from breeding programs has significantly increased (Zhao et al., 2017); however, the susceptibility of different kiwifruit genotypes to HCO_3^- is not fully known.

The objective of the present study was to investigate the physiological responses of six kiwifruit genotypes from three species, *Actinidia chinensis var. deliciosa (*DA), *Actinidia chinensis var. chinensis* (CK), and *Actinidia arguta* (AA) to bicarbonate stress in soilless culture under the greenhouse condition.

2. Materials and methods

2.1 Plant materials

Seeds of 6 kiwifruit populations from *Actinidia chinensis var. deliciosa* (DA), *Actinidia chinensis var. chinensis* (CK), and *Actinidia arguta* (AA) according to Table 1 were obtained and transferred to Lab at the University of Guilan, Rasht, Iran. After the harvest, fruits were stored at 23 °C, and the ripening stage was controlled every week. Seed extraction is carried out when ensuring the fruit is ripe and soft. The fruit pulp was extracted in a juice extractor. The seeds were washed with water and dried and stored at 4 °C until required for germination studies (Maghdouri et al., 2021).

2.2 Growth conditions and treatments

For disinfection, the seeds were soaked in a solution of 2 mg L^{-1} fungicide (Maxim XL 035) for one min and immediately transferred onto paper cloths to remove excess water at room temperature. Seeds were placed into Petri dishes with moist Whatman No. 1 filter paper and incubated at 4 °C for five weeks (Windauer et al., 2016; Sekhukhune et al., 2018). After that, the stratified seeds were placed in a chamber with alternating temperature and light 24 °C (16 h)/ 5 °C (8 h) and then transferred to a growth chamber with constant temperature (20 °C) and continuous light for germination. Here, their germination was followed for 8 weeks (Windauer et al., 2016; Lawas and Anderson, 1980). The germinated seeds were transferred to greenhouse conditions with relative humidity 80%–90% and temperature 25–30 °C with supplemental light and sown in 45 cell trays. The growing media contained different ratios of perlite and peat moss (pH = 5.5–6.5, salinity = 1 mS/cm, moisture content = 40%–60%).

After two months, the seedlings were transferred to the disinfected pots containing the same ratio of coco peat and perlite (Maghdouri et al., 2021). Seedlings were maintained at 20–25 °C, under supplemental light with special bulbs for growth, humidity 85% to 90%, the complete seedlings were fertilized with macro/microelements for seven months. Plants were irrigated with tap water as described in Table 2. All seedlings cut back when reached 150 cm.

This study was conducted as a factorial experiment in a completely randomized design with three replications, each replication was three pots. Bicarbonate treatment was performed by the method as described by Martinez-Cuenca et al., (2013). The pots were fed with a complete nutrient solution and irrigated with different levels of HCO_3^- (0, 170, 350, and 550 mg L^{-1}) from the source of ammonium bicarbonate (NH_4HCO_3). To eliminate the effect of different amounts of nitrogen in different bicarbonate treatments, the amount of nitrogen in irrigation water was adjusted in equal amounts using ammonium nitrate salt. The seedlings were irrigated for six weeks at three days' intervals. Distilled water was used as a control.

2.3 Measurement of leaf abscission, chlorosis, and necrosis percentage

Leaf abscission, chlorosis, and necrosis were measured according to Martinez-Cuenca et al., (2013) method. The data was expressed as a percentage.

2.4 Measurement of chlorophylls and carotenoids

Photosynthesis pigments such as chlorophylls and carotenoids were measured according to Ranganna, (1997). Fresh leaf (0.5 g) was ground through liquid

Table 1. List of kiwifruit genotypes and their important characteristics.

Species	Ploidy level	Flesh colour	Symbol
Actinidia chinensis var. *chinensis* (cv. 'Red 2')	2*x*	Red	CK_1
Actinidia chinensis var. *chinensis*	4*x*	Red	CK_2
Actinidia chinensis var. *chinensis*	2*x*	Red	CK_3
Actinidia chinensis var. *deliciosa* (cv. 'Bruno')	6*x*	Green	DA_1
Actinidia chinensis var. *deliciosa* (cv. 'Green 11')	6*x*	Green	DA_2
Actinidia arguta (cv. 'Hortgem Tahi')	4*x*	Green	AA

Table 2. The quality analysis of irrigation water used in the experiment.

