

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/236189497>

# Photosynthetic response of Cannabis sativa L. to variations in photosynthetic photon flux densities, temperature and CO<sub>2</sub> conditions

Article in *Physiology and Molecular Biology of Plants* · October 2008

DOI: 10.1007/s12298-008-0027-x · Source: PubMed

CITATIONS

62

READS

10,448

4 authors:



**Suman Chandra**

University of Mississippi

119 PUBLICATIONS 3,598 CITATIONS

[SEE PROFILE](#)



**Hemant Lata**

University of Mississippi

106 PUBLICATIONS 2,026 CITATIONS

[SEE PROFILE](#)



**Ikhlas Khan**

University of Mississippi

807 PUBLICATIONS 20,341 CITATIONS

[SEE PROFILE](#)



**Mahmoud A Elsohly**

University of Mississippi

365 PUBLICATIONS 12,535 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Plant antioxidants [View project](#)



Analytical Applications [View project](#)



# Photosynthetic response of *Cannabis sativa* L. to variations in photosynthetic photon flux densities, temperature and CO<sub>2</sub> conditions

Suman Chandra<sup>1</sup>, Hemant Lata<sup>1</sup>, Ikhlas A. Khan<sup>1,2</sup> and Mahmoud A. Elsohly<sup>1,3</sup>

<sup>1</sup>National Center for Natural Product Research, School of Pharmacy, University of Mississippi, MS-38677, USA.

<sup>2</sup>Department of Pharmacognosy, University of Mississippi, MS-38677, USA.

<sup>3</sup>Department of Pharmaceutics, School of Pharmacy, University of Mississippi, University, MS 38677, USA.

## ABSTRACT

Effect of different photosynthetic photon flux densities (0, 500, 1000, 1500 and 2000  $\mu\text{mol m}^{-2}\text{s}^{-1}$ ), temperatures (20, 25, 30, 35 and 40 °C) and CO<sub>2</sub> concentrations (250, 350, 450, 550, 650 and 750  $\mu\text{mol mol}^{-1}$ ) on gas and water vapour exchange characteristics of *Cannabis sativa* L. were studied to determine the suitable and efficient environmental conditions for its indoor mass cultivation for pharmaceutical uses. The rate of photosynthesis ( $P_N$ ) and water use efficiency (WUE) of *Cannabis sativa* increased with photosynthetic photon flux densities (PPFD) at the lower temperatures (20–25 °C). At 30 °C,  $P_N$  and WUE increased only up to 1500  $\mu\text{mol m}^{-2}\text{s}^{-1}$  PPFD and decreased at higher light levels. The maximum rate of photosynthesis ( $P_{N\text{ max}}$ ) was observed at 30 °C and under 1500  $\mu\text{mol m}^{-2}\text{s}^{-1}$  PPFD. The rate of transpiration (E) responded positively to increased PPFD and temperature up to the highest levels tested (2000  $\mu\text{mol m}^{-2}\text{s}^{-1}$  and 40 °C). Similar to E, leaf stomatal conductance ( $g_s$ ) also increased with PPFD irrespective of temperature. However,  $g_s$  increased with temperature up to 30 °C only. Temperature above 30 °C had an adverse effect on  $g_s$  in this species. Overall, high temperature and high PPFD showed an adverse effect on  $P_N$  and WUE. A continuous decrease in intercellular CO<sub>2</sub> concentration (Ci) and therefore, in the ratio of intercellular CO<sub>2</sub> to ambient CO<sub>2</sub> concentration (Ci/Ca) was observed with the increase in temperature and PPFD. However, the decrease was less pronounced at light intensities above 1500  $\mu\text{mol m}^{-2}\text{s}^{-1}$ . In view of these results, temperature and light optima for photosynthesis was concluded to be at 25–30 °C and ~1500  $\mu\text{mol m}^{-2}\text{s}^{-1}$  respectively. Furthermore, plants were also exposed to different concentrations of CO<sub>2</sub> (250, 350, 450, 550, 650 and 750  $\mu\text{mol mol}^{-1}$ ) under optimum PPFD and temperature conditions to assess their photosynthetic response. Rate of photosynthesis, WUE and Ci decreased by 50 %, 53 % and 10 % respectively, and Ci/Ca, E and  $g_s$  increased by 25 %, 7 % and 3 % respectively when measurements were made at 250  $\mu\text{mol mol}^{-1}$  as compared to ambient CO<sub>2</sub> (350  $\mu\text{mol mol}^{-1}$ ) level. Elevated CO<sub>2</sub> concentration (750  $\mu\text{mol mol}^{-1}$ ) suppressed E and  $g_s$  ~ 29% and 42% respectively, and stimulated  $P_N$ , WUE and Ci by 50 %, 111 % and 115 % respectively as compared to ambient CO<sub>2</sub> concentration. The study reveals that this species can be efficiently cultivated in the range of 25 to 30 °C and ~1500  $\mu\text{mol m}^{-2}\text{s}^{-1}$  PPFD. Furthermore, higher  $P_N$ , WUE and nearly constant Ci/Ca ratio under elevated CO<sub>2</sub> concentrations in *C. sativa*, reflects its potential for better survival, growth and productivity in drier and CO<sub>2</sub> rich environment. [Physiol. Mol. Biol. Plants 2008; 14(4) : 299–306] E-mail : [suman@olemiss.edu](mailto:suman@olemiss.edu)

