

उच्च तापमान एवम् कार्बन डाइऑक्साइड का मक्का में मेडिस  
पर्ण झुलसा रोग की संभावित सहनशीलता पर प्रभाव

**Effect of Elevated Temperature and Carbon Dioxide on  
Tolerance Potential of Maize to Maydis Leaf Blight  
Disease**

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# Effect of Elevated Temperature and Carbon Dioxide on Tolerance Potential of Maize to Maydis Leaf Blight Disease

A Thesis

By

**RAM KUMAR**

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## **CERTIFICATE**

This is to certify that the thesis entitled “**Effect of Elevated Temperature and Carbon Dioxide on Tolerance Potential of Maize to Maydis Leaf Blight Disease**” submitted to the Post Graduate School, ICAR-Indian Agricultural Research Institute, New Delhi in partial fulfillment of the requirements for the award of the degree of **Master of Science in Environmental Sciences** embodies the results of *bona fide* research work carried out by **Mr. Ram Kumar (Roll no. 20420)** under my supervision and guidance, and that no part of the thesis has been submitted by him for any other degree or diploma. It is further certified that any help or information received during the work on this thesis has been duly acknowledged by him.

Date: July 21, 2015

Place: New Delhi 110 012

**Usha Mina**

(Chairperson, Advisory committee)

*Dedicated to my  
Chairperson  
and Advisory committee  
members*



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---

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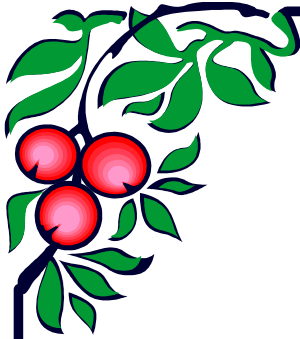
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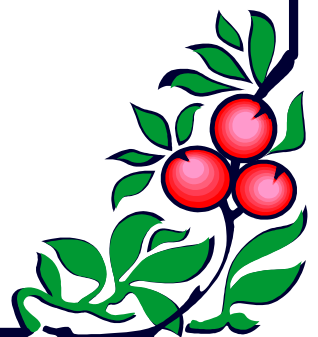
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## **Chapter-1**

# **INTRODUCTION**



# Introduction

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Maize (*Zea mays* L.), as one of the major staple food crop contributes to food and economic security in most of the developed and developing countries (CIMMYT, 2014). It contributes maximum among the food cereal crops, i.e. 40 % annually in the global food production (DMR, 2012). Among the world's maize growing countries, USA is the largest producer which contributes nearly 35% of the total maize production followed by China with more than 20% production with same acreage as of USA (Dass *et al.*, 2012). Together with rice and wheat, maize provides 30% of the food calories to more than 4.5 billion people in 94 developing countries (Renard, 2014). Globally, maize is grown on more than 160 Mha area across 166 countries in a wide variety of soil, climate, biodiversity and management practices (Meena *et al.*, 2014). It is considered to be originated at Central America and Mexico (Wilkes, 1979). Its production in India occurred around the beginning of the seventeenth century, during the early days of the East India Company. The importance of maize lies in its wide industrial applications besides serving as human food and animal feed. Maize is primarily used for feed (60%), followed by human food (24%), industrial (starch) products (14%), beverages and seeds (1% each) (DMR, 2012). Among the major maize producing states in India, Andhra Pradesh tops the list with the contribution of 21.64% to the total Indian maize production followed by Karnataka (15.43%) and Bihar (10.48%) (Anon., 2014a). The growing demand of maize in poultry and livestock sectors, non- vegetarian population and changing food habits suggests that in India demand of maize will increase from current level of 22 mt to 45 mt by 2030 (DMR, 2013). To meet the growing demand, enhancement of maize yield in coming years across all the growing locations in India is the big challenge in the era of climate change.

As a result of climate change, the global surface temperature increase by the end of the 21st century is likely to exceed 1.5°C relative to 1850 and CO<sub>2</sub> concentration has been projected to reach 560 ppmv by 2050 (IPCC, 2013). Many studies have reported the effect of elevated temperature (Naveed *et al.*, 2014; Rahman *et al.*, 2013; Cairns *et al.*, 2012; Suwa *et al.*, 2010) and CO<sub>2</sub> (Vaughan *et al.*, 2014; Hussain *et al.*, 2013; Twine *et al.*, 2013; Chun *et al.*, 2011) on various

physiological and biochemical characters of maize, but effect of elevated temperature and CO<sub>2</sub> in combination on disease tolerance potential of maize needs a systematic study.

