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GENOTYPIC VARIATIONS IN REPRODUCTIVE MORPHOLOGY AND ANATOMY OF HOT CHILLI (*Capsicum chinense* Jacq.) GROWN UNDER ELEVATED CO_2 AND TEMPERATURE

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ABSTRACT

Two cultivars of hot chilli (*Capsicum chinense* Jacq.) cv. 'Manipur' and cv. 'Assam' were used to study the impact of elevated carbon dioxide (eCO_2) and elevated temperature (eTemp) on some of their reproductive morphological features and anatomy. Hot chilli were grown under carbon dioxide temperature gradient tunnels (CTGT) wherein different concentration of CO_2 and temperature were maintained. The study revealed significant variations in pollen number, diameter and viability under variable regimes of eCO_2 and eTemp. Highest pollen number, diameter and viability was found in the plant grown under CTGT II. Significant increase in xylem and phloem number in cv. 'Manipur' under CTGT II revealed better adaptability of crop due to improved solute transport to the growing regions. Anomaly in flower development was evidenced in CTGT III causing improper fertilization due to elongated and curved style that might obstruct fertlization, although a genotypic variation existed. Therefore, hot chilli cv. 'Manipur' can be grown under eCO_2 (550 ppm) and eTemp (2°C higher than ambient conditions).

Keywords: Elevated CO_2, temperature, hot chilli, phloem, pollen, stigma bending, water status, xylem, viability

INTRODUCTION

Seed formation is dependent on successful pollination and fertilization of ovules by pollen grains. This accomplishment relies on the receptivity of stigma and viability of pollen grains. Reproductive success is highly regulated by various abiotic factors; some of the factors that have been predicted to change in future include CO_2, temperature and UV-B radiation (Koti *et al.*, 2005). Sensitivity of male gametophyte development to the environmental fluctuations and suboptimal conditions limiting sexual reproduction has been reported (Kim *et al*., 2001).

Male gametophytes are sensitive to temperature stress during flower bud development and anthesis (Bennici, 2019). Plant reproduction as affected by high temperature has been focused on heat induced failure in both male and female reproduction in crop leading to sterility (Sage *et al*., 2015). According to Petty *et al*. (2004) CO_2 being a potent greenhouse gas plays role in regulating earth's surface temperature through greenhouse effect by absorbing infrared radiation and re-radiation to earth causing warming. Thus, the beneficial effects of rise in CO_2 level are linked to the increase in atmospheric temperature which may lead to aberrations in crop. It is projected that due to increase in CO_2 concentration, the global mean air temperature would be elevated ranging from 1.4 to 5.8°C by 2100 (Houghton *et al*., 1990). The interaction effects between various climate variables have revealed

This paper is a part of Ph.D. thesis research submitted to the Assam Agricultural University, Jorhat – 785 013, Assam, India that different climatic stress factors may aggravate the rate and direction as compared to the individual climate stress factors (Reddy and Hodges, 2000). The expected increase in yield obtained due to the elevated CO_2 was not obtained in the plants grown at combined elevated CO_2 and temperature (Wheeler *et al*., 2000; Prasad *et al*., 2003). The role of high temperature in fruit setting of tomato has been reported (Paupiere *et al*., 2017).

Sorghum plants grown at different CO_2 concentration and temperature regimes show inhibited panicle emergence at temperature (40/30 and 44/34°C) and decreased pollen production, pollen viability, seed-set and seed yield at temperature greater than 36/26°C. Elevated CO_2 and temperatures increase vegetative growth and decrease seed yield, thereby lower harvest index (Prasad *et al*., 2006). A significant decrease in the performance of reproductive traits was observed in tomato under long term moderate heat (32 and 26°C day/night) (Xu *et al*., 2017). The present study was aimed to understand the reproductive morphology and anatomy of hot chilli (*Capsicum chinense* Jacq.) grown under a combination of elevated CO_2 and temperature.

MATERIALS AND METHODS

The present study was conducted in the Department of Crop Physiology, AAU, Jorhat (India) during 2012-2015. Mature seeds of two hot chilli cultivars *viz*., cv. 'Manipur' and 'Assam' were collected from Indian states of Manipur and Assam, respectively.