**Key words :** *Cannabis sativa*, Photosynthesis, Transpiration, Water use efficiency

**Abbreviations :** PPFD - Photosynthetic photon flux density,  $P_N$  - Photosynthesis,  $R_d$  - Dark respiration,  $P_{N\text{ max}}$  - Maximum rate of photosynthesis, E - Transpiration,  $g_s$  - Leaf stomatal conductance, Ci - Leaf internal CO<sub>2</sub> concentration, Ci/Ca - Internal to ambient CO<sub>2</sub> concentration, WUE - Water use efficiency

The ability of a species to acclimate and adapt to environmental variations is directly/indirectly associated with its ability to modulate photosynthesis and water vapour exchange (Pearcy, 1977; Berry and Downton, 1982; Stoutjesdijk and Barkman, 1992; Ayuko *et al.*, 2008; Dieleman and Meinen, 2008; Kruse *et al.*, 2008), which

in turn affects biochemical and physiological processes in the leaf and, consequently the physiology and productivity of whole plant. Studies on gas exchange characteristics may provide valuable information on functioning of plants in variable environment. Photosynthesis, being the primary source of carbon and energy, plays a prominent role in the logistics of plant growth. There is a close correlation between

**Correspondence and Reprint requests :** Suman Chandra

productivity and yield of the plants with their photosynthetic rate, in the given environment, as more than 90% of dry matter of live plants is derived from photosynthetic CO<sub>2</sub> assimilation (Zelitch, 1975). Therefore, photosynthesis is a valuable physiological tool to evaluate the response of plants to environmental stresses and for the rapid selection of plants for a particular environmental condition (Joshi and Palni, 2005; Monclus *et al.*, 2006) or selection of suitable environmental conditions for a particular plant species.

Furthermore, elevated CO<sub>2</sub> may increase photosynthetic carbon assimilation and may accelerate plant growth and potentially improve productivity. Indeed, a doubling in CO<sub>2</sub> concentration increases crop yield by 30% or more, in experiments conducted under close environmental conditions such as green houses and growth chambers (Kimball, 1983a, b; 1986; Cure, 1985; Poorter, 1993; Idso and Idso, 1994). Therefore, in the present study, *C. sativa* plants were exposed to a range of CO<sub>2</sub> concentration to understand their response in term of their photosynthetic capacity to the range of elevated CO<sub>2</sub> labels.

*Cannabis sativa* L. is widely distributed around the world. Originally indigenous to temperate regions of Asia, it now grows in a variety of habitats ranging from sea level in tropical areas to alpine foot hills of Himalayas. *Cannabis* has a long history of the medicinal use in Middle East and Asia, with references as far back as the 6<sup>th</sup> century B.C. This species was introduced in the Western Europe medicine in the early 19<sup>th</sup> century A.C. to treat epilepsy, tetanus, rheumatism, migraine, asthma, trigeminal neuralgia, fatigue, and insomnia (Doyle and Spence, 1995; Zuardi, 2006). *C. sativa* contains cannabinoids, a unique class of terpenophenolic compounds, which accumulates mainly in glandular trichomes of the plant (Hammond and Mahlberg, 1977). Over 70 cannabinoids have been isolated from *Cannabis sativa*, the major biologically active compound being  $\Delta^9$ -tetrahydrocannabinol, commonly referred as THC (Mechoulam and Ben-Shabat, 1999). Besides its psychoactivity, THC possesses analgesic, anti-inflammatory, appetite stimulant and anti-emetic properties making this cannabinol a very promising therapeutic drug, especially for cancer and AIDS patients (Sirikantaramas *et al.*, 2005). The pharmacologic and therapeutic potency of preparations of *Cannabis sativa* L. and its main active constituent  $\Delta^9$ -tetrahydrocannabinol (THC) has been extensively reviewed by researchers (Mechoulam, 1986; Formukong *et al.*, 1989; Grinspoon and Bakalar, 1993; Mattes *et al.*, 1993; 1994; Brenneisen *et al.*, 1996).

THC has a tremendous commercial value in the pharmaceutical market. Since *C. sativa* is a natural and inexpensive source of THC (as compared to producing it synthetically), efforts to select *Cannabis* varieties with high THC content are underway. However, due to the allogamous (cross fertilization) nature of the species, it is very difficult to maintain the chemical profile of selected high THC-producing genotypes under field conditions. Since this plant is also used as an illicit drug, its cultivation in open field must be done in secured areas and is highly regulated in the USA and some other parts of the world. Considering these limitations, indoor cultivation of a selected high yielding genotype/clone under controlled environmental conditions is the most suitable way to maintain its potency and efficacy while circumventing the regulatory problems. The objective of this study was to determine the effect of light intensity, temperature and CO<sub>2</sub> conditions on gas and water vapour exchange characteristics of *C. sativa* L. to establish suitable and efficient environmental conditions for its indoor cultivation.

