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Evaluation of blackgram (*vigna mungo* (L.) hepper) genotypes under high temperature and interaction with elevated carbon dioxide

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Abstract

The crop productivity reduction was caused by the hot weather condition in several climatic zones. The reproductive stages are very sensitive to the heat stress in many plants. In recent days, the temperature drastically increased due to global warming. In this century, the atmospheric carbon dioxide concentrations increased from 300 to 399 ppm and it may further increase during the next century. The objectives of this study were to determine the responses of blackgram genotypes to ambient and elevated carbon dioxide (CO_2) under high temperature and optimal temperature, test the pollen viability, pollen germination, fertility coefficient and yield. Pulses are vulnerable to heat stress and results in substantial yield loss. In this study, the impact of elevated carbon dioxide and high temperature interaction was studied in blackgram. Based on Temperature Induction Response (TIR), six genotypes representing the three categories of heat tolerance (highly heat tolerant, moderately heat tolerant and heat susceptible) were screened. The six selected blackgram genotypesare i) VBG-07-001, VBG-06-010 (Heat tolerant) ii) VBN-6, COBG-11-02 (Moderately heat tolerant) and iii) COBG-11-03, VBG-08- 003 (Heat susceptible). These six genotypes were chosen to study the impact of elevated CO_2 and high temperature interaction. The observations on pollen viability, pollen germination, number of flowers, total flowers shed and fertility coefficient has shown that there is a beneficial effect when the crop is subjected to 700 ppm of carbon dioxide with ambient temperature. Interestingly, it was also seen that tolerant genotypes VBG-07-001, VBG-006-010 registered higher yield of 16.3 and 15.8 respectively under elevated CO_2 and ambient temperature.

Keywords: Blackgram, Carbon dioxide, High temperature, Pollen viability, Pollen Germination, Fertility coefficient.

Introduction

The role of increased carbon dioxide concentration in the atmosphere is a matter of concern as the atmospheric temperature is simultaneously expected to increase. Both temperature and carbon dioxide play major role and the impact on agriculture are viewed seriously because of the global warming and associated climate change. The most worrying part of the prediction is the estimated increase in winter and summer temperatures by 3.2°C and 2.2°C respectively by 2050. The physiology of plants grown at elevated CO_2 levels is altered in several ways. Plants that utilize C_3 photosynthesis typically show larger growth responses to elevated CO_2 than C_4 plants. Pulses are very sensitive to drought, water logging and high temperature. Recently, high temperature is implicated as a major limiting factor for yield decline particularly when flowering and anthesis coincides with temperature rise[1]. India is the largest producer and consumer of blackgram in the world.

In contrast, the effects of elevated CO_2 and the interactions between elevated CO_2 and higher mean growth temperature on plant responses to acute heat stress have been examined in only a few studies, and the results have been variable. Therefore, it is important to select genotypes having tolerance mechanism for both temperature and elevated CO_2 so that the future challenge on global warming can be addressed.

Reproductive phase have been more vulnerable to heat stress that effects the time to flowering, fertilization, pod, seed and yield[1,2]. Several studies on high temperature and elevated CO_2 have shown contrasting results. Elevated carbon dioxide and high temperature were increased the flower production in soybean[3, 4]. High temperatures including short episodes of extreme events during the plant reproductive period have been shown to cause extensive damage to grain and fruit yield in many crops[5-9].

In rice, floret sterility was increased due to high temperature above 33°C with + 300 $molmol^{-1}$carbon dioxide due to increased canopy[10]. Heinemann *et al.* reported that, increased CO_2 level in atmosphere not only improved the shoot and root biomass but also yield and its components in soybean[11]. Prasad *et al.* reported that, elevated CO_2 increased seed yield up to 24 per cent in kidney bean[4]. The field grown peanut plants produced more biomass and higher pod yield at 1000 $\mu mol\ mol^{-1}$ CO_2 than at ambient carbon dioxide[12]. Lower seed yield at high temperature under both ambient and elevated CO_2

conditions was shown to be due to decreased pollen viability in groundnut and bean[4].