Growth conditions

Fine and sterilized field soil was used as a medium to germinate hot chilli seeds of above genotypes. The 40 cm dia. earthen pots were filled with a mixture of soil and organic manure (50:50; 7 kg pot^{-1}). Prior to sowing, the seeds were treated with fungicide Captan (@ 2.5 g kg^{-1} seed. The seedlings at 3-4 leaf stage were transplanted in 40 cm dia. earthen pots which were filled with topsoil, sand and cow dung (1:1:1). The pots were fertilized with N, P and K @ 120, 60 and 60 kg ha^{-1}, respectively. The half dose of N (urea) and full dose of P (single super phosphate) and K (muriate of potash) were applied as basal dose and rest half N top dressed in 2 splits, 30 and 60 days after transplanting. Hand weeding, irrigation and pesticide were applied as and when required. The pots were kept inside a controlled environment i.e. carbon dioxide temperature gradient tunnel (CTGT) [Genesis Technologies, Maharashtra, India] established in the Department of Crop Physiology, AAU, Jorhat.

Treatments and growth chamber

The experiment was conducted in a factorial completely randomized design comprising of 2 factors (elevated CO_2 and elevated temperature) and each treatment was replicated three times. The four treatments included were T_1 = Field condition (ambient CO_2 condition and ambient temperature condition), T_2 = CTGT I (380 ppm CO_2 + ambient temperature), T_3 = CTGT II (550 ppm CO_2 + 2°C higher than ambient from flower bud initiation till maturity) and T_4 = CTGT III (750 ppm CO_2 + 4°C higher than ambient from flower bud initiation till maturity). The elevated CO_2 was maintained throughout the crop growth period from 9 am to 2 pm regularly. The elevated temperature was maintained through Infra-Red Heater regulated by SCADA software. Three CTGTs were used in this experiment. CTGT structure consisted of a rectangular block, fabricated by metallic sheet pipe, covered with poly carbonate sheet (100 µ gauge) and installed in the experimental field. The poly carbonate sheets showed >85% transmission of light. The tunnel was fitted with 4 RTD (remote terminal unit) or data logger**,** temperature sensors, humidity transmitters and infrared heaters. Air compressor was used to maintain uniformity of CO_2 supply. To record ambient data, a temperature sensor and a humidity transmitter were placed outside the chamber. Monitoring and controlling of CO_2 in CTGTs was fully automatic and the desired CO_2 levels were maintained throughout the experiment. Data logger, SCADA software and personal computer were used to monitor and control appropriate CO_2 levels in each chamber. SCADA software facilitated setting of various concentration of CO_2 in different CTGT chambers at a time. Computer was used for software run and to store daily data. Most control actions were performed automatically by RTUs. Control signals were given to solenoid valves of particular chamber to close or open the CO_2 supply to CTGT chamber. The CO_2 in chambers was controlled by a 12-channel measurement and control system using ADC WA 526 IRGA (Infra-Red Gas Analyzer (LICOR; model: LI 6400).

The system sampled each chamber and delivered (pure) CO_2 to each chamber via 12 solenoid valves, according to the calculations based on the difference between reading and set point. Outlets of CO_2 regulator were connected manifold in which CO_2 passed through distributing system to the respective CTGT. Manifold was used to distribute CO_2 into hexagonal rings and solenoid valve was connected with each outlet of manifold. The command to cut and pass CO_2 was linked by software to the solenoid valves.

Anatomical studies

To study stem anatomy, fine sections were cut from the tender portions of stem. The sections were stained with safranin; and xylem and phloem tissues were observed under light microscope (Olympus, model 10132) at 10X magnification. The calibration of microscope was done with the help of stage and ocular micrometers; and xylem and phloem number, size, pollen number and diameter were determined using a microscope. The anatomical studies of xylem, phloem and pollen were conducted by using a light microscope (Setia *et al*., 1997). Mature anthers were collected from five numbers of flower before anthesis to determine the number of pollen grains. Pollen numbers were counted by placing anthers in a water drop on a glass slide and squashed with a needle, and then pollen grains were stained with safranin (0.1%) and counted under a microscope (Bennett, 1999). The number of pollen grains from anthers of each genotype under each treatment was counted. The diameter of pollen grains was measured and calculated with the help of stage and occular micrometers. The viability of pollen was assessed on the basis of tetrazolium test that helped to distinguish fresh and dead pollens using 1% 2,3,5-triphenyltetrazolium chloride (TTC).

To study the floral anatomy, floral parts were dissected with the help of a blade and needle, and observed under a trinocular microscope (Zeiss, Stemi 2000 C) at 4X and photographs were taken.

