

Effects of Alkali Stress and Growing Media on Growth and Physiological Characteristics of Gerbera Plants

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ABSTRACT

In order to determine the best growing media for *Gerbera jamesonii* under alkaline conditions, a factorial experiment was conducted with two factors, namely: (i) Growing medium, and (ii) Concentrations of bicarbonate (NaHCO_3) in nutrient solution. Results showed that increasing the concentration of NaHCO_3 from 0 to 40 mM in nutrient solution significantly decreased plant growth, maximal quantum yield of PSII photochemistry (F_v/F_m), photosynthesis Performance Index (PI), Glutamine Synthetase (GS) activity, Leaf Relative Water Content (LRWC), Chlorophylls (Chl a, b and total Chl) and carotenoids. Under alkalinity stress, reduction in vegetative growth, F_v/F_m , PI, LRWC, GS activity and photosynthesis pigments content was the lowest in Coconut Fiber (CF) substrate compared to the other substrates. Furthermore, plants grown in CF substrate had higher soluble sugars and proline content than those in other substrates. On the other hand, plants grown on Perlite (P) substrate had the highest reduction in growth and physiological characteristic in alkaline conditions. The alleviation of alkalinity-induced growth inhibition of plants by CF substrate may be related to improvement of photosynthesis, enhancement of GS enzyme activity and osmotic regulation. It is concluded that the use of CF substrate could provide a useful tool to improve alkalinity tolerance of gerbera plants under NaHCO_3 stress.

Keywords: Alkalinity, Glutamine synthetase activity, Growing media, Osmotic adjustment, Photosynthetic pigments.

INTRODUCTION

The availability of good quality water for agricultural use is becoming scarce (Shannon *et al.*, 2008). Water quality is an important factor for production of greenhouse crops (Valdez-Aguilar and Reed, 2007). Alkalinity is the most important water quality parameter because of its impact on soil or growing medium solution pH (Petersen, 1996). Bicarbonate (HCO_3^-) and Carbonates (CO_3^{2-}) are the main ions that cause alkalinity of irrigation water. Water with high alkalinity could adversely influence the pH of the growing medium,

destroy the root cell structure, interfere with nutrient uptake, and cause nutrient deficiencies which reduce plant growth (Chen *et al.*, 2011; Yang *et al.*, 2009). High bicarbonate-induced alkalinity has been reported to cause leaf chlorosis, which is usually associated with Fe deficiency due to a reduction in Fe availability at high pH (Alhendawi *et al.*, 1997; Römheld, 2000). The most obvious effect of Fe chlorosis is a decrease in photosynthetic pigments, resulting in a relative enrichment of carotenoids over Chlorophylls (Chl), and leading to the yellow color characteristic of chlorotic leaves (Abadia and Abadia, 1993, Morales *et al.*, 1998). Valdez-Aguilar and

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Reed (2007) estimated a 10% decrease in chlorophyll concentration in vinca plants when irrigation water contained 6.8 mM NaHCO_3 . The reduction in chlorophyll concentration is often accompanied by a marked reduction of chlorophyll fluorescence levels (Nedunchezian *et al.*, 1997), and by a reduction in photosynthesis rate (Marschner, 1995; Molassiotis *et al.*, 2006). Abiotic stresses that affect PSII efficiency lead to a characteristic decrease in F_v/F_m (Krause and Weis, 1991). The F_v/F_m is a measure of the light energy transfer in dark adapted samples or the photochemical quantum yield of open PSII centers (De Ell and Toivonen, 2003). Reduction in photosynthesis performance index (PI) by bicarbonate treatment in tomato has been reported by Mohsenian *et al.* (2012). PI is a more complex parameter reflecting overall efficiency of light absorption as well as both light and dark redox reactions (Strauss *et al.*, 2006). Therefore, PI is a potential indicator of current physiological status of a plant, reflecting the disturbance of photosynthetic apparatus by environmental stresses (Clark *et al.*, 2000). Mohsenian *et al.* (2012) reported that high alkalinity (10 mM NaHCO_3) treatment induced significant decreases in LRWC in the stressed tomato plants compared with those in the control plants. Reduction in leaf RWC indicates loss of turgor that resulted in limited water availability for cell extension process (Katerji *et al.*, 1997) and consequently causes plant growth reduction (Marschner, 1995).

Significant decrease in plant growth due to alkalinity stress has been reported for tomato (Wang *et al.*, 2011), barley (Yang *et al.*, 2009), wheat (Yang *et al.*, 2008) peach (De La Guardia and Alcantara, 2002), cotton (Chen *et al.*, 2011), and rose (Cartmill *et al.*, 2007). The alkalinity tolerance of plants depends on plant species, the age of the plant, type and volume of growing medium (Whipker *et al.*, 1996), length of the crop period, and buffering capacity of growing medium (Kessler, 1999). Thus, the use of suitable substrate for potted plant production

may reduce losses in plant production caused by alkalinity.

No information was found about the effects of high alkalinity in the rooting medium on growth, physiological, and biochemical responses of gerbera in different substrates. Therefore, the main objective of this study was to compare growth characteristics of gerbera plants in different substrates under alkali stress.

