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Growth and Chemical Composition of Cherry Rootstock Gisela 5 Cultured Under Different Bicarbonate Concentrations *in vitro*

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In vitro response of Gisela 5 cherry rootstock to increasing concentrations of HCO_3^- (0, 5, 10, 20, 30, 40, 50 mM) in MS medium was investigated. With increasing concentration of HCO_3^-, dry biomass and shoot length were negatively affected. At 50 mM HCO_3^- treatment, the reduction of dry biomass production was 48 % whereas shoots length exhibited 4 %. Leaf injury symptoms were more severe at HCO_3^- levels ≥ 20 mM. Viability loss was monitored in explants by increasing HCO_3^- levels. Chlorophyll content (SPAD units) of leaves declined with increasing HCO_3^- concentrations of the culture medium. As for ion concentration, increasing HCO_3^- levels of the culture medium caused K and Ca concentration of explants to decrease, whereas Na, Mg and Mn concentrations increased. When HCO_3^- level increased, the content of Fe did not directly affected. Cu and Zn concentrations in the explants were not significantly changed by HCO_3^- levels.

Key Words: Bicarbonate, Cherry, Tissue culture.

INTRODUCTION

High concentrations of bicarbonate ion in soil solution and around plant roots caused by excessive amounts of calcareous materials is one of the main nutritional problems of fruit orchards. Heavy soil texture, soil compaction, high moisture content, deficient drainage, a high level of microbial activity, as well as a high rate of root respiration would increase the partial pressure of CO_2 in soil which, in turn, would mean high concentrations of HCO_3^- in soil solution[1]. On the other hand, high concentrations of bicarbonate ion in irrigation water are also considered as one of the common factors in increasing the bicarbonate content of soil solutions[2].

The physiological problems caused by bicarbonate ion are quite complicated[1,3,4]. Researchers have presented various mechanisms to explain such problems. One of the obvious effects of excessively high bicarbonate ion concentrations in the root zone is the problem in translocation of

calcium ions from plant roots to shoots and its accumulation in the roots with precipitation as $CaCO_3$. Production and accumulation of organic acids within the roots would be another adverse physiological effect of too much bicarbonate ion in the root zone[1]. Similarly, high concentrations of bicarbonate in the rhizosphere are associated with high pH levels in cytoplasm, high rates of root respiration, cytochrome oxidase enzyme activity and reduction in the chlorophyll content of the leaves[1]. Various researchers[5-7] working with differing concentrations of bicarbonate ion in solution culture found a positive and significant correlation between the extent of leaf chlorosis and bicarbonate ion concentrations. On the other hand, the findings[2] make it imperative to investigate the concentrations of bicarbonate ion in irrigation water that can cause serious nutritional problems in agricultural crops. Shahabi *et al.*[1] investigated the effects of varying concentrations of bicarbonate in the irrigation water on nutritional disorders of some apple cultivars, grown under greenhouse conditions. They concluded that the increasing levels of bicarbonate ion increased the leaf concentrations of N, P and K, but lowered the concentrations of Fe, Mg and Mn and did not affect Ca, Cu, or Zn levels. Brancadore *et al.*[8] investigated the resistance of various peach and grape varieties to bicarbonate ion in solution culture. They concluded that bicarbonate ion caused Fe chlorosis and thus that resistance to Fe chlorosis depends on the variety and also the genetic factors of the plants. In the case of the Mazani variety grown under conditions of high bicarbonate ion concentrations in the rhizosphere, the roots had become more resistant to bicarbonate's adverse effects than the roots developed in a rhizosphere containing Fe but no bicarbonate ion.

Gisela 5 cherry rootstock is among the best dwarfing, precocious and productive rootstocks for modern, intensive sweet cherry growing. Recently it has replaced *Prunus avium* and *Prunus mahaleb* rootstock in cherry growing area[9,10]. Despite its thorough testing in many different scientific investigations, the studies on the responses of Gisela 5 to environmental stresses are limited and the responses of bicarbonate ion remain unclear.

Different techniques and approaches have been developed, alone or in sequence, from the most conventional, as pot or hydroponic culture, to more recent approaches, as *in vitro* culture and molecular investigations[11]. Tissue culture can be an attractive system to unravel the physiological and biochemical aspects undergoing the different genotypic responses *vs.* bicarbonate enriched conditions[11-13]. The objective of this study was to determine the effect of HCO_3^- on dry weight, shoot length, chlorophyll content and chemical composition of *in vitro* produced shoots of Gisela 5 rootstock. The understanding of the physiological and biochemical responses to bicarbonate ion stress in this rootstock can be important for the cherry growing area affected bicarbonate ion.

EXPERIMENTAL

Plant material and growth conditions: Shoot-tips from healthy shoots of 3-year- old plants of Gisela 5 were established on MS (M 5519, Sigma Chemical Co., St. Louis, Missouri, USA) medium supplemented with sucrose (30 g L^{-1}) and solidified with agar (7g L^{-1}) and media pH adjusted to 5.8 before autoclaving (for 20 min at 121ºC). The explants were established and proliferated on the above medium containing 1.0 mg L^{-1} 6-benzylaminopurine (BAP), 0.1 mg L^{-1} indole-3 butyric acid (IBA) and 0.1 mg L^{-1} gibberellic acid (GA_3). The proliferated cultures were subcultured at 4 week intervals and all cultures were maintained at 25 ± 1ºC under a 16 h photoperiod with white light (51 µmol m^{-2} s^{-1}).