Physiological studies

Water saturation deficit was calculated using the following formula:

$$\text{Water saturation deficit (WSD)} = 100 - \text{RWC}$$

The relative water content (RWC) was calculated by following equation (Weatherly and Barrs, 1962) and expressed as percentage. For calculation of RWC the following method was utilized. Twenty discs were taken from the leaves of each treatment and weighed. Leaf discs were submerged in water in Petri-dishes for 4 h. These were blotted and their saturated weight measured. Discs were dried at 80°C till constant weight. RWC was calculated by following equation of Weatherly and Barrs (1962) and expressed in percentage.

$$\text{Relative leaf water content} = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

Statistical analysis

For statistical analysis of data, the method of analysis of variance was followed (Panse and Sukhatme, 1967). The critical difference between the treatments were calculated at 5% probability level.

RESULTS AND DISCUSSION

A significant difference in pollen number and diameter was noted amongst the hot chilli cultivars. An increase of 11% in both pollen number and diameter was observed in cv. ‘Manipur’ as compared to

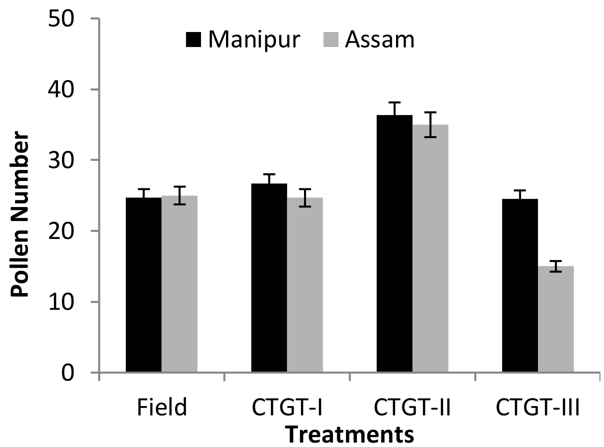


Fig. 1: Comparative differences in the number of pollen grains among various treatments and hot chilli cultivars

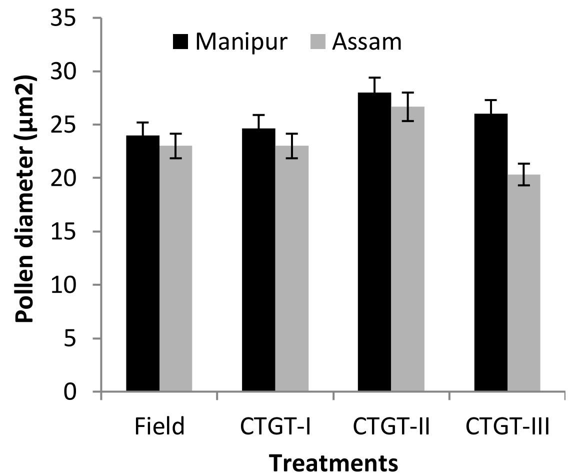


Fig. 2: Comparative differences in the diameter of pollen grains among various treatments and hot chilli cultivars

to cv. 'Assam' (Fig. 1 and 2). Pollens collected from CTGT II recorded 45 and 15% increase in their number and diameter, respectively, over field condition. A significant difference in pollen number and diameter was also noted in their cultivar x treatment interaction. In interaction effect, cv. 'Manipur' showed a significant 13.5 and 5.0% increase in pollen diameter at CTGT II and CTGT III, respectively, over field condition.

In our experiment, due to the interaction of various treatments and cultivars, the highest viable pollens were recorded at CTGT II, followed by CTGT-I and the least viable pollens were observed at CTGT III (Fig. 3). On the other hand, maximum non-viable pollens were found at CTGT III in cv. 'Assam', whereas minimum nonviable pollens were observed at CTGT II in cv. 'Manipur' (Fig. 4). A differential response of eCO_2 and eTemp was noted in pollen characteristics (Fig. 5).

Due to variations in carbon dioxide and temperature treatments, a significant alteration in xylem (proto + meta) and phloem (proto + meta) number was noted (Fig. 6). An increase of 35 and 17% was seen in xylem and phloem number, respectively, at CTGT II; whereas a decrease of 9 and 22% was recorded in xylem and phloem number, respectively, in CTGT III (Fig.7 and 8). Hot chilli cv.