MATERIALS AND METHODS

Plant Material, Treatments, and Growth Conditions

The experiment was conducted in a greenhouse at the Agri-college of Vali-e-Asr University of Rafsanjan (30° 23' 06" N, 55° 55' 30" E), at 1,523 m asl. Seedling of gerbera (*Gerbera jamesonii* L. cv. Dafne) plants were produced by tissue culture preparation (Florist Schrusse Company, the Netherlands), then, at the four-leaf stage, they were transferred into pots filled with different substrates. The plants were grown in a greenhouse with 11 hours light phase (at 24±2°C) and 13 hours dark phase (at 20±2°C). Greenhouse temperature was controlled using cool air flowing into greenhouse from central cooler. The relative humidity was 57.4–68.2%. Seven different substrates were used to study the effect of alkali stress on the growth characteristic of gerbera. The substrates studied were as follows: (v/v, 100% Coconut Fiber (CF), 75% Vermicompost+25% Perlite (VP), 25% Zeolite+75% Perlite (ZP), 75% Peat+25% Perlite (PP), 75% Coco Chip+ 25% Perlite (CCP), 75% Coconut Fiber+25% Perlite (CFP) and 100% Perlite (P). Plants were grown in 4 L pots that were filled with the growing media. The basic nutrient solution used in the experiment was a modified Hoagland and Arnon formulation. This nutrient solution consisted of: 5 mM $\text{Ca}(\text{NO}_3)_2$, 6 mM KNO_3 , 1 mM KH_2PO_4 , 2 mM MgSO_4 , 0.1 mM NaCl , 20 μM Fe-EDDHA, 7 μM MnSO_4 , 0.7 μM ZnCl_2 , 0.8

μM CuSO_4 , $2 \mu\text{M}$ H_3BO_3 , and $0.8 \mu\text{M}$ Na_2MoO_4 . One plant was grown in each pot and was irrigated three times a day with 200 mL of this solution due to: (i) Adequate assurance of leaching from the bottom of pots, and (ii) Avoidance of salt accumulation. Solutions were prepared 24 h before use to allow pH stabilization. pH's were recorded before renewal. Average initial pH's were 7.1, 8.1, and 8.4 for solutions containing 0, 20, and 40 mM NaHCO_3 , respectively. Three weeks after transplanting, sodium bicarbonate (NaHCO_3) was added to nutrient solutions in different concentrations of 0, 20 and 40 mM. The solutions were renewed every two days. The experiment was conducted for 150 days.

Substrate Characterization

Physical properties of the substrates such as total porosity, air-filled porosity, shrinkage, bulk density, and container capacity were determined according to Raviv and Lieth (2008). Electrical conductivity and pH were determined by a conductivity meter (Model AZ, 86503) and a pH meter (Model WTW LF 90) in an extract with substrate: water ratio of 1:2 after shaking the suspension at 2.5 Hz in an end-over-end shaker for 2 hours.

Plant Growth Measurement

At the end of the experiment, the leaf number produced for each treatment was recorded. The plant roots and shoots were harvested and weighed for determination of Shoot Fresh Mass (SFM) and Root Fresh Mass (RFM).

Physiological Indices Measurements

Physiological indices were determined at the end of the experiment. Chlorophylls (Chl a, b and total Chl) and carotenoids were extracted with 80% aqueous acetone (v/v) and were quantified using Arnon (1949) method. After filtering, absorbance of centrifuged extracts was measured at 480, 510, 645, 652 and 663

nm using a spectrophotometer (U-2000, Hitachi Instruments, Tokyo, Japan).

Second leaves from top (young leaves) were used for the measurement of maximal quantum yield of PS II photochemistry (F_v/F_m) and Performance Index (PI) using a Plant Efficiency Analyzer, Handy PEA (Hansatech Instruments Ltd., Norfolk, UK). Leaves were maintained in darkness for 15 minutes before taking the data on chlorophyll fluorescence. The fully expanded fourth leaf from the top was used for measuring Leaf Relative Water Content (LRWC) as described by Weatherley (1950) and calculated according to the formula:

$$\text{LRWC} = [(FM - DM) / (FM \text{ at full turgor} - DM)] \times 100,$$

Where, *FM* and *DM* are Fresh and Dry Mass, respectively.

Leaf soluble sugar content was measured according to Irigoyen *et al.* (1992). Free proline contents in leaves and roots were determined according to Bates *et al.* (1973).

For determination of glutamine synthetase activity, frozen organ tissue was pulverized in a mortar under liquid nitrogen. Glutamine Synthetase (GS, EC 6.3.1.2) was extracted with TriEthanolAmine (TEA) 100 mM, EDTA 1 mM, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 10 mM, glutamate 5 mM, glycerol 10% v/v, triton X100 0.1% and DiThioThreitol (DTT) 6 mM. After centrifugation (21,000 \times g, 15 minutes, 4°C) enzyme activity was assayed for 30 minutes at 30°C in TEA 100 mM, glutamate 70 mM, hydroxylamine, HCl 6 mM, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 20 mM, EDTA 4 mM, ATP 10 mM and terminated by addition of acidic Fe(III)Cl_3 . The γ -glutamylhydroxamate produced was quantified spectrophotometrically (Finnemann and Schjoerring, 1998).

