Comparative Performance of *Vigna unguiculata l. (walp)* and *Vigna radiata l. (wilckzck)* (fabaceae) in Elevated Carbondioxide Concentrations

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ABSTRACT

Morphological analysis and change in the chlorophyll content, total carbohydrates and reducing sugars in two specific beans; black eyed peas (*Vigna unguiculata L. Walp*) and mung bean (*Vigna radiata L. Wilckzck*) were studied after fumigation with high percentage of carbondioxide (2% CO_2+98% air and 3% CO_2+97% air). Morphological analysis was carried out on the basis of vigor index and specific leaf area. Vigor index increased in both crops in control as well as treated samples. Specific leaf area also increased but decreased later in all treated samples except 2% CO_2 treated plants fumigated for 15 minutes in black-eyed peas. In mung bean specific leaf area was increased in all treatments but slightly decreased in 3% treated samples fumigated for 20 minutes. Carbohydrate content in 2&3% CO_2 treated samples for 15 & 20 minutes was found to increase over the control samples in both crops but decreased in 3% CO_2 treated samples fumigated for 15 minutes in the last week of the experiment and also in both treated samples fumigated for 20 minutes during last week. Reducing sugar in both crops showed the same trend as was observed in case of carbohydrates. Reducing sugars were markedly decreased during the last week in both the treated samples fumigated with CO_2 for 20 minutes.

Key words: Chlorophyll, Total carbohydrates, Reducing sugar, Black eyed beans, Mung beans

INTRODUCTION:

Atmospheric CO_2 concentration has risen from pre-industrial value of about 280 cm^3 m^{-3} to present concentration of 372 cm^3 m^{-3} and is expected to cross 700 cm^3 m^{-3} by the end of this century [22,

15]. Increased CO_2 concentration in the atmosphere affects the plant physiology, morphogenesis, and photosynthesis. Plant response to elevated carbon dioxide concentration is dependent on species, on plant development stage and can be modified by a number of factors, including light, nutrient and water availability [21]. Numerous experimental studies have been conducted to investigate the effect of elevated atmospheric CO_2 concentration on economically important agricultural crops [12, 13, 23, 4, 6]. Plant responses to elevated CO_2 are fundamentally medicated by photosynthesis [7, 20], and can potentially lead to a suite of morphological and growth changes.

Increasing plant productivity in response to rising CO_2 concentrations is largely dictated by photosynthesis, respiration, carbohydrate production, and the subsequent incorporation of that carbohydrate into biomass [32, 9]. Short-term exposure of elevated CO_2 for plants generally leads to increased rates of leaf-level photosynthesis due to enhanced activity of ribulose-1.5-bisphosphate carboxylase/oxygenase (Rubisco) [17]. The response to elevated CO_2 results in an increase in leaf area, biomass accumulation or individual plant size [11, 24]. As the result of elevated CO_2, carbohydrates are accumulated in plant tissues, as their use intensity is lower than the production under these conditions [18, 35].

Green manure legumes may improve microbial biomass and soil organic fertility. Being N_2 fixers, legumes are believed to increase the N fertility of soil [28] and improve the soil quality when used as green [2]. Mungbean (*Vigna radiata* L.wilczek) is an important legume of dry land agriculture. Legumes are being used in annual crop rotations on an increasingly large area of heavy clay soils in many regions of Pakistan [1]. It is a summer pulse crop with short duration (70-90 days) and high nutritive value. The seeds contain 22-28 % protein, 60-65 % carbohydrates, 1-1.5 % fat, 3.5-4.5 % fibers and 4.5-5.5 ash, it has many effective uses, green pods in cooking as peas, sprout rich in vitamins and amino acids. This crop can be used for both seeds and forage since it can produce a large amount of biomass and then recover after grazing to yield abundant seeds [14]. Cowpea (*Vigna unguiculata* L. Walp.) is an important grain legume crop used as a fodder crop

for livestock, as a green vegetable, and for dry beans [32]. It is grown to obtain seeds and pods for human consumption, as a source of green manure and organic material on unproductive soils, primarily in semi-arid regions.

