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# Growth Efficiency of Greengram (*Vigna radiata* L. Wilczek) Under Elevated Carbondioxide Condition

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

The $CO_2$ concentration in the atmosphere is rising and anticipated to be doubled by the end of the current century. Agricultural crop production is one of the key sectors that might be affected by rising atmospheric $CO_2$ through its effect on photosynthetic rates and thus productivity. It was reported that $C_3$ plants respond to elevated $CO_2$ by modification of morpho-physiological traits. The crop selected for the present study was Green gram (*Vigna radiate* L. Wilczek). Though it is an important crop, the availability of pulses has declined. So, a study of the plant responses to high atmospheric $CO_2$ is important since it regulates productivity and quality. Moreover information about genotypic variation of crops under elevated $CO_2$ is lacking in legumes. The general aim of the study is test whether Green gram can adapt to such a change and to explore mechanisms underlining the adaptive response.

Six genotypes of green gram used in the study were SML1827, SML832, SML1831, PM1533, Pusa M-19-31, and Pant M-5. Three different levels of $CO_2$ concentration namely 390ppm, 600 ppm and 750ppm under open top chambers along with an ambient concentration were maintained to assess the response of growth, physiological and yield parameters. The purpose of Open Top Chamber was to study the response of plants in high $CO_2$ environment with precise control and regulation of desired $CO_2$, temperature and humidity.

The results obtained for this experiment showed that elevated $CO_2$ has a positive effect on crop growth and development. Results indicated that 600ppm $CO_2$ enhanced some growth parameters

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*viz*. leaf area, number of branches per plant, number of effective root nodules and total biomass of plant which ultimately influenced the yield. Under 750 ppm $CO_2$, An opposite trend was recorded where yield was significantly reduced. Genotypes like Pant M-5, Pusa M-19-31 could be considered as better genotypes when grown under elevated levels of $CO_2$ as they have better N acquisition capability because of greater nodule formation in addition to biomass accumulation. Therefore, such genotypes may be utilized as future breeding materials for adaptation to the changed climatic condition.

*Keywords: Biomass; climate change; elevated $CO_2$; root nodulation; harvest index; leaf nitrogen.*

## 1. INTRODUCTION

The $CO_2$ concentration in the atmosphere is rising and is anticipated to be doubled by the end of the present century [1,2]. This is a likely consequence of $CO_2$ emission from fossil fuel combustion and land use changes. The elevated $CO_2$ is responsible for global warming and would also change the carbon balance in the biosphere by affecting the photosynthetic carbon assimilation in plants [3]. The agricultural crop production is one of the key sectors that might be affected by the rising atmospheric $CO_2$ with consequence on the global food security through its effect on photosynthetic rates and thus productivity. However, there is no consensus on the quantitative effects of increased $CO_2$ on plant processes and growth due to differences in response at different stages of growth, species of crops and because of growth limiting environmental factors. The extent of growth and yield responses of plants to elevated $CO_2$ depends on the photosynthetic pathway. Several studies were designed to elucidate the physiological mechanisms underlying the positive response of plants to rising atmospheric $CO_2$ concentration [4,5,6], though information about genotypic variation in the response of crops is lacking, especially in legumes. However, Uprety et al. [6] noticed an enhanced growth of green gram plant in response to elevated $CO_2$ and the growth improvement was related to a high water use efficiency, photosynthetic activity and nutrient use efficiency. It was reported that $C_3$ plants (e.g. wheat, rice, oilseeds, pulses) respond to elevated $CO_2$ by reducing the oxygenase activity of RuBP carboxylase oxygenase enzyme, changes in stomatal conductance, root growth and water use efficiency [7]. At the plant level, $CO_2$ elevation increases photosynthesis, growth, development and yield of a wide range of cultivated crops [8, 9,10].

The normal sowing time for kharif green gram in Assam is mid August to mid September whereas for summer green gram it is mid February to mid March. Green gram accounts for about 10-12% of total pulse production in the country. Green gram is cultivated during warm and wet season in North India whereas in South India in mild winter season. Warm and humid climate with a temperature range of 25 to 35°C with moderate rains of 850 to 1000mm is considered best for green gram production. Though pulses play a vital role in the Indian diet, the per capita availability of pulses has declined from 60.7 g $day^{-1}$ in 1951 to 47.2 g $day^{-1}$ in 2014 as against the FAO/WHO's recommendation of 80 g $day^{-1}$. The low productivity and quality degradation may be one of the major causes of decline the pulse productivity in present day context of climate change due to more increase in atmospheric $CO_2$ with concomitant decrease in N concentration of leaves *i.e.* C: N ratio. Owing to the importance of green gram, the present investigation was undertaken to study the response of green gram genotypes to elevated $CO_2$ conditions. Number of advanced lines of green gram were taken as study material. The mechanisms for adaptation were also explored.

## 2. MATERIALS AND METHODS

The experiment was conducted under 3 Open Top Chambers (OTCs) measuring 2.5 x2.5 $m^2$ and an ambient condition during 2019 in Kharif season. The OTC was fabricated with a metallic sheet (MS) pipe and installed in the experimental field. The OTC is covered with poly carbonate sheet of 100 micron gauge, which has good transmission of photosynthetically active and UV radiations having more than 85 % transmission of light. For recording the ambient data, a temperature sensor and a humidity transmitter were placed outside the chamber.The small plots in the OTCs were than laid out in Factorial Randomized Block Design.

To impose elevated $CO_2$ levels, the OTCs were used. The $CO_2$ concentrations in respective chambers were maintained by using DATA

LOGGER and SCADA software for automatic control.