Statistical Analysis

The two-way Analysis Of Variance (ANOVA) was performed using the SAS



program. If ANOVA determined that the effects of the treatments were significant ($P \leq 0.05$ for F-test), then the treatment means were separated by *LSD* test.

RESULTS AND DISCUSSION

Physical and Chemical Characteristics of the Media

The physical and chemical characteristics of the media are described in Table 1. The highest values of porosity were observed in CFP (73%) and CF (71%) substrates. An increase in total pore space will often increase oxygen transport and increase root penetration, these, in turn, will influence plant growth (Hernandez-Apaolaza and Guerrero, 2008). CF substrate also had the highest values of container capacity (54.41%) and shrinkage (25%) as compared to other growing media. Differences in container capacity among media could be due to their total porosity and types of pores. All the substrates showed acceptable values of shrinkage according to the suggested reference level ($< 30\%$ vol) (Abad *et al.*, 2001). Shrinkage may be desirable in some transplant mixes used in tray cells as the plugs are to shrink loose from the surrounding tray which facilitates transplanting (Raviv and Lieth, 2008). The highest (39.35%) air-filled porosity was measured in ZP substrate. However, the lowest (16.59%) values of air-filled porosity

were recorded on CF substrate. VP substrate exhibited the highest (0.36 g cm^{-3}) amount of bulk density, whereas CCP exhibited the lowest (0.07 g cm^{-3}) amount of bulk density. High value of bulk density has the disadvantage of increasing the transportation costs and reducing porosity and air capacity (Corti *et al.*, 1998). The pH of PP substrate was higher (8.1) than that of the other media. The lowest pH (6.2) recorded was related to the CF substrate. The pH and EC values of leached solutions over time showed changes between substrates and NaHCO_3 levels (data not shown). At the end (day 150) of the experiment and in the highest NaHCO_3 concentration (40 mM) tested, leached solution pH increased about 1.89, 1.33, 2.27, 2.21, 2.82, 2.95 and 2.34 pH units in VP, PP, CCP, CF, CFP, P, and ZP substrates, respectively, compared to the control. Except for CF substrate, the pH of the studied substrates exceeded the acceptable limit for an ideal substrate (Abad *et al.*, 2001). According to Abad *et al.* (2001), the optimal pH range of media and mixes for growing ornamental plants in containers is 5.3–6.5. Electrical Conductivity (EC) of the substrates increased with increase in NaHCO_3 level. Electrical conductivity showed a general tendency to increase from day 15 to day 150. The highest EC values were found in treatment 40 mM NaHCO_3 with PP substrate (data not shown). Except for perlite substrate, EC values of the other substrates were higher than the suggested reference level ($\leq 0.5 \text{ dS m}^{-1}$) (Abad *et al.*, 2001).

Table 1. Physical and chemical properties of the studied growing media.

Substrate ^a	Porosity (%)	Air-filled porosity (%)	Container capacity (%)	Bulk density (g cm^{-3})	Shrinkage (%)	pH	EC (dS m^{-1})
VP	59	21.09	37.91	0.36	16.6	7.5	4.04
PP	62	17.35	44.65	0.27	10	8.1	4.35
CCP	64	35.12	28.88	0.07	20	6.7	1.4
CF	71	16.59	54.41	0.11	25	6.2	1.1
CFP	73	27.16	45.84	0.14	20	6.6	0.74
P	52	24.4	27.60	0.13	13.3	7.25	0.12
ZP	59	39.35	19.65	0.30	16.6	7.4	0.67

^a 100% Coconut Fiber (CF), 75% Vermicompost+25% Perlite (VP), 25% Zeolite+75% Perlite (ZP), 75% Peat+25% Perlite (PP), 75% Coco Chip+25% Perlite (CCP), 75% Coconut Fiber+25% Perlite (CFP) and 100% Perlite (P).

Effect of Substrate and Sodium Bicarbonate on Plant Growth

Researchers have demonstrated that plants respond to elevated NaHCO_3 concentrations in soil or in growing medium solution with decreased shoot and root growth (Alhendawi *et al.*, 1997; Campbell and Nishio, 2000). This might be due to either HCO_3^- or Na^+ . Sugar beet (Campbell and Nishio, 2000), tomato (Mohsenian *et al.*, 2012), rose (Cartmill *et al.*, 2007), vinca, chrysanthemum, hibiscus, rose, ivy

geranium (Valdez-Aguilar and Reed, 2007), exhibited stunted growth when growing in either soil or nutrient solution containing a high concentration of HCO_3^- . In the present experiment, significant depression in plant growth parameters in bicarbonate treated gerbera plants was observed, and that effect varied as a function of substrate (Table 2). The results obtained from this experiment showed that the shoot and root fresh mass and leaf number were highly influenced by NaHCO_3 , substrate, and their interaction (Table 2). Shoot and root fresh mass and leaf number decreased significantly in response