MATERIAL AND METHOD:

Effect of increased CO_2 concentration for different time periods on *Vigna radiata* and *Vigna unguiculata* was investigated. Seeds of both species were sterilized with 0.1% mercuric chloride and soak in distilled water for two hours. Soaked seeds were sown in plastic pots containing 1Kg soil saturated with full strength Hoagland solution. Plants of both species at four leaf stage were subjected to 2% and 3% CO_2 concentration for 15 and 20 minutes, while control plants were kept without any treatment. CO_2 fumigation was performed in cabinets twice a week for each plant. All plants were harvested after 4 weeks treatment. Chlorophyll, total carbohydrates and total reducing sugars in control as well as in treated plants were estimated on weekly basis. Vigor index and specific leaf area in control and treated plants were calculated weekly by the following formulas.

$$\text{Vigor Index (V.I)} = \frac{\text{Root length in cm + Shoot length in cm}}{\text{Standard Germination}}$$

$$\text{Specific Leaf Area (SLA)} = \frac{\text{Leaf Area}}{\text{Dry Mass in mg}}$$

Total Carbohydrates:

Total carbohydrates were extracted and estimated by Yemm and Willis (1956).

Anthrone reagent was prepared by dissolving 0.4g of Anthrone in 200ml of conc. Sulphuric acid with constant shaking. The acid solution was then cooled. 60ml of distilled water and 15ml of 95% ethanol was taken in dark coloured flask which was placed in an ice bath. Acid solution was transferred drop by drop in a dark colored flask with constant shaking.

0.2g of fresh sample of leaves was crushed in a mortar with 10ml of distilled water. The crushed material was then centrifuged at 1000rpm for about 10 minutes. Supernatant was then collected in another test tube and residue was discarded.

1ml of plant extract was taken in a test tube and 5ml of Anthrone reagent was added to it. The tubes were then heated in a water bath for about 30 minutes and

immediately cooled in ice cold water. Optical density was recorded at 620nm against reagent blank. Anthrone reagent was taken as the reagent blank. Amount of carbohydrates was determined in microgram per ml. amount was then calculated in microgram per milligram fresh weight.

Reducing Sugars:

Reducing sugars were extracted and estimated by Nelson Somogyii's method.

Reagents:

Reagent A (Cu Reagent)

Reagent a:

25g of sodium carbonate + 25g of sodium tartarate + 200g of sodium sulphate in 700ml of distilled water and finally made up to 1000ml with distilled water.

Reagent b: (Arseno Molybdate Reagent)

5g of Cu sulphate in 100ml of distilled water with 1 drop of conc. Sulphuric acid.

25 parts of Cu reagent (a) and 1 part of Cu reagent (b) were mixed for the preparation of mixed Cu reagent.

Reagent B:

1) 25g of ammonium molybdate in 450 ml of distilled water.
2) 3gm of sodium arsenate in 25ml of distilled water.

The two solutions were mixed together along with 21ml of conc. Sulphuric acid and the final volume was made up to 500ml with distilled water. The reagents were stored in brown bottles and incubated at 37oC for 48 hrs.

0.2g of fresh sample of leaves was crushed in a mortar with a little quantity of distilled water. The crushed material was then centrifuged at 1000rpm for about 10 minutes. Supernatant was then collected in another test tube and residue was discarded.

1ml of supernatant was taken separately of each treated and control samples and to it 1ml of mixed Cu reagent was added. Now test tubes were kept in a boiling water bath for 20 minutes. The test tubes were then cooled and about 1ml of Arseno molybdate reagent was added. Reagent blank was prepared by taking 1ml of distilled water instead of plant extract and was treated in the same manner as the rest of the test tubes. Optical density was recorded at 500nm. Amount was determined in microgram per ml. amount was then calculated in microgram per milligram fresh weight.