Heat stress severely affects the reproductive development like flower production, pollination and fertilization leads to cause yield reduction in many legume plants groundnut and kidney bean[4,13,14]. Lower seed-set under heat stress can be caused either by poor anther dehiscence, hence low numbers of germinating pollen grains on the stigmaor because of decreased pollen viability or ovule function[10,15-17]. Ultimately, high temperature effects on pre-anthesis are related to anther development, pollen sterility and pollen production. Grain yield reduction was due to high temperature effects on pre-anthesis, post-anthesis development and pollination. Among the genotypes reproductive phase heat tolerance variation can able to predict with the help of pollen biology. Pollen sterility is one of the key factors limiting legume yield under high temperature[7]. Pollen production was reduced at high temperature compared with ambient temperature in soybean[18]. Therefore, pre-anthesis flower abortion is caused by male sterility resulting from abnormal pollen development and anther indehiscence[19]. In legumes, flowers are produced, but only a few set pods. Degree of flower shedding varies between 60–92percent in soybean[20,21]. The high proportion of reproductive abscission is due to most of the later-formed flower that mostly abscise in legumes[22-24]. Because of the flowers and pods of the raceme may not receive enough assimilates from the leaf due to inadequate phloem tissue development in distal (top) part of the raceme resulting abscission of flowers and immature pods in legumes[20,25]. It is widely accepted that yield of leguminous crops can be increased, if abscission could be reduced. Hence, in the present study the impact of elevated carbon dioxide and temperature on six blackgram genotypes was studied in the control carbon dioxide chamber in which modules are available for systematic control of temperature and carbon dioxide regime.

Materials and methods

The carbon dioxide chamber was designed by M/s. Jai scientific and diagnostic supplies with the internal dimension of 60cm W x 60cm D x 100cm H. The chamber was internally made up of 20 SWG (standard wire gauge) Stainless steel sheet and externally made up of 20 SWG MS sheeting with 70mm thick glass wool insulation. The chamber consists of six stainless steel weld mesh portable trays. Six flourescent lamps were attached on either sides of the chamber. The chamber was suitable for use on 230V, single phase AC supply. The CO_2set point ranged from 350-1000ppm, temperature set point ranged from +35 to 75˚C and humidity controller ranged from 50 to 80 per cent RH. The door was made up of insulation glass viewing window. Temperature, carbon dioxide and humidity were controlled by Process Precision Instrument (PPI). Air circulation was provided by a fan driven by the Fraction HP motor to maintain uniform temperature throughout the chamber with 1kW sheathed air heater for attaining the temperature. The CO_2 gas cylinder had a dimension of 7 Cubic meter capacity with two pressure gauge (0-250 and 0-350 kg/cm^2) and ferrel. The cylinder had a capacity to supply CO_2 gas continuously for 7 to 8 days. Humidity inside the chamber was created by injecting water vapour from low pressure boiler with constant water level tank and 2 kW heater. In CO_2 chamber, the temperature gradually increased starting from 10:00 AM to 12:00 PM IST and then slowly declined. The relative humidity (65 per cent) was maintained throughout the experiment. Elevated CO_2 and temperature treatments began at reproductive stage form 29th day to 34th day.

Blackgram genotypes were chosen for the study. Based on the Temperature Induction Response a set of nineteen blackgram genotypes were screened for intrinsic thermo tolerance. Out of the 19 genotypes, six blackgram genotypes representing the three categories of heat tolerance (highly heat tolerant, moderately heat tolerant and heat susceptible) were screened. The six selected blackgram genotypes are i) VBG-07-001, VBG-06-010 (Heat tolerant) ii) VBN-6, COBG-11-02 (Moderately heat tolerant) and iii) COBG-11-03, VBG-08- 003 (Heat susceptible). These six genotypes were chosen to study the impact of elevated CO_2 and high temperature interaction. The blackgram genotypes were subjected to the following temperature and CO_2 regimes in the CO_2 chamber. i. At ambient CO_2 and ambient temperature (398 µmol mol^{-1}, Max/Min Temp 35/20°C), ii. At elevated CO_2 and ambient temperature (700 µmol mol^{-1}, Max/Min Temp 35/20°C), iii. At elevated CO_2 and elevated temperature (700µmol mol^{-1}, Max/Mini Temp 44/25°C).

It is estimated that by the end of this century, CO_2 concentration would be around 700 µmol mol^{-1} . Due to its role as a greenhouse gas, this increase of CO_2 will lead to an increase of 4°C in atmospheric mean temperature. Hence, 700 µmol mol^{-1} is taken as the elevated CO_2 level in the study. Blackgram seedlings were transferred to the CO_2 chamber along with the pots. Three plants/ pot and four replications were maintained for each of the treatment. Observations on pollen viability, germination and flower shedding and fertility coefficient were recorded during the period of treatment and correlated with yield components.

Pollen viability: For determination of pollen viability, three to 10 flower buds were fixed in 1 acetic acid: 3 ethanol (v/v)one day before flowering. Six to ten anthers from each flower bud were squeezed and stained using acetocarmine[26]. At least 100 pollen grains for each flower bud were used to determine pollen viability. Pollen viability was estimated as the ratio of number of stained pollens to total number of pollen grains and expressed as per cent.

Pollen germination: Flower buds were collected randomly from each genotype in the morning hours. Pollen was dusted on to the petriplate containing pollen germination medium (15 g sucrose, 0.03 g calciumnitrate, 0.01 g boric acid and 5g agar dissolved in 100mL of distilled water. The contents were heated in a microwave oven to obtain a clear solution and poured into petriplates. The plates were sealed and incubated at room temperature.