Table 2. Interactive effects of NaHCO_3 levels and different substrates on Shoot Fresh Mass (SFM), Root Fresh Mass (RFM), leaf number, proline, and Glutamine Synthetase (GS) activity ($\mu\text{mol glutamyl hydroxamat g}^{-1} \text{FM h}^{-1}$) in leaf and root of studied plants.^a

Substrate ^b	NaHCO_3 (mM)	SFM (g plant ⁻¹)	RFM (g plant ⁻¹)	Leaf number (leaf plant ⁻¹)	Proline ($\mu\text{mol g}^{-1} \text{FM}$)	Leaf GS activity	Root GS activity
VP	0	24.12 gh [†]	10.02 f-i	21.25 e	33.82 jkl	8.20 d	3.45 bc
	20	8.33 j-m	7.33 jkl	8.75 ghi	54.54 f-i	6.19 ghi	2.87 fgh
	40	5.65 m	7.06 kl	5.50 j	76.48 cde	4.45 j	2.19 j
PP	0	17.18 hi	11.48 efg	10.00 gh	29.76 l	7.26 ef	3.15 de
	20	13.06 i-l	10.36 fgh	7.25 hij	52.44 f-k	5.62 i	2.69 h
	40	8.88 j-m	8.08 i-l	5.25 j	68.36 def	3.65 k	1.95 k
CCP	0	42.69 e	9.42 g-j	20.75 e	32.70 kl	7.45 e	3.45 bc
	20	14.62 ij	8.58 h-k	10.00 gh	53.17 f-j	5.61 i	2.71 gh
	40	7.57 klm	5.88 lm	8.75 ghi	82.60 cd	3.78 k	2.25 ij
CF	0	137.25 a	25.58 a	43.75 a	43.95 g-l	10.91 a	3.97 a
	20	121.75 b	21.56 b	38.50 b	121.75 c	9.42 bc	3.59 b
	40	112.25 c	20.26 b	32.50 c	243.36 a	8.92 c	3.26 cd
CFP	0	106.25 ab	16.43 c	38.25 b	35.56 i-l	9.81 b	3.59 b
	20	65.75 d	9.36 g-j	24.25 d	62.80 efg	6.77 fg	2.83 fgh
	40	36.25 ef	6.71 kl	16.00 ef	117.70 b	4.90 j	2.43 i
P	0	36.26 ef	12.17 def	15.00 f	33.87 jkl	7.81 de	3.42 bc
	20	16.92 i	9.92 ghi	11.00 g	42.60 h-l	5.70 hi	2.93 fg
	40	5.44 m	4.42 m	5.00 j	65.81 def	3.33 k	1.74 l
ZP	0	29.91 fg	14.25 cd	14.25 f	35.56 i-l	9.44 bc	3.45 bc
	20	13.80 ijk	13.22 de	8.75 ghi	60.56 e-h	6.32 gh	3.03 ef
	40	6.53 lm	8.14 h-k	6.50 ij	88.57 c	4.65 j	2.31 ij
ANOVA	df	Mean square					
Substrate	6	21292.77**	308.12**	1527.32**	9089.48**	26.95**	1.29**
NaHCO_3	2	6584.34**	214.88**	1034.82**	36422.00**	106.69**	9.97**
Substrate \times NaHCO_3	12	397.41**	9.90**	39.58**	3918.70**	1.18**	0.12**

^a Mean separation was done by the LSD test and the same letter(s) in each column indicates non-significant difference at $P < 0.05$. ^b The Substrates are: 100% Coconut Fiber (CF), 75% Vermicompost+25% Perlite (VP), 25% Zeolite+75% Perlite (ZP), 75% Peat+25% Perlite (PP), 75% Coco Chip+25% Perlite (CCP), 75% Coconut Fiber+25% Perlite (CFP) and 100% Perlite (P). ** Significant ($P \leq 0.01$).



to an increase of alkalinity (high-pH) in the nutrient solution. This is in agreement with the results of Campbell and Nishio (2000) who reported the shoots and roots biomass of plants decreased with increasing NaHCO_3 concentrations in soil or in growing medium solution. Plant species and cultivars may differ in their tolerance to HCO_3^- stress. Root physiology and nutrient solubility are affected by the buffering capacity of HCO_3^- , which is related to an increase in substrate pH (Cartmill *et al.*, 2007). High-pH stress can lead to lack of protons, the destruction or inhibition of transmembrane electrochemical-potential gradients in root cells, damage contents of photosynthetic pigments and the loss of normal physiological root functions such as absorption of water and ions (Wang *et al.*, 2011). This may be the main reason explaining that growth of gerbera under alkalinity stress was less than unstressed. Plants grown on CF substrate showed the lower reduction in vegetative growth than those grown into other substrates under the same alkalinity stress (Table 2). Also, under alkalinity treatments, plants grown on Perlite (P) substrate had the highest reduction in growth characteristic in comparison with

plants grown in normal conditions. Alkalinity tolerance of gerbera plants grown in CF substrate might be due to the enhanced water and ions uptake and leaf chlorophyll concentration.

