INFLUENCE OF CO_2 ENRICHMENT AND ASSOCIATED HIGH TEMPERATURE ON REPRODUCTIVE PHYSIOLOGY OF TOMATO (*Solanum lycopersicum* L.)

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Influence of CO_2 enrichment and associated high temperature on reproductive physiology of tomato (*Solanum lycopersicum* L.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Date : 29/08/2019

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Certified that this thesis entitled **"Influence of CO_2 enrichment and associated high temperature on reproductive physiology of tomato (*Solanum lycopersicum* L.)"** is a record of research work done independently by Ms. Lakshmi G. Ajay (2017-11-098) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associate ship to her.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
@	At the rate of
µg	Microgram
µm	Micrometer
°C	Degree Celsius
m^{-2}	Per metre square
CD	Critical difference
cm	Centimeter
ml	Millilitre
M	Molar
ppm	Parts per million
o	Degree
m	Meter
µ	Micro
CRD	Completely Randomized Design
DNA	Deoxyribo nucleic acid
rpm	Rotations per minute
et al.	and other Co workers
OD	Optical density
Fig.	Figure
g	Gram
i.e.	That is
KAU	Kerala Agricultural University
mm	Millimeter
viz.	Namely
OTC	Open Top Chamber

IPCC	Inter-governmental Panel on Climate Change
NOAA	National Oceanographic and Atmospheric Administration
TIRT	Temperature Induction Response Technique
mm	Milli meter
ha	Hectare
FACE	Free Air CO_2 enrichment
µmol	Micromoles
mmol	Millimoles
pCO_2	Partial pressure of CO_2
µL	Microliter
kDa	Kilo Dalton
µ Enst.	Micro Einstein
mg	Milligram
nm	Nanometer
s	Seconds
A_{663}	Absorbance at 663nm
A_{645}	Absorbance at 645nm
A_{480}	Absorbance at 480nm
A_{510}	Absorbance at 510nm
A_{520}	Absorbance at 520nm
A_{460}	Absorbance at 460nm
FW	Fresh weight

INTRODUCTION

1. INTRODUCTION

Climate change is a subject of paradox. With the increasing greenhouse gases and associate increase in temperature, most of the studies reveal this changing climate is a threat to the future humanity. This changing climate is having a profound effect on the agricultural systems which is a matter of concern for worldwide food security and to feed the growing population. Among all the factors influencing climate change, it is the effect of elevated CO_2 and associated high temperature is causing the major issue of concern.

During the last 150 years, atmospheric carbon dioxide concentration has increased from about 280 ppm to the current level of 400ppm and its concentration is predicted to increase to about 500 ppm within the next 50 years. CO_2 as the main plant nutrient will have its direct influence on plant growth and development. But this steadily rising trace gas will have its impact on global temperature due to its radioactive forcing character and longer residence time in the atmosphere. Predictions suggest that global temperature will increase by 1.8-4.0°C by the end of this century. Since the temperature is a major determinant of the timing and duration of key developmental phases of any crop, including flowering (Bahuguna and Jagadish, 2015), interaction effects of increasing CO_2 and associated high temperature are likely to have significant impacts on the reproductive physiology of any crop. However, as a basic substrate for the life sustaining chemical reaction, the photosynthesis, the increasing CO_2 is having a positive impact on plant community and agriculture sector.

Tomato (*Solanum lycopersicum* L.), is world's largest vegetable crop cultivated and serves as a protective food. It is a typical day neutral plant which is mainly self - pollinated. It is a warm season crop which is reasonably heat and drought resistant and can be grown under a wide range of climatic and soil conditions. But many works have shown that higher temperature can significantly reduce the fruit setting and yield in tomato plants. Considering this effect, the impact of climate change, especially due to the CO_2 enrichment and associated high temperature stress on the tomato plants is an

issue to be focused on. The average productivity of tomato in our country is merely 15.8 t/ha while that of USA is 58.8 t/ha. In this scenario achieving higher yields by coping up with the changing climate is still challenging and worthy to be considered seriously.

Carbon dioxide enrichment studies carried out in the Department of Plant Physiology under elevated CO_2 in the Open Top Chamber (OTC) system has shown drastic modifications in reproductive phase, in terms of time of flowering, number of flowers, floral characters and pollen viability in the case of tomato. Because of these modifications the advantage of higher dry matter accumulation during vegetative phase shall not be reflected in yield.

The optimum temperature range for tomato plants is 25-30 °C. A higher temperature condition (30 ^{0}C $\pm$ 2 ^{0}C day and 22 ^{0}C $\pm$ 2 ^{0}C night) was found to cause decreased fruit set in tomatoes. This was found to be associated with lesser pollen viability, style elongation and lack of formation of endothecium, essential for stamen and pollen theca opening (Rudich et al., 1977). However the increased fruit weight was found to be associated with increased atmospheric CO_2 (Dheeraj., 2015). Hence, once the problems due to the associated high temperature is resolved the overall yield of this crop can be increased.

In the present study, efforts were made to analyze the growth and development of tomato under the influence of elevated CO_2 and associated high temperature stress giving emphasis to reproductive physiology in terms of flower development, pollen viability and fruit development. The improper floral development as noticed in the previous programmes conducted under elevated CO_2 conditions was also addressed using growth regulators, nutrients and temperature induction stress technique.

The whole programme was divided into two experiments. The first experiment included the standardization of TIRT for two popular varieties of tomato in Kerala, namely Vellayani Vijay and Anagha and their evaluation under elevated CO_2 conditions (500 ppm). TIRT is a technique by which thermo-tolerance of the plants can

be improved by exposing plants to a mild stress (induction) before exposing to a lethal stress so that it can withstand the lethal stress due to the adaptation. This technique was used to improve the performance of the tomato varieties under the elevated CO_2 conditions and associated high temperature.

The second experiment consist of evaluation of the impact of various growth regulators and nutrient application on improving the reproductive physiology of tomato under CO_2 enrichment. CO_2 enrichment was done using open top chamber (OTC) facility in College of Agriculture, Vellayani. OTC is one of the recent technologies with a holistic approach to study the impact of rising atmospheric CO_2 in agricultural systems. CO_2 was released into these chambers from CO_2 cylinders in a controlled manner. Sensors which were placed inside the OTC measured parameters like air temperature, CO_2 concentration, humidity, light intensity, leaf temperature etc. on real time basis. Potted plants were kept within these chambers and response to elevated CO_2 environment was studied.

Considering the positive impact of increasing CO_2, recognizing various effective means to improve the photosynthetic efficiency, reproductive physiology and finally yield efficiency is intended to be achieved through the present study. The results of this study provided insights into our understanding of how environmental and endogenous cues interfere with already well defined floral development pathway. The results of this study also help to design improved production technologies for a changing climate scenario.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

India is one of the countries which is at high risk of food insecurity due to weather extremes caused by climate change. Oman, India, Bangladesh, Saudi Arabia, and Brazil are on top of the list of countries being affected by climate change and the associated increase in temperature (Philosophical Transaction of the Royal Society, 2019). Climate change is expected to cause extremes of both rainfall and drought with different effects in different parts of the world. Such weather extremes may increase vulnerability to food insecurity.

Climate change refers to any change in climate which occurs over a specified period, either as a result of natural variations or human activities (anthropogenic causes) (IPCC, 2001). Climate change is marked by changes in temperature, patterns of rainfall, and a rise in sea level. Climate change has affected all sectors of life. Coming to agriculture, the effect of climate change is felt most strongly in the production of food crops.

Carbon dioxide, an important heat-trapping (greenhouse) gas, is being fueled by human activities such as deforestation and burning fossil fuels, as well as natural processes such as respiration and volcanic eruptions. They are the largest contributors to human induced warming and therefore one of the most critical sign spot to keep a watch on. This ever increasing trace gas result in rise in temperature which in turn affects the physiology of crop plants. The present research study was conducted as an investigation on improving adaptations of tomato under elevated CO_2 and associated by applying various nutrient and growth regulator treatments and temperature induction response techniques.

Under the changing weather conditions, crops do not grow properly. The increasing concentration of CO_2 and associated high temperature has both negative as well as positive effects on plant growth. Besides the positive direct impact of CO_2, increasing air temperature can interfere with the growth of crops and can lead to a

decrease in overall production (Furuya and Koyama, 2005). According to Lars (2007). An increase in temperature causes an increase in transpiration which finally reduces the productivity of the crops. An experiment by Peng *et al.* (2004) leads to a conclusion that for every 10^0C increase in temperature, the crop production decreased by up to 10 percent.

As far as India is concerned, the changing climate and associated rise in CO_2 has a great influence on the national economy as well. This is because the agriculture sector contributes to a major part of the national economy. Agriculture is not only a source of food but also a source of raw materials for many agro-based industries. Kerala, being a fragile and closed eco-system, the agriculture production scenario is being affected greatly by the variation in climate. The impact of weather aberrations was found to have ill effects on perennial as well as seasonal crops in nature which is reflected upon the state's economy (Rao *et al.*, 2008).

High-temperature stress associated with increasing CO_2 raises concerns about the reproductive physiology and flowering of the crops. It is mainly associated with reduced pollen viability and lower fruit setting percentage. Another consequence of exposure to these stresses is the increase in root shoot ratios and leaf relative conductivity. Numerous studies have shown that plants responded in a large set of parallel changes in growth and morphological and physiological responses when they were exposed to elevated conditions of CO_2 (Kim et al., 2001).

Currently, all over the world, many technologies are being used to study the impact of rising CO_2 on agricultural systems. Technologies such as SPAR (Soil Plant Atmosphere Research), FACE (Free Air CO2 enrichment) and OTC (Open Top Chamber) are being used for crop response studies in this field. In India, studies have been reported from IARI New Delhi, CRIDA Hyderabad, IGFRI Jhansi, NPL New Delhi, CRRI Cuttack, BHU, etc. CO_2 enrichment studies in Kerala are being carried out in CPCRI Kasargode and the College of Agriculture Vellayani.

The least expensive and the most generally used technology for estimating the effect of elevated atmospheric CO_2 concentration in plants is the Open Top Chamber (OTC) facility. It consists of metal constructions with transparent vertical sidewalls and a frustum on the top. An opening in the middle of the frustum allows air exchange to reduce temperature and humidity built up in the chamber. Sensors are placed inside the chambers to measure different microclimatic parameters like air temperature, humidity, light intensity, etc. on a real-time basis. The actual concentration of CO_2 within the chamber is measured by the CO_2 analyzer and is controlled by computer-supported regulation of inlet valves. OTC system provides a unique opportunity to examine the growth and productivity of crop plants under elevated CO_2.

Tomato (*Solanum lycopersicon* L.) of family Solanaceae, is the native of Peruvian and Mexican region and occupies an important place among the important vegetable crops grown in our country. Tomato is a herbaceous plant growing to 1-3 m in height with a weak woody stem. The flowers are yellow and the fruits of cultivated varieties vary in size from cherry tomatoes (1–2 cm diameter) to beefsteak tomatoes (10 cm diameter). Most cultivars produce red fruits when ripe. Tomato is one of the most important "protective foods" because of its special nutritive value and widespread production. It is one of the most versatile vegetables with wide usage in Indian culinary tradition. It is considered as an important commercial and dietary vegetable crop having a very good post-harvest potential. It is rich in vitamin A, vitamin C and also ranks first in their nutrient contribution to the human diet. The average productivity of tomato in our country is merely 15.8 t/ha while that of the USA is 58.8 t/ha.

Tomato production as compared to other vegetables has always been associated with the requirement of an abundance of water. All developmental stages of tomato plants are highly vulnerable to temperature changes, however, developing anthers and pollen grains are more sensitive than vegetative organs. It has shown dramatic improvements in production due to alterations in the mechanisms under various stress conditions (Tigchelaar and Foley, 1991). In tomato, early reproductive processes

including micro, mega sporogenesis, pollination, pollen tube growth, fertilization, and early embryo development were all affected by high-temperature stress as explained by Srivastava *et al.* (2014).

This susceptibility of the tomato plant to various stress situation was evident from the results of various works by scientists. Noto and Malfa, (1985) opinioned that tomato plants when exposed to higher temperature resulted in an increase in the time period from sowing to flowering. This increased crop duration is considered as an important contributing factor for the failure of tomato plants to set fruits under high temperatures. Under elevated CO_2 conditions, plant experiences high-temperature stress and associated water stress. Similar effects on the reproductive morphology of the plant are also seen under such condition. In this scenario achieving better yields by coping up with the challenging stress is worthy to be considered seriously.

Under this background it is necessary to find out alternative methods that help in mitigating the negative impacts of the CO_2 enrichment so that maximum utilization of the available carbon can be done, mitigating the impact of associated stress. In this study, an attempt is being made to cope up with all the ill effects caused by the increasing CO_2 and the associated high temperature on the reproductive physiology and yield of tomato plants through the application of various growth regulators, nutrients as well as temperature induction response technique (TIRT).

Effect of CO_2 enrichment on growth parameters of tomato.

Plant growth is affected by both external and internal factors. CO_2 is the most important substrate for photosynthesis, any increase in this CO_2 content influences the growth rate of the plant species. Geissler *et al.* (2009) concluded that in the case of terrestrial plants, any increase in CO_2 resulted in an increased rate of photosynthesis , but the growth responses of the plants varied from 0-50% gain per season depending on the plant age, duration of crop and growth conditions (Beismann *et al.*, 2002).

According to Curtis and Wang (1998), elevated CO_2 resulted in increased plant biomass, total leaf area, and root mass.

Mamata *et al.* (2014) concluded that there was a significant difference in the plant height, number of leaves and branches and leaf area under enriched CO_2 conditions compared to control conditions especially at the flowering stage of tomato. In an experiment in pea plants conducted in OTC with elevated CO_2 conditions, irrespective of varieties the plants showed an increased plant height at 50% flowering stage and maturity (Meena *et al.*, 2016). Pritchard *et al.* (2001) reviewed that plant height increased under elevated CO_2 conditions. Studies showed that elevated CO_2 (700 $\mu L\ L^{-1}$) increased the plant height in Pima cotton (Reddy *et al.*, 1995)

The growth of plants under elevated CO_2 was found to alter plant structure by altering both primary and secondary meristems of shoots and roots (Pritchard *et al.*, 2001). Minu and Manju (2015) reported that elevated CO_2 had improved the plant height by 18 %, leaf production by 84 %, leaf area by 174.4 % in an experiment in pepper. A higher number of branches were observed at elevated CO_2 (700ppm) compared to elevated CO_2 (550ppm) and control (ambient) conditions (Mamata *et al.*, 2014). Alterations in developmental processes at the shoot apex and within the vascular cambium contributed to increased plant height, altered branching characteristics, and increased stem diameters. Under elevated CO_2 conditions, the total biomass increased by increasing the number of leaves and branches in populous clones even though the branches were shorter (Ceulemans *et al.*, 1995 in Populus; Bhattacharya, 1985 in cowpea). However, Paez *et al.* (1983) reported in *Pisum sativum* that, additional CO_2 did not have any impact on the number of branches. Brown and Higginbotham (1986) reported that elevated CO_2 conditions resulted in increased height in boreal tree seedlings. Bhattacharya (1985), reported that there was a significant increase in the number of leaves in sweet potato under elevated CO_2 conditions. A similar report was given by Pal (2004) in berseem. According to Alexandre (2012), the leaf growth rate of *Zostera noltii* did not show any significant increase under elevated CO_2.

Leaf area per unit leaf dry weight is called specific leaf area and is the inverse of specific leaf weight. According to Tsukaya (2006), leaf size is determined by cell production and expansion, which are controlled in a coordinated manner during leaf organogenesis. It is this leaf that allows more light energy to be absorbed enabling better photosynthetic performance. Many research groups have highlighted the role of elevated CO_2 concentration on cell division and expansion of leaves. Heath and Kerstiens (1997) concluded that leaf size increased under elevated CO_2 conditions in Beech and Oak and at the same time certain other reports say that there was a decrease in specific leaf area in different plant species (Norby and Neill, 1991). During high-temperature conditions there occurs a drought-induced reduction in the leaf area as a result of leaf expansion by reduced photosynthesis and turgor pressure (Rucker *et al.*, 1995). Meng, (2013) found out that it was not the elevated CO_2 that affected the specific leaf area but the associated drought conditions that resulted in a decrease in specific leaf area in *Jatropha curcas*. In Norway spruce, the elevated CO_2 treatment decreased specific leaf area but did not affect SLA of Scots pine (Sallas *et al.*, 2003). At the same time, an increased leaf area was found in mustard by about 23% under drought conditions (Mishra *et al.*, 1999).

Effect of growth regulator, nutrient application and temperature induction response technique on growth parameters of tomato under CO_2 enrichment

Application of growth regulators and extra nutrients find application in enhancing the improved growth parameters under elevated CO_2 conditions. Carbon dioxide is one of the inorganic substrates that is used by plants for its primary plant proceses. During the long term exposure, availability is a major factor that contribute to plant responses under elevated CO_2.

Reports have shown that the whole plant nutrient uptake increased under elevated CO_2 condition but tissue concentration of these elements decreased (Kim *et al.*, 2001; Ainsworth and Long, 2005). Mishra *et al.* (1999) studied the effect of

different levels of nitrogen and reported significantly more height and the number of leaves in plants supplied with 150 % of the recommended dose of nitrogen compared to normal nitrogen supplied plants in tomato. An experiment conducted by Verma *et al.* (1993) revealed that the application of 90 Kg N/ha brought significant improvement in the number of leaves per plant in okra. Mahapatra and Adhya (1996) studied the effect of different levels of nitrogen on pointed gourd in a field study and concluded that the maximum nitrogen level resulted in significantly longer vines, an increased number of branches and leaf area.

In an experiment conducted by Misra *et al.* (2012), a positive impact of boron (45μM) on growth parameters of seed geranium, barley and water fern was observed. Much work have not been taken up on the impact of boron and zinc on alleviating the stress caused due to elevated CO_2. However, works have been conducted on the impact of these micronutrients on growth and activity of various plants under normal environment. Patil *et al.* (2008), reported that the application of boric acid at 100 ppm resulted in the maximum number of primary branches. Meena *et al.* (2015) reported that boron (100 ppm) significantly increased the plant height and number of branches. Acharya *et al.* (2015) conducted an experiment on the effect of boron and zinc application on seedlings of onion and concluded that the treatment of zinc and boron resulted in a significant increase in growth, fresh weight and total dry matter production compared to control. Ahmed *et al.* (2011), in an experiment in potato, concluded that 300 ppm zinc significantly increased the vegetative growth like plant height, stem and number of leaves and leaf area.

Goswami *et al.* (2013), in an experiment on the effect of plant growth substances on the growth of pomegranate, found out that NAA at 50 mg/l at the time of pruning resulted in increased number of shoots per plant. Thaware *et al.* (1991) revealed that NAA at 20ppm had a positive impact on the morphological characters of cowpea such as height, branches per plant, leaves per plant, etc under various stress conditions.

Salicylic acid is a phenolic compound, which is enhancing the plant growth and development (Arfan *et al.*, 2007). SA was found to be ameliorating the damaging effects of heavy metal stress in rice (Misra *et al.*, 1999), and salt stress in wheat (Arfan *et al.*, 2007). There have been very little discussion on the effect of SA under elevated CO2 condition. Ghasenzadeh *et al.* (2012) reported that application of SA under elevated CO2 resulted in increased growth of ginger plants. Nezhad *et al.* (2014) reported the number of branches increased by a spray of 1.5 mM SA in mung bean. Increased plant height and number of branches was reported by application of SA in mung bean by Nezhad *et al.* (2014) (1.5 mM salicylic acid) and Vaisnad and Talebi (2015) in chickpea (1 mM Salicylic acid). Zamaninejad *et al.* (2013) reported that the foliar spray of SA at 1.5 mM resulted in an increase in leaf area in maize plant compared to plants that were not treated with SA. Aldesuquy *et al.* (2012) reported that there was significant increase in plant height in wheat cultivars under drought stress, and when salicylic acid was applied at 0.05 M, an improvement in plant height was noted.

Effect of CO_2 enrichment on physiological and biochemical parameters of tomato.

Under enriched CO_2 conditions, plants alter their anatomy and leaf morphology to cope up with the changing conditions and to cope up with stress (Mc Lellan, 2000). Plant growth is stimulated by the elevation of CO_2. As photosynthesis increases, more plant biomass accumulates per unit of water consumed, and economic yield is enhanced. Elevated CO_2 generally affects C_3 photosynthesis more than C_4. A two-fold increase in current ambient CO_2 concentration stimulated the growth of C_4 plants from 10–20% whereas in C_3 plants the increase was about 40–45% (Ghannoum *et al.*, 2000; Beismann *et al.,* 2002).

Total chlorophyll content

The total productivity or capacity of a plant can be determined by the total chlorophyll per unit area. Environmental factors, as well as nutrient availability,

influences the total amount of chlorophyll present in the leaf tissue. (Otitoju and Onwurah, 2010). Various studies revealed different results in the case of chlorophyll content of the leaf tissue. Some studies showed an increase in the pigment while others showed decreased pigments.

In cucumber seedlings, leaf chlorophyll content decreased with age both under ambient (380 ppm) and elevated CO_2 (760 ppm) (Li *et al.*, 2008). Mamata *et al* (2014) concluded that as the CO_2 concentration increased there was a relative decrease in the chlorophyll content in tomato, especially during the flowering stage. The total chlorophyll content in the highest CO_2 treated plants (e [CO_2]-700ppm) was 15 % lower than control and 14.5% lower than e[CO_2]-550ppm.

Total soluble protein

Ibrahim and Jaafar (2012) reported that there was a decrease in total soluble protein in *Elaeis guineensis* (Oil Palm) as the level of CO_2 was increased from 400 to 1200 μmol mol−1. 10 % of the total dry mass of plant roots and shoot is constituted by amino acids which make up the protein. Under elevated CO_2 conditions, a large decline was noticed in the Rubisco protein up to 60% (Besford *et al.*, 1990; Rowland-Bamford *et al.*, 1991). Under elevated CO_2 concentration of 700 μmol mol-1, a decrease in the total soluble protein of penultimate leaves of barley and flag leaves of wheat were reported with an increase in the leafage (Richard and James, 1997).

Contrary to this, Joseph *et al.* (2009) reported that the soluble protein increased to 31% under 800 μl CO_2 per liter in soybeans (*Glycine max*) compared to the control plants at 330 μl CO_2 per liter. The soluble protein recorded was found to be higher in leaves of *Stylosanthes hamata* grown under 600ppm CO_2 (Baig *et al.*, 2012).

Total carbohydrate

Carbohydrates are molecules that are chemically bound to other molecules or physically associated or present as isolated molecules (Wang *et al.*, 2003; Ibrahim and

Jaafar, 2012). The carbohydrate group includes sugars, starches, and cellulose. CO_2 enrichment has a positive correlation with the concentration of total carbohydrates in plants (Ibrahim and Jaafer, 2012).

Accumulation of carbohydrates has been observed in many studies during plant growth under CO_2 enrichment (Makino *et al.*, 1999). Increased carbon uptake, resulted from initial stimulation of photosynthesis by elevated CO_2, altered the balance of supply and capacity to use carbohydrates. As a result of this, the non-structural carbohydrate concentrations invariably increased within leaves grown under elevated CO_2 (Drake *et al.,* 1997). A marked increase in foliar carbohydrate content was common at elevated CO_2, even when plants were free from the artificial restriction of sink development (Long *et al.*, 2004).

Elevated CO_2 levels increased carbohydrate by 52% in *Alpine tundra* (Moore *et al.*, 1999). Exposure of C_3 plants to elevated CO_2 frequently resulted in an immediate increase in the rate of CO_2 assimilation. However, a reduction in photosynthetic capacity often occurred after prolonged periods of elevated CO_2 (Stitt, 1991; Griffin and Seemann, 1996). This down-regulation or acclimation of photosynthesis is generally accompanied by a large increase in leaf carbohydrates.

A high concentration of starch has been found in mature tomato leaves exposed to elevated levels of CO_2 (Yelle *et al.,* 1989). Observations of the increased foliar carbohydrate content in plants grown under elevated CO_2 were well documented, including soybean, in which growth at elevated CO_2 resulted in a 45% increase in total non-structural carbohydrate (Ainsworth *et al.,* 2002; Rogers *et al.,* 2004). The favorable response to CO_2 enrichment might be due to increased sugar production thereby triggering some biochemical changes. Lilley *et al.* (1996) reported that elevated CO_2 conditions produced an average increase in total non-structural carbohydrate contents by 28% for clover and 16% for phalaris.CO_2 enrichment improved the carbohydrate contents of tomato fruit compared to fruits exposed to ambient CO_2

concentration, which might be due to enhanced translocation of photosynthates with elevated enzyme activities (Islam *et al.*, 2004).

Transpiration rate

Transpiration refers to the loss of water from the aerial parts of plants as water vapour. It is known as a necessary evil simply because, besides being a wasteful process in terms of water loss, it is a mechanism that enables plants to pull up water and nutrients from the root and supply for photosynthesis, supply minerals and cooling of the leaf which helps the plant cell to remain turgid. The rate of transpiration is affected by both internal (plant factor) and external factors (light, temperature, humidity, wind, atmospheric pressure and water supply).

Gruda (2005) reported that an increase in the atmospheric CO_2 levels enhanced the water use efficiency due to reduced stomatal conductance which stimulated light-saturated photosynthesis, particularly in C3 plants. Centritto (1999), in his experiment in cherry plants, recorded that there was a significantly higher rate of transpiration under elevated CO_2 than control. But in another experiment by Bazzaz (1990) plants showed a decreased stomatal conductance under elevated CO_2 conditions. This finally resulted in reduced transpiration per unit leaf area and higher soil water content compared with non- CO_2 enriched plant communities (Field *et al.*, 1995).

A reduction in transpiration rate was also reported in winter wheat and barley due to a partial closure of stomata and a decrease in stomatal conductance when grown under elevated CO2 (Morison and Gifford, 1983; Bunce, 2000). Elevated levels of CO_2 in *Podophyllum hexandrum* showed decreased levels of stomatal conductivity and specific leaf area (Chaturvedi *et al.*, 2009). Growth in elevated CO_2 caused a decrease in stomatal conductance in some tree species (Medlyn *et al.*, 2001).

Photosynthetic rate

Prasad *et al.* (2005) reported an increased rate of photosynthesis in various crop species with both short-term and long-term exposure to elevated CO_2. At the same time

studies by Ghilidyal and Natu (2000) showed that there was stimulation in photosynthesis due to CO_2 enrichment which did not sustain during long term exposure. These responses are known as photosynthetic acclimation or down-regulation which may occur due to certain biochemical, morphological and physiological adjustments under prolonged exposure of plants to high CO_2. However, certain species showed little or no acclimation of photosynthesis even when they were grown for the long term under CO_2 enrichment and had excess levels of carbohydrates. These species accumulated a greater amount of carbohydrates in the leaves, especially as starch in chloroplast without influencing their photosynthetic capacity.

Higher rate of photosynthesis, due to exposure to elevated CO_2 has been reported in various legume crop species like soybean (Vu *et al.,* 2001), dry bean (Prasada *et al.,* 2002) and peanut (Clifford *et al.,* 2000). At elevated CO_2 conditions, it was observed that photosynthesis increased with increasing temperature from about 28/22 to 40/30 °C but at ambient CO_2 it decreased above 32/22°C. This suggests greater and wider temperature optima for photosynthesis of plants grown at elevated CO_2. Long *et al.* (1991) showed that temperature optimum for light-saturated photosynthesis increased by 5°C with an increase in atmospheric CO_2 from 350 to 650 μmol mol^{-1}. Elevated CO_2 brought about a significant increase in the rate of photosynthesis in mung bean varieties at pre-flowering, flowering and post-flowering stages (Haque *et al.*, 2011).

Water use efficiency

Plant water use efficiency is strongly affected by stomatal density (Woodward and Kelly, 1995). Reduced stomatal opening under high CO_2 conditions leads to improved water use efficiency (Guy and Reid 1986; Clifford *et al.,* 2000).

Tardieu and Simonneau (1988) indicated that stomatal behavior remained regulated by the concentration of abscisic acid (ABA) under stress conditions. When water loss by transpiration cannot be compensated by the water absorption, a hormonal

imbalance was produced, increasing the proportion of abscisic acid. This resulted in the potassium concentration in guard cells to decrease and they lose turgor and stomata was partially closed. Partial closure of stomata has been reported by Garcia- Brugger *et al.* (2006) in tomato, when the proportion of ABA was greater than that of cytokinins and resulted in increased water use efficiency.

Elevated CO_2 resulted in higher water use efficiency in peach (*Prunus persica*) seedlings (Centritto *et al.*, 2002). Water use efficiency was found to be higher in *Brassica juncea* plants when exposed to elevated CO_2 concentration of about 600 ± 20 µmol mol^{-1}(Rabha and Uprety, 1998).

Chlorophyll fluorescence

The Chlorophyll fluorescence (Fv/Fm) is a parameter that allows the detection of any damage to PSII and possible photoinhibition (Ahmed *et al.*, 1992). Stress in plants decreased the capacity and efficiency of photosynthesis through changes in gas exchange, pigment composition, chloroplast development and declined chlorophyll fluorescence (Farooq *et al.*, 2008). Changes in the proportion of photochemical and energy-dependent quenching lead to alteration of fluorescence kinetics under drought stress (Zlatev and Yordanov, 2004). Chlorophyll fluorescence emitted from the chloroplast thylakoid membrane is often used as a very sensitive intrinsic indicator of the photosynthetic reaction in photosystem II (Ahmed *et al.*, 1992). Analysis of chlorophyll fluorescence and measurement of the Fv/Fm ratio can be useful in determining damage to light reaction systems in photosynthetic mechanisms under any stress.