Bicarbonate treatments: For HCO_3^- treatment (supplied as $NaHCO_3$), three-node-shoot explants were exposed to increasing concentrations of $NaHCO_3$ (0, 5, 10, 20, 30, 40 and 50 mM) on MS solid medium. According to $NaHCO_3$ concentrations, pH of media was 5.80, 6.95, 7.20, 7.47, 7.70, 7.72, 7.74, respectively. After 4 weeks, the explants were collected and washed for 2 min with distilled water to remove medium, dried on filter paper and either used or dried at 70ºC for later use.

Measurements: The growth response of Gisela 5 to HCO_3^- was measured in terms of both dry weight and shoot length. Dry matter of explants was obtained by heated at 70ºC for 24 h. Data are expressed as per cent of control at each level of HCO_3^-.

The relative chlorophyll content was measured with a portable leaf chlorophyll meter (SPAD 502, Minolta Co. Ltd., Japan) and the data represented were the means of one reading from each explant of each replicate.

Explants were also scored for visible symptoms of bicarbonate injury on a scale, ranging from **1** to **5** (**1**, no symptoms; **2**, light green colour; **3**, green shoot-tip and browning of leaf edge; **4**, intensive brown leaf; **5**, dead). Following this, bicarbonate injury index (BI Index) was calculated according to the following formula:

BI Index = Σ ($n_i \times i$)/N, where n_i is the number of explants receiving the mark i (from **1** to **5**) and N is the total number of explants in each bicarbonate concentration.

To determine the mineral composition, 0.3-0.4 g of previously dried, ground and homogenized plant material was placed in a platinum crucible. Crucible was covered during the ashing as a precaution against contamination. Dry ashing was accomplished in a muffle furnace at 550°C for 6 h. After a 10 min cooling period, the ash was dissolved in 2 mL concentrated HNO_3 (65 %) and heated on a hot plate for 10 min. The digested material was transferred into a volumetric flask and made up to 25 mL with distilled water, which was used for rinsing the platinum crucible. The digested material was diluted to 25 mL with the rinsing solutions prior to analyses.

The concentration of Na, Mg, K, Mn, Ca, Cu, Zn and Fe in the explants were determined by atomic absorption spectrophotometer (Unicam, 929 Model AA).

Statistics: The experiments were set up in a completely randomized design. There were four explants in each 250 mL jar (containing 40 mL medium), 3 jars in each replicate and 3 replicates in each treatment. Analysis of variance was performed on the data and significant differences among treatment means were calculated by LSD test at $p < 0.05$.

RESULTS AND DISCUSSION

Table-1 shows both growth parameters (dry biomass and shoot length) were differently affected by the increasing bicarbonate levels. Dry biomass was markedly reduced in the 50 mM HCO_3^- treatment (shoot dry weight of bicarbonate treated explants was about 48 % of that control plants), while shoot length was slightly decreased. Increases in the concentration of HCO_3^- in the nutrient medium decreased dry weight of shoots in peach[14,15], barley, sorghum and maize[6], tobacco[16], tomato[17] and rice[18]. Whereas, shoot growth was not affected by bicarbonate in lupins[3]. On the other hand, Zribi and Gharsalli[19] reported that the growth response of pea plants to the bicarbonate treatments differed according to the cultivar. Similar results were also reported for grapevine cultivars[20,21].

TABLE-1
EFFECT OF HCO_3^- CONCENTRATIONS ON THE GROWTH, CHLOROPHYLL CONTENTS AND INJURY OF GISELA 5 GROWN *in vitro*

$NaHCO_3$ (mM)	pH of Media	Dry Biomass Production (% of control)	Shoot length (% of control)	Relative chlorophyll content (SPAD)	Leaf Injury Index
0	5.83	100.00 a*	100.00 a	41.74 a	1.00 d
5	6.95	105.00 a	96.87 b	39.30 a	1.27 d
10	7.20	77.66 b	96.76 b	18.49 b	3.05 c
20	7.47	61.53 cd	96.25 b	10.29 bc	4.19 b
30	7.70	60.20 cd	95.62 b	10.16 bc	4.27 b
40	7.72	65.69 c	95.41 b	4.46 c	4.38 ab
50	7.74	52.22 d	95.40 b	0.49 c	4.88 a

*Values not associated with the same are significantly different ($p < 0.05$).

Chlorophyll contents of leaves declined as HCO_3^- concentration of the culture medium increased from 0 to 50 mM (Table-1). Marginal chlorosis was noted on the leaves when HCO_3^- concentration was above 10 mM, which later resulted in brown and dead leaves. Shahabi *et al.*[1] reported that different bicarbonate levels in the irrigation water significantly reduced

chlorophyll development in seedlings of apple cultivars. Ksouri *et al.*[21] found that chlorophyll concentration of leaves of grapevine cultivated on bicarbonate-enriched medium decreased. Similar results were also found in peach rootstocks[11,14].