Effect of Substrate and Sodium Bicarbonate on Physiological Indices

Increasing the concentration of NaHCO_3 from 0 to 40 mM in the nutrient solution significantly increased the proline content in leaves (Table 2). The highest proline value ($243.36 \mu\text{mol g}^{-1}\text{ FM}$) was recorded in plants grown in the CF substrate at 40 mM NaHCO_3 level. Soluble sugars content was significantly ($P < 0.01$) affected by NaHCO_3 and substrate, but not by their interaction. The highest soluble sugars values ($1,400 \mu\text{g g}^{-1}$) were observed in plants grown on CF substrate (Figure 1). As shown in Figure 1, the lowest soluble sugars values ($1,225 \mu\text{g g}^{-1}$) were observed in plants grown on PP substrate. Regarding the effect of sodium bicarbonate on soluble sugars content, increasing the concentration of NaHCO_3 from 0 to 40 mM in the nutrient solution decreased the soluble sugars content

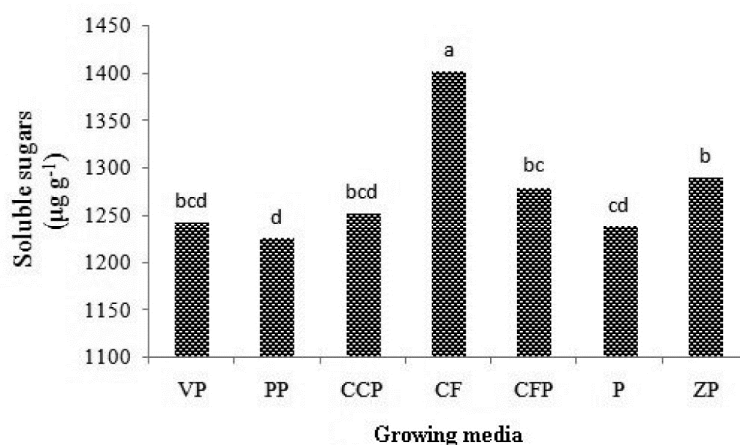


Figure 1. Effect of the different substrates [100% Coconut Fiber (CF), 75% Vermicompost+25% Perlite (VP), 25% Zeolite+75% Perlite (ZP), 75% Peat+25% Perlite (PP), 75% Coco Chip+25% Perlite (CCP), 75% Coconut Fiber+25% Perlite (CFP) and 100% Perlite (P)], on soluble sugars contents of gerbera plants. Different letters indicate significant differences according to *LSD* test ($P < 0.05$).

significantly, however, differences between the two levels of NaHCO_3 (20 and 40 mM) were not significant (Figure 2). During stress conditions, plants need to maintain internal water potential below that of nutrient solution and maintain turgor and water uptake for growth (Ahmad and Sharma, 2008). This requires an increase in osmotically active solutes either through uptake of inorganic ions or synthesis of metabolically compatible solutes (Munns and Tester, 2008). At the same time, plants also synthesize compatible low molecular weight organic solutes, such as betaine, proline, free sugars and polyalcohols in the cytoplasm to prevent cytoplasm dehydration (Wang *et al.*, 2011). Proline increased with increasing concentration of NaHCO_3 in the present study (Table 2). These results corroborated those previously obtained for tomato (Wang *et al.*, 2011), mulberry (Ahmad and Sharma, 2010) and barley plants (Yang *et al.*, 2009). Proline is considered to be a compatible solute. At high concentrations, compatible solutes function in osmotic adjustment (Colla *et al.*, 2010). In the present study, the highest proline content was measured in the plants grown on CF substrate treated with 40 mM of NaHCO_3 . These findings agree with the results of the experiment conducted by Yang *et al.* (2009). Additionally, in accordance with the present result, Ganeg Don *et al.*

(2010) also reported that gerbera plants had higher proline content under sodium chloride (NaCl) stress. Soluble sugars are considered to be a compatible solute and their major functions are osmoprotection, osmotic adjustment, carbon storage, and radical scavenging (Qun *et al.*, 2010). The soluble sugars values also decreased with increasing concentration of NaHCO_3 in nutrient solution (Figure 2). Yang *et al.* (2009) proposed that the decreased content of soluble sugars after 60 mM NaHCO_3 treatment was not a response to osmotic stress or ion toxicity, and that the high-pH might result from abnormal metabolism caused by intercellular ion imbalance from damage to root function by high alkali-stresses. In the present study, plants grown on CF substrate had higher soluble sugars and proline content in the leaves compared to plants grown on other substrates. Benefits of accumulation of soluble sugars and proline mentioned above might be part of the reason for the increased alkalinity tolerance of gerbera grown on CF substrate. Under the same alkalinity stress, the Glutamine Synthetase (GS) activity of plants grown on CF substrate was significantly higher than that of plants grown in the other substrates. The GS activity in leaves and roots of gerbera plants decreased significantly as NaHCO_3 levels increased (Table 2). The highest reduction in GS