Pan *et al.* (2018) observed in tomato that even though heat stress decreased the efficiency of photosystem II by reduction in Fv/Fm value, under elevated CO_2 condition this was compensated and in effect an improvement in functioning of photosystem II (Fv/Fm) was noticed. Roden and Ball (1996) observed that there was a reduction in Fv/Fm ratio under elevated CO_2 conditions in Eucalyptus sp. This

reduction in Fv/Fm was correlated with the increased levels of non-structural carbohydrates.

Lin *et al.*, (2013) in an experiment on oak tress concluded that photochemical efficiency of photosystem II increased with in increasing rate of nitrogen application.

Relative Water content

Relative water content is a useful indicator of the state of the water balance of *Araucacia* sp. (Yamasaki and Dilenberg, 1999). It is considered as a measure of plant water status, reflecting the metabolic activity in tissues and is used as a most meaningful parameter for dehydration tolerance studies. Leaf water status is intimately related to several leaf physiological variables, such as leaf turgor, growth, stomatal conductance, transpiration, photosynthesis, and respiration (Kramer and Boyer, 1995).

Elevated CO_2 can lead to an increase in plant water potential and a delay in the onset of water stress, thus improving growth. It has been reported that elevated CO_2 increased biomass production in peach (*Prunus persica*) seedlings by 31% (Centritto *et al.*, 2002) and resulted in higher integrated water use efficiency under water-stressed conditions. No significant difference was reported in relative water content when alfalfa plants were grown under CO_2 enrichment (700 μmol mol^{-1}) and associated high temperature.

Salehpour *et al.* (2009) reported an increase in the RWC by the application of extra nitrogen in lentils. Rostami and Movahedi (2016) reviewed an increased root growth by application of NAA as foliar spray in *Valeriana officinalis*. This in turn increased the RWC of the plants.

Superoxide dismutase

Plant cells involve complex antioxidant defense mechanisms against oxidative stress generated under challenging stress conditions (Matsuura and Fett-Neto, 2013).

There are mainly non-enzymatic and enzymatic antioxidants that help plants cope up with the stress conditions by quenching the reactive oxygen species (ROS). Non-enzymatic antioxidants include vitamin C, vitamin E, glutathione, flavonoids, alkaloids, carotenoids, etc. Enzymatic antioxidants include catalase, superoxide dismutase peroxidase and metallothionein (Seki *et al.*, 2001).

A study by Pritchard *et al.* (2000) showed that the additional CO_2 fixed in an enriched CO_2 condition was invested in the making of additional antioxidants. Lin and Wang (2002) observed that activities of superoxide dismutase and catalase were much higher in CO_2 enriched wheat than in ambiently grown wheat.

However, in some other cases, a reduction in antioxidants during exposure to elevated CO_2 conditions was observed by many researchers. Three months' exposure to an elevated CO_2 concentration of 720μL L–1 in OTC reduced the activities of superoxide dismutase and catalase by an average of 23% and 39% respectively in soybean (Pritchard *et al.*, 2000). Polle *et al.* (1997) reported that two years of atmospheric CO_2 enrichment reduced the activities of several key anti-oxidative enzymes including catalase and superoxide dismutase in beech seedlings.

In a two-year field experiment under elevated CO_2 (336μmol mol-1) in OTC, a decreased activity of SOD was observed in the leaves of soybean (Booker and Fiscus, 2005). Lin and Wang (2002) reported that the activity of SOD declined significantly after temperature stress for 10 days, in two spring wheat cultivars (*Triticum aestivum* L, Longchun 292 and Longchun 8139), regardless of ambient or doubled CO_2.

Peroxidase

The results of Yusake *et al* (2007) suggested that CO_2 enrichment reduced the oxidative stress caused by reactive oxygen species to *P. subcordiformis*, and reduced the lipid peroxidation. UV-B and CO_2 in combination significantly decreased MDA content compared to UV-B alone. MDA is a product of lipid peroxidation and thus indicates a decreased lipid peroxidation under higher CO_2. Doubling of the present atmospheric CO_2 partial pressure altered the CO_2/O_2 ratio at the Rubisco fixation site

and caused a 50% decrease in the ratio of photorespiration. Therefore, the decrease in lipid peroxidation of *P. subcordiformis* at elevated CO_2 was expected to be due to the favor carboxylation reaction Rubisco, limited photorespiration and less photo-reduction of dioxygen. In this case, few electrons were transported to dioxygen during photosynthesis and reduced the damage potential of active oxygen to the membrane system.

Chlorophyll stability index

The Chlorophyll stability index (CSI) indicates how well chlorophyll performs under stress conditions. The leaf chlorophyll content is a good indicator of photosynthesis and chlorophyll stability index (CSI) is an indicator of stress tolerance capacity of plants. A higher CSI means that the stress experienced by that plant did not have much impact on the chlorophyll content of that plant and that it can withstand stress through better availability of chlorophyll leading to increased photosynthetic rate and higher productivity (Mohan *et al.*, 2000). Terzi and Kadioglu (2006) reported that in *Ctenanthe setose* (Rosc.) CSI decreased during the early stages of drought stress but increased in later stages, approaching the control levels.

Proline content

Proline is a versatile metabolite playing a role in the protection of plants against environmental stresses. The amount of proline produced by a plant is an indication of the stress condition experienced by the plant. The stress may be due to changing climate conditions, including drought stress or elevated temperature and CO_2 or a combination of all these.

Predicting the effect of future climate conditions on proline metabolism is complicated by the multitude of environmental variables involved. Drought and UV radiation increased proline content in durum wheat, but not in the presence of elevated CO_2 (Balouchi *et al.*, 2009). Because of the effects of combined perturbations of different environmental factors that are not always additive, it is necessary to

investigate their interaction and combined impact to understand the effect of future climate scenarios (Miller *et al.*, 2009). The extent of proline accumulation also varied among plant species (Reddy *et al.*, 2004; Kishor *et al.*, 2005). As per the study conducted by El Gawad *et al.* (2015) in temperate grassland species, drought and elevated temperature generally caused a pronounced increase in proline content and concomitant decrease in its immediate precursor P5C. In most cases, elevated CO_2 reduced the stress-induced proline increase.

Effect of growth regulator, nutrient application and temperature induction response technique on physiological and biochemical parameters of tomato under CO_2 enrichment.

Plant growth regulators and nutrient application find application during various crop growth stages and influence the physiological and biochemical parameters on the plant under elevated CO_2 condition. This is because nutrient limitation may be exacerbated by CO_2 enrichment and restrained carbon sequestration (Woodward, 2002). Manzoor *et al.* (2015) reported a reduction in chlorophyll content in maize under stress conditions. He also reported that 5 mM foliar spray of SA increased the chlorophyll content significantly under the stress condition. It was also reported that in mustard plants the total chlorophyll content increased with the application of 0.5 mM of SA (Nazar *et al.*, 2015).

Javanmardi and Rasuli (2017) reported that 2000mg/l zinc sulfate resulted in higher tuber crude protein content of 8.37 % tuber dry weight which was over twice that of control treatment.

Ghasemzadeh *et al.* (2012) in his work in ginger reported that application of SA under elevated CO_2 resulted in improvement in the photosynthetic capacity, antioxidant activity and flavonoid production compared to control plants without SA application. Chandra *et al.* (2011) concluded that the exogenous application of SA increased the amount of total soluble protein of cowpea plants. Salicylic acid is a

regulator of photosynthesis as it affects chloroplast and leaf structure, stomatal closure, chlorophyll and carotenoid contents (Mateo *et al.*, 2006). In mustard, the application of SA has been found to increase the photosynthetic net CO_2 assimilation (Zhang *et al.*, 2007). Reports show that exogenous application of 1.5 mM Salicylic acid resulted in a significant increase in the SOD content at the post-anthesis stage of chickpea under drought stress (Patel *et al.*, 2005). Habibi (2012) reported a significant increase in SOD activity in barley when it was sprayed with 500 µM SA. Similar reports were given by Hosseini *et al.* (2015) in Lolium grass where an increase in SOD activity was initiated by application of 0.75 and 1.5 mM concentration of SA. Several studies have shown that low concentration of SA may increase the activation of the antioxidants (Farooq *et al.,* 2008) but high concentration may result in negative results or susceptibility to the stress condition (O'Hara *et al.*, 2012). Many scientists have reported that with the application of SA, there was a reduction in the MDA content in plants which denotes a corresponding increase in the peroxidase content and hence more tolerance than normal conditions (Kang and Saltveit, 2002 in cucumber; Tasgin *et al.*, 2006 in wheat). Kabir *et al.* (2014) reported a reduction in the levels of lipid peroxidation and more profound growth processes in plants applied with SA compared to non-stressed tomato plants.

Hemantaranjan *et al.* (2014) conducted an experiment on the effect of exogenous application of SA on chickpea and concluded that the highest proline content was observed in plants treated with 1.5 mM SA. Afshari *et al.* (2013), in an experiment in cowpea reported that 300 micro M SA resulted in highest proline concentration under water stress.

Xu-Cheng *et al.* (2011) reported that no significant impact was noted on the chlorophyll fluorescence of wheat under elevated CO_2 condition. Nwadukwe and Chude (1994) concluded from their experiment that water use efficiency and yield of tomato plants were higher for those with higher nitrogen treatments. Chatterjee and Mallick (2008) concluded from their work that water use efficiency increased

substantially with an increase in the amount of nitrogen applied to tomato plants. According to Chatterjee and Mallick (2004) application of nitrogen had a positive impact on the transpiration rate of tomato plants. The least rate of transpiration was observed in plants treated with the least amount of fertilizers. Similarly, some micronutrients like Zinc, Iron, Manganese, Copper, Boron, and Magnesium have an important role in the physiology of tomato crop and are required for plant activities such as transpiration, meristematic development, chlorophyll formation, photosynthesis, gossypol, tannin and phenolic compounds development. They help in stress alleviation under enriched CO_2 condition.

Thermo-tolerant genotype *Gossypium hirsutum* (H-28), identified by the TIR technique, showed increased cell viability and protein synthesis capacity during alleviation from high-temperature stress (Kheir *et al.*, 2012). A higher chlorophyll stability index and a strong anti-oxidant enzyme system with lesser lipid peroxidation was reported in rice landraces like Norungan when subjected to a gradual temperature increase from 38-48°C for 3 hrs (induction temperature), immediately followed by lethal or challenging temperature at 54 °C for 3 hours (Vijayalakshmi *et al.*, 2015). According to Chandola (2016), the lethal temperature for tomato was found to be 48 °C for 2 hrs, and the induction temperature range was found to be 38 °C to 46 °C.

Effect of CO_2 enrichment on observations related to the flowering of tomato.

Flowering is a crucial determinant for plant reproductive success and seed-set. Increasing temperature and elevated carbon-dioxide are key climate change factors that could affect plant fitness and flowering related events. Addressing the effect of these environmental factors on flowering events such as time of anthesis (TOA) and flowering time (duration from germination till flowering) is critical to understand the adaptation of plants/crops to change climate

Elevated CO_2 and associated high temperature advanced flowering time in certain crops. Days to first flowering was delayed in tomato under low light conditions

compared to open field, as more leaves were initiated before the inflorescence emergence (Ho., 1996). The temperature also affects floral initiation, floral development, fruit set, and fruit growth. Elevated CO_2 advanced the time of flowering and maturity of the mungbean genotypes (Haque *et al.*, 2011). Rusterholz and Erhardt (1998) reported that elevated CO_2 enhanced early flowering and flower bud development in *C. jacea* and *B. officinalis* and produced more flowers under elevated CO_2.

Pollen viability

Elevated [CO_2] (720 µmol mol^{-1}) increased stand-level ragweed-pollen production by 61% (Wayne *et al.*, 2002), but did not affect peanut pollen viability (Prasad *et al.*, 2003). By contrast, high temperature reduced pollen viability in peanut (Prasad *et al.*, 2003). According to Koti *et al.* (2004), when +T and +CO_2 acted together, the combined effect on pollen number and pollen germination was additive, being higher than the single stress effects. This is in accordance with the studies of Prasad *et al.* (2002, 2003), where they showed that elevated [CO_2] did not counteract the negative effects of high temperature on pollen production and viability. Suzuki *et al.* (2001) reported that the CO_2 enrichment and associated high temperature had a negative effect on the pollen viability of green bean (*Phaseolus vulgaris* L.) which could be due to degeneration of tapetum layer.

The findings of Harsant *et al.* (2013) also confirms that increased temperature resulted in declined pollen viability in the C3 model grass Brachypodium distachyon. Pollen viability was found to be the least (8.37 % decrease) under elevated CO_2 conditions compared to control conditions in pea plants (Meena *et al.*, 2016).

Pollen morphology

Meena *et al.* (2016) reported that high-temperature stress due to elevated CO_2 affected the pollen development. Pollen produced by flowers in soybean grown under

elevated CO_2 conditions appeared shriveled without apertures and with disturbed exine ornamentation (Koti *et al.*, 2004).

In soybean, 20% more pollen was found to be produced by plants grown at e[CO_2]. Genotypes varied significantly in their response to elevated [CO_2] for pollen production. Elevated CO_2 either increased (7% and 20% in DG 5630RR and PI 471938, respectively), decreased (15% and 17% in D 88-5320 and DP 4933RR, respectively) or had no effect (D 90-9216 and Stalwart III) on the pollen number and morphology. Pollen produced in plants grown under +CO_2 treatment, when germinated, had longer pollen tubes than that of the control pollen. CO_2 conditions produced smaller flowers and fewer pollen grains per flower together with lower pollen germination (Koti *et al.*, 2004). Pollen sterility and pollen production at high temperatures was also associated with early degeneration of the tapetal layer of pollen (Porch and Jahn, 2001).

Effect of growth regulator, nutrient application and temperature induction response technique on observations related to flowering in tomato under CO_2 enrichment

Warade and Singh (1977) reported that planofix (NAA 4.5 %) prevented flower drop and increased fruit set in chillies . He also reported that, 200 ppm Planofix sprayed during the bloom stage increased the fruit set to 70.5 %. Kailash (2013) reported that 45 ppm NAA, when sprayed to cowpea plants, resulted in more days to first flowering. Rehman *et al.* (1995) studied the effect of different doses of NPK on cucumber and concluded that 140 N than recommended dose resulted in significant delay in days to flowering, fruit setting and maturity.

According to Trilok and Prasad, 2017, boron application has resulted in early days to flower formation, more number of flowers per plant, number of flowers per inflorescences, number of inflorescences per plant, days taken for formation of fruit per plant, higher yield per plant and fruit yield (t/ha) in tomato.

As the concentration of CO_2 increases the temperature automatically rises. According to Kaur *et al.* (2017), high temperature coinciding with reproductive phase in *Capsicum annum* was most detrimental to its yield and productivity. Floral abscission under high temperature is associated with increased biosynthesis of ethylene and ABA with a decline in auxin. Elevated concentration of atmospheric CO_2 can lead to a progressive limitation of nutrients that can quickly limit the initial increase in plant production (Newton *et al.*, 2010). Stimulation of plant growth under elevated CO_2 depends heavily on the availability of non-carbon resources (Mc Carthy *et al.*, 2010).

The significant effect of NAA in preventing flower drop by suppressing the formation of the abscission layer was studied in legumes by Subhaiah and Chamy (1984). Sharma and Dey (1986) opinioned that the foliar application of NAA at flower initiation stage reduced the shedding of flowers by 56 percent. Foliar spray of 25 ppm NAA recorded significantly higher seed yield by 21to 22 percent than control through increased flower production, clusters per plant, pod setting percentage and pods per plant in mung bean (Patil *et al.,* 2005).

The application of zinc and boron each at 100 ppm caused early flowering as well as showed maximum number of flowers and fruit yield. Thus, the study indicated that the application of boron and zinc either solely or in combination was quite beneficial for vegetative growth, flowering, and fruiting as well as quality improvement of tomato fruits (Sati *et al.*, 2017).

However, no much reports have been there about the interactive effect of the various nutrient application and growth regulators on flowering and yield parameters of various crops under elevated CO_2.

Effect of CO_2 enrichment on yield parameters in tomato.

The beneficial effects of CO_2 enrichment on yield and quality of crops including tomato have been extensively reported by numerous studies (Islam *et al.*,

1996 in tomato; Bindi *et al.*, 2001 in grapes; Högy and Fangmeier, 2009 in potato). An increase in RUBISCO content and its activity resulted in enhanced leaf photosynthesis, which probably lead to accumulated concentration of carbon-based compounds in response to e[CO_2] environment due to the source–sink balance hypothesis (Patil *et al.*, 2005). In an experiment on the combined effects of CO_2 and temperature on the grain yield, Hemantaranjan *et al.* (2014) observed that a temperature of 27 °C or higher applied mid-way through anthesis could result in a high number of sterile grains and resulted in considerable yield losses. Increased number of flowers and fruits together with higher fruit set led to higher fruit yield in tomato was observed at elevated conditions of CO_2 concentrations (700ppm and 550 ppm) compared to control conditions, the highest yield being obtained under 700ppm of CO_2 (Mamata *et al.*, 2014).

CO_2 plays a crucial role in the physiology of plants by affecting the leaf photosynthesis, plant growth, and crop yield. Previous studies have concluded that plant photosynthesis, stomatal aperture, biomass production, yield, and water use efficiency was modulated by elevated CO_2 environment (Ainsworth and Long, 2005; Pazzagli *et al.*, 2016). For instance, more carbohydrates could be transferred into fruits due to the increased photosynthesis in plants grown under CO_2 enriched environment, which could enhance yield and increase the concentrations of starch, sugars, ascorbic acid, etc resulting in quality improvement also (Islam *et al.*, 1996)

Wei *et al.* (2013) reviewed that tomato plants grown under elevated CO_2 possessed greater fruit yield than those grown under ambient CO_2 treatment. This was consistent with the findings by Domis *et al.* (2002); Ainsworth and Long (2005), and Pazzagli *et al.* (2016), where e[CO_2] environment enhanced the yield or biomass production of the crop. The reduction in oxygenase activity of Rubisco at e[CO_2] plants lead to decreased photorespiration rate and in turn increased net photosynthesis (Ainsworth and Long, 2005)

Effect of growth regulator, nutrient application and temperature induction response technique on yield parameters in tomato under CO_2 enrichment

Ghosh *et al.* (2009) recorded that the application of NAA at 50 % flowering of guava and at a subsequent interval of 21 days resulted in highest fruit set, fruit retention and fruit yield per plant than other treatments. Dubey *et al.* (2003) studied the effect of various concentrations of NAA (125, 250 and 750 mg/l) applied at the full bloom stage on the yield of guava. NAA at 250 mg/l gave the highest yield with quality fruits.

Singh and Agarwal (2015) studied the effect of external application of growth regulators and nutrients in ber and he found out that 10 mg/l NAA at full bloom stage increased the fruit set, fruit weight, fruit size, and yield.

Sati *et al.* (2017) experimented the effect of foliar application of zinc on the yield and quality of potato. He concluded that there was a significant increase in the tuber per plant and tuber yield per hectare for those plants sprayed with 15 ppm zinc. According to Patil *et al.* (2008), the application of boric acid at 100 ppm resulted in maximum fruit yield and total yield per plant. Salam *et al.* (2010) reported that Boron and zinc played an important role in the quality parameters of tomato. His result confirmed that the application of zinc and boron improved the lycopene content of the tomato fruits. Besides the main effect, the application of additional NPK was also found to have a positive influence on the total lycopene content.

Ekinci *et al.*, (2012) reported a significant increase in the yield of tomato with the application of SA. Number of berries significantly increased in pepper (Hayat et al., 2010) and cucumber (Elvwan and Hamahyomy, 2009) as a result of foliar application of SA. Javaheri *et al.* (2012) reported that there was a significant increase in vitamin C and lycopene of tomato fruits along with improvement in the number of fruits with application of exogenous SA.

Tabassum *et al.* (1995) studied the effect of different doses of NPK on cucumber and concluded that 140% N than recommended dose resulted in significantly

higher vine length, number of fruits, increased average fruit weight and total yield. Mahapatra *et al.*, (1996) studied the effect of different levels of nitrogen on pointed gourd in a field study and reported increased fruit length, average fruit weight and yield in treatment with maximum N. Agarwal and Verma. (1970) reported that application of 90 Kg N/ha in okra brought significant improvement in the number of fruits per plant and fruit yield over lower levels of nitrogen. Chattopadhyay and Sahana (2000) studied the effect of five levels of nitrogen and four levels of phosphorus on the quality and yield of okra. The result showed that as the amount of nitrogen increased, the quality also improved. Mishra *et al.* (2000) reported improved fruit yield (87 t ha^{-1}) and dry matter production in plants supplied with 150 % of the recommended dose of nitrogen compared to normal nitrogen supplied plants in tomato. Singadhupe *et al.* (2003) found out that 120 kg ha^{-1} nitrogen produced higher yield compared to control plants with normal recommended nitrogen.

MATERIALS AND METHOD

3. MATERIALS AND METHOD

The experiment entitled 'Influence of CO_2 enrichment and associated high temperature on reproductive physiology of tomato (*Solanum lycopersicum* L.)' was undertaken with the main objective to study the effect of CO_2 enrichment and associated high temperature on flowering and fruiting in tomato and their improvement through growth regulators and nutrient applications and through temperature induction response technique.

The programme was divided into two experiments. Experiment 1 consisted of standardization of temperature induction response technique (TIRT) of two varieties namely Vellayani Vijay and Anagha and their evaluation. The second experiment consisted of CO_2 enrichment studies. This was to evaluate the impact of growth regulators and nutrient applications on reproductive physiology of tomato under elevated CO_2 condition.

EXPERIMENT DETAILS

Experiment 1

Part 1: Standardization of Temperature Induction Response Technique (TIRT) for Tomato varieties Vellayani Vijay and Anagha.

This experiment was aimed at standardizing the lethal temperature and the induction temperature for tomato varieties Vellayani vijay and Anagha. This was done using the incubator facility at the Department of Agricultural Entomology. Initially the lethal temperature for both the varieties were found out. For this a set of five day old tomato seedlings were subjected to different temperatures (between 40-50 °C) for different durations (1h-2h). The temperature treatment that resulted in 95% mortality or above was selected as lethal temperature.

The second part consisted of standardization of the temperature induction temperature for tomato. For this, a set of five day old tomato seedlings were subjected to different temperatures (between 35-46 °C) for different durations (1h – 2 h) and then were exposed to the lethal temperature. The temperature treatments and their durations

in which maximum recovery was observed at recovery temperature after exposing to lethal temperature were selected as the induction temperatures range.

The performance of induced seedlings of Vellayani Vijay under elevated CO_2 was evaluated in the second part of the experiment.

Part 2: Evaluation of Temperature Induced seedlings

The temperature induced seedlings were planted in pots. After establishment, one set was kept under elevated CO_2 conditions (500 ppm) in Open Top Chamber and the other was kept in open field as control.

Season: The experiments were conducted from October 2018 to January 2019.

Layout of the Experiment and Treatments:

The experiment consisted of two treatments with 4 replications, laid out in CRD under two conditions (OTC and Control).

T1: With induction

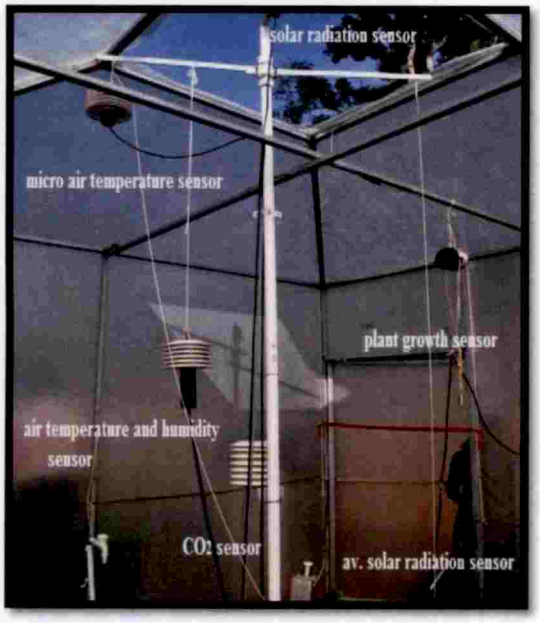
T2: Without induction

Conditions for experiment:

1. OTC with elevated CO2 concentration (500ppm).
2. Open condition

Open Top Chamber for CO_2 enrichment

Technology used for creating CO_2 enriched environment was Open Top Chambers (OTC). Open Top Chambers (OTC) were square type chambers constructed to maintain near natural conditions and elevated CO_2 conditions for experimental purposes. The basic structure of OTC was built of metal frame and installed in the experimental field. OTCs were covered with a 200 micron UV poly sheet .The chamber was constructed with 3 x 3 x 3 dimension, 450slope and $1m^2$ opening at the top. Two such chambers were built in the experimental field; one serves to impose CO_2 enrichment and the other serves as control chamber to study the chamber effects. Elevated CO_2 was released into the chamber from a CO_2 cylinder in a controlled manner. Measurements of microclimatic parameters (temperature, humidity and light)



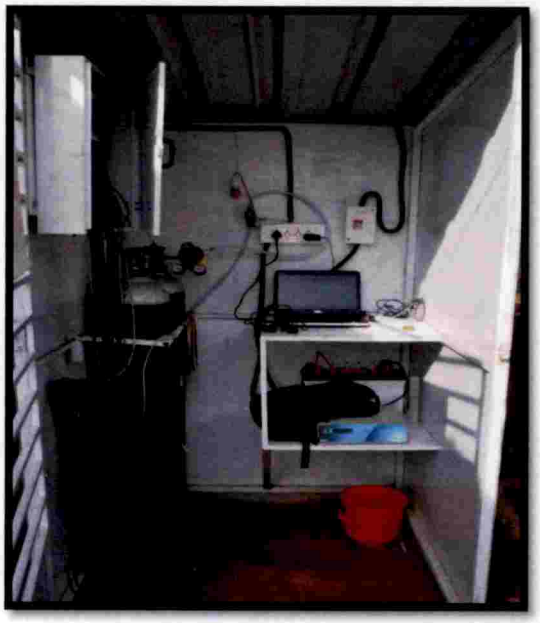


Plate 1. Open Top Chamber for CO_2 enrichment

were done within and outside the OTCs with the help of sensors on a real time basis. On an average basis, mean temperature of 46.15°C relative humidity of 65.96% and solar radiation of 384.65µ Enst. were recorded inside the chambers during the experimental period. Potted plants were kept within these chambers for a period of three months and observations were taken. The elevated CO_2 concentration of 500 ppm was selected by referring IPCC (2007) which suggested that atmospheric concentrations of carbon dioxide has been steadily rising with an average annual increase rate of about 2 ppm and continued to rise to 500 - 1000 ppm by the year 2100.

Preparation and planting

The experiment was conducted in pots filled with potting mixture containing farm yard manure, sand and soil in the ratio of 1:1:1. The temperature induced seedlings of both the varieties were transplanted into these pots. Aftercare and management was done as per package of practices recommendations (KAU 2016) and observations related to growth, physiological and biochemical parameters, and observations related to flowering and yield parameters were recorded. Among these, observations related to growth, physiological and biochemical parameters were recorded once, at 60 DAS.

OBSERVATIONS

Growth Parameters

1. Plant height (cm)

The height of the treatment plants were measured at flowering stage using scale in centimeters

2. No: of branches

Number of primary branches per plant was counted at flowering stage and noted.

3. No: of leaves

Average leaves per branch was counted and recorded.

4. Specific leaf area (cm^2g^{-1})

From each plant, fully expanded third leaf (from main stem apex) was collected. Leaflets were separated, petioles were discarded and area was measured. Leaflets were dried at 800°C for 2 days and the dry weight was taken. SLA was calculated using the formula:

$$\text{SLA } (cm^2\ g^{-1}) = \frac{\text{Leaf area}}{\text{Dry weight}}$$

(b) Physiological and Biochemical parameters

The physiological and biochemical parameters were observed twice (60 DAS and 75 DAS) during the entire crop period.

1. Total chlorophyll (mg g^{-1} fresh weight)

Chlorophyll content of leaf samples were estimated as per the procedure described by Hiscox and Israelstam (1979). A weighed quantity of leaf sample (0.5g) was taken from third fully expanded leaf and it was cut into small bits. These bits were put into test tubes and incubated overnight at room temperature with 10 ml DMSO: 80% acetone mixture (1:1 v/v). The coloured solution was transferred into a measuring cylinder and made up to 25 ml with the DMSO-acetone mixture. The absorbance was measured at 663, 645, 480 and 510nm. The chlorophyll content was measured by substituting the absorbance values in the given formula:

$$TotalChl(a+b) = (8.02 \times A_{663} - 20.2 \times A_{645}) \times \frac{V}{1000} \times \frac{1}{freshweight}$$

2. Total soluble protein (mg g^{-1})

The total soluble protein of leaf samples were estimated using simple protein dye binding assay of Bradford (1976) using bovine serum albumin (BSA) as the standard. One hundred milligram of CBB 250 was dissolved in 50 ml of 95% ethanol. To this 100 ml of 85% (w/v) ortho-phosphoric acid was added. The resulting solution was diluted to a final volume of 200 ml with distilled water. 0.1g of leaf samples was

taken from third fully opened leaves and was ground to a thin paste and soluble protein was extracted with 10 ml of phosphate buffer (pH 7.8).