## 2.1 Plant Material and Treatments

Six genotypes of green gram *viz*. SML 1827, SML 832, SML 1831, PM 1533, Pusa M-19-31 and Pant M-5 were tested. There were three levels of elevated $CO_2$ applied along with a control and replicated four times. Forty seeds were sown in each plot at a depth of 2–2.5 cm and thinned to twenty plants per plot at the three-leaf stage. Plants were maintained under fully watered conditions with a complete nutrition throughout the crop growth cycle. Half dose of nitrogen and full dose of phosphorus and potassium were applied as basal doses. The crop was top dressed with remaining half dose of N at 45 days after sowing. There were no major pest or disease problems.

Two independent sets of plants of 6 genotypes were maintained as $T_1$=Ambient $CO_2$ Condition, $T_2$=OTC I(390 ppm), $T_3$=OTCII(600 ppm), $T_4$ =OTCIII (750 ppm). Plants from each treatment were tagged and samples were taken during experimentation which was utilized for studying various plant parameters *viz.* on leaf area, plant height at flowering stage, node number, number of branches per plant at flowering stage, chlorophyll content, number of effective root nodules per plant at 55 days and total plant biomass at harvest and harvest index. Plant samples were collected at 15.00 h when the sunshine was 1200 µmol $mol^{-1}$.

Leaf chlorophyll was estimated by non-maceration method using Dimethyl Sulphoxide (DMSO), where absorption of the chlorophyll extract was measured at 663 nm and 645 nm in a spectrophotometer. The chlorophyll content was determined by using the Arnon formula and expressed as mg $g^{-1}$ leaf fresh weight. Chl a: b was then calculated from contents of chlorophyll a and b. The total nitrogen was estimated by the Wet Kjeldahl digestion process.

# 3. RESULTS AND DISCUSSION

## 3.1 Leaf Area ($cm^2$ $plant^{-1}$)

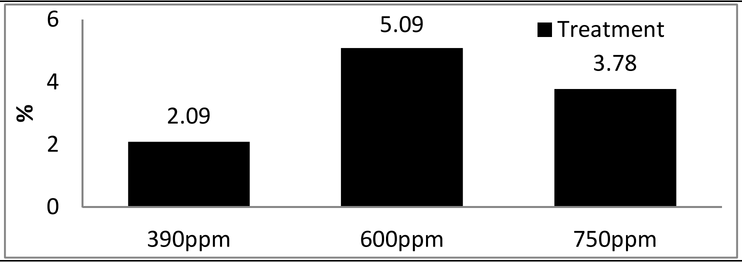
The Data presented in the Table 1 revealed that the leaf area at 50 DAS showed significant variation among the genotypes and treatments. The percent increase in leaf area was higher in treatment 600 ppm of $CO_2$ than in plants grown under 750 ppm of $CO_2$ (Fig. 1). Genotype Pant M-5 recorded the highest leaf area in treatment OTC-II (670.92 $cm^2$ $plant^{-1}$) followed by Pant M-5(662.14 $cm^2$ $plant^{-1}$) under $T_4$, whereas lowest leaf area was recorded in SML 832(437.21 $cm^2$ $plant^{-1}$) under $T_1$ (Table 1). It has been reported that greater leaf area and dry matter production was obtained when plants were grown under $CO_2$ enrichment at initial growth stages in barseem [11] and soybean [12]. Our result was in conformity with the findings. The increased leaf growth, larger leaf size due to elevated $CO_2$ might be considered as a reason behind increased leaf area in our study. Similar findings have been reported by Taylor et al., [13] in poplar; Tricker et al., [14] in Populus, Dermody et al., [15] in soybean. In addition to leaf size, the increase in leaf area under elevated $CO_2$ has been attributed to increase number of leaves [16]. Greater carbon assimilation might have influenced the growth of the green gram plant positively; therefore it might have helped in leaf ontogeny and ultimately in leaf area development.

## 3.2 Plant Height

The plant height showed significant variation amongst the genotypes as well as amongst the treatments and the interaction between the genotypes and treatments were also significant (Table 2). Among the treatments, the highest plant height was recorded in OTC-II (96.38 cm) and lowest (50.89 cm) was in the ambient condition. The percent increase in plant height was 89.39 % recorded under OTC-II over control (Fig 2). Genotype Pant M-5 showed the highest plant height (109.82 cm) followed by Pusa M-19-31 (105.07 cm) under OTC-II, whereas lowest plant height was recorded in SML 832 (47.07 cm) under ambient condition. Although an increase in plant height was observed under 600 ppm of $CO_2$, a reduction in plant height was observed in 750 ppm of $CO_2$. These results are in confirmity with the findings of Vanaja et al., [17] who reported that leaf area and plant height were significantly increased at 600 ppm $CO_2$ in black gram grown under open top chambers. The decrease in plant height might be due to reduction in photosynthesis because of smaller leaf area at 750 ppm $CO_2$. Similarly Brodribb et al., [18] reported a greater stomatal closure and lower photosynthesis in soybean under 700 ppm $CO_2$.

**Table 1. Effect of elevated $CO_2$ on leaf area ($cm^2$ $plant^{-1}$)**

| **Treatment (T)** | |
|---|---|
| AMB | 530.86 |
| OTC-I(390ppm) | 541.97 |
| OTC-II (600 ppm) | 557.90 |
| OTC-III (750ppm) | 550.92 |
| S Ed | 0.31 |
| CD (0.05%) | 0.37 |
| **Genotype (G)** | |
| G1(SML 1827) | 475.30 |
| G2 (SML 832) | 455.88 |
| G3 (SML 1831) | 596.93 |
| G4 (PM 1533) | 497.21 |
| G5 (Pusa M-19-31) | 590.13 |
| G6(Pant M-5) | 656.50 |
| S Ed | 0.37 |
| CD (0.05%) | 0.46 |



