AcademicPres

Available online: www.notulaebiologicae.ro

Print ISSN 2067-3205; Electronic 2067-3264

Not Sci Biol, 2018, 10(3):400-409. DOI: 10.15835/nsb10310286

Notulae Scientia Biologicae

Original Article

Differential Growth Responses of Wheat Seedlings to Elevated CO_2

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Abstract

Intraspecific variations in wheat growth responses to elevated CO_2 was evaluated using 20 Iranian bread wheat (*Triticum aestivum* L.) cultivars. The plants were grown in the modified Hoagland nutrient solution at a greenhouse until 35 days of age using two levels of CO_2 (~380 and 700 μmol mol^{-1}). The shoot and root dry weights of the wheat cultivars exhibited average enhancements of 17% and 36%, respectively, under elevated CO_2. This increase was associated with higher levels of chlorophyll *a* (25%), chlorophyll *b* (21%), carotenoid (30%), leaf area (54%) and plant height (49.9%). The leaf area ($r = 0.69^{**}$), shoot N content ($r = 0.62^{**}$), plant height ($r = 0.60^{**}$) and root volume ($r = 0.53^{*}$) were found to have important roles in dry matter accumulation of tested wheat cultivars under elevated CO_2 concentration. However, responses to elevated CO_2 were considerably cultivar-dependent. Based on the stress susceptibility index (SSI) and stress tolerance index (STI), the wheat cultivars exhibiting the best response to elevated CO_2 content were 'Sistan', 'Navid', 'Shiraz', 'Sepahan' and 'Bahar', while the ones with poor responses were 'Omid', 'Marun', 'Sorkhtokhm' and 'Tajan'. The findings from the present experiment showed significant variation among the Iranian wheat cultivars in terms of their responses to elevated air CO_2, providing the opportunity to select the most efficient ones for breeding purposes.

Keywords: climate change; CO_2 enrichment; genetic diversity; Nitrogen use efficiency; stress susceptibility index

Abbreviations: NUE: Nitrogen use efficiency; SSI: Stress susceptibility index; STI: Stress tolerance index

Introduction

Wheat is one of the most important crops as it provides approximately 20% of the energy and 25% of the protein requirements of the world's population of 6.6 billion (Pocketbook, 2015; Reddy and Hodges, 2000). It also ranks first among cultivated field crops in Iran with an average per capita consumption of about 220 kg, consumed both directly and indirectly (Khajehpour, 2013). A 60% increase is imminent in the demand for wheat by a world population of 9 billion by 2050 (Fischer *et al.*, 2014). Clearly, any contribution to greater production of wheat and its reduced production costs will benefit human food security.

Based on the records of monitoring stations at Mauna Loa in Hawaii, the annual mean growth rate of carbon dioxide concentration increased from 0.94 in 1959 to 3.05 μmol mol^{-1}/yr, whereas the atmospheric CO_2 has risen from 315 to 400 μmol mol^{-1} over the past 56 years (Tans, 2016).

Current projections indicate that atmospheric CO_2 will continue to rise to 450–1.000 μmol mol^{-1} by the year 2100 (IPCC, 2014). Increasing carbon dioxide concentration improves photosynthesis in C_3 plants such as wheat via prevention of photorespiration; thus, wheat yield is expected to increase under elevated CO_2 assuming that other growth factors remain within optimal limits (Amthor, 1997). Bourgault *et al.* (2013) found that average leaf area of wheat plants was increased by 30% under elevated CO_2 concentration of 700 μmol mol^{-1} compared with its normal ambient level (400 μmol mol^{-1}). Pal *et al.* (2005) reported that wheat plants recorded a greater photosynthetic rate, plant height, leaf surface area and plant dry mass at all growth stages (40, 60, and 90 d after sowing) under an elevated CO_2 of 600 ± 50 μmol mol^{-1} than those grown under the ambient CO_2 of 350 ± 50 μmol mol^{-1}.

Although increasing concentrations of CO_2 are expected to have a positive effect on the performance of C_3 crops, a wide variation is observed within the species. Amthor (2001) reviewed 50 studies investigating the effects of carbon dioxide on wheat growth to conclude that, regardless of the approach adopted to control CO_2, a great variation, ranging from −20 to +250%, can be observed in the influence CO_2 on grain yield. Musgrave and Strain (1988) investigated the effect of CO_2 enrichment (1.000 vs 350 μmol l^{-1}) on two wheat cultivars in a growth chamber

Received: 17 Apr 2018. Received in revised form: 10 Sep 2018. Accepted: 11 Sep 2018. Published online: 27 Sep 2018.

and reported that growth and assimilation rates were more pronounced in 'Yecora Rojo' than in the 'Sonoita' cultivar. Seneweera *et al.* (2010) evaluated eight wheat cultivars in the Australian Grains Free Air Carbon Dioxide Enrichment (AGFACE) facility and showed that the largest (by 30%) and smallest (4%) relative increases in dry mass due to CO_2 enrichment (550 vs 380 μmol mol^{-1}) were observed in the 'Gladius' and 'Janz' cultivars, respectively.

Mitterbauer *et al.* (2014) reported significant differences in the responses to elevated CO_2 (~700 vs ~400 μmol mol^{-1}) among 101 barely genotypes grown in open-top field chambers. In their experiment, the changes in grain yield ranged from –48 to +175% and those in the aboveground biomass varied from +45 to +166%. Uprety *et al.* (2003) examined the influence of elevated CO_2 concentrations of 575 – 620 μmol mol^{-1} on two rice varieties and concluded that the positive effects on most traits were more pronounced in 'Pusa Basmati-1' than in the 'Pusa-677' cultivar. Kazemi *et al.* (2018) reported that the effects of elevated CO_2 on the growth of rice plants depended on both variety and the salinity level.