To determine the pollen tube germination, germinated pollen grains on the petriplate were viewed under a light microscope (LEICA) and counted after 40 hours of incubation. Percentage of pollen germination in each field view was calculated by the number of germinated pollen grains by the total number of pollen grains and was expressed in per cent[27].

Flower shedding and fertility coefficient: Three plants were randomly selected and tagged for daily count of opened and shedding flowers on main stem as well as on the branches. Flower count began from the date of opening of first flower and continued after every 12 hours i.e. at 6.00 and 18.00 hours daily until flowering ceased for first flush. The flower extent (total number of flowers produced and shed) was computed separately for each genotype. Fertility coefficient was estimated as:

$$\text{Fertility coefficient} = \frac{\text{Number of pods plant}^{-1}}{\text{Number of opened flowers plant}^{-1}} \text{ X } 100$$

Result and discussion

Pollen viability (%): The impact of elevated carbon dioxide and high temperature interaction on pollen viability was studied in the six select genotype representing the three categories of heat tolerant (Table-1, Figure-1). The mean pollen viability registered 41.3 % under ambient carbon dioxide and temperature irrespective of the genotypes. Interestingly, it is evident that the pollen viability has shown 3.6 per cent increase under ambient temperature and elevated carbon dioxide situation irrespective of the genotypes. This treatment has recorded the mean pollen viability 44.9 per cent irrespective of the genotypes. Under the situation of elevated carbon dioxide and elevated temperature the pollen viability registered the lowest value of 33.5 per cent irrespective of the genotypes. Among the genotypes VBG-07-001 and VBG-06-010 have recorded more than 55 % of pollen viability under elevated carbon dioxide and ambient temperature. The same genotypes has shown least reduction in pollen viability under both elevated carbon dioxide and elevated temperature indicating the superiority of the genotype for thermo tolerance.

Pollen viability has shown that there is a beneficial effect when the crop is subjected to 700 ppm of carbon dioxide with ambient temperature. This indicated that blackgram is beneficial in observing more carbon dioxide under the ambient temperature condition. Interestingly, it was seen that tolerant genotypes VBG-07-001, VBG-006-010 invariably register highest pollen viability of 58.5 and 55.3 respectively. The advantageous situation of elevated carbon dioxide in pollen viability and fertilization is documented in peanut[28].

Pollen germination (%): Considering the pollen tube growth it is almost the representative of pollen viability trend (Table-2, Figure-2). The mean pollen germination showed the higher value under ambient temperature with elevated carbon dioxide in respective of the genotypes. Under elevated carbon dioxide and elevated temperature showed drastic reduction in pollen germination irrespective of the genotypes. Again VBG-07-001 and VBG-06-010 showed a sustains of pollen germination even under elevated carbon dioxide and temperature.

Table-1: Effect of high temperature and carbon dioxide on pollen viability (%) in blackgram genotypes

Sl. No.	V. No	Genotypes	Temperature and CO_2 concentration		
			35˚C/20˚C 350ppm	35˚C/20˚C 700ppm	44˚C/25˚C 700ppm
1.	V10	VBG – 07 – 001	53.7	58.5	48.1
2.	V9	VBG – 06 – 010	51.3	55.3	42.4
3.	V23	VBN – 6	42.6	47.8	33.1
4.	V26	COBG – 11 – 02	40.5	43.4	30.3
5.	V27	COBG – 11 – 03	31.2	34.2	25.3
6.	V17	VBG – 08 – 003	28.4	30.2	22
Mean			41.3	44.9	33.5
SEd			0.543	0.384	0.941
CD (P=0.05)			1.102*	0.779*	1.909*

Table-2: Effect of high temperature and carbon dioxide on Pollen germination (%) in blackgram genotypes

Sl. No.	V. No	Genotypes	Temperature and CO_2 concentration		
			35˚C/20˚C 350ppm	35˚C/20˚C 700ppm	44˚C/25˚C 700ppm
1.	V10	VBG – 07 – 001	64.7	66.5	49.4
2.	V9	VBG – 06 – 010	62.1	63.3	43.5
3.	V23	VBN – 6	58.2	60.2	40.2
4.	V26	COBG – 11 – 02	57.5	60.0	38.5
5.	V27	COBG – 11 – 03	54.7	59.8	35.3
6.	V17	VBG – 08 – 003	53.3	59.0	33.8
Mean			58.4	61.5	40.1
SEd			0.639	0.452	1.107
CD (P=0.05)			1.297*	0.917*	2.246

Similar trend as that of pollen viability was observed in pollen germination also. The favorable effect of elevated carbon dioxide is maintaining high pollen germination in peanut and grain sorghum[28]. Again the tolerant genotypes showed advantageous position of response to elevated carbon dioxide at 700 ppm. More pollen germination was observed in elevated carbon dioxide[29].