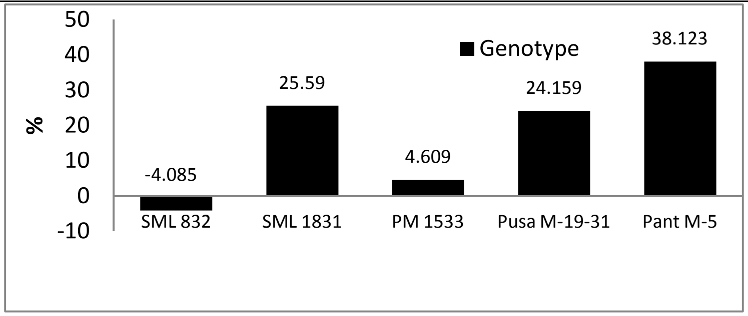
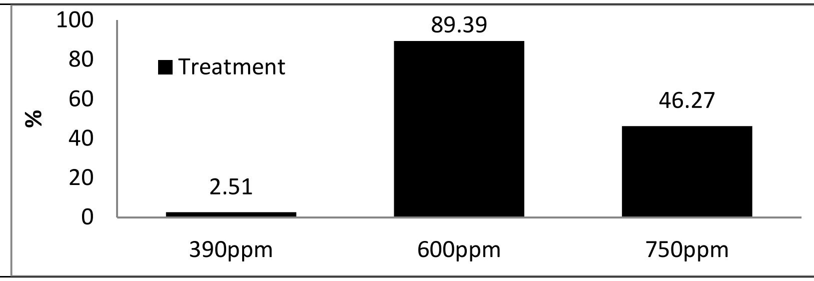




**Fig. 1. Percent increase/decrease on leaf area at 50 DAS as compared to (a ) ambient)and genotype SML 1827(b)**

| **T x G** | **SML 1827** | **SML 832** | **SML 1831** | **PM 1533** | **Pusa M-19-31** | **Pant M-5** |
|---|---|---|---|---|---|---|
| AMB condition | 465.19 | 437.21 | 582.79 | 480.23 | 576.88 | 642.85 |
| OTC-I (390ppm) | 472.32 | 452.32 | 596.99 | 493.98 | 586.08 | 650.11 |
| OTC-II (600ppm) | 483.85 | 470.89 | 606.00 | 513.81 | 601.89 | 670.92 |
| OTC-III (750ppm) | 481.85 | 463.09 | 601.94 | 500.83 | 595.67 | 662.14 |
| S Ed | 0.46 | | | | | |
| CD (0.05%) | 0.91 | | | | | |
| CV | 0.12 | | | | | |

**Table 2. Effect of elevated $CO_2$ on plant height (cm)**

| | |
|---|---|
| **Treatment (T)** | |
| AMB | 50.89 |
| OTC-I(390ppm) | 52.16 |
| OTC-II (600 ppm) | 96.38 |
| OTC-III (750ppm) | 74.44 |
| S Ed | 1.71 |
| CD (0.05%) | 3.42 |




| | |
|---|---|
| **Genotype (G)** | |
| G1(SML 1827) | 59.90 |
| G2 (SML 832) | 85.03 |
| G3 (SML 1831) | 86.20 |
| G4 (PM 1533) | 91.05 |
| G5 (Pusa M-19-31) | 93.93 |
| G6(Pant M-5) | 96.738 |
| S Ed | 2.105 |
| CD (0.05%) | 4.201 |

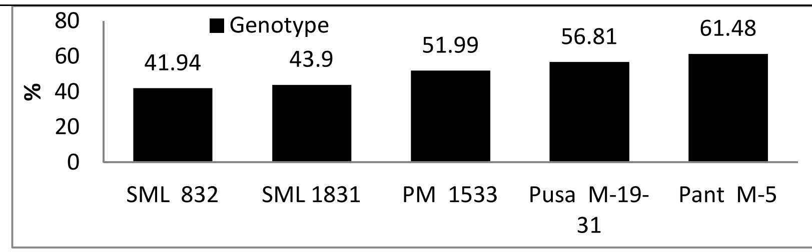
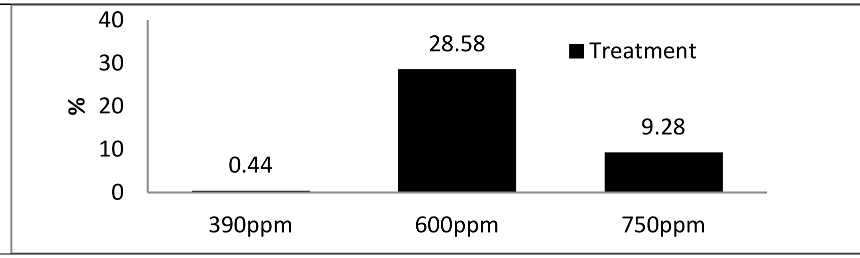



**Fig. 2. Percent increase/decrease on plant height as compared to ambient(a) and genotype SML1827(b)**

| T x G | SML 1827 | SML 832 | SML 1831 | PM 1533 | Pusa M-19-31 | Pant M-5 |
|---|---|---|---|---|---|---|
| AMB condition | 45.375 | 47.075 | 53.575 | 55.225 | 52.675 | 51.425 |
| OTC-I (390ppm) | 64.450 | 68.175 | 76.275 | 79.450 | 77.875 | 76.375 |
| OTC-II (600ppm) | 70.300 | 99.850 | 95.275 | 98.000 | 105.075 | 109.825 |
| OTC-III (750ppm) | 59.500 | 70.700 | 72.900 | 76.500 | 81.300 | 85.750 |
| S Ed | 4.201 | | | | | |
| CD (0.05%) | 8.384 | | | | | |
| CV | 8.046 | | | | | |

