# Initial growth responses of blackgram (*Vigna mungo* L. Hepper) under elevated $CO_2$ and moisture stress

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**ABSTRACT**

An attempt was made to understand the interactive effect of the elevated $CO_2$ and moisture stress on germination and initial growth responses of blackgarm (*Vigna mungo* L. Hepper). Four open top chambers (OTCs) were used for different conditions: irrigated with ambient $CO_2$ (365 ppm), irrigated with elevated $CO_2$ (600 ppm), moisture stress with ambient $CO_2$ and moisture stress with elevated $CO_2$. The percentage of germination, germination speed, emergence index, and vigor index were measured on the $5^{th}$ and $6^{th}$ day after sowing (DAS). Plants were harvested at different time intervals, i.e. on 7, 14, 21 and 28 DAS; leaf area and dry weights of the seedlings were recorded. It was observed that moisture stress in general reduced the germination in all the conditions and affected seedling growth of blackgram. Germination percentage, emergence index, germination speed and vigor index were increased with elevated $CO_2$ under both irrigated and moisture stress conditions. Plants grown with elevated $CO_2$ were taller and attained a greater leaf area along with more biomass than ambient $CO_2$ levels under irrigated and moisture stress conditions at all time intervals. The increase in the germination, larger leaf area and dry matter of root, shoot and leaf proved that $CO_2$ enrichment of the atmosphere will be beneficial for the crops for better establishment and greater productivity.

**Keywords:** elevated $CO_2$; blackgram; moisture stress; germination; biomass; leaf area

Atmospheric $CO_2$ concentration, which was about 280 μmol/mol before the industrial revolution, is now 360 μmol/mol and is increasing by 1.8 μmol/mol/year (IPCC 2001) due to man-made activities. Much of the changes in atmospheric $CO_2$ occurred over the last fifty years (1953 to 2003) and the increase in $CO_2$ was about 64 ppm (Krull et al. 2005) during this period. This will affect the vegetation, as elevated $CO_2$ substantially increases photosynthesis and thereby the growth and total biomass, particularly in $C_3$ plants (Drake et al. 1997). Increased concentrations of elevated $CO_2$ have already resulted in a global increase of temperatures, and this trend is expected to continue. Changes in temperature are likely to alter precipitation worldwide, decreasing in many areas (IPCC 2001). Together with the higher evapotranspiration from soils, it can result in a prolonged water deficit (Samarakoon and Gifford 1995, Ellsworth 1999). Decreasing water supply causes moisture stress and affects the growth of the plants. Under the present global scenario of $CO_2$ increase according to the Intergovernmental Panel on Climate Change (IPCC 1996), changes in temperature and water availability affect growth and many key metabolic processes in plants. Particularly, there is limited quantitative understanding of the effects of interactions of elevated $CO_2$ and water deficit (Samarakoon and Gifford 1995).

The crops and weeds responded differently to germination and emergence under elevated $CO_2$ conditions. Ziska and Bunce (1993) reported that doubling of $CO_2$ concentration resulted in an increase in the rate and final percentage of germination for alfalfa, and weed species *Amaranthus hybridus* and *Chenopodium album*. Several researchers reported that the response of pulse crops was stronger under elevated $CO_2$ conditions compared with other cereals and oil seeds. Cowpea showed positive growth responses to elevated $CO_2$ grown in controlled environmental chambers (Mbikayi et al. 1983). Greengram grown under $CO_2$ enrichment was taller and attained a greater leaf area along with dry matter than ambient $CO_2$ grown plants at initial growth stages (Srivastava et al. 2001). Under elevated

$CO_2$ (600 ppm) and well-watered conditions the response of blackgram was higher than sorghum and sunflower at initial growth stages (Vanaja et al. 2006). Blackgram [*Vigna mungo* (L.) Hepper] is an important source of protein and is cultivated as short duration rainfed crop in semiarid areas of South Asian countries. This paper tried to quantify the response of blackgram to elevated $CO_2$ and moisture stress in terms of germination and growth at initial stages.