Flower shedding and fertility coefficient (%): The floral biology in relation to global warming is an interesting subject which will help us to identify a stable genotype in turns of sustaining high yield under the changing climate scenario. The six genotypes respectively three categories were evaluated for total number of flowers, total flowers shed and fertility coefficient under three treatments (Table-3). It is seen from the table that, the earlier trend on pollen viability and pollen germination exactly showed the same trend. In fact the elevated carbon dioxide under ambient temperature showed less number of flowers shed per plant indicating that the advantages position of blackgram response to elevated carbon dioxide. The total number of flowers was observed to be high under ambient temperature and elevated carbon dioxide with 72.9 flowers per plant irrespective of the genotypes. In fact there was almost an increase of 10 flowers per plant due to this treatment indicating the beneficial effect of elevated carbon dioxide. Coming to the interaction of elevated carbon dioxide and high temperature further reduction of total number flowers per plant noticed with a mean number of 43.1 irrespective of the genotypes. Considering the total flowers shed per plant showed interesting observation that elevated carbon dioxide under ambient temperature registered the mean value of lower number of flowers with only 28.6 flowers irrespective of the genotypes. The impact of high temperature and elevated carbon dioxide triggered more flower shedding indicating high number of flower shed with 33.3 flowers irrespective of the genotypes. The fertility coefficient which the ratio of total pods indicated that the mean fertility coefficient of 25.2 recorded under elevated carbon dioxide with ambient temperature. This indicated that relative beneficial influence of increasing atmospheric carbon dioxide has a positive response in a crop like blackgram.

Considering the floral behavior among the genotypes designated that heat tolerant genotypes VBG-07-001 and VBG-06-010 recorded more flower retention. Thus, showing high rate of fertility coefficient in significant manner. Considering total number of flowers produced much difference could not be discernible with respect to the genotypic variability. However, the heat tolerant genotypes showed less number of flowers shed under elevated carbon dioxide showing genotypic superiority. Heat stress during reproductive development in legumes is generally allied with lack of pollination and abscission of flower buds, flowers and pods leading to substantial yield loss[30]. Fluctuation in day and night temperature during reproductive phase inducing more flowers shedding which adversely affects yield potential of the pigeonpea crop[31].

The impact of elevated carbon dioxide on reproductive characters as indicated that carbon dioxide concentration at 700 ppm under ambient temperature seems to be optimum in recording more number of flowers with consequent high fertility coefficient. This might be due to number of flower shedding invariably lower in the case of tolerant genotypes under 700 ppm carbon dioxide. There are earlier reports that elevated carbon dioxide would be an advantageous position provided the atmospheric temperature is kept at normal.

Yield: The impact of elevated carbon dioxide and high temperature interaction on the yield of grain (Table-4) was evaluated in the six blackgram genotypes. The mean yield recorded 11.4 g plant^{-1} under ambient carbon dioxide and temperature irrespective of the genotypes. The yield was recorded to be high under ambient temperature and elevated carbon dioxide with 13.3 g plant^{-1} irrespective of the genotypes. Coming to the interaction of elevated carbon dioxide and high temperature further reduction of yield was noticed with a mean of 8.4 g plant^{-1} irrespective of the genotypes. Considering the yield among the genotypes designated that heat tolerant genotypes VBG-07-001 and VBG-06-010 recorded higher yield.