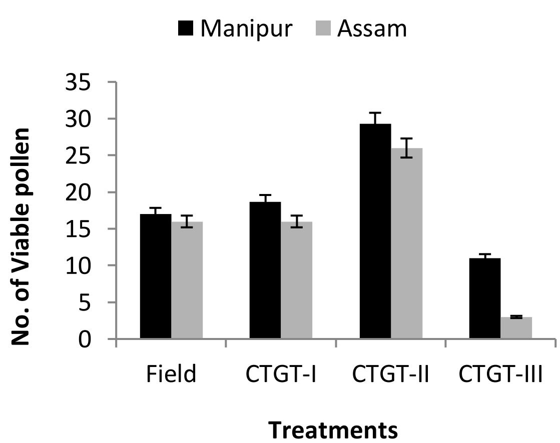
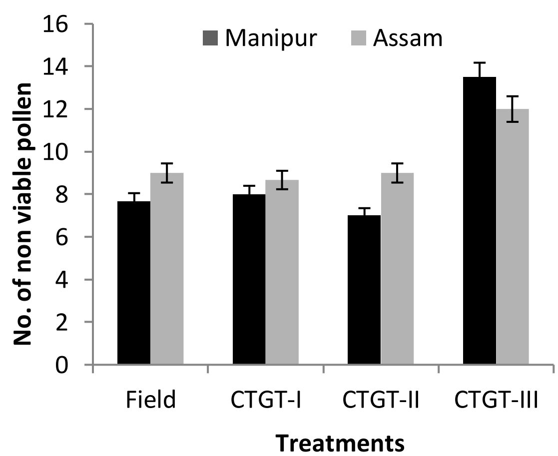


Fig. 3: Comparative differences in the number of viable pollen grains per microscopic field amongst various treatments and hot chilli cultivars



Fig, 4: Comparative differences in the number of non-viable pollen grains per microscopic field amongst the various treatments and hot chilli cultivars

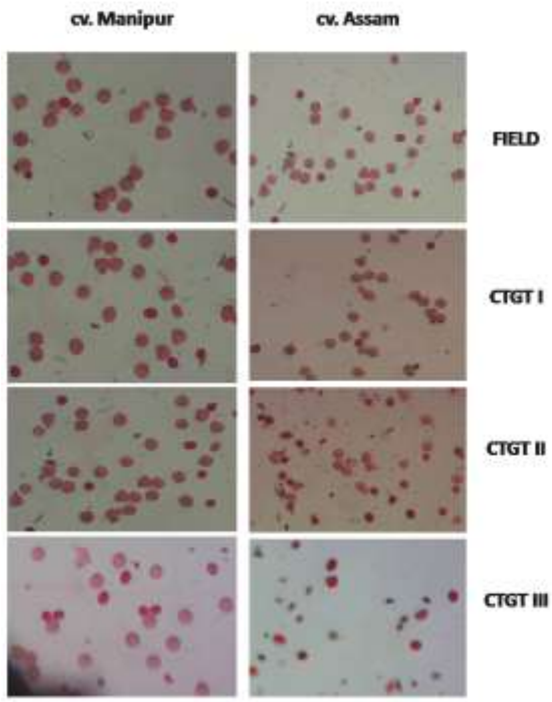


Fig. 5: Differential response of elevated CO_2 and temperature in pollen characteristics of two hot chilli cultivars

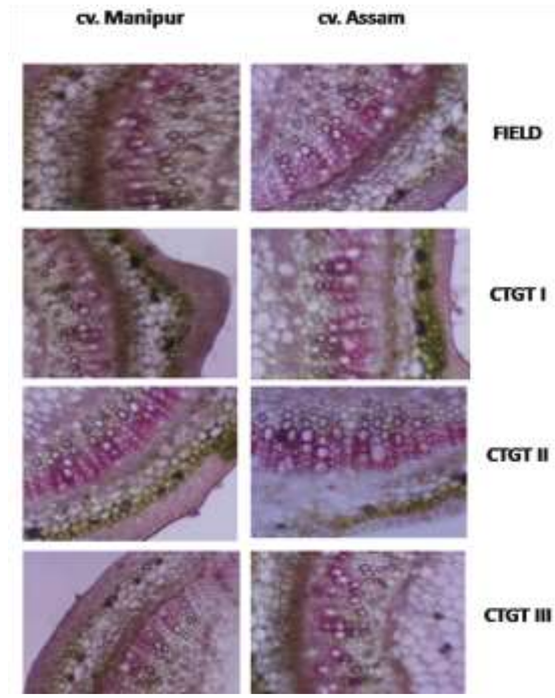


Fig.6: Differential response of elevated CO_2 and temperature conducting vessel characteristics of two hot chilli cultivars

