Biomass production and nutritional levels of berseem (Trifolium alexandrium) grown under elevated CO₂


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Abstract

There is little available information about the effect of elevated CO₂ on the growth and mineral nutrients of fodder crops. To investigate the changes in vegetative biomass and mineral concentration of berseem (Trifolium alexandrium L.), an important forage legume, was grown in ambient (360 μl l⁻¹) as well as elevated (600 μl l⁻¹) CO₂ conditions from germination onwards in open top chambers. Elevated CO₂ increased the leaf size, plant height and fresh and dry mass of shoots. There was more partitioning of photosynthates towards the growth of new branches than towards the growth of leaves. Leaf nitrogen, soluble proteins, calcium, iron and nitrate reductase (NR) activity decreased in elevated CO₂ while leaf carbon and phosphorus contents increased. The results suggest that berseem grown in elevated CO₂ throughout the crop season can produce more fodder in less time. The study concludes that elevated CO₂ may increase the fodder production by 30–35% but will adversely affect the nutritional quality of the forage due to reduction in nitrogen, protein, calcium and iron concentration in leaves on a unit dry weight basis. On a unit area basis, however, there will be an increase in total nutrient content, including nitrogen, due to increased fodder biomass in elevated CO₂.

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Keywords: Growth; Nitrate reductase activity; Nitrogen; Phosphorus; Photosynthesis

1. Introduction

The increasing atmospheric CO₂ concentration (Idso, 1980; Lawlor and Mitchell, 1991) in recent times has led to many assumptions and experiments on the possible effects of CO₂ on the growth and development of plants. Since the current ambient level of atmospheric CO₂ (about 360 μl l⁻¹) is a limiting factor for maximum photosynthesis (Tolbert and Zelitch, 1983), any increase in CO₂ above ambient level has the potential to increase the rate of photosynthesis, especially in C₃ plants. The increased rate of photosynthesis will affect the growth of plants, as shown by the increased growth and yield in many crop species grown under elevated levels of CO₂ (Sionit et al., 1982; Sasek and Strain, 1991; Sharma and Sengupta, 1990; Das et al., 2000). However, the response of plants to elevated CO₂ differs from one species to another.

Increased biomass in elevated CO₂ due to greater carbon concentration is not always supplemented by an increase in the concentration of essential nutrient...
elements. Reduction in nutrient concentration of plants has been reported due to increased CO$_2$ concentration (Conroy, 1992; Overdieck, 1993; Manderscheid et al., 1995). The main reason may be that the uptake of nutrient from the soil is not increased to similar extent as the gain in carbon under such conditions, thereby leading to changes in the nutritional quality of the crops.

There have been a few studies on the effects of elevated CO$_2$ on fodder crops (Gorisson and Cotrufo, 2000; Wagner et al., 2001; Morgan et al., 2001). Berseem is an important winter forage as it is nutritive and succulent. It contains more than twenty percent crude protein and has seventy percent dry matter digestibility (Singh, 1989). In India, it is grown in irrigated areas as rabi (winter) fodder crop. This investigation documents the effects of elevated CO$_2$ on foliage growth and nutrient levels of berseem plants.

2. Materials and methods

2.1. Plant material and growth conditions

Berseem (Trifolium alexandrium L. cv. Pusa Jayant) was grown in soil inside open top chambers (1.6 m diameter and 1.8 m height) lined with transparent PVC sheet (120 µm thickness) under natural conditions. The seeds were treated with Rhizobium trifoli before sowing. Farmyard manure at the rate of 15 t ha$^{-1}$ along with 25 kg N ha$^{-1}$ and 55 kg P$_2$O$_5$ ha$^{-1}$ was mixed in the soil before sowing and irrigation was given as and when required. Pure CO$_2$ gas used for enrichment was mixed with ambient air (360 µl l$^{-1}$) and injected into the chamber through a regulator and a circulating pump. The flow of CO$_2$–air mixture was adjusted with the help of a flow meter to get the target concentration of CO$_2$ (600 ± 50 µl l$^{-1}$) above the plant canopy level inside the open top chamber. Similar open top chambers were used as control (ambient CO$_2$) wherein free air was injected inside instead of CO$_2$–air mixture. There were three replicate chambers each for elevated and ambient CO$_2$ exposure with a randomized experimental design. The period of CO$_2$ enrichment was 80 days from 8:00 a.m. to 5:00 p.m. from the onset of seed germination. The level of CO$_2$ was monitored daily using the LI-6200 photo-