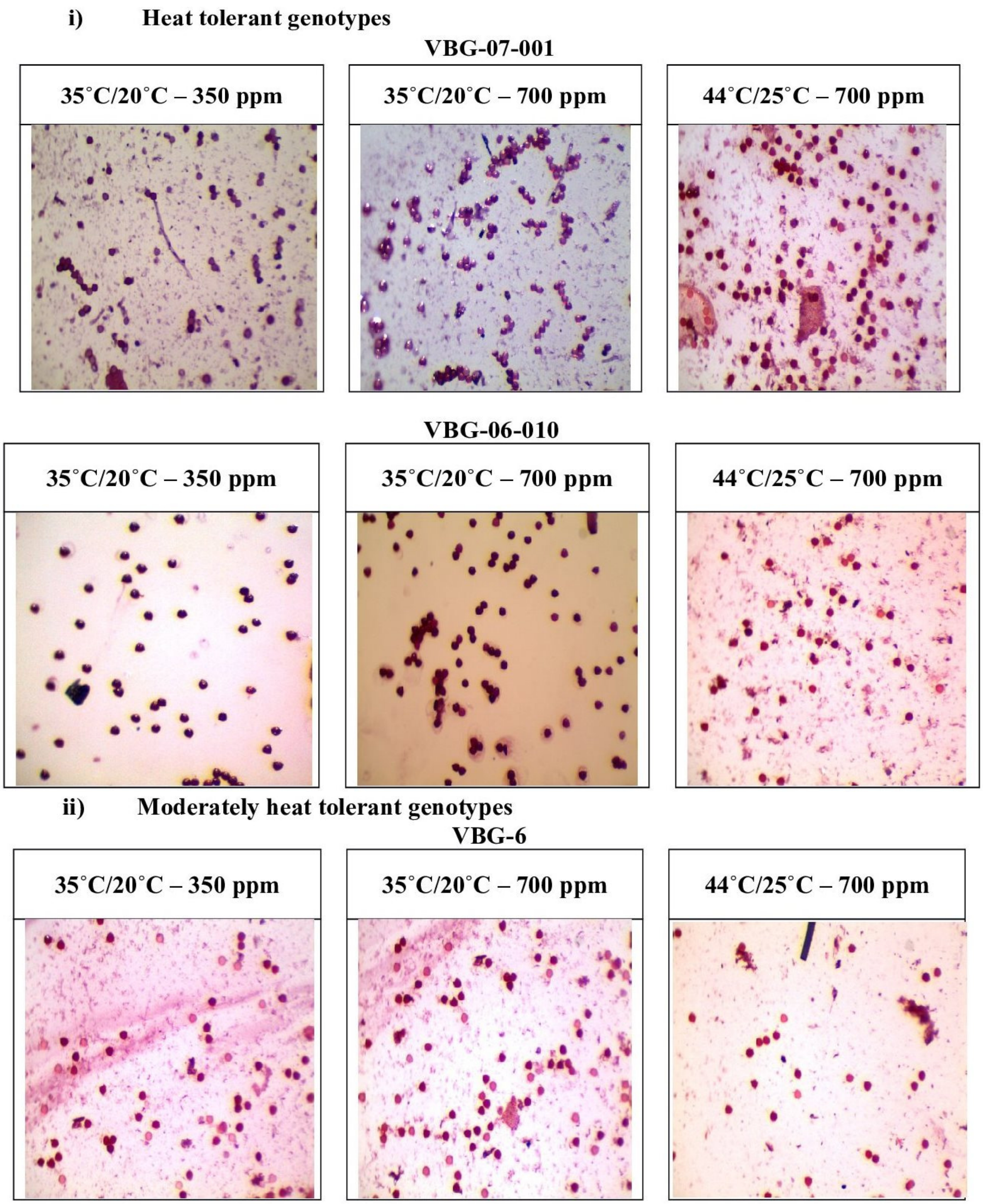

Figure-1(a): Effect of high temperature and carbon dioxide on Pollen Viability (%) in blackgram genotypes.

