Elevated CO₂ influences photosynthetic characteristics of *Avena sativa* L cultivars

**R.K. Bhatt,** M.J. **Baig** and H.S. Tiwari

1Indian Grassland and Fodder Research Institute, Jhansi - 284 003, India
2Central Rice Research Institute, Cuttuck - 753 006, India

(Received: May 12, 2009; Revised received: February 02, 2010; Accepted: February 26, 2010)

**Abstract:** The impact of elevated CO₂ concentration on the growth, photosynthesis and biomass production was investigated in three oat (*Avena sativa*) cultivars viz. Kent, JHO-822 and JHO-851 by growing under three environmental conditions i.e. elevated CO₂ at 600 ± 50 µmol mol⁻¹ (C₂e), OTC with ambient CO₂ (C₀) and under open field condition (C₉). Plant height and leaf area increased in the elevated CO₂ grown plants. JHO-822 attained maximum height under C₂e followed by Kent and JHO-851. The specific leaf mass (SLM) and specific leaf area (SLA) were also influenced significantly when the plants were grown under C₂e. Kent showed highest SLM under C₀, corresponding lower value of SLA. The accumulation of soluble protein in the oat leaves decreased under C₂e except JHO-822 where marginal increase in soluble protein was recorded under C₂e. JHO-822 showed an increase in Chl a, b and total in C₂e over C₀, whereas other two cultivars did not follow any specific trend in the pigment accumulation. Our results confirmed that the net photosynthetic rate (Pn) increased by 37% in Kent followed by JHO 822 under elevated CO₂ over the control. This strong association of Pn with gₛ was evidenced by a positive significant correlation (r=0.885**). A clear stimulatory effect at elevated CO₂ was detected in all the cultivars in terms of green and dry matter production than at ambient CO₂ and C₀. A large increase in Pn in the present investigation was accompanied by relatively small decrease in gₛ which limits the water loss through transpiration rate. The elevated CO₂ induced changes in gₛ and reduction in transpiration.

**Key words:** Biomass production, Oat, OTC, Specific leaf mass, Photosynthesis, Stomatal conductance

PDF of full length paper is available online

**Introduction**

Factors associated with global environmental change, particularly in elevated atmospheric CO₂ and temperature, changes in the mean and variance of regional perception, and land-use changes, are predicted to have profound effects on ecosystem functioning in the future. There is evidence that some factors are already affecting current ecosystems. There is strong evidence that plants have already responded to the 25% increase in atmospheric CO₂ that has occurred since the onset of the Industrial revolution (Dippery et al., 1995; Duquesnay et al., 1998). Further more, atmospheric CO₂ concentrations are projected to double from the current concentration of 360 to 700 µmol mol⁻¹ within the next 80 yrs, which will further stimulate ecosystem responses. In addition, similar increases in CO₂ are expected to occur in all ecosystems, making this change unique among global change factors. Because the predicted increase in atmospheric CO₂ may affect biological processes at many levels of organization (Mooney et al., 1999), it is important to continue studying the direct effects of elevated CO₂ ranging from the molecular to the global.

The current level of atmospheric CO₂ (360 µmol mol⁻¹) is a limiting factor for maximum photosynthetic rate (Tolbert and Zelitch, 1983), any increase in CO₂ above ambient level has the potential to increase the rate of photosynthesis, more particularly in C₃ plants. Effect of elevated CO₂ on C₃ photosynthetic rates have been the subject of many CO₂ enrichment studies. Most of these studies showed that photosynthetic rate is increased following initial exposure to elevated CO₂ (hours to days). Increases in photosynthetic rate are brought about by increased availability of CO₂ at the chloroplasts and reduction in photorespiration resulting from an increased ratio of CO₂ to O₂ (Farquhar and Sharkey, 1982). The increased rate of photosynthesis has been shown to increase growth and yield in many crop species grown under elevated CO₂ (Das et al., 2000). However the response of plants to elevated CO₂ differs from one species to another.

There have been a few studies on the effects of elevated CO₂ on fodder crops (Gorisson and Cotrufo, 2000; Wagner et al., 2001; Morgan et al., 2001). Oat (*Avena sativa*) is widely recognized as one of the major cultivated C₃ fodder as well as grain crop which are nutritive as well as highly palatable. The crude protein percent in oat genotypes varies from 7.4 -16.4% and the dry matter digestibility ranges from 7.6 to 8.4% (Pathak and Jakhmola, 1983). In this piece of work an attempt has been made to study the effect of elevated CO₂ on growth, biomass production and assimilatory functions in oat cultivars.