Characteristics	Value
pH	6.55
EC	319 μs cm^{-1}
TDS	204 mg L^{-1}
SAR	0.4 meq L^{-1}
Total hardness	99.89 mg L^{-}
Total alkalinity	155 mg L^{-1}
Bicarbonate	170 mg L^{-1}
Carbonate	0
Chloride	12.46 mg L^{-1}
Calcium	31.76 mg L^{-1}
Magnesium	5 mg L^{-1}
Sodium	9.13 mg L^{-1}

nitrogen, and then 10 mL of 80% cold acetone was added to it. The resulting solution was filtered through filter paper and the extraction was carried out again to completely discolour the tissue. The final extract volume was increased to 20 mL and the absorption was recorded at 646.8 (chlorophyll a), 663.2 (chlorophyll b), and 470 nm (carotenoids) by the UV/VIS spectrophotometer model T80 (PG Instruments Ltd.). The following equations were exerted to estimate chlorophyll content in mg per 100 g fresh weight along with carotenoid content in mg per 1000 g dry weight (Equations 1–4):

$$Chl.a\ (mg/mL) = 12.25\ OD_{663.2} - 2.79\ OD_{646.8} \quad \text{Equation 1}$$

$$Chl.b\ (mg/mL) = 21.50\ OD_{646.8} - 5.10\ OD_{663.2} \quad \text{Equation 2}$$

$$TChl\ (mg/mL) = 18.71\ OD_{646.8} + 7.15\ OD_{663.2} \quad \text{Equation 3}$$

$$Carotenoids = (1000\ OD_{470} - 1.8Chl.a - 85.2Chl.b)/198 \quad \text{Equation 4}$$

Where OD, Chl.a, Chl.b, and TChl are the amount of light absorbed by the device, chlorophyll a, chlorophyll b, and total chlorophyll, respectively.

2.5 Measurement of mineral content

The nutrients measured in this experiment included leaf iron, zinc, and manganese. Initially, two middle mature leaves, one from the main stem and one from the other stems, were selected and maintained in an oven at 65 °C for 48 h to be dried. To prepare the ash, 0.5 g of each sample was placed in an electric oven with an increasing temperature of 500 °C for 4 h. After turning off the furnace and reaching room temperature, the ash of each sample was dissolved with 10 mL of HCl solution (1N) and transferred to a 50 mL volumetric flask, and eventually, its volume reached to 50 mL via distilled water. Finally, the relevant values were read through a 220 FS Varian atomic absorption spectrometer. All these steps were performed according to the methods of element analysis of Page et al., (1982). To prepare the standard iron, manganese, and zinc solutions, the iron sulphate, manganese nitrate, and pure zinc were used, respectively.

2.6 Statistical analysis

Data were analysed via the variance analysis (ANOVA) and Duncan's multiple range tests in SAS 9.0 (2003) and presented as mean. The effect of HCO_3^- treatment was separated at $p < 0.05$. Tables and graphs were drawn withExcel 2013 software.

3. Result and discussion

Nutrient imbalances and their physiological consequences are common responses to high HCO_3^- in plants, such as grapevines (Cambrollé et al., 2015; Assimakopoulou et al., 2016). A range of nutrient disorders is also reported in kiwifruit orchards (Lu et al., 2016). Therefore, understanding the association between HCO_3^- stress and nutrient homeostasis helps guarantee kiwifruit production sustainability in the calcareous soil.

3.1 Leaf abscission

With increasing bicarbonate content in irrigation water, the percentage of leaf abscission increased. The highest value (11.41%) was found at 550 mg L^{-1} bicarbonate and the lowest one (0.56%) was found in control (Figure 1a). The results showed that AA (*A. arguta*) seedlings with 16.6% had the highest percentage of leaf abscission when exposed to high bicarbonate concentration of irrigation water and the lowest leaf abscission percentage was found in DA_1 (*A. deliciosa* cv. 'Bruno') seedlings with 1.19% (Figure 1b). The comparison leaves abscision in bicarbonate irrigated seedlings and control showed that AA was more sensitive than others with 15.3% and 32.5% increase in leaf abscision than control when irrigated with 170 and 540 mg L^{-1} bicarbonate (Figure 2).

Leaf abscission is the result of physiological and morphological responses to environmental stresses (Fathi et al., 2019). Plant genotypes show different reactions to stress conditions due to their physiological characteristics (Sircelj et al., 2007). Previous studies showed that with increasing bicarbonate content in irrigation water the synthesis of abscisic acid and the flow of abscisic acid from the phloem to the xylem, which reduces stomatal conduction and consequently reduces transpiration and mesophilic efficiency. As a result of closing the stomata, the relative water content of the leaf decreases (Lu et al., 2016). It is obvious that by reducing leaf area and leaf fall, the plant loses less water through transpiration, so limiting the transpiration level may be one of the plant's responses to stress conditions (Lu et al., 2005).