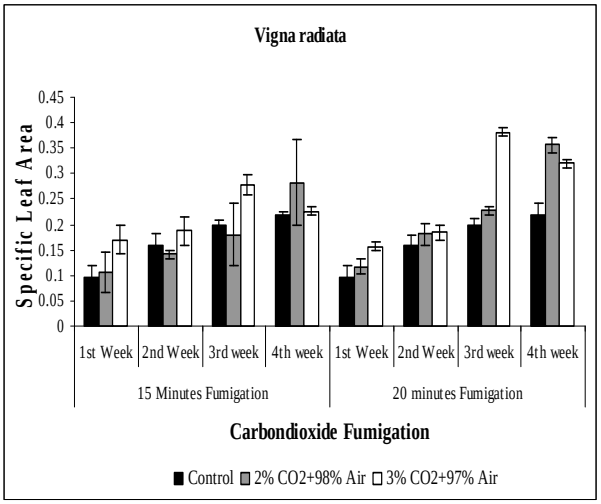
Total Chlorophyll:

Total chlorophyll was extracted and estimated by the method of Maclachlam and Zalik. 1963.

0.2g of leaves was crushed in 80% acetone. The two samples were centrifuged at 1600rpm for 10 minutes. The supernatant was collected and residue was washed with acetone and centrifuged again. This process was repeated thrice in order to have complete extraction. The supernatant was made up to a fixed volume with acetone. Optical density was recorded at 645nm and 663nm against reagent blank.

RESULTS AND DISCUSSION:

Stimulation of photosynthesis and plant growth is the direct effects of CO_2, which is beneficial for plants [2, 12]. Elevated CO_2 often increases leaf area, but the extent of stimulation depends on species and environmental variables [31]. Cell division and cell expansion may be affected, mainly due to increased substrate (sucrose) availability and perhaps also due to differential expression of specific genes [24, 26]. Specific leaf area of both *Vigna radiata* and *Vigna unguiculata* was significantly ($p< 0.001$) increased with time (Fig. 1). *Vigna unguiculata* exposed to CO_2 (2%) for 15 minutes exhibited increase while the plants exposed to 3% CO_2 concentration showed decrease in this parameter. All plants exposed to CO_2 (2&3%) for 20 minutes exhibited significant ($p<0.001$) decrease as compare to control plants. Our observations for *Vigna radiata* exhibited significant ($p<0.001$) increase in this parameter in both CO_2 concentrations as compared to the control. [36] observed that two weeks exposure of Carbon dioxide (900µmol/m³) 55% increase in leaf area and 33% increase in the specific leaf weight of tomato plants.



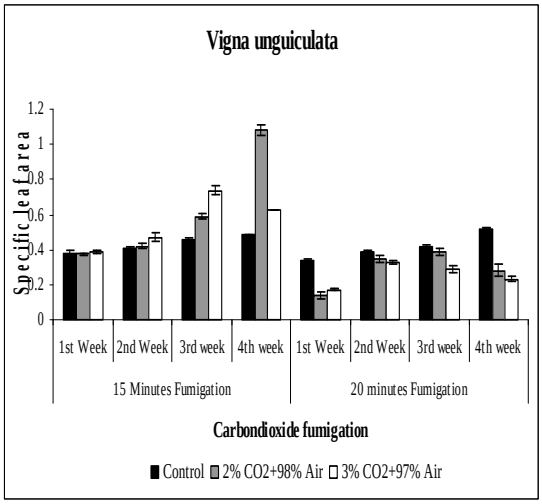
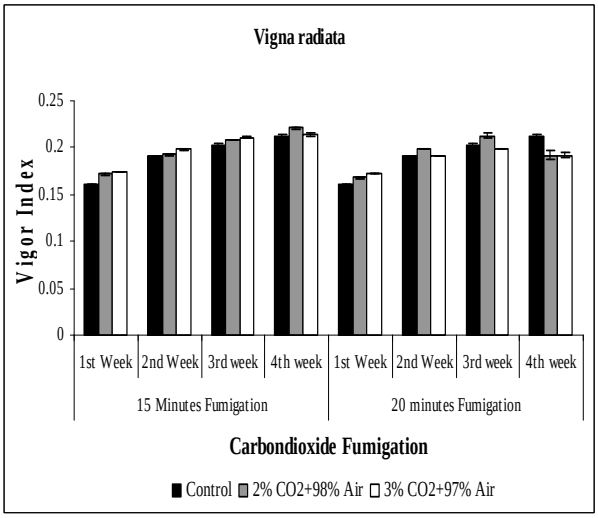


Fig. 1. Effect of different carbondioxide concentrations on specific leaf area of *Vigna radiata* and *Vigna unguiculata*.