The extract was centrifuged at 5000 rpm for 10 minutes. To the 20µl of the supernatant a known volume (5 ml) of diluted dye binding solution was added. The solution was mixed well and allowed to develop a blue colour for at least 5 min but no longer than 30 min and the absorbance was measured at 596 nm. The protein content was calculated using the BSA standard in the range of (10-100µg). The protein content was expressed as mg g^{-1} fresh weight.

3. Total carbohydrate (mg g^{-1})

The total carbohydrate of leaf samples were estimated using Anthrone method. Carbohydrates was first hydrolyzed into simple sugars using dilute hydrochloric acid. Glucose was dehydrated to hydromethyl furfuralcin hot acid medium. A green compound was formed whose absorbance was measured at 630nm.

0.1 g of leaf samples each was taken in a boiling tube and was hydrolyzed using 5 ml of 2.5 N HCl by keeping in a boiling water bath for 3 hours. This was neutralized with solid sodium carbonate until effervescence ceased. The volume was 100 mL and centrifuged. 0.5 mL of supernatant was collected for analysis. Standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1mL. 0 served as the blank. All samples were made upto 1 mL in all tubes (samples and standard) by adding distilled water. 4 mL anthrone reagent was added and heated for 8 minutes in boiling water bath. It was cooled rapidly and the green colour was read at 630 nm. The standard graph was plotted by plotting concentration of standards on X axis and absorbance on Y axis. The amount of carbohydrate of sample was calculated from the graph.

$$\text{Amount of carbohydrate present in 100 mg of sample} = \frac{\text{mg of glucose}}{\text{volume of test}} \times 100$$

4. Transpiration rate (mmole H_2O m^{-2} sec^{-1})

Transpiration rate was measured using portable photosynthetic system (CIRAS-3 SW) available in Department of Plant Physiology, College of Agriculture, Vellayani.

5. Photosynthetic rate (µmol CO_2 m^{-2} sec^{-1})

Photosynthetic Rate was measured using portable photosynthetic system (CIRAS-3 SW) available in Department of Plant Physiology, College of Agriculture, Vellayani.

6. Water use efficiency (mmol CO_2 mol^{-1} H_2O)

Water use efficiency was measured using portable photosynthetic system (CIRAS-3 SW) available in Department of Plant Physiology, College of Agriculture, Vellayani.

7. Chlorophyll fluorescence (Fv/Fm)

Chlorophyll fluorescence was measured using portable photosynthetic system (CIRAS-3 SW) available in Department of Plant Physiology, College of Agriculture, Vellayani.

8. Relative water content (%)

Relative water content was estimated as per Barr and Weatherly (1962) by measuring the fresh weight, turgid weight and dry weight of known number of leaf discs from the experimental plants. After measuring the fresh weight of the sample, it was submerged in distilled water for 3 hours and then the turgid weight was taken. The dry weight of the sample was measured after keeping the samples in oven at 80°C for 3 consecutive days. The RWC was calculated using the following formula:

$$RWC = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

9. Super Oxide Dismutase (µmol min^{-1} mg $protein^{-1}$)

Superoxide dismutase (SOD) activity was quantified following the method described by Kakkar *et al.* (1984). Leaf samples (0.5g) from third fully opened leaves were ground with 3.0 ml of potassium phosphate buffer, centrifuged at 2000rpm for 10 minutes and the supernatants were used for the assay. The assay mixture contained 1.2ml of sodium pyrophosphate buffer, 0.1ml of PMS, 0.3ml of NBT, 0.2 ml of the enzyme preparation and water in a total volume of 2.8 ml. The reaction was initiated by the addition of 0.2ml of NADH.

The mixture was incubated at 30°C for 90 second and arrested by the addition of 1.0ml of glacial acetic acid. The reaction mixture was then shaken with 4.0ml of n-butanol, allowed to stand for 10 minute and centrifuged. The intensity of the chromogen in the butanol layer was measured at 560 nm. One unit of enzyme activity is defined as the amount of enzyme that gave 50% inhibition of NBT reduction in one minute.

10. Lipid peroxidation (mmol g^{-1})

The peroxidase activity in plants was estimated following the method described by Reddy *et al.* (1995). Leaf sample of 200 mg was homogenised in 1 ml of 0.1 M phosphate buffer (pH 6.5) and centrifuged at 5000 rpm for 15 minute at 40C. To 3.0ml of pyrogallol solution, 0.1ml of the enzyme extract was added and adjusted to read zero at 430 nm. The enzyme reaction was started by adding 0.5 ml of one percent hydrogen peroxide (H_2O_2) into sample cuvettes and change in absorbance was measured every 30 second up to 3 minute. One unit of peroxidase is defined as the change in absorbance/minute at 430nm.

11. Chlorophyll Stability Index (%)

Chlorophyll stability index shows the stability of chlorophyll molecule under stress condition. This is calculated using the formula:

$$CSI = \frac{\text{Total chlorophyll content of (treatment – control)}}{\text{Total chlorophyll content of control}} \times 100$$

12. Proline content (μM g^{-1} tissue)

Prolines are precipitated as a protein-sulphosalicylic acid complex during extraction of tissue with sulphosalicylic acid. The extracted proline is made to react with ninhydrin under acidic conditions to form a red colour which is measured colorimetrically at 520nm.

0.5 g of tissue was homogenized in a pestle and mortar with 10 ml of 3% aqueous sulphosalicylic acid and filtered through Whatman No. 2 filter paper. Extraction and pooling the filtrate was repeated. To 2 ml of filtrate, 2 ml each of glacial acetic acid and ninhydrin was added and mixed. It was then kept in boiling water bath for 1 h and then the reaction was terminated by placing on ice bath. 4 ml of toluene was added and mixed vigorously for 20-30 sec. The chromophore (toluene) layer was aspirated and warmed to room temperature. The absorbance of red colour was measured at 520 nm against a reagnet blank. The amount of proline in the sample was calculated using a standard curve prepared from pure proline (range 0.1-36 μ mole) and expressed on fresh weight basis of sample.

$$\mu M \text{ of proline/ g tissue} = \frac{(\mu g \text{ proline/ ml}) \times \text{ml toluene}}{115.5} \times \frac{5}{\text{g sample}}$$

EXPERIMENT 2

CO_2 enrichment studies: Impact of application of growth regulators and nutrient applications on reproductive physiology of tomato var. Vellayani Vijay under elevated CO_2 condition.

Pot culture experiment was conducted with tomato var. Vellayani Vijay at Department of Plant Physiology, College of Agriculture, Vellayani. The Open Top Chamber (OTC) technique, as described in the previous experiment was used for this experiment. One month old tomato seedlings were transplanted into pots. One set of established seedlings were kept as control plants under open condition and the other

set was kept under CO_2 enrichment condition. Plants were maintained under well irrigated conditions

Location:

The field experiment was conducted in Open Top Chambers located at College of Agriculture Vellayani, situated at 8°5'N latitude and 7609'E longitude and an altitude of 29 m above mean sea level.

Season:

The experiments were conducted from October 2018 to January 2019

Planting Material:

One month old tomato plants of variety Vellayani Vijay was transplanted into pots and used for the study. The planting material was procured from Department of Vegetable Science, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala.

Layout of the Experiment

The experiment was laid out in CRD with eight treatments with three replications under two conditions (OTC and Control).

Treatments

T1: 50 ppm NAA

T2: 50 ppm salicylic acid

T3: 50 ppm Boron

T4: 50 ppm Boron + 50 ppm Zinc

T5: POP 125% N: 100% P: 100%K

T6: POP 125% N: 125% P: 125%K

T7: Control (water spray)

T8: Absolute control

Application of treatments

Treatments T1 to T4 were given as foliar spray at 40,55 and 70 DAS. The additional nutrients were given as equal splits along with the normal recommendation for tomato (Package of Practices Recommendations, 2016)

Conditions:

1. OTC with elevated CO2 concentration (500ppm)
2. Open condition

Preparation and Planting

The experiment was conducted in pots filled with potting mixture containing farm yard manure, sand and soil in the ratio of 1:1:1. The seedlings were transplanted at 1 month old stage. The experiment was laid out in CRD. The potted plants were kept in OTC and in open conditions (control) during the experimental period. Aftercare and management was done as per package of practices recommendations (KAU 2016).

OBSERVATIONS

(a) Growth parameters

1. Plant height (cm)
2. No: of branches.
3. No: of leaves
4. Specific leaf area (cm^2g^{-1})

All the above observations related to growth parameters were recorded twice at 60 and 75 DAS. The materials and method used for all the estimation was as described earlier in experiment 1.

(b) Physiological and biochemical parameters

1. Total chlorophyll (mg g^{-1} fresh weight)
2. Total soluble protein (mg g^{-1})
3. Total carbohydrate (mg g^{-1})
4. Transpiration rate (mmole H_2O m^{-2} sec^{-1})
5. Photosynthetic rate (µmol CO_2 m^{-2} sec^{-1})
6. Water use efficiency (mmol CO_2 mol^{-1} H_2O)
7. Chlorophyll fluorescence (Fv/Fm)
8. Relative water content (%)
9. Super Oxide Dismutase (activity g^{-1} min^{-1})
10. Peroxidase (units min^{-1} g^{-1})

11. Chlorophyll Stability Index (%)

12. Proline content (μM g^{-1} tissue)

All the above mentioned observations related to physiological and biochemical parameters were recorded twice at 60 and 75 DAS. The materials and method used for all the estimation was as described earlier in experiment 1.

(c) Observation related to flowering

1. Days to first flowering

The number of days from transplanting to the production of first flower was recorded.

2. Days to 50% flowering

The number of days from transplanting to production of first flower in 50 % of the replication was recorded.

3. No: of flower clusters per plant

The total number of flower clusters during the peak flowering time was observed per plant.

4. Flowers per cluster

The total number of flowers of 5 clusters per plant was recorded and average taken.

5. Pollen viability (%)

Acetocarmine stain indicate the amount of starch development in pollen grains, and thus indicate the viability of pollen. To test viability, fresh pollen was dusted on a glass slide, and covered with cover slip. The pollen was kept in the staining solution for 30 minutes. Then it was observed under 10 X, 40 X and 100X in compound microscope. A red colour was developed in the viable pollen while non-viable ones remained colourless.

6. Pollen morphology

The exine morphology was studied using scanning electron microscopy (SEM) and the images were taken to study its morphological difference.

(d) Yield parameters

1. Days to fruiting

The number of days taken from transplanting to appearance of first fruit was recorded.

2. Fruit weight (g)

Total weight of five fruits per replication was observed at each harvest and the mean was calculated to get individual fruit weight.

3. No: of fruits per plant

The number of fruits per harvest from each plant was added to get the total number of fruits from each plant.

4. Fruit setting (%)

The number of fruits formed from the total number of flowers produced was recorded and the mean was calculated.

$$\frac{\text{Number of fruits}}{\text{Number of flowers}} \quad X \quad 100$$

5. Intensity of fruiting (%)

The total number of fruits were recorded till the last harvest of the crop and the intensity of fruiting was calculated as:

$$\frac{\text{Number of fruits per cluster}}{\text{Number of flowers per cluster}} \quad X \quad 100$$

6. Intensity of fruit drop (%)

The intensity of fruit drop was calculated in percentage as:

$$\frac{\text{Number of fruits dropped}}{\text{Number of fruits dropped + Number of flowers}} \quad X \quad 100$$

7. Yield per plant (g plant^{-1})

Fruit yield per plant was calculated for all the replications by adding the yield of individual harvest and expressed in grams per plant. The average was taken as the yield of each treatment.

8. Lycopene (mg g^{-1} fresh weight)

The carotenoids in the sample are extracted in acetone and then taken up in the petroleum ether. Lycopene has absorption maxima at 473nm and 503 nm. One mole of lycopene when dissolved in one litre light petroleum (40-60°C) and measured in a spectrophotometer at 503nm in 1cm light path gives an absorbance of 17.2 x 104. Therefore, a concentration of 3.1206µg lycopene/ml gives unit absorbance.

One fully ripened tomato fruit per plant (sample) was taken and pulped. 5-10g of this pulp was weighed and extracted repeatedly with acetone until the residue was colourless. The acetone extract was pooled and transferred to a separating funnel containing about 20ml petroleum ether and mixed gently. About 20ml of 5% sodium sulphate solution was added and the separating funnel was shaken gently. 20ml more of petroleum ether was added to the separating funnel for clear separation of two layer. The two phases were separated and lower aqueous phase was re-extracted with additional 20ml petroleum ether until the aqueous phase was colourless. The petroleum ether extracts were pooled and washed with a little distilled water. The washed petroleum ether extract was poured into a brown container with about 10g anhydrous sodium sulphate and kept aside for 30 minutes. The petroleum ether extract was decanted into a 100ml volumetric flask through a funnel containing cotton wool. Sodium sulphate slurry was washed with petroleum ether until it was colourless and transferred the washings to the volumetric flask. The volume was made up and absorbance measured in a spectrophotometer at 503nm using petroleum ether as blank.

Absorbance (1 unit) = 3.1206µg lycopene/ml

$$\text{mg lycopene in 100g sample} = \frac{\text{Absorbance X 31.206}}{\text{Weight of sample (g)}}$$

RESULTS

4. RESULTS

The present investigation entitled "Influence of CO_2 enrichment and associated high temperature on reproductive physiology of tomato (*Solanum lycopersicum* L.)" was undertaken with the main objective to study the effect of CO_2 enrichment and associated high temperature on flowering and fruiting in tomato and their improvement through growth regulators and nutrient applications and through temperature induction response technique.

The result based on statistically analyzed data pertaining to the experiment conducted during the course of investigation is presented below.

EXPERIMENT 1

Experiment 1 consisted of two parts of which Part 1 consisted of standardization of Temperature Induction Response Technique (TIRT). In this experiment standardization of lethal temperature and Induction temperature was done.

To standardize the lethal temperature, five day old tomato seedlings were subjected to various high temperature treatments in different combinations of time as in table 1. Among all the treatments 48°C for 2 h was selected as the lethal temperature which resulted in 100 % mortality in both Vellayani Vijay and Anagha.

Standardization of induction temperature was done by subjecting five day old tomato seedlings to a set of sub lethal temperature in combination with different time intervals. The temperature range that showed maximum recovery after exposing to lethal temperature was selected as the induction temperature. The temperature treatment with 38 °C (1h) - 43 °C (1h) and then to lethal temperature 48°C for 2 h showed maximum recovery of 87 % for vellayani vijay and 83 % for Anagha. Hence this sequence of temperatures and time was selected as the induction temperature for both the varieties of tomato (Table 2).

Table 1. Recovery responses of seedlings exposed to lethal temperature

Sl no:	Temperature (°C)	Time (h)	Recovery (%)		Mortality (%)	
			Vellayani Vijay	Anagha	Vellayani Vijay	Anagha
1	42	1	10	10	0	0
2	42	2	10	10	0	0
3	44	1	10	10	0	0
4	44	2	8	9	20	10
5	46	1	7	8	30	20
6	46	2	5	6	50	40
7	48	1	2	3	80	70
8	48	2	0	0	100	100
9	Control		10	10	0	0
SE. m ±			0.41	0.41		
CD (0.05)			1.23	1.23		

Table 2. Recovery responses of induced seedlings after recovery period

Sl no:	Induction Temperature (°C)	Vellayani Vijay		Anagha	
		No: of seedlings recovered (mean)	Recovery %	No: of seedlings recovered (mean)	Recovery %
1	36 °C (1h)- 42 °C (1h)- 48 °C (2 h)	6.6	66	6	60
2	36 °C (2h)- 42 °C (2h)- 48 °C (2 h)	5.6	56	5	50
3	38 °C (1h)- 43 °C (1h)- 48 °C (2 h)	8.7	87	8.3	83
4	38 °C (2h)- 43 °C (2h)- 48 °C (2 h)	4	40	4.3	43
5	Control (Ambient temperature)- 48 °C (2 h)	0	0	0	0
SE. m ±		0.45		0.49	
CD (0.05)		1.43		1.58	

Plate 2. Recovery responses (after 3 days) of seedlings exposed to lethal temperature.

Plate 2 (a): Control seedlings (ambient temperature) - after recovery

Plate 2 (b): Seedlings exposed to lethal temperature (48 ^{o}C for 2 h) - after recovery

Plate 3. Recovery responses (after 3 days) of induced and non-induced seedlings

Plate 3 (a): Control seedlings exposed to lethal temperature - after recovery

Plate 3 (b): Temperature induced exposed to lethal temperature- after recovery

Part 2 of the experiment consisted of evaluation of performance of the temperature induced seedlings in both open and CO_2 enriched condition. The growth parameters, physiological and biochemical parameters were taken into consideration and the results based on the statistically analyzed data pertaining to the respective parameters are given below.

1.1 Effect of TIRT on growth parameters in tomato under CO_2 enrichment.

1.1.1 Plant height

The effect of TIRT on plant height is presented in the table 3. Between both the treatments highest mean value (33.16 cm) for plant height was recorded by T2 (without induction) at 60 DAS. Comparing both the conditions, plants showed highest mean value (31.83 cm) inside OTC condition.

1.1.2 No: of branches

As in table 4, no significant difference was seen in number of branches was observed between the two treatments under both the conditions at 60 DAS.

1.1.3 No: of leaves

The mean value for no: of leaves were found to be significantly higher (9.50) in plants with induction compared to control. No: of leaves was found to be significantly higher (9.67) in the open conditions as presented in the table 5.

1.1.4 Specific leaf area

Table 6 shows the result of specific leaf area of the temperature induced seedlings. Specific leaf area recorded highest mean significant value (283.50 cm^2g^{-1}) for plants with induction. Specific leaf area recorded higher value (322.83 cm^2g^{-1}) in open condition.

Table 3. Effect of TIRT on plant height (cm) in tomato under CO_2 enrichment at 60 DAS

Treatments	Open condition	OTC condition	Mean
T1(with induction)	27.00	29.33	28.16
T2(without induction)	32.00	34.33	33.16
Mean	29.50	31.83	
	T	E	T X E
SE m ±	0.63	0.63	0.89
CD (0.05)	2.10	2.10	NS

OTC- Open top chamber

Table 4. Effect of TIRT on number of branches in tomato under CO_2 enrichment at 60 DAS

Treatments	Open condition	OTC condition	Mean
T1(with induction)	0.00	0.33	0.16
T2(without induction)	0.00	0.33	0.16
Mean	0.00	0.33	
	T	E	T X E
SE m ±	0.16	0.16	0.24
CD (0.05)	NS	NS	NS

OTC- Open top chamber

Table 5. Effect of TIRT on number of leaves in tomato under CO_2 enrichment at 60 DAS

Treatments	Open condition	OTC condition	Mean
T1(with induction)	7.33	9.00	8.17
T2(without induction)	8.67	10.33	9.50
Mean	8.00	9.67	
	T	E	T X E
SE m ±	0.29	0.29	0.41
CD (0.05)	0.96	0.96	NS

OTC- Open top chamber

1.2 Effect of TIRT on biochemical and physiological parameters in tomato under CO2 enrichment.

1.2.1 Total Chlorophyll content

Total chlorophyll content of the leaves were estimated and the result is presented in the table 7. It shows significantly higher (1.74 mg g^{-1}) chlorophyll content in temperature induced plants compared to control plants. A significant increase in total chlorophyll content (1.64 mg g^{-1}) was also noticed under elevated CO_2 conditions compared to control plants (Table 7).

1.2.2 Total soluble protein

The result of study of total soluble protein content of temperature induced seedlings showed no significant difference inside OTC compared to control plants. Similarly TIRT did not show any significant increase in total soluble protein content at 60 DAS (Table 8).

1.2.3 Total Carbohydrate

The result of total carbohydrate content is presented in the table 9. Highest mean value (36.71 mg g^{-1}) for total carbohydrate content was exhibited by plants that had undergone temperature induction. A significantly higher total carbohydrate (35.50 mg g^{-1}) was obtained under elevated CO_2 conditions compared to control (Table 9).

1.2.4 Transpiration rate

TIRT resulted in a significant reduction in transpiration rate (18.07 mmol $H_2O^{-2}sec^{-1}$) when compared to the control plants. A similar reduction in transpiration rate was noticed in plants kept under OTC conditions (Table 10).

1.2.5 Photosynthetic rate

Photosynthetic rate was found to increase in plants that had undergone temperature induction. Significantly higher mean value for photosynthetic rate was noticed in T1 (22.13 μmol CO_2 m^{-2} sec^{-1}) compared to the control plants (Table 11).

Table 6. Effect of TIRT on specific leaf area ($cm^2\ g^{-1}$) in tomato under CO_2 enrichment at 60 DAS

Treatments	Open condition	OTC condition	Mean
T1(with induction)	331.00	236.00	283.50
T2(without induction)	314.67	209.00	261.83
Mean	322.83	222.50	
	T	E	T X E
SE m ±	1.14	1.14	1.62
CD (0.05)	3.78	3.78	5.35

OTC- Open top chamber

Table 7. Effect of TIRT on total chlorophyll content (mg g^{-1} fresh weight) in tomato under CO_2 enrichment at 60 DAS

Treatments	Open condition	OTC condition	Mean
T1(with induction)	1.51	1.98	1.74
T2(without induction)	1.10	1.31	1.21
MEAN	1.30	1.65	
	T	E	T X E
SE m ±	0.06	0.06	0.09
CD (0.05)	NS	0.51	NS

OTC- Open top chamber

Table 8. Effect of TIRT on total soluble protein (mg g^{-1}) in tomato under CO_2 enrichment at 60 DAS

Treatments	Open condition	OTC condition	Mean
T1(with induction)	27.00	29.33	28.17
T2(without induction)	32.00	34.33	33.17
Mean	29.50	31.83	
	T	E	T X E
SE m ±	0.64	0.64	0.90
CD (0.05)	2.10	2.10	NS

OTC- Open top chamber

Table 9. Effect of TIRT on total carbohydrate (mg g^{-1}) in tomato under CO_2 enrichment at 60 DAS

Treatments	Open condition	OTC condition	Mean
T1(with induction)	30.78	42.63	36.71
T2(without induction)	25.32	28.36	26.84
MEAN	28.05	35.50	
	T	E	T X E
SE m ±	1.29	1.29	1.83
CD (0.05)	4.30	4.30	6.08

OTC- Open top chamber

Table 10. Effect of TIRT on transpiration rate (mmol H_2O m^{-2} sec^{-1}) in tomato under CO_2 enrichment at 60 DAS

Treatments	Open condition	OTC condition	Mean
T1(with induction)	20.56	15.58	18.07
T2(without induction)	23.66	18.66	21.16
MEAN	22.11	17.12	
	T	E	T X E
SE m ±	0.57	0.57	0.80
CD (0.05)	1.88	1.88	2.66

OTC- Open top chamber

Table 11. Effect of TIRT on photosynthetic rate (µmol CO_2 m^{-2} sec^{-1}) in tomato under CO_2 enrichment at 60 DAS

Treatments	Open condition	OTC condition	Mean
T1(with induction)	21.11	23.15	22.13
T2(without induction)	17.96	19.18	18.57
Mean	19.54	21.16	
	T	E	T X E
SE m ±	0.54	0.54	0.77
CD (0.05)	1.80	NS	NS

OTC- Open top chamber

1.2.6 Water use efficiency

Significantly higher value (4.98 mmol CO_2 mol^{-1} H_2O) for water use efficiency was exhibited by plants that had undergone temperature induction. Plants kept inside OTC showed significantly higher water use efficiency (5.09 mmol CO_2 mol^{-1} H_2O compared to open condition (Table 12).

1.2.7 Chlorophyll fluorescence

Under OTC condition, significant increase in chlorophyll fluorescence (Fv/Fm) (1.64 mg g^{-1}) was also noticed. Chlorophyll fluorescence (Fv/Fm) was significantly higher (0.82) in temperature induced plants compared to control plants (Table 13).

1.2.8 Relative water content

From table 14 it is clear that no significant increase in relative water content was found inside OTC compared to control plants. Similarly TIRT did not show any significant increase in relative water content at 60 DAS.

1.2.9 Superoxide dismutase

The SOD value of the leaves were calculated and is presented in table 15. It shows that SOD recorded significantly higher mean value (0.21 activity g^{-1} min^{-1}) for plants that had undergone temperature induction. CO_2 enrichment conditions resulted in a significantly higher SOD value (0.21 activity g^{-1} min^{-1}) compared to open conditions.

1.2.10 Peroxidase

Peroxidase value did not show any significant increase for temperature induced seedlings neither did CO_2 enrichment showed significant improvement in the peroxidase activity.

Table 12. Effect of TIRT on water use efficiency (mmol CO_2 mol^{-2} H_2O) in tomato under CO_2 enrichment at 60 DAS

Treatments	Open condition	OTC condition	Mean
T1(with induction)	3.81	6.15	4.98
T2(without induction)	3.00	3.96	3.48
Mean	3.41	5.06	
	T	E	T X E
SE m ±	0.26	0.26	0.37
CD (0.05)	0.87	0.87	NS

OTC- Open top chamber

Table 13. Effect of TIRT on chlorophyll fluorescence (Fv/Fm) in tomato under CO_2 enrichment at 60 DAS

Treatments	Open condition	OTC condition	Mean
T1(with induction)	0.87	0.77	0.82
T2(without induction)	0.81	0.61	0.71
Mean	0.84	0.69	
	T	E	T X E
SE m ±	0.03	0.03	0.04
CD (0.05)	0.09	0.09	NS

OTC- open top chamber

Table 14. Effect of TIRT on relative water content (%) in tomato under CO_2 enrichment at 60 DAS

Treatments	Open condition	OTC condition	Mean
T1(with induction)	93.59	90.82	92.20
T2(without induction)	91.06	89.69	90.38
Mean	92.32	90.25	
	T	E	T X E
SE m ±	0.63	0.63	0.89
CD (0.05)	NS	2.07	NS

OTC- Open top chamber

Table 15. Effect of TIRT on superoxide dismutase (activity g^{-1} min^{-1}) in tomato under CO_2 enrichment at 60 DAS

Treatments	Open condition	OTC condition	Mean
T1(with induction)	0.19	0.22	0.20
T2(without induction)	0.12	0.20	0.16
MEAN	0.16	0.21	
	T	E	T X E
SE m ±	0.01	0.01	0.01
CD (0.05)	0.04	0.04	0.04

OTC- Open top chamber

Table 16. Effect of TIRT on peroxidase (unit min^{-1} g^{-1}) in tomato under CO_2 enrichment at 60 DAS

Treatments	Open condition	OTC condition	Mean
T1(with induction)	20.51	22.89	21.70
T2(without induction)	20.28	20.70	20.49
MEAN	20.39	21.80	
	T	E	T X E
SE m ±	0.40	0.399	0.56
CD (0.05)	NS	1.320	NS

OTC- Open top chamber

1.2.11 Chlorophyll stability index

Chlorophyll stability index increased significantly (145.97 %) in temperature induced plants. However, no significant increase in the activity of peroxidase was noticed under CO_2 enrichment (Table 17).

1.2.12 Proline

TIRT resulted in increased proline content in general. Significantly higher mean value for proline content was noticed in T1 (1.32 µM g-1 tissue) compared to the control plants. Besides this, a significant increase in proline content (1.68 µM g-1 tissue) was noticed in plants exposed to elevated CO_2.

Table 17. Effect of TIRT on chlorophyll stability index (%) in tomato under CO_2 enrichment at 60 DAS

Treatments	Open condition	OTC condition	Mean
T1(with induction)	153.70	138.23	145.97
T2(without induction)	100.00	100.00	100.00
Mean	126.85	119.12	
	T	E	T X E
SE m ±	7.44	7.44	10.52
CD (0.05)	24.63	NS	NS

OTC- Open top chamber

Table 18. Effect of TIRT on proline content (µM g^{-1} tissue) in tomato under CO_2 enrichment at 60 DAS

Treatments	Open condition	OTC condition	Mean
T1(with induction)	0.68	1.97	1.32
T2(without induction)	0.36	1.39	0.88
Mean	0.52	1.68	
	T	E	T X E
SE m ±	0.04	0.04	0.06
CD (0.05)	0.15	0.15	NS

OTC- Open top chamber

EXPERIMENT 2: CO_2 enrichment studies - Impact of application of growth regulators and nutrient application on reproductive physiology of tomato.

The impact of various growth regulators and nutrient application on tomato under elevated CO_2 condition on tomato variety Vellayani Vijay was studied in the present study. The method used for subjecting the plants to elevated CO2 environment was Open Top Chamber. The growth parameters, physiological and biochemical parameters, parameters related to flowering and yield parameters were evaluated. The results based on the statistically analyzed data pertaining to the experiment are presented below.

2.1 Effect of growth regulators and nutrient applications on growth parameters of tomato under CO2 enrichment at 60 DAS and 75 DAS

2.1.1. Plant height

The plant height was measured at two intervals (60 and 75 DAS) and the data is presented in table 19. All treatments had significant influence on plant height. At 60 DAS, significantly higher mean value for plant height (33.33 cm) was exhibited by T3 (50 ppm Boron) compared to control. Similar trend was seen at 75 DAS also. Elevated CO2 resulted in significantly higher plant height (90.66 cm) at 75 DAS compared to open condition (57.04 cm). T7 showed resulted in shortest plants (29.00 cm) at 60 DAS and in T1 (66.00 cm) at 75 DAS (Table 19).

2.1.2 Number of branches

The effect of growth regulators and nutrient application on number of branches was studied and the data is presented in table 20. At 60 DAS the highest mean value (0.83) for number of branches was observed for T5 (POP 125% N: 100% P: 100 % K). Similar result was observed at 75 DAS with T5 with the highest mean value (5.50).