**Table 3. Effect of elevated $CO_2$ on number of branches plant$^{-1}$**

| **Treatment (T)** | |
|---|---|
| AMB | 4.290 |
| OTC-I(390ppm) | 4.309 |
| OTC-II (600 ppm) | 5.516 |
| OTC-III (750ppm) | 4.688 |
| S Ed | 0.425 |
| CD (0.05%) | 0.850 |




| **Genotype (G)** | |
|---|---|
| G1(SML 1827) | 5.813 |
| G2 (SML 832) | 4.236 |
| G3 (SML 1831) | 5.063 |
| G4 (PM 1533) | 4.987 |
| G5 (Pusa M-19-31) | 4.813 |
| G6(Pant M-5) | 6.250 |
| S Ed | 0.521 |
| CD (0.05%) | NS |

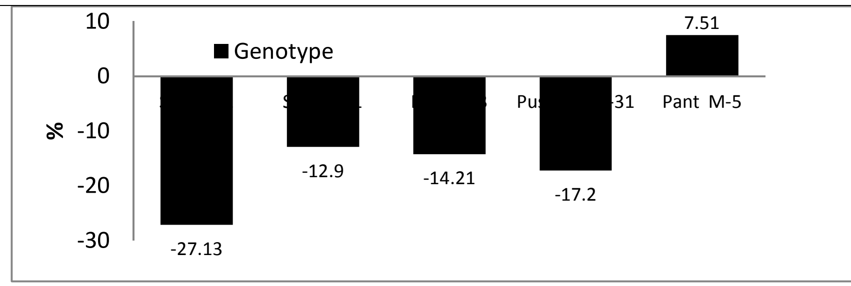
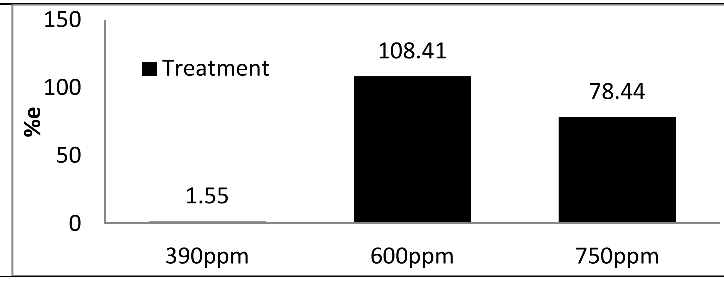



**Fig. 3. Percent increase/decrease on number of branches per plant as compared to ambient (a)and SML 1827(b)**

| **T x G** | **SML 1827** | **SML 832** | **SML 1831** | **PM 1533** | **Pusa M-19-31** | **Pant M-5** |
|---|---|---|---|---|---|---|
| AMB condition | 4.000 | 4.040 | 4.150 | 4.750 | 4.350 | 4.950 |
| OTC-I (390ppm) | 4.350 | 4.156 | 4.750 | 5.000 | 4.800 | 4.650 |
| OTC-II (600ppm) | 6.250 | 4.500 | 5.500 | 5.350 | 5.250 | 6.250 |
| OTC-III (750ppm) | 4.750 | 4.250 | 4.280 | 4.850 | 4.500 | 5.500 |
| S Ed | 1.041 | | | | | |
| CD (0.05%) | NS | | | | | |
| CV | 27.291 | | | | | |

**Table 4. Effect of elevated $CO_2$ on number of effective root nodules per plant**

| Treatment (T) | |
|---|---|
| AMB | 32.667 |
| OTC-I(390ppm) | 33.175 |
| OTC-II (600 ppm) | 68.083 |
| OTC-III (750ppm) | 58.292 |
| S Ed | 0.792 |
| CD (0.05%) | 1.543 |




| Genotype (G) | |
|---|---|
| G1(SML 1827) | 46.188 |
| G2 (SML 832) | 42.063 |
| G3 (SML 1831) | 49.938 |
| G4 (PM 1533) | 56.063 |
| G5 (Pusa M-19-31) | 55.500 |
| G6(Pant M-5) | 64.375 |
| S Ed | 0.969 |
| CD (0.05%) | 1.889 |

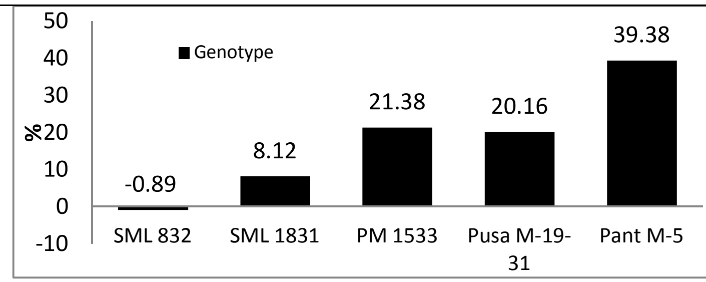



**Fig. 4. Percent increase/decrease on number of effective root nodules as compared to ambient(a) and SML 1827(b)**

| T x G | SML1827 | SML 832 | SML 1831 | PM 1533 | Pusa M-19-31 | Pant M-5 |
|---|---|---|---|---|---|---|
| AMB condition | 28.500 | 31.250 | 30.000 | 33.500 | 34.750 | 38.000 |
| OTC-I (390ppm) | 30.750 | 32.000 | 34.500 | 31.750 | 33.500 | 35.750 |
| OTC-II (600ppm) | 68.750 | 51.000 | 66.500 | 74.500 | 67.750 | 80.000 |
| OTC-III (750ppm) | 46.750 | 44.000 | 58.750 | 64.500 | 62.000 | 73.750 |
| S Ed | 1.889 | | | | | |
| CD (0.05%) | 3.779 | | | | | |
| CV | 5.083 | | | | | |

**Table 5. Effect of elevated $CO_2$ on total plant biomass (g plant $^{-1}$) at harvest**

| Treatment (T) | g plant $^{-1}$ |
|---|---|
| AMB | 14.097 |
| OTC-I(390ppm) | 14.940 |
| OTC-II (600 ppm) | 18.788 |
| OTC-III (750ppm) | 17.330 |
| S Ed | 0.073 |
| CD (0.05%) | 0.146 |
| **Genotype (G)** | |
| G1(SML 1827) | 12.544 |
| G2 (SML 832) | 14.174 |
| G3 (SML 1831) | 15.583 |
| G4 (PM 1533) | 17.280 |
| G5 (Pusa M-19-31) | 19.109 |
| G6(Pant M-5) | 20.393 |
| S Ed | 0.090 |
| CD (0.05%) | 0.179 |