Elevated CO2 is reported to stimulate the growth and yield of plants [5]. Vigor index of both species showed significant ($p<0.001$) increase with time in all control as well as in treated plants (Fig. 2). Different CO_2 fumigation (2&3%) also showed significant ($p<0.001$) increase in this parameter as compare to control plants in *Vigna unguiculata*. In case of *Vigna radiata* plants exposed to CO_2 (2%) for 15 minutes exhibited increase while those exposed to 3% CO_2 concentration showed decrease in this parameter. Plants treated with CO_2 (2&3%) for 20 minutes exhibited non significant increase in this parameter as compared to control.



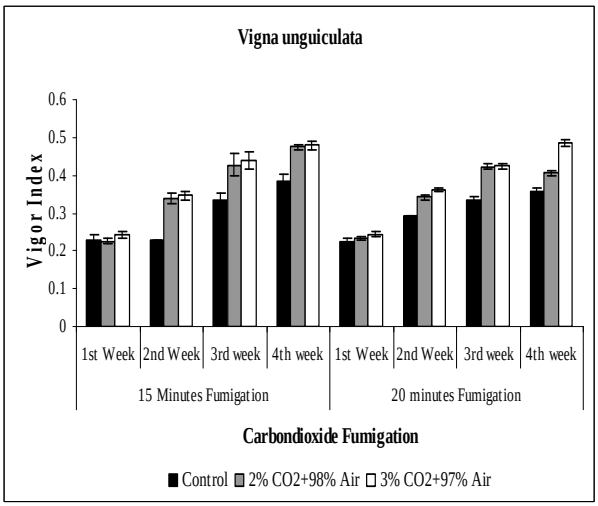


Fig. 2. Effect of different carbondioxide concentrations on vigor index of *Vigna radiata* and *Vigna unguiculata*.

In both species amount of chlorophyll was significantly increased ($p<0.001$) with time in elevated CO_2 concentration for 15 and 20 minutes, while showed significant ($p<0.001$) decrease in treated plants as compare to their respective controls (Table 1). The chlorophyll

content decreases with CO_2 fumigation resulting in the chlorosis. The chloroplast however get locked and deformed by the large number of starch granules. Finally this leads to retardation of photosynthesis. The net effect of too high carbondioxide enrichment with these plants is not growth enhancement but growth retardation [8, 19].

Table 1. Effect of different carbondioxide fumigation concentrations on total chlorophyll (µg/mg fresh wt.) of *Vigna unguiculata* and *Vigna radiata*.

Vigna unguiculata

	15 Minutes Fumigation				20 minutes Fumigation			
	1st Week	**2nd Week**	**3rd week**	**4th week**	**1st Week**	**2nd Week**	**3rd week**	**4th week**
Control	1.94 ± 0.153 -	2.49 ± 0.115	2.68 ± 0.092	2.776 ± 0.055	2.44 ± 0.083	2.983 ± 0.088	2.216 ± 0.063	3.363 ± 0.027 -
2% CO_2+98% Air	1.663 ± 0.034 -	2.7 ± 0.029	2.497 ± 0.02	1.657 ± 0.055	2.123 ± 0.04	2.406 ± 0.044	1.613 ± 0.031	1.136 ± 0.103 -
3% CO_2+97% Air	2.143 ± 0.155 -	2.88 ± 0.036	2.136 ± 0.093	1.536 ± 0.08	2.276 ± 0.003	2.313 ± 0.063	1.13 ± 0.03	0.986 ± 0.031 -
$LSD_{0.05}$	1.91	2.827	2.798	1.92	3.494	2.309	1.741	0.245