**Materials and Methods**

**Plant materials and growth condition:** Oat (*Avena sativa*) cultivars JHO 822, JHO 851 and Kent were grown inside the open top chambers (OTCs) 3 m diameter and 10 m height) lined with transparent PVC sheets (0.125 mm thickness). Seeds were sown in line with 25 cm spacing between lines in OTCs and open field condition as well which acted as control. The lands were fertilized with the fertilizer N:P:K (60:40:40) kg ha⁻¹ in two splits, half of the dose as basal before sowing and the rest half at the active tillering stage i.e.
at 35 days after sowing. Irrigation was given as and when required. Pure CO$_2$ gas was used for the enrichment of the cropping environment. Rubber pipes with small holes throughout were circulated inside the OTC, which acted as the elevated CO$_2$ environment at the canopy height and the same was connected to the gas cylinders containing pure CO$_2$ gas. The flow of the CO$_2$ was adjusted with a flow meter to get the exact concentration of CO$_2$ (600 ± 50 µmol mol$^{-1}$). Similarly OTCs were used as control where the crop was grown under ambient CO$_2$ (360 µmol mol$^{-1}$). The crop was also grown in open field with ambient CO$_2$ (360 µmol mol$^{-1}$).

There were three replicate chambers and open plots each for elevated and ambient CO$_2$ exposure with a Complete Randomized Design. The period of CO$_2$ enrichment was 90 days from 08:00 to 17:00 from 2nd leaf stage of the crop. The periodical monitoring of CO$_2$ inside the chamber was done by using IRGA.

**Measurements of photosynthesis and related parameters:** The net photosynthetic rate ($P_n$) was measured at the 50% flowering stage of the crop with a portable photosynthesis system Li-6200 (LI-COR, Inc, Lincoln, NE, USA). $P_n$ was recorded in the fully expanded second leaf between 10:00 to 11:30 hr when the photosynthetic active radiation (PAR) ranged between 1200-1400 µmol m$^{-2}$ s$^{-1}$. For measurement of growth characters, oat plants of one m$^2$ were harvested from each chamber as well as from open field condition. The leaves and stem portion were separated after the recording of the tiller number and the plant height. All the plant parts were dried at 80°C for determining the dry mass. The leaf area was measured by using the LI-3000 area meter (LI-COR). The fresh and dry masses of the leaf samples were recorded. Specific leaf mass (SLM), leaf thickness expressed as the dry mass of leaf blade per unit leaf area (g cm$^{-1}$) and the specific leaf area (SLA), expressed as the ratio of unit leaf area by unit leaf mass (cm$^2$ g$^{-1}$) (Yoshida et al., 1976).

**Biochemical analysis:** To determine chlorophyll content fully expanded leaf from top was collected at random from three plants and after cleaning the leaves were cut into small pieces (2-3 mm$^2$), placed in dimethyl sulfoxide (DMSO) at 60°C for 4 hr in oven, the pigments extracted to the organic solvent, DMSO was measured colorimetrically with an UV-VIS spectrophotometer (UNCAM, USA) at 645 and 663 nm using DMSO as a reference. Chlorophyll (a, b and total) contents in fresh mass basis were calculated using the method of Hiscox and Israelstam (1979). For soluble protein estimation fresh leaves were ground in a pre-chilled pestle and mortar with 1:2 (m/v) 50 mM phosphate buffers, pH 7.0. Homogenate was centrifuged at 4°C for 20 min. at 15000 g. This extract was used for estimating soluble protein following the procedure of Lowry et al. (1951).

