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BRIEF COMMUNICATION

Photosynthesis and nutrient composition of spinach and fenugreek grown under elevated carbon dioxide concentration

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Abstract

The effect of elevated carbon dioxide concentration on the changes in the biomass, photosynthesis and nutrient composition was investigated in two leafy vegetables. Spinach (*Spinacia oleracea* L.) and fenugreek (*Trigonella foenum-graecum* L.) plants were grown in open top chambers under either ambient (ACO_2, 350 ± 50 µmol mol^{-1}) or elevated (ECO_2, 600 ± 50 µmol mol^{-1}) CO_2 concentration and analyzed 40, 60 and 80 days after exposure. The plants grown in ECO_2 had higher net photosynthetic rate and lower stomatal conductance when compared with the plants grown in ACO_2. ECO_2 also changed the nutrient composition: a lower N, Mg and Fe contents and higher C and Ca contents were observed in the leaves of plants exposed to ECO_2 than in those grown at ACO_2.

Additional key words: calcium, iron, magnesium, net photosynthetic rate, nitrate reductase, stomatal conductance.

The effects of elevated atmospheric carbon dioxide concentration on plant growth and tissue composition have been studied extensively with C_3 species as photosynthesis in these plants is unsaturated at the present atmospheric CO_2 concentrations and hence the response to elevated CO_2 concentration is usually positive (Drake *et al.* 1997). CO_2 being the primary substrate for photosynthesis influences several plant physiological processes directly as a result of this primary response, and a range of secondary responses, including growth, dry matter allocation, and nutrient composition and assimilation may change (Stitt and Krapp 1999, Schortemeyer *et al.* 1999, Uprety *et al.* 2003, Pal *et al.* 2005).

Plants often respond to the increased CO_2 concentration by increasing the growth (Conroy *et al.* 1992, Centritto *et al.* 1999, Drake *et al.* 1997). Decreased N concentration can also be due to lower specific leaf area in elevated CO_2 (Centritto *et al.* 2002). Leaf nitrogen and some proteins decrease in leaves exposed to high CO_2 (Rogers *et al.* 1996, Uprety *et al.* 2002, Moynul Haque *et al*. 2006). It has been demonstrated that CO_2-induced reduction in tissue nitrogen concentration was due to accelerated plant growth under elevated CO_2 (Coleman *et al.* 1993). Photosynthetic apparatus acclimates to elevated CO_2 by reducing the enzymatic components (*e.g.* Rubisco) while being able to sustain photosynthetic rates comparable to those in plants exposed to ambient CO_2 (Sage *et al.* 1989).

Our previous studies in berseem (*Trifolium alexandrium*) crop indicated that under elevated CO_2 there was an increase in nutrients such as N, P, Ca and C and some of the micronutrients like Cu, Fe and Zn on a leaf area unit but all these nutrients showed a decline when calculated on a dry mass unit (Pal *et al.* 2004). In the present study we examined the influence of elevated CO_2 on shoot biomass, photosynthesis and foliar nutrient content in spinach and fenugreek.

The seeds of spinach (*Spinacia oleracea* L.) and fenugreek (*Trigonella foenum-graecum* L.) cv. Pusa Early Bunching were grown in soil as per recommended agricultural practices inside open top chambers (1.6 m diameter and 1.8 m high) lined with transparent 120 µm thick PVC sheet. The plants were grown under ambient (ACO_2, 350 ± 50 µmol mol^{-1}) or elevated (ECO_2, 600 ± 50 µmol mol^{-1}) CO_2. There were three replicated chambers each for elevated and ambient CO_2 exposure in a randomized experimental design. The concentration of CO_2 inside the chambers was measured regularly using a portable photosynthetic system (*LI-6200*, *LI-COR*,

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Abbreviations: ACO_2 - ambient CO_2; DAE - days after exposure; ECO_2 - elevated CO_2; g_s - stomatal conductance; P_N - net photosynthetic rate.

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Lincoln, USA) and was monitored by the flow meter as described by Pal *et al*. (2004).