2.2. Measurements of growth characters and photosynthesis

For the measurement of growth characters, berseem plants were harvested at 40, 60 and 80 days after initiation of CO$_2$ exposure (DAE). Five plants from each chamber were harvested at each stage for analysis of plant growth. The leaves and stem portion were separated and the number of branches and leaves per plant were counted. All the plant parts were dried at 80°C for determining dry weight. The leaf blade area was measured using a LI-3100 area meter (LI-COR, Inc., Lincoln, NE, USA). The fresh and dry weights of the samples were recorded following standard procedures. Specific leaf weight (SLW) was calculated as leaf weight/leaf area (Gardner et al., 1988). The rate of photosynthesis was measured at growth CO$_2$ concentration (i.e. ambient or elevated) with a portable LI-6200 photosynthesis system at 40, 60 and 80 DAE. Photosynthetic rate was recorded in top most fully expanded leaves between 10:00 and 11:30 a.m. when the photosynthetically active radiation (PAR) ranged between 1000 and 1300 µmol m$^{-2}$ s$^{-1}$.

2.3. Biochemical analysis

Nitrate reductase (NR) activity in leaves was estimated in vivo according to the method of Klepper et al. (1971) and modified by Nair and Abrol (1973). Fresh leaf samples were taken in a test tube containing phosphate buffer (0.1 M, pH 7.5), and KNO$_3$ (0.4 M). Nitrate was infiltrated into the leaf tissues using a vacuum pump and samples were incubated at 33°C in a shaking water bath for 30 min in dark. The reaction was stopped by keeping the samples at 70°C for 5 min. One ml of sulfanilamide (0.1% in 1N HCl) and 1 ml N-1-naphthyl ethylene diamine dihydrochloride (NEDD, 0.01%) each was added to 0.1 ml of aliquot. Absorbance was recorded at 540 nm using Beckman spectrophotometer. The NR activity was expressed as µmol NO$_3^-$ formed g$^{-1}$ fresh weight h$^{-1}$. The total soluble protein content was estimated following the method of Bradford (1976). Fresh leaf samples were ground in chilled extraction buffer [50 mM Tris (pH 7.4), 50 mM ethylene diamine tetra acetic acid]
(EDTA, pH 8.0), 10 mM dithiothreitol (DTT) and 100 mM PMSF] at 4°C. Ground samples were centrifuged at 12,000 rpm for 15 min at 4°C. A 20 μl leaf extract was poured in test tube containing 5.0 ml diluted Bradford dye and absorbance was recorded at 595 nm using a Beckman spectrophotometer. Dried plant samples were ground and sieved for analysis of nitrogen, carbon, phosphorus, calcium and iron. Organic carbon content was estimated by wet digestion following the modified method of Walkley and Black (1934) and expressed as percentage of shoot dry mass. For total nitrogen analysis, the samples were digested by the Kjeldahl method and analyzed with an auto analyzer (Technicon, USA). For estimation of phosphorus, calcium and iron the leaf samples were digested using a diacid mixture (Bhargava and Raghupathi, 1993). Phosphorus was estimated following the procedure given by Jackson (1973). Calcium and iron contents were estimated following the method of Bhargava and Raghupathi (1993) using an atomic absorption spectrophotometer (Model GBC 902, GBC Scientific Equipment Pvt. Ltd., Victoria, Australia).