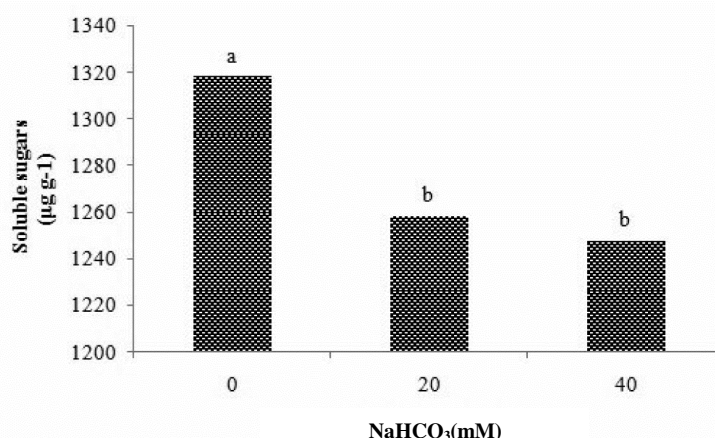


Figure 2. Effect of the different NaHCO_3 concentrations on soluble sugars content of gerbera plants. Different letters indicate significant differences according to *LSD* test ($P < 0.05$).



activity of the leaves and roots were observed in plants grown on perlite substrate treated with 40 mM NaHCO_3 . In higher plants, GS is a key enzyme involved in the assimilation of inorganic nitrogen into organic forms (Teixeira *et al.*, 2005). GS expression is affected by biotic and abiotic factors such as salinity and drought (Teixeira and Pereira, 2007), Na_2SO_4 stress (Santos *et al.*, 2002), nitrogen deficiency (Finnemann and Schjoerring, 1998) and microbe infection (Perez-Garcia *et al.*, 1998). GS is an octameric enzyme, contains bound Mg^{2+} in its structure. Mg^{2+} is essential for the GS activity (Kertész *et al.*, 2002). Bicarbonate ions interfere with the uptake of macro elements, in particular P, K, and Mg (Pissaloux *et al.*, 1995). In the current study, decreasing GS activity may be due to reduction of Mg and K concentrations under alkalinity condition. On the other hand, we propose that the higher GS activity of gerbera plants grown in CF substrate may be due to the better uptake of Mg and K (data not shown). Roosta and Schjoerring (2008) showed that high K^+ supply enhanced total C and stimulated GS and PEPCase activities in leaves and roots of cucumber plants. The Chlorophylls (Chla, Chlb, and total Chl) and Carotenoids (Car) contents were significantly affected by NaHCO_3 levels, substrate combination and $\text{NaHCO}_3 \times$ substrate interaction (Table 3). The chlorophylls contents decreased in response to an increase of alkalinity (high-pH) in the nutrient solution, while at 40 mM NaHCO_3 in the nutrient solution, plants grown in CF substrate showed the lowest percentage decrease in chlorophylls contents among those grown in the other substrates (Table 3). Except for plants grown on CF substrate, the addition of bicarbonate induced a significant reduction in carotenoid contents. In the plants grown in CF substrate, measured carotenoid contents were not significantly affected by bicarbonate application (Table 3). Chlorophyll and carotenoid are the main photosynthetic pigments of higher plants. Our results are in agreement with several

reports of decreased contents of chlorophylls and carotenoids under alkaline stress as reported for a number of plant species (Yang *et al.*, 2009; Yang *et al.*, 2011). Under high levels of alkalinity, the contents of Chl and Car in the barley plants decreased sharply with increased alkalinity stress in comparison to salinity stress (Yang *et al.*, 2009). These results indicate that high pH might decrease contents of photosynthetic pigments. The decreasing photosynthetic pigment concentrations under alkaline conditions may be attributed to enhanced activity of the Chl-degrading enzyme chlorophyllase (Reddy and Vora, 1986), the precipitation of Mg^{2+} in high pH, hence inhibiting Chl synthesis (Shi and Zhao, 1997), the disturbance of the balance of certain ions (*e.g.* Na^+) (Elstner, 1982) and iron deficiency (De La Guardia and Alcantara, 2002). Iron is essential for the proper functioning of multiple metabolic and enzymatic processes such as electron transport, nitrogen fixation, Chl biosynthesis and photosynthesis during plant growth and development (Briat, 2007; Jeong and Guerinot, 2009). The solubility of Fe is known to decrease by the increase of pH associated with increasing concentrations of carbonates (Bloom, 2000). The reduction in Chl concentration during the growing season can reduce plant growth, vigor, and tolerance to stress conditions (Yang *et al.*, 2011). In the present study, higher pigments in plants grown in CF substrate could be due to the high ability of this substrate in providing Fe and Mg for plant under alkaline conditions (data not shown). Chlorophyll fluorescence analysis has proven to be a sensitive method for the detection and quantification of changes induced in the photosynthetic apparatus (Mehta *et al.*, 2010). The activity of PSII was investigated in the present study using chlorophyll fluorescence technology. Except for plants grown on CF substrate, in the other substrates, an obvious decrease in F_v/F_m and PI values was observed with 20 mM NaHCO_3 compared with plants grown under unstressed conditions (Table 3).