The leaf area and dry mass were measured on five replicate plants per chamber 40, 60 and 80 d after exposure (DAE). The leaf blade area was measured using *LI-3100* area meter (*LI-COR*). All the plant parts were dried at 80 °C and then kept at 60 °C until the constant dry mass was recorded. Net photosynthetic rates and stomatal conductance were measured at growth CO_2 concentration (ambient or elevated) with a portable *LI-6200* photosynthetic system. The photosynthetic rate was recorded for the top most fully expanded leaves between 10:00 and 11:30 when photosynthetically active radiation ranged between 1000 and 1300 µmol m^{-2} s^{-1}. The temperature and the relative humidity ranged between 18 - 23 ºC and between 65 - 70 %, respectively, during the sampling period.

The dried plant samples were ground and sieved for the analysis of total reduced nitrogen (N), carbon (C) content and other nutrients such as calcium (Ca), magnesium (Mg) and iron (Fe). The organic C content was estimated by wet digestion following the modified method of Walkley and Black (1934). The analysis of total N was done with an autoanalyzer (*Technicon*, Tarrytown, USA) by Kjeldahl method. For the estimation of Ca, Mg and Fe the samples were digested using a diacid mixture (Bhargava and Raghupati 1993) and were estimated using an atomic absorption spectrophotometer (*Model GBC 902*, *GBC Scientific Equipment*, Melbourne, Australia). The experiment was conducted following the completely randomized design with each treatment replicated three times. Statistical analysis of the data was done following the method of analysis of variance (*ANOVA*) (Panse and Sukhatme 1967). The critical difference (CD) values between the treatments were calculated at 5 % probability levels.

Continuous exposure to ECO_2 increased the growth of both spinach and fenugreek plants. At 60 DAE, a significant increase in the number (data not shown) and the area of leaves was observed in both the vegetable crops under ECO_2. The increase in the leaf area in fenugreek was 49, 29.5 and 17.7 % at 40, 60 and 80 DAE, respectively, whereas in spinach the increase in the leaf area was only 12.6, 18.5 and 6.5 % at respective days of sampling (Table 1). The leaf dry mass increased significantly in both spinach (16.4 %) and fenugreek (37 %) plants grown under ECO_2 at 80 DAE (Table 1). The increase in the stem biomass was also more in fenugreek as compared to that in spinach (Table 1). An increase of 15.2 % in the stem dry mass was observed in spinach and an increase of 27.4 % was observed in fenugreek plants grown under ECO_2 as compared to plants grown under ACO_2 at 40 DAE. At 80 DAE stem dry mass increased by more than one fold in fenugreek whereas in spinach the increase in stem dry mass at 80 DAE was only 12.3 %. Our earlier report in wheat (Pal *et al*. 2003/4) and berseem (Pal *et al.* 2004) also showed that ECO_2 increased growth. The overall increase in growth of leafy vegetables was due to the increased P_N as also observed in many other crop species (Drake *et a*l. 1997, Pal *et al*. 2005). In mung bean, the extra carbon fixed under elevated CO_2 was translocated to the growing organs within 48 h (Sharma and Sengupta 1990).

Elevated CO_2 markedly increased P_N in both spinach and fenugreek. The P_N of spinach was higher than that of fenugreek. An increase of 29 % was recorded in spinach at 40 DAE and an increase of up to 49 % in the P_N was observed in fenugreek grown under ECO_2 as compared to the plants grown under ACO_2 (Table 1). It was also noted that the P_N of the ACO_2 grown plants was constant with time while that of the ECO_2 grown plants decreased with time. Stomatal conductance (g_s) decreased significantly in both spinach and fenugreek grown under ECO_2 (Table 1). The increase in P_N has been reported in various crop species by various researchers under ECO_2 (Aben *et al*. 1999, Uprety and Mahalaxmi 2000). In the present study, however, complete acclimation was not observed but the relative increase caused by ECO_2 decreased with time.

The organic C content, which constitutes both the structural and non-structural components, increased

Table 1. Effect of ambient (ACO_2) and elevated (ECO_2) CO_2 concentration on growth and photosynthesis of spinach and fenugreek at different growth stages. Values are mean ± SD (n = 5), * - P < 0.05.