In tropical and temperate regions, 61 diseases in maize have been reported (Payak and Sharma, 1981). Of these diseases, maydis leaf blight (MLB), also known as southern corn leaf blight, caused by the fungus *Bipolaris maydis* (Nisikada and Miyake) Shoemaker is a major disease of maize. It has potential to reduce the grain yield up to the extent of 70% in susceptible cultivars (Nasir *et al.*, 2012). MLB was first reported by Drechsler (1925) on maize in Florida and teosinte in the Philippines. In India, the disease was first reported by Munjal and Kapoor (1960) from the specimens collected from Malda region (West Bengal) by Butler in 1905. Maydis leaf blight is also known as southern corn leaf blight due to its prevalence in the southern region of United States of America. MLB has been reported from most maize growing regions of the world and in our country it has been found in almost all maize growing areas.

Symptoms of MLB vary according to the causal race and host germplasm. Pathogen causing MLB has been reported to have three races, viz. race 'T', race 'O' and race 'C', of which, 'O' is prevalent in India (Singh *et al.*, 2014). Lesions produced by race 'O' are initially small and diamond shaped. They elongate as they mature, but growth of lesions are restricted by leaf veins. Ultimate lesions are rectangular (2–6 × 3–22 mm), restricted by leaf veins, and tan in colour. Lesions caused by isolates of race 'O' are restricted to leaves. Lesions caused by race 'T' are oval and slightly larger (6–12 × 6–27 mm) than those caused by race 'O'. Lesion borders are characterized by dark, brown borders. Race 'T' causes lesions on all above ground parts of the plant including stem, sheaths and ears. It can also cause ear rots.

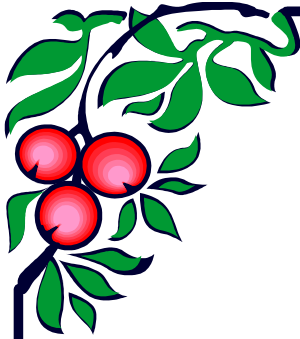
Warm (21° to 32°C temperature) and high humidity (more than 80%) conditions are most favourable for incidence of MLB. In India, MLB disease occurs in *kharif* (June to September) maize when the climate is hot and humid and is prevalent in Jammu & Kashmir, Himachal Pradesh, Sikkim, Meghalaya, Punjab, Haryana, Rajasthan, Delhi, Uttar Pradesh, Bihar, Madhya Pradesh, Gujarat, Maharashtra, Andhra Pradesh, Karnataka and Tamil Nadu (Shekhar and Kumar, 2013). The fungi overwinters in infected crop debris and therefore regions where infected crop debris overwinters and where maize is cultivated continuously, are at high risk.

Since weather determines the development of MLB disease, the increasing temperature and CO<sub>2</sub> levels due to climate change may affect the development of disease in coming decades and also it can change the morphological, physiological and biochemical characteristics of maize, which may alter ecological interaction between the MLB pathogen and maize.

In India, although studies related to impact of elevated temperature and CO<sub>2</sub> on growth and yield of maize has been done, however impact of elevated temperature and CO<sub>2</sub> on response of maize crop to diseases has to be undertaken.

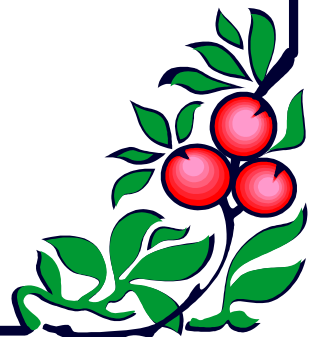
Keeping these views in mind, the present research work entitled “Effect of elevated temperature and carbon dioxide on tolerance potential of maize to maydis leaf blight disease” was undertaken with the following objectives:

1. To assess impact of elevated temperature and carbon dioxide on maydis leaf blight disease development on resistant and susceptible genotypes of maize.
2. To assess impact of elevated temperature and carbon dioxide on maydis leaf blight disease tolerance potential of maize.