## MATERIAL AND METHODS

**Plant material and growth conditions.** The seeds of blackgram cv. T-9 were sown in four open top chambers (OTCs) of 3 × 3 m, lined with transparent PVC (polyvinylchloride) sheet having 90% transmittance of light. The seeds were sown directly in the soil (alfisol) to study the effect of elevated $CO_2$ level (600 ± 50 ppm) on germination and initial plant growth up to four weeks. Among the four OTCs, two OTCs were maintained at 600 ± 50 ppm and the elevated levels of $CO_2$ at crop canopy level was maintained by continuously injecting 100% $CO_2$ into plenum where it will mix with air from air compressor before entering into the chamber. Other two OTCs were maintained at ambient $CO_2$ level (365 ppm) without any external $CO_2$ supply and served as control chambers. The air sample from each chamber was drawn at 3-minute intervals into non-dispersive infrared (NDIR) $CO_2$ analyzer (California Analytical) and the set ppm of $CO_2$ concentration (600 ± 50 ppm) was maintained with the help of solenoid valves, rotameters, PCs, Program Logic Control (PLC) and Supervisory Control and Data Acquisition (SCADA) software. Throughout the experimental period continuous measurement of relative humidity and temperature of all the OTCs was possible with the sensors fitted inside the chambers. The temperature of chambers with elevated levels of $CO_2$ remained nearly the same as in the control chambers. The light intensity in chambers was 80–95% of the outside field. However gentle washing of polythene cover was frequently required to maintain transparency. For each $CO_2$ level one OTC was maintained stress free and other moisture stressed. Each chamber had four 1 × 1 m plots, each plot with five rows of crop with ten plants in each row. The experimental site was sandy loam in texture, neutral in pH (6.8), low in available nitrogen (225 kg/ha), phosphorus (10 kg/ha) and medium to high in available potassium (300 kg/ha). The seeds were sown at field capacity and after sowing the moisture stress was imposed with holding the water in two OTCs and in other two the plots were maintained stress free by irrigating at regular intervals. The moisture content of the stressed and control plots were measured gravimetrically and expressed as percentage of moisture. The control plots were maintained at filed capacity 16–18% of moisture and stressed plots at 7–9% soil moisture.

**Germination studies.** Germination measurements were made on the 5$^{th}$ and 6$^{th}$ day from the date of sowing according to ISTA (1976). Standard practices were followed to calculate percentage of germination: germination speed = % germination/days of completion of germination, emergence index = DN/N where DN = emergence (number of seed germination on particular day) and N = days of emergence, vigor index = % of germination × shoot length.

**Plant growth.** Plants were harvested at weekly intervals with three replications of each treatment, with ten plants for each replication, i.e. thirty plants for each treatment. Roots were carefully washed from the soil and plants were separated into roots, stems and leaves. The measurements were recorded on length of shoot and root for individual plants, i.e. 10 × 3 observations for each parameter, whereas leaf area, stem dry weight, root dry weight and leaf dry weights were recorded for 10 plants/replication, i.e. 2 × 3 observations for each parameter. The root length was recorded on the main root of each plant and root volume of 10 plant roots was measured as ml of water displaced and expressed as ml/10 plants. The leaf area at different time intervals was measured with photo-electronic leaf area meter (LI-3100, LI-COR) and expressed as $cm^2$/10 plants. The dry weights of stem, root and leaf were recorded after thorough drying of the plant material in hot air oven at 65°C and expressed as g/10 plants. All the data were statistically analyzed using a two-way analysis of variance (ANOVA) to test the significance of treatments and their interactions.

## RESULTS AND DISCUSSION

The mean values of germination characters of blackgram studied at 5 and 6 DAS under elevated $CO_2$ (600 ± 50 ppm) and chamber control (365 ppm) for four treatments were presented in Table 1 and growth responses at 28 DAS in Table 2, while the ANOVA of the above characters at different time intervals in Table 3.