## MATERIAL AND METHODS

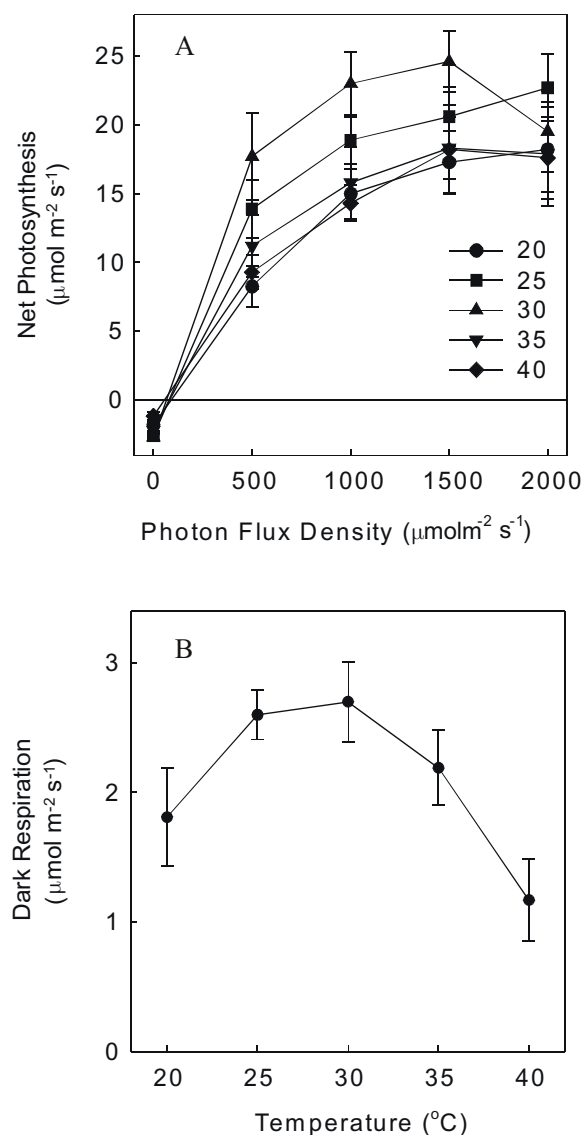
To study the photosynthetic response of *C. sativa* under different PPFD and temperature levels, leaves of twenty vegetatively propagated, four month old plants from a single mother plant of high yielding Mexican variety were exposed to a range of PPFD (0, 500, 1000, 1500 and 2000  $\mu\text{mol m}^{-2}\text{s}^{-1}$ ) and temperature conditions (20, 25, 30, 35 and 40 °C) under controlled humidity ( $55 \pm 5\%$ ) and CO<sub>2</sub> ( $350 \pm 5 \mu\text{mol mol}^{-1}$ ) concentration to determine suitable environmental conditions for its optimum photosynthetic assimilation. Thereafter, leaves were acclimated under optimum light and temperature conditions and exposed to different CO<sub>2</sub> concentrations (250, 350, 450, 550, 650 and 750  $\mu\text{mol mol}^{-1}$ ) to study the effect of CO<sub>2</sub> on photosynthetic and water vapour characteristics of this species. All the measurements were carried out on five upper undamaged, fully expanded and healthy leaves of each plant with the help of a closed portable photosynthesis system (Model LI-6400; LI-COR, Lincoln, Nebraska, USA) equipped with light, temperature, humidity and CO<sub>2</sub> controls. Different PPFD were provided with the help of an artificial light source (Model LI-6400-02; light emitting silicon diode; LI-COR), fixed on the top of the leaf chamber and were recorded with the help of quantum sensor kept in range of 660-675 nm, mounted at the leaf level. The rate of dark respiration was measured by maintaining the leaf cuvette at zero irradiance. To avoid any radiation from

outside the leaf chamber was covered with a black cloth through the respiratory measurements. Temperature of the cuvette was controlled by the integrated Peltier coolers, which is controlled by the microprocessor. Different concentrations of CO<sub>2</sub> were supplied to the cuvette of climatic unit (LI-6400-01, LI-COR Inc., USA) by mixing pure CO<sub>2</sub> with CO<sub>2</sub> free air and were measured by infrared gas analyzer. All the measurements for gas and water vapour exchange were first recorded at lowest PPFD and temperature condition and then subsequently to the increasing levels of these parameters. Similarly, leaves under optimum PPFD and temperature conditions were first exposed to the lowest level of CO<sub>2</sub> concentration followed by elevated levels. Air flow rate (500 mmol s<sup>-1</sup>) and relative humidity (55 ± 5%) were kept nearly constant throughout the experiment. Since steady state photosynthesis is reached within 30–45 min, the leaves were kept for about 45–60 min under each set of light conditions before the observations were recorded. Four gas exchange parameters viz., photosynthetic rate (P<sub>N</sub>), transpirational water loss (E), stomatal conductance for CO<sub>2</sub> (g<sub>s</sub>) and intercellular CO<sub>2</sub> concentration (C<sub>i</sub>) were measured simultaneously at steady state condition under various permutations and combinations of light and temperature. Water use efficiency (WUE) was calculated as a ratio of the rate of photosynthesis and transpiration. A correlation and multiple regression analysis of data was performed on the basis of multiple linear hypothesis P<sub>N</sub>, E, g<sub>s</sub>, C<sub>i</sub>, C<sub>i</sub>/C<sub>a</sub> and WUE as a dependent variable on PPFD, temperature and different CO<sub>2</sub> concentrations using SYSTAT-11 (Systat Software Inc. San Jose, CA, USA) statistical software.

## RESULTS AND DISCUSSION

Both photosynthetic assimilation and biomass production are temperature- and light-dependent processes. The potential for photosynthetic acclimation to growth temperature is quite variable between species. Generally, variations in P<sub>N</sub> reflect adjustment to the respective growth environment and also to the resistance to climate rigors. Although plants can exhibit a high degree of plasticity with respect to temperature response of photosynthesis, there is a general consensus that the optimum temperature for photosynthesis for an individual plant species reflects the environmental temperature range for which the species is genetically and physiologically adapted (Berry and Bjorkman, 1980). On other hand, response of photosynthesis to PPFD has been a long standing interest. At the leaf surface, low PPFD might be a limiting factor and high PPFD may be a threat to the plant metabolism if the irradiance

exceeds the demand of photosynthesis (Osmond, 1994; Aguirre-von Wobeser *et al.*, 2000). Therefore, determination of the conditions for optimum gas and water vapour exchange processes is a prerequisite for growing any species indoor. According to our data on *C. sativa*, temperature optima for P<sub>N</sub> was observed at 30 °C. In general, temperature higher than 30 °C had an adverse effect on P<sub>N</sub> (Fig. 1A). At 25 °C, rate of photosynthesis increased with increasing PPFD, but this trend peaked with 1500 μmol m<sup>-2</sup>s<sup>-1</sup> PPFD at 30 °C, and decreased at higher light intensities. Similar effect of



**Fig. 1.** A. Variations in net photosynthesis in *C. sativa* with varying photosynthetic photon flux densities (PPFD) and temperature conditions. B. The temperature dependence of Dark respiration in *Cannabis sativa*.