Although at 40 mM NaHCO₃ level, F_v/F_m and PI values decreased significantly in all plants compared with plants grown under unstressed conditions, plants grown in CF substrate showed the lowest reduction in F_v/F_m and PI values. A decrease in F_v/F_m can be due to the development of slowly relaxing quenching processes and photo damage to PSII reaction centers, both of which reduce the maximum quantum efficiency of PSII photochemistry (Baker

and Rosenqvist, 2004). In the present experiment, plants grown in CF substrate showed the lowest reduction in Chl a content and had the highest F_v/F_m in leaves under NaHCO₃ treatment. The most popular parameter of JIP test is the Performance Index (PI). The photosynthetic performance index is an indicator of plant vitality (Mehta *et al.*, 2010). Moreover, PI is found to be a very sensitive parameter in different crops and in most of environmental stresses (Strasser *et*

Table 3. Interactive effects of NaHCO₃ levels and different substrates on the pigments, maximal quantum yield of PSII photochemistry (F_v/F_m), photosynthesis Performance Index (PI) and Leaf Relative Water Content (LRWC) (%) of gerbera plants.

Substrate ^b	NaHCO ₃ [mM]	Chl a [†]	Chl b	Total Chl	Carotenoid	F_v/F_m	PI	LRWC
		(mg g ⁻¹ FW)				(%)		
VP	0	0.520c	0.308d	1.18d	0.259e	0.747abc	2.88b	51.89c
	20	0.237fgh	0.157ghi	0.504gh	0.111ijk	0.545hi	0.56hij	40.55ef
	40	0.232fgh	0.143ghi	0.497gh	0.093jkl	0.477j	0.26k	29.15gh
PP	0	0.281fg	0.146ghi	0.613fg	0.174g	0.667def	0.85fg	40.56ef
	20	0.209gh	0.115hij	0.459gh	0.128hi	0.637ef	0.59ghi	30.93gh
	40	0.109i	0.055j	0.225hi	0.07 l	0.445j	0.32jk	22.59i
CCP	0	0.544c	0.318d	1.228d	0.307d	0.690cde	2.22c	53.56bc
	20	0.201ghi	0.108ij	0.434gh	0.120ij	0.605fg	1.34d	45.62de
	40	0.158hi	0.091ij	0.354hi	0.098jk	0.540hi	0.85fg	26.10hi
CF	0	0.696a	0.698a	2.155a	0.448a	0.775a	3.69a	62.77a
	20	0.603bc	0.601b	1.888b	0.443a	0.742abc	3.65a	61.18a
	40	0.560c	0.578b	1.592c	0.425ab	0.705bcd	2.93b	53.85bc
CFP	0	0.663ab	0.406c	1.559c	0.386c	0.760ab	3.66a	58.14ab
	20	0.396d	0.181fgh	0.772ef	0.197fg	0.697b-e	2.95b	49.15cd
	40	0.164hi	0.085ij	0.355hi	0.113ijk	0.545hi	0.57hij	39.18f
P	0	0.411d	0.229ef	0.917e	0.255e	0.637ef	1.19de	52.99bc
	20	0.177hi	0.107hi	0.407hi	0.118 ij	0.480ij	0.40ijk	33.18f
	40	0.149hi	0.089ij	0.344hi	0.090jkl	0.417j	0.26 k	21.72i
ZP	0	0.678ab	0.629b	2.013ab	0.414b	0.705bcd	2.69b	54.14bc
	20	0.385de	0.256de	0.910e	0.216f	0.612fg	1.03ef	46.39cd
	40	0.303ef	0.193efg	0.625fg	0.149h	0.570gh	0.69gh	31.18gh
ANOVA	df	Mean square						
Substrate	6	0.244**	0.387**	2.92**	0.138**	0.06**	12.31**	988.67**
NaHCO ₃	2	0.692**	0.358**	4.99**	0.226**	0.14**	17.03**	3250.59**
Substrate× NaHCO ₃	12	0.022**	0.019**	0.17**	0.009**	0.013**	1.69**	71.38**

^a Mean separation was done by the LSD test and the same letter(s) in each column indicates non significant difference at $P < 0.05$. ^b Gerbera were grown on seven substrates of: 100% Coconut Fiber (CF), 75% Vermicompost+25% Perlite (VP), 25% Zeolite+75% Perlite (ZP), 75% Peat+25% Perlite (PP), 75% Coco Chip+25% Perlite (CCP), 75% Coconut Fiber+25% Perlite (CFP) and 100% Perlite (P). ** Significant ($P \leq 0.01$).