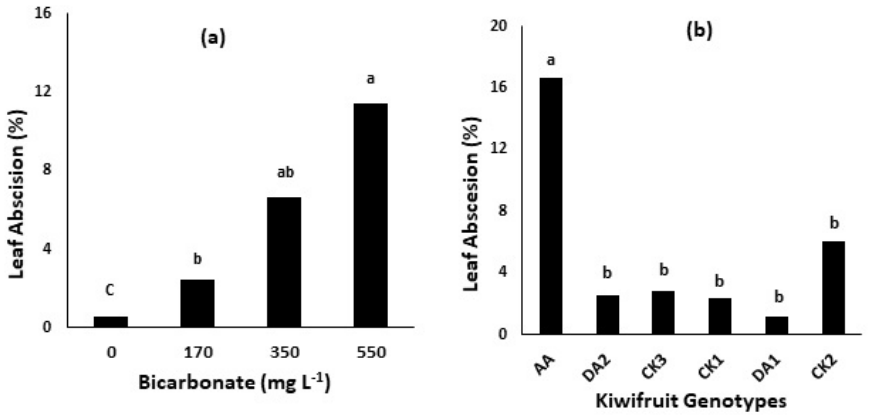


Figure 1. Effect of different bicarbonate contents (a) in irrigation water on leaf abscission percentage of different kiwifruit genotypes (b).

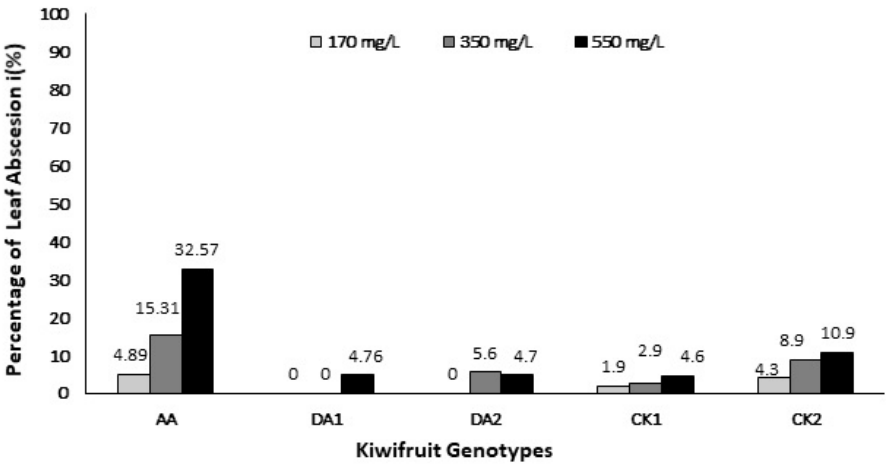


Figure 2. The effect of HCO^{3-} in irrigation water on leaves abscission in different kiwifruit genotypes as percent relative to control.

3.2 Leaf chlorosis and necrosis

The main response of kiwifruit genotypes to a high level of bicarbonate in irrigation water or soil is leaf chlorosis (Figure 3). Leaf chlorosis in response to high concentration of biocarbonate ions in kiwifruit species, chinensis (A), and deliciosa (B). The results showed that with increasing bicarbonate ions in irrigation water the percentage of kiwifruit leaf chlorosis increased. The highest leaf chlorosis (26.33%) was found at 550 mg L^{-1} bicarbonate and the lowest percentage was found in the control (14.51%) treatment with (Figure 4a). The responses of kiwifruit seedlings to leaf chlorosis when exposed to bicarbonate stress were completely dependent on genotypes (Figure 3b). The genotype CK_1 seedlings showed the highest chlorosis percentage (38.46%) and the lowest leaf chlorosis percentage was found in DA_2 genotype at 6.95% (Figure 4b). The comparison leaves chlorosis in bicarbonate irrigated seedlings and control showed that CK2 was more sensitive than others with a 19% increase in leaf chlorosis than control when irrigated with 540 mg L^{-1} bicarbonate (Figure 5) and the lowest chlorosis was found in AA seedlings. Furthermore, the results also showed that kiwifruit leaf necrosis was significantly affected by bicarbonate content in irrigation water. With increasing bicarbonate levels the percentage of leaf necrosis also increased. The highest leaf necrosis was found in 550 mg L^{-1} with 20.4% and the lowest one was found in the control (Figure 6). No significant differences were found between kiwifruit genotypes for leaf necrosis.

Previous studies suggested the use of chlorosis-resistant cultivars and rootstocks as a sustainable solution to reduce the effects of calcareous chlorosis (Pirmoradian, 2019).



Figure 3. Leaf chlorosis in response to high concentratio of biocarbonae ions in kiwifruit species, *A.chinensis* (a), and *A.deliciosa* (b).