Although variation in growth response to elevated CO_2 among wheat cultivars is documented, no information is yet available in this regard on Iranian bread wheat cultivars. Accordingly, the present study was conducted to evaluate the growth responses of Iranian bread wheat cultivars to increasing atmospheric CO_2.

Materials and Methods

Plant material

The local names and some agronomic characteristics of the studied wheat cultivars are given in Table 1. Seeds were provided by Agricultural and Natural Resources Research Center of Isfahan Province, Iran.

Experimental procedures

The experiment was conducted in 2013 under greenhouse conditions at Isfahan University of Technology (32° 33′ N; 51° 31′ 45′ E, 1602 m above sea level), Iran. For the purposes of this study, 20 Iranian bread wheat cultivars (Table 1) were grown under two different environments (ambient CO_2 of 380 ± 50 μmol mol^{-1} and enriched CO_2 of 700 ± 50 μmol mol^{-1}). The average air temperature throughout the experiment fluctuated between 25 and 32 °C and relative humidity ranged from 50 to 70%.

For the CO_2 treatments, two separate plastic containers, each 24 m^3, were initially designed and prepared. One container was equipped with an automated CO_2 gas injection system. The device was set at 700 ± 50 μmol mol^{-1} using interchangeable CO_2 cylinders of 10 kg. The other container was used as the control treatment and contained only ambient air CO_2.

Planting seeds and seedling growth

Seeds of the selected wheat cultivars were first sterilized in a solution of 2% sodium hypochlorite for 2 min, washed properly with water and planted in seedling trays filled with cocopeat. Wheat seedlings were kept under the same conditions up to the two-leaf stage before they were transplanted into the pores made on polystyrene layers floating on each pan. Seedling roots were completely placed in distilled water in the pan for three days when distilled water was replaced with the modified Hoagland nutrient solution to supply all the nutrients necessary for plant growth (Hoagland and Arnon, 1950).

After one week, the ventilation system was established for the solutions in the pans. During the study period, the acidity (pH) and electrical conductivity (EC) of the control treatment were set to 7.5 and 2.5 dS/m, respectively. To avoid extensive changes in the composition of the nutrient solution, it was renewed once a week. The CO_2 treatments were effected from the 3-leaf stage onwards.

Table 1. Main characteristics of the investigated Iranian bread wheat cultivars

Cultivars	Growth type*	Grain yield (t ha^{-1})	1.000 seed weight (g)	Height (cm)	Year introduced	Planting areas**
'Sistan'	S	4.32	48	90-95	1991	Wd & T
'Bahar'	S	6.67	38	95	2007	T
'Navid'	I	5.0	41	108	1990	C
'Shiraz'	S	7.29	40	101	2002	T
'Shoeleh'	S	3.00	43	115	1958	T
'Pishtaz'	S	6.5	44	94	2002	T
'Kavir'	S	4.14	38	85-90	2006	Wd & T
'Sepahan'	S	10.0	40	95-100	2006	T
'Karaj'	W	5.0	44.5	115	1973	C
'Ghods'	I	6.0	42	97.5	1989	T
'Gaspard'	W	6.14	39	85-90	1994	C
'Chamran'	S	5.5-6.5	39	90-100	1997	Wd & T
'Tajan'	S	6.30	38	95-100	1995	T
'Alamoot'	W	6.40	36	100	1995	C
'Khoshki11'	S	5.00	35	-	-	Wd
'Shahriyar'	W	6.72	38	105	2002	C
'Marun'	S	3.04	39.5	94	1991	Wd
'Sorkhtokhm'	S	3.50	25.5	-	1941	Wd
'Omid'	W	6.8	40	110	1956	C
'Marvdasht'	S	6.7	36	102	1999	T

*S: spring; W: winter; I: intermediate; **Wd: warm and dry; C: cold; T: Temperate

Measurements

After 35 days (mid-tillering stage), wheat seedlings were harvested and separated into roots and shoots. The green leaf area was measured using a digital leaf area meter (Model GA-5, OSK Company, Tokyo, Japan). Plant height was measured from crown to the extended tip of the newest fully developed leaf, using a metric ruler. Root volume was determined using the water displacement method (Pang *et al*., 2011). Plant root and shoot samples were oven dried at 70 °C for 48 h and their dry weights were separately recorded.

Nitrogen concentration of the shoot tissues was measured using the Berthelot reaction, in which a phenolic compound (salicylates) in the presence of ammonia and hypochlorite turns blue-green (Novozamsky *et al*., 1974). The absorption rate was measured by a spectrophotometer at a wavelength of 660 nm.

The nitrogen content and nitrogen use efficiency (Cheng *et al*., 2011) were determined using the following formulas:

Nitrogen content (g plant $^{-1}$) = Nitrogen concentration (g kg $^{-1}$) × dry weight (kg plant $^{-1}$)

Nitrogen User Efficiency (NUE) = Dry weight (g)/ Nitrogen content (g)

Stress susceptibility index (SSI) (Fischer and Maurer, 1978) and stress tolerance index (STI) (Fernandez, 1992) were calculated as follows:

$$\mathrm{SSI} = \left[1 - \left(\mathrm{Y_{si}}/\mathrm{Y_{pi}}\right)\right]/\mathrm{SI}$$

$$\mathrm{SI} = \left[1 - \left(\mathrm{Y_S}/\mathrm{Y_p}\right)\right]$$

$$\mathrm{STI} = \left(\mathrm{Y_{si}} \times \mathrm{Y_{pi}}\right)/(\mathrm{Yp}^2)$$

where, Ypi = total dry biomass weight of individual cultivars in the absence of stress, Ysi = total dry biomass weight of individual cultivars in the presence of stress, Ys = average total dry biomass weight of all the cultivars in the presence of stress, and Yp = average total dry biomass weight of all the cultivars in the absence of stress. An elevated CO_2 of 700 ± 50 μmol mol^{-1} was considered as a non-stress treatment and the ambient concentration of CO_2 equal to 380 ± 50 μmol mol^{-1} was regarded as a stress treatment.