al., 2000; Jiang *et al.*, 2006), which is in accordance with our results achieved on gerbera plants under alkalinity stress. In this experiment, *PI* value significantly decreased with increasing NaHCO_3 concentration (Table 3). Mohsenian *et al.* (2012) found that alkali stress decreased *PI* in grafted and ungrafted tomato plants. Similar results are reported by Deng *et al.* (2010) under conditions of mixed salinity-alkalinity stress. The same authors stated that nonstomatal limitation, i.e. decreased photosynthetic activity in PSII plays an important role in decreased photosynthetic rate at high salinity-alkalinity (Deng *et al.*, 2010). The nonstomatal factors mainly depend on the cumulative effects of leaf water and osmotic potential, biochemical constituents (Sultana *et al.*, 1999), contents of photosynthetic pigments (Yang *et al.*, 2008), ion toxicities in the cytosol (James *et al.*, 2006), etc. We can conclude that reduction of photosynthetic pigments under NaHCO_3 treatments might be a part of the reason for *PI* reduction. Our results showed that the gerbera plants grown on CF substrate exhibited a higher value for total Chl content and greater *PI* than those grown in the other substrates (Table 3). The derived *PI* illustrated the enhanced vitality of gerbera plants grown in CF substrate. Leaf Relative Water Content (LRWC) was significantly ($P < 0.01$) affected by NaHCO_3 , substrate, and $\text{NaHCO}_3 \times \text{substrate}$ interaction (Table 3). Sodium bicarbonate at 20 mM concentration had no significant effect on LRWC in plants grown on CF substrate. At 40 mM NaHCO_3 , the lowest reduction in LRWC was observed in plants grown in CF substrate (Table 3). LRWC was used as a measure to estimate the stress response (Jain and Chattopadhyay, 2010). During stress conditions, plants usually accumulate inorganic ions in vacuoles to decrease cell water potential. Reduction in leaf RWC indicates loss of turgor that resulted in limited water availability for cell extension process (Katerji *et al.*, 1997). High alkalinity (40 mM NaHCO_3) treatment induced significant decreases in LRWC compared to the control (Table 3). These results corroborated those previously obtained in

mulberry (Ahmad and Sharma, 2010) and tomato (Mohsenian *et al.*, 2012). The decrease in water content under alkali stress might result from the destructive effect of high pH on root function and water uptake or accumulation of solutes (Yang *et al.*, 2008). In the present experiment, under 40 mM NaHCO_3 treatment, plants grown on CF substrate had the lowest reduction in RWC of leaves in comparison with plants grown in normal conditions. Less reduction in LRWC for CF grown plants may be due to sufficient osmotic adjustment in plants under stress conditions. Therefore, less LRWC reduction in plants grown on CF substrate results in higher tolerance of these plants to alkalinity stress (Balaguer *et al.*, 2002).

CONCLUSIONS

Results showed that the studied vegetative growth parameters (shoot and root fresh mass and leaf number) in plants grown on Coconut Fiber (CF) substrate were higher than those grown in the other substrates under the same alkalinity stress. Under alkalinity stress, reduction in F_v/F_m , *PI*, LRWC, GS activity and photosynthesis pigments contents was the lowest in CF substrate. Also, plants grown in CF substrate had the higher soluble sugars and proline content than those grown in the other substrates. It is concluded that use of CF substrate could provide a useful tool to improve alkalinity tolerance of gerbera plants under NaHCO_3 stress. On the other hand, plants grown on P substrate had the highest reduction in growth and physiological characteristic in alkaline conditions. Therefore, P is not suggested for use under alkali stress.

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اثرات تنش قلیائیت و بسترهای مختلف کاشت بر رشد و ویژگیهای فیزیولوژیکی گیاهان ژربرا

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چکیده

به منظور تعیین بهترین بستر کشت برای کاهش اثرات مضر تنش قلیائیت روی ژربرا، آزمایشی به صورت فاکتوریل با دو فاکتور بستر کشت و سطوح مختلف قلیائیت اجرا شد. گیاهان با محلول غذایی شامل سه pH (۷/۱، ۸/۱ و ۸/۴) متفاوت تغذیه می شدند. رشد رویشی، F_v/F_m ، PI، فعالیت آنزیم گلوتامین سنتتاز (GS)، محتوای نسبی آب برگ (LRWC)، محتوای کارتنوئیدها و کلروفیل (a, b) و کل گیاهان در واکنش به افزایش قلیائیت در محلول غذایی کاهش یافت. افزایش غلظت بیکربنات سدیم از ۰ به ۴۰ میلی مولار در محلول غذایی به طور معنی داری مقدار پرولین گیاهان را افزایش داد. گیاهان رشد یافته در بستر کاشت کوکوپیت (CF) کمترین میزان کاهش در رشد رویشی، F_v/F_m ، PI، فعالیت آنزیم گلوتامین سنتتاز (GS)، محتوای نسبی آب برگ (LRWC) و محتوای رنگیزه‌های فتوسنتزی تحت تنش قلیائیت در مقایسه با شاهد داشتند. همچنین، گیاهان رشد یافته در بستر کاشت کوکوپیت (CF) بالاترین میزان قندهای محلول و پرولین را نسبت به گیاهان رشد یافته در سایر بسترهای کاشت داشتند. از سوی دیگر، گیاهان رشد یافته در بستر پرلایت بیشترین کاهش در رشد رویشی و پارامترهای فیزیولوژی داشتند. بی اثر بودن تنش قلیائیت بر گیاهان رشد کرده در بستر کوکوپیت (CF) احتمالاً مربوط به بهبود فتوسنتز، افزایش فعالیت آنزیم GS و تنظیم اسمزی می باشد. بنابراین، نتیجه گیری می شود که استفاده از بستر کوکوپیت (CF) می تواند یک مکانیسم مناسب برای بهبود مقاومت به قلیائیت گیاهان ژربرا تحت شرایط تنش بیکربنات سدیم باشد.