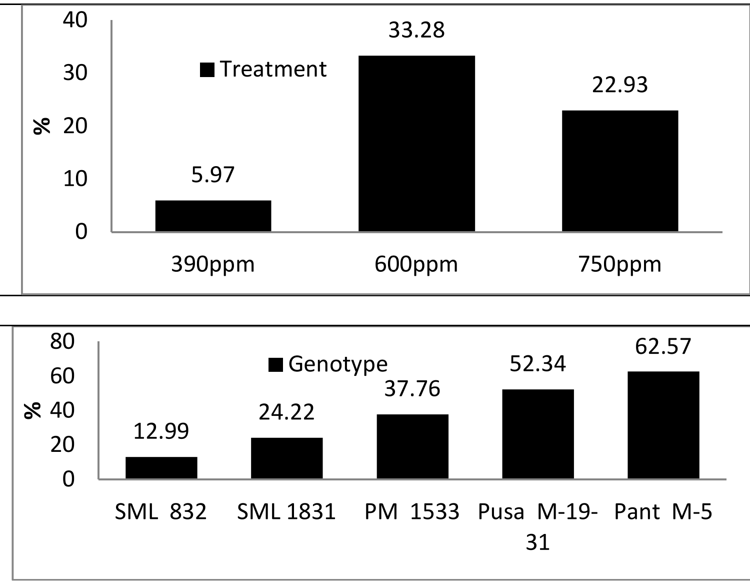
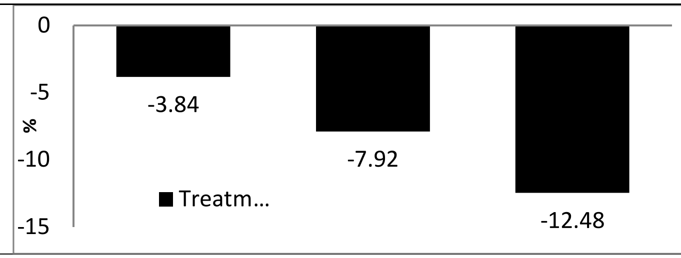



**Fig. 5. Percent increase/decrease on whole plant biomass at harvest as compared to ambient(a) and genotype SML 1827(b)**

| T x G | SML 1827 | SML 832 | SML 1831 | PM 1533 | Pusa M-19-31 | Pant M-5 |
|---|---|---|---|---|---|---|
| AMB condition | 11.313 | 12.628 | 13.948 | 14.550 | 15.785 | 16.360 |
| OTC-I (390ppm) | 11.855 | 13.000 | 14.330 | 16.710 | 14.563 | 19.580 |
| OTC-II (600ppm) | 14.223 | 15.668 | 17.443 | 19.403 | 22.265 | 23.738 |
| OTC-III (750ppm) | 12.785 | 14.400 | 15.610 | 18.458 | 20.823 | 21.903 |
| S Ed | 0.179 | | | | | |
| CD (0.05%) | 0.358 | | | | | |
| CV | 1.53 | | | | | |

**Table 6. Effect of elevated $CO_2$ on leaf chlorophyll a/b ratio**

| Treatment (T) | chl. a:b ratio |
|---|---|
| AMB | 2.475 |
| OTC-I(390ppm) | 2.380 |
| OTC-II (600 ppm) | 2.279 |
| OTC-III (750ppm) | 2.166 |
| S Ed | 0.008 |
| CD (0.05%) | 0.014 |
| **Genotype (G)** | |
| G1(SML 1827) | 2.200 |
| G2 (SML 832) | 2.979 |
| G3 (SML 1831) | 2.296 |
| G4 (PM 1533) | 2.278 |
| G5 (Pusa M-19-31) | 2.772 |
| G6(Pant M-5) | 2.909 |
| S Ed | 0.010 |
| CD (0.05%) | 0.017 |



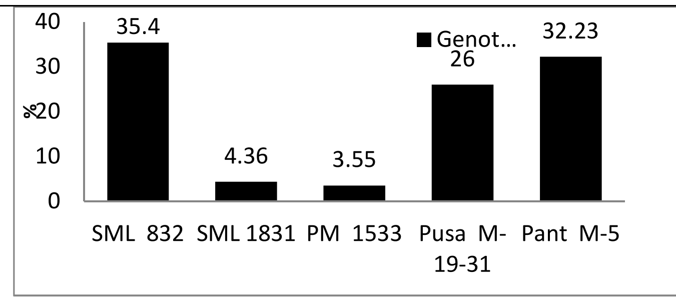
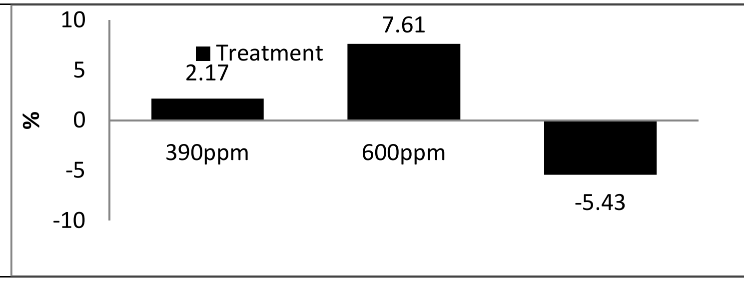