## **Chapter-2**

# **REVIEW OF LITERATURE**



## Review of Literature

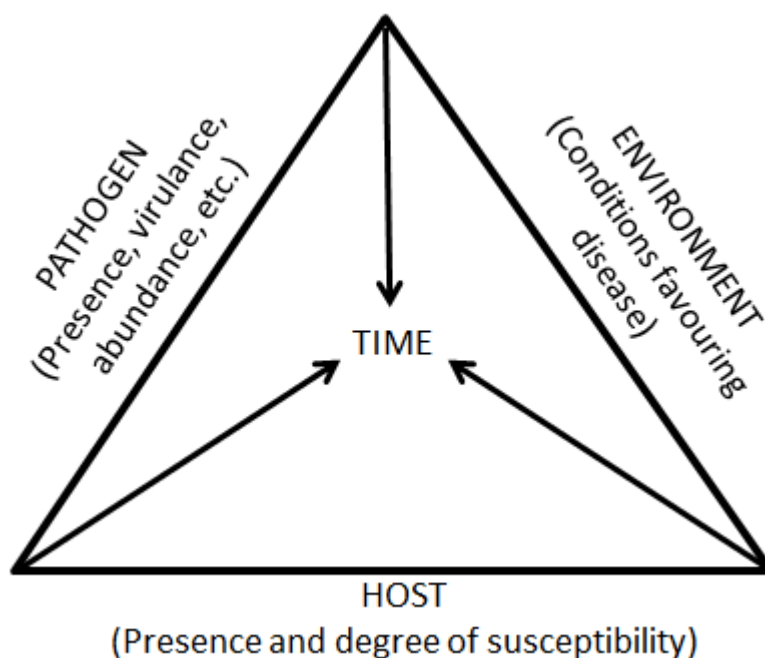
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Global food production has to must increase by 50% to meet the projected demand of the world's population by 2050 (Chakraborty and Newton, 2011), but due to effect of climate change the production is going to be affected severely (Sakschewski *et al.*, 2014). Recent studies have shown that global atmospheric carbon dioxide (CO<sub>2</sub>) has increased by 40% since pre-industrial times, primarily from fossil fuel emissions and secondarily from net land use change emissions. Rising of atmospheric CO<sub>2</sub> has caused the global average surface temperature to increase by 0.89°C ± 0.2°C over the 1901–2012 period, while relative to the average from year 1850 to 1900, global surface temperature change by the end of the 21<sup>st</sup> century is projected to likely exceed 1.5°C for different Representative Concentration Pathways (RCPs) like RCP 4.5, RCP 6.0 and RCP 8.5 (IPCC, 2013).

Both elevated temperature and carbon dioxide affects growth, physiology and yield parameters of plants (Sicher and Bunce, 2015). Many studies have been done to assess the effect of elevated temperature and carbon dioxide level on various parameters of the crop plants in different regions of the world. Studies report that both, natural and cultivated plants are affected by elevated CO<sub>2</sub> (Norby and Luo, 2004; Jablonski *et al.*, 2002; Kimball, 1983) and high temperature (Morecroft and Paterson, 2006; Körner, 2006). Elevated atmospheric CO<sub>2</sub> has positive effects on crop growth and productivity, both in terms of quantity and quality, by increasing photosynthesis and water use efficiency and decreasing transpiration through reducing stomatal conductance (Long *et al.*, 2004). Contrary to this, elevated temperature has negative impact on growth of plants including respiratory losses (Aiken, 2009), poor germination rate (Peacock and Heinrich, 1984) and decreased grain yield potential (Zinn *et al.*, 2010; Prasad *et al.*, 2006).

Crop species are directly affected by increased atmospheric CO<sub>2</sub>, which changes the physical structure of plants and carbon : nitrogen balance (Torbert *et al.*, 2004) and in turn, affects growth, yield (Kimball *et al.*, 2002), tolerance to drought stress (Robredo *et al.*, 2007) and susceptibility to pests and herbivores (Heagle, 2003). Despite abundant evidence about the direct effect of elevated temperature and carbon

dioxide on crop plants, we still need a more thorough understanding of their impact on plant diseases in agricultural ecosystem to predict and plan for disease pressure under future climatic condition. For a plant disease to occur, a pathogen, a host plant and favourable environmental conditions must be present over a certain period of time (Agrios, 2005). This is known as disease triangle (Figure 2.1). Thus, environmental conditions are a prime factor in disease development on host plant, so crop plant response to disease and pest will be certainly affected due to climate change (elevated temperature and carbon dioxide level) over a period of time.



**Figure 2.1: The plant disease triangle**

### **2.1 Effect of elevated temperature on crops**

Elevated temperature has detrimental effect on crop growth and productivity. Effect of elevated temperature on different crop species has been assessed in different crops (wheat, pearl-millet, maize, sugarcane, tomato, groundnut, rice, sorghum, soybean etc.) and has been summarized in Table 2.1.

