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Elevated CO_2 influences photosynthetic characteristics of *Avena sativa* L cultivars

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Abstract: *The impact of elevated CO_2 concentration on the growth, photosynthesis and biomass production was investigated in three oat (Avena sativa L) cultivars viz. Kent, JHO-822 and JHO-851 by growing under three environmental conditions i.e. elevated CO_2 at $600 \pm 50\ \mu$ mol mol^{-1} (C_{600}), OTC with ambient CO_2 (C_{OTC}) and under open field condition (C_a). Plant height and leaf area increased in the elevated CO_2 grown plants. JHO-822 attained maximum height under C_{600} 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 C_{600}. Kent showed highest SLM under C_{600} corresponding lower value of SLA. The accumulation of soluble protein in the oat leaves decreased under C_{600} except JHO-822 where marginal increase in soluble protein was recorded under C_{600}. JHO-822 showed an increase in Chl orophyll a, b and total in C_{600} over C_a, whereas other two cultivars did not follow any specific trend in the pigment accumulation. Our results confirmed that the net phosynthetic rate (P_N) increased by 37% in Kent followed by JHO 822 under elevated CO_2 over the control. This strong association of P_N with g_s was evidenced by a positive significant correlation (r=0.885**). A clear stimulatory effect at elevated CO_2 was detected in all the cultivars in term of green and dry matter production than at ambient CO_2 and C_{OTC}. A large increase in P_N in the present investigation was accompanied by relatively small decrease in g_s, which limits the water loss through transpiration rate. The elevated CO_2 induced changes in g_s 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 CO_2 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 CO_2 that has occurred since the onset of the Industrial revolution (Dippery *et al.,* 1995; Duquesnay *et al.,* 1998). Further more, atmospheric CO_2 concentrations are projected to double from the current concentration of 360 to 700 μ mol mol^{-1} within the next 80 yrs, which will further stimulate ecosystem responses. In addition, similar increases in CO_2 are expected to occur in all ecosystems, making this change unique among global change factors. Because the predicted increase in atmospheric CO_2 may affect biological processes at many levels of organization (Mooney *et al.,* 1999), it is important to continue studying the direct effects of elevated CO_2 ranging from the molecular to the global.

The current level of atmospheric CO_2 (360 μ mol mol^{-1}) is a limiting factor for maximum photosynthetic rate (Tolbert and Zelitch, 1983), any increase in CO_2 above ambient level has the potential to increase the rate of photosynthesis, more particularly in C_3 plants. Effect of elevated CO_2 on C_3 photosynthetic rates have been the subject of many CO_2 enrichment studies. Most of these studies showed that photosynthetic rate is increased following initial exposure to elevated CO_2 (hours to days). Increases in photosynthetic rate are brought about by increased availability of CO_2 at the chloroplasts and reduction in photorespiration resulting from an increased ratio of CO_2 to O_2 (Farquhar and Sharkey, 1982). The increased rate of photosynthesis has been shown to increase growth and yield in many crop species grown under elevated CO_2 (Das *et al.,* 2000). However the response of plants to elevated CO_2 differs from one species to another.

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). Oat (*Aven sativa* L.) is widely recognized as one of the major cultivated C_3 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 CO_2 on growth, biomass production and assimilatory functions in oat cultivars.

Materials and Methods

Plant materials and growth condition: Oat (*Avena sativa* L.) 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.*

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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 μ mol mol^{-1}). Similarly OTCs were used as control where the crop was grown under ambient CO_2 (360 μ mol mol^{-1}). The crop was also grown in open field with ambient CO_2 (360 μ mol 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 chamberwas 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 μ mol 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^{o}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 was recorded. Specific leaf mass (SLM), leaf thickness expressed as the dry mass of leaf blade per unit leaf area (g cm^{-2}) 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 sulphoxide (DMSO) at 60^{o}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 centrifuge at 4^{o}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 μ mol 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 through out 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, incase 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 less 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 pigment 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 cultivars did not show any accumulation of pigments (Fig. 2A,B). This implies that leaves grown at high CO_2 can efficiently capture the photons for photosynthesis grow at ambient CO_2. In our experiment *Avena sativa* cv. JHO 822 showed an increase in Chl content under elevated CO_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 *et al.* (1994) found a decrease of D1 and D2 in PS II core complex during the long term exposure to high CO_2 in *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 (P_N and Chl *a/b*). These parameters are