Vigna radiata

	15 Minutes Fumigation				20 minutes Fumigation			
	1st Week	**2nd Week**	**3rd week**	**4th week**	**1st Week**	**2nd Week**	**3rd week**	**4th week**
Control	1.262 ± 0.123 -	1.725 ± 0.111	2.223 ± 0.085	2.508 ± 0.085	1.262 ± 0.132	1.725 ± 0.111	2.223 ± 0.085	2.508 ± 0.071 -
2% CO_2+98% Air	1.391 ± 0.237 -	1.745 ± 0.022	2.339 ± 0.164	2.339 ± 0.164	1.391 ± 0.237	1.745 ± 0.022	2.339 ± 0.164	2.514 ± 0.152 -
3% CO_2+97% Air	1.671 ± 0.031 -	1.915 ± 0.069	1.559 ± 0.032	1.056 ± 0.032	1.671 ± 0.031	1.915 ± 0.069	1.559 ± 0.032	1.056 ± 0.099 -
$LSD_{0.05}$	1.982	1.952	2.013	2.22	2.004	2.062	1.705	1.601

Sugars have important hormone–like functions as primary messengers due to their essential role in plant growth, development and metabolic links with primary physiological processes [25]. In *Vigna unguculata* amount of reducing sugar was significantly ($p<0.001$) increased with time (Table 2). Plants treated with 2% CO_2 showed slightly increase while those plants exposed to 3% CO_2 showed decrease in this parameter as compare to control plants. When plants were fumigated with both concentrations for 20 minutes they exhibited significant ($p<0.01$) reduction as compare to control plants. In *Vigna radiata* those plants treated with 2% CO_2 concentration for 15 minutes showed increase while those plants treated with 3% CO_2 concentration for 15 minutes showed decrease in this parameter. When plants treated with 3% CO_2 for 20 minutes exhibited significant ($p<0.05$) reduction in this parameter. In treated plants photosynthetic rate is much higher, as a matter of fact it is so high that some plants cannot handle the normal processing of the large amounts of formed sugars anymore. Out of necessity these plants make starch from them, which is stored as granules in the leaves and which is meant as an energy and carbon reserve stock. [19]. This suggests that carbohydrates other than glucose accumulate in tissues increasing carbohydrate content.

Table 2. Effect of different carbondioxide concentrations on reducing sugars (µg/mg fresh wt.) of *Vigna unguiculata* and *Vigna radiata*.

Vigna unguiculata

	15 Minutes Fumigation				20 minutes Fumigation			
	1st Week	**2nd Week**	**3rd week**	**4th week**	**1st Week**	**2nd Week**	**3rd week**	**4th week**
Control	3.033 ± 0.28 -	3.426 ± 0.214	3.617 ± 0.233	3.517 ± 0.186	2.347 ± 0.103	2.083 ± 0.044	3.43 ± 0.174	4.217 ± 0.088 -
2% CO_2+98% Air	2.547 ± 0.16 -	3.83 ± 0.147	4.066 ± 0.101	4.116 ± 0.088	2.437 ± 0.094	2.733 ± 0.093	3.233 ± 0.13	2.8 ± 0.104 -
3% CO_2+97% Air	2.68 ± 0.096 -	3.2 ± 0.132	3.166 ± 0.187	2.55 ± 0.057	2.43 ± 0.062	3.116 ± 0.187	2.166 ± 0.166	1.583 ± 0.159 -
$LSD_{0.05}$	1.851	1.803	2.027	2.704	1.931	2.341	1.808	2.369

Vigna radiata

	15 Minutes Fumigation				20 minutes Fumigation			
	1st Week	**2nd Week**	**3rd week**	**4th week**	**1st Week**	**2nd Week**	**3rd week**	**4th week**
Control	2.733 ± 0.073 -	3.133 ± 0.088	3.316 ± 0.101	3.266 ± 0.101	2.733 ± 0.073	3.133 ± 0.088	3.316 ± 0.101	3.266 ± 0.101 -
2% CO_2+98% Air	2.992 ± 0.058 -	3.3 ± 0.076	3.497 ± 0.085	3.83 ± 0.065	3.133 ± 0.093	3.417 ± 0.088	3.1 ± 0.18	2.553 ± 0.029 -
3% CO_2+97% Air	3.067 ± 0.06	3.51 ± 0.107	3.283 ± 0.117	3.1 ± 0.18	3.217 ± 0.093	3.397 ± 0.087	2.5 ± 0.058	2 ± 0.087
$LSD_{0.05}$	1.987	2.052	2.079	2.128	2.02	1.847	2.183	1.908