**Table 2.1: Impact of elevated temperature on crops**

Parameter	Plant species	Impact	References
Seed germination, seedling emergence and its establishment	Wheat, Pearl-millet, Maize	↓	Grass and Burris, 1995; Ashraf and Hafeez, 2004; Wahid <i>et al.</i> , 2007
Biomass, leaf area, photosynthesis and enzyme activity	Maize	↓	Kim <i>et al.</i> , 2007
Shoot dry weight, net assimilation rate (NAR) and relative growth rate (RGR)	Sugarcane, Pearl-millet, Maize	↓	Ashraf and Hafeez, 2004; Wahid, 2007
Increased flower abortion and decreased seed size	Groundnut	↓	Talwar <i>et al.</i> , 1999
Photosynthesis, leaf area, shoot and grain biomass, sugar content of kernels	Wheat	↓	Shah and Paulsen, 2003
Cell water relations	Sugarcane, Tomato, Wheat, Sorghum	↓	Wahid and Close, 2007; Mazorra <i>et al.</i> , 2002; Machado and Paulsen, 2001
Activity of RUBISCO in C <sub>3</sub> and C <sub>4</sub> plants	Maize, Wheat, Cotton, Jojoba, Tobacco	↓	Salvucci and Crafts-Brandner, 2004; Crafts-Brandner and Salvucci, 2000,2002; Law and Crafts-Brandner, 1999
Structure of thylakoid membrane	Maize	↓	Karim <i>et al.</i> , 1997
Enhances chlorophyllase activity and decreased photosynthetic pigments concentrations	Mouse ear cress	↓	Todorov <i>et al.</i> , 2003
Respiratory losses of photosynthates and increased crop pest interactions	Wheat, Soybean, Rice, Sorghum, Maize	↓	Aiken, 2009

↑- Positive impact; ↓- Negative impact

## 2.2 Effect of elevated CO<sub>2</sub> on crops

The primary response of plants to elevated CO<sub>2</sub> is increase in photosynthetic rate and reduced stomatal conductance (Ainsworth and Rogers, 2007). The increase in photosynthetic rate occurs because Rubisco is not saturated at ambient CO<sub>2</sub> level in C<sub>3</sub> plants. Numerous studies have been done to assess the effect of rising atmospheric CO<sub>2</sub> level and the studies indicate that rate of leaf stomatal conductance will decrease, primarily as an adaptive response to conserve water (Buckley & Schymanski, 2014). Plants generally exhibit reduced stomatal conductance in elevated CO<sub>2</sub> level. Lower stomatal conductance results in reduced water loss from transpiration and C<sub>3</sub> plants shows a greater response to elevated CO<sub>2</sub> compared to C<sub>4</sub> plants (Grossman and Rice, 2014). Ainsworth and Long (2005) reported that exposure to elevated CO<sub>2</sub> level resulted in 31% increase in photosynthetic rate and 28% increase in diurnal photosynthetic carbon assimilation. Increasing atmospheric CO<sub>2</sub> concentration will increase the photosynthetic rate in C<sub>3</sub> plants but C<sub>4</sub> plants are less responsive to elevated concentration of CO<sub>2</sub> as they have evolved with the mechanism to concentrate CO<sub>2</sub> in the leaf mesophyll (Ghannoum, 2009). At elevated CO<sub>2</sub> a 'fertilization effect' stimulates plant growth and productivity and changes plant morphology, canopy architecture, anatomy, physiology, chemical composition and gene expression profile (Braga *et al.*, 2006; Matros *et al.*, 2006; Sinha *et al.*, 2009). Morphological changes at elevated CO<sub>2</sub> including increased plant height, number of branches and tillers, and number, thickness and area of leaves all lead to an enlarged canopy. These and other changes in canopy size, density and architecture influence canopy microclimate (Sauer *et al.*, 2007). Under elevated CO<sub>2</sub> level dry mass per unit of leaf N increases. For example, in rice, the critical N concentrations were 4% at ambient CO<sub>2</sub> level, but when it was doubled the critical level decreased to 2.8% (Aben *et al.*, 1999). Response of plants to elevated CO<sub>2</sub> concentration on various parameters has been summarized in Table 2.2. It can be concluded that both C<sub>3</sub> and C<sub>4</sub> plants are benefitted by increase in concentration of CO<sub>2</sub> level but positive effect is more pronounced in case of C<sub>3</sub> plants than C<sub>4</sub> plants, but findings are also available which shows that C<sub>4</sub> plants are not at all benefitted. For example, Leaky *et al.* (2009) reported that when maize was grown without drought stress at high concentration of CO<sub>2</sub> level (550 ppm), then there was no effect on photosynthetic rate, plant growth, yield and biomass.