Statistical analysis

The Bartlett's test was initially conducted for homogeneity of error variances. The null hypothesis in terms of non-significant differences between the variances of the errors in the two environments (i.e., the ambient CO_2 concentration of 380 ± 50 μmol mol^{-1} and the elevated one of 700 ± 50 μmol mol^{-1}) was not rejected.

Based on the uniformity of the error variances, the combined analysis of variances was performed as (20 wheat cultivars) experiment in a completely randomized design with three replications using SAS v9.1. The least significant difference (LSD) test was employed for mean comparisons at α level = 0.05.

In addition, the Ward method was employed to identify cluster groups in dendrograms using the measured values of the different traits of the 20 Iranian bread wheat cultivars. Clustering was accomplished on the basis of changes (in %) in the values of variables for the elevated (700) vs. ambient (380 μmol mol^{-1}) CO_2 concentrations.

Results

Shoot nitrogen concentration, N content and NUE

Shoot nitrogen concentration, nitrogen content and NUE were found to be significantly ($P < 0.01$) affected by elevated CO_2 and cultivar (Table 2). The elevated vs. ambient CO_2 decreased shoot nitrogen concentration by 10% on the average, but increased N content and NUE by 26 and 14%, respectively. The highest and lowest mean shoot N concentrations of 108 and 3.40 mg g^{-1} DW were observed in 'Alamoot' and 'Omid' as were the highest and lowest shoot N contents of 125 and 3.60 mg shoot^{-1}. This is while the highest and lowest NUE values of 415 and 9.35 were measured for 'Omid' and 'Alamoot' cultivars, respectively (Table 2).

Finally, shoot NUE was significantly ($P < 0.01$) affected by the interaction of CO_2 and cultivar (Table 2). Under both elevated and ambient CO_2, the highest and lowest NUE values belonged to 'Omid' and 'Alamoot' cultivars, respectively (Table 2).

Chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoid concentrations

Leaf Chl*a*, Chl*b* and carotenoid concentrations were significantly ($P < 0.01$) affected by elevated CO_2 and wheat cultivar (Table 3). The elevated vs. ambient CO_2 increased leaf Chl*a*, Chl*b* and carotenoid concentrations by 25, 21, and 30%, respectively (Table 3).

Leaf Chl*a*, Chl*b* and carotenoid concentrations were significantly ($P < 0.01$) affected by the interaction of CO_2 and wheat cultivar (Table 3). The highest and lowest mean Chl*a* concentrations were observed in 'Alamoot' and 'Tajan', respectively, under both ambient (0.478 and 0.218) and elevated (0.508 and 0.255 mg g^{-1}FW) CO_2. Chl*b* concentration recorded their highest and lowest values in 'Ghods' and 'Tajan' cultivars, respectively, under both ambient (0.229 and 0.139) and elevated (0.260 and 0.180 mg g^{-1}FW) CO_2. This is while 'Ghods' and 'Tajan' cultivars recorded the highest (0.402 mg g^{-1}FW) and lowest (0.300) mean values of carotenoid concentration under the ambient CO_2 and 'Ghods' (0.495 mg g^{-1}FW) and 'Marvdasht' (0.400 mg g^{-1}FW) under the elevated CO_2 (Table 3).

Leaf area, plant height and root volume

Leaf area, plant height and root volume were found to be significantly ($P < 0.01$) affected by elevated CO_2 and wheat cultivar (Tables 4). The elevated vs. ambient CO_2 increased leaf area, plant height and root volume by 54, 49.4, and 51%, respectively (Table 4). The highest and lowest mean values of 148 and 95 cm^{2}plant^{-1} were measured in 'Marun' and 'Omid', respectively, for leaf area; those of 63.1 and 42.6 cm were recorded in 'Marun' and 'Gaspard' cultivars for plant height and those of 15.7 and 10.1 cm^3 in 'Navid' and 'Omid' cultivars, respectively, for root volume (Table 4). Interaction of CO_2 and wheat cultivar also had significant ($P < 0.01$) effects on leaf area, plant height and root volume (Table 4). The highest and lowest mean values of 133 and 72 cm^2 plant^{-1} were observed for leaf area in 'Marun' and 'Navid', respectively, under the ambient CO_2, while values of 198 and 113 cm^2 plant^{-1}were recorded in

'Navid' and 'Omid' cultivars under the elevated CO_2 (Table 4). The values of 55.6 and 33.0 cm were obtained as the highest and lowest means in 'Marun' and 'Gaspard' for plant height under the ambient CO_2. Under the elevated CO_2, however, 'Marun' and 'Shahriyar' cultivars recorded the highest and lowest mean values of 69.7 and 50.7 cm, respectively. Maximum (13.4 cm^3 $plant^{-1}$) and minimum (7.8 cm^3 $plant^{-1}$) mean values of root volume belonged to 'Marun' and 'Tajan' cultivars under the ambient CO_2 while the same values for the same trait were 20.8 and 11.1 cm^3 $plant^{-1}$ under the elevated CO_2, which belonged to 'Navid' and 'Omid' cultivars, respectively (Table 4).