Leaf injury developed only when HCO_3^- levels reached 10mM. As the HCO_3^- level increased from 10 to 50 mM, the severity of the injury increased. Table-1 indicates that 10 mM HCO_3^- treatment induced green-shoot tip and browning of leaf edge, whereas above 10 mM HCO_3^- stimulated intensive brown leaves. The burned appearance suggested that, in this case, the leaf damage may have been caused with HCO_3^- toxicity, as observed in tobacco plants[16]. Coulombe[22] and Mengel and Geurtzen[23] reported that leaf injury in the $NaHCO_3$ experiment may be attributed to toxicity of HCO_3^-.

Table-2 shows the ion concentration of the explants treated with increasing bicarbonate concentrations in the culture medium. K concentration declined markedly as HCO_3^- concentration increased. Similar findings were observed in peach rootstock[14]. K is an essential cation for plants and is required for the regulation of ion transport and osmotic regulation[24]. Ca concentration increased initially as HCO_3^- levels increased, but decreased again at the two highest levels of HCO_3^-. Decreasing Ca concentration with increasing HCO_3^- level was reported in peach, lettuce and celery[14,24,25]. As mentioned, bicarbonate effects may have appeared as decreases in the rates of absorption, as translocation of nutrients, or as reduced activities of those elements.

TABLE-2
EFFECTS OF HCO_3^- ON ION CONCENTRATIONS (mg/g)
OF GISELA 5 GROWN *in vitro*

$NaHCO_3$ (mM)	K	Ca	Na	Mg	Mn	Fe	Cu	Zn
0	31.75 a*	5.91 d	1.20 e	1.38 c	0.121 b	0.231 b	0.008	0.095
5	27.94 a	6.01 d	1.33 de	1.23 c	0.137 ab	0.231 b	0.006	0.074
10	18.79 b	6.66 cd	1.99 d	1.49 c	0.129 b	0.339 a	0.008	0.120
20	17.10 bc	8.47 ab	3.02 c	1.87 b	0.157 a	0.198 b	0.007	0.127
30	15.44 bc	9.72 a	4.32 b	2.29 a	0.155 a	0.217 b	0.005	0.096
40	16.50 bc	7.80 bc	4.70 b	2.09 ab	0.148 ab	0.217 b	0.004	0.079
50	14.10 c	6.98 cd	5.65 a	1.95 ab	0.159 a	0.339 a	0.005	0.103

*Values not associated with the same are significantly different ($p < 0.05$).

Increased HCO_3^- caused significant increases in the Na, Mg, Mn content of the explants (Table-2). Increase in Na contents of the explants may be attributed to the increases in $NaHCO_3$ concentration of the medium. Similar results were found by Bie *et al.*[24]. Shi *et al.*[14] and Pearce *et al.*[26] demonstrated that excess HCO_3^- did not change Mg content in peach

and tobacco leaves. Cinelli and Viti[27] found that so high bicarbonate conditions did not affect foliar Mn content in the Myrobalan clones and GF 677 rootstock. But, Shahabi *et al.*[1] and Shi *et al.*[14] reported that increased HCO_3^- concentrations resulted in lower concentrations of Mn in leaves. The results of the present study do not agree with the above research findings. Higher Mn concentrations are usually found in chlorotic plants grown in calcareous soils[28,29] and Mn has been reported to inactivate Fe metabolic activity by decreasing Fe^{2+} concentrations and resulting in lower chlorophyll concentrations[30].

Fe concentration of the explants did not decrease linearly as the HCO_3^- level increased (Table-2). The higher Fe concentrations in the 10 mM and 50 mM HCO_3^- were an unexpected results since Fe content was generally reported to decrease with increasing bicarbonate concentrations. Many researchers[8,14,31] reported that high HCO_3^- ion concentration makes Fe insoluble or hampers Fe uptake, possible also affecting translocation of this element within the plant itself. Brown and Jolley[32] reported that there was no correlation between Fe chlorosis in grapes and high bicarbonate concentrations. Pearce *et al.*[26] demonstrated that Fe concentration in the tobacco seedlings was not significantly affected by HCO_3^-. Cu and Zn concentrations in the explants were not significantly affected by HCO_3^- levels (Table-2). Similar results were found working with apple seedling[1].

In conclusion, in Gisela 5 rootstock, tissue culture technique was used as a rapid screening method to identify growth rate, chlorophyll content and ion accumulation associated with bicarbonate enriched conditions. Affected by HCO_3^- Gisela 5 explants grown *in vitro* showed the typical necrosis within few days after the beginning of the experiments. Necrosis started old leaves as yellowing and evolved in a complete browning of the entire shoot. Viability loss was observed in Gisela 5 due to HCO_3^-. By increasing HCO_3^- concentrations of the culture medium Ca and K concentrations of explants decreased, whereas Na, Mg and Mn concentrations increased. This is the first report documenting some physiological and biochemical responses of Gisela 5 to HCO_3^-. Further research on this subject should be conducted to clarify the effects of HCO_3^-.

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