Parameter	Plant	40 DAE ACO_2	40 DAE ECO_2	60 DAE ACO_2	60 DAE ECO_2	80 DAE ACO_2	80 DAE ECO_2
Leaf d.m.	spinach	1.24 ± 0.06	1.47 ± 0.03*	1.49 ± 0.10	1.65 ± 0.08*	1.58 ± 0.15	1.84 ± 0.07*
[g $plant^{-1}$]	fenugreek	0.71 ± 0.03	0.96 ± 0.05*	0.80 ± 0.08	1.11 ± 0.14*	0.89 ± 0.07	1.22 ± 0.10*
Stem d.m.	spinach	1.38 ± 0.12	1.59 ± 0.11*	1.62 ± 0.08	1.84 ± 0.15*	1.70 ± 0.09	1.91 ± 0.11*
[g $plant^{-1}$]	fenugreek	0.77 ± 0.05	0.98 ± 0.04*	0.88 ± 0.05	1.62 ± 0.06*	0 92 ± 0.09	1.98 ± 0.08*
Leaf area	spinach	495.74 ± 12.50	558.40 ± 6.55*	610.52 ± 15.06	724.30 ± 8.56*	732.45 ± 12.15	780.24 ± 11.06*
[cm^2]	fenugreek	166.60 ± 7.86	248.00 ± 6.57*	220.50 ± 9.95	285.60 ± 8.86*	247.30 ± 11.23*	291.10 ± 13.45*
P_N	spinach	13.05 ± 0.56	16.40 ± 0.18	12.66 ± 0.82	19.52 ± 0.09	15.78 ± 0.48	16.47 ± 0.40
[µmol m^{-2} s^{-1}]	fenugreek	9.70 ± 0.08	13.45 ± 0.32	11.26 ± 0.06	16.81 ± 0.46	10.47 ± 0.12	15.62 ± 0.21
g_s	spinach	0.89 ± 0.05	0.84 ± 0.01	1.05 ± 0.05	0.91 ± 0.06	0.73 ± 0.06	0.69 ± 0.08
[cm s^{-1}]	fenugreek	0.74 ± 0.01	0.72 ± 0.06	0.81 ± 0.01	0.70 ± 0.02	0.43 ± 0.03	0.40 ± 0.10

Table 2. Effect of ambient (ACO_2) and elevated (ECO_2) CO_2 concentration on nutrients of spinach and fenugreek leaves at different growth stages. Values are mean of ± SD (n = 5), * - P < 0.05.

Parameter	Plant	40 DAE ACO_2	ECO_2	60 DAE ACO_2	ECO_2	80 DAE ACO_2	ECO_2
C	spinach	52.15 ± 2.47	56.20 ± 2.50	58.20 ± 2.50	65.93 ± 4.75*	61.38 ± 3.93	72.03 ± 2.95*
[%(d.m.)]	fenugreek	47.27 ± 1.28	54.07 ± 1.47	51.55 ± 3.68	59.90 ± 2.59*	53.25 ± 3.00	63.88 ± 3.58*
N	spinach	4.08 ± 0.32	4.65 ± 0.17	3.31 ± 0.31	2.91 ± 0.39*	6.26 ± 0.36	5.21 ± 0.14*
[%(d.m.)]	fenugreek	6.18 ± 0.27	7.15 ± 0.40*	6.04 ± 0.35	5.64 ± 0.41*	8.99 ± 0.12	6.96 ± 0.56*
C:N	spinach	12.78 ± 0.29	12.08 ± 0.35	17.58 ± 0.25	22.65 ± 0.38*	9.80 ± 0.20	13.82 ± 0.16*
	fenugreek	7.64 ± 0.17	7.56 ± 0.23	9.52 ± 0.13	10.62 ± 0.09*	5.92 ± 0.07	9.17 ± 0.14*
Ca	spinach	63.59 ± 3.84	72.13 ± 4.79*	63.00 ± 6.82	67.58 ± 5.75	55.08 ± 1.49	57.35 ± 0.98
[μg g^{-1}(d.m.)]	fenugreek	18.58 ± 2.15	42.32 ± 5.70*	40.54 ± 0.83	63.70 ± 3.64*	45.94 ± 3.25	59.01 ± 0.74*
Mg	spinach	5.61 ± 0.62	2.99 ± 0.01*	9.61 ± 2.53	5.77 ± 0.91*	3.92 ± 0.57	1.54 ± 0.80*
[μg g^{-1}(d.m.)]	fenugreek	4.16 ± 0.89	1.45 ± 0.14*	7.26 ± 1.02	4.26 ± 0.68*	3.60 ± 0.74	2.55 ± 0.26*
Fe	spinach	11.09 ± 1.07	5.05 ± 0.29*	10.01 ± 1.32	6.94 ± 0.78*	8.92 ± 0.32	3.98 ± 0.73*
[μg g^{-1}(d.m.)]	fenugreek	3.57 ± 0.17	2.39 ± 0.81 ^{NS}	6.81 ± 0.77	3.59 ± 0.68*	4.63 ± 0.81	2.89 ± 0.27*