PPFD was observed at temperatures higher than 30 °C. Maximum rate of photosynthesis ( $P_{N\max}$ ) was  $24.60 \mu\text{mol m}^{-2}\text{s}^{-1}$  at 30 °C and under  $1500 \mu\text{mol m}^{-2}\text{s}^{-1}$  PPFD. The interaction of PPFD and temperature demonstrates that high PPFD and higher temperature together (PPFD  $\times$  temperature) had an adverse effect on  $P_N$ . In general, effect of PPFD ( $r = 87$ ) was more prominent in regulating  $P_N$  in *Cannabis sativa* as compared to temperature ( $r = 46$ ).

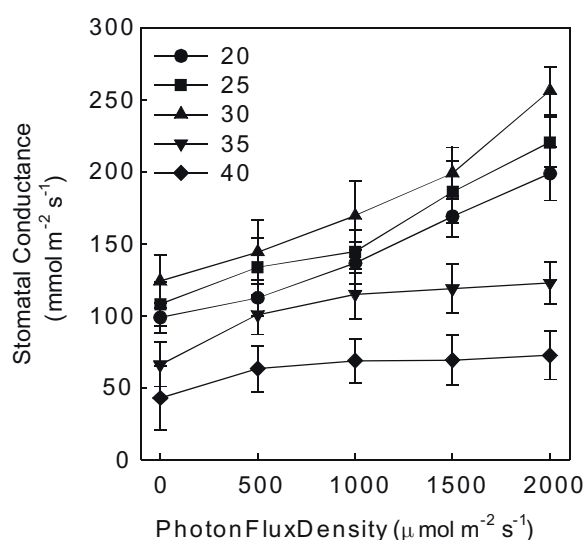
An increase in  $R_d$  ( $\mu\text{mol m}^{-2}\text{s}^{-1}$  PPFD) was observed with increasing temperature up to 30 °C and decreased at higher temperature (Fig. 1B). Working on two different populations of *Podophyllum hexandrum*, Singh and Purohit (1997) reported a linear increase in  $R_d$  with temperature (up to 40 °C) in alpine population whereas; in temperate population,  $R_d$  increased with temperature up to 30 °C and decreased at higher levels. 2 to 10 fold increase in  $R_d$  was reported by Joshi and Palni (1998) in different tea leaves with increase in temperature from 20 to 40 °C; higher temperature however, was associated with clones having higher photosynthetic rates. In *C. sativa*, decrease in  $R_d$  followed a trend similar to  $P_N$ , with varying temperatures. Reduced  $P_N$ , and increased  $R_d$  are reported to limit the productivity in some plant species at higher temperatures (Alexander *et al.*, 1995; Thornton *et al.*, 1995).

Stomatal conductance was commensurate to PPFD levels, irrespective of temperature (Fig. 2). A positive correlation ( $r = 56$ ) was observed between PPFD and  $g_s$

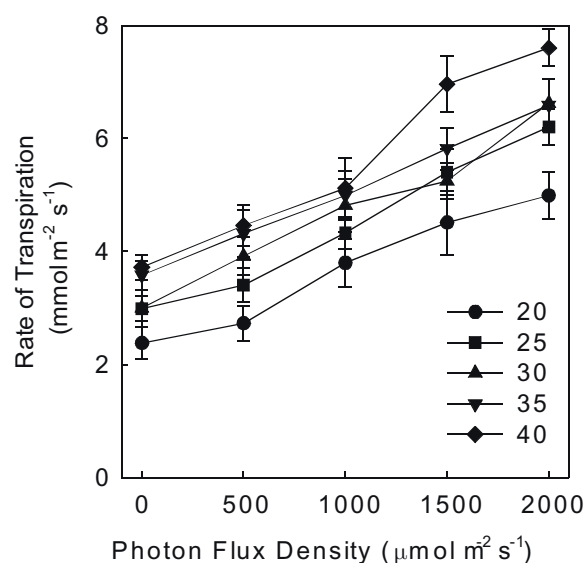
in *C. sativa*. On other hand,  $g_s$  increased with increasing temperature up to a maximum value at 30 °C and decreased at higher temperatures under all the PPFD labels. Maximum value of  $g_s$  was recorded at 30 °C and highest level of PPFD ( $2000 \mu\text{mol m}^{-2}\text{s}^{-1}$ ).

In contrast to  $g_s$ ,  $E$  increased in response to both higher temperature and high PPFD. Lowest value of  $E$  ( $2.38 \pm 0.28 \text{ mmol m}^{-2}\text{s}^{-1}$ ) was observed at 20 °C under  $0 \mu\text{mol m}^{-2}\text{s}^{-1}$  PPFD, whereas highest value ( $7.60 \pm 0.33 \text{ mmol m}^{-2}\text{s}^{-1}$ ) was recorded at 40 °C under  $2000 \mu\text{mol m}^{-2}\text{s}^{-1}$  (Fig. 3). Transpiration rate is known to depend on  $g_s$  (Alexander *et al.*, 1995), and it seems to be major factor driving  $E$  in the present study. An increase in  $E$  and decrease in  $g_s$  is reported in many plant studies (Rawson *et al.*, 1977; Schulze *et al.*, 1972).