Finally, enhancements were observed in leaf area, plant height, and root volume in all the wheat cultivars examined as a result of increased CO_2 although the extent of the enhancements varied with cultivar (Table 4). The highest and lowest increases were obtained as 175 and 23% for leaf area in 'Navid' and 'Marun', 89 and 25% for plant height in 'Sistan' and 'Marun', respectively, 96% for root volume in 'Tajan' and 'Navid' and 7% in 'Marvdasht' (Table 4).

Table 2. Effects of two [CO_2] (380 ± 50 vs. 700 ±50 µmol mol^{-1}) on nitrogen concentration (mg g^{-1}DM), nitrogen content (mg $shoot^{-1}$) and NUE in the shoots of 20 Iranian bread wheat cultivars

Cultivars	N concentration (mg g^{-1}DM)				N content (mg $shoot^{-1}$)				NUE			
	[CO_2] (µmol mol^{-1})		%	Mean	[CO_2] (µmol mol^{-1})		%	Mean	[CO_2] (µmol mol^{-1})		%	Mean
	380	700			380	700			380	700		
'Sistan'	17	15.5	-9	16.3	13.6	24.6	81	19.1	58.8	64.6	10	61.7
'Bahar'	12	9.7	-19	10.9	12.5	15.8	26	14.2	83.2	103	24	93.2
'Navid'	52.5	46.7	-11	49.6	51.5	89.2	73	70.4	19.0	21.4	13	20.2
'Shiraz'	23.3	21.5	-8	22.4	19.6	31.6	61	25.6	42.9	46.5	9	44.7
'Shoeleh'	14.8	10.1	-32	12.5	13.6	14.4	6	14.0	67.6	99.3	47	83.5
'Pishtaz'	66.7	67.5	1	67.1	62.7	90.5	44	76.6	15.0	14.8	-1	14.9
'Kavir'	96.7	101	4	98.9	84.1	116	38	100	10.3	9.9	-4	10.1
'Sepahan'	77.5	75	-3	76.3	59.7	94.5	58	77.1	12.9	13.3	3	13.1
'Karaj'	12.9	18.1	40	15.5	12.9	26.6	106	19.8	77.5	55.3	-29	66.4
'Ghods'	88.3	84.2	-5	86.3	79.5	108	36	93.8	11.3	11.9	6	11.6
'Gaspard'	8.2	5.8	-29	7.0	6.6	7.0	6	6.80	121	173	43	147
'Chamran'	32.5	24.7	-24	28.6	38.7	35.1	-9	36.9	30.7	40.5	32	35.6
'Tajan'	35.8	33.3	-7	34.6	39.0	40.6	4	39.8	27.9	30.0	8	29.0
'Alamoot'	107	108	1	107	116	133	15	125	9.3	9.2	-1	9.3
'Khoshki11'	47.5	45.8	-4	46.7	51.3	57.7	12	54.5	21.1	21.8	4	21.4
'Shahriyar'	5.6	4.4	-21	5.0	4.9	4.80	-2	4.85	180	225	25	202
'Marun'	4.3	3.3	-23	3.8	6.1	5.10	-16	5.60	233	306	31	269
'Sorkhtokhm'	39.2	35	-11	37.1	46.6	46.6	0	46.6	25.5	28.5	12	27.0
'Omid'	4.1	2.7	-34	3.4	4.00	2.70	-33	3.4	245	374	53	310
'Marvdasht'	104	95.8	-8	99.9	114	120	5	117	9.6	10.4	8	10.0
Mean	42.5^{a**}	40.4^{b}	-10	41.5	41.8^{b}	53.2^{a}	26	47.5	65.1^{a}	82.9^{b}	14	74.0
$LSD_{5\%}$	0.13				0.10				3.80			
	Source of variation											
[CO_2]	<0.01				<0.01				<0.01			
Cultivar (C)	<0.01				<0.01				<0.01			
[CO_2] *C	<0.01				<0.01				<0.01			

Table 3. Effects of two [CO_2] (380 ± 50 vs. 700 ± 50 µmol mol^{-1}) on chlorophyll a (mg g^{-1}FW), chlorophyll b (mg g^{-1}FW) and carotenoid concentrations (mg g^{-1}FW) in the leaves of 20 Iranian bread wheat cultivars

Cultivars	Chlorophyll *a* (mg g^{-1}FW)				Chlorophyll *b* (mg g^{-1}FW)				Carotenoid (mg g^{-1}FW)			
	[CO_2] (µmol mol^{-1})		%	Mean	[CO_2] (µmol mol^{-1})		%	Mean	[CO_2] (µmol mol^{-1})		%	Mean
	380	700			380	700			380	700		
'Sistan'	0.288	0.442	53	0.365	0.172	0.237	38	0.205	0.345	0.448	30	0.397
'Bahar'	0.372	0.452	22	0.412	0.173	0.195	13	0.184	0.330	0.432	31	0.381
'Navid'	0.343	0.442	29	0.393	0.205	0.240	17	0.223	0.348	0.482	39	0.415
'Shiraz'	0.274	0.342	25	0.308	0.213	0.222	4	0.218	0.327	0.490	50	0.409
'Shoeleh'	0.253	0.343	36	0.298	0.173	0.207	20	0.190	0.332	0.437	32	0.385
'Pishtaz'	0.270	0.297	10	0.284	0.173	0.228	32	0.201	0.330	0.457	38	0.394