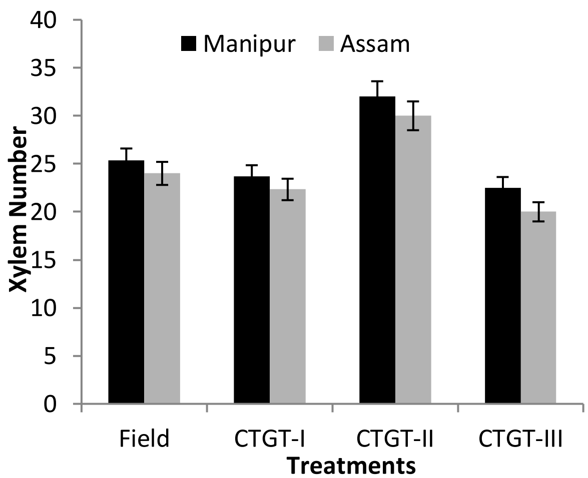


Fig. 7: Comparative differences in xylem number amongst the various treatments and hot chilli cultivars

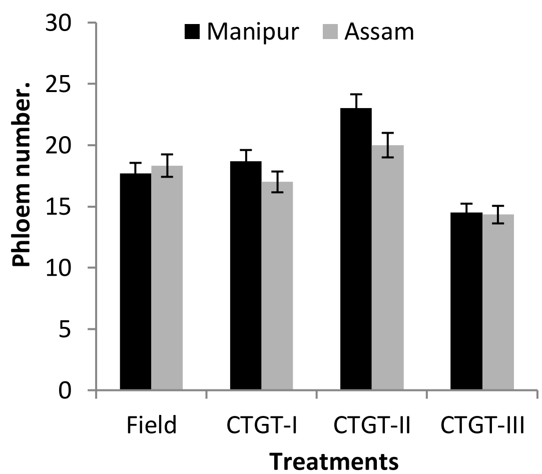


Fig. 8: Comparative differences in phloem number amongst the various treatments and hot chilli cultivars

'Manipur' showed 5% increase over cv. 'Assam'. The interaction between treatment and cultivars revealed insignificant difference in xylem number, but significant variations were observed in xylem and phloem size (Fig. 9). CTGT II depicted significant increase in xylem and phloem size in comparison to other treatments.

A significant variation in water saturation deficit (WSD) was noticed within the cultivars and with the eCO_2 and eTemp. treatments (Table 1). Highest WSD was observed at CTGT III. The interaction between treatments and cultivars was insignificant.

The floral morphology of hot chilli, cv. 'Assam' revealed some abnormal flower formation where the flowers collected from CTGT III showed elongation of style as compared to the stamens

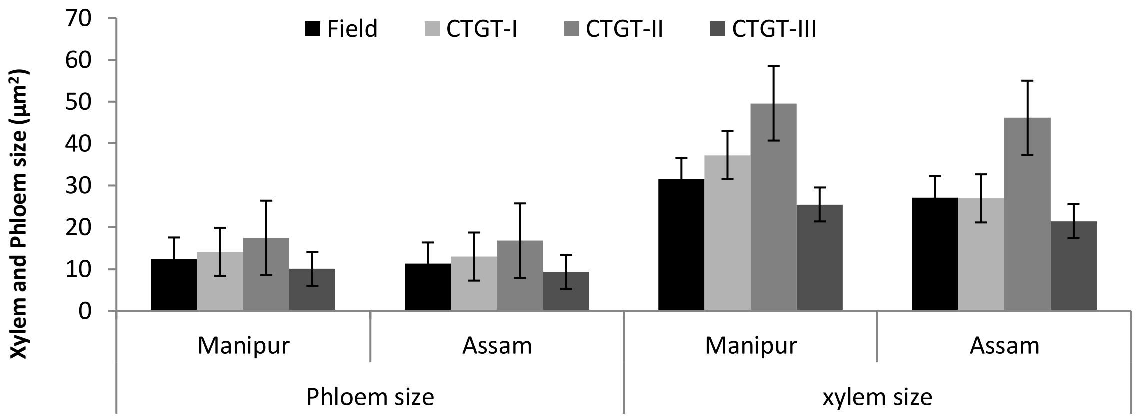


Fig. 9: Variation in xylem and phloem size amongst different treatments and cultivars