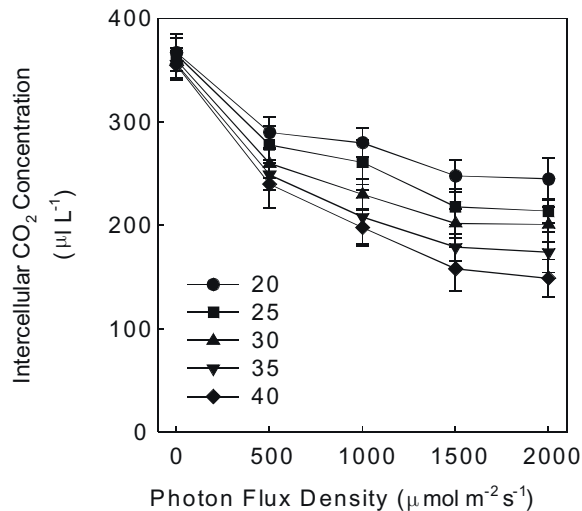
Intercellular  $\text{CO}_2$  concentration ( $C_i$ ) decreased with increase in PPFD and temperatures up to highest level tested (PPFD up to  $2000 \mu\text{mol m}^{-2}\text{s}^{-1}$  and temperature up to 40 °C (Fig. 4). Highest  $C_i$  ( $367 \text{ ml L}^{-1}$ ) was observed at lowest PPFD and temperature conditions i.e. 20 °C and  $0 \mu\text{mol m}^{-2}\text{s}^{-1}$  PPFD and, thereafter lowest  $C_i$  ( $149 \text{ ml L}^{-1}$ ) was recorded at highest PPFD and temperature conditions. However, the decrease was less pronounced at light intensities above  $1500 \mu\text{mol m}^{-2}\text{s}^{-1}$ . Effect of temperature on depression of  $C_i$  was more prominent above 30 °C. Higher temperature and higher light together had a significant adverse effect on  $C_i$  of this species. Photosynthetic data particularly on  $C_i$  and  $g_s$ ,



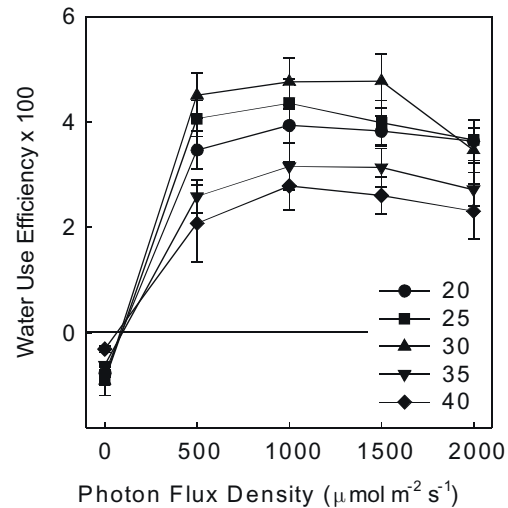
**Fig. 2.** Variations in stomatal conductance in *C. sativa* with varying photosynthetic photon flux densities (PPFD) and temperature conditions.



**Fig. 3.** Variations in rate of transpiration in *C. sativa* with varying photosynthetic photon flux densities (PPFD) and temperature conditions.



**Fig. 4.** Variations in intercellular CO<sub>2</sub> concentration in *C. sativa* with varying photosynthetic photon flux densities (PPFD) and temperature conditions.



**Fig. 5.** Variations in water use efficiency in *C. sativa* with varying photosynthetic photon flux densities (PPFD) and temperature conditions.

indicates that both stomatal and mesophyll factors seems to be involved in the mechanism of control of photosynthesis by temperature and light in *C. sativa*.

Similar to  $C_i$ , a gradual decrease in  $C_i/C_a$  ratio was also observed with increasing PPFD and temperature conditions (Table 1). About 32 %, 41 %, 44 %, 50 % and 57 % decrease in  $C_i/C_a$  ratio was observed at 20, 25, 30, 35 and 40 °C respectively when plants were exposed from 0 to 2000  $\mu\text{mol m}^{-2}\text{s}^{-1}$  PPFD. Similarly, about 3 %, 17 %, 29 %, 37 % and 39 % depression was observed under 0, 500, 1000, 1500 and 2000  $\mu\text{mol m}^{-2}\text{s}^{-1}$  PPFD when plants were exposed to 40 °C as compared to 25 °C. Although essentially a biochemical process, photosynthesis is often regarded as a diffusive process. The rate of diffusion of CO<sub>2</sub> is largely controlled by two

factors,  $g_s$  and CO<sub>2</sub> concentration gradient between carboxylation site and ambient air ( $C_a$ ). This CO<sub>2</sub> concentration gradient at given  $g_s$  and  $C_a$  is established predominantly by  $C_i$ , which is a result of mesophyll efficiency. Therefore, the diffusive entry of CO<sub>2</sub> into leaf is a reflection of intrinsic mesophyll capacity. Sheshshayee *et al.* (1996) have reported  $C_i/g_s$  ratio as an indicator of mesophyll efficiency and a representation of mesophyll control on  $P_N$ . Our data also represent highest mesophyll efficiency (i.e. lowest  $C_i/g_s$  ratio) around 30 °C and 1500  $\mu\text{mol m}^{-2}\text{s}^{-1}$  PPFD. Values of  $C_i/g_s$  ratio increased with temperature higher than 30 °C, which further confirms that a combination of 30 °C temperature and 1500  $\mu\text{mol m}^{-2}\text{s}^{-1}$  PPFD may be best suitable for the indoor cultivation of *C. sativa*.