**Fig. 6. Percent increase/decrease on leaf chlorophyll a/b ratio as compared to ambient(a) and genotype SML1827(b)**

| T x G | SML 1827 | SML 832 | SML 1831 | PM 1533 | Pusa M-19-31 | Pant M-5 |
|---|---|---|---|---|---|---|
| AMB condition | 2.050 | 2.930 | 2.175 | 2.150 | 2.690 | 2.853 |
| OTC-I (390ppm) | 2.203 | 2.178 | 2.278 | 2.258 | 2.608 | 2.699 |
| OTC-II (600ppm) | 2.320 | 2.130 | 2.098 | 2.125 | 2.893 | 2.808 |
| OTC-III (750ppm) | 2.228 | 1.780 | 2.033 | 2.278 | 2.098 | 2.580 |
| S Ed | 0.017 | | | | | |
| CD (0.05%) | 0.033 | | | | | |
| CV | 0.914 | | | | | |

**Table 7. Effect of elevated $CO_2$ on leaf nitrogen (%) content**

| **Treatment (T)** | |
|---|---|
| AMB | 0.92 |
| OTC-I(390ppm) | 0.94 |
| OTC-II (600 ppm) | 0.99 |
| OTC-III (750ppm) | 0.87 |
| S Ed | 0.32 |
| CD (0.05%) | 0.68 |
| **Genotype (G)** | |
| G1(SML 1827) | 0.87 |
| G2 (SML 832) | 0.92 |
| G3 (SML 1831) | 0.86 |
| G4 (PM 1533) | 0.95 |
| G5 (Pusa M-19-31) | 0.98 |
| G6(Pant M-5) | 1.02 |
| S Ed | 0.22 |
| CD (0.05%) | 0.48 |



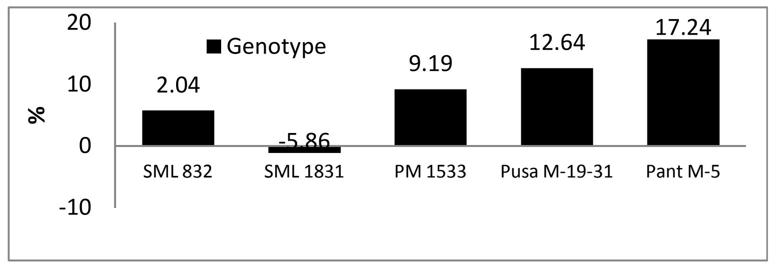
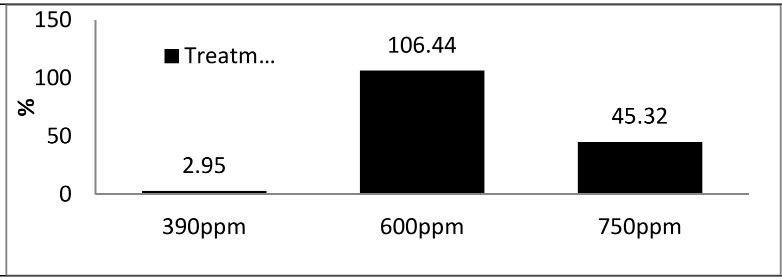




**Fig. 7. Percent increase/decrease on leaf nitrogen content as compared to ambient(a) and genotype SML1827(b)**

| **T x G** | **SML 1827** | **SML 832** | **SML 1831** | **PM 1533** | **Pusa M-19-31** | **Pant M-5** |
|---|---|---|---|---|---|---|
| AMB condition | 0.84 | 0.91 | 0.87 | 0.94 | 0.96 | 0.99 |
| OTC-I (390ppm) | 0.87 | 0.93 | 0.84 | 0.97 | 0.98 | 1.04 |
| OTC-II (600ppm) | 0.92 | 0.96 | 0.91 | 0.99 | 1.06 | 1.08 |
| OTC-III (750ppm) | 0.83 | 0.86 | 0.80 | 0.89 | 0.91 | 0.94 |
| S Ed | 1.11 | | | | | |
| CD (0.05%) | 1.68 | | | | | |
| CV | 1.96 | | | | | |

**Table 8. Effect of elevated $CO_2$ on number of pods plant$^{-1}$ at harvest**

| Treatment (T) | |
|---|---|
| AMB | 23.958 |
| OTC-I(390ppm) | 24.667 |
| OTC-II (600 ppm) | 49.458 |
| OTC-III (750ppm) | 34.815 |
| S Ed | 0.598 |
| CD (0.05%) | 1.233 |




| Genotype (G) | |
|---|---|
| G1(SML 1827) | 32.063 |
| G2 (SML 832) | 38.375 |
| G3 (SML 1831) | 36.812 |
| G4 (PM 1533) | 40.313 |
| G5 (Pusa M-19-31) | 41.938 |
| G6(Pant M-5) | 47.312 |
| S Ed | 0.717 |
| CD (0.05%) | 1.510 |

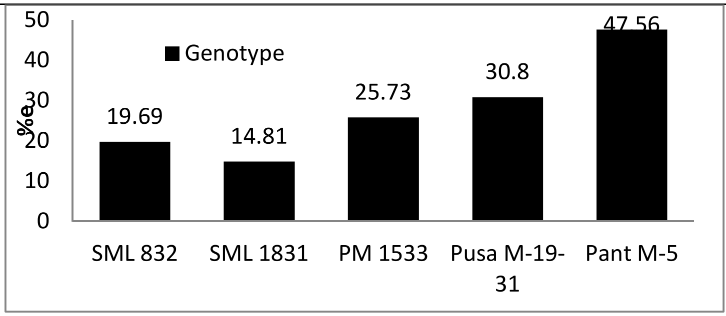
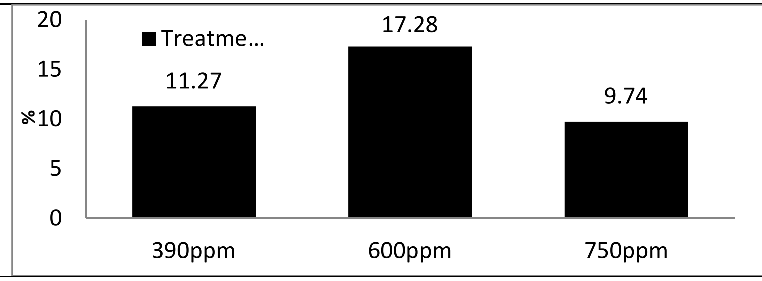