Effect of elevated CO2 on the number of branches in tomato showed a significant increase in number of branches under CO_2 enriched condition (6.92) compared to open condition (0.50) at 75 DAS (Table 20).

Plate 4. Plants maintained inside Open Top Chamber

Plate 5. Plants kept in open (control) condition

2.1.3 Number of leaves

The number of leaves per plant was recorded at 60 and 75 DAS. Treatments did not show any significant variation in number of leaves at 60 DAS. However, at 75 DAS application of 25 % extra N (T5) resulted in significantly higher (46.67) number of leaves (Table 21).

Carbon dioxide enrichment resulted in a significant increase in number of leaves compared to open condition. The effect was more prominent at 75 DAS recording significant increase in the number of leaves (46.92) (Table 21).

2.1.4 Specific leaf area

Specific leaf area (SLA) was calculated at two intervals (60 DAS and 75 DAS) and the data is presented in table 22. The results showed significantly higher SLA for 25 % extra NPK (T6) (383.33 cm^2 g^{-1}) compared to absolute control (315.03 cm^2 g^{-1}) at 60 DAS. The least value was exhibited by T3 (244.90 cm^2 g^{-1}). A similar trend was observed at 75 DAS with significantly higher mean value was observed for T6 (347.98 cm^2 g^{-1}) (Table 22).

Under elevated CO_2 conditions, an improvement in the specific leaf area was observed. A significantly higher SLA was observed under elevated CO_2 condition at 60 DAS (347.01 cm^2 g^{-1}) and 75 DAS (321.59 cm^2 g^{-1}) (Table 22).

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Table 19. Effect of growth regulators and nutrient application on plant height (cm) in tomato under CO_2 enrichment at 60 DAS and 75DAS.

	60 DAS			75 DAS		
	Open condition	OTC condition	Mean	Open condition	OTC condition	Mean
T1 (50ppm NAA)	36.00	29.33	32.67	55.33	76.67	66.00
T2 (50ppm Salicylic acid)	33.33	29.00	31.17	62.33	85.67	74.00
T3 (50 ppm boron)	34.67	32.00	33.33	55.33	115.33	85.33
T4 (50ppm B+ 50ppm Zn)	36.33	28.00	32.17	48.67	91.00	69.83
T5 (POP 125%N: 100P: 100K)	35.00	29.00	32.00	45.00	96.67	70.83
T6 (POP 125 N: 125P: 125 K)	30.33	28.33	29.33	70.00	78.33	74.17
T7 (water spray)	30.00	28.00	29.00	66.33	75.33	70.83
T8 (absolute control)	31.00	30.66	30.83	53.33	106.33	79.83
Mean	33.33	29.29		57.04	90.66	
	T	E	T X E	T	E	T X E
SE m ±	0.69	0.34	0.98	0.62	0.31	0.87
CD (0.05)	2.01	1.01	2.84	1.78	0.89	2.52

OTC- Open top chamber

Table 20. Effect of growth regulators and nutrient application on number of branches in tomato under CO_2 enrichment at 60 DAS and 75DAS.

	60 DAS			75 DAS		
	Open condition	OTC condition	Mean	Open condition	OTC condition	Mean
T1 (50ppm NAA)	0.00	0.00	0.00	0.67	4.33	2.50
T2 (50ppm Salicylic acid)	0.00	0.00	0.00	0.00	7.00	3.50
T3 (50 ppm boron)	0.00	0.00	0.00	0.00	6.33	3.17
T4 (50ppm B+ 50ppm Zn)	0.00	0.67	0.33	1.33	5.00	3.17
T5 (POP 125%N: 100P: 100K)	0.33	1.33	0.83	1.00	10.00	5.50
T6 (POP 125 N: 125P: 125 K)	0.00	0.33	0.17	0.33	10.33	5.33
T7 (water spray)	0.00	0.67	0.33	0.00	6.67	3.33
T8 (absolute control)	0.00	0.33	0.17	0.67	5.67	3.17
Mean	0.04	0.42		0.50	6.92	
	T	E	T X E	T	E	T X E
SE m ±	0.14	0.07	0.20	0.38	0.19	0.54
CD (0.05)	0.42	0.21	NS	1.11	0.55	1.56

OTC- Open top chamber

Table 21. Effect of growth regulators and nutrient application on number of leaves in tomato under CO_2 enrichment at 60 DAS and 75DAS.

	60 DAS			75 DAS		
	Open condition	OTC condition	Mean	Open condition	OTC condition	Mean
T1 (50ppm NAA)	11.33	9.00	10.17	22.33	38.00	30.17
T2 (50ppm Salicylic acid)	9.33	9.33	9.33	22.33	49.33	35.83
T3 (50 ppm boron)	9.33	9.00	9.17	23.33	46.67	35.00
T4 (50ppm B+ 50ppm Zn)	10.00	9.33	9.67	25.00	44.33	34.67
T5 (POP 125%N: 100P: 100K)	9.33	9.00	9.17	29.33	64.33	46.67
T6 (POP 125 N: 125P: 125 K)	7.00	9.33	8.17	30.33	57.33	43.83
T7 (water spray)	9.00	9.00	9.00	23.33	43.33	33.33
T8 (absolute control)	8.33	10.33	9.33	20.33	32.00	26.17
Mean	9.21	9.29		24.54	46.92	
	T	E	T X E	T	E	T X E
SE m ±	0.59	0.29	0.83	0.64	0.32	0.91
CD (0.05)	NS	NS	NS	1.85	0.93	2.62

OTC- Open top chamber

Table 22. Effect of growth regulators and nutrient application on specific leaf area ($cm^2\ g^{-1}$) in tomato under CO_2 enrichment at 60 DAS and 75DAS.

	60 DAS			75 DAS		
	Open condition	OTC condition	Mean	Open condition	OTC condition	Mean
T1 (50ppm NAA)	191.54	370.30	280.92	175.32	334.30	254.81
T2 (50ppm Salicylic acid)	252.27	370.92	311.59	235.51	353.92	294.72
T3 (50 ppm boron)	193.20	296.60	244.90	172.35	275.09	223.72
T4 (50ppm B+ 50ppm Zn)	232.76	337.74	285.25	207.58	332.56	270.07
T5 (POP 125%N: 100P: 100K)	297.64	298.55	298.10	276.74	278.38	277.56
T6 (POP 125 N: 125P: 125 K)	326.42	462.24	383.33	297.58	398.40	347.99
T7 (water spray)	198.83	336.64	267.74	173.65	314.47	244.06
T8 (absolute control)	304.98	303.08	315.03	177.47	285.58	231.52
Mean	249.70	347.01		214.53	321.59	
	T	E	T X E	T	E	T X E
SE m ±	0.86	0.43	1.22	0.88	0.44	1.24
CD (0.05)	2.50	1.25	3.53	2.54	1.27	3.60

OTC- Open top chamber

2.1 Effect of growth regulators and nutrient applications on physiological and biochemical parameters of tomato under CO2 enrichment at 60 DAS and 75 DAS

2.2.1 Total chlorophyll content

Effect of various treatments on the total chlorophyll content of tomato at 60 and 75 DAS is presented in the table 23. Higher value for total chlorophyll content was seen in T3, both at 60 DAS (1.90 mg g^{-1} FW) and at 75 DAS (1.55 mg g^{-1} FW) compared to control.

The total chlorophyll content increased significantly under elevated CO_2 conditions at 60 (1.73 mg g^{-1} FW) and 75 DAS (1.52 mg g^{-1} FW) compared to open conditions. Lowest value for chlorophyll content was observed in T8 (absolute control).

2.2.2 Total soluble protein

The impact of application of various growth regulators and nutrient application on the total soluble protein (TSP) content of tomato was studied and the result is presented in table 24. Significantly higher mean value for TSP content was observed in plants under treatment T3 (24.06 mg g^{-1}) which was on par with values of T2, T4, T5 and T6 at 60 DAS. At 75 DAS, highest mean value for total soluble protein was observed for T2 (11.93 mg g^{-1}) which was on par T5 and T6.The lowest TSP content was recorded in T8 both at 60 DAS (18.47 mg g^{-1}) and 75 DAS (10.10 mg g^{-1}).

CO_2 enrichment showed significant reduction in the TSP content. At 60 DAS, TSP of 20.61 mg g^{-1} was recorded under CO_2 enrichment compared to open condition (24.37 mg g^{-1}). Similarly, at 75 DAS those plants kept inside OTC recorded lower TSP content (10.42 mg g^{-1}) compared to open.

2.2.3 Total Carbohydrate

Effect of various growth regulators and nutrient application on total carbohydrate content of tomato plants was estimated at 60 and 75 DAS. A significantly higher value for total carbohydrate was observed under treatment T5 (59.31 mg g^{-1}) at 60 DAS, and under treatment T6 (47.88 mg g^{-1}) at 75 DAS compared to control.

Plants exposed to elevated CO_2 showed a higher value for total carbohydrate content compared to control. This was evident both at 60 DAS (55.89 mg g^{-1}) and at 75 DAS (50.07 mg g^{-1}).

2.2.4 Transpiration rate

Application of various growth regulators and additional nutrients resulted in a decrease in transpiration rate under elevated CO_2 condition as well as under open conditions. At 60 DAS, transpiration rate was found to be significantly lower in T5 (18.17 mmol H_2O $m^{-2}sec^{-1}$) compared to control (21.74 mmol H_2O $m^{-2}sec^{-1}$). At 75 DAS, transpiration rate was found to be higher under all the treatments. Among the treatments, T5 recorded highest significant value (18.27 mmol H_2O m^{-2} sec^{-1}) and the lowest value was recorded for the absolute control T8 (15.57 mmol H_2O m^{-2} sec^{-1}).

Considering the effect of elevated CO_2 conditions on the transpiration rate, a significant reduction was noted both at 60 DAS (15.37 mmol H_2O m^{-2} sec^{-1}) and at 75 DAS (11.94 mmol H_2O m^{-2} sec^{-1}) compared to open condition.

2.2.5 Photosynthetic rate

Photosynthetic rate was recorded twice at 60 DAS and 75 DAS. Effect of various treatments on photosynthetic rate was recorded as in the table 27. Photosynthetic rate increased with the application of various treatments and significantly higher value was noted in T5 at 60 DAS (24.10 µmol CO_2 m^{-2} sec^{-1}) and at 75 DAS (21µmol CO_2 m^{-2} sec^{-1}). The lowest photosynthetic rate was recorded in T8 both at 60 DAS and 75 DAS.

Significant increase in photosynthetic rate was observed in plants exposed to elevated CO_2 compared to control plants. The photosynthetic rate recorded at 60 DAS was 22.77 µmol CO_2 m^{-2} sec^{-1} at 60 DAS and at 75 DAS was 20.58 µmol CO_2 m^{-2} sec^{-1} under elevated CO2 condition.

Table 23. Effect of growth regulators and nutrient application on total chlorophyll content (mg g^{-1} fresh weight) in tomato under CO_2 enrichment at 60 DAS and 75DAS.

	60 DAS			75 DAS		
	Open condition	OTC condition	Mean	Open condition	OTC condition	Mean
T1 (50ppm NAA)	1.29	1.36	1.33	0.95	1.22	1.08
T2 (50ppm Salicylic acid)	1.02	2.10	1.56	0.90	1.94	1.42
T3 (50 ppm boron)	1.12	2.68	1.90	1.03	2.06	1.55
T4 (50ppm B+ 50ppm Zn)	1.58	1.55	1.57	1.24	1.25	1.25
T5 (POP 125%N: 100P: 100K)	1.35	1.67	1.51	1.14	1.46	1.30
T6 (POP 125 N: 125P: 125 K)	1.29	1.46	1.38	1.06	1.40	1.23
T7 (water spray)	0.91	1.75	1.33	0.87	1.62	1.25
T8 (absolute control)	0.88	1.26	1.07	0.83	1.16	1.00
Mean	1.18	1.73		1.00	1.51	
	T	E	T X E	T	E	T X E
SE m ±	0.01	0.01	0.01	0.01	0.00	0.01
CD (0.05)	0.03	0.01	0.04	0.02	0.01	0.03

OTC- Open top chamber

Table 24. Effect of growth regulators and nutrient application on total soluble protein (mg g^{-1}) in tomato under CO_2 enrichment at 60 DAS and 75DAS

	60 DAS			75 DAS		
	Open condition	OTC condition	Mean	Open condition	OTC condition	Mean
T1 (50ppm NAA)	23.99	19.50	21.75	12.59	10.37	11.48
T2 (50ppm Salicylic acid)	24.35	21.74	23.04	13.20	10.67	11.93
T3 (50 ppm boron)	25.20	22.91	24.06	13.09	9.60	11.35
T4 (50ppm B+ 50ppm Zn)	24.34	22.38	23.36	11.96	11.47	11.72
T5 (POP 125%N: 100P: 100K)	24.51	23.11	23.81	13.59	10.04	11.82
T6 (POP 125 N: 125P: 125 K)	25.50	21.77	23.64	12.67	11.03	11.85
T7 (water spray)	23.56	20.09	21.78	11.89	10.82	11.36
T8 (absolute control)	23.47	13.38	18.47	10.80	9.39	10.10
Mean	24.37	20.61		12.48	10.42	
	T	E	T X E	T	E	T X E
SE m ±	0.43	0.22	0.61	0.59	0.29	0.83
CD (0.05)	1.25	0.62	1.77	1.70	0.85	NS

OTC- Open top chamber

Table 25. Effect of growth regulators and nutrient application on total carbohydrate (mg/g) in tomato under CO_2 enrichment at 60 DAS and 75DAS

	60 DAS			75 DAS		
	Open condition	OTC condition	Mean	Open condition	OTC condition	Mean
T1 (50ppm NAA)	36.90	50.51	43.71	32.26	46.20	39.23
T2 (50ppm Salicylic acid)	43.48	50.84	47.16	42.36	50.23	46.29
T3 (50 ppm boron)	36.93	52.21	44.57	24.14	45.93	35.04
T4 (50ppm B+ 50ppm Zn)	35.04	50.15	42.60	42.92	49.87	46.40
T5 (POP 125%N: 100P: 100K)	45.28	73.35	59.31	32.49	55.40	43.95
T6 (POP 125 N: 125P: 125 K)	44.69	65.47	55.08	36.90	58.86	47.88
T7 (water spray)	45.64	53.90	49.77	43.85	49.12	46.49
T8 (absolute control)	46.29	50.73	48.51	43.32	44.96	44.14
Mean	41.78	55.90		37.28	50.07	
	T	E	T X E	T	E	T X E
SE m ±	1.07	0.54	1.51	1.64	0.82	2.33
CD (0.05)	3.10	1.55	4.38	4.76	2.38	6.73

OTC- Open top chamber

Table 26. Effect of growth regulators and nutrient application on transpiration rate (mmol H_2O m^{-2} sec^{-1}) in tomato under CO_2 enrichment at 60 DAS and 75DAS

	60 DAS			75 DAS		
	Open condition	OTC condition	Mean	Open condition	OTC condition	Mean
T1 (50ppm NAA)	28.65	17.73	19.64	21.67	12.32	17.00
T2 (50ppm Salicylic acid)	22.59	16.51	19.55	20.69	13.18	16.93
T3 (50 ppm boron)	23.14	14.49	18.82	21.00	11.81	16.41
T4 (50ppm B+ 50ppm Zn)	21.93	14.89	18.41	21.54	12.94	17.24
T5 (POP 125%N: 100P: 100K)	22.02	14.32	18.17	23.27	13.27	18.27
T6 (POP 125 N: 125P: 125 K)	23.79	15.09	19.45	23.00	11.92	17.46
T7 (water spray)	21.56	14.05	17.81	21.68	10.19	15.93
T8 (absolute control)	27.64	15.85	21.75	21.28	9.86	15.57
Mean	23.92	15.37		21.77	11.94	
	T	E	T X E	T	E	T X E
SE m ±	1.15	0.58	1.63	0.40	0.20	0.56
CD (0.05)	1.21	0.60	1.71	0.42	0.21	0.59

OTC- Open top chamber

Table 27. Effect of growth regulators and nutrient application on photosynthetic rate (μmol CO_2 m^{-2} sec^{-1}) in tomato under CO_2 enrichment at 60 DAS and 75DAS

	60 DAS			75 DAS		
	Open condition	OTC condition	Mean	Open condition	OTC condition	Mean
T1 (50ppm NAA)	19.60	27.40	23.50	15.12	24.00	19.56
T2 (50ppm Salicylic acid)	15.63	24.13	19.88	12.08	21.50	16.79
T3 (50 ppm boron)	18.83	20.60	19.72	15.44	16.77	16.11
T4 (50ppm B+ 50ppm Zn)	19.77	21.57	20.67	17.39	20.27	18.83
T5 (POP 125%N: 100P: 100K)	20.80	27.40	24.10	18.58	24.67	21.62
T6 (POP 125 N: 125P: 125 K)	16.30	22.00	19.15	14.68	21.60	18.14
T7 (water spray)	19.80	21.37	20.58	15.12	19.37	17.24
T8 (absolute control)	18.20	17.70	17.95	12.77	16.50	14.64
Mean	18.62	22.77		15.15	20.58	
	T	E	T X E	T	E	T X E
SE m ±	1.80	0.90	2.55	0.62	0.31	0.88
CD (0.05)	1.68	0.84	2.38	0.58	0.29	0.82

OTC- Open top chamber

2.2.6 Water use efficiency

Variation in water use efficiency of tomato plants kept under elevated CO_2 condition in response to the application of various growth regulators and additional nutrients was studied at two time intervals of 60 DAS and 75 DAS and the result is presented in table 28. The significantly higher value for water use efficiency was observed in plants treatment T5 (5.83 mmol CO_2 mol^{-1} H_2O) which was on par with T6 (5.67 mmol CO_2 mol^{-1} H_2O) at 60 DAS. At 75 DAS, similar trend was observed with T5 and T6 showing significantly higher water use efficiency (4.67 mmol CO_2 mol^{-1} H_2O) compared to control plants.

Elevated CO_2 was found to influence water use efficiency positively. Plants kept inside OTC was found to have significantly higher water use efficiency at 60 DAS (5.75 mmol CO_2 mol^{-1} H_2O) and 75 DAS (5.09 mmol CO_2 mol^{-1} H_2O).

2.2.7 Chlorophyll fluorescence (Fv/Fm)

Chlorophyll fluorescence denotes the stability of chlorophyll molecule at stress condition. It is represented by Fv/Fm value. The Fv/Fm value of tomato plants under elevated CO_2 condition that had undergone various treatments were recorded and the data is given in the table 29. The significantly higher value (0.84) for Fv/Fm was shown by T6 at 60 DAS. Similar trend was followed at 75 DAS.

Fv/Fm was found to improve in an atmosphere of enriched CO_2 at 60 DAS (0.82) and 75 DAS (0.65).

2.2.8 Relative water content

Relative water content (RWC) of tomato leaves were recorded at 60 and 75 DAS to study the effect of various growth regulators and additional nutrients and the result is presented in the table 30. At 60 DAS, the increase in RWC was not significant. It was noted to be significantly higher in T5 (96.87 %) compared to control (95.71 %) at 75 DAS. The lowest value for RWC was recorded for T6, both at 60 DAS (90.73 %) and 75 DAS (89.48 %).

RWC was found to decrease with effect to CO_2 enrichment. There was a significant reduction in RWC at both 60 DAS (93.48 %) and 75 DAS (92.06 %) compared to open condition.

2.2.9 Superoxide dismutase (SOD)

Table 31 shows the effect of Superoxide dismutase activity (SOD) in the leaves of tomato plants at 60 DAS and 75 DAS under elevated CO_2 condition. Activity of SOD increased with the application of various treatments and significantly higher value for SOD was noted in T6 (0.27 activity g^{-1} min^{-1}) which was on par with T3 and T5 at 60 DAS. Same trend followed at 75 DAS with significantly higher value recorded by T6 (0.23 activity g^{-1} min^{-1}) which was on par with T5.

Significant increase in SOD activity was found in plants exposed to elevated CO_2 compared to control plants. Under OTC condition a significantly higher SOD activity was observed at 60 DAS (0.27 activity g^{-1} min^{-1}) and at 75 DAS (0.22 activity g^{-1} min^{-1}).

2.2.10 Peroxidase

The impact of various treatments on peroxidase activity in leaves of tomato plants were recorded and the result is presented in table 32. A significant increase in peroxidase activity was observed by under treatment T6 at 60 DAS (45.61 unit min^{-1} g^{-1}) and at 75 DAS (44.43 unit min^{-1} g^{-1}) compared to control. Lowest activity for peroxidase was exhibited by T8 at 60 DAS (34.67 unit min^{-1} g^{-1}) and at 75 DAS (33.40 unit min^{-1} g^{-1}).

Significant increase in peroxidase activity was observed under elevated CO_2. Peroxidase activity under elevated CO2 condition at 60 DAS was 47.12 unit min^{-1} g^{-1} and at 75 DAS was 45.87 unit min^{-1} g^{-1}.

Table 28. Effect of growth regulators and nutrient application on water use efficiency (mmol CO_2 mol^{-2} H_2O) in tomato under CO_2 enrichment at 60 DAS and 75DAS.

	60 DAS			75 DAS		
	Open condition	OTC condition	Mean	Open condition	OTC condition	Mean
T1 (50ppm NAA)	2.33	7.00	4.67	1.33	6.33	3.83
T2 (50ppm Salicylic acid)	2.67	5.33	4.00	1.67	5.33	3.50
T3 (50 ppm boron)	2.00	5.00	3.50	1.00	5.33	3.17
T4 (50ppm B+ 50ppm Zn)	3.33	5.67	4.50	1.67	4.33	3.00
T5 (POP 125%N: 100P: 100K)	3.67	8.00	5.83	2.67	6.67	4.67
T6 (POP 125 N: 125P: 125 K)	4.00	7.33	5.67	3.00	6.33	4.67
T7 (water spray)	3.67	4.00	3.83	2.67	3.33	3.00
T8 (absolute control)	3.33	3.67	3.50	2.33	3.00	2.67
Mean	3.13	5.75		2.04	5.08	
	T	E	T X E	T	E	T X E
SE m ±	1.12	0.56	1.58	0.39	0.19	0.55
CD (0.05)	0.99	0.50	1.41	0.34	0.17	0.49

OTC- Open top chamber

Table 29. Effect of growth regulators and nutrient application on chlorophyll fluorescence (Fv/Fm) in tomato under CO_2 enrichment at 60 DAS and 75DAS.

	60 DAS			75 DAS		
	Open condition	OTC condition	Mean	Open condition	OTC condition	Mean
T1 (50ppm NAA)	0.55	0.87	0.71	0.46	0.64	0.55
T2 (50ppm Salicylic acid)	0.64	0.83	0.74	0.55	0.60	0.58
T3 (50 ppm boron)	0.64	0.77	0.70	0.55	0.54	0.54
T4 (50ppm B+ 50ppm Zn)	0.70	0.86	0.78	0.61	0.63	0.62
T5 (POP 125%N: 100P: 100K)	0.73	0.89	0.81	0.64	0.66	0.65
T6 (POP 125 N: 125P: 125 K)	0.80	0.87	0.84	0.71	0.80	0.76
T7 (water spray)	0.51	0.73	0.62	0.42	0.66	0.54
T8 (absolute control)	0.50	0.72	0.61	0.41	0.65	0.53
Mean	0.63	0.82		0.54	0.65	
	T	E	T X E	T	E	T X E
SE m ±	0.01	0.00	0.01	0.01	0.00	0.01
CD (0.05)	0.02	0.01	0.03	0.02	0.01	0.03

OTC- Open top chamber

Table 30. Effect of growth regulators and nutrient application on relative water content (%) in tomato under CO_2 enrichment at 60 DAS and 75DAS.

	60 DAS			75 DAS		
	Open condition	OTC condition	Mean	Open condition	OTC condition	Mean
T1 (50ppm NAA)	90.93	94.69	92.81	89.52	93.38	91.45
T2 (50ppm Salicylic acid)	97.27	96.91	97.09	96.20	95.31	95.75
T3 (50 ppm boron)	96.17	89.36	92.77	95.35	87.66	91.50
T4 (50ppm B+ 50ppm Zn)	94.43	94.25	94.34	93.33	92.64	92.99
T5 (POP 125%N: 100P: 100K)	98.37	97.89	98.13	97.23	96.51	96.87
T6 (POP 125 N: 125P: 125 K)	97.09	84.37	90.73	96.02	82.95	89.48
T7 (water spray)	91.75	93.39	92.57	89.38	92.60	90.99
T8 (absolute control)	96.99	96.98	96.99	95.98	95.44	95.71
Mean	95.38	93.48		94.13	92.06	
	T	E	T X E	T	E	T X E
SE m ±	NS	NS	NS	1.90	0.95	2.68
CD (0.05)	1.97	0.98	2.78	0.68	0.34	0.96

OTC- Open top chamber

Table 31. Effect of growth regulators and nutrient application on superoxide dismutase (activity g^{-1} min^{-1}) in tomato under CO_2 enrichment at 60 DAS and 75DAS.

	60 DAS			75 DAS		
	Open condition	OTC condition	Mean	Open condition	OTC condition	Mean
T1 (50ppm NAA)	0.10	0.25	0.18	0.09	0.21	0.15
T2 (50ppm Salicylic acid)	0.11	0.28	0.20	0.08	0.25	0.17
T3 (50 ppm boron)	0.17	0.34	0.25	0.14	0.26	0.20
T4 (50ppm B+ 50ppm Zn)	0.22	0.25	0.24	0.20	0.19	0.20
T5 (POP 125%N: 100P: 100K)	0.20	0.30	0.25	0.17	0.24	0.21
T6 (POP 125 N: 125P: 125 K)	0.20	0.33	0.27	0.17	0.27	0.22
T7 (water spray)	0.09	0.23	0.16	0.06	0.17	0.12
T8 (absolute control)	0.22	0.21	0.22	0.19	0.14	0.17
Mean	0.17	0.27		0.14	0.22	
	T	E	T X E	T	E	T X E
SE m ±	0.01	0.00	0.01	0.01	0.00	0.01
CD (0.05)	0.02	0.01	0.03	0.02	0.01	0.03

OTC- Open top chamber

Table 32. Effect of growth regulators and nutrient application on peroxidase (unit min^{-1} g^{-1}) in tomato under CO_2 enrichment at 60 DAS and 75DAS

	60 DAS			75 DAS		
	Open condition	OTC condition	Mean	Open condition	OTC condition	Mean
T1 (50ppm NAA)	22.17	47.76	34.97	21.07	46.00	33.53
T2 (50ppm Salicylic acid)	29.25	42.77	36.01	26.47	41.82	34.14
T3 (50 ppm boron)	24.30	46.27	35.28	23.10	45.07	34.08
T4 (50ppm B+ 50ppm Zn)	24.13	50.17	37.15	22.97	48.40	35.68
T5 (POP 125%N: 100P: 100K)	31.93	49.60	40.77	31.27	48.30	39.79
T6 (POP 125 N: 125P: 125 K)	37.48	53.73	45.61	36.30	52.57	44.43
T7 (water spray)	30.02	45.50	37.76	28.75	44.63	36.69
T8 (absolute control)	28.20	41.14	34.67	26.60	40.20	33.40
Mean	28.44	47.12		27.07	45.87	
	T	E	T X E	T	E	T X E
SE m ±	0.31	0.16	0.44	0.38	0.19	0.53
CD (0.05)	0.09	0.45	1.27	1.09	0.55	1.55

OTC- Open top chamber

2.2.11 Chlorophyll stability index

The impact of various growth regulator application and additional nutrients on chlorophyll stability index (CSI) is presented in table 33. At 60 DAS the highest significant value (137.64%) was observed for T3. Similar result was observed at 75 DAS with T3 with highest significant mean value (124.88 %) which was on par T2 (123.23%).

A significant increase in chlorophyll stability index was noticed under CO_2 enriched condition compared to open condition at 60 DAS (127.10 %) and 75 DAS (124.16 %).

2.2.12 Proline

Influence of application of various treatments on the proline content of tomato leaves under elevate CO_2 content was estimated and the result was presented in the table 34. At 60 DAS, significantly higher proline content was recorded by T5 (0.22 μM g^{-1} tissue). However at 75 DAS, significantly higher value for proline content was observed in plants under treatment T4 (0.15 μM g^{-1} tissue) which was on par with T2, T6 and T5.

Plants kept inside OTC was found to have significantly higher proline content at 60 DAS (0.24 μM g^{-1} tissue) and 75 DAS (0.18 μM g^{-1} tissue)

Table 33. Effect of growth regulators and nutrient application on chlorophyll stability index (%) in tomato under CO_2 enrichment at 60 DAS and 75DAS.