**Table 1.** Effect of different photosynthetic photon flux density and temperature conditions on  $C_i/C_a$  ratio in the leaves of *Cannabis sativa*.

Light Intensities ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ )	Temperature (°C)				
	20	25	30	35	40
000	1.04 ± 0.12	1.04 ± 0.14	1.02 ± 0.11	1.01 ± 0.09	1.01 ± 0.07
500	0.82 ± 0.05	0.79 ± 0.06	0.74 ± 0.06	0.71 ± 0.06	0.68 ± 0.05
1000	0.80 ± 0.06	0.75 ± 0.04	0.66 ± 0.06	0.59 ± 0.04	0.57 ± 0.06
1500	0.71 ± 0.04	0.62 ± 0.06	0.58 ± 0.05	0.51 ± 0.05	0.45 ± 0.04
2000	0.70 ± 0.06	0.61 ± 0.05	0.57 ± 0.05	0.50 ± 0.04	0.43 ± 0.03



**Table 2.** Effect of different levels of CO<sub>2</sub> on net photosynthesis (P<sub>N</sub>), transpiration (E), stomatal conductance (g<sub>s</sub>), internal CO<sub>2</sub> concentration (Ci), Ratio of internal to external CO<sub>2</sub> concentration (Ci/Ca) and water use efficiency (WUE) on the leaves of *Cannabis sativa*.

CO <sub>2</sub> levels (μmol mol <sup>-1</sup> )	P <sub>N</sub> (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	E (mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	g <sub>s</sub> (mmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	Ci (μl L <sup>-1</sup> )	Ci/Ca ratio	WUE × 100
250	12.48 ± 1.76	5.69 ± 0.47	202.76 ± 19.78	138.00 ± 11.42	0.55	2.19
350	24.64 ± 2.24	5.31 ± 0.35	195.99 ± 18.40	202.00 ± 14.00	0.47	4.64
450	24.76 ± 1.89	5.76 ± 0.44	189.78 ± 16.97	260.00 ± 19.34	0.58	4.30
550	26.54 ± 2.12	4.87 ± 0.38	148.37 ± 13.99	330.00 ± 22.47	0.60	5.46
650	30.48 ± 2.76	4.65 ± 0.76	136.08 ± 12.36	385.00 ± 33.24	0.61	6.56
750	36.80 ± 3.18	3.75 ± 0.33	112.76 ± 10.32	435.00 ± 37.23	0.58	9.81

At 20 and 25 °C, WUE increased with increase in PPFD up to 2000 μmol m<sup>-2</sup>s<sup>-1</sup> (Fig. 5). On the other hand, WUE increased only up to 1500 μmol m<sup>-2</sup>s<sup>-1</sup> PPFD at 30 °C and decreased thereafter at higher light levels. Temperature higher than 30 °C had an adverse effect on WUE of this species. The maximum WUE was observed at 30 °C and under 1500 μmol m<sup>-2</sup>s<sup>-1</sup> PPFD. Photosynthesis appears to have a greater influence than E over regulating water use efficiency in *C. sativa*. A highly significant positive correlation was observed between WUE and P<sub>N</sub> (r = 0.92). Together, high temperature and high PPFD had an adverse effect on the WUE in *C. sativa*.

Increasing atmospheric CO<sub>2</sub> is a global environmental concern. Atmospheric CO<sub>2</sub> has risen from pre- industrial value of ~ 280 μmol mol<sup>-1</sup> to present concentration of ~ 372 μmol mol<sup>-1</sup> and is expected to exceed 700 μmol mol<sup>-1</sup> by the end of century (Prentice *et al.*, 2001; Long *et al.*, 2004). Since ambient CO<sub>2</sub> concentration as a substrate is still a limiting factor for photosynthesis in C<sub>3</sub> plants, attempts are being made to study how changes in atmospheric CO<sub>2</sub> concentration will affect crops (Bowes, 1993; Drake *et al.*, 1997; Long *et al.*, 2004). This study on *Cannabis sativa* shows that P<sub>N</sub>, WUE and Ci decreased by 50 %, 53 % and 10 % respectively, and Ci/Ca, E and g<sub>s</sub> increased by 25 %, 7 % and 3 %, respectively, when measurements were made at 250 μmol mol<sup>-1</sup> as compared to ambient CO<sub>2</sub> (~350 μmol mol<sup>-1</sup>) level (Table 2). An average of 30 to 33 % increase in P<sub>N</sub> and productivity of C<sub>3</sub> plants with doubling atmospheric CO<sub>2</sub> concentration has been already reported by Kimball 1983a, b; 1986; Idso and Idso 1994; Bazzaz and Gabutt, 1988; Cure and Acock, 1986. In *C. sativa*, a doubling of

CO<sub>2</sub> concentration (750 μmol mol<sup>-1</sup>) suppressed E and g<sub>s</sub> ~29 % and 42 % respectively, and stimulated P<sub>N</sub>, WUE and Ci by 50%, 111 % and 115 % respectively as compared to ambient CO<sub>2</sub> concentration. Doubling CO<sub>2</sub> level had a significant effect on all these parameters. Suppression in g<sub>s</sub> and consequently in E (Emaus *et al.*, 1993; Thomas *et al.*, 1994) and improvement in P<sub>N</sub> and WUE and Ci (Kimball 1983a, b; 1986; Idso and Idso 1994, Morison, 1993) under elevated CO<sub>2</sub> concentration is reported in many other plant species. Higher WUE under elevated CO<sub>2</sub>, primarily because of decreased g<sub>s</sub> and E, may enable this species to survive under drought conditions. This species maintained nearly constant values of Ci/Ca with increasing CO<sub>2</sub> concentration despite the increase in P<sub>N</sub> and WUE, and decrease in g<sub>s</sub> and E, represents a close coordination between stomatal and mesophyll functions (Morison, 1993) and reported to improve growth and productivity of plant (Jones, 1992).