**Fig. 8. Percent increase/decrease on number of pods at harvest as compared to ambient(a) and genotype SML1827(b)**

| T x G | SML 1827 | SML 832 | SML 1831 | PM 1533 | Pusa M-19-31 | Pant M-5 |
|---|---|---|---|---|---|---|
| AMB condition | 19.750 | 25.250 | 20.750 | 25.500 | 21.500 | 31.000 |
| OTC-I (390ppm) | 20.250 | 18.000 | 23.500 | 22.500 | 23.500 | 26.250 |
| OTC-II (600ppm) | 41.750 | 48.500 | 43.250 | 49.500 | 54.250 | 59.500 |
| OTC-III (750ppm) | 36.500 | 31.750 | 32.350 | 31.890 | 35.500 | 37.500 |
| S Ed | 1.510 | | | | | |
| CD (0.05%) | 3.020 | | | | | |
| CV | 5.362 | | | | | |

**Table 9. Effect of elevated $CO_2$ on Harvest index (%)**

| Treatment (T) | % |
|---|---|
| AMB | 23.474 |
| OTC-I(390ppm) | 26.119 |
| OTC-II (600 ppm) | 27.531 |
| OTC-III (750ppm) | 25.762 |
| S Ed | 0.124 |
| CD (0.05%) | 0.247 |




| Genotype (G) | |
|---|---|
| G1(SML 1827) | 27.222 |
| G2 (SML 832) | 24.825 |
| G3 (SML 1831) | 25.404 |
| G4 (PM 1533) | 23.717 |
| G5 (Pusa M-19-31) | 25.841 |
| G6(Pant M-5) | 27.321 |
| S Ed | 0.152 |
| CD (0.05%) | 0.303 |

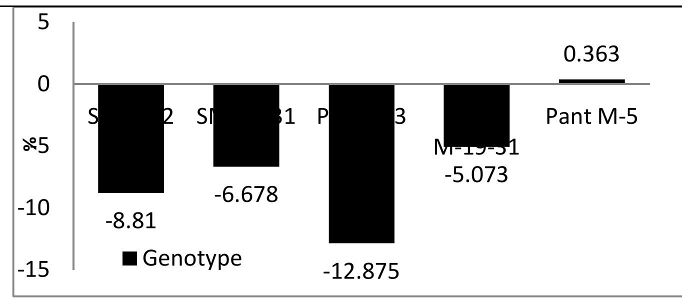



**Fig. 9.Percent increase/decrease on harvest index as compared to ambient (a)and genotype SML 1827(b)**

| T x G | SML 1827 | SML 832 | SML 1831 | PM 1533 | Pusa M-19-31 | Pant M-5 |
|---|---|---|---|---|---|---|
| AMB condition | 23.000 | 23.420 | 23.663 | 22.205 | 23.515 | 25.043 |
| OTC-I (390ppm) | 28.453 | 25.078 | 25.863 | 23.713 | 25.730 | 27.880 |
| OTC-II (600ppm) | 29.040 | 26.108 | 26.705 | 25.760 | 27.888 | 29.685 |
| OTC-III (750ppm) | 27.748 | 24.695 | 25.388 | 23.190 | 26.230 | 27.320 |
| S Ed | 0.303 | | | | | |
| CD (0.05%) | 0.605 | | | | | |
| CV | 1.659 | | | | | |

### 3.3 Number of Branches per Plant

The data presented in the Table 3 revealed that the number of branches showed significant variation due to the treatment. Increased number of branches was recorded in Pant M-5 and SML1831 under OTC-II which might be due to the more number of nodes, higher rate of photosynthesis and increase in leaf area as compared to OTC-III. In black gram, Allen [19] observed that branch number increased under elevated $CO_2$ condition. According to him the extra carbon in plant leaves induced by elevated $CO_2$ resulted in more number of branches, leaf area, number of nodes and branch number.

### 3.4 Number of Effective Root Nodules

Significant variations in root nodules were noticed amongst the genotypes as well as due to treatments and interaction between the genotypes and the treatment (Table 4.). On an average, highest number (68.08) of effective root nodules were recorded under OTC-II compared to ambient condition (32.66). Therefore, under OTC-II, the percent increase in effective root nodules was 108.41% over control (Fig 4). Among the interaction, highest value of effective nodulation was recorded in genotype Pant M-5(80.00) followed by PM 1533 (74.50) under OTC-II, whereas the lowest value was recorded in SML 1827 (28.50) under the ambient $CO_2$ condition (Table 4). Greater leaf area in genotype Pant M-5 might be due to an increase in photosynthesis leading to a greater carbon gain under OTC-II which might have helpedin the formation of nodules. Some studies have reported that elevated $CO_2$ increases nodule number and biomass in chickpea, field pea [20], and common bean [21,22].

### 3.5 Total Plant Biomass at Harvest

Amongst the treatments, highest total plant biomass(18.78 g) was recorded in OTC-IIfollowed by OTC-III (17.33 g). The highest percent increases in total plant biomass was recorded in OTC-II (Table.5)when compared to control. The genotype Pant M-5recorded highest total plant biomass (23.73 g) under OTC-II whereas the lowest value was recorded in SML 1827 (11.31 g) under ambient condition. (Fig.5). This result is in conformity with that of Rogers et al., [23] in higher plants and Wittwer, [24] in *Trifolium repens* L. Similarly, thelargest proportion of the biomass produced under elevated $CO_2$ is found belowground in black gram [25]. In irrigated soybean plants, long term exposure of elevated $CO_2$ can enhance leaf area and plant biomass by maintaining photosynthetic activity as compared to those grown under ambient $CO_2$ [26]. Elevated $CO_2$ increased total biomass and grain yield of black gram at 650ppm concentration in OTC [27].