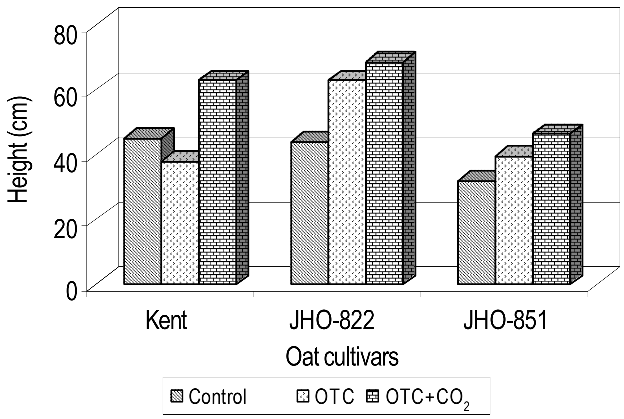

Fig. 1: Height of oat cultivars affected by elevated CO_2

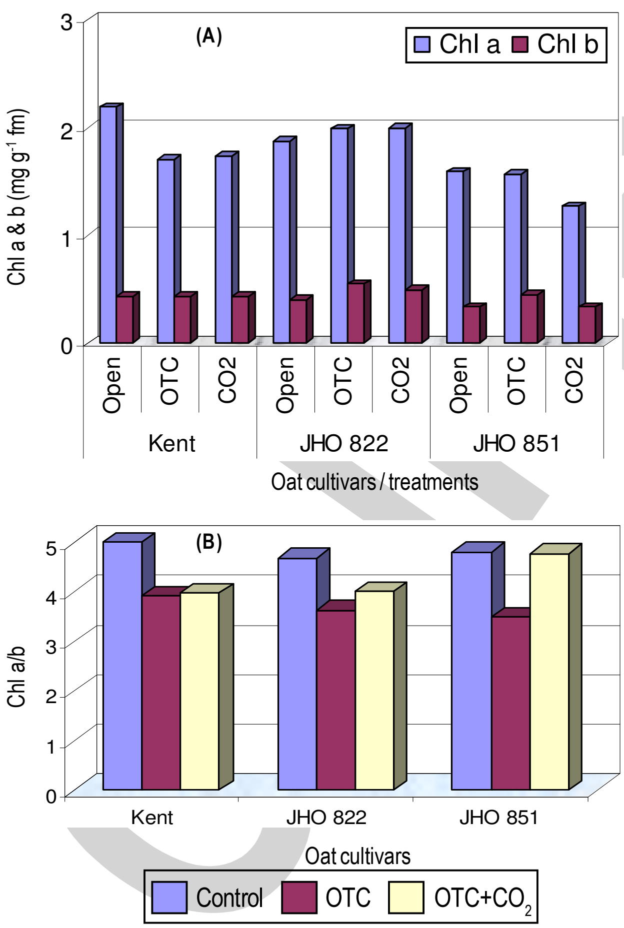

Fig. 2: Chlorophyll *a,b* content (A) and *a/b* ratio (B) as influenced by elevated CO_2 in the oat cultivars