2.4. Statistical analysis

The experiment was conducted as a completely randomized design with each treatment replicated three times. Statistical analysis of the data was done following the methods of analysis of variance (ANOVA) (Panse and Sukhatme, 1967). The critical difference (CD) values between the treatments were calculated at 5% probability levels.

### Table 1

<table>
<thead>
<tr>
<th>Days of exposure</th>
<th>CO2 level (μl l⁻¹)</th>
<th>Plant height (cm per plant)</th>
<th>Stem fresh weight (g per plant)</th>
<th>Stem dry weight (g per plant)</th>
<th>Total shoot biomass (g per plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>350 ± 20</td>
<td>21.5 ± 0.4</td>
<td>2.88 ± 0.11</td>
<td>0.31 ± 0.01</td>
<td>0.53 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>600 ± 50</td>
<td>31.5 ± 0.5*</td>
<td>4.98 ± 0.16*</td>
<td>0.55 ± 0.01*</td>
<td>1.05 ± 0.02*</td>
</tr>
<tr>
<td>60</td>
<td>350 ± 20</td>
<td>30.2 ± 0.7</td>
<td>8.20 ± 0.37</td>
<td>0.90 ± 0.02</td>
<td>1.39 ± 0.03</td>
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<tr>
<td></td>
<td>600 ± 50</td>
<td>41.1 ± 0.6*</td>
<td>12.37 ± 0.52*</td>
<td>1.35 ± 0.02*</td>
<td>1.98 ± 0.04*</td>
</tr>
<tr>
<td>80</td>
<td>350 ± 20</td>
<td>40.1 ± 0.8</td>
<td>12.45 ± 0.48</td>
<td>1.41 ± 0.03</td>
<td>2.05 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>600 ± 50</td>
<td>56.9 ± 0.9*</td>
<td>19.74 ± 0.56*</td>
<td>2.13 ± 0.03*</td>
<td>2.87 ± 0.06*</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. (n = 3).
* P < 0.05: a significant difference between CO2 levels at specific days of exposure.

3. Results

3.1. Stem and leaf growth

Long-term exposure to elevated CO2 (600 μl l⁻¹) in open-top chambers increased the growth of berseem plants. Plant height and leaf area increased in elevated CO2 grown plants. The effect was greatest during initial exposure (40 days), where a 46% increase in stem length was observed (Table 1). At 60 and 80 DAE, a 36 and 42% increase, respectively, in plant height was observed, accompanied by an increase in both fresh and dry weights of the stem. At 40 DAE, the increase in fresh and dry weights was about 73 and 77%, respectively. The elevated CO2 plants maintained higher stem weights until later stages of growth (about 58 and 51% increases in fresh and dry weights, respectively, were recorded at 80 days). The fodder biomass (fresh weight) produced by elevated CO2 grown plants after 60 days was equivalent to that produced by 80-day-old ambient CO2 plants (Table 1). These results suggest that when berseem plants are grown in elevated CO2 throughout the growing season, rapid and enhanced vegetative growth can increase fodder biomass by approximately 2.25 t ha⁻¹.

The leaf growth was also significantly higher in plants grown in elevated CO2 (Table 2). The observed increases in leaf area were: 98% at 40 DAE, 79% at 60 DAE and 46% at 80 DAE. The number of leaves on a per plant basis increased as a result of exposure to elevated CO2. The secondary branches produced more leaves than the primary branches (data not included). At 40 DAE, there was no significant difference in
the number of leaves produced by primary and secondary branches but at 60 and 80 DAE, the number of leaves produced by secondary branches increased significantly under elevated CO$_2$. The leaf fresh weight showed an increase of about 108% at 40 DAE but increase was comparatively less at 60 and 80 DAE (58 and 35% higher than at ambient CO$_2$). The dry weight of leaves of elevated CO$_2$ grown plants was significantly higher than that of the ambient CO$_2$ grown plants. Maximum increase (118%) in dry weight was at 40 DAE compared to 60 DAE (29%) and 80 DAE (13%) (Table 2).