	60 DAS			75 DAS		
	Open condition	OTC condition	Mean	Open condition	OTC condition	Mean
T1 (50ppm NAA)	114.81	92.73	103.77	91.92	95.10	93.51
T2 (50ppm Salicylic acid)	90.45	154.03	122.24	87.41	159.05	123.23
T3 (50 ppm boron)	78.42	196.88	137.65	80.61	169.17	124.89
T4 (50ppm B+ 50ppm Zn)	141.12	113.86	127.49	120.77	102.49	111.63
T5 (POP 125%N: 100P: 100K)	120.04	122.63	121.33	110.68	119.42	115.05
T6 (POP 125 N: 125P: 125 K)	114.64	107.62	111.13	102.94	115.03	108.99
T7 (water spray)	80.83	129.05	104.94	84.81	132.97	108.89
T8 (absolute control)	100.00	100.00	100.00	100.00	100.00	100.00
Mean	105.04	127.10		97.39	124.16	
	T	E	T X E	T	E	T X E
SE m ±	0.87	0.43	1.23	1.03	0.52	1.46
CD (0.05)	2.51	1.26	3.55	2.99	1.49	4.23

OTC- Open top chamber

Table 34. Effect of growth regulators and nutrient application on proline content ($\mu M\ g^{-1}$ tissue) in tomato under CO_2 enrichment at 60 DAS and 75DAS

	60 DAS			75 DAS		
	Open condition	OTC condition	Mean	Open condition	OTC condition	Mean
T1 (50ppm NAA)	0.13	0.21	0.17	0.05	0.14	0.10
T2 (50ppm Salicylic acid)	0.15	0.24	0.20	0.08	0.20	0.14
T3 (50 ppm boron)	0.12	0.24	0.18	0.05	0.17	0.11
T4 (50ppm B+ 50ppm Zn)	0.15	0.26	0.21	0.08	0.22	0.15
T5 (POP 125%N: 100P: 100K)	0.15	0.29	0.22	0.06	0.21	0.13
T6 (POP 125 N: 125P: 125 K)	0.15	0.26	0.20	0.06	0.21	0.14
T7 (water spray)	0.12	0.23	0.17	0.05	0.18	0.11
T8 (absolute control)	0.09	0.21	0.15	0.06	0.13	0.09
Mean	0.13	0.24		0.06	0.18	
	T	E	T X E	T	E	T X E
SE m ±	0.01	0.00	0.01	0.01	0.01	0.02
CD (0.05)	0.02	0.01	0.02	0.03	0.02	NS

OTC- Open top chamber

2.3 Effect of growth regulators and nutrient applications on observations related to flowering n tomato under CO_2 enrichment at 60 DAS and 75 DAS

2.3.1 Days to first flowering

The impact of treatments on days to first flowering was recorded and is presented in table 35. Application of various growth regulators and additional nutrients did not show significant variation in days to first flowering. However, a significant delay (2 days) in days to first flowering was noticed in plants kept inside OTC with elevated CO_2 conditions.

2.3.2 Days to 50 % flowering

Days to 50 % flowering as influenced by various treatments under elevated CO2 condition was recorded and is presented in table 36. No significant difference in days to 50 % flowering was noticed among different treatments. However, a significant delay (3 days) was observed in days to 50% flowering under elevated CO_2 conditions compared to plants under open condition.

2.3.3 Number of flower clusters per plant

The effect of application of growth regulators and additional nutrients on number of flower clusters per plant was recorded and is as presented in table 37. A significant increase in the number of flower clusters per plant was observed with the application of various treatments. Among the treatments maximum number of flower clusters was noticed in plants under treatment T6 (6.33) was on par with T1 (6.00), T3 (5.83) and T2 (5.33) compared to control (3.67).

Elevated CO_2 resulted in a significant increase in the number of flower clusters per plant. Number of flower clusters per plant was recorded to be 6.08 under elevated CO_2 compared to control condition with mean number of flower clusters as 4.29. Within OTC, T6 resulted in highest number of flower clusters per plant.

2.3.4 Number of flowers per cluster

The effect of various treatments on number of flowers per cluster under the influence of elevated CO_2 was recorded and is given in table 38.There was a significant improvement in the number of flowers per cluster with the application of various treatments. The significantly higher number of flowers per cluster was noticed in T5 (5.42) which was on par with T4 (5.28), T3 (5.17), and T7 (5.00). Lowest number of flowers was reported in T8 (3.65)

2.3.5 Pollen viability

The impact of application of various plant growth regulators and nutrient application on pollen viability (%) under elevated CO2 was calculated and the result is presented in table 39. Pollen viability was found to reduced drastically under elevated CO_2 (17.58 %) compared to that under open condition (99.62 %). All the treatments effectively improved the pollen viability under OTC conditions.

Under CO_2 enrichment, application of T3 was most effective in improving the pollen viability to a significantly higher percent (30.28 %) compared to the absolute control (10.34 %).

2.3.5 Pollen morphology

The impact of various treatments on pollen morphology was studied using scanning electron microscopy (SEM) images. The SEM image of pollen under 150 X in open condition (Plate 9 (a)) shows visible round intact pollen. This was more clearly visible in a magnified image (Plate 9 (c)) of single pollen (3500 X).

Similarly the SEM images of pollen under elevated CO_2 showed no visible round pollen grains (150 X) (Plate 9 (b)). A higher magnification (1500 X) clearly shows the distorted structure of individual pollen grain (Plate 9 (d)).

Table 35. Effect of growth regulators and nutrient application on days to first flowering in tomato under CO_2 enrichment.

	Open condition	OTC condition	Mean
T1 (50ppm NAA)	45.00	47.00	46.00
T2 (50ppm Salicylic acid)	45.00	47.00	46.00
T3 (50 ppm boron)	45.00	47.00	46.00
T4 (50ppm B+ 50ppm Zn)	45.00	47.00	46.00
T5 (POP + 25%N)	45.00	47.00	46.00
T6 (POP 125 N: 125P: 125 K)	45.00	47.00	46.00
T7 (water spray)	45.00	47.00	46.00
T8 (absolute control)	45.00	47.00	46.00
Mean	45.00	47.00	
	T	E	T X E
SE. m ±	0.87	0.44	1.23
CD (0.05)	NS	1.26	NS

OTC- Open top chamber

Table 36. Effect of growth regulators and nutrient application on days to 50 % flowering in tomato under CO_2 enrichment.

	Open condition	OTC condition	Mean
T1 (50ppm NAA)	49.00	52.00	50.50
T2 (50ppm Salicylic acid)	49.00	52.00	50.50
T3 (50 ppm boron)	49.00	52.00	50.50
T4 (50ppm B+ 50ppm Zn)	49.00	52.00	50.50
T5 (POP + 25%N)	49.00	52.00	50.50
T6 (POP 125 N: 125P: 125 K)	49.00	52.00	50.50
T7 (water spray)	49.00	52.00	50.50
T8 (absolute control)	49.00	52.00	50.50
Mean	49.00	52.00	
	T	E	T X E
SE. m ±	0.65	0.32	0.91
CD (0.05)	NS	0.93	NS

OTC- Open top chamber

Table 37. Effect of growth regulators and nutrient application on number of flower clusters per plant in tomato under CO_2 enrichment.

	Open condition	OTC condition	Mean
T1 (50ppm NAA)	5.33	6.67	6.00
T2 (50ppm Salicylic acid)	4.33	6.33	5.33
T3 (50 ppm boron)	5.33	6.33	5.83
T4 (50ppm B+ 50ppm Zn)	4.67	5.33	5.00
T5 (POP + 25%N)	4.00	5.67	4.83
T6 (POP 125 N: 125P: 125 K)	4.67	8.00	6.33
T7 (water spray)	3.00	6.00	4.50
T8 (absolute control)	3.00	4.33	3.67
Mean	4.29	6.08	
	T	E	T X E
SE. m ±	1.35	0.68	1.91
CD (0.05)	0.47	0.23	0.66

OTC- Open top chamber

Table 38. Effect of growth regulators and nutrient application on flowers per cluster in tomato under CO_2 enrichment.

	Open condition	OTC condition	Mean
T1 (50ppm NAA)	6.33	2.52	4.43
T2 (50ppm Salicylic acid)	7.33	1.33	4.33
T3 (50 ppm boron)	8.33	2.00	5.17
T4 (50ppm B+ 50ppm Zn)	6.89	3.67	5.28
T5 (POP + 25%N)	9.33	1.50	5.42
T6 (POP 125 N: 125P: 125 K)	7.33	1.67	4.50
T7 (water spray)	8.00	2.00	5.00
T8 (absolute control)	6.00	1.30	3.65
Mean	7.45	2.00	
	T	E	T X E
SE. m ±	1.21	0.60	1.71
CD (0.05)	0.42	0.21	0.59

OTC- Open top chamber

Table 39. Effect of growth regulators and nutrient application on pollen viability in tomato under CO_2 enrichment.

	Open condition	OTC condition	Mean
T1 (50ppm NAA)	99.60	10.52	55.06
T2 (50ppm Salicylic acid)	99.23	11.50	55.37
T3 (50 ppm boron)	99.93	30.28	65.11
T4 (50ppm B+ 50ppm Zn)	99.93	23.26	61.60
T5 (POP + 25%N)	99.90	12.50	56.20
T6 (POP 125 N: 125P: 125 K)	99.97	23.48	61.72
T7 (water spray)	99.33	19.32	59.33
T8 (absolute control)	99.10	10.34	54.72
Mean	99.62	17.58	
	T	E	T X E
SE. m ±	1.83	0.92	2.59
CD (0.05)	0.63	0.32	0.90

OTC- Open top chamber

Plate 6. Floral deformities inside Open Top Chamber

Plate 6 (a). Stigma exertion

Plate 6 (b). Drying of flowers

Plate 7. Pollen viability test using acetocarmine stain

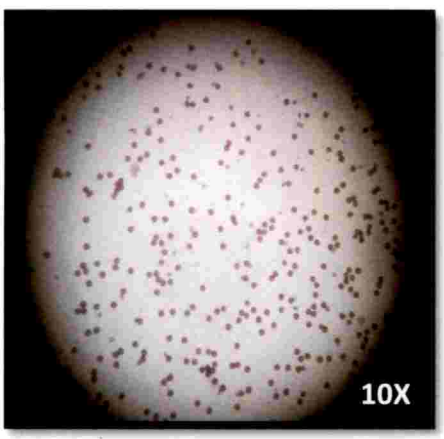


Plate 7 (a). Viable pollen under open condition

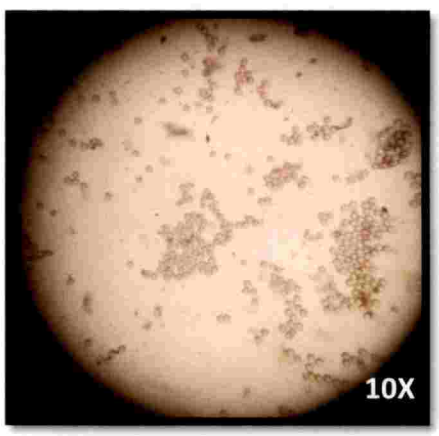


Plate 7 (b). Non- viable pollen under elevated CO_2 condition

Plate 8: Pollen viability under CO_2 enriched condition

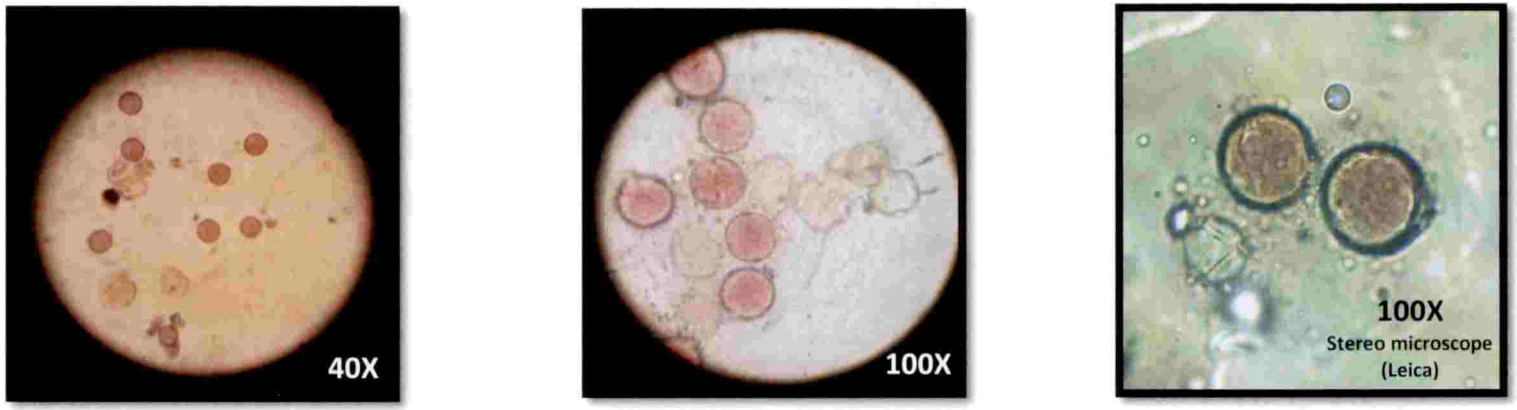


Plate 8 (a): Effect of Boron (50 ppm) on pollen viability under elevated CO_2 condition

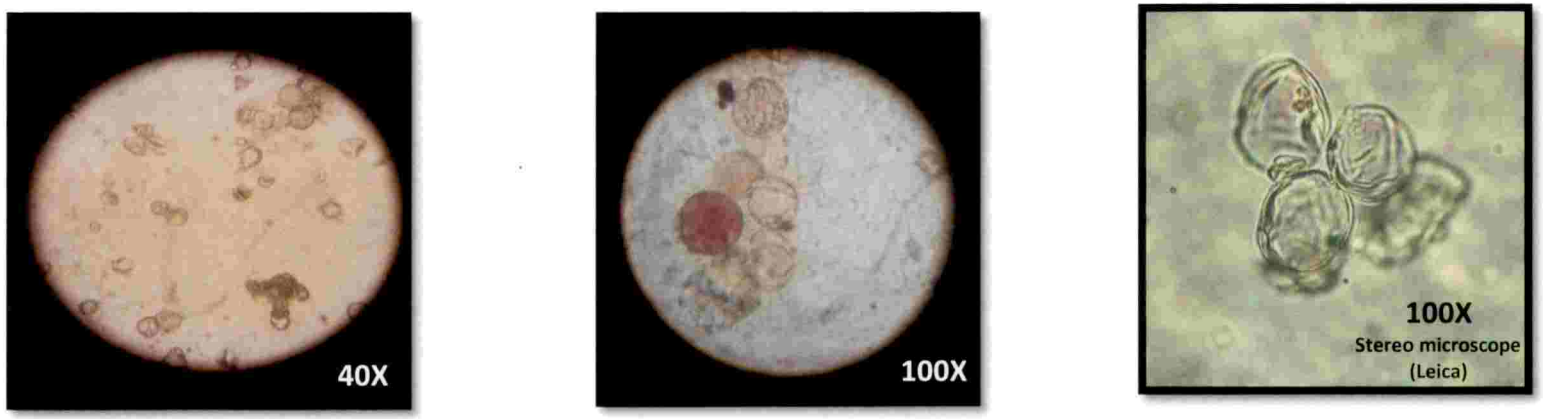


Plate 8 (b): Pollen viability of control plants under elevated CO_2 condition

Plate 9: Scanning electron microscopic (SEM) images of tomato pollen

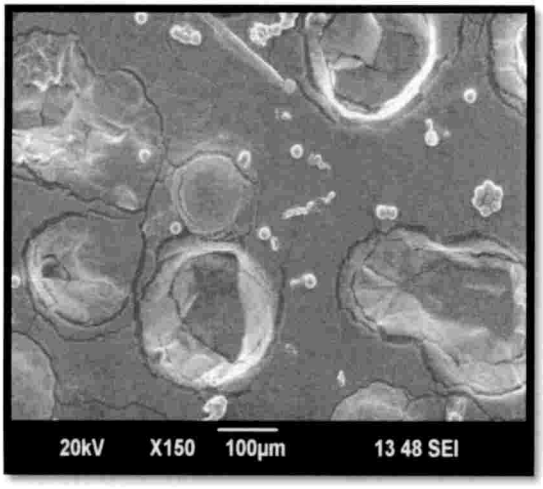


Plate 9 (a): SEM images of pollen under open condition (150 X)

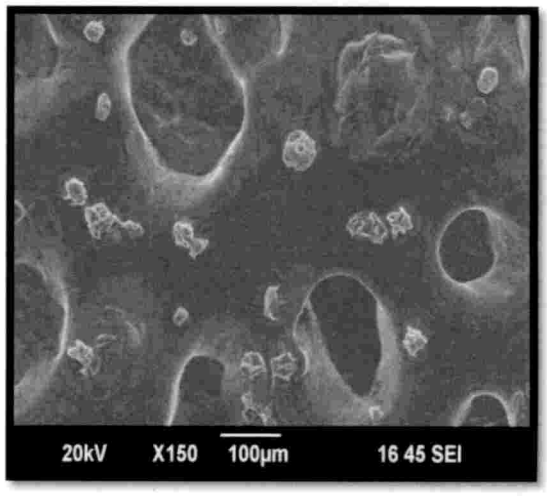


Plate 9 (b): SEM images of pollen under elevated CO_2 condition (150 X)

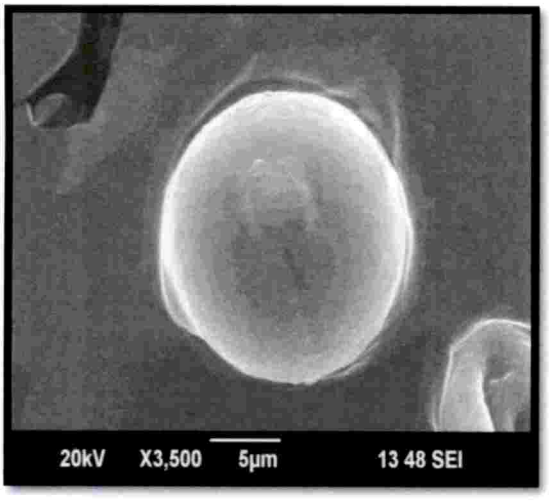


Plate 9 (c): SEM images of pollen under open condition (3,500 X)

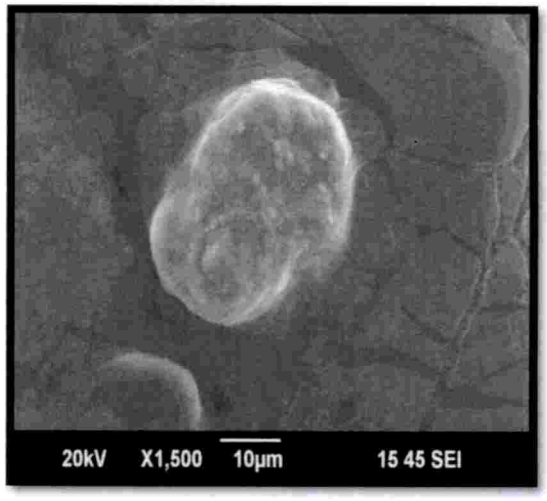


Plate 9 (d): SEM images of pollen under elevated CO_2 condition (1,500 X)

2.4 1 Effect of growth regulators and nutrient applications on yield parameters in tomato under CO_2 enrichment at 60 DAS and 75 DAS

2.4.1 Days to fruiting

The impact of various growth regulators on the days to fruiting was recorded and the result is presented in table 40. Foliar application of 50 ppm B + 50 ppm Zn resulted in early fruiting (67.33 DAS). The treatment T8 and T7 delayed days to fruiting significantly (73.67 DAS). Fruiting was earlier in plants kept in open condition (47.71 DAS) compared to plants kept under CO_2 enriched condition (52.50 DAS).

2.4.2 Fruit weight

Influence of various treatments on fruit weight was evaluated and result is presented in table 41.Water spray was found to improve the fruit weight significantly (34.81 g) compared to control (12.60 g).

Under elevated CO2 condition, T7 resulted in significantly higher fruit weight (42.86 g) compared to control (13.35 g). All treatments, except T1 and T2 resulted in significant improvement in fruit weight.

2.4.3 No: of fruits per plant

Table 42 represents the impact of growth regulators and nutrient application on number of fruits per plant under elevated CO_2. All treatments had significant influence on number of fruits per plant. Among the treatments, application of 50 ppm B + 50 ppm Zn resulted in significantly higher number of fruits (11.17) which was on par with T4 (10.50) and T3 (9.33) compared to control (5.67).

Exposure to elevated CO_2 was found to significant influence number of fruits per plant. A drastic reduction in number of fruits was noticed in OTC condition (2.96) compared to open condition (13.67).

2.4.4 Fruit setting %

Application of growth regulators and nutrients influenced the fruit setting percent in tomato plants. The results is presented in table 43. Various treatments improved fruit setting percent. Application of 50 ppm B (38.52 %) resulted in significant increase in fruit setting percent which was on par with T4 (35.86 %), T5 (36.87 %) and T6 (35.25 %). Application of 50 ppm NAA resulted in lowest fruit setting percent.

Fruit setting % was found to drastically decrease under enriched CO_2 condition. A reduction in fruit setting percent was seen inside OTC (7.76 %) compared to open condition (61.46 %).

2.4.5 Intensity of fruiting

Impact of various treatments on growth regulators and nutrient application on intensity of fruiting was estimated and is represented in table 44. The intensity of fruiting was significantly higher in T6 (56.37 %) compared to control (46.19 %). Intensity of fruiting was least in plants treated with 50 ppm NAA (25.03 %).

A significant decrease in intensity of fruiting was noticed under elevated CO_2 conditions (26.49 %) compared to control (62.44 %). Within OTC, significantly higher intensity of fruiting was observed under treatment T4 (40.20 %) compared to control (31.77 %).

2.4.6 Intensity of fruit drop

Intensity of fruit drop as influenced by the application of various growth regulators and nutrient application was calculated and is presented in table 45. Intensity of fruit drop increased under CO_2 enrichment compared to open field. Application of additional NPK resulted in least intensity of fruit drop (29.94 %) compared to control (34.12 %). Fruit drop was found to be highest in plants treated with 50 ppm NAA (41.09 %).

Table 40. Effect of growth regulators and nutrient application days to fruiting in tomato under CO_2 enrichment.

	Open condition	OTC condition	Mean
T1 (50ppm NAA)	47.67	0.00	23.83
T2 (50ppm Salicylic acid)	47.67	0.00	23.83
T3 (50 ppm boron)	48.00	68.00	58.00
T4 (50ppm B+ 50ppm Zn)	47.67	67.33	57.50
T5 (POP + 25%N)	47.67	68.33	58.00
T6 (POP 125 N: 125P: 125 K)	47.67	69.67	58.67
T7 (water spray)	47.67	73.00	60.33
T8 (absolute control)	47.67	73.67	60.67
Mean	47.71	52.50	
	T	E	T X E
SE. m ±	1.28	0.64	1.81
CD (0.05)	0.44	0.22	0.62

OTC- Open top chamber

Table 41. Effect of growth regulators and nutrient application on fruit weight (g) in tomato under CO_2 enrichment.

	Open condition	OTC condition	Mean
T1 (50ppm NAA)	23.01	0.00	11.51
T2 (50ppm Salicylic acid)	13.89	0.00	6.95
T3 (50 ppm boron)	18.42	18.22	18.32
T4 (50ppm B+ 50ppm Zn)	21.16	37.06	29.11
T5 (POP + 25%N)	21.02	14.28	17.65
T6 (POP 125 N: 125P: 125 K)	26.27	17.46	21.86
T7 (water spray)	26.75	42.86	34.81
T8 (absolute control)	11.85	13.35	12.60
Mean	20.30	17.90	
	T	E	T X E
SE. m ±	3.49	1.74	4.93
CD (0.05)	1.21	0.60	1.70

OTC- Open top chamber

Table 42. Effect of growth regulators and nutrient application on number of fruits per plant in tomato under CO_2 enrichment.

	Open condition	OTC condition	Mean
T1 (50ppm NAA)	11.00	0.00	5.50
T2 (50ppm Salicylic acid)	14.00	0.00	7.00
T3 (50 ppm boron)	13.67	5.00	9.33
T4 (50ppm B+ 50ppm Zn)	16.00	5.00	10.50
T5 (POP + 25%N)	15.33	7.00	11.17
T6 (POP 125 N: 125P: 125 K)	12.67	2.33	7.50
T7 (water spray)	12.67	3.00	7.83
T8 (absolute control)	10.00	1.33	5.67
Mean	13.67	2.96	
	T	E	T X E
SE. m ±	2.59	1.30	N A
CD (0.05)	0.90	0.45	1.27

OTC- Open top chamber

Table 43. Effect of growth regulators and nutrient application on fruit setting (%) in tomato under CO_2 enrichment.

	Open condition	OTC condition	Mean
T1 (50ppm NAA)	59.19	0.00	29.59
T2 (50ppm Salicylic acid)	68.20	0.00	34.10
T3 (50 ppm boron)	65.51	11.50	38.50
T4 (50ppm B+ 50ppm Zn)	57.41	14.32	35.86
T5 (POP + 25%N)	56.35	17.38	36.87
T6 (POP 125 N: 125P: 125 K)	63.08	7.41	35.25
T7 (water spray)	61.89	7.36	34.62
T8 (absolute control)	60.03	4.14	32.08
Mean	61.46	7.76	
	T	E	T X E
SE. m ±	3.55	1.77	5.02
CD (0.05)	1.23	0.61	1.73

OTC- Open top chamber

Table 44. Effect of growth regulators and nutrient application on intensity of fruiting (%) in tomato under CO_2 enrichment.

	Open condition	OTC condition	Mean
T1 (50ppm NAA)	50.06	0.00	25.03
T2 (50ppm Salicylic acid)	65.66	0.00	32.83
T3 (50 ppm boron)	54.44	40.20	47.32
T4 (50ppm B+ 50ppm Zn)	67.20	38.48	52.84
T5 (POP + 25%N)	64.33	32.70	48.51
T6 (POP 125 N: 125P: 125 K)	78.12	34.61	56.37
T7 (water spray)	59.10	34.22	46.66
T8 (absolute control)	60.61	31.77	46.19
Mean	62.44	26.50	
	T	E	T X E
SE. m ±	2.00	1.00	2.82
CD (0.05)	0.69	0.35	0.98

OTC- Open top chamber

Table 45. Effect of growth regulators and nutrient application on intensity of fruit drop (%) in tomato under CO_2 enrichment.

	Open condition	OTC condition	Mean
T1 (50ppm NAA)	32.19	50.00	41.10
T2 (50ppm Salicylic acid)	25.56	50.00	37.78
T3 (50 ppm boron)	31.00	40.35	35.68
T4 (50ppm B+ 50ppm Zn)	28.00	44.72	36.36
T5 (POP + 25%N)	27.56	37.41	32.49
T6 (POP 125 N: 125P: 125 K)	21.30	38.58	29.94
T7 (water spray)	32.45	43.39	37.92
T8 (absolute control)	24.79	43.46	34.12
Mean	27.86	43.49	
	T	E	T X E
SE. m ±	3.39	1.69	4.79
CD (0.05)	1.17	0.59	1.66

OTC- Open top chamber

2.4.7 Yield per plant

The impact of growth regulators and additional nutrients on the yield per plant in tomato under elevated CO_2 was recorded and is presented in table 46. Among treatments, significantly higher yield was recorded in plants under treatment T5 (190.85 g plant^{-1}) compared to control (61.83 g plant^{-1}). The lowest yield was recorded in plants treated with 50 ppm SA (56.71 g plant^{-1}).

A significant reduction in yield per plant was noticed inside OTC chamber (35.83 g plant^{-1}) compared to open condition (194.06 g plant^{-1}). However, this was improved by the application of various treatments. The significantly higher yield inside OTC was observed in T5 (73.24 g plant^{-1}) compared to control (13.19 g plant^{-1}).

2.4.8 Lycopene

Table 47 shows the variation in lycopene content under elevated CO_2 condition in effect to various treatments. Among treatments, highest lycopene content was recorded in plants under treatment T3 (19.78 µg g^{-1} fresh weight) which was on par with T6 (19.77 µg g^{-1} fresh weight), T1 (19.11 µg g^{-1} fresh weight), T5 (18.74 µg g^{-1} fresh weight), T2 (18.51 µg g^{-1} fresh weight). The lowest lycopene content was recorded in T8 (15.67 µg g^{-1} fresh weight).

Lycopene content was found to be decreasing under elevated CO_2 condition (16.35 µg g^{-1} fresh weight) compared to plants under open condition (19.85 µg g^{-1} fresh weight). This reduction in lycopene was compensated by application of various growth regulators and additional nutrients. T3 resulted in significantly higher lycopene content (19.23 µg g^{-1} fresh weight) compared to control (13.35 µg g^{-1} fresh weight).

Table 46. Effect of growth regulators and nutrient application on yield per plant (g plant^{-1}) in tomato under CO_2 enrichment.

	Open condition	OTC condition	Mean
T1 (50ppm NAA)	154.43	0.00	77.22
T2 (50ppm Salicylic acid)	113.42	0.00	56.71
T3 (50 ppm boron)	127.54	52.08	89.81
T4 (50ppm B+ 50ppm Zn)	227.44	69.14	148.29
T5 (POP + 25%N)	308.45	73.25	190.85
T6 (POP 125 N: 125P: 125 K)	270.42	39.99	155.21
T7 (water spray)	240.28	39.00	139.64
T8 (absolute control)	110.46	13.19	61.83
Mean	194.06	35.83	
	T	E	T X E
SE. m ±	1.90	0.95	2.69
CD (0.05)	0.66	0.33	0.93

OTC- Open top chamber

Table 47. Effect of growth regulators and nutrient application on lycopene ($\mu g\ g^{-1}$ fresh weight) in tomato under CO_2 enrichment.