**Table 2.2: Impact of elevated CO<sub>2</sub> on crops**

Parameter	Plant species and type (C <sub>3</sub> /C <sub>4</sub> )	Response to elevated CO <sub>2</sub>	References
<b>Growth parameters</b>			
Plant height	Maize (C <sub>4</sub> )	↑	Driscoll <i>et al.</i> , 2006
Leaf number and LAI	Soybean (C <sub>3</sub> )	↑	Ainsworth and Long, 2005; Ainsworth <i>et al.</i> , 2002
Shoot biomass	Sugarcane (C <sub>4</sub> )	↑	de Graaff <i>et al.</i> , 2006; De Souza <i>et al.</i> , 2008
Root biomass	Wheat (C <sub>3</sub> ), Ryegrass (C <sub>3</sub> ), Rice (C <sub>3</sub> )	↑	Kimball, 2011
Total biomass	<i>Populus tremuloides</i> (C <sub>3</sub> )	↑	Karnosky <i>et al.</i> , 2003
<b>Physiological parameters</b>			
Stomatal conductance	Soybean (C <sub>3</sub> )	↓	Buckley & Schymanski, 2014; Ainsworth & Long, 2005
Water use efficiency	Wheat (C <sub>3</sub> )	↑	Tiedemann and Firsching, 2000
Photosynthesis	Sugarcane (C <sub>4</sub> )	↑	De Souza <i>et al.</i> , 2008
Photorespiration	Rice (C <sub>3</sub> ), Soybean (C <sub>3</sub> )	↑	Fukayama <i>et al.</i> , 2009; Ainsworth <i>et al.</i> , 2006
<b>Bio-chemical parameters</b>			
Sugar and starch content	Soybean (C <sub>3</sub> ), <i>Populus tremuloides</i> (C <sub>3</sub> )	↑	Hamilton <i>et al.</i> , 2005; Karnosky <i>et al.</i> , 2003
Leaf Phenolics	<i>Populus tremuloides</i> (C <sub>3</sub> )	↑	Noormets <i>et al.</i> , 2010
C:N ratio of leaf tissue	Field pea (C <sub>3</sub> ), Barley (C <sub>3</sub> ), Soybean (C <sub>3</sub> )	↑	Lam <i>et al.</i> , 2013; Ainsworth & Long, 2005
<b>Yield</b>	Field pea (C <sub>3</sub> ), Barley (C <sub>3</sub> )	↑	Lam <i>et al.</i> , 2013
<b>Defence related</b>			
ROS scavenging network	Populus (C <sub>3</sub> ), Soybean (C <sub>3</sub> )	↑	Taylor <i>et al.</i> , 2005; Ainsworth <i>et al.</i> , 2006
Senescence	Sugarcane (C <sub>4</sub> ), <i>Populus tremuloides</i> (C <sub>3</sub> )	↓	De Souza <i>et al.</i> , 2008; Cseke <i>et al.</i> , 2009
Defence	Rice (C <sub>3</sub> ), Soybean (C <sub>3</sub> )	↑	Fukayama <i>et al.</i> , 2009; Leakey <i>et al.</i> , 2009
Flavonoid biosynthesis	Populus (C <sub>3</sub> ), Soybean (C <sub>3</sub> )	↑	Taylor <i>et al.</i> , 2005; Ainsworth <i>et al.</i> , 2006