'Kavir'	0.270	0.385	43	0.328	0.158	0.205	30	0.182	0.320	0.427	33	0.374
'Sepahan'	0.318	0.362	14	0.340	0.163	0.198	21	0.181	0.330	0.415	26	0.373
'Karaj'	0.244	0.382	57	0.313	0.150	0.208	39	0.179	0.320	0.418	31	0.369
'Ghods'	0.414	0.458	11	0.436	0.229	0.260	14	0.245	0.402	0.495	23	0.449
'Gaspard'	0.234	0.284	21	0.259	0.160	0.193	21	0.177	0.313	0.425	36	0.369
'Chamran'	0.358	0.396	11	0.377	0.177	0.233	32	0.205	0.360	0.445	24	0.403
'Tajan'	0.218	0.255	17	0.237	0.139	0.180	29	0.160	0.300	0.410	37	0.355
'Alamoot'	0.478	0.508	6	0.493	0.218	0.233	7	0.226	0.378	0.457	21	0.418
'Khoshki11'	0.344	0.430	25	0.387	0.203	0.228	12	0.216	0.348	0.472	36	0.410
'Shahriyar'	0.400	0.500	25	0.450	0.187	0.242	29	0.215	0.372	0.442	19	0.407
'Marun'	0.301	0.408	36	0.355	0.196	0.235	20	0.216	0.368	0.442	20	0.405
'Sorkhtokhm'	0.275	0.302	10	0.289	0.193	0.210	9	0.202	0.343	0.438	28	0.391
'Omid'	0.333	0.456	37	0.395	0.168	0.233	39	0.201	0.335	0.452	35	0.394
'Marvdasht'	0.310	0.373	20	0.342	0.187	0.199	6	0.193	0.353	0.400	13	0.377
Mean	0.315^{b**}	0.391^{a}	25		0.182^{b}	0.219^{a}	21		0.343^{b}	0.444^{a}	30	
LSD 5%		0.019				0.01				0.02		
	Source of variation											
$[CO_2]$		<0.01				<0.01				<0.01		
Cultivar (C)		<0.01				<0.01				<0.01		
$[CO_2]$ *C		<0.01				<0.01				<0.01		

Table 4. Effects of two $[CO_2]$ (380 ± 50 vs. 700 ± 50 µmol mol^{-1}) on leaf area (cm^2 plant^{-1}), plant height (cm) and root volume (cm^3 plant^{-1}) of 20 Iranian bread wheat cultivars

Cultivars	Leaf area (cm^2 plant^{-1})				Plant height (cm)				Root volume (cm^3 plant^{-1})			
	$[CO_2]$ (µmol mol^{-1})		%	Mean	$[CO_2]$ (µmol mol^{-1})		%	Mean	$[CO_2]$ (µmol mol^{-1})		%	Mean
	380	700			380	700			380	700		
'Sistan'	88	166	89	127	35.2	66.5	88.9	50.9	8.4	13.8	65	11.08
'Bahar'	110	175	59	143	45.5	63.2	38.9	54.4	9.6	13.9	45	11.74
'Navid'	72	198	175	135	42.4	65.7	55.0	54.1	10.6	20.8	96	15.70
'Shiraz'	87	151	74	119	36.5	62.5	71.2	49.5	9.9	17.8	79	13.86
'Shoeleh'	96	154	60	125	37.8	57.7	52.6	47.8	10.7	15.2	42	12.95
'Pishtaz'	101	145	44	123	38.8	55.0	41.8	46.9	10.3	16.7	62	13.50
'Kavir'	94	144	53	119	35.5	55.7	56.9	45.6	10.3	16.9	64	13.60
'Sepahan'	94	133	41	114	36.3	52.8	45.5	44.6	10.7	14.4	35	12.55
'Karaj'	106	155	46	131	41.7	61.0	46.3	51.4	11.5	17.8	55	14.65
'Ghods'	95	138	45	117	37.3	54.8	46.9	46.1	10.2	16.7	64	13.45
'Gaspard'	87	129	48	108	32.7	52.5	60.6	42.6	8.3	14.6	77	11.43
'Chamran'	113	152	35	133	46.0	61.8	34.3	53.9	10.2	14.6	43	12.40
'Tajan'	85	133	56	109	33.0	56.3	70.6	44.7	7.8	15.2	96	11.48
'Alamoot'	99	142	43	121	39.7	61.0	53.7	50.4	10.8	15.9	47	13.35
'Khoshki11'	104	146	40	125	41.3	60.0	45.3	50.7	13.0	16.0	23	14.50
'Shahriyar'	84	119	42	102	35.8	50.7	41.6	43.3	10.5	14.0	33	12.25
'Marun'	133	164	23	149	55.6	69.7	25.4	62.7	13.4	17.0	27	15.20
'Sorkhtokhm'	105	148	41	127	45.8	62.8	37.1	54.3	12.3	17.0	38	14.65
'Omid'	78	113	45	96	36.5	53.5	46.6	45.0	9.1	11.1	22	10.09
'Marvdasht'	107	136	27	122	46.3	59.7	28.9	53.0	12.4	13.3	7	12.85
Mean	96.9^{b**}	147^{a}	54		39.5^{b}	59.1^{a}	49.4		10.5^{b}	15.6^{a}	51.2	
LSD 5%		4.70				4.80				1.80		
	Source of variation											
$[CO_2]$		<0.01				<0.01				<0.01		
Cultivar (C)		<0.01				<0.01				<0.01		
$[CO_2]$ *C		<0.01				<0.01				<0.01		

Shoot, root and shoot/ root dry weight (S/R) ratio

Shoot and root dry weights were both significantly ($P < 0.01$) affected by the elevated CO_2 treatment and cultivar (Table 5). The S/R ratio was also significantly ($P < 0.01$) affected by cultivar (Table 5). Shoot and root dry weights exhibited increases of 39% and 18% in the elevated CO_2 treatment (Table 5). The highest and lowest values of shoot dry weight were obtained to be 1.49 and 0.98 g plant^{-1} for 'Marun' and 'Shahriyar'; root dry weight were 0.255 in 'Marun' and 0.17 g plant^{-1} in 'Omid', 'Gaspard' and 'Shoeleh'; S/R ratio were 8.07 and 5.38 in the 'Sistan' and 'Shahriyar' cultivars, respectively (Table 5).