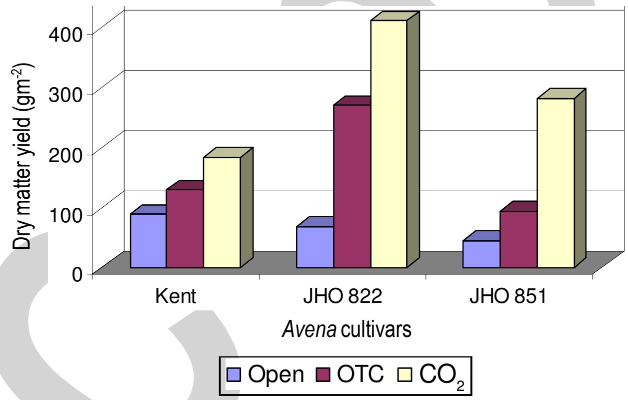

Fig. 3: Dry matter yield in oat cultivars as influenced by elevated CO_2

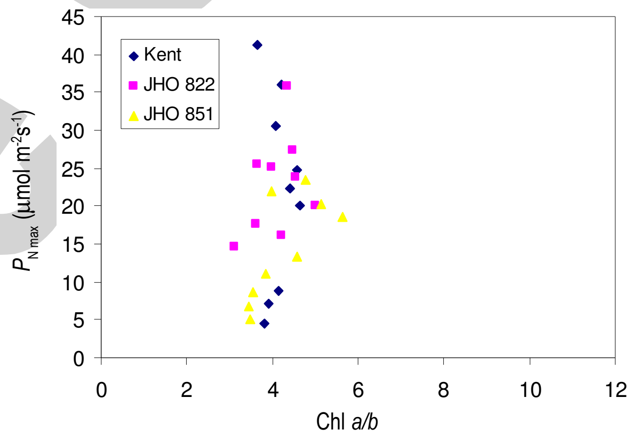

Fig. 4: Difference in chlorophyll a/b ratio plotted against the difference in maximal net photosynthetic rate, P_{Nmax}

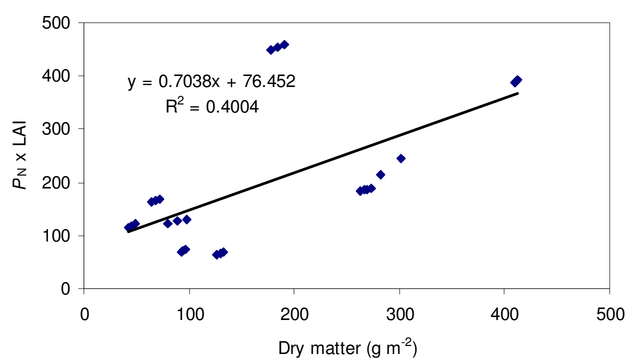

Fig. 5: Canopy photosynthesis plotted against dry matter yield in different cultivars of oat under elevated CO_2

Table - 1: Variation in leaf size, specific leaf mass and specific leaf area as influenced by elevated CO_2

Cultivars	Leaf length (cm)						SLM (mg cm^{-2})			SLA (cm^2 g^{-1})		
	1st Leaf (flag leaf)			2nd Leaf								
	OPEN	OTC	OTC+CO_2	OPEN	OTC	OTC+CO_2	OPEN	OTC	OTC+CO_2	OPEN	OTC	OTC+CO_2
KENT	43.92±2.59	59.36±2.27	60.52±4.29	60.38±2.22	52.72±4.95	65.46±5.00	4.94±0.52	5.02± 0.48	6.17±1.01	202.4±5.94	199.4±2.25	161.9±4.32
JHO 822	46.38±4.31	47.48±4.68	53.44±4.32	52.52±1.82	47.04±4.13	60.56±2.19	5.05±0.65	5.37±0.76	6.11±0.76	197.9±4.74	186.1±3.34	163.8±2.91
JHO 851	43.22±2.77	48.32±4.66	63.12±1.94	54.32±2.58	51.28±1.77	60.20±3.89	3.99±0.37	4.76±0.54	3.93±0.54	250.5±2.51	210.1±5.65	254.2±3.98

OPEN =Open field condition, OTC =Open top chamber with ambient CO_2, OTC+CO_2 = Open top chamber with elevated with ambient CO_2, 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 CO_2

	P_N (µ moles m^2s^{-1})			g_s (mol $m^{-2}s^{-1}$)			Transpiration (µ moles m^2s^{-1})			LAI		
	OPEN	OTC	OTC+CO_2	OPEN	OTC	OTC+CO_2	OPEN	OTC	OTC+CO_2	OPEN	OTC	OTC+CO_2
KENT	22.39	6.80	35.96	0.811	0.258	0.958	15.53	8.25	10.52	5.64	9.87	12.64
JHO 822	23.73	16.06	28.79	0.899	0.631	1.327	14.99	16.97	15.54	6.98	11.67	13.54
JHO 851	18.98	6.79	18.24	0.775	0.211	0.569	13.48	7.99	15.22	6.24	10.56	11.76
L.S.D.		T =10.235			T= 0.3085			T= 3.8812			T= 0.7865	
p<0.05		V = 7.122			V= 0.3774			V= 2.6356			V= 1.7635	
		VxT = 7.958			VxT = NS			VxT = 2.9448			VxT = 1.2135	