**Results and Discussion**

**Stem and leaf growth:** Long-term exposure to elevated CO$_2$ (600 ± 50 µmol mol$^{-1}$) in open-top chambers increased the growth of oat cultivars. Plant height and leaf area increased in elevated CO$_2$ grown plants. Among the oat cultivars JHO 822 attained maximum height (68.5 cm) followed by Kent (62.7 cm) and JHO 851 (46.3 cm) under elevated CO$_2$ (Fig. 1). The rate of growth and branching increased in some tree species exposed to elevated CO$_2$ (Curtis and Wang, 1998). Long-term exposure of *Avena sativa* L. cultivars to elevated CO$_2$ in OTCs resulted in a significant growth enhancement, which continued throughout the period of elevated CO$_2$ exposure. This increase in growth may be due to the greater amounts of carbon assimilation. This result supports the observations of Sharma and Sengupta (1990), which showed that the extra carbon fixed by the plants due to CO$_2$ enrichment translocated towards the growing axis. A significant increase in the leaf length was observed in oat cultivars under elevated CO$_2$ (Table 1). In case of 1st leaf (flag leaf) the cultivar JHO 851 showed highest value followed by Kent and JHO 822, however, in case of 2nd leaf the highest value was observed in Kent followed by other two cultivars as JHO 822 and JHO 851. Leaf width varies from 1.8 to 2.24 cm in 1st leaf and 1.66 to 2.1 cm in 2nd leaf in all the cultivars. The specific leaf mass and specific leaf area was also influenced significantly when the plants were grown under high concentration of CO$_2$ (Table 1). JHO-822 and Kent showed highest SLM corresponding to lower value of SLA indicating that with high SLM the dry matter accumulation per unit leaf area was more and corresponding leaf expansion was less showing a lower value of SLA. However JHO 851 showed less SLM and high SLA. High CO$_2$ stimulated leaf proliferation and number of leaves per plant; however, SLA of plants grown in C$_{600}$ was considerably decreased due to increase in total biomass. In our experiment, high CO$_2$ concentrations stimulated allocation of more biomass to leaves as was established by higher SLM. According to Poorter et al. (1979) this pronounced increase in SLM is due to changes in leaf chemical composition, mainly due to the accumulation of total non-structural saccharides. Much of the increase in leaf mass per area was probably due to the accumulation of starch (Cave et al., 1981; Mauney et al., 1979). As leaf number increases, leaf area index (leaf area/land area) may also increase, resulting in higher carbon assimilation on an ecosystem level. Jach and Ceulemans (1999) found evidence for these responses in *Pinus sylvestris* seedlings grown at elevated CO$_2$ and they predicted that the increase in LAI would result in more rapid canopy closure. These results indicate that changes in growth form response to elevated CO$_2$ may have a substantial effect on light interception. In our finding we also confirmed that the LAI in all the genotypes increased significantly when the crop was subjected to elevated CO$_2$ environment (Table 2).

**Soluble protein and photosynthetic pigments:** The accumulation of soluble protein in the oat leaves decreased under elevated CO$_2$ except JHO 822 where marginal increase was recorded under C$_{600}$. However, under OTC at ambient CO$_2$ both the cultivars JHO 822 and JHO 851 showed a significant increase in the soluble protein content of the leaves. 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).

The accumulation of photosynthetic pigments was influenced by the elevated CO$_2$ in the cv JHO 822 alone with an increase in chlorophyll a, b and total chlorophyll over the control. Other two
Avena sativa photosynthesis under elevated CO\textsubscript{2}

cultivars did not show any accumulation of pigments (Fig. 2A,B). This implies that leaves grown at high CO\textsubscript{2} can efficiently capture the photons for photosynthesis grow at ambient CO\textsubscript{2}. In our experiment Avena sativa cv. JHO 822 showed an increase in Chl content under elevated CO\textsubscript{2}, suggesting an increase in efficiency of radiant energy capture through a shift in carbon allocation with time.

In our experiment reduction in Chl amount in the cultivar Kent and JHO 851 is an indicator of structural damage of PS II and not all reaction centres opened for primary chemistry. Wilkins \textit{et al.} (1994) found a decrease of D1 and D2 in PS II core complex during the long term exposure to high CO\textsubscript{2} in \textit{P. avium}. The variability in Chl content among the species was much profound and possibly arising from content of water and amount of non-photosynthesising tissues. There was substantial variation between species in the extent and nature of alteration in photosynthetic characteristics. This is demonstrated in Fig. 4 \textit{(P\textsubscript{N} and Chl a/b)}. These parameters are