Interaction of CO_2 and cultivar had significant ($P < 0.01$) effects on shoot and root dry weights, as well as S/R ratio (Table 5). The highest and lowest values of shoot dry weight were determined to be 1.42 and 0.770 g plant^{-1} in 'Marun' and 'Sepahan', respectively, under the ambient CO_2, but 1.91 and 1.01 g plant^{-1} in 'Navid' and 'Omid' under the elevated CO_2, respectively (Table S5). Root dry weight recorded the highest and lowest values of 0.250 and 0.150 g plant^{-1} in 'Marun' and 'Omid-Gaspard', respectively, under the ambient CO_2, but under the elevated CO_2 'Sistan' recorded the highest root dry weight of 0.270, while 'Omid' and 'Shoeleh' recorded the lowest value of 0.180 g plant^{-1}. S/R ratio recorded its highest value of 6.53 in 'Omid' and its lowest value of 4.53 in 'Sepahan' under the ambient CO_2. Values of 9.10 and 5.14 were recorded for this same trait in 'Navid' and 'Shahriyar' cultivars, respectively, under the elevated CO_2 treatment (Table 5).

All the investigated wheat cultivars exhibited enhanced shoot and root dry weights in the elevated CO_2 treatment; the enhancements, however, varied with cultivar such that the highest and lowest increases of 99% and 3% were observed in 'Sistan' and 'Omid', respectively, for shoot dry weight. Root dry weight had its highest increase of 69% in 'Sistan' and its lowest increase of 4% in 'Marun'. S/R ratio decreased in some cultivars but increased in others under the elevated CO_2 treatment. The highest and lowest decreases of 14% and 0% belonged to 'Omid' and 'Tajan', respectively, while the highest and lowest increases of 86% and 4% were recorded for 'Navid' (Table 5).

Discussion

The current atmospheric carbon dioxide concentration of 400 μmol mol^{-1} is considered as a limiting factor to photosynthesis rate in C_3 plants. Increased CO_2 concentration can, therefore, improve photosynthesis primarily due to the associated enhancement in Rubisco carboxylation capacity (Long *et al.*, 2004; Kant *et al.*, 2012), which, in turn, reduces CO_2 losses to photorespiration (Barnaby and Ziska, 2012).

Table 5. Effects of two [CO_2] (380 ± 50 vs. 700 ± 50 μmol mol^{-1}) on shoot (g plant^{-1}), root (g plant^{-1}) and shoot/ root dry weight ratio of 20 Iranian bread wheat cultivars

Cultivars	Shoot dry weight (g plant^{-1})				Root dry weight (g plant^{-1})				Shoot/ root dry weight ratio			
	[CO_2] (μmol mol^{-1})		%	Mean	[CO_2] (μmol mol^{-1})		%	Mean	[CO_2] (μmol mol^{-1})		%	Mean
	380	700			380	700			380	700		
'Sistan'	0.80	1.59	99	1.195	0.160	0.270	69	0.215	5.00	5.89	18	5.44
'Bahar'	1.04	1.63	57	1.335	0.170	0.220	29	0.195	6.12	7.41	21	6.76
'Navid'	0.98	1.91	95	1.445	0.200	0.210	5	0.205	4.90	9.10	86	7.00
'Shiraz'	0.84	1.47	75	1.155	0.160	0.200	25	0.180	5.25	7.35	40	6.30
'Shoeleh'	0.92	1.43	55	1.175	0.160	0.180	13	0.170	5.75	7.94	38	6.85
'Pishtaz'	0.94	1.34	43	1.140	0.180	0.220	22	0.200	5.22	6.09	17	5.66
'Kavir'	0.87	1.15	32	1.010	0.180	0.200	11	0.190	4.83	5.75	19	5.29
'Sepahan'	0.77	1.26	64	1.015	0.170	0.200	18	0.185	4.53	6.30	39	5.41
'Karaj'	1.00	1.47	47	1.235	0.220	0.230	5	0.225	4.55	6.39	41	5.47
'Ghods'	0.90	1.29	43	1.095	0.180	0.200	11	0.190	5.00	6.45	29	5.73
'Gaspard'	0.80	1.21	51	1.005	0.150	0.190	27	0.170	5.33	6.37	19	5.85
'Chamran'	1.19	1.42	19	1.305	0.190	0.230	21	0.210	6.26	6.17	-1	6.22
'Tajan'	1.09	1.22	12	1.155	0.170	0.190	12	0.180	6.41	6.42	0	6.42
'Alamoot'	1.08	1.23	14	1.155	0.200	0.220	10	0.210	5.40	5.59	4	5.50
'Khoshki11'	1.08	1.26	17	1.170	0.190	0.230	21	0.210	5.68	5.48	-4	5.58
'Shahriyar'	0.88	1.08	23	0.980	0.170	0.210	24	0.190	5.18	5.14	-1	5.16
'Marun'	1.42	1.56	10	1.490	0.250	0.260	4	0.255	5.68	6.00	6	5.84
'Sorkhtokhm'	1.19	1.33	12	1.260	0.220	0.230	5	0.225	5.41	5.78	7	5.60
'Omid'	0.98	1.01	3	0.995	0.150	0.180	20	0.165	6.53	5.61	-14	6.07
'Marvdasht'	1.10	1.25	14	1.175	0.210	0.230	10	0.220	5.24	5.43	4	5.34
Mean	0.994^{b**}	1.36^{a}	39		0.184^{b}	0.215^{a}	18		5.41^{b}	6.33^{a}	18	
LSD 5%	0.019				0.01				0.02			
Source of variation												
[CO_2]	<0.01				<0.01				<0.01			
Cultivar (C)	<0.01				<0.01				<0.01			
[CO_2] *C	<0.01				<0.01				<0.01			