Table 1. Interactive effect of elevated $CO_2$ and moisture stress on germination measurements over chamber control of blackgram

| Germination measurements | Water status | | | | | |
|---|---|---|---|---|---|---|
| | irrigated | | | moisture stress | | |
| | chamber control (365 ppm) | elevated $CO_2$ (600 ppm) | percentage increase | chamber control (365 ppm) | elevated $CO_2$ (600 ppm) | percentage increase |
| Percent of germination (%) | 95 | 100 | 5.26 | 80 | 98 | 22.0 |
| Emergence index | 15.8 | 16.7 | 5.69 | 13.3 | 16.3 | 22.5 |
| Vigor index | 34.2 | 41.0 | 19.8 | 26.6 | 35.0 | 31.5 |
| Germination speed | 15.8 | 16.6 | 5.06 | 13.3 | 15.6 | 17.2 |

Germination studies in blackgram showed a positive response under elevated $CO_2$ whereas moisture stress delayed the germination. The percentage increase of germination at 6 DAS under elevated $CO_2$ over ambient condition was 5.2% and 22%, with irrigated and moisture stress respectively. The emergence index was also increased with elevated $CO_2$ both under irrigated and stress and the increment was 5.6% and 22% over chamber control, respectively. The vigor index was increased by 20% and 31% with elevated $CO_2$ for irrigated and stress conditions, respectively, when compared to the ambient level. The speed of germination was higher with elevated $CO_2$ levels both under irrigated (5%), and stress conditions (17%) as compared to ambient variants (Table 1).

The growth response of blackgram was positive to elevated $CO_2$ both under irrigated and moisture stress conditions. The lengths of root and shoot decreased under moisture stress both with elevated level of $CO_2$ and ambient conditions when compared with respective irrigated

Table 2. Interactive effect of elevated $CO_2$ and moisture stress on growth components over chamber control of blackgram at 28 DAS

| Plant measurements | Water status | | | | | |
|---|---|---|---|---|---|---|
| | irrigated | | | moisture stress | | |
| | chamber control (365 ppm) | elevated $CO_2$ (600 ppm) | percentage increase | chamber control (365 ppm) | elevated $CO_2$ (600 ppm) | percentage increase |
| **Geometry** | | | | | | |
| Leaf area ($cm^2$/10 plants) | 913.45 | 1067.81 | 16.89 | 658.43 | 837.74 | 27.23 |
| Shoot length (cm) | 8.93 | 10.0 | 11.98 | 8.49 | 9.33 | 9.89 |
| Root length (cm) | 9.78 | 13.26 | 35.58 | 10.04 | 10.94 | 8.96 |
| Root volume (ml) | 2.0 | 3.5 | 75.0 | 1.5 | 3.0 | 100 |
| Dry weights (g/10 plants) | | | | | | |
| Leaf | 3.72 | 5.31 | 42.74 | 3.42 | 4.38 | 28.07 |
| Stem | 1.22 | 1.66 | 36.06 | 0.91 | 1.31 | 43.96 |
| Root | 0.30 | 0.42 | 40.00 | 0.28 | 0.36 | 28.57 |
| Total plant | 5.24 | 7.39 | 41.03 | 4.61 | 6.05 | 31.24 |
| Root:shoot ratio | 0.062 | 0.060 | –3.22 | 0.062 | 0.065 | 4.85 |

Table 3. ANOVA for various characters at 28 DAS of blackgram crop with elevated $CO_2$ (600 ppm), and chamber control (365 ppm) under irrigated and moisture stress conditions

| Characteristics | *DF* | Mean sum of squares | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | shoot length | root length | leaf area | total dry weight | stem dry weight | root dry weight | leaf dry weight | root:shoot weight |
| Replications | 2 | 1.23* | 0.230 | 3704* | 0.003 | 0.005 | 0.002 | 0.001 | 0.00 |
| Conditions | 1 | 11.33** | 18.22** | 75524** | 3.74** | 0.153** | 0.027** | 1.898** | 0.00 |
| Treatments | 1 | 3.739** | 8.99** | 230518** | 1.84** | 0.152** | 0.003 | 0.829* | 0.001* |
| Time intervals | 3 | 67.73** | 81.5** | 1678248** | 75.7** | 3.584** | 0.179** | 40.860** | 0.173** |
| Conditions × treatments | 1 | 0.263 | 0.235 | 314.0 | 0.16 | 0.005 | 0.001 | 0.094 | 0.00 |
| Conditions × time intervals | 3 | 0.543 | 1.234** | 20169** | 2.05** | 0.128** | 0.005** | 1.042** | 0.005** |
| Treatments × time intervals | 3 | 0.431 | 0.131 | 42957** | 0.54 | 0.068** | 0.001 | 0.205 | 0.00 |
| Conditions × treatments × time intervals | 3 | 0.003 | 1.839** | 87.33 | 0.07 | 0.003 | 0.000 | 0.070 | 0.00 |
| Error | 30 | 0.343 | 0.194 | 892.91 | 0.191 | 0.010 | 0.001 | 0.140 | 0.00 |