Table 1: Variations in water saturation deficit amongst various treatments and cultivars

	Water saturation deficit	
Treatments		
Field	20.28	
CTGT-I (Amb)	22.53	
CTGT II	26.64	
CTGT III	49.68	
SE±	1.30	
$CD_{0.05}$	2.76	
Cultivars		
Manipur	31.06	
Assam	28.51	
SE±	0.92	
$CD_{0.05}$	1.95	
Treatment x cultivar	cv. 'Manipur'	cv. 'Assam'
Field	20.25	20.31
CTGT-I (Amb)	24.52	20.54
CTGT II	27.69	25.58
CTGT III	51.78	47.59
SE±	1.84	
$CD_{0.05}$	ns	

*Field condition= ambient CO_2 condition and ambient temperature condition; CTGT I = 380 ppm CO_2 + ambient temperature; CTGT II = 550 ppm CO_2 + 2°C higher than ambient from flower bud initiation till maturity; CTGT III = 750 ppm CO_2 + 4°C higher than ambient from flower bud initiation till maturity

(Fig. 10). The elongation of style due to high temperature treatment at CTGT III might have prevented pollination processes. Some other anatomical aberrations like bending of style were noticed under elevated temperature. The bending of style might have prevented the pollen entry into egg cells, thereby prevented the fertilization of egg cells.

The study revealed that high temperature had adverse effect on pollen development *viz.*, pollen diameter & number and their viability. The variation amongst the cultivars found in present study appears due to some defragmentation of metabolic processes related to reproductive growth. Bokszczanin and Fragkostefanakis (2013) opined that high temperature induces structural changes in biomolecules which may affect the basic metabolic processes *viz*., photosynthesis, respiration and redox regulation including proline accumulation. Lansac *et al*. (1996) reported that proline being an osmo-protectant accumulates under various abiotic stresses and is considered as a key factor in pollen viability. Morphological characterization and analysis of descriptors related to pollen like shape, position, types of apertures, decoration of exine etc. are required to separate the related species (Iwanami and Yoshio, 1988). Although the variations in pollens characters are genetically determined yet environmental factors especially high temperature play crucial role in developmental processes. In our study, cv. 'Manipur' maintained good pollen characters in terms of size, shape and viability under elevated carbon-dioxide and temperature. Mesihovic *et al.* (2016) showed that the variation in heat sensitivity of pollen exists among plant species under variable high temperature

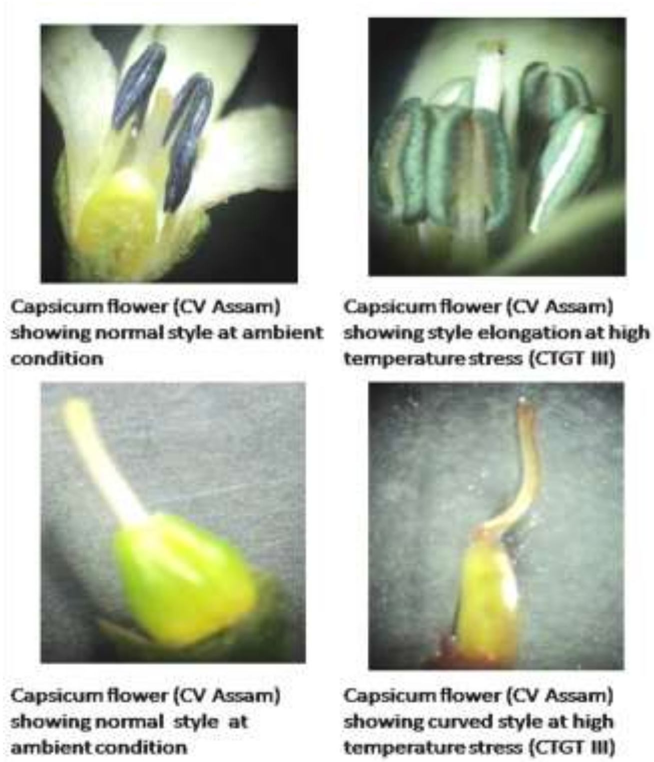


Fig. 10: Abnormal floral reproductive organs grown under ambient condition and at elevated CO_2 and temperature