	Open condition	OTC condition	Mean
T1 (50ppm NAA)	19.79	18.43	19.11
T2 (50ppm Salicylic acid)	19.57	17.45	18.51
T3 (50 ppm boron)	20.34	19.23	19.78
T4 (50ppm B+ 50ppm Zn)	19.20	13.77	16.49
T5 (POP + 25%N)	21.15	16.33	18.74
T6 (POP 125 N: 125P: 125 K)	21.40	18.14	19.77
T7 (water spray)	19.80	13.68	16.74
T8 (absolute control)	17.59	13.75	15.67
Mean	19.86	16.35	
	T	E	T X E
SE. m ±	1.39	0.70	1.97
CD (0.05)	0.48	0.24	0.68

OTC- Open top chamber

DISCUSSION

5. DISCUSSION

Travelling through Earths recent history of atmosphere, we can find a significantly higher amount of CO_2. Carbon dioxide, an important heat-trapping (greenhouse) gas, is being fueled by human activities such as deforestation and burning fossil fuels, as well as natural processes such as respiration and volcanic eruptions. Reports by Global Project forecast 2017 shows that CO_2 emissions have climbed by 6.3 % in India between 2017 and 2018 which is three times compared to the last year (Rashid *et al.*, 2018). Latest report by NASA in April, 2019 shows that the CO_2 concentration has reached 411 ppm. The temperature data released by Copernicus Climate Change Service shows that the global average surface air temperature was 14.7°C, recording the year 2018 as fourth warmest year ever so recorded (Copernicus climate change service, ECMWF, 2018).

Tomato is the second most important vegetable crop in India accounting 15.8 t/ha average productivity. However, it is highly sensitive to fluctuations in temperature, water availability etc. The most sensitive stage is just after transplanting, at flowering and fruit developmental stages (Vijitha and Mahendran, 2010). Developing anthers and pollen grains, pollen tube growth, fertilization and early embryo growth are most affected under high temperature stress conditions

In this background, efforts were made in Department of Plant Physiology, College of Agriculture, Vellayani to understand the deleterious effect of this ever increasing CO_2 and associated high temperature on crop plants. The results of those studies showed a positive impact of the increased atmospheric CO_2 on the vegetative growth of plants which was not reflected on the final yield (Chatti and Manju, 2018). This was attributed mainly to the impact of the high temperature on floral morphology and pollen viability, which affected the fruit set. So, in the present study, efforts were made to address the negative impacts on the pollen viability and floral morphology

through the application of various growth regulators, nutrient application and temperature induction response technique.

EXPERIMENT 1

The entire programme was divided into two experiments. Part one of the first experiment consisted of standardization of TIRT for tomato and the second part was evaluation of the temperature induced seedlings under CO_2 enriched condition for assessing any improvement in stress tolerance. Acquired thermos-tolerance could be gained through induction. The technique of exposing young seedlings to sub lethal and lethal temperatures has been validated in many crop species (Kheir *et al.,* 2012 in cotton; Gangappa *et al.,* 2006 in groundnut).

This acquired resistance for different abiotic stresses have been made use of for dealing with various stresses like high temperature, dry spell, saltiness, chilling and so on. From this perspective, a protocol called Temperature Induction Response Technique (TIRT) has been developed as an effective tool for improving stress tolerance. So the primary objective of the first experiment was standardization of the lethal temperature as well as the induction range for two popular tomato varieties of Kerala, namely Vellayani Vijay and Anagha.

In the first experiment, lethal and induction temperatures for Vellayani Vijay and Anagha were identified. For this 5 day old tomato seedlings were subjected to a range of temperature for different duration. Result showed that as the temperature increased from 42- 48 °C, the percentage recovery of seedlings after treatment decreased thereby increasing the mortality rate. Seedlings exposed to 48 °C for 2 h showed 100 % mortality in both the varieties. Hence, exposure to 48 °C for 2 h was identified as the lethal temperature for both the varieties. A similar study was done by Chandola (2015) where 48°C for 2 h was selected as the lethal temperature in tomato utilizing thirty genotypes. A comparative study was reported in banana, where 55°C for 2h was chosen as lethal temperature with 11% recovery (Suseela *et al.*, 2017).

Sdhakar *et al.* (2012) revealed lethal temperature of rice as 52°C for 3 h with 98 % mortality. A study by Srikanthbabu *et al.* 2002 showed that the lethal temperature for pea was 48°C for 1h with 10% survivability. A lethal temperature of 54°C for 3 h was chosen as best challenging temperatures for screening of rice seedlings for heat tolerance (Vijayalakshmi *et al.*, 2015). Gangappa *et al.* (2006) observed lethal temperature in groundnut as 55 °C.

Following standardization of lethal temperatures, induction cycles were optimized as 38 °C (1h) - 43 °C (1h) and then to lethal temperature 48°C for 2 h. This was standardized based on the recovery response of induced seedlings after exposure to lethal temperature.

Impact of temperature induction response in resisting the stress condition occurring due to elevated CO_2 was studied by analyzing the performance of the temperature induced seedlings under elevated CO_2 conditions. The growth, physiological and biochemical parameters of the induced seedlings in comparison with non-induced were evaluated under elevated CO_2 and ambient conditions.

Impact of Temperature Induction on growth, physiological and biochemical parameters of tomato under CO_2 enrichment

Plants are seen to overcome stress by adopting several physiological and biochemical mechanisms, in order to acclimatize to the stressful situation. The induced plants showed such adaptations in improving photosynthetic parameters as well as enhanced defense mechanism. Acquired tolerance is ubiquitous and has been demonstrated in several species (Senthil *et al.*, 2007).

Results showed that plants without temperature induction showed significantly higher values for plant height, number of branches and number of leaves compared to induced plants under both the conditions. This was contradictory to the findings of Chandola (2015) in tomato, where plant height was found to increase in temperature induced plants as compared to non-induced plants. However, SLA was found to be

significantly higher in induced plants compared to non-induced plants. This may be attributed to the extra carbon dioxide available for plants for the expansion of leaf area for better photosynthesis.

In the present study, induced plants showed significantly higher value for total chlorophyll content (1.74 mg g^{-1}) compared to control plants. Normally plants under stress produce less of the photosynthetic pigment compared to non- stressed plants. But here, even the stressed plant under elevated CO_2 conditions showed better chlorophyll content. This may be attributed to better antioxidant mechanism developed in induced plants that helped them to perform better. Hence, the ability of plants to utilize the nutrients for the formation of various pigments was possible which was reflected in the total chlorophyll content.

Kheir *et al.* (2012) reported an increase in the total soluble protein content in plants that were pre exposed to TIR at early stages. Contrary to this, in the present study the total soluble protein was found to decrease by 15.07 % in plants that had undergone temperature induction compared to control plants. Similar results was reported by Pal (2004) where soluble protein content decreased under CO_2 induced stress condition in berseem grown under elevated CO_2. This reduction in TSP may be attributed to the reduction in Rubisco content under stress, which constitute major part of total soluble protein in plants.

A study by Fender and Connel (1990) in tomato showed an improvement in HSP levels in plants that recovered from a mild heat stress compared to normal plants. In a similar study by Pareek *et al.* (1997) reported that when the rice seedlings were exposed to 45 °C for eight hours resulted in accumulation of HSP 90 and HSP 104 resulting in a better plant performance under lethal heat stress conditions. Due to the production of these HSPs, plants are seen to tolerate a lethal temperature level (Gangappa., 2006). The HSPs are known to have an important biochemical role during the heat stress as they act as molecular chaperons by helping in assisting proteolytic

degradation, protection of various cell organelles a the folding of polypeptides thus preventing incorrect folding (Gangappa., 2006). Similar tolerance was obtained in plants that had undergone temperature induction in the current experiment.

The result of the present study clearly showed an improvement in the total carbohydrate by 36.7 % in case of temperature induced plants, compared to control plants. A reduced transpiration rate by 14.67 % with a higher photosynthetic rate (19.17 %) was also observed in the temperature induced plants, resulting in an increased water use efficiency by 43.10 %. This may be attributed to the acquired thermo-tolerance and due to the accumulated heat shock proteins (HSP) which enabled the plant to perform better.

Relative water content was found to increase by 2.02 % in case on temperature induced seedlings in comparison to non- induced control seedlings. RWC is the most appropriate measurement that shows the plant water status. A higher value for RWC indicate the plants ability to sustain water levels irrespective of the stress condition. This means that the induced plants are able to maintain water and perform its normal activities in a better manner compared to the non-induced plants.

Similarly, the defense mechanism of the temperature induced seedlings were found to be enhanced by the increased production of certain enzymatic antioxidants like superoxide dismutase, peroxidase and osmo-protectant like proline. An increase in SOD activity (27.50%), peroxidase activity (5.8 %), proline content (51.31%) was noticed in the temperature induced seedlings in comparison to control plants. This increased antioxidant activity shows that plants that has undergone temperature induction has improved stress tolerance mechanism. The antioxidants help in scavenging the ROS produced during the stress situation and thus help the plant to overcome the problems cased due to high temperature stress (Hameed *et al.*, 2015). Thus this accumulation and maintenance of higher levels of antioxidants in temperature

induced seedlings can be a better protection mechanism in plant cell (Chutipaijit., 2016).

Sairam *et al.* (1997) reported that both drought stress and temperature stress decreased membrane stability, chlorophyll content and chlorophyll stability index in all wheat genotypes. Chlorophyll stability index is an indicative of maintenance of photosynthetic pigments even under stressful situation (Nahakpam, 2018). In the present study, chlorophyll stability index (145 %) was noted to be significantly higher in the temperature induced plants. The higher chlorophyll stability index in temperature induced plants shows their capacity to withstand stress through better availability of chlorophyll and hence better photosynthetic rate and higher dry matter accumulation (Bahuguna *et al.*, 2015).

The results revealed that TIRT is a robust and powerful technique in improving the stress tolerance and photosynthetic mechanism of plants which can be attributed to enhanced stress protein production. These stress proteins bring about required changes in the cell metabolism for enhanced performance under a similar stress condition. There are many reports on increased expression of these heat shock proteins and their localization in various parts during induction period (Brothers *et al.*, 1993). On the other hand, plants without temperature induction resulted in poor performance under stress. From this, it can be concluded that TIRT is a powerful technique that can be used to produce acquired resistance in plants under heat stress, in order to ensure a better performance even under the stressful situation.

EXPERIMENT 2

In the second experiment, flowering and fruiting in tomato under elevated CO_2 environment, as influenced by growth regulators and nutrient applications, were evaluated. The experiment was laid out in CRD with eight treatments and three replications. The treatments comprised of 50 ppm NAA (T1), 50 ppm Salicylic acid (T2), 50 ppm Boron (B) (T3), 50 ppm Boron (B) + 50 ppm Zinc (Zn) (T4), as foliar

spray (at 40,55 and 75 DAS), nutrient application of POP 125% N: 100% P: 100% K (T5), POP 125% N: 125% P: 125% K (T6), a control (water spray) (T7) and an absolute control (T8). The effect of these treatments on growth parameters, physiological and biochemical parameters, parameters related to flowering, yield and quality parameters in tomato were analyzed at two intervals (60DAS and 75 DAS) under elevated CO_2 conditions as well as open condition. The results are as discussed below.

Effect of growth regulators and nutrient application on growth parameters of tomato under CO_2 enrichment.

Plant morphogenesis and physiology is influenced by the changing environmental condition. Among the changes in the environment, the increased CO_2 is having a profound influence on the growth parameters of the plants. A positive influence of increased CO_2 on the growth parameters is evident from the results of the present study. Elevated CO_2 generally stimulates C_3 photosynthesis more than C_4. For C_3 plants the positive responses by CO_2 enrichment are mainly attributed by the competitive inhibition of photorespiration (Loomis and Durst, 1992). The various growth parameters considered under this study including plant height, number of branches, number of leaves and specific leaf area were found to increase significantly under elevated CO_2 condition.

The plant height was found to increase significantly under CO_2 enrichment at 75 DAS. 58.94 % increase in plant height was observed under elevated CO_2 at 75 DAS. Among the treatments, application of 50 ppm boron significantly increased plant height by 8.11 % and 6.89 % at 60 DAS and 75 DAS respectively. The increase in plant height could be related to increase in the number of nodes and stimulation of elongation of the internodes. The result was in agreement with the findings of Naz *et al.* (2012), where boron positively influenced the growth parameters including plant height in tomato variety, Rio Grand. A similar result was observed in the work of Bomesh *et al.* (2016) in greenhouse cucumber where he found that application of boron has resulted in improved vine length. Boron along with calcium is used by plants in synthesis of cell

wall and is essential for cell division. Boron also plays an important role in cell strength and development, sugar transport, and hormone development. It also interacts with specific pathways of nitrogen, phosphorus and calcium, rather stimulating or inhibiting certain metabolic pathways. All these roles of boron may have contributed to the better growth response of the plants supplied with boron spray.

Number of leaves, leaf anatomy and leaf size were observed to alter under elevated CO_2 condition and its magnitude depend on many genetic factors (Pritchard *et al.,* 1999). In the present study, number of leaves did not show significant increase at 60 DAS but increased significantly at 75 DAS under elevated CO_2 condition. Irrespective of the conditions, application of extra nitrogen resulted in significant increase in plant height (78.36 %) at 75 DAS. The result was in agreement to the findings of Yelle *et al.* (1989) where number of leaves significantly increased by addition of extra nitrogen under enriched CO_2 conditions. Besides having a positive influence on the number of leaves, elevated CO_2 also resulted in more branching in tomato plants in the present investigation. Irrespective of the condition, application of extra nitrogen increased the branching by 73.67 % at 75 DAS compared to control. This was in agreement with the work of Mahapatra *et al.* (1996) in pointed gourd and Haque *et al.* (2011) in tomato, where they found out that extra nitrogen resulted in improved branching under elevated CO_2 conditon.

Specific leaf area (SLA) is an indicator of leaf thickness. Under elevated CO_2 condition, SLA increased by 38.97 % and 49.91 % at 60 and 75 DAS respectively. Irrespective of conditions, application of extra NPK resulted in 50.30% increase in the specific leaf area in tomato at 75 DAS. in his study on the effect of nitrogen application in drip irrigated tomato concluded that, a higher amount of nitrogen resulted in an improvement in the specific leaf area. This may be due to the higher availability of nitrogen, which has resulted in progressive enhancement of vegetative growth and leaf expansion.

Effect of growth regulators and nutrient application on physiological and biochemical parameters of tomato under CO_2 enrichment

The effect of growth regulators and nutrient application on physiological and biochemical parameters like total chlorophyll content, total soluble protein, total carbohydrate, transpiration rate, photosynthetic rate, water use efficiency, chlorophyll fluorescence, relative water content (RWC), superoxide dismutase (SOD), peroxidase, chlorophyll stability index (CSI) and proline content were analyzed under elevated CO_2 conditions and the results are as discussed below.

Plant productivity depends on the chlorophyll content of that plant, as it is the primary pigment which helps in photosynthesis. In the present study, the total chlorophyll content was found to increase by 46.61 % under CO_2 enrichment compared to open condition at 60 DAS. Under elevated CO_2 condition, there occurs enlargement of chloroplast and increase in the number of chloroplasts present in leaf tissue, which results in a higher chlorophyll content in plants under such conditions.

The amount of chlorophyll in leaf tissues is also influenced by nutrient availability and environmental stresses. In the present study, under the elevated CO_2 condition, various treatments improved the chlorophyll content significantly among which, boron 50 ppm was found to exhibit the highest percent increase in chlorophyll content (55 %) compared to control. This is totally in agreement with the findings of Ahmed *et al.* (2011) in cotton where an improvement in the chlorophyll content was achieved by the application of 50 ppm boron. This may be due to the impact of boron on nitrogen metabolism and also positive influence on uptake of other plant nutrients essential for the production of chlorophyll molecule (Ahmed *et al.*, 2011). The increase in chlorophyll content as a result of application of extra nitrogen may be explained by the larger size and number of chloroplasts due to enhanced availability of nutrients.

The total soluble proteins comes under the primary plant metabolites, which is affected by the overall plant mechanism. The CO_2 enrichment was found to have a

profound negative impact on the total soluble protein content. This was as explained by Lin and Wang (2002) where a reduction in total soluble protein was observed in wheat cultivars under CO_2 enrichment. This may be due to a reduction in ribulose - 1,5- bisphosphate carboxylase/ oxygenase (RUBISCO) protein and hence an overall reduction in protein content was noted.

In the present experiment, total soluble protein was found to be decreasing within OTC compared to control. The result was in accordance with the works in amaranthus by Chatti and Manju (2015), in sunflower by Tezara *et al.,* (2002), in maize by Driscoll *et al.*, (2005) and in pine tree by Schwanz, P and Polle, A., (2001), where they found reduction in protein content with CO_2 enrichment. But this was found to be improved by the application of various growth regulators and nutrients. All the treatments were found to increase the total soluble protein compared to control. The result clearly shows that application of 50 ppm Boron resulted in an increase of TSP by 30.27 %. However this was on par with plants under treatment with 50 ppm SA, 50 ppm B + 50ppm Zn, 25 % additional nitrogen and 25 % additional NPK. Boron is having a prominent role in plant nitrogen metabolism and indirectly affects the protein synthesis. Reduced nitrate reductase activity was reported in boron deficient sugarbeer by Mateo *et al.* (2006) which clearly shows that boron has a role in protein metabolism. It was also reported that adequate levels of Boron increased the nitrate reductase activity, based initially on protein synthesis (Shelp and Liu, 1992). In the present experiment, exposure to increased CO_2 and associated high temperature may have reduced the absorption of boron from soil and in turn when applied as foliar spray could be easily utilized by plants .Subsequently, the role of boron in protein synthesis could have positively influenced the increased total soluble protein content in the present experiment.

Several studies have shown that elevated CO_2 has enhanced the accumulation of carbohydrates in plants. It was reported that there was an increase in the carbohydrate accumulation in tomato plants when exposed to elevated CO_2 conditions

(800 µmol mol^{-1}) (Li *et al.*, 2013). The results of the present study was in agreement to this, showing that the total carbohydrate increased under elevated CO_2 conditions compared to open condition. 52% increase in total soluble carbohydrate content was observed in beech leaves by Landolt *et al.* (1997). Wang et al. (2003), in his work on strawberry reported a higher carbohydrate accumulation under elevated CO_2 condition. Carbohydrates content accumulate in plant tissues, under CO_2 enrichment, since their usage intensity is lower than their production under these conditions (Moore *et al.*, 1998).

The carbohydrate content of the tomato leaves was most pronounced in plants treated with extra NPK which resulted in 8.49 % increase when compared to control plants. This may be attributed to additional nutrients that help the plant to use them for the plant process irrespective of the stress condition. NPK is essential for synthesis of diverse compounds needed of plant metabolism. The increased nitrogen content has got a positive impact on the adventitious root system which contributes to the total carbohydrate pool (Blazich, 1988). Nitrogen supply highly influences the allocation of carbon and its partitioning in plants (Kaiser *et al.*, 1997). Significant increase in the fructose and sucrose levels were obtained in plants supplied with high NPK (Druege *et al.*, 2000). The increased carbohydrate content in plants treated with extra NPK can thus be attributed to higher carbon allocation and partitioning increasing the carbohydrate accumulation.

Reduction in transpiration rate was observed in plants in response to enriched CO_2 condition, brought about by partial closure of the stomata and decreased the stomatal conductance (Morison and Gifford, 1983 and Bunce, 2000). This is clear from the results of Apple *et al.*, (2000) on Douglas fir seedlings grown for three years in environmental chambers under CO_2 concentration of 530 ppm + 3.5°C, where, 12% reduction of transpiration rate was noticed.

Similar results were obtained in the present study where, the plants under elevated CO_2 showed decreased transpiration rate. A significant reduction in transpiration rate under such a situation was observed by the application of extra nitrogen resulting better plant performance. Extra nitrogen increased the transpiration rate by 17.37 % compared to control. These results were found to be in agreement with the studies conducted by, in eggplant, by Tezara *et al.* (2002) and Chaturvedi *et al.* (2009) in sunflower and *Podophyllum hexandrum* respectively.

Nitrogen is an important nutrient which determines the production of various enzymes and hormones that regulate proper functioning of the plant and help in coping up with the stressful situation (Barberon *et al.*, 2016). Wilkinson *et al.*, (2007) observed that there was a decrease in the closure of stomata and leaf elongation during stress condition in maize plants supplied with more of nitrogen. Along with increased absorption of water, this inhibition of closure of stomata helps in increasing the transpiration rate of the plants and thus help the plant to cope up with the stress.

Application of extra nitrogen helps in regulating the root hydraulics/ aquaporins through local and systemic signaling induced by nitrate (Cramer *et al.*, 2009; *Li G. et al.*, 2016), root anatomy by depositing lignin and suberin regulated by ammonium and nitrate (Ren *et al.*, 2015; Barberon *et al.*, 2016; Ranathunge *et al.*, 2016; Gao *et al.*, 2017) or by transportation of nitrogen containing molecules (Wang *et al.*, 2016) which helps in coping with the stressful situation and result in proper functioning of the plants.

Elevated CO_2 may result in high temperature stress that cause damage to the photosynthetic mechanism of the plants. There occurs an interactive effect of plant water status and protection of photosynthesis under elevated CO_2 and associated high temperature. If proper plant water status is maintained in the plants, then they can utilize the excess CO_2 for carboxylation especially in C3 plants like tomato. This improved efficiency is obtained by avoidance of the wasteful process of photo

oxidation due to the CO_2 enriched atmosphere which prevents the oxidation of Rubisco (Leakey *et al.*, 2009). .

In the present study, elevated CO_2 conditions resulted in a significantly higher photosynthetic rate compared to plants in open condition. This effect was evident at both 60 DAS and 75 DAS. The result was in total agreement with the observations of Lawlor and Mitchell (2000) and Ziska *et al.* (2004) in wheat.

In addition to this, all the treatments showed positive impact on the photosynthetic rate of the plants and among the treatments, application of extra nitrogen increased photosynthetic rate by 47.77 % recording the highest percentage increase among all. Similar result was obtained by Zhou *et al.* (2011) in cucumber, where increased application of nitrogen resulted in higher photosynthetic rate in cucumber. This can be attributed to the role of nitrogen on the production of proteins of Calvin cycle and thylakoids. There is a strong positive relation between the nitrogen and RuBP carboxylase and chlorophyll content. With increasing nitrogen content, the total soluble proteins also increase. It influences the growth of the plants which is finally reflected in the photosynthetic capacity and the final yield. However, this was in contradiction to the findings of Guo *et al.* (2007) and Li *et al.* (2009), where N did not show any significant impact on photosynthetic rate in rice plants.

Water use efficiency is the ratio of water used by the plants in metabolism to the water lost by the plants through transpiration. From a physiological point of view, increased WUE represents one of the most important plant responses under elevated CO_2 (Rogers *et al.*, 1994). Reduced stomatal opening result in improved water use efficiency (Guy and Reid, 1986; Clifford *et al.*, 2000) reducing the water stress for plants (Kimball, 1983).

Usually increased CO_2 result in improved WUE as the water being taken up is not lost by transpiration due to closed stomata under high temperature stress. So using the available water, more photosynthates can be produced increasing the water use

efficiency. In the present experiment, similar results were obtained where plants exposed to elevated CO_2 resulted in higher water use efficiency.

Application of growth regulators and nutrients also had significant impact on water use efficiency of tomato plants. Among treatments, application of extra nitrogen increased the WUE by 75.18 % which was on par with WUE of plants treated with extra NPK. This is attributed to the effect of nitrogen and phosphorous on crop yield and WUE (Pendall *et al.*, 2004). Additional nutrients enhanced the root growth and thereby more water uptake from deeper layers of the soil. It also results in rapid growth of plant canopy, providing shade on the lower surface and reducing the transpiration which improves the WUE. This effect was demonstrated in the study of Kathju *et al.* (2001) where application of 80 Kg N h^{-1} in pearl millet resulted in significant increase in the water use efficiency.

Chlorophyll fluorescence is a phenomenon in which plants re-emit the excess energy in the form of light. This phenomenon is measured using the ratio Fv/ Fm and this value is directly proportional to the photosynthetic efficiency of the plants. Under CO_2 enriched condition, there was a significant increase in the Fv/ Fm value, which shows that the overall photosynthetic efficiency of the plants had improved under CO_2 enrichment.

Nutrient application has been found to have positive impact on preventing the photo inhibition. Nitrogen did not significantly influence the Fv/ Fm ratio in cucumber and rice (Zhou *et al.*, 2011). However in the current study, plants treated with extra NPK increased chlorophyll fluorescence by 41.91% compared to control. Extra nutrients help in better growth, water use efficiency and antioxidant production which helps in coping up with the stress experienced by plants under elevated CO_2 conditions. This might have prevented the photo inhibition and also improved the photochemical efficiency, expressing a higher Fv/ Fm value.

Elevated CO_2 concentration and associated high temperature is having a direct impact on the water status of plants through its direct impact on stomatal mechanism and characteristics. The water status of the leaves influences various physiological parameters including stomatal conductance, transpiration rate, photosynthetic rate, respiration etc. and finally influence the growth and development (Yamasaki and Dilenberg, 1999). The results clearly shows that plants exposed to elevated CO_2 showed decreased RWC by 2.02 % at 60 DAS and by 2.18 % at 75 DAS compared to open condition. This was contradictory with the results of Rogers *et al.* (1984) who reported that exposure to increased CO_2 resulted in improved RWC in soybean. Similar results were obtained in *Brassica juncea* by Rabha and Uprety (1998) and Yusuke *et al.* (2007) in ginger (*Zingiber officinale* Roscoe) under water stress. Application of extra nitrogen was found to improve the RWC by 96.87 % even under CO_2 enrichment. Nitrogen helps in better root formation and hence better absorption of water from deeper layers of plant thus maintaining a better water status and contributing to improved RWC.

Chlorophyll stability denotes how stable the chlorophyll is even under stress conditions. Hence, it shows the ability of a plant to perform better and carry out photosynthesis under stress situation, hence considered as a desirable trait for resistant plants. Here, CO_2 enrichment has resulted improved CSI as a result of increased total chlorophyll content and a better defense mechanism available for the plants. 15 % increase in CSI was noticed in plants supplied with additional nitrogen as this is contributing positively to total chlorophyll content of the plants and hence improved CSI.

Effect of growth regulators and nutrient application on antioxidant mechanism in tomato under CO_2 enrichment.

Exposure of plants to various abiotic stresses lead to the production of reactive oxygen species (ROS) which are highly reactive and damages the proteins,

carbohydrates, lipids and DNA which produces an oxidative stress to the plants (Gill and (Gill and Tuteja, 2010). Antioxidants are compounds produced by plants in response to the production of these ROS, which protects the cell from these damages by quenching the ROS. These antioxidants may be enzymatic and non-enzymatic. Among these enzymatic antioxidants such as superoxide dismutase, peroxidase and osmo-protectants like proline were estimated in the treatment plants in the present experiment so as to get an idea about the status of defense machinery of the plants. Elevated CO_2 showed improvement in the defense mechanism of the plants by improving the antioxidant production in general.

Oxidative damage occurs when plant experiences high temperature stress. CO_2 enrichment result in an enhanced photosynthetic rate and increased carbohydrate production which helps in providing raw materials for production of higher amount of antioxidants. The additional CO_2 fixed in an enriched CO_2 condition is invested in making of additional antioxidants (Pritchard *et al.*, 2001) many of which are secondary metabolites. The results were in accordance with earlier findings of Lin and Wang (2002). Lin and Wang (2002) reported increased SOD and catalase activities in CO_2 enriched condition than in ambient conditions in wheat.

Similarly, in the present study, a significant increase in enzymatic anti-oxidants was observed under elevated CO_2 condition.

The SOD activity increased by 28.57 % and 37.50 % at 60 AS and 75 DAS respectively by the application of extra NPK. Peroxidase activity increased by 31.56 % and 33.02 % by the application of additional NPK, compared to control at 60 and 75 DAS respectively. Proline content increased by 59.78 % with addition of extra nitrogen. This was on par with the proline content with the application of extra NPK and 50 ppm B + 50ppm Zn.

Under stress condition especially in CO_2 enriched situation, availability and acquisition of nitrogen was found to decrease (Mc Donald *et al.*, 2002). Deficiency of

nitrogen triggers the production of ROS. With the addition of extra nitrogen, positive signaling of N- induced antioxidant enzyme genes get activated resulting in higher levels of antioxidants such as SOD, CAT, GPX and APX which will quench the reactive oxygen species produced as a result of the stress condition and thus protects the plants. Huang *et al.* (2004) reported that N deficiency reduces the activity of SOD, exacerbating lipid peroxidation in rice. Nitrogen is also necessary in the biosynthesis of certain aromatic amino acids such as phenyl alanine, tyrosine and tryptophan which are essential components of antioxidant responses in plants (Nybakken *et al.*, 2013). This might be the reason for enhanced antioxidant activity in treatments with extra nitrogen.

Effect of growth regulators and nutrient application on flowering in tomato under CO_2 enrichment.

One of the objectives of the present programme was to study the effect of CO_2 and associated high temperature on flowering in tomato and their improvement through the application of growth regulators and nutrient applications. In this regard, observations on parameters related to flowering time, no: of flowers and pollen characters. Plants which were exposed to CO_2 enrichment showed distorted flowers and pollen which affected the fruit setting. Many of the treatments listed in the programme significantly influenced reproductive physiology of tomato.

In the current experiment, a significant delay in flowering (2 days) was observed under elevated CO_2 condition. The number of flowers per cluster increased by 73.16 % inside OTC conditions compared to open condition. 55.55 % increase in flowers per cluster was observed by the application of extra nitrogen and 182.08 % increase in flowers per cluster by application of 50 ppm B + 50 ppm Zn compared to plants in open condition and OTC conditions respectively. Among treatments, addition of extra nitrogen resulted in increased number of flowers per cluster (48.40 %) and addition of extra NPK increased number of flower clusters (72.74%) compared to control. This may be attributed to the favoured metabolic process and auxin activity.