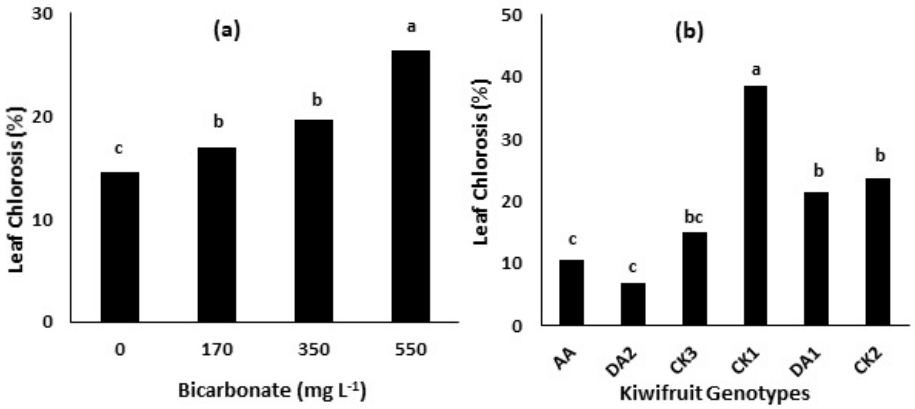


Figure 4. Effect of different bicarbonate contents(a) in irrigation water on leaf chlorosis percentage of different kiwifruit genotypes(b).

Because the high level of bicarbonate (HCO_3^-) inhibits root growth and reduces the transfer of cytokinin from the roots to the shoots. Cytokinin is essential for protein production and chloroplast development and results in chlorosis (Pirmoradian, 2019). Mengel (1994) suggested chlorosis induced by bicarbonate is a result of the transfer of bicarbonate into the central cylinder of the root and alkalinisation of crude sap and leaf apoplasts. In contrast, Romheld (2000) stated that the inactivation of iron in leaves is a secondary effect that occurs after the occurrence of iron chlorosis in leaves. In fact, high concentrations of $HCO3^-$ in the soil lead to a decrease in the uptake and availability of sufficient iron to grow the tree canopy and reduce or stop the growth, and the observation of higher concentrations of iron in chlorinated leaves is the result of reduced growth occurred by bicarbonate. Also, the smaller size of

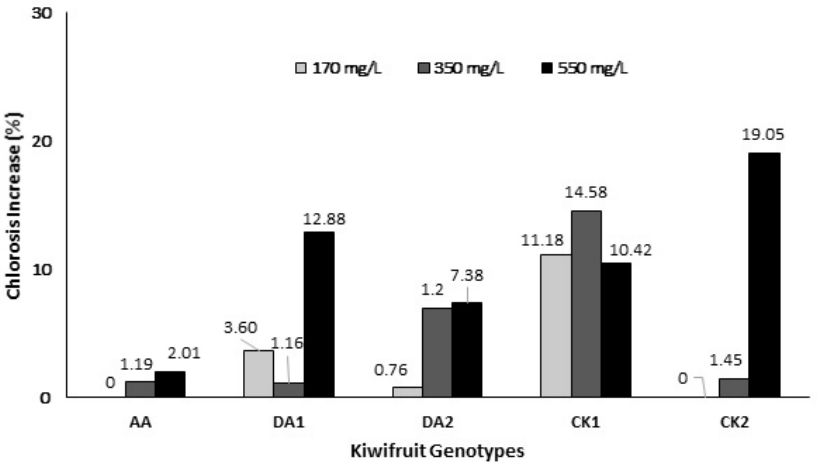


Figure 5. The effect of HCO^{3-} in irrigation water on leaves chlorosis in different kiwifruit genotypes as percent relative to control.

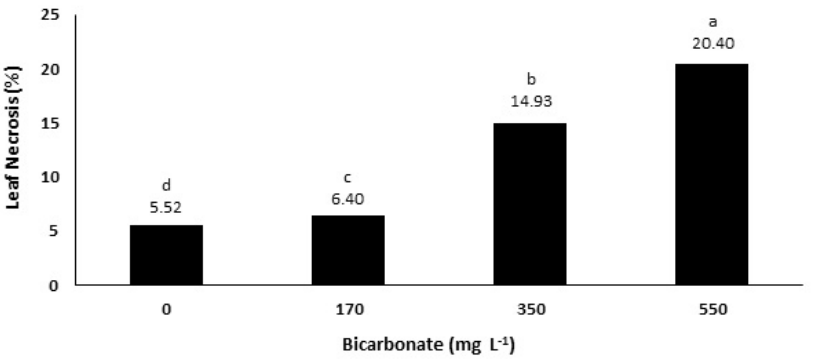


Figure 6. Effect of different bicarbonate contents in irrigation water on leaf necrosis of kiwifruit plants.

chlorinated leaves is due to the limited use of iron, and this issue shows more iron in the texture of chlorinated leaves than green leaves (Pirmoradian, 2019).