The specific leaf weight (SLW) was slightly higher in CO$_2$ enriched berseem plants at 40 DAE (10%) but was less by 39 and 27%, at 60 and 80 DAE, respectively, compared to that of ambient CO$_2$ grown plants (Table 2). The total shoot biomass was also higher in elevated CO$_2$ grown plants and a maximum increase of 98% was recorded at 40 DAE. The increase in shoot biomass at 60 and 80 DAE was about 42 and 40%, respectively (Table 2).

### 3.2 Net photosynthesis, nitrate reductase (NR) activity and soluble proteins

The photosynthetic rate of elevated CO$_2$ grown plants was higher than that of the ambient grown plants at all the stages (Fig. 1). About 107 and 92% increases in net photosynthetic rate were observed at 40 and 80 DAE, respectively. The NR activity declined in elevated CO$_2$ grown plants despite increased photosynthesis (Fig. 2). The reduction in NR activity was 20% at 40, 60 and 80 DAE. The total soluble proteins in the leaves decreased under elevated CO$_2$ (Fig. 3). The maximum reduction in total soluble protein concentration (26.2%) was found at 80 DAE.

### 3.3 Macro- and micronutrients of leaves

Percent nitrogen in the leaves declined in elevated CO$_2$ grown plants (Table 3). A reduction of 27–33% in nitrogen was observed at 40, 60 and 80 DAE. However, the total nitrogen increased (20–25%) when
expressed on a unit land area basis. Elevated CO$_2$ also altered the concentration of other nutrient elements. The organic carbon content, which constitutes both structural and non-structural components, increased in elevated CO$_2$ grown plants. The increase in organic carbon content was 21, 24 and 33% at 40, 60 and 80 DAE, respectively. The increase in carbon concentration and reduction in nitrogen concentration increased the C/N ratio of berseem leaves, which was the highest (44%) at 60 DAE (Table 3). The phosphorus content of the leaves was higher in elevated CO$_2$ grown plants (Table 3). An increase of 12, 8 and 9% in phosphorus content was observed at 40, 60 and 80 DAE, respectively. The calcium and iron contents of berseem leaves were not significantly affected by elevated CO$_2$ and the plants were able to maintain about the same level of these two nutrients as the plants grown in ambient CO$_2$ (Table 3).

Table 3

<table>
<thead>
<tr>
<th>Days of exposure</th>
<th>CO$_2$ level (µl/l)</th>
<th>Organic carbon (%)</th>
<th>Nitrogen (%)</th>
<th>C:N ratio</th>
<th>Phosphorus (%)</th>
<th>Calcium (%)</th>
<th>Iron (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>350 ± 20</td>
<td>53.35 ± 1.42</td>
<td>5.46 ± 0.14</td>
<td>9.84 ± 0.24</td>
<td>0.51 ± 0.01</td>
<td>2.07 ± 0.04</td>
<td>113 ± 3.26</td>
</tr>
<tr>
<td></td>
<td>600 ± 50</td>
<td>64.77 ± 1.38 (NS)</td>
<td>3.89 ± 0.11</td>
<td>13.80 ± 0.26*</td>
<td>0.57 ± 0.01*</td>
<td>2.03 ± 0.03 (NS)</td>
<td>111 ± 2.82 (NS)</td>
</tr>
<tr>
<td>60</td>
<td>350 ± 20</td>
<td>54.25 ± 1.33</td>
<td>4.69 ± 0.14</td>
<td>11.57 ± 0.19</td>
<td>0.40 ± 0.01</td>
<td>1.94 ± 0.03</td>
<td>107 ± 2.26</td>
</tr>
<tr>
<td></td>
<td>600 ± 50</td>
<td>67.40 ± 1.46*</td>
<td>3.43 ± 0.09*</td>
<td>16.73 ± 0.28*</td>
<td>0.43 ± 0.01*</td>
<td>1.92 ± 0.03 (NS)</td>
<td>105 ± 2.33 (NS)</td>
</tr>
<tr>
<td>80</td>
<td>350 ± 20</td>
<td>55.20 ± 1.27</td>
<td>4.53 ± 0.12</td>
<td>12.23 ± 0.24</td>
<td>0.35 ± 0.01</td>
<td>1.73 ± 0.03</td>
<td>109 ± 2.01</td>
</tr>
<tr>
<td></td>
<td>600 ± 50</td>
<td>73.71 ± 1.39*</td>
<td>3.05 ± 0.08*</td>
<td>21.52 ± 0.43*</td>
<td>0.38 ± 0.01*</td>
<td>1.71 ± 0.03 (NS)</td>
<td>103 ± 1.94 (NS)</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. (n = 3). NS: non-significant.
* $P < 0.05$: a significant difference between CO$_2$ levels at specific days of exposure.
4. Discussion