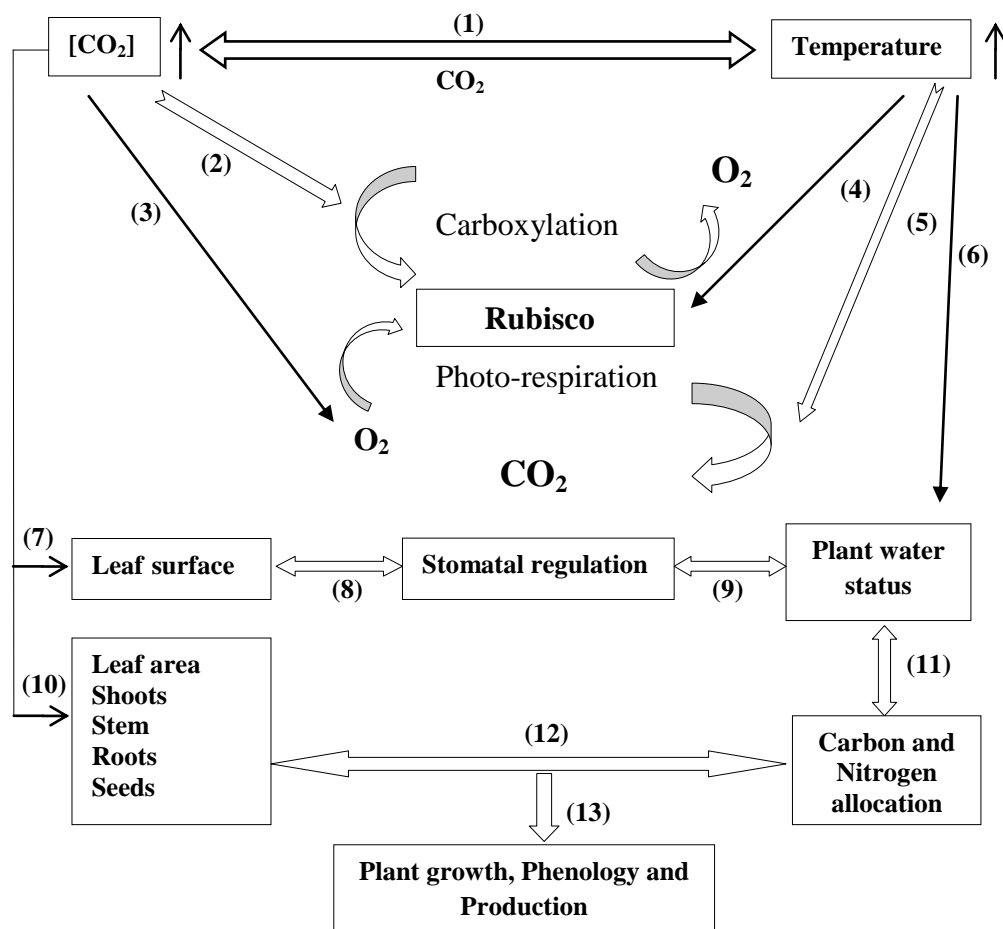
(↑- Positive response; ↓- Negative response)

### 2.3 Combined effects of elevated temperature and CO<sub>2</sub> on crops

Understanding how plants respond to simultaneous increases in atmospheric CO<sub>2</sub> and temperature is a central topic of climate change. Crop responses to elevated CO<sub>2</sub> are dependent on temperature (Wheeler *et al.*, 1994). Both CO<sub>2</sub> and temperature as environmental factors affect crop yields, in terms of seed quantity and quality. These two factors affect various aspects of plants such as growth, physiological and biochemical process from ecosystem, and even to the biome level (Milcu *et al.*, 2012). The increase in plant growth and leaf area under elevated CO<sub>2</sub> may be confounded by the above optimum temperature. Baker *et al.* (1989) reported that in soybean, increased plant growth with high CO<sub>2</sub> concentration decreases remarkably with high temperature. Lower stomatal conductance under elevated CO<sub>2</sub> may provoke heat damage in plants under high temperatures (Warren *et al.*, 2011).

Wang *et al.* (2012) studied the combined effect of elevated temperature and CO<sub>2</sub> by grouping the different plant species according to photosynthetic pathway (C<sub>3</sub> and C<sub>4</sub>), functional types, growth forms and economic purposes. They found that at elevated CO<sub>2</sub> (>560 ppm) there was enhanced photosynthesis at ambient temperature, elevated temperature (ambient temperature + 1.4–6°C) and heat stress (ambient temperature + >8°C) in C<sub>3</sub> species. On the other hand, in C<sub>4</sub> species elevated CO<sub>2</sub> (>560 ppm) had no effect at ambient temperature, a positive effect at elevated temperature (ambient temperature + 1.4–6°C) and a negative effect at heat stress (ambient temperature + >8°C) condition. Positive effect of elevated CO<sub>2</sub> on net photosynthesis was greater for legumes than for non-legumes in heat stress, for non-crops than crops at elevated temperature and for woody and herbaceous species at elevated temperature and heat stress. Total, above and below-ground biomasses were increased by elevated CO<sub>2</sub> for most species groups at all temperatures except for C<sub>4</sub> species and below-ground biomass of legumes at heat stress condition. A general schematic illustration of the combined effects of elevated CO<sub>2</sub> and temperature on plant growth, physiology and nutrient allocation is shown in Figure