3.3 Chlorophylls and carotenoid content

The results showed that with increasing bicarbonate ion levels in irrigation water both total leaf chlorophyll and chlorophyll significantly decreased in seedlings of all kiwifruit genotypes (Figure 7). The lowest total chlorophyll and chlorophyll b were found when seedlings were irrigated with 540 mg L^{-1}.

There was a significant difference between kiwifruit genotypes for total chlorophyll and chlorophyll b content when exposed to bicarbonate stress (Figure 8). The highest total chlorophyll and chlorophyll b was found in AA seedlings from *A. arguta*. In contrast, genotype CK_2 from *A. chinensis* showed the lowest total chlorophyll and chlorophyll b content when exposed to bicarbonate stress.

Similarly, Deng et al., (2010) demonstrated a significant decrease in chlorophyll content in alkaline stressed plants. However, Covarrubias and Rombolà, (2013) indicated that HCO3- increased chlorophyll content in the leaf. Previous studies confirm that bicarbonate stress has been found to lead to a lower photosynthetic rate due to the damaged chlorophyll biosynthesis, which happened because of lower iron solubility and/or low iron translocation in the growing environment (Roosta, 2011). Iron is needed for the biosynthesis of the heme structure (Nikolic and Kastori, 2000), the critical section of chlorophyll pigments. Therefore, if iron is not inadequate or available in plant tissues, chlorophyll synthesis is damaged. In addition to Fe, the decreased chlorophyll content in $HCO3^-$ treatment may be attributed to the decomposition of chlorophyll structure by chlorophyllase enzyme, which catalyses chlorophyll by removing the phytol group (Roosta, 2011).

No significant difference was found between the control and 170 mg L^{-1} bicarbonate on leaf carotenoids content, but thereafter with increasing bicarbonate content carotenoids content significantly decreased (Figure 9a).

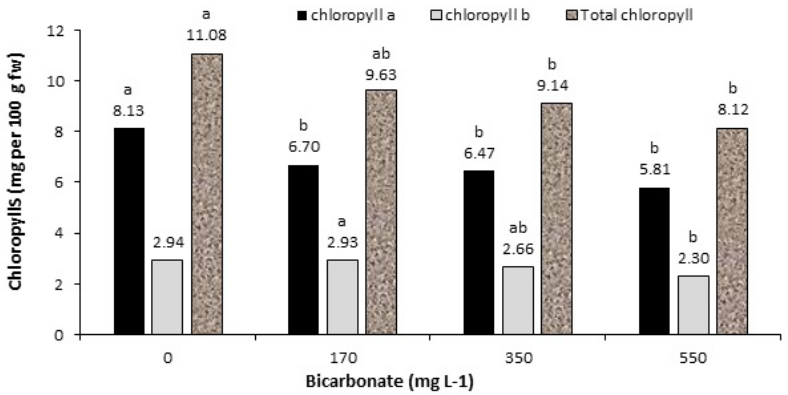


Figure 7. Effect of different bicarbonate contents in irrigation water on leaf chlorophylls content of different kiwifruit genotypes.

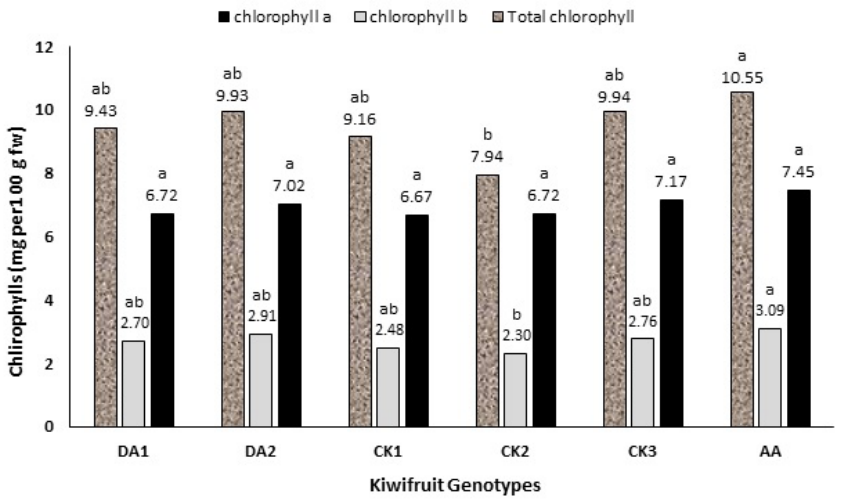


Figure 8. The comparison of kiwifruit genotypes for leaf chlorophylls under bicarbonate treatments.