P_N = Photosynthetic rate, g_s = Stomatal conductance, LAI = Leaf area index, OPEN =Open field condition, OTC =Open top chamber with ambient CO_2, OTC+CO_2 = Open top chamber with elevated with ambient CO_2, LSD = Least significant difference, T = Treatment, V = Cultivar, Values significant at p<0.05 level

Table - 3: Leaf soluble protein and, fresh and dry biomass (% increase over control) in oat cultivars as influenced by elevated CO_2

	Soluble protein (mg g^{-1} fw)			Fresh and dry biomass (% increase over control)			
				Fresh		Dry	
	OPEN	OTC	OTC+CO_2	OTC	OTC +CO_2	OTC	OTC +CO_2
KENT	5.93	5.27	4.89	-	115.60	45.93	107.35
JHO-822	5.43	6.49	5.74	182.46	432.48	29.55	502.44
JHO-851	7.31	9.73	7.61	179.06	878.64	106.79	517.31

OPEN =Open field condition, OTC =Open top chamber with ambient CO_2, OTC+CO_2 = Open top chamber with elevated with ambient CO_2, 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 P_N under elevated CO_2 are often associated with altered ribulose-1,5-biphosphate carboxylase/ oxygenase content (Stitt, 1986).

Photosynthesis and biomass production: P_N increased by 37% in Kent followed by JHO 822 under C_{600} as compared to C_a, however, no significant change was observed in JHO 851. Increased P_N during the growth period of the crop could be interpreted in terms of high CO_2 induced transient activation of photosynthesis as a stress response (Lichtenthaler, 1996). The P_N decreased under OTC (without elevated CO_2) in all the cultivars (Table 3). The stomatal conductance followed similar pattern as P_N. The reduction in P_N under C_{OTC} occurred may be due to lower stomatal conductance, which also declined under C_{OTC}. Lesson and Rozema (1990) and Hertog *et al*. (1993) also reported that rates of photosynthesis also increased due to elevated CO_2.

According to Harley *et al.* (1992) stomatal conductance (g_s) decreases in elevated CO_2. Of course these effects depend on water supply (Palanisamy, 1999). In our experiment there were no depression effects on g_s by C_{600}, rather there were slight increase in g_s was marked except the cultivar JHO 851 in which the decrease in g_s was noticed in comparison to the C_a. However a decrease in g_s was marked in the crops grown under OTC with ambient CO_2. Uniform change in physiological parameters could be explained by transitory state of plant organism under high CO_2 preceding another stable level of plant metabolism. The degree of responsiveness of g_s in the treatments differed. C_{600} stimulated g_s more than C_a and C_{OTC} in both the cultivars, Kent and JHO-822. Established g_s values tended to preserve during the experiment and at many measuring data, enhanced g_s was associated with high P_N 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 g_s and C_i. Plants from ambient CO_2 (both open and OTC) exhibited a typical response to increasing CO_2 concentration (high g_s followed by high P_N with increasing CO_2). Plants at C_{600} did not reach saturation, indicating that net photosynthetic rate regeneration capacity increased relative to RuBP carboxylase-regeneration. Sage *et al.* (1988) 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.

In all the cultivars the dry matter yield increased significantly under elevated CO_2 (C_{600}) (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 CO_2 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 CO_2 and it is assumed that the increase may be due to the marginal increase in the temperature in the chamber.

C_{600} stimulated total dry biomass accumulation. Steady increase of dry matter is a common physiological response to high CO_2 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 P_N, but to the change in SLA, which is in agreement with our results. The canopy photosynthesis (P_N x LAI) plays a crucial role in terms of biomass production under elevated CO_2. The canopy P_N increased in all the cultivars of *Avena* as compare to C_a and C_{OTC}. The value under C_{OTC} declined except the cv JHO 822. The cv. Kent and JHO 851 maintained high P_N and P_N x LAI under the elevated CO_2. The correlation between P_N x LAI and dry matter yield was depicted in the Fig. 5. The growth at different CO_2 concentrations led to a different biomass partitioning between organs.

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