Long-term exposure of berseem plants to elevated CO$_2$ (600 μL L$^{-1}$) in open-top chambers resulted in a significant enhancement in the growth of these plants which was maintained throughout the period of CO$_2$ exposure (Tables 1 and 2). This increase in growth appeared to be due to the partitioning of greater amounts of assimilated carbon towards the growing organs. Our earlier observations showed that the extra carbon fixed by the plants due to CO$_2$ enrichment translocates to the growing axis (Sharma and Sengupta, 1990). The growth enhancement effect at the early stage of CO$_2$ exposure was greater in the leaves (118%) compared to the stem (77%). Under elevated CO$_2$, berseem plants produced more leaves on the secondary branches, which caused increased leaf biomass. An increase in biomass due to increase in the number of branches/leaves has also been reported in Japanese honey-suckle (Sasek and Strain, 1991) and in sweet potato (Bhattacharya et al., 1985) under elevated CO$_2$.

SLW, which is an indicator of leaf thickness, showed a marginal increase at 40 DAE but a decrease at 60 and 80 DAE. This indicates that exposure to elevated carbon caused an initial increase in leaf thickness but at later stages further expansion of the leaves resulted in thinner leaves. Reduction in leaf thickness has been reported in various crop species such as soybean (Cure et al., 1987) and beans (Campbell et al., 1988). The reduction of leaf thickness was due to less accumulation of starch and a faster rate of leaf expansion. It is worthwhile to note that although the leaves became thinner, the total fodder biomass increased. In addition to the increase in dry matter production, CO$_2$ enrichment caused an acceleration in biomass (fodder) production. This may lead to more than one cutting of berseem fodder if plants are grown under high CO$_2$ conditions throughout the crop season. Morgan et al. (2001) reported increased regrowth and dry mass of a C$_3$ grass species (Paspalum smitti) and of a forage legume (Medicago sativa) when grown under elevated CO$_2$. Increased fodder productivity (30–45%) of white clover, and increased yield in rice when grown under elevated CO$_2$ has also been reported by Saebo and Mortensen (1995) and Uprety et al. (2002), respectively.

The overall increase in the growth of berseem crop under elevated CO$_2$ is the result of increased net photosynthesis as observed in many other crop species (Kimball, 1983; Sengupta, 1988; Sharma and Sengupta, 1990; Drake et al., 1997). The enhancement in photosynthesis was largest at 40 DAE (107%). Increased net photosynthesis under elevated CO$_2$ might have resulted in greater accumulation of assimilates, which resulted in the production of more biomass. In mung bean, the extra carbon fixed under elevated CO$_2$ was translocated to the growing organs within 48 h (Sharma and Sengupta, 1990).