Therefore, the lowest carotenoid content was found when seedlings were irrigated with 540 mg L^{-1} (1.67 mg/g). There was a significant difference between kiwifruit genotypes for total carotenoid content (Figure 7). The lowest carotenoid content was found in AA (1.6 mg/g FW) and DA_1 (1.7 mg/g FW), respectively. No significant decrease in total carotenoid content was found in the other genotypes (Figure 9b). When bicarbonate content in irrigation water to 170 mg L^{-1}, leaves carotenoids content increased slightly in AA, DA1, and CK2, and decreased in DA2 and CK1 genotypes. But thereafter, with increasing bicarbonate content all genotypes showed a significant suppression in carotenoid synthesis (Figure 10).

Although carotenoid pigments are typically less sensitive to stress than chlorophyll (Shahsavandi and Eshghi, 2021), their content declined just in AA (1.6 mg/g FW) and DA_1 genotypes. Similarly, Shahsavandi and Eshghi, (2021) demonstrated declining in carotenoids in the HCO_3^- stressed-grapevine plants. Unfavourable environmental conditions such as bicarbonate stress leaf carotenoid content decreased, which is a key function in the mechanisms involved in protecting the photosynthetic apparatus (Sabir et al., 2010). When carotenoids are damaged, plants cannot protect themselves against extreme temperatures, scavenging reactive oxygen species, and over excitation in strong light (Shahabi et al., 2005). It seems that by damaging the carotenoids, HCO_3^- actually disables a part of the antioxidant system, thus making the plant more sensitive to abiotic stresses.

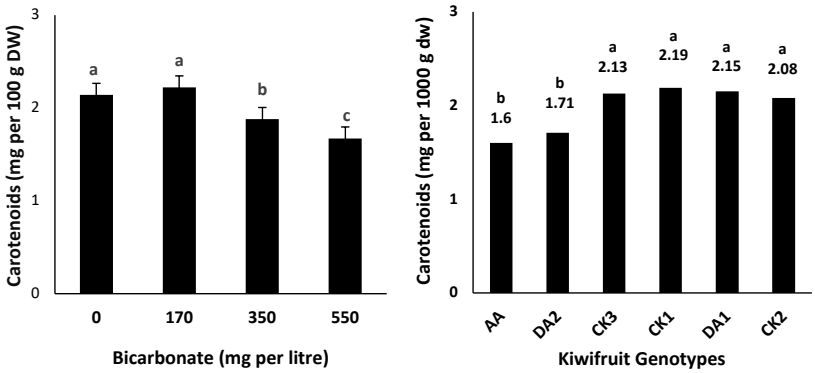


Figure 9. Effect of different bicarbonate contents(a) in irrigation water on leaf carotenoid content of different kiwifruit genotypes(b).

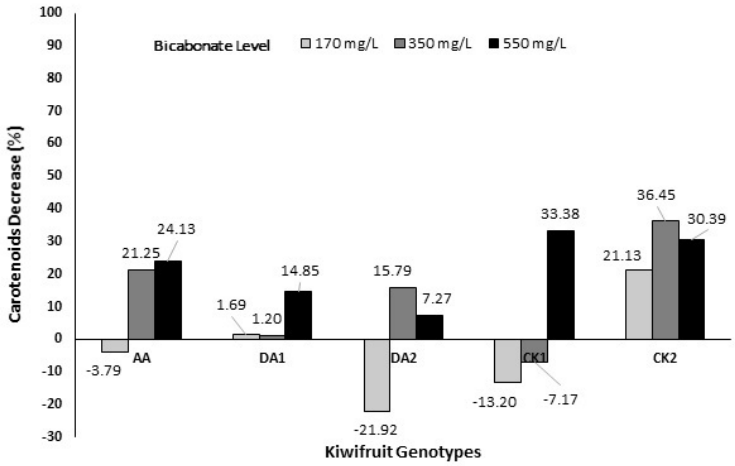


Figure 10. The effect of HCO3$^-$ in irrigation water on leaves carotenoid in different kiwifruit genotypes as percent relative to control.

3.4 Leaf mineral nutrient content

As observed in this study, the leaf iron content of all kiwifruit genotypes significantly decreased with increasing bicarbonate concentration in irrigation water. Seedlings of different genotypes included a range of iron content in the leaves in response to irrigation water HCO_3^- (Table 3). The lowest percentage of iron content reduction in the presence of HCO_3^- was recorded in the DA_1 (Bruno) genotype and the highest rate of iron decline was found in CK_1. In fact, 170, 350, and 550 mg L^{-1} HCO_3^- levels decreased iron by 3.28%, 4.29%, and 4.72%, respectively, in comparison to the control (Table 3; Figure 11a).