Although increased concentration of CO_2 stimulates photosynthesis, this stimulation is often lost during prolonged exposure to elevated carbondioxide, leading to an attenuation of the potential gain in yield. Carbohydrates are the energy source for most plant physiological processes such

as respiration and cell growth. In *Vigna unguculata* and *Vigna radiata* amount of carbohydrates was significantly ($p<0.001$) increased with time (Table 3). Partitioning of current photosynthate to starch and sucrose is reported to be dependent on the tissue's source-sink balance. In CO2 enrichment studies, where photosynthesis and growth are often equally enhanced, the carbohydrate status of leaves exposed to elevated CO_2 concentrations should reflect a flow of carbon to both starch and sucrose [33, 27]. Plants treated with 2% CO_2 showed slightly increase while those plants exposed to 3% CO_2 showed decrease in this parameter as compare to control plants. When plants were fumigated with both concentrations for 20 minutes they exhibited significant ($p<0.01$) reduction as compare to control plants. In *Vigna radiata* plants treated with 2% CO_2 concentration for 15 and 20 minutes showed significant ($p<0.001$) increase while those plants exposed to 3% CO_2 showed increase in 15 minutes fumigation and exhibited decrease in 20 minutes exposure in this parameter. It is evident that the majority of the additional carbohydrate provided by CO_2 enrichment was stored in the shoots as leaf starch with relatively little being partitioned to the roots and nodules. Although additional photosynthate was produced under short-term CO_2 enrichment, there was no concomitant increase in SNA for those plants. The additional photosynthate being retained in the shoot material, predominately as starch, was possibly the result of inefficient partitioning into sucrose and reduced translocation of the sucrose to other metabolic sinks [29, 30].

Table 3. Effect of different carbondioxide fumigation concentrations on total carbohydrates (µg/mg fresh wt.) of *Vigna unguiculata* and *Vigna radiata*.

Vigna unguiculata

	15 Minutes Fumigation				20 minutes Fumigation			
	1st Week	**2nd Week**	**3rd week**	**4th week**	**1st Week**	**2nd Week**	**3rd week**	**4th week**
Control	3.667 ± 0.352 -	4.366 ± 0.092	4.426 ± 0.163	4.433 ± 0.116	5.45 ± 0.175	6.116 ± 0.088	6.117 ± 0.09	5.3 ± 0.133 -
2% CO_2+98% Air	3.2 ± 0.284 -	4.766 ± 0.148	5.283 ± 0.145	5.617 ± 0.174	6.367 ± 0.072	5.967 ± 0.044	2.983 ± 0.072	2.6 ± 0.144 -
3% CO_2+97% Air	4.617 ± 0.219 -	4.75 ± 0.251	4.55 ± 0.152	6.617 ± 0.06	6.483 ± 0.06	4.2 ± 0.152	3.51 ± 0.087	2.1 ± 0.115 -
$LSD_{0.05}$	1.981	2.04	2.342	1.957	2.07	2.124	2.033	1.943

Vigna radiata

	15 Minutes Fumigation				20 minutes Fumigation			
	1st Week	**2nd Week**	**3rd week**	**4th week**	**1st Week**	**2nd Week**	**3rd week**	**4th week**
Control	3.878 ± 0.077 -	4.15 ± 0.058	4.267 ± 0.088	4.467 ± 0.044	3.878 ± 0.077	4.15 ± 0.058	4.267 ± 0.088	4.467 ± 0.044 -
2% CO_2+98% Air	4.467 ± 0.073 -	4.7 ± 0.087	5.467 ± 0.06	6.4 ± 0.058	5.6 ± 0.058	5.63 ± 0.174	5.667 +0.093	5.31 +0.12
3% CO_2+97% Air	4.983 +0.044	5.391 +0.106	5.133 +0.044	4.943 +0.087	5.817 +0.109	5.466 +0.217	4.717 +0.073	4 ± 0.189 -
$LSD_{0.05}$	2.082	2.97	2.765	3.568	2.842	2.564	1.876	1.549

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