Besides this, nitrogen is the most imperative element for proper growth and development of plants which significantly increases the yield and its quality by playing vital role in biochemical and physiological functions (Leghari *et al.*, 2016). This was in accordance with the work of Nakamoto *et al.*, 2001 and Zheng *et al.*, 2002 where an increase in the number of flowers were noted under elevated CO_2 conditions in soybean.

The effect of elevated CO_2 and associated high temperature on flowering responses is specific to plant species. The enhanced growth rates, increased plant growth at flowering stage and raised tissue sugar status resulted from CO_2 enrichment (Springer and Ward, 2007). The accumulation of excess foliar sugar under elevated CO_2 delayed flowering in several species. In Arabidopsis, the sustained expression of the floral repressor gene FLC was reported to be associated with delayed flowering under e[CO_2] (Springer *et al.*, 2008). Elevated CO_2 delayed flowering in Arabidopsis plants with a 41 and 105% increase in foliar sucrose and starch content, respectively (Bae and Sicher, 2004), indicating differential response to foliar sugar levels below and above threshold limits. Application of extra nutrients and growth regulators did not show any significant difference in the days to flowering and days to 50 % flowering.

Considering the pollen viability percentage, an overall decrease of 82.28 % was noted under elevated CO_2 conditions compared to open condition. This was attributed due to the increased temperature inside the chamber associated with CO_2 enrichment which resulted in decreased viability. This was in accordance with the results of work conducted by Prasad *et al.* (2003), where elevated CO_2 and associated high temperature reduced pollen viability in peanut. Similar studies associated with the interactive effect of elevated CO_2 and high temperature on legumes showed positive interaction on the vegetative growth which was not reflected on the reproductive phase of the crop legumes (Ahmed *et al.*, 1992; Prasad *et al.*, 2002, 2003). This result coincides with the results of Prasad *et al.* (2002, 2003) where they showed a decrease in seed yield at

elevated CO_2 condition which was reported to be associated with decreased pollen viability.

No significant difference in pollen viability was observed under open condition with the application of various treatments. But under elevated CO_2 condition, T3 (50ppm B) showed an increase of 192.90 % in pollen viability compared to absolute control. This may be attributed to the role of boron on the anther development, pollen germination and pollen tube growth (Loomis and Durst, 1992). Due to the higher CO_2 concentration and associated high temperature, normally the pollen fertility is seen to decrease inside OTC. The present study showed that this could be mitigated most effectively by foliar application of 50 ppm boron at 60 DAS and 75 DAS in tomato. Similarly, additional NPK increased viability percentage by 127.12% followed by B spray (125.05%) compared to absolute control under elevated CO_2 conditions. Firon *et al.* (2009) suggested that the reduction of pollen viability in tomato was due to the lower soluble sugar concentration in the developing pollen grains

Similarly pollen morphology was also studied using scanning electron microscopy (SEM). The images showed distorted pollen under elevated CO_2 conditions. Contrary to this large and intact pollen was observed under open condition. The distorted structure of pollen inside OTC may be attributed to the high temperature stress damage to the exine membranes of the pollen that lead to deterioration of its structure. The observed result is in accordance with the work of Prasad and Djanaguiraman (2014) where wheat pollen collapsed and was desiccated when the plants were exposed to 35/25°C day/night temperatures during anthesis. When soybean plants were exposed to 38/28°C day/night temperatures in full bloom stage, morphological abnormalities was observed in their pollen (Djanaguiraman et al., 2013). Likewise, abnormalities in common bean pollen were observed when plants were exposed to 24 days of high temperature (32/27°C day/night) before anthesis, but no abnormalities were observed when high temperature exposure was given 1, 5, 9 and 13 days before anthesis (Porch & Jahn, 2001). The abnormalities in pollen morphology

observed under elevated CO_2 and associated high temperature could be due to damage in the tapetum during pollen development (Suzuki *et al.*, 2001) as a result of programmed cell death (Djanaguiraman *et al.*, 2013)

Effect of growth regulators and nutrient application on yield parameters in tomato under CO_2 enrichment

Since the previous studies conducted under elevated CO_2 condition indicated a drastic reduction in fruit setting in the case of crops like tomato and chilli, yield parameters were also studied in the present programme.

The effect of CO_2 enrichment was significant in the case of the yield parameters of tomato. A decreased fruit weight of 11.8 % under elevated CO_2 condition was noted compared to the open condition. Similarly a decrease in number of fruits by 78.6 % and in total yield by 81.54 % were found inside OTC compared to open conditions irrespective of the treatments. This concurs with the study of Prasad *et al.* (1999) in which they reported that in peanut there existed a strong negative linear relationship between day temperature over the range of 28-48 °C and fruit number and pollen production and viability percentage of pollen under elevated CO_2 conditions.

Fruit weight under open condition was significantly high in cases of water spray (125.71%), additional NPK (121.61%) and 50 ppm NAA (94.14%) compared to absolute control. Similar result was obtained under elevated CO_2 conditions where water spray (221.12%) showed the highest percentage increase compared to absolute control. This concurs with the work of Romeo- Aranda *et al.* (2002) who found out that misting or water spray increased water use efficiency from 84 % to 100% in tomato plants grown under greenhouse conditions as was estimated from the ratio of net CO_2 assimilation to transpiration. Under the OTC chamber, extremely high VPD_{air} was observed which was lowered considerably by the water spray, thereby improving the growth and fruit yield in tomato plants. This was in conformity with the work of Barker

(1990) where lower VPD resulted in lower transpiration rates and thus increased water use efficiency, which was reflected in high yield of greenhouse tomatoes.

Considering the effect of various treatments on the total number of fruits, 50 ppm B + 50 ppm Zn showed the highest percentage increase of 60 % followed by additional N with 53.33% compared to absolute control under open conditions. Under elevated CO_2 conditions highest percentage increase in yield was observed in additional N (425.13 %) followed by 50 ppm B + 50 ppm Zn and 50 ppm B with 275.5% increase. Thus irrespective of the conditions, highest percentage yield was observed in additional N (97.06 %) followed by 50 ppm B + 50 ppm Zn (85.30 %) and 50 ppm B (64.71%). The result clearly shows the effect of 25 % extra nitrogen than recommended dose has a significant impact on the yield attributes such as number of fruits, fruit weight and total yield. This result is in agreement with Singandhupe *et al.* (2003) who recorded increased yield attributes with increase in N in field grown tomato. The reason for this is that extra nitrogen helps to increase photosynthetic activities, protein synthesis and better translocation of assimilates due to suitable environmental condition that activates enzyme that result in better yield.

The significant impact of boron spray on yield can be due to its effect on the photosynthetic rate and better translocation of photosynthates from source to sink (Duli zhao and Derrick, 2003).

The effect of extra nitrogen favoured the metabolism and auxin activity which accelerated the photosynthetic rate and in turn increased the supply of carbohydrate to plant system resulting in increased number of fruits, average fruit weight and yield per plant (Everaarts and Boou, 2000). This concurs with the study of Carmo *et al.* (2017) where, higher amount of nitrogen resulted in an increased plant height, number of branches and yield parameters like number of fruits, average fruit weight and total yield per plant. Higher nitrogen resulted in higher yield and yield parameters.

In the present programme, a significant positive impact was observed by application of various growth regulators and additional nutrients on the reproductive physiology of tomato under elevated CO_2 condition. The problems associated with CO_2 enrichment on reproductive physiology could be alleviated effectively by the application of various treatments. Improvement in the final yield was brought about by increasing the number of flowers, pollen viability, number of fruits and fruit weight.

Among all the treatments, additional nitrogen application has resulted in significantly higher number of fruits, fruit weight, final yield and lycopene content. It promoted vegetative growth, flowering and fruit setting in tomato. The nitrogen use efficiency was found to be higher under elevated CO_2 condition. Due to availability of more CO_2 for photosynthesis, the availability of nutrients could have been the limiting factor. One additional nutrients was supplied, the efficiency of plant performance was increased resulting in better vegetative growth, flowering, fruiting and final yield.

Similarly, application of 50 ppm Boron spray was found to be effective in improving the reduced pollen viability under elevated CO_2 condition and reducing the floral deformities. Thus it helped in increasing the fruit setting percentage and thus playing a positive role in overall yield. Boron spray could have helped in proper development of pollen grains and for pollen germination. It may also have contributed to proper cell development and cell wall formation which is essential for the proper functioning of the whole plant. All these roles of boron may have contributed to the better growth and development of the plants under elevated levels of CO_2.

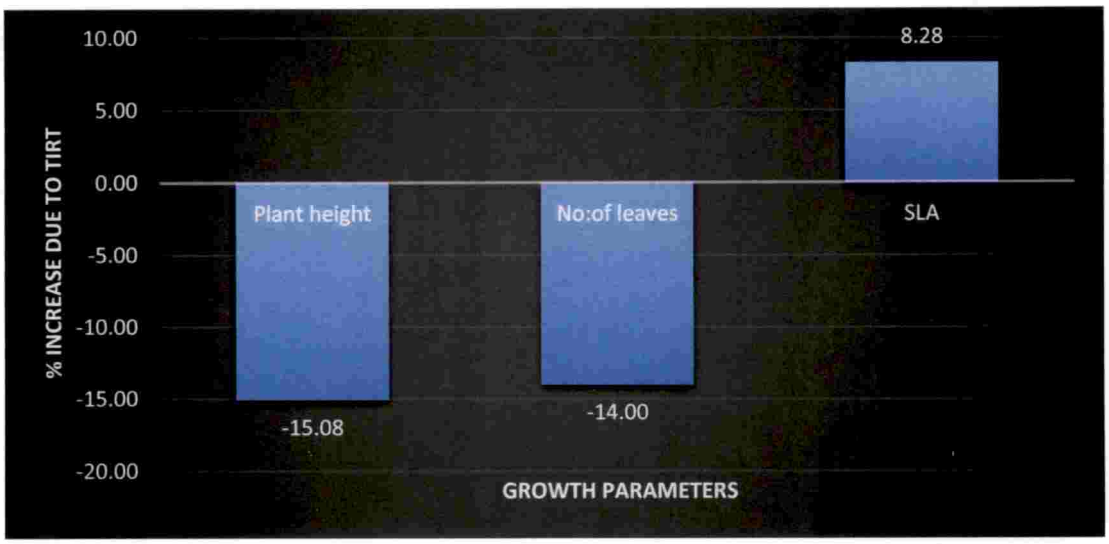


Figure 1. Percentage increase in growth parameters due to TIRT in tomato at 60 DAS

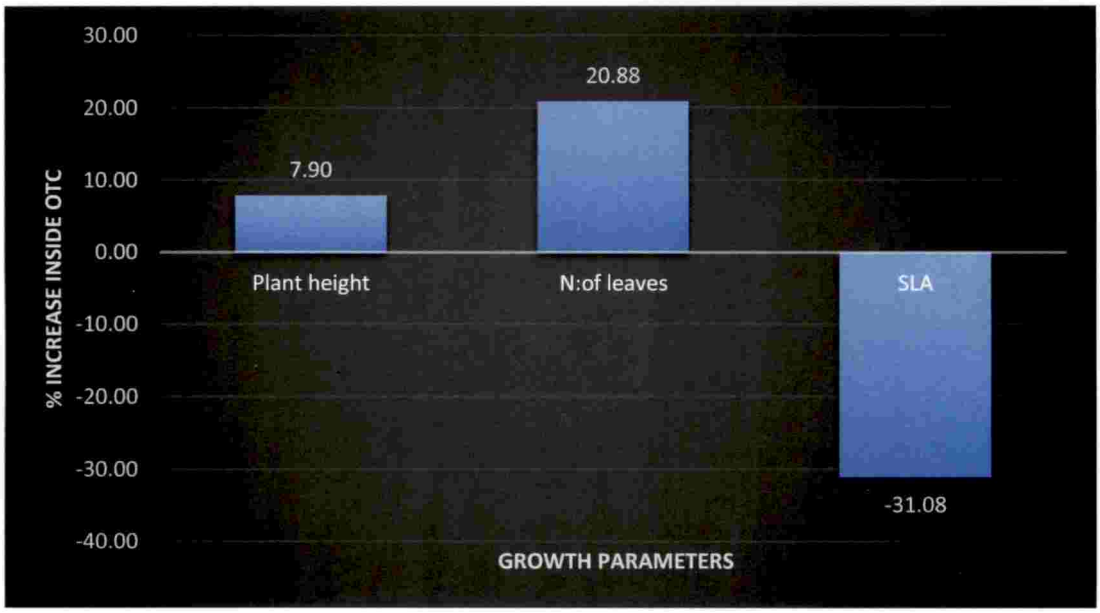


Figure 2. Percentage increase in growth parameters under elevated CO_2 condition in tomato at 60 DAS.

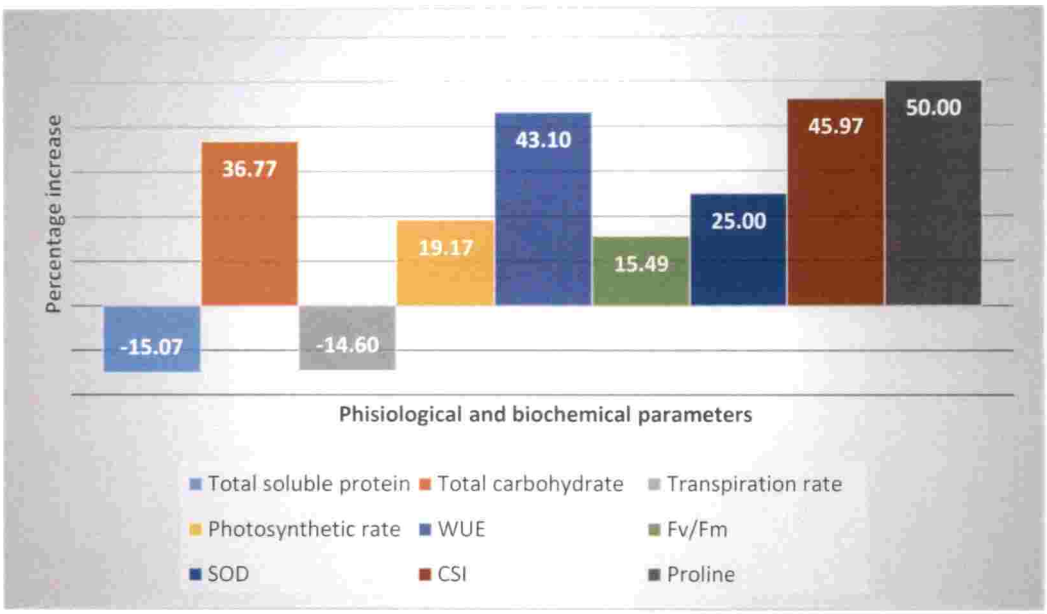


Figure 3. Percentage increase in physiological and biochemical parameters due to TIRT in tomato at 60 DAS

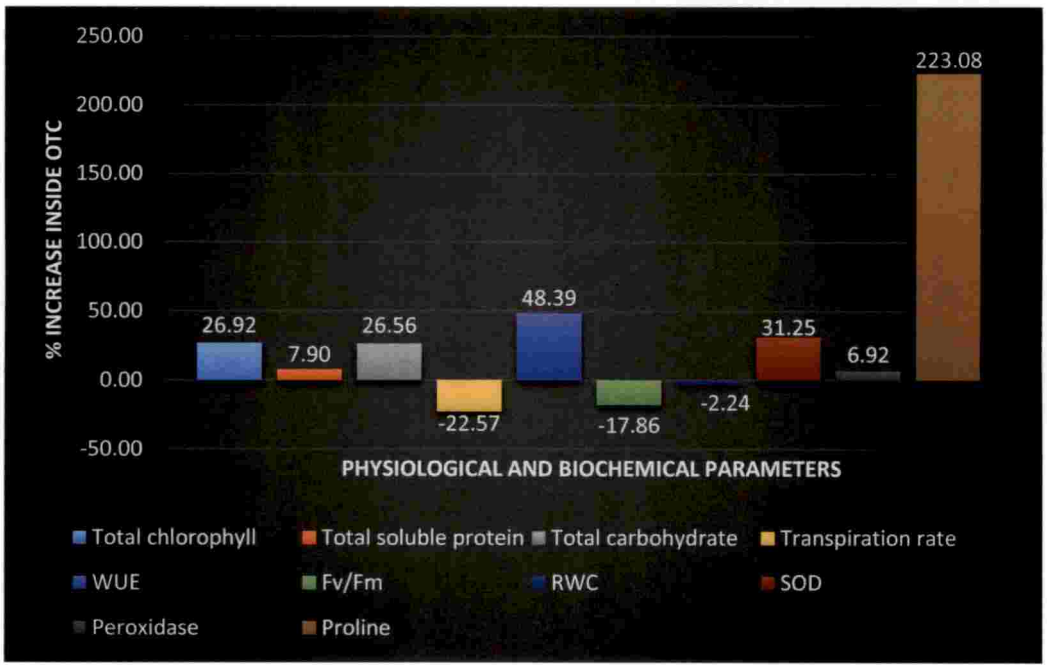


Figure 4. Percentage increase in physiological and biochemical parameters under elevated CO_2 condition in tomato at 60 DAS.

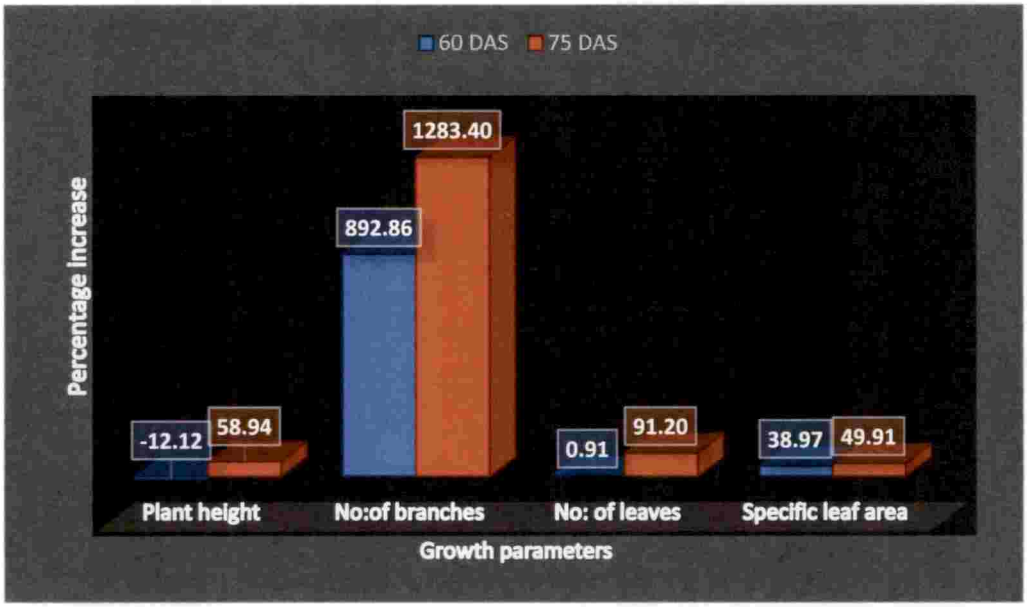
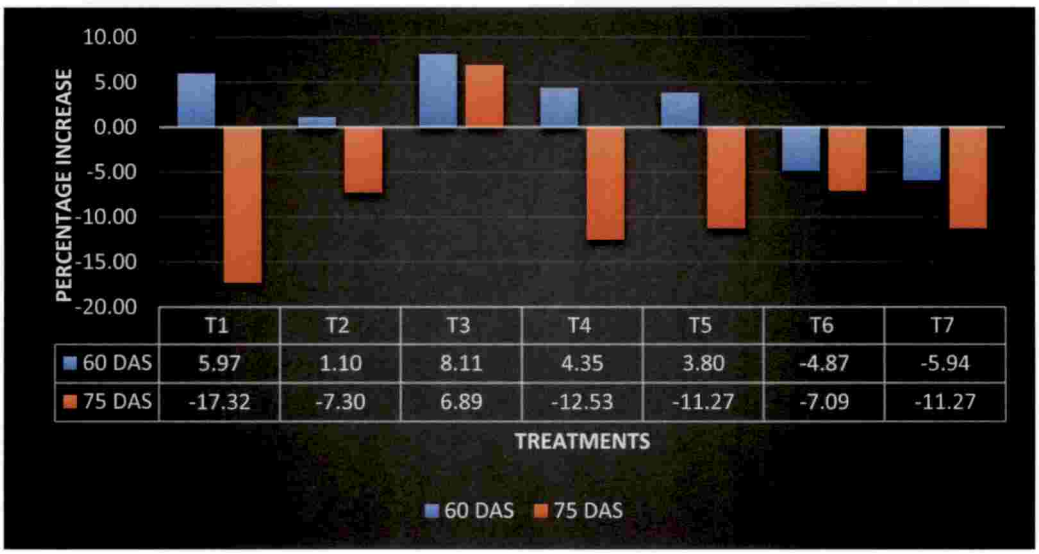


Figure 5. Effect of CO_2 enrichment on growth parameters percentage increase at 60 DAS and 75 DAS



	T1	T2	T3	T4	T5	T6	T7
60 DAS	5.97	1.10	8.11	4.35	3.80	-4.87	-5.94
75 DAS	-17.32	-7.30	6.89	-12.53	-11.27	-7.09	-11.27

Figure 6. Effect of growth regulators and nutrient applications on percentage increase in plant height at 60 DAS and 75 DAS.

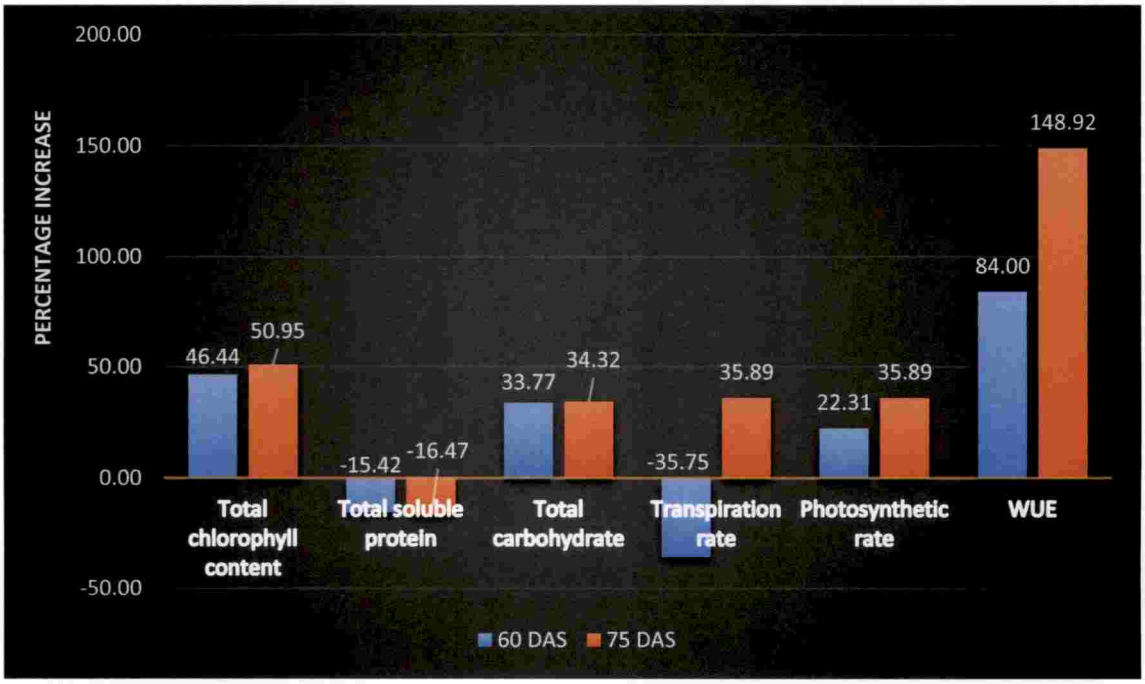


Figure 7. Effect of CO_2 enrichment on physiological and biochemical parameters percentage increase at 60 DAS and 75 DAS.

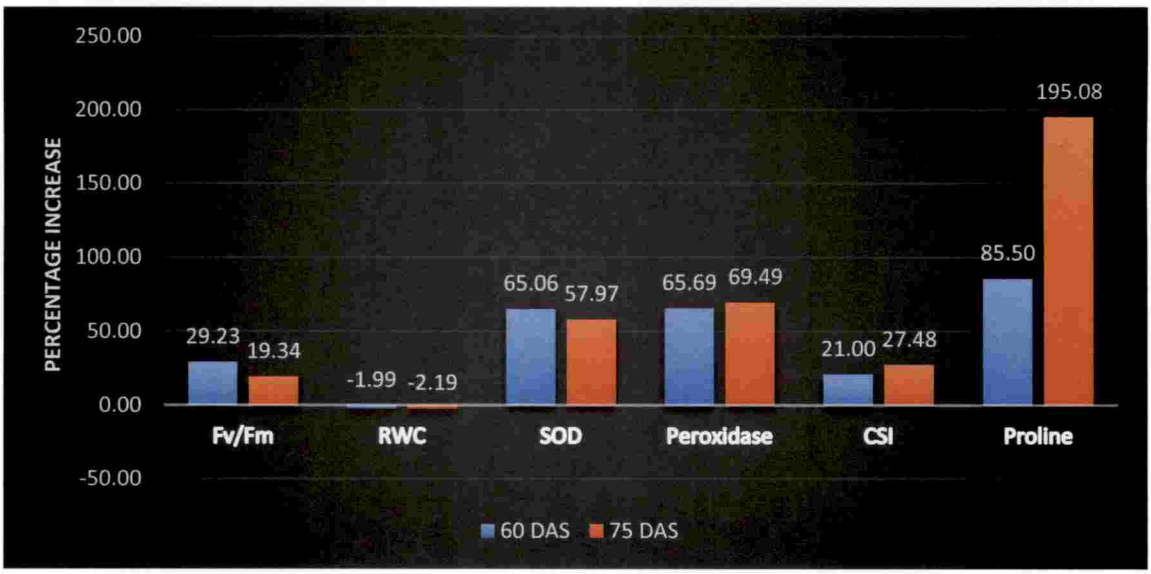


Figure 8. Effect of CO_2 enrichment on physiological and biochemical parameters percentage increase at 60 DAS and 75 DAS.

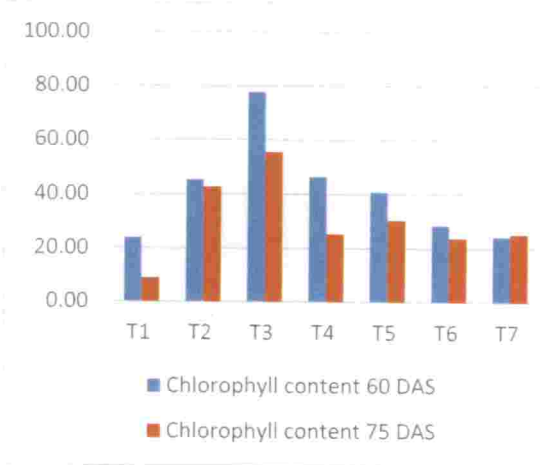


Figure 9. Effect of growth regulators and nutrient applications on percentage increase in chlorophyll content (%) at 60 DAS and 75 DAS.

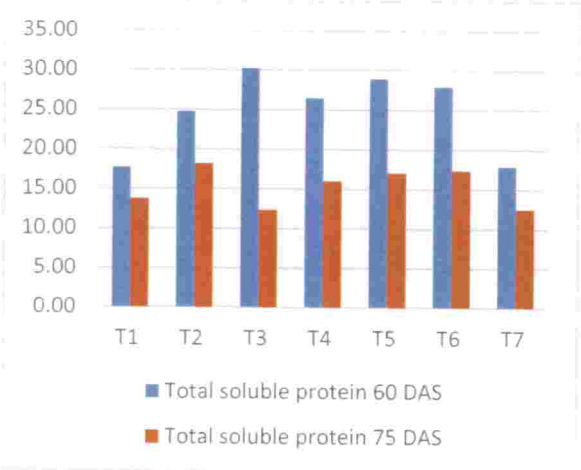


Figure 10. Effect of growth regulators and nutrient applications on percentage increase in total soluble protein (%) at 60 DAS and 75 DAS.

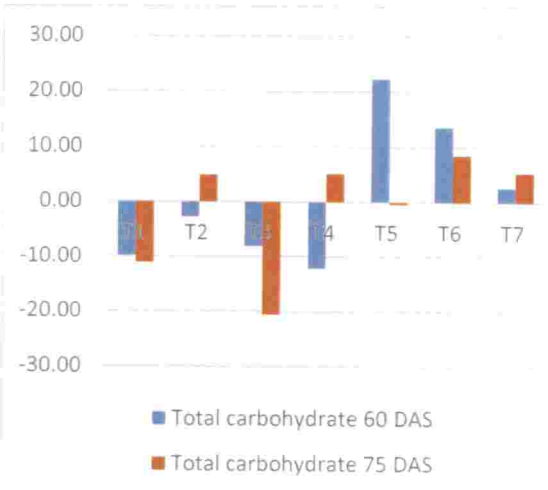


Figure 11. Effect of growth regulators and nutrient applications on percentage increase in total carbohydrate (%) at 60 DAS and 75 DAS.