In view of our results, it is concluded that *C. sativa* can utilize a fairly high level of PPFD and temperature for its gas and water exchange processes, and can perform much better if grown at ~ 1500 μmol m<sup>-2</sup> s<sup>-1</sup> PPFD and around 25 to 30 °C temperature conditions. Furthermore, higher P<sub>N</sub>, WUE and nearly constant Ci/Ca ratio under elevated CO<sub>2</sub> concentration, reflects its potential for improved growth and productivity in drier and CO<sub>2</sub> rich environment.

#### ACKNOWLEDGMENTS

This research was supported by National Institute of Drug Abuse (NIDA), USA, Contract # NO1DA-0-7707.

## REFERENCES

- Aguirre-von Wobeser E, Figueroa FL and Calello-Pasini A (2000). Effect of UV-B radiation in photoinhibition of marine macrophytes in culture systems. *J. Appl. Phycol.*, 12: 159-168.
- Alexander JD, Donnelly JR and Shane JB (1995). Photosynthetic and transpirational response of red spruce an understory tree to light and temperature. *Tree Physiol.*, 15: 393-398.
- Ayuko U, Tadahiko M and Amane M (2008). Effects of temperature on photosynthesis and plant growth in the assimilation shoots of a rose. *Soil Sci. Plant Nutrition*, 54: 253-258.
- Bazzaz FA and Garbutt K (1988). The response of annuals in competitive neighborhoods: Effect of elevated CO<sub>2</sub>. *Ecology*, 69: 937-946.
- Berry J and Bjorkman O (1980). Photosynthetic response and adaptation to temperature in higher plants. *Ann. Rev. Plant Physiol.*, 31: 491-543.
- Berry JA and Downton WJS (1982). Environmental regulation of photosynthesis. In: Development carbon metabolism and plant productivity, vol. II (ed. Govindjee), Academic press, New York, pp. 263-343.
- Bowes G (1993). Facing the inevitable: Plant and increasing atmospheric CO<sub>2</sub>. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 44: 309-332.
- Brenneisen R, Egli A, ElSohly MA, Henn V and Spiess Y (1996). The effect of orally and rectally administered D<sup>9</sup>-tetrahydrocannabinol on spasticity. A pilot study with two patients. *Internat. J. Clin. Pharmacol. Therap.*, 34: 446.
- Cure JD (1985). Carbon dioxide doubling response: A crop survey. In: Direct effect of CO<sub>2</sub> on vegetation (Eds. Strain BR and Cure JD), US Department of Energy Washington, pp: 99-116.
- Cure JD and Acock B (1986). Crop response to carbon dioxide doubling: A literature survey. *Agric. For. Meteorol.*, 38: 127-145.
- Dieleman JA and Meinen E (2008). Interacting effects of temperature integration and light intensity on growth and development of single-stemmed cut rose plants. *Scientia Hort.*, 113: 182-187.
- Doyle E and Spence AA (1995). Cannabis as a medicine? *Brit. J. Anaesth.*, 74: 359-361.
- Drake BG, Gonzalez-Meler MA and Long SP (1997). More efficient plants: A consequence of rising CO<sub>2</sub>? *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 48: 609-639.
- Eamus D, Berryman DA and Duff GA (1993). Assimilation, stomatal conductance, specific leaf area and chlorophyll responses to elevated CO<sub>2</sub> of *Maranthas corymbosa*, a tropical monsoon rain forest species. *Aust. J. Plant Physiol.*, 20: 741-755.
- Formukong EA, Evans AT and Evans F (1989). The medicinal uses of *Cannabis* and its constituents. *J. Phytother. Res.*, 3: 219-231.
- Grinspoon L and Bakalar JB (1993). Marihuana, the forbidden medicine. Yale University Press, New Haven.
- Hammond CT and Mahlberg PG (1977). Morphogenesis of capitate glandular hairs of *Cannabis sativa* (Cannabaceae). *Amer. J. Bot.*, 64: 1023-1031.
- Idso KE and Idso SB (1994). Plant responses to atmospheric CO<sub>2</sub> in the face environmental constituents: A review of past ten years' research. *Agric. Forest Meteorol.*, 69: 153-203.
- Jones HG (1992). Plants and microclimate: Quantitative approach to environmental plant physiology. II<sup>nd</sup> ed., Cambridge University Press, Cambridge.
- Joshi SC and Palni LMS (1998). Clonal variation in temperature response of photosynthesis in tea. *Plant Sci.*, 13: 225-232.
- Joshi SC and Palni LMS (2005). Greater sensitivity of *Hordeum himalayens* Schult. to increasing temperature causes reduction in its cultivated area. *Curr. Sci.*, 89: 879-882.
- Kimball BA (1983a). Carbon dioxide and agricultural yield: An assemblage and analysis of 430 prior observations. *Agron. J.*, 75: 779-788.
- Kimball BA (1983b). Carbon dioxide and agricultural yield: An assemblage and analysis of 770 prior observations. Water conservationlab report 14, US water conservation lab. USDA-ARS, Phoenix, AZ, pp. 71.
- Kimball BA (1986). Influence of elevated CO<sub>2</sub> on crop yield. In: Carbon dioxide enrichment of greenhouse crops. Vol. 2: Physiology yield and economics (eds Enoch HZ and Kimball BA), CRC Press, Inc. Boca Raton, FL., pp. 105-115.
- Kruse J, Hopmans P and Adams MA (2008) Temperature responses are a window to the physiology of dark respiration: differences between CO<sub>2</sub> release and O<sub>2</sub> reduction shed light on energy conservation. *Plant Cell Environ.*, 31: 901-914
- Long SP, Ainsworth EA, Rogers A and Ort DR (2004). Rising atmospheric carbon dioxide: Plant face the future. *Annu. Rev. Plant Biol.*, 55: 591-6287.
- Mattes RD, Shaw LM, Eding-Owens J, Egelman K and ElSohly MA (1993). Bypassing the first pass effect for therapeutic use of cannabinoids. *Pharmacol. Biochem. Behav.*, 44: 745-747.
- Mattes RD, Egelman K, Shaw LM and ElSohly MA (1994). Cannabinoids appetite stimulation. *Pharmacol. Biochem. Behav.*, 49:187.
- Mechoulam R (1986). Cannabinoids as therapeutic agents. CRPS Press, Boca Raton.
- Mechoulam R and Ben-Shabat A (1999). From gan-zi-gun-nu to anandamide and 2- arachidonoylglycerol: the ongoing story of *Cannabis*. *Nat. Prod. Rep.*, 16: 131-143.
- Monclus R, Dreyer E, Villar M, Delmotte FM, Delay D, Petit JM, Barbaroux C, Thiec DL, Brechet C and Brignolas F (2006). Impact of drought and productivity and water use efficiency in 29 genotypes of *Populus deltoids* x *Populus nigra*. *New Phytol.*, 169: 765-777.
- Morison JIL (1993). Response of plants to CO<sub>2</sub> under water limited conditions. *Check Vegetatio.*, 104/105: 193-209.