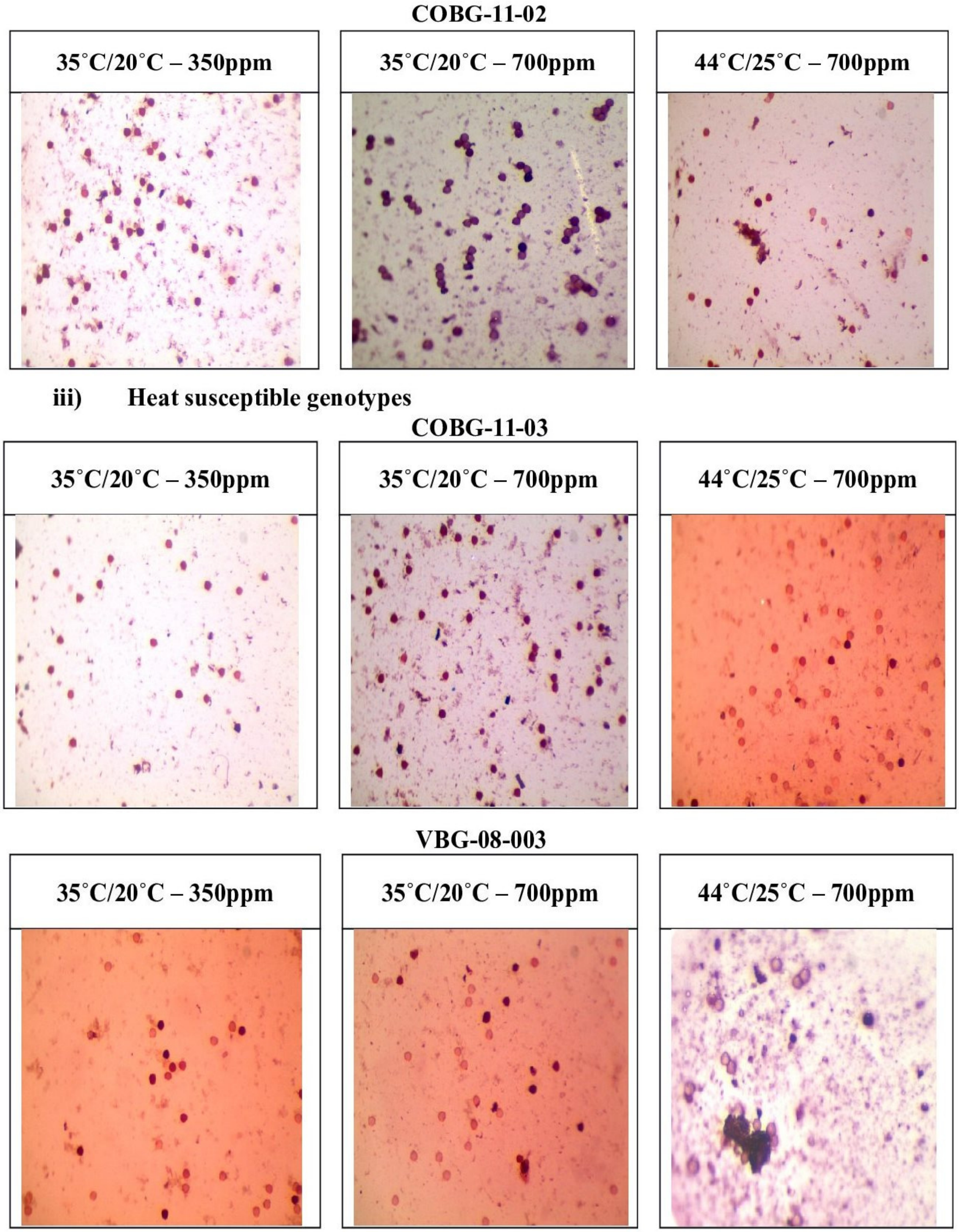

Figure-1(b): Effect of high temperature and carbon dioxide on Pollen Viability (%) in blackgram genotypes.