In line with our observations, Ksouri et al., (2005) revealed that HCO_3^- stress possesses an adverse effect on iron concentration, which was associated with HCO_3^- concentration and grape genotype. Generally, increasing the HCO_3^- ion concentration declines the iron solubility and hampers the iron uptake by the root and its movement to shoot and leave (Nikolic and Kastori, 2000). There are two opinions endeavoring to elucidate the HCO_3^- -derived Fe decrease. One of these opinions explains that Fe is absorbed however it is inactivated via the root tissue alkalinization; while the other states that HCO_3^- in the root medium impedes iron acquisition (Wang et al., 2020). Overall, increasing HCO_3^- declined the iron accumulation, which happened in both low and high alkalinity (Luis and David, 2008).

With increasing HCO_3^- in irrigation water, the amount of leaf manganese (Mn) was decreased in all genotypes. In similar research, Wang et al., (2019) reported that HCO_3^- treatment reduced Zn accumulation in kiwifruit roots. In a recent study, the lowest reduction percentage of leaf manganese in the presence of HCO_3^- was recorded in the CK3 genotype. In fact, 170, 350, and 550 mg L^{-1} HCO_3^- levels declined Mn accumulation by 3.28%, 4.29%, and

Table 3. The interaction effect of genotype and bicarbonate ion levels on iron, manganese, and zinc content of kiwifruit leaves.

Genotypes	Bicarbonate (mg L^{-1})	Fe (mg kg^{-1} dw)	Mn (mg kg^{-1} dw)	Zn (mg kg^{-1} dw)
AA	0	94.70 [a]	131.32 [c]	56.30 [d]
	170	69.89 [h]	119.84 [d]	50.13 [e]
	350	65.83 [i]	82.21 [j]	45.31 [fg]
	550	65.28 [i]	67.94 [m]	39.61 [h]
DA2	0	84.62 [b]	100.82 [g]	70.66 [a]
	170	70.99 [g]	85.92 [i]	56.32 [d]
	350	65.99 [i]	78.77 [k]	56.44 [d]
	550	58.26 [l]	68.22 [m]	43.57 [g]
CK3	0	78.23 [e]	130.49 [c]	66.05 [b]
	170	64.62 [j]	120.86 [cd]	61.85 [c]
	350	60.50 [k]	115.09 [e]	57.48 [d]
	550	56.80 [m]	110.38 [f]	53.08 [e]
CK1	0	69.81 [h]	84.31 [i]	53.09 [e]
	170	63.76 [j]	81.63 [j]	50.24 [e]
	350	45.96 [n]	78.58 [k]	43.99 [g]
	550	40.46 [p]	59.68 [n]	42.68 [g]
DA1	0	83.81 [b]	147.19 [b]	68.71 [a]
	170	81.09 [c]	108.57 [f]	58.82 [d]
	350	80.23 [c]	101.20 [g]	63.52 [c]
	550	79.88 [d]	94.06 [h]	62.09 [c]
CK2	0	73.00 [f]	151.63 [a]	50.51 [e]
	170	66.16 [i]	101.83 [g]	50.12 [e]
	350	60.92 [k]	101.33 [g]	47.12 [f]
	550	51.43 [n]	72.60 [l]	43.20 [g]

* For each column, values with similar letters are not significantly different ($p < 0.01$).

4.72%, respectively, compared to the control (Table 3; Figure 11b).

The mechanisms by which HCO_3^- leads to such nutrient shortage are very similar to those for iron. HCO_3^- impedes the absorption of Zn by the root and restricts the Zn translocation from root to shoot. Similar to the recent study's findings, a high HCO_3^- level led to a decrement in Mn accumulation in olive seedlings (De la Guardia and Vara, 2002). Mn plays a role as an activator for enzymes responsible for the tri-carboxylic acid cycle and is a necessary trace element for plant growth and development (Jarrahi et al., 2013), therefore HCO_3^- -derived Mn deficiency must be alleviated in kiwifruit.

The results showed that different kiwifruit genotypes decreased zinc (Zn) levels in response to increasing water HCO_3^-. In the DA_1 genotype, zinc content was decreased by 14.40%, 7.54%, and 9.62% at the levels of 170, 350, and 550 mg L^{-1} HCO_3^-, respectively, over the control (Table 3; Figure 11c). In line with our observations, the previous reports indicated that HCO_3^- restricts the Zn uptake by roots and its transfer from root to branch (Shahabi et al., 2005; Çelik et., 2006).

Lower Zn concentration in kiwifruit leaves at high HCO_3^- levels can be a part of the reason for shoot growth decrement at high pH, as reported by Ksouri et al., (2005) in grapevine. Leaf apoplast pH is known as a key factor in modulating the availability of elements such as zinc in the leaves; therefore, HCO_3^- can modulate the Zn accumulation by changing pH in the apoplast (Nikolic and Römheld, 2002).