- Osmund CB (1994). What is photoinhibition? Some insights from comparisons of shade and sun plant. In: Photoinhibition of photosynthesis, from molecular mechanisms to the field. (Eds. Baker NR and Bowyer NR), BIOS Sci. Publ., Oxford. pp. 1-24.
- Pearcy RW (1977). Acclimation of photosynthetic and respiratory carbon dioxide exchange to growth temperature in *Atriplex tentiformis* (Torr.) Wats. *Plant Physiol.*, 59: 795-799.
- Poorter H (1993). Inter-specific variation in the growth response of plant to an elevated CO<sub>2</sub> concentration. In: CO<sub>2</sub> and Biosphere (Eds. Rozema J, Lambers H, Van de Geijn SC and Cambridge ML), Kluwer Academic Publication, Boston, MA., pp: 77-97.
- Prentice IC, Farquhar GD, Fasham MJR, Goulden M, Heinmann M, Jaramillo VJ, Khashgi HS, Le Querere C, Scholes RJ and Wallace DWR (2001). The carbon cycle and atmospheric carbon dioxide. In: Climatic change 2001: The scientific basis. Contribution of working group 1 to the third assessment report of the intergovernmental panel of climatic change (Eds. Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linden PJ and Xiaosu D), Cambridge University Press, Cambridge, pp. 183-238.
- Rawson HM, Begg JR and Woodward RG (1977). The effect of atmospheric humidity on photosynthesis, transpiration and water use efficiency of leaves of several plant species. *Planta*, 134: 5-10.
- Schulze ED, Lange OL, Buschbom U, Kappen L and Evenari M (1972). Stomatal response to change in humidity in plants grown in the desert. *Planta*, 108: 250-270.
- Sheshshayee MS, Krishna Prasad BT, Natraj KN, Sankar AG, Prasad and Udayakumar M (1996). Ratio of intercellular CO<sub>2</sub> concentration of mesophyll efficiency. *Curr. Sci.*, 70: 672-675.
- Singh A and Purohit AN (1997). Light and temperature effects on physiological reactions on alpine and temperate populations of *Podophyllum hexandrum* Royle. *J. Herbs Spices Med. Plants*, 5: 57-66.
- Sirikantaramas S, Taura F, Tanaka Y, Ishikawa Y, Morimoto S and Shoyama Y (2005). Tetrahydrocannabinolic acid synthase, the enzyme controlling marijuana psychoactivity is secreted into the storage cavity of the glandular trichomes. *Plant Cell Physiol.*, 46: 1578-1582.
- Stoutjesdijk P and Barkman JJ (1992). Microclimate, Vegetation and Fauna., Opulus Press Pub., Sweden.
- Thomas RB, Lewis JD and Strain BR (1994). Effect of leaf nutrient status on photosynthetic capacity in loblolly pine (*Pinus taeda* L.) seedling grown in elevated CO<sub>2</sub>. *Tree physiol.*, 14: 947-960.
- Thornton MK, Malik NJ and Dwyer RB (1995). Relationship between gas exchange characteristics and productivity of potato clones grown at different temperatures. *Check A. Potato J.*, 73: 63-77.
- Yao X, Liu Q and Han C (2008). Growth and photosynthetic responses of *Picea asperata* seedlings to enhanced ultraviolet-B and to nitrogen supply. *Brazilian J. Plant Physiol.*, 20: 11-18.
- Zelitch I (1975). Improving the efficiency of photosynthesis. *Science*, 188: 626-633.
- Zuardi AW (2006). History of Cannabis as a medicine: a review. *Rev. Bras. Psiquiatr.*, 28: 153-157