**significance at 1% level, *significance at 5% level

conditions (Figures 1a, b). The shoot length with elevated $CO_2$ was higher at different DAS and at 28 DAS it was higher by 12% under irrigated and 9.8% under stress conditions, as compared to the ambient level $CO_2$. The increment in root length was smaller when compared with the increment in root volume under elevated $CO_2$. Under irrigated conditions the percentage increase of root length with elevated $CO_2$ was 35%, whereas for root volume it was 75%. Under moisture stress conditions the response of elevated $CO_2$ for root length was only 8.96% whereas the root volume doubled (100%) (Figure 1h). This was mainly due to more lateral roots and root hair formation with elevated $CO_2$ levels. Leaf area was decreased drastically under stress and that was mitigated with elevated $CO_2$ (Figure 1c) and showed an increase of 17% under irrigated and 27% under stress conditions at 28 DAS.

The response of total dry weight (Figure 1d) to elevated $CO_2$ was also significant and showed a positive response at different time intervals. Elevated $CO_2$ increased total dry weights by 41% (irrigated) and 31% (stress) at 28 DAS. The responses of leaf, stem and root dry weight (Figures 1e–g) were also positive and significant with elevated $CO_2$ both under irrigated and stress conditions. The percentage increase in dry weight of leaf was 43% and 28%; stem 36% and 44%; root 40% and 28% under irrigated and stress conditions, respectively (Table 2) at 28 DAS. The percentage of root:shoot ratio with elevated $CO_2$ was negative (–3.2%) under irrigated conditions and positive (4.8%) with moisture stress.

The results of the present investigation clearly showed that with elevated $CO_2$ level the percentage of germination, speed of germination, emergence index and vigor index were positively increased. The increment of the above-mentioned parameters was higher under moisture stress conditions than under irrigated conditions. The mechanism by which elevated $CO_2$ affects germination and emergence is unknown. This could be due to the thin seed coat, which turns translucent, and underlined cotyledons can photosynthesize in blackgram as it was reported in *Kalmia* (Heichel and Jaynes 1974). Ziska and Bunce (1993) observed that small seeds have a greater surface to volume ratio and hence a greater diffusing capacity for $CO_2$. On other hand elevated $CO_2$ levels may stimulate the internal ethylene production (Esashi et al. 1986); Ziska and Bunce (1993) were speculating that the influence of the elevated $CO_2$ in stimulating the germination and emergence might be ethylene. To determine the actual effect of elevated $CO_2$ on germination/emergence and to understand the underlined mechanisms further a more detailed study is needed.