Altogether, in most of the studied genotypes, manganese was more sensitive to the increasing HCO_3^-

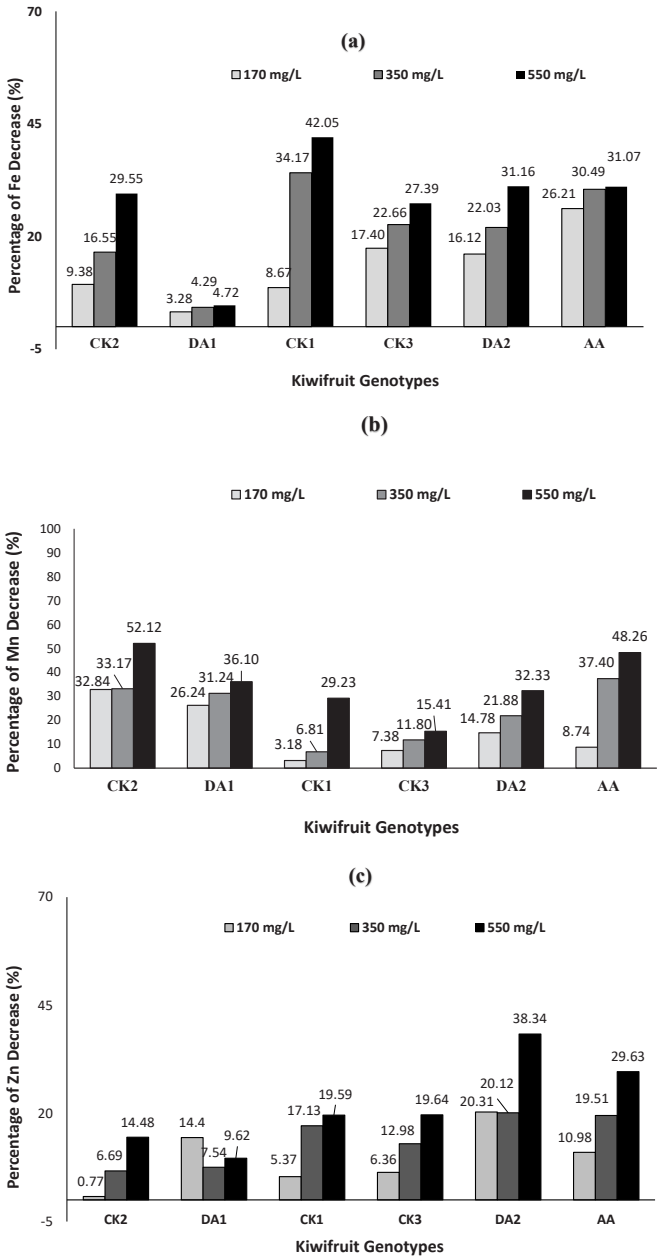


Figure 11. The effect of $HCO3^-$ in irrigation water on decreasing Fe(a), Mn(b), and Zn(c) contents as percent relative to control.

concentrations and had a higher reduction percentage compared to iron and zinc. It seems that the HCO_3^- impact may be derived from the changed expression of several genes involved in Fe acquisition, including *PEPC1*, *HA1*, *FRO2*, *FRO1*, and *IRT1*, and the activity of the corresponding enzymes (PEPCase, H^+- ATPase, and FC-R) (Martínez-Cuenca et al., 2013). Moreover, Wang et al., (2020) exhibited that HCO_3^- slowdowns the release of hemicellulose Fe in the kiwifruit cell wall, which might be associated with the imbalance between C/N metabolisms and low H^+ extrusion. Wang et al., (2019) also reported a decrease in the active Fe as well as an increase in Zn and Mn due to the above-mentioned mechanisms.

5. Conclusions

From our results, the kiwifruit genotypes were found different in their sensitivity to HCO_3^- content of irrigation water. The superiority of 'Bruno' over other genotypes

may be related to maintaining iron and zinc in leaves. Moreover, manganese was found more sensitive to the increasing HCO_3^- concentrations relative to iron and zinc. These findings would assist to monitor the physiological mechanisms of HCO_3^- derived stress and introduce potential material in future breeding schedules. Our observations presented experimental evidence regarding the higher tolerance of Bruno to HCO_3^- stress when compared with other cultivars, and thereby revealed the potential of this genotype in future breeding schedules.

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Author contributions

Methodology, Z.A., M.G.; data curation, Z.A.; writing-original draft preparation, M.G. and A.A; conceptualization, M.G.; investigation, M.G. and A.A; resources, M.G.; writing-review and editing, M.G., and A.A; supervision, M.G.; project administration, M.G.; software, M.F.S.; formal analysis, Z.A, M.G., and M.F.S. All authors have read and approved the final manuscript.

Data availability statement

"Not applicable"

Conflicts of interest

The authors declare no competing financial interest.

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