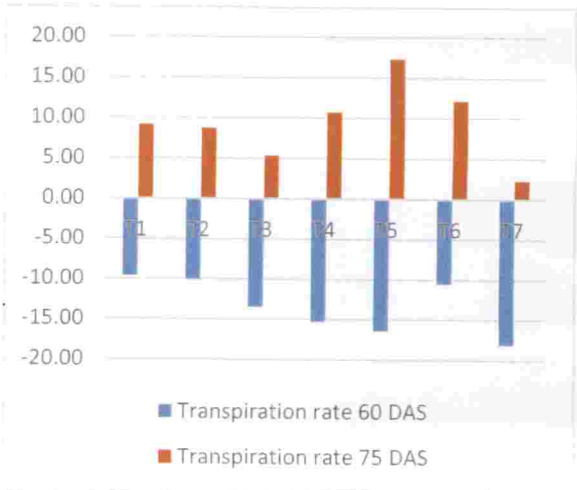


Figure 12. Effect of growth regulators and nutrient applications on percentage increase in transpiration rate (%) at 60 DAS and 75 DAS.

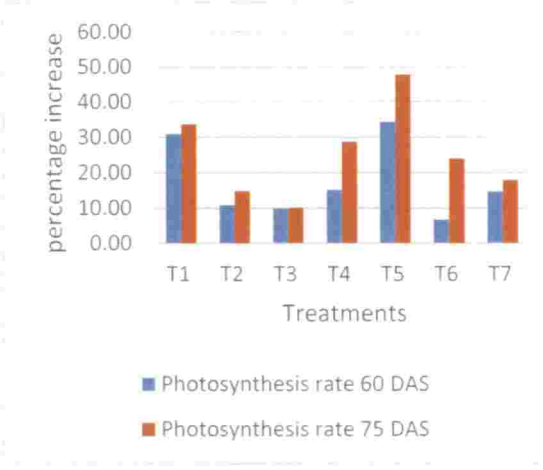


Figure 13. Effect of growth regulators and nutrient applications on percentage increase in photosynthetic rate (%) at 60 DAS and 75 DAS.

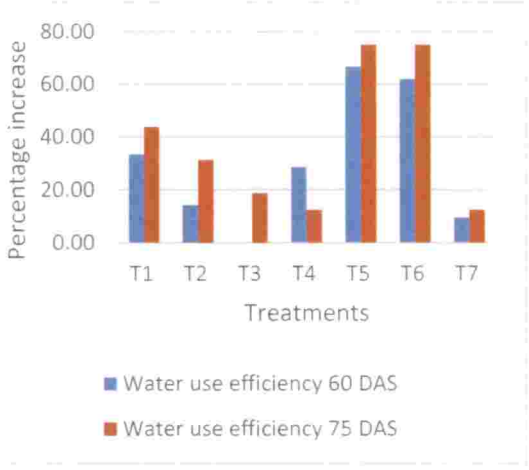


Figure 14. Effect of growth regulators and nutrient applications on percentage increase in water use efficiency (%) at 60 DAS and 75 DAS.

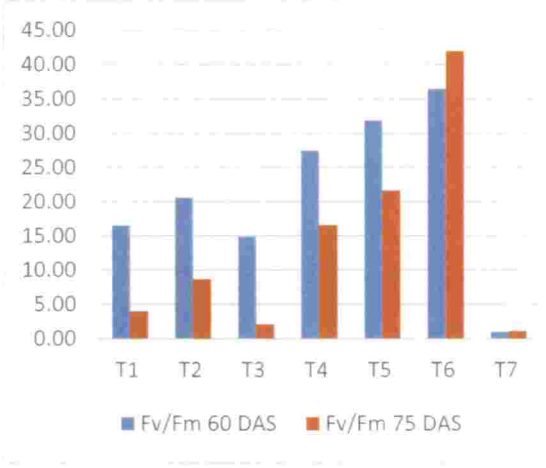


Figure 15. Effect of growth regulators and nutrient applications on percentage increase in chlorophyll fluorescence (%) at 60 DAS and 75 DAS.

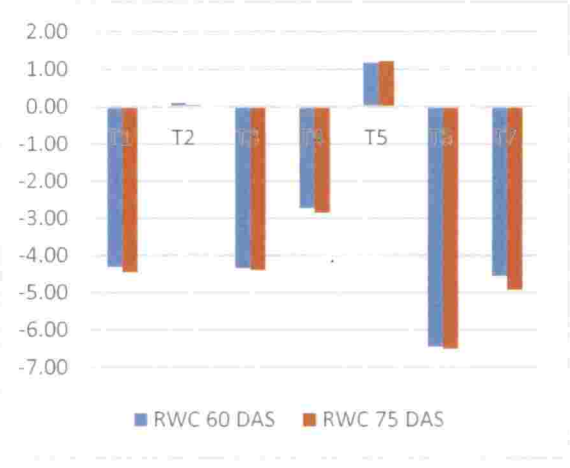


Figure 16. Effect of growth regulators and nutrient applications on percentage increase in relative water content (%) at 60 DAS and 75 DAS.

Figure 17. Effect of growth regulators and nutrient applications on percentage increase in SOD activity (%) at 60 DAS and 75 DAS.

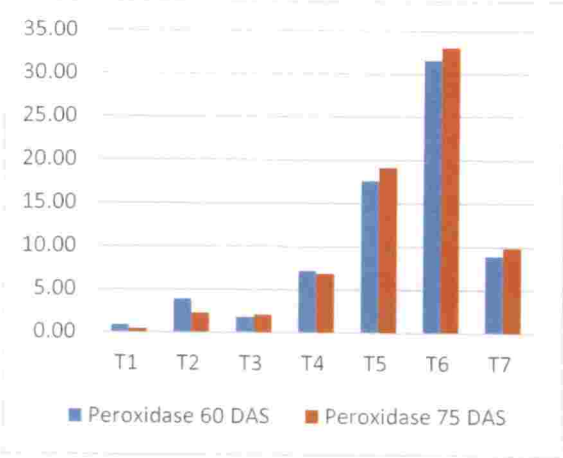


Figure 18. Effect of growth regulators and nutrient applications on percentage increase in peroxidase activity (%) at 60 DAS and 75 DAS.

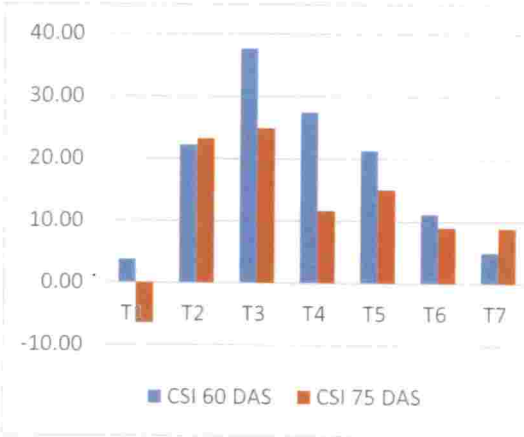


Figure 19. Effect of growth regulators and nutrient applications on percentage increase in CSI (%) at 60 DAS and 75 DAS.

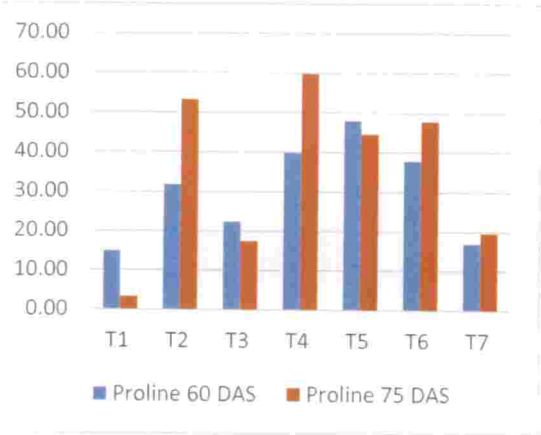


Figure 20. Effect of growth regulators and nutrient applications on percentage increase in proline content (%) at 60 DAS and 75 DAS.

Figure 21. Impact of CO_2 enrichment on days to 50 % flowering

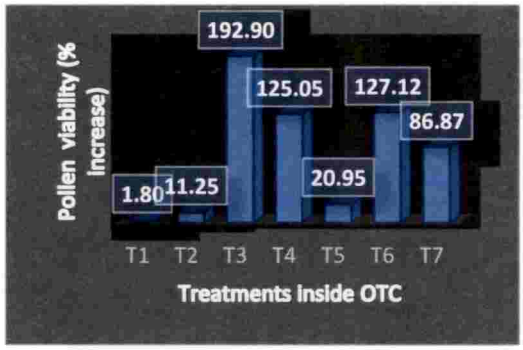


Figure 22. Effect of growth regulators and nutrient applications on percentage increase in pollen viability under elevated CO_2 condition.

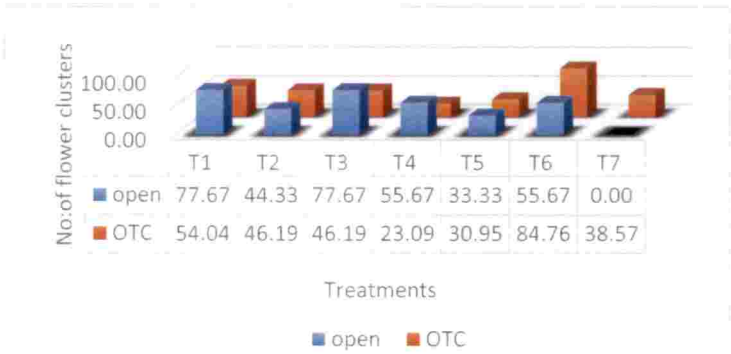


Figure 23. Effect of growth regulators and nutrient applications on percentage increase in no: of flowers under elevated CO_2 and Open conditions

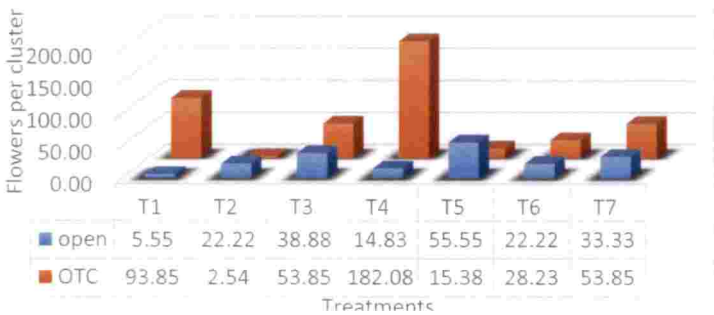


Figure 24. Effect of growth regulators and nutrient applications on percentage increase in no: of flowers under elevated CO_2 and Open conditions

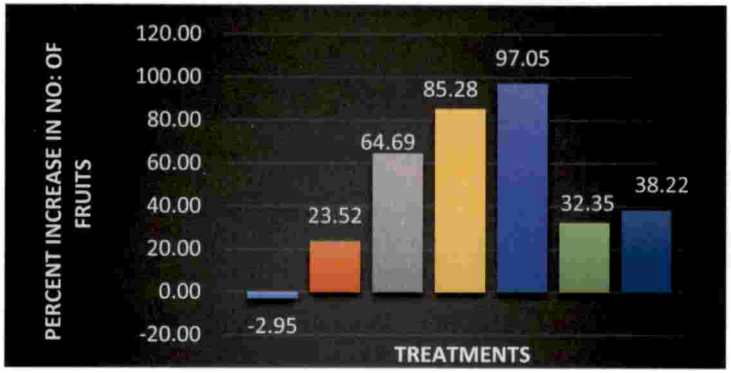


Figure 25. Effect of growth regulators and nutrient applications on percentage increase in no: of fruits under elevated CO_2 condition.

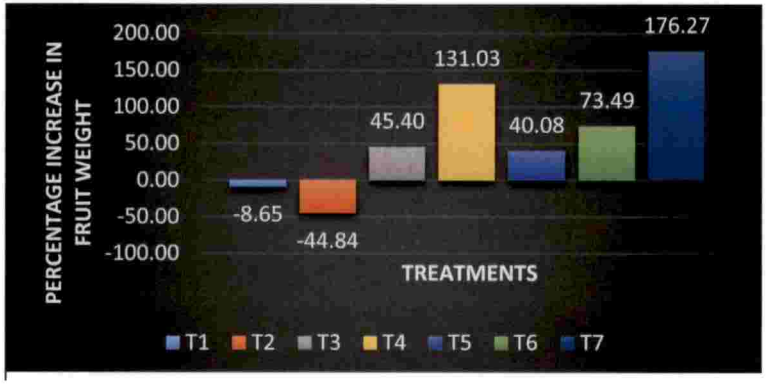


Figure 26. Effect of growth regulators and nutrient applications on percentage increase in fruit weight under elevated CO_2 condition.

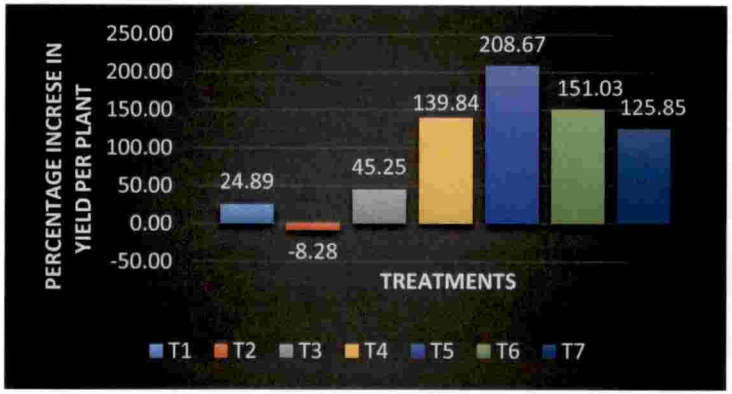


Figure 27. Effect of growth regulators and nutrient applications on percentage increase in yield per plant under elevated CO_2 condition.

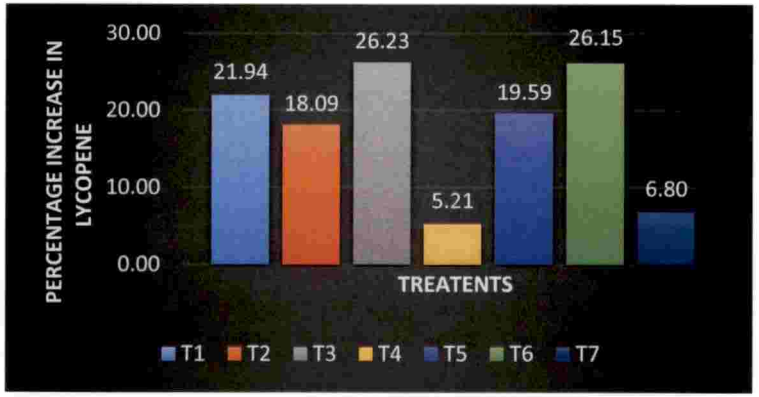


Figure 28. Effect of growth regulators and nutrient applications on percentage increase in lycopene content under elevated CO_2 condition.

SUMMARY

6. SUMMARY

Global climate change is considered to be the most serious environmental threat that the earth is facing. It has been the centre of debate among the global scientific community recently. The major factor contributing to this climate change is the increasing greenhouse gases, especially CO_2 which is contributing to global warming. Concentration of CO_2 has increased from the pre-industrial level of 300ppm to the current level of 411ppm as of June, 2019 in the atmosphere. As per predictions, the increase in greenhouse gases such as CO_2, methane and N_2O will result in an increase in mean temperature by 2-3 °C by 2050. The increasing temperature is a serious stress that affect the plant community and agricultural sector negatively. However, the elevated CO_2 levels in the atmosphere is having a positive impact as it is the primary substrate for photosynthesis. Hence the concern about the possible consequences of the increasing CO_2 and the associated rise in temperature is still under consideration.

Tomato is an economically important crop. Tomato (*Solanum lycopersicum* L.) is the world's largest vegetable crop and is known as protective food because of its special nutritive value and also because of its widespread production. It is considered as an important commercial and dietary vegetable crop having a very good post-harvest potential. It is rich in vitamin A, vitamine C and also ranks first in their nutrient contribution to human diet. It comes first among the canned vegetable products.It is an ideal research material for physiological, cellular, biochemical, molecular and genetic investigations because it is easy to cultivate, has short lifecycle and high productivity. Tomato is very sensitive to water deficits during transplanting, flowering and fruit developmental stages (Vijitha and Mahendran, 2010).

Under this background, various studies have been conducted in Department of Plant Physiology, College of Agriculture, Vellayani, pertaining to elevated CO_2 induced modifications in growth, phenology and stress response in crop plants. The results of these studies revealed that elevated CO_2 has a positive impact on the growth

parameters of the plants, which was not reflected in the reproductive phase and final yield. In this context, the objective of the present study entitled "Influence of CO_2 enrichment and associated high temperature on reproductive physiology of tomato (*Solanum lycopersicum* L.)" was proposed with the objectives of studying the effect of CO_2 enrichment and associated high temperature on flowering and fruiting in tomato and their improvement through growth regulators and nutrient applications and through temperature induction response technique.

Two pot culture experiments were conducted in this programme. First experiment consisted of two parts. Part 1 being standardization of Temperature Induction Response Technique (TIRT) for two popular tomato varieties of Kerala namely Vellayani Vijay and Anagha. TIRT is a technique in which plants are being pre exposed to a mild stress before exposing to a lethal stress. Exposing the plants to a mild stress was expected to enhance the plants defense mechanism and help the plants survive the lethal stress level. Hence, lethal temperature and induction temperatures were standardized for both the varieties. Five day old tomato seedlings grown in petri plates were taken for this experiment. The seedlings were exposed to different temperatures (35-50 ^{0}C) for different durations (1h to 2h). The temperature of 48 °C for 2 h (100% mortality) was identified as the lethal temperature for both the varieties. Recovery responses after exposing to lethal temperature was also studied. The temperature treatment, 38 °C (1h)- 43 °C (1h)- 48 °C (2h), which resulted in maximum recovery percent, after exposure to lethal temperature was identified as the induction temperature treatment for both the varieties. The recovery percentage for Vellayani Vijay and Anagha after temperature induction was observed to be 87% and 83% respectively. No varietal difference in lethal as well as induction temperatures was observed for both the varieties.

Part 2 consisted of evaluation of the induced seedlings of variety Vellayani Vijay. The experiment was laid out in CRD with two treatments and four replications under two conditions - OTC with elevated CO_2 condition and open field (control).

Induction resulted in significant per cent increase (8.2 %) in specific leaf area compared to control. However, temperature induction did not show any significant impact on growth parameters like plant height, number of branches and number of leaves. Among the photosynthetic parameters, temperature induction resulted in significantly higher chlorophyll content (1.74 mg g^{-1} fresh weight), total carbohydrate (36.71 mg g^{-1}), photosynthetic rate (22.13 µmol CO_2 m^{-2} sec^{-1}), water use efficiency (4.98 mmol CO_2 mol^{-2} H_2O), chlorophyll fluorescence (0.82) and relative water content (92.20 %) compared to plants without temperature induction. 44.3 % increase in total chlorophyll content and 19.17 % increase in photosynthetic rate indicates a higher photosynthetic efficiency for the induced seedlings compared to control. The induced plants showed enhanced defense mechanism with a significantly higher SOD activity (0.21 activity $g^{-1}min^{-1}$), chlorophyll stability index (145.968 %) and proline content (1.324 µM g^{-1} tissue) compared to non-induced plants. The results showed that temperature induction has improved the photosynthetic efficiency and stress tolerance mechanism of tomato plants.

Second experiment was designed to evaluate the response of tomato variety Vellayani Vijay to various growth regulators and nutrient applications under carbon dioxide enrichment. The experiment consisted of eight treatments with three replications laid out in CRD. The treatments comprised of 50 ppm NAA (T1); 50 ppm Salicylic acid (T2); 50 ppm Boron (T3); 50 ppm Boron + 50 ppm Zinc (T4) as foliar spray (at 3 intervals 40, 55, 75 DAS); nutrient application of POP 125% N: 100% P: 100%K (T5); POP 125% N: 125% P: 125%K (T6); a control (water spray) (T7) and an absolute control (T8).

Experimental result revealed that all the treatments had significant effects on growth parameters, physiological and biochemical parameters, parameters relating to flowering and yield. Plant height (58.94 %), number of branches, number of leaves (91.19 %) and specific leaf area (49.90 %) were found to increase significantly under elevated CO_2 condition at 75 DAS. Among the treatments foliar spray with 50ppm

boron recorded higher plant height at 60 DAS and 75 DAS. However nutrient application POP 125% N: 100% P: 100%K and POP 125% N: 125% P: 125%K recorded significantly higher mean value for number of branches, number of leaves and specific leaf area at 60 DAS and 75 DAS resulting in better vegetative growth.

A significant increase in physiological and biochemical parameters such as total chlorophyll content (51.00 %), total carbohydrate (34.31 %), photosynthetic rate (35.84 %), water use efficiency (149.01 %), chlorophyll fluorescence (20.37 %), superoxide dismutase (57.14 %), chlorophyll stability index (27.49 %) and proline content (200 %) was recorded inside OTC with elevated CO_2 (500 ppm). However, a significant decrease were noted in transpiration rate (45.15 %) and total soluble protein content (16.50 %) under elevated CO_2 condition.

Irrespective of the condition, foliar spray of 50 ppm boron recorded significantly higher values for chlorophyll content (1.55 mg g^{-1}) and chlorophyll stability index (124.88 %) compared to control plants at 60 DAS and 75 DAS.

Comparing the effect of various treatments, irrespective of the condition, nutrient application of POP 125% N: 100% P: 100%K (T5) recorded significantly higher photosynthetic rate (21.62 µmol CO_2 m^{-2} sec^{-1}) and water use efficiency (4.67 mmol CO_2 mol^{-2} H_2O). Addition of extra nutrients, POP 125% N: 125% P: 125% K (T6) also boosted the activity of SOD (29.41 %), peroxidase (33.02 %) and increased the proline content (55.56 %) and thus enhancing the defense mechanism of the plants. Significantly higher values for chlorophyll fluorescence (0.75), relative water content (96.87 %), superoxide dismutase (0.23 activity g^{-1} min^{-1}) and peroxidase (44.43 unit min^{-1} g^{-1}) compared to control was observed for plants treated with extra nutrients irrespective of the condition.

Flowering was delayed (3 days) under elevated CO_2 conditions. There was an increase in total number of flower clusters inside OTC. However, a significant reduction in pollen viability (%) was also noticed under elevated CO_2 conditions.

50ppm boron spray resulted in increased pollen viability (30.28%) and significantly higher fruit setting percentage (38.52%) compared to control.

Days to fruiting was found to be delayed inside OTC. Water spray has significantly increased the fruit weight by 125 % in open condition and by 221 % under elevated CO_2. POP 125% N: 100% P: 100%K resulted in increased number of fruits. Lowest intensity of fruit drop (29.94 %) was recorded for POP 125% N: 125% P: 125%K.

Yield per plant was significantly higher for POP 125% N: 100% P: 100%K (190.85g/plant), POP 125% N: 125% P: 125%K (155.21g/plant) and 50ppm boron spray (148.29g/plant) compared to control. Under elevated CO_2 condition POP 125% N: 100% P: 100%K, 50 ppm boron + 50 ppm zinc, 50ppm boron spray was found to significantly increase the yield per plant by 455.19 %, 424.08% and 294.73% respectively. All these three treatments also resulted in an increase in the lycopene content and thus improving quality of the fruit.

Considering the photosynthetic efficiency parameters and defense mechanism, foliar spray with 50 ppm B+ 50 ppm Zn at 40, 55 and 75 DAS and an additional application of 25% nitrogen resulted in better plant performance. Considering the yield per plant, application of 25 % extra nitrogen proved to give the best results.

In the present study, CO_2 enrichment was found to have a deleterious influence on flowering and fruiting in tomato mainly due to reduced pollen viability and floral deformities. TIRT was proved to improve photosynthetic efficiency and stress tolerance mechanism. Foliar spray with 50 ppm B + 50 ppm Zn at 40, 55 and 75 DAS and addition of 25% extra nitrogen than recommended dose in equal splits were found to improve yield and quality to a great extent. This was achieved through improvement in pollen viability, fruit set and individual fruit weight and also through a reduction in fruit drop. These treatments can be exploited further, individually or in combination, in any crop management programme for tomato under increasing CO_2 and associated

high temperature conditions. The study also highlights a need for reassessment of critical nutrient requirements for individual crops in the changing global climatic scenario.

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INFLUENCE OF CO_2 ENRICHMENT AND ASSOCIATED HIGH TEMPERATURE ON REPRODUCTIVE PHYSIOLOGY OF TOMATO (*Solanum lycopersicum* L.)

by

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ABSTRACT

The study entitled "Influence of CO_2 enrichment and associated high temperature on reproductive physiology of tomato (*Solanum lycopersicum* L.)", was undertaken with the objective to study the effect of CO_2 enrichment and associated high temperature on flowering and fruiting in tomato and their improvement through growth regulators and nutrient applications and through temperature induction response technique. The experiments were conducted using the Open Top Chamber (OTC) facility at the Department of Plant Physiology, College of Agriculture, Vellayani during 2017-2019.

The first part of Experiment I was designed for the standardization of Temperature Induction Response Technique (TIRT) for two popular varieties of tomato namely Vellayani Vijay and Anagha by identifying lethal and induction temperatures. Five day old tomato seedlings grown in petri plates were exposed to different temperatures (35-50 ^{0}C) for different durations (1h to 2h). The temperatures of 48°C for 2 h and 38°C (1h)- 43 °C (1h)- 48 °C (2h) proved to be the lethal and induction temperatures respectively for both the varieties based on the recovery responses.

In the second part of Experiment I growth performance of temperature induced seedlings of variety Vellayani Vijay was evaluated under elevated CO_2 (500 ppm) and ambient conditions. The experiment was laid out in CRD with four replications. Growth parameters like plant height, number of branches and number of leaves were not significantly affected by temperature induction but significantly higher value was recorded in the case of specific leaf area (283.50 $cm^2 g^{-1}$) compared to control (261.83 $cm^2 g^{-1}$). Among the physiological and biochemical parameters, temperature induction resulted in significantly higher values for total chlorophyll content (1.74 mg g^{-1}), total carbohydrate (36.71 mg g^{-1}), photosynthetic rate (22.13 µmol CO_2 m^{-2} sec^{-1}), water use efficiency (4.98 mmol CO_2 mol^{-1} H_2O), chlorophyll fluorescence (Fv/Fm) (0.82), relative water content (92.20 %), superoxide dismutase (0.21 activity $g^{-1}min^{-1}$),

chlorophyll stability index (145.97 %) and proline content (1.32 μM g^{-1} tissue) compared to plants without induction.

In the second experiment, flowering and fruiting in tomato under elevated CO_2 environment, as influenced by growth regulators and nutrient applications, were evaluated. The experiment was laid out in CRD with eight treatments and three replications. The treatments comprised of 50 ppm NAA (T1), 50 ppm Salicylic acid (T2), 50 ppm Boron (B) (T3), 50 ppm Boron (B) + 50 ppm Zinc (Zn) (T4), as foliar spray (at 40,55 and 75 DAS), nutrient application of POP 125% N: 100% P: 100% K (T5), POP 125% N: 125% P: 125% K (T6), a control (water spray) (T7) and an absolute control (T8).

Plant height, number of branches, number of leaves and specific leaf area were found to increase significantly under elevated CO_2 condition. Among the treatments, T3 recorded taller plants (85.33 cm). However number of branches (5.50), number of leaves (46.67) were significantly higher in T5. T6 recorded significantly higher value (347.99 cm^2 g^{-1}) for specific leaf area.

All the physiological and biochemical parameters except transpiration rate and total soluble protein content showed significantly higher values inside OTC. T3 significantly higher values for chlorophyll content (1.55 mg g^{-1}) and chlorophyll stability index (124.88 %) among treatments. T5 recorded highest values for photosynthetic rate (21.62 μmol CO_2 m^{-2} sec^{-1}) and water use efficiency (4.67 mmol CO_2 mol^{-2} H_2O). T6 resulted in significantly higher values for chlorophyll fluorescence (0.75), relative water content (96.87 %), superoxide dismutase (0.23 activity g^{-1} min^{-1}) and peroxidase (44.43 unit min^{-1} g^{-1}) compared to control.

Flowering was delayed under elevated CO_2 condition. There was an increase in total number of flower clusters inside OTC. However a significant reduction in pollen viability (82.35 %) was noticed under elevated CO_2 conditions. T3 resulted in a higher pollen viability (30.28 %) and fruit setting percentage (38.52 %) compared to control

under elevated CO_2 condition. Days to fruiting was found to be delayed inside OTC. T7 and T4 significantly increased the fruit weight. T5 resulted in less fruit drop leading to increased number of fruits.

Yield per plant was significantly higher for T5 (190.85 g plant^{-1}), T6 (155.21 g plant^{-1}) and T3 (148.29 g plant^{-1}) compared to control (61.83 g plant^{-1}). Under elevated CO_2 condition, T5, T4and T3 were found to increase the yield per plant. T3, T1 and T6 resulted in an increase in the lycopene content, thus improving quality of the fruit.

In the present study, CO_2 enrichment was found to have a deleterious influence on flowering and fruiting in tomato mainly due to reduced pollen viability and floral deformities. TIRT was proved to improve photosynthetic efficiency and stress tolerance mechanism. Foliar spray with 50 ppm B + 50 ppm Zn at 40, 55 and 75 DAS and addition of 25% extra nitrogen than recommended dose in equal splits were found to improve yield and quality to a great extent. This was achieved through improvement in pollen viability, fruit set and individual fruit weight and also through a reduction in fruit drop. These treatments can be exploited further, individually or in combination, in any crop management programme for tomato under increasing CO_2 and associated high temperature conditions. The study also highlights a need for reassessment of critical nutrient requirements for individual crops in the changing global climatic scenario.

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