---

**Fig. 1:** Height of oat cultivars affected by elevated CO\textsubscript{2}

**Fig. 2:** Chlorophyll \textit{a,b} content \textit{(A)} and \textit{a/b} ratio \textit{(B)} as influenced by elevated CO\textsubscript{2} in the oat cultivars

**Fig. 3:** Dry matter yield in oat cultivars as influenced by elevated CO\textsubscript{2}

**Fig. 4:** Difference in chlorophyll \textit{a/b} ratio plotted against the difference in maximal net photosynthetic rate, \textit{P}\textsubscript{\textit{Nmax}}

**Fig. 5:** Canopy photosynthesis plotted against dry matter yield in different cultivars of oat under elevated CO\textsubscript{2}
### Table 1: Variation in leaf size, specific leaf mass and specific leaf area as influenced by elevated CO₂

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>1st Leaf (flag leaf)</th>
<th>2nd Leaf</th>
<th>SLM (mg cm⁻²)</th>
<th>SLA (cm² g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OPEN</td>
<td>OTC</td>
<td>OTC+CO₂</td>
<td>OPEN</td>
</tr>
<tr>
<td>KENT</td>
<td>43.92±2.59</td>
<td>59.36±2.27</td>
<td>60.52±4.29</td>
<td>60.38±2.22</td>
</tr>
<tr>
<td>JHO 822</td>
<td>46.38±4.31</td>
<td>47.48±4.68</td>
<td>53.44±4.32</td>
<td>52.52±1.82</td>
</tr>
<tr>
<td>JHO 851</td>
<td>43.22±2.77</td>
<td>48.32±4.66</td>
<td>63.12±1.94</td>
<td>54.32±2.58</td>
</tr>
</tbody>
</table>

OPEN = Open field condition, OTC = Open top chamber with ambient CO₂, OTC+CO₂ = Open top chamber with elevated ambient CO₂, SLM = Specific leaf mass, SLA = Specific leaf area, Mean values±SD (n=6)

### Table 2: Photosynthetic rate, stomatal conductance, transpiration rate and variation in leaf area index as influenced by elevated CO₂

<table>
<thead>
<tr>
<th></th>
<th>Pₙ (µ moles m⁻²)</th>
<th>gₛ (mol m⁻²s⁻¹)</th>
<th>Transpiration (µ moles m⁻²s⁻¹)</th>
<th>LAI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OPEN</td>
<td>OTC</td>
<td>OTC+CO₂</td>
<td>OPEN</td>
</tr>
<tr>
<td>KENT</td>
<td>22.39</td>
<td>6.80</td>
<td>35.96</td>
<td>0.811</td>
</tr>
<tr>
<td>JHO 822</td>
<td>23.73</td>
<td>16.06</td>
<td>28.79</td>
<td>0.899</td>
</tr>
<tr>
<td>JHO 851</td>
<td>18.98</td>
<td>6.79</td>
<td>18.24</td>
<td>0.775</td>
</tr>
</tbody>
</table>

L.S.D. = Least significant difference, T = Treatment, V = Cultivar, Values significant at p<0.05 level

Pₙ = Photosynthetic rate, gₛ = Stomatal conductance, LAI = Leaf area index, OPEN = Open field condition, OTC = Open top chamber with ambient CO₂, OTC+CO₂ = Open top chamber with elevated ambient CO₂, LSD = Least significant difference, T = Treatment, V = Cultivar, Values significant at p<0.05 level
Avena sativa photosynthesis under elevated CO₂

Table 3: Leaf soluble protein and, fresh and dry biomass (% increase over control) in oat cultivars as influenced by elevated CO₂

<table>
<thead>
<tr>
<th>Soluble protein (mg g⁻¹ fw)</th>
<th>Fresh and dry biomass (% increase over control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPEN</td>
<td>OTC</td>
</tr>
<tr>
<td>KENT</td>
<td>5.93</td>
</tr>
<tr>
<td>JHO-822</td>
<td>5.43</td>
</tr>
<tr>
<td>JHO-851</td>
<td>7.31</td>
</tr>
</tbody>
</table>

OPEN = Open field condition, OTC = Open top chamber with ambient CO₂, OTC+CO₂ = Open top chamber with elevated ambient CO₂. LSD = Least significant different for soluble protein, significant at p<0.05 level, Treatment (T) = 0.589, Cultivar (V) = 2.564, VxT = 1.895

commonly used when monitoring stress sensitive photosynthetic characteristics. The changes in Pₙ under elevated CO₂ are often associated with altered ribulose-1,5-biphosphate carboxylase/ oxygenase content (Stitt, 1986).