Table 6. Average values of SSI and STI in Iranian bread wheat cultivars. The values are based on total dry biomass (shoot plus root dry weights)

Cultivar	CO_2^*	
	SSI	STI
'Sistan'	1.93	0.72
'Bahar'	1.38	0.91
'Navid'	1.77	1.01
'Shiraz'	1.60	0.68
'Shoeleh'	1.32	0.70
'Pishtaz'	1.13	0.71
'Kavir'	0.89	0.57
'Sepahan'	1.42	0.56
'Karaj'	1.13	0.84
'Ghods'	1.10	0.65
'Gaspard'	1.28	0.54
'Chamran'	0.65	0.92
'Tajan'	0.43	0.72
'Alamoot'	0.47	0.75
'Khoshki11'	0.59	0.77
'Shahriyar'	0.74	0.55
'Marun'	0.33	1.23
'Sorkhtokhm'	0.38	0.89
'Omid'	0.20	0.55
'Marvdasht'	0.46	0.79

*The elevated [CO_2] of 700 ± 50 µmol mol^{-1} serves as the non-stress treatment while the ambient CO_2 of 380 ± 50 µmol mol^{-1} serves as the stress treatment.

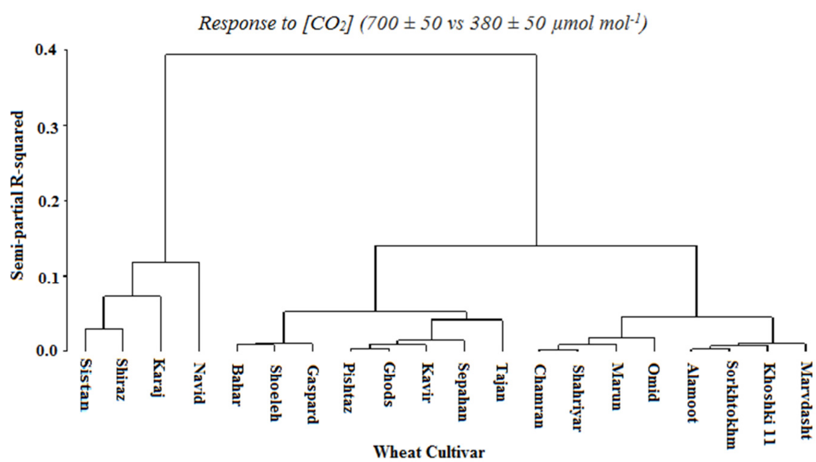

Fig. 1. Hierarchical cluster analysis: Dendrogram using the Ward Method and the variables consisting of nitrogen concentration, nitrogen content, NUE, leaf area, plant height, root volume, chlorophyll *a*, chlorophyll *b*, carotenoid concentration, shoot dry weight, root dry weight and shoot/ root dry weight (S/R) ratio in 20 Iranian bread wheat cultivars. Based on changes (%) in the measured values of the variables for an elevated [CO_2] concentration of 700 vs the ambient concentration of 380 µmol mol^{-1}

The current results indicated that under elevated CO_2 (~700 vs. ~380 µmol mol^{-1}), root and shoot dry weights experienced average enhancements of 36 % and 17%, respectively in Iranian wheat cultivars. This was associated with enhanced chlorophyll *a* (25%), chlorophyll *b* (21%), carotenoid (30%), leaf area (54%) and height (49.9%) in the plants grown under a CO_2 enriched atmosphere (Tables 3 and 4). This suggests that leaf area had a comparatively greater contribution to the positive response of wheat cultivars to elevated CO_2 (Table 3). These findings are also supported by those reported by other researchers. Van der Kooi *et al. (*2016) stated that both biomass and yield of C_3 crops have steadily increased when grown under elevated CO_2. Ainsworth and Rogers (2007) reported an average increase of 40% in photosynthetic rate in a variety of plant species grown under elevated CO_2 in the range of 475 - 600 µmol mol^{-1}. Cai *et al.* (2016) examined the influence of elevated CO_2 concentrations (~550 vs. ~370 µmol mol^{-1}) in semi-arid environments and concluded that average yield stimulation was 24% in 'Horsham' and 53% in 'Walpeup'. Madhu and Hatfield (2014) reported that roots become more abundant, longer, thicker and faster growing when crops are grown under high CO_2 conditions. Other studies reported that elevated CO_2 could lead to faster development of the root system in winter wheat (*Triticum aestivum* L.) (Chaudhuri *et al.*, 1990) or higher root weight in sorghum (*Sorghum bicolor* L. Moench) (Chaudhuri *et al.*, 1986), improved root length, surface area, volume and tip numbers in two *Lolium* species (Jia *et al.*, 2011) and increased root length (110%) and root dry weight (143%) in soybean (Rogers *et al.*, 1992).