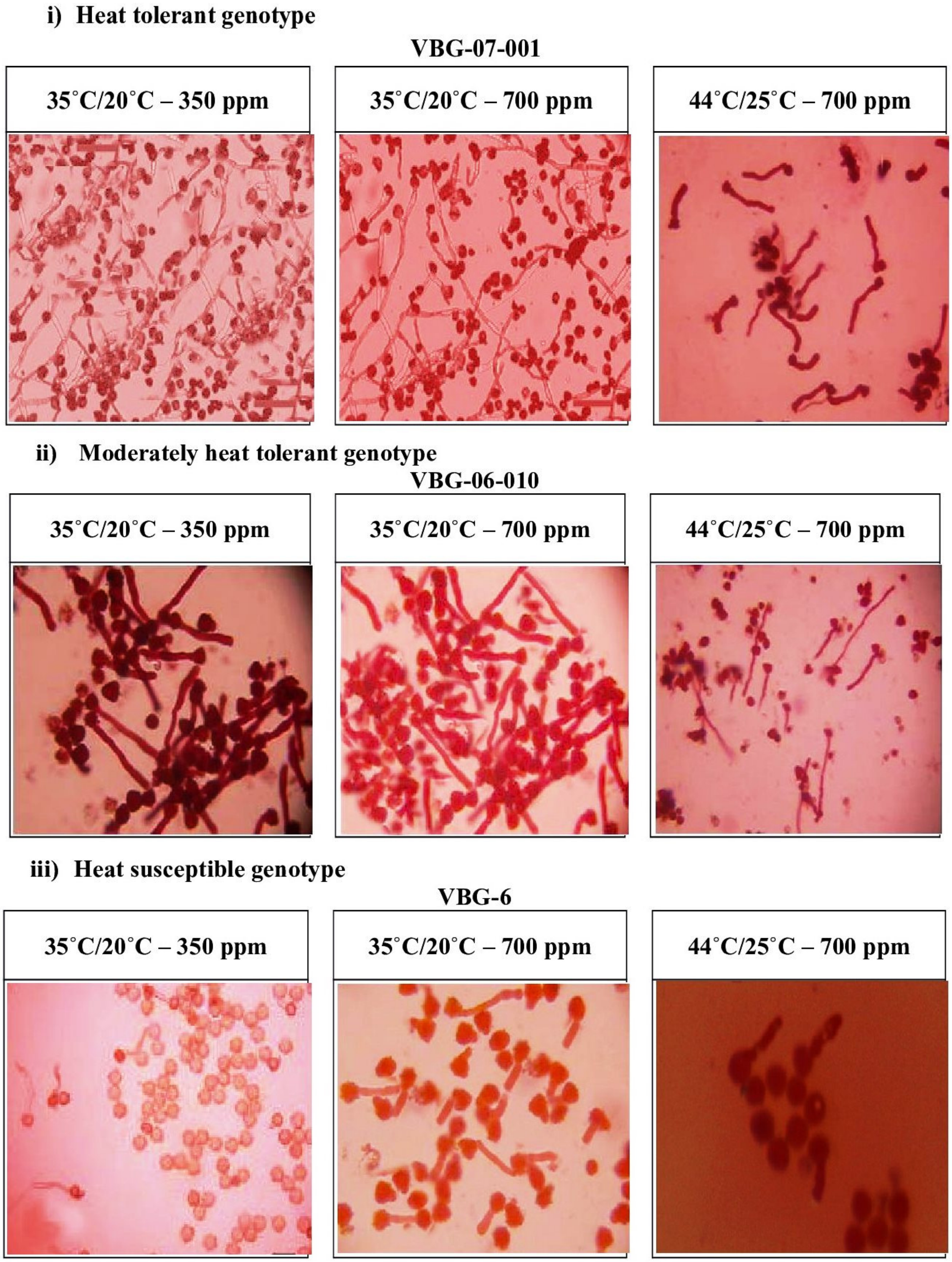

Figure-2: Effect of high temperature and carbon dioxide on Pollen germination (%) in blackgram genotypes

Table-3: Effect of high temperature and carbon dioxide on flower shedding and fertility coefficient in blackgram genotypes (Mean of 3 plants).

Sl N	V. No	Genotypes	Total no. of flowers $plant^{-1}$			Total no. of flowers shed $plant^{-1}$			Total no. of pods $Plant^{-1}$			Fertility coefficient (%)		
			35/20 °C 350 ppm	35/20 °C 700 ppm	44/25 °C 700 ppm	35/20 °C 350 ppm	35/20 °C 700 ppm	44/25 °C 700 ppm	35/20 °C 350 ppm	35/20 °C 700 ppm	44/25 °C 700 ppm	35/20 °C 350 ppm	35/20 °C 700 ppm	44/25 °C 700 ppm
1.	V10	VBG-07- 001	64.1	74.3	46.5	40.1	34.3	43.4	20.3	24.3	13.4	26.3	30.3	20.5
2.	V9	VBG-06-010	65.3	75.4	45.3	41.1	33.5	42.1	19.1	23.5	12.1	25.4	29.6	20.1
3.	V23	VBN-6	63.0	73.1	43.6	29.3	28.1	31.1	15.7	18.1	11.1	21.5	23.8	18.9
4.	V26	COBG-11-02	63.1	72.4	42.4	26.5	26.0	29.1	14.6	16.0	10.1	21.1	21.8	18.2
5.	V27	COBG-11-03	62.5	70.3	40.3	25.7	25.4	28.0	12.3	15.4	8.0	19.4	23.6	15.9
6.	V17	VBG-08-003	60.3	71.9	40.2	23.2	24.1	26.3	11.5	14.1	6.3	19.1	22.4	14.3
Mean			63.1	72.9	43.1	31.0	28.6	33.3	15.6	18.6	10.2	22.1	25.3	18.0
SEd			0.841	0.595	1.458	0.363	0.257	0.630	0.396	0.280	0.686	0.245	0.173	0.425
CD (P=0.05)			1.707 *	1.207 *	2.957 NS	0.737 *	0.521 *	1.278 *	0.795 *	0.562 *	1.377 *	0.497 *	0.352 *	0.862 *

Table-4: Effect of high temperature and carbon dioxide on yield (g $plant^{-1}$) in blackgram genotypes (Mean of 3 plants)

Sl. No.	V. No	Genotypes	Yield per plant(g $plant^{-1}$)		
			35/20°C 350ppm	35/20°C 700ppm	44/25°C 700ppm
1.	V10	VBG – 07 – 001	15.2	16.3	12.1
2.	V9	VBG – 06 – 010	13.4	15.8	10.2
3.	V23	VBN – 6	11.1	13.6	8.2
4.	V26	COBG – 11 – 02	12.4	14.9	9.3
5.	V27	COBG – 11 – 03	9.8	10.7	6.5
6.	V17	VBG – 08 – 003	6.7	8.2	4.1
Mean			11.4	13.3	8.4
SEd			0.07	0.07	0.05
CD (P=0.05)			0.15*	0.15*	0.10*

In the present study the elevated carbon dioxide up to 700 ppm was beneficial in recording higher yield than the rest of genotypes. Again the tolerance genotypes showed high grain yield under ambient temperature indicating that in blackgram particularly heat tolerant group would respond favorably to increased atmospheric carbon dioxide. Reports indicate that carbon dioxide and temperature interaction highly influence the crop growth, grain yield as well. Yield reduction in green gram under heat stress can be caused by accelerate the ending of crop cycle[32].