Photosynthesis and biomass production: Pₙ increased by 37% in Kent followed by JHO 822 under Cmuş as compared to Cₘ however, no significant change was observed in JHO 851. Increased Pₙ during the growth period of the crop could be interpreted in terms of high CO₂ induced transient activation of photosynthesis as a stress response (Lichtenthaler, 1996). The Pₙ decreased under OTC (without elevated CO₂) in all the cultivars (Table 3). The stomatal conductance followed similar pattern as Pₙ. The reduction in Pₙ under Cₘ occurred may be due to lower stomatal conductance, which also declined under CₒTC. Lesson and Rozema (1990) and Hertog et al. (1993) also reported that rates of photosynthesis also increased due to elevated CO₂.

According to Harley et al. (1992) stomatal conductance (gₛ) decreases in elevated CO₂. Of course these effects depend on water supply (Palanisamy, 1999). In our experiment there were no depression effects on gₛ by CₒTC, rather there were slight increase in gₛ was marked except the cultivar JHO 851 in which the decrease in gₛ was noticed in comparison to the Cₘ. However a decrease in gₛ was marked in the crops grown under OTC with ambient CO₂. Uniform change in physiological parameters could be explained by transitory state of plant organism under high CO₂, preceding another stable level of plant metabolism. The degree of responsiveness of gₛ in the treatments differed. CₒTC stimulated gₛ more than Cₘ and CₒTC in both the cultivars, Kent and JHO-822. Established gₛ values tended to preserve during the experiment and at many measuring data, enhanced gₛ was associated with high Pₙ and the association was depicted as the significant positive correlation (r=0.885**).

Differences in plant growth conditions led to a different stomata response when comparing gₛ and Cₘ. Plants from ambient CO₂ (both open and OTC) exhibited a typical response to increasing CO₂ concentration (high gₛ followed by high Pₙ with increasing CO₂). Plants at CₒTC did not reach saturation, indicating that net photosynthetic rate regeneration capacity increased relative to RuBP carboxylase-regeneration. Sage et al. (1998) suggested that this pattern might not reflect the acclimation, but excess of starch accumulation and subsequent distortion of the chloroplasts that cause a stress response.

Table - 3: Leaf soluble protein and, fresh and dry biomass (% increase over control) in oat cultivars as influenced by elevated CO₂

In all the cultivars the dry matter yield increased significantly under elevated CO₂ (CₒTC) (Fig. 3). JHO 851 showed maximum increase in dry biomass which is in agreement with other results (Teramura et al., 1990; Dev Kumar et al., 1998; Van de Staaij et al., 1993; Hertog et al., 1993; Uprety et al., 2000). There was a 5-fold increase in dry biomass with a 2-fold increase CO₂ level. The percent increase in fresh and dry biomass yield due to the different environmental conditions was depicted in the Table 3. The higher biomass production was also recorded in the oat cultivars under OTC, with ambient CO₂ and it is assumed that the increase may be due to the marginal increase in the temperature in the chamber.

CₒTC stimulated total dry biomass accumulation. Steady increase of dry matter is a common physiological response to high CO₂ concentration (Mott, 1990; Righetti et al., 1996; Atkinson et al., 1997). Van der Werf (1996) considers that high carbon gain per plant is attributed not to high SLM or Pₛ but to the change in SLA, which is in agreement with our results. The canopy photosynthesis (Pₙ x LAI) plays a crucial role in terms of biomass production under elevated CO₂. The canopy Pₙ increased in all the cultivars of Avena as compare to Cₘ and CₒTC. The value under CₒTC declined except the cv. JHO 822. The cv. Kent and JHO 851 maintained high Pₙ and Pₛ x LAI under the elevated CO₂. The correlation between Pₙ x LAI and dry matter yield was depicted in the Fig. 5. The growth at different CO₂ concentrations led to a different biomass partitioning between organs.

Acknowledgments
The authors are thankful to the Director, Indian Grassland and Fodder Research Institute, Jhansi for facilities and Indian Council for Agricultural Research (ICAR), for providing financial support.

References