Compared to the ambient CO_2, however, elevated levels led to reduced shoot N concentration by an average value of 10% in the different studied wheat cultivars. This is while previous studies reported a range of 10-15% (Ainsworth and Long, 2005; Seneweera, 2010). The reduced N concentration could be attributed to such factors as nitrogen dilution in plant tissue, lower transpirational N flow as a result of reduced stomatal conductance, reduced N uptake due to soil-root source effects, reduced N demand due to the down-regulation of photosynthetic enzymes, reduced N assimilation capacity and declining electron flows for nitrate (Taub and Wang, 2008; Kant *et al.*, 2012).

The effect of increased carbon dioxide on NUE was observed to vary with cultivar (Table 2). Under elevated CO_2, NUE was increased from 4% to 53% in most of the investigated cultivars; however, cv. 'Karaj' exhibited a significant decrease in this trait (Table 2). Elevated CO_2 (~550 vs ~330 μmol mol^{-1}) reportedly increased photosynthesis at all canopy levels and enhanced nitrogen use efficiency in the spring wheat plants under FACE plots (Arp, 1991). It has also been shown that elevated CO_2 concentrations (700 and 350 μmol mol^{-1}) enhance both N and agronomic N use efficiencies in the spring wheat, and that this effect is more pronounced under lower, rather than high, levels of N application (Li *et al.*, 2003). However, the reduced plant wheat biomass under an enriched CO_2 atmosphere observed in some experiments might imply a decrease in N use efficiency.

Plant growth response, as realized by the different traits measured, to elevate CO_2 was found to depend on cultivar (Tables 2-5). With increasing CO_2 concentration, changes were observed from -34 to 40% in N concentration, from -33 to 106% in N content, from -29 to 53% in NUE (Table 2), from 23 to 175% in leaf area, from 25.4 to 88.9% in plant height, from 7 to 96% in root volume (Table 3), from 6 to 57% in Chl*a*, from 4 to 39% in Chl*b*, from 13 to 50% in carotenoid (Table 4), from 3% to 99% in shoot dry weight, from 4% to 69% in root dry weight and from -14 to 80% in shoot/root dry weight ratio. Cluster analysis, which separates genotypes into groups exhibiting a high homogeneity within each group and heterogeneity across the classified groups (Jaynes *et al.*, 2003) was used to classify the cultivars into two major clusters according to the percentage changes of the measured traits in response to the elevated CO_2 concentration. Cluster I consist of 4 cultivars, while cluster II includes 16 that are further divided into two sub-clusters each consisting of 8 wheat cultivars (Fig. 1).

The development of crop varieties that enjoy a high potential for fixing CO_2 in their photosynthetic process is considered as an appropriate solution in the face of rising atmospheric CO_2 and achieving food security (Kant *et al.*, 2012). The present findings of the experiment showed significant variation among the Iranian wheat cultivars in terms of their response to elevated air CO_2, providing the opportunity to select the most efficient ones for breeding purposes.

Based on the SSI and STI indices, that are probably used for the first time to identify the response of wheat cultivars to the increase of carbon dioxide, the wheat cultivars exhibiting the best response to elevated CO_2 content were 'Sistan', 'Navid', 'Shiraz', 'Sepahan' and 'Bahar', while the ones with poor responses were 'Omid', 'Marun', 'Sorkhtokhm' and 'Tajan' (Table 6). This is confirmed by other studies (Musgrave and Strain, 1988; Amthor, 2001; Bourgault *et al.*, 2013) that have disclosed the capacity of wheat germplasm for enhanced adaptability to elevated CO_2 content. Barnaby and Ziska (2012) found that plants exhibit both inter- and intra-species differences in their molecular, genetic and physiological responses to rising air CO_2. Manderscheid and Weigel (1997) also reported of spring wheat cultivars, introduced between 1890 and 1988, that differed in their response to atmospheric CO_2 enrichments of as high as 689 μmol mol^{-1}. They found that the differences were mostly due to differences in tillering rate, spike number and grain number per spike, especially in old cultivars as compared to modern ones.

It has been claimed that the variation among wheat cultivars in response to elevated CO_2 could be explained by differences in net photosynthesis (P_N), stomatal conductance (gs), transpiration (E) and water use efficiency (WUE), as well as their antioxidant enzyme systems (Bencze *et al.,* 2014). In the present experiment, leaf area (r = 0.69**), shoot N content (r = 0.62**), plant height (r = 0.60**) and root volume (r = 0.53*) were found to have important roles in dry matter accumulation in wheat cultivars under elevated CO_2 concentrations.

Conclusions

The investigated Iranian bread wheat cultivars in the present experiment exhibited large variations in their response to the atmospheric CO_2, suggesting that the cultivars enjoy the considerable genetic capacity for yield improvements under elevated CO_2 concentration. In addition, under increasing CO_2 concentration, traits such as leaf area, shoot N content, plant height and root volume were found to have important roles in dry matter accumulation. However, further experiments are required in the field under natural conditions to verify these findings.

Acknowledgements

Isfahan University of Technology (IUT) is acknowledged for funding this project. The authors are grateful to the staff of the Department of Agronomy and Plant Breeding, especially to Mr. Emadi for his assistance in the climate chamber control operations. The authors would also like to extend their gratitude to Dr. Ezzatollah Roustazadeh from ELC, IUT, for editing the final English manuscript.

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