Analysis of protein profile in leaves: Protein was extracted from the leaves of the tolerant and susceptible genotypes kept under 35°C, 350 ppm; 35°C, 700 ppm; 44°C, 700 ppm. SDS-PAGE analysis of the protein profiles of two blackgram genotypes (Heat Tolerant - VBG-07-001 and Heat Susceptible - VBG-08-003) under high temperature and elevated CO_2 conditions showed significant difference among the banding patterns. Here again, two major protein bands were observed, one at 45 KDa and the other at 24 KDa. A protein band at 29 KDa was also observed in all the treatment conditions but with varying band intensity. The heat tolerant genotype showed greater band intensity at 45 KDa as well as at 24 KDa than the heat susceptible genotype. It was observed that. The protein bands of plants kept at 35°C, 700 ppm was more intense than the plants kept under 35°C, 350 ppm and 44°C, 700 ppm (Figure-3). The results thus indicate that the important proteins related to heat tolerance and elevated CO_2 may have been upregulated, which needs further investigation.

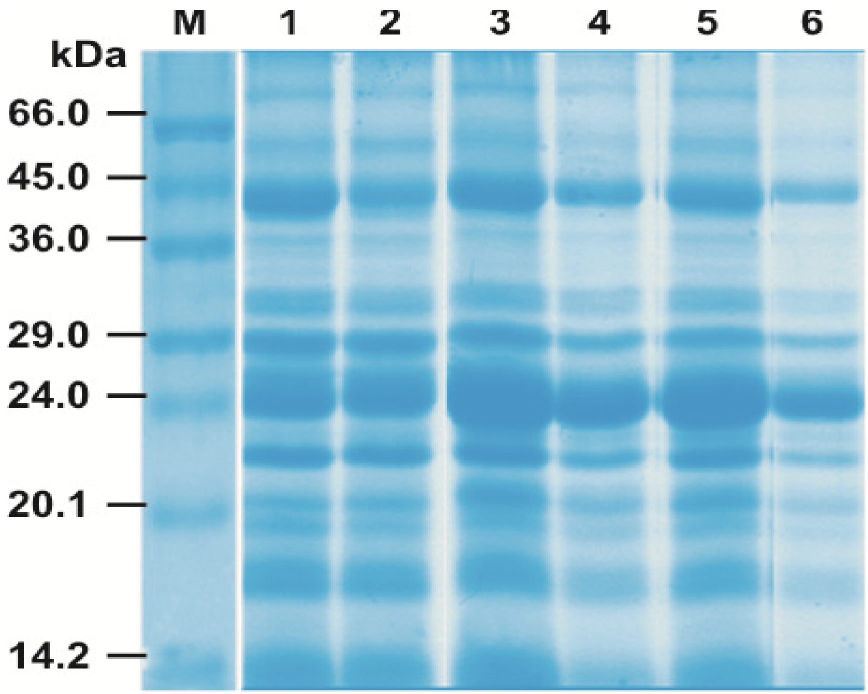

Figure-3: SDS – PAGE analysis for Protein profile in leaves under high temperature and CO_2 interaction
M - Marker, Sample **1 -** VBG-07-001 @ 350 ppm CO_2 and 35/20˚C, Sample **2 -** VBG-08-003 @ 350 ppm CO_2 and 35/20˚C, Sample **3 -** VBG-07-001 @ 700 ppm CO_2 and 35/20˚C, Sample **4 -** VBG-08-003 @ 700 ppm CO_2 and 35/20˚C, Sample **5 -** VBG-07-001 @ 700 ppm CO_2 and 44/25˚C, Sample **6 -** VBG-08-003 @ 700 ppm CO_2 and 44/25˚C

Reports are available on accumulation of specific protein due to heat stress. For an exampleBansod and Malodedemonstrated accumulation of specific protein accumulation in *Vigna mungo*[33].

Conclusion

The impact of elevated CO_2 and temperature on selected six blackgram genotypes was studied in the control CO_2 chamber in which modules are available for systematic control of temperature and CO_2 regime. Pollen viability has shown that there is a beneficial effect when the crop is subjected to 700 ppm of CO_2 with ambient temperature. This indicated that blackgram is beneficial in observing more CO_2 under the ambient temperature condition. Interestingly, it was seen that tolerant genotypes VBG-07-001, VBG-006-010 invariably register highest pollen viability of 58.5 and 55.3 respectively. Again the tolerant genotypes showed advantageous position of response to elevated CO_2 at 700 ppm.

The impact of elevated CO_2 on reproductive characters as indicated that CO_2 concentration at 700 ppm under ambient temperature seems to be optimum in recording more number of flowers with consequent high fertility coefficient. This might be due to number of flower shed is invariably lower in the case of tolerant genotypes under 700 ppm CO_2. It is concluded that, the present investigation has paved way for identifying superior genotypes tolerant to high temperature and elevated CO_2.

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