regimes and it also depends on the stages of pollen development**.** Further, high temperature stress at later stages of pollen development leads to microspore abortion, causing reduced number of mature pollen grains during anthesis, leading to lesser number of viable pollens for germination. In present study, the elevated CO_2 and high temperature (CTGT III, 750 ppm + 4^{o}C) lead to the injury of pollen shape and size and even stigma. However, the intensity of injury was less in CTGT II (550 ppm +2^{o}C). Some ameliorative effect was observed in cv. 'Manipur' as compared to cv. 'Assam' which indicated adaptive behavior within the species. Better pollen quality in cv. 'Manipur' under stress condition seems due to the maintenance of metabolites like carbohydrate. High temperature stress reportedly causes starch deficiency in sorghum micro-spores leading to reduced pollen germination and seed setting (Jain *et al*., 2007) where carbohydrate metabolism and unloading in anther and pollen play an important role in pollen viability. Tolerant genotypes could maintain high pollen starch and sugar level as compared to sensitive varieties (Pressman *et al.*, 2002; Firon *et al.*, 2006) and role of sugar partitioning under stress is well documented (Ruan *et al*., 2010). Flowering under high temperature regimes could disturb pollen development (Tang *et al*., 2008, Sakata *et al*., 2010) in sensitive varieties.

From present study, it is evident that high temperatures lead to water scarcity in CTGT III during pollen development. Under medium range of stress at CTGT II, some anatomical modification in plants may occur in terms of xylem and phloem characters which are attributed to the more number of xylem and phloem with greater area of both that help in maintaining the water status and metabolites during pollen development. Results indicated water saturation deficit (WSD) was minimum in CTGT II. However, variation among the cultivars existed. Das (2003) reported that in *Brassica* spp., elevated CO_2 significantly reduced the pith size as a result of increase in area and number of secondary xylem and phloem. Such alteration in xylem and phloem anatomy could increase the stem and root girth. Increased vessel transactional area was an important contributing factor for increase in stem girth. Such increase of radial width due to increase in xylems (meta + proto) and phloem (meta + proto) under elevated CO_2 implies higher transport of nutrients and water from soil to maintain higher water and nutrient status during growth period. Domec *et al.* (2017) reported more strengthening of xylem conduits and increase in wall thickness under elevated CO_2 condition. In our study, a significant difference in WSD due to various CO_2 and temperature treatments could be as a results of improper flower development that prevented proper pollination and fruit formation under CTGT III. Mangena (2017) reported drastic changes in morphological and anatomical features in soybean which resulted in flower abortion. In present study some abnormal flower formation was observed when the plants were grown at CTGT III. The abnormal flower formation included bending of style (Fig. 10) which appears to have prevented the pollen passage to the ovary causing poor fertilization. This anomaly in flower development was evidenced in CTGT III causing improper fertilization due to the elongated style as compared to stamens and the curved style that might have obstructed the passage of pollen to the egg cells in hot chilli although a variation in genotypic response also existed. The elongation of style than that of stamens could also be one of the reasons for hindrances in pollination. Similar results were observed by Giorno *et al*. (2013) in tomato wherein one of the most noticeable effect of high temperatures was the production of an exserted style (i.e. stigma was elongated beyond the anther cone), which could have prevented pollination. Another cause of pollen damage might be the heat-induced male sterility and susceptibility of pollen to oxidative damage than that of pistil (Djanaguiraman *et al.*, 2018). The effects of high temperature on development of flowers, anthers and pollen viability were observed in heat-sensitive and heat-tolerant lines (Bita and Gerats, 2013). The variation of response was noted in the two cultivars tested in present study under varying level of CO_2 and temperature. Hot chilli cv. 'Manipur' was found less sensitive to elevated CO_2 and temperature as compared to cv. 'Assam'.

Conclusion: The present study revealed various morpho-anatomical variations in the reproductive parts that affected the productivity in hot chilli. The cv. 'Manipur' showed some degree of tolerance against high temperature and CO_2 levels which could be attributed to the modification of anatomical features like xylem phloem characters that help in maintaining the water status of plant system. This might have helped in metabolite allocation to the newly dividing cells of reproductive parts. This better performing cultivar may be used as a future breeding material for advanced research on the development of new plant types suitable for changing climatic condition.

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