An increase in photo-assimilate production due to enhanced net photosynthesis under elevated CO$_2$ may also affect other plant metabolic processes, especially nitrogen metabolism. This is because the oxidation of photosynthetic intermediates generates reductants for nitrate assimilation (Plaut and Littan, 1974). The NR activity decreased under elevated CO$_2$ despite the increase in the rate of net photosynthesis. These findings are in contrast to the results reported by Hocking and Meyer (1991), who observed increased NR activity in maize plants grown under elevated CO$_2$. Reduction in NO$_3$-nitrogen concentration has been reported in wheat (Hand, 1984). Low NR activity may be due to less availability of NR enzyme protein, as we found low soluble protein in elevated CO$_2$-exposed berseem plants. There is probably a change in nitrogen metabolism due to reduction in NR activity under elevated CO$_2$ conditions.

The leaf soluble protein content declined in berseem plants grown under elevated CO$_2$. Although, Nie et al. (1995) did not find an effect in the amount of any major protein of the photosynthetic apparatus, several other reports showed a decline in soluble proteins of leaves grown in elevated CO$_2$ (Campbell et al., 1988; Stitt, 1991; Akin et al., 1995). While Rubisco is the major fraction of soluble protein in the leaves, its reduction under elevated CO$_2$ did not alter the photosynthetic activity proportionately.

A reduction in leaf nitrogen concentration with elevated CO$_2$ has been reported in different crop species (Hocking and Meyer, 1991; Conroy, 1992; Rogers et al., 1996; Ziska et al., 1996). This reduction in nitrogen may be either due to the dilution effect as a result of greater carbohydrate accumulation (Rogers et al., 1996) or due to the acceleration of plant growth, but not due to the increased nitrogen use efficiency (Coleman et al., 1993). Our study suggests that the reduction in leaf nitrogen may be due to a greater
increase in leaf area as a result of extra carbon fixed by higher photosynthesis and lack of proportionate gain in nitrogen by the plants. If calculated on a unit land area basis, an increase of 20−25% in nitrogen content occurs.

Exposure of berseem plants to elevated CO₂ altered the composition of other nutrient elements also. Organic carbon increased due to elevated CO₂. The extra carbon fixed by the plants was partitioned to other growing sinks to support their higher meristematic activity (Sharma and Sengupta, 1990). Increase in carbon concentration and reduction in nitrogen content increased the C:N ratio of berseem leaves. High C:N ratio due to elevated CO₂ has been reported in various investigations (Gifford et al., 2000; Farage et al., 1998). As a result, Lincoln et al. (1993) reported 22−80% increase in the rate of consumption by herbivorous insect fed on enriched CO₂ grown foliage.

The phosphorus content of the leaves increased significantly in elevated CO₂ plants by about 12% increase at 40 DAE. An increase in phosphorus content was reported in the straw of wheat plants by Manderscheid et al. (1995). The calcium and iron contents of berseem leaves decreased marginally at all stages of CO₂ exposure. Reduction of calcium and iron contents under elevated CO₂ was also reported in other crop plants (Porter and Grodzinski, 1989; Conroy, 1992; Overdieck, 1993). Fangmeier et al. (1999) suggested that the reduction in nitrogen and other elements associated with it occurred due to the changes in the demand by green leaf tissues of elevated CO₂ grown plants. However, the exact mechanism involved in the alteration of element concentration in leaves is not known.

5. Conclusions

This study concludes that the overall effect of elevated CO₂ (600 µl l⁻¹) on berseem crop is similar to that in many other C₃ plant species. However, in most of the crops, the final yield (seed) is the most important factor, whereas, in berseem being a forage legume, vegetative biomass is the most important factor. Thus, the nutritional composition of leaf biomass is of utmost significance so far as the quality of fodder for the cattle is concerned. The present study showed that under elevated CO₂ the vegetative biomass produced per unit time (both fresh and dry mass) increased significantly. This suggests that under elevated CO₂, fodder production of berseem crop will be 30−35% greater annually on a per unit area basis as compared to that of ambient CO₂ grown crop. However, the nutritional value of the crop may decline due to a reduction in the concentration of various major and micronutrients on a per unit mass basis. High C:N ratio due to the reduction in nitrogen content and increased carbon content per unit mass of leaves and the lower protein content will deteriorate the nutritional quality of the forage.

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References