### 3.6 Leaf Chlorophyll a/b Ratio

The data presented in the Table 6 revealed that leaf chl. a:b ratio showed significant variation amongst the genotypes as well as amongst the treatments. On an average plant grown under ambient $CO_2$ recorded higher leaf chl. a:b ratio (2.475) compared to control. The highest percent of leaf chl. a: b ratio decrease with OTC-III (Fig. 6). The highest chl a:b (2.853) value was recorded in genotype Pant M-5under OTC-III,whereas the lowest value was recorded in SML 1827(1.780) under OTC-III (Table 6). Delucia et al. 1985, also reported that elevated $CO_2$ (800 ppm), reduced leaf chlorophyll a, b content, and the ratio of chlorophyll a/b changed with reductions in nitrogen content of leaf. Although, the ratio of chl. a: b ratio decreased under elevated $CO_2$, the higher value of Chlorophyll a/b ratio was maintained in genotype Pant M-5 under OTC-II. But the highest decrease was recorded at 750 ppm of $CO_2$ indicating sustained activity of chlorophyll pigment at certain level of elevated $CO_2$. The increase might be associated with the protection of photosynthetic system under stress conditions due to an increase of N in leafs of OTC-II grown plant. Similar findings have been reported by Langjun et al. [28] in *Festuca spp* under stress condition (750 ppm). During high temperature stress Chl b is converted to Chla and this explains the increase of the ratio Chl a/b in 600-650 ppm in maize leaves together with the depression of chlorophyll content [29]. Jeong et al. [30] also reported that leaf Nitrogen, Carbon, chlorophyll contents and C:N ratio in the leaves of seven rare and endangered species of plant were found to be influenced by elevation and duration of $CO_2$ exposure and temperature as well as the interaction among those factors.

### 3.7 Leaf Nitrogen (N) Content (%)

Amongst the treatments, the highest leaf N content (0.99%) was recorded in OTC-II and the lowest (0.87%) was recorded in OTC-III(Table 7). OTC–II recorded a higher percent increasein leaf N (7.6%) over control (Fig. 7). A negative trend was observed in OTC-III grown plants. The genotype Pant M-5 recorded the highest leaf N content (1.08%) followed by Pusa M-19-31(1.06%) under OTC-II, whereas the lowest N (0.80%) was recorded in SML 1831under OTC-III (Table 7). Nutrient uptake might have been affected by higher $CO_2$ concentrations. At higher level of $CO_2$ (750ppm of $CO_2$), less amount of leaf N was recorded. This could be because of N dilution due to accelerated growth under high the high CO2 level, although genotypic variation existed.

Increase in leaf N may be related with higher amount of nodulation which might have fixed more amount of atmospheric N and maintained the leaf N in the genotypePant M-5. Higher leaf N might be linked with some important C and nitrogen assimilating enzyme. Kimball 2011; Kimball 1983 Miyagi et al. [21], observed that the nitrate reductase activity decreases under elevated $CO_2$.

### 3.8 Number of Pods at Harvest

The highest number of pods per plant (49.45) was recorded under OTC-II (Table 8) as compared to ambient condition (23.95). Percent increase (106.44%) in number of pod was highest with OTC-II over ambient (Fig. 8). Significant variation was also recorded due to interaction between treatment and genotype (Table 8). Pant-M-5 recorded the highest pod number (59.50) amongst genotypes under OTC-II whereas the lowest value (18.00) was recorded in SML 832 under ambient $CO_2$ (Table 8). This result was in conformity with the findings of Drake et al., [31] where the number of pods in black gram decreased at elevated $CO_2$ concentration (800 ppm). A genotypic variation was also noticed by some workers. Increase of dry matter and seed yield was recorded in narrow leafed lupin by Palta and Ludwig, [32] and Hao et al., [33] with elevated $CO_2$ in soybean cultivars. The main reason behind the decrease in number of pods per plant at 750 ppm could be a decrease in biomass production due to a decrease in photosynthesis. However, biomass production was higher under OTC-II.

### 3.9 Harvest Index

The data presented in the Table 9 revealed there were significant variation among the genotypes and treatments in terms of harvest index. The percent increase in harvest index was more in OTC-II (17.28%) than in ambient $CO_2$ (Fig 9). PantM-5 recorded the highest (29.69 %) harvest index followed by SML 1827 (29.04%) under OTC-II and the lowest (22.21%) was recorded in PM 1533 under ambient $CO_2$ (Table 9). At 750 ppm $CO_2$, harvest index decreased, which could be due to less amount of photosynthates accumulation. Growths in reproductive and vegetative biomass are usually increased by elevated $CO_2$. In our study, the harvest index was typically lower under 750 ppm $CO_2$ than under 600 ppm $CO_2$. However, the harvest index increased under elevated $CO_2$ when concentration was lower than 700 ppm. Vanaja et al., [17] also reported a significant increase in harvest index at 600 ppm than control. This result was in conformity with the finding of Allen et al., [34].

## 4. CONCLUSION

From this above discussion it was clear that some genotypes could show positive response to elevated $CO_2$ and it was possible only in the genotypes which were able to maintain better morpho-physiological characteristics and biomass production with an efficient nodulation providing optimum N status in the plant. This helped in maintaining the pigment system with greater leaf area and might have enhanced the photosynthetic rate leading to greater harvest index in some genotypes. Genotypes like Pant M-5, Pusa M-19-31 could be considered as efficient genotypes when grown under elevated levels of $CO_2$. Such genotypes could be utilized as breeding material for resistance breeding under future high $CO_2$ environment

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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