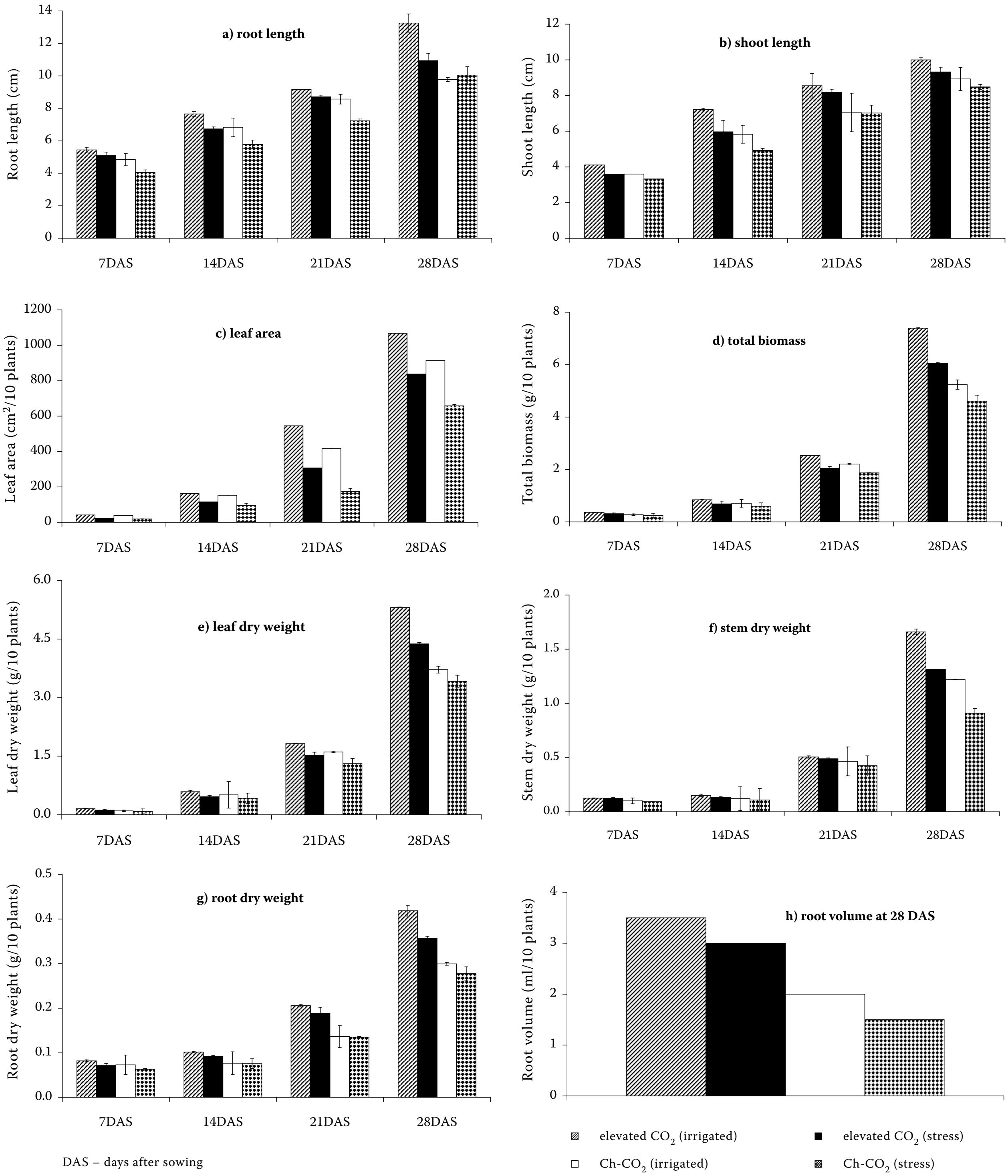


Figure 1. Response of blackgram to elevated $CO_2$ and moisture stress at different days after sowing

The percentage of increase in root length was higher under elevated than ambient $CO_2$ both under irrigated and stress conditions. Increased root length implies the probability of deeper soil penetration and access to deeper reservoirs of soil moisture. This deeper rooting would be an advantage if climates became drier. Root volume was also increased due to more lateral roots and root hair formation under elevated $CO_2$ condition. $CO_2$ enrichment enhances the root growth much more by increasing its length, volume and weight (Rogers et al. 1992). Increased root biomass due to high $CO_2$ also indicates greater portioning of assimilates to the roots. The comparison of partitioning of dry matter allocation to above- and under-ground portions showed that under elevated

$CO_2$ the response of root shoot ratio was negative under irrigated condition, whereas it was positive under moisture stress condition. The results indicate that proportioning of assimilates was greater to the roots than to the shoots under stress (Table 2). This will provide the plants with a greater support for the growth when grown under high $CO_2$ and moisture stress environment.

High $CO_2$ caused a significant increase in growth both in terms of leaf area and dry matter production. The increase in stem and leaf growth with elevated $CO_2$ caused an overall increase in total biomass in blackgram under irrigated and moisture stress conditions. Similar reports with *Vigna radiata* L. also showed an increase in total biomass under elevated $CO_2$ conditions (Srivastava et al. 2001); it resulted in a greater leaf growth, which in turn produced greater photosynthetic surface area at the early stages of plant and when combined with higher root growth, it can facilitate better initial establishment and growth under moisture stress environment.


## Acknowledgements

This work was carried out in AP-Cess program and authors are grateful to ICAR for the financial support. The support and guidance provided by the Director, CRIDA is highly acknowledged.


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Received on April 4, 2006


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