significantly in the ECO_2 grown plants (Table 2). Maximum increase of 17.3 and 19.9 % was at 80 DAE in the leaves of spinach and fenugreek, respectively. Increase in the C content has been reported by Van Ginkel *et al*. (1997) in root, stem and leaves of *Lolium perenne* in response to ECO_2. In contrast to C, the N concentration in the leaves declined in ECO_2 grown plants compared with ACO_2 grown plants. The reduced N in the leaves of ECO_2 grown spinach and fenugreek increased in the initial stages but by 80 DAE the N concentration declined in the leaves of both spinach and fenugreek by 17 and 22.4 %, respectively, under ECO_2 (Table 2). The decrease in N concentration has been reported under ECO_2 in wheat (Pal *et al*. 2003/4), berseem (Pal *et al.* 2004) and soybean (Reeves *et al.* 1994). The increase in the C content and decline in the N concentration increased the C:N ratio in the leaves of both the vegetables, which was the highest at 60 DAE (Table 2) as the N concentration was the lowest at 60 DAE. High C:N ratio due to ECO_2 has been reported in various investigations (Farage *et al*. 1998, Gifford *et al*. 2000, Pal *et al*. 2004).

The ECO_2 also affected the content of other macro and micronutrients. Spinach leaves had higher concentration of Ca, Mg and Fe as compared to fenugreek (Table 2). Ca increased in the leaves of both the vegetables under ECO_2 but the increase was more in fenugreek, *i.e.*, 48, 57 and 28.4 %, respectively at 40, 60 and 80 DAE as compared to spinach which showed an increase of 13.4, 7.2 and 4.1 % on the respective days of sampling in the leaves of the plants growing under ECO_2. A significant reduction in Mg concentration (62 and 29 %) was observed in the leaves of spinach and fenugreek, respectively at 80 DAE to ECO_2. The Fe content of the leaves also significantly declined in the leaves of the plants grown under ECO_2. In spinach, the maximum decline of Fe content in the leaves (55 %) was recorded at 80 DAE, and in fenugreek, 47 % decline in Fe content was recorded at 60 DAE (Table 2). Baxter *et al*. (1994) reported increase in P, Mg and K content in three grass species grown under ECO_2. In our study the C and Ca increased and the N, Mg and Fe among micronutrients declined in both the vegetables under ECO_2. A reduction in plant nutrient concentration is often found at ECO_2 (Schaffer *et al*. 1997). In our study Ca concentration increased in the leaves of both the vegetables.

The present study concludes that the overall effect of ECO_2 on both the leafy vegetables is similar to that in many other crop species. However, in most of the crops, the final yield (seed) is important factor, whereas in spinach and fenugreek vegetative biomass and its nutritional composition is of utmost significance as these are consumed. The decline in the concentration of important micronutrient Fe and increase in the C:N ratio in the leaves may affect the nutritional quality of the vegetables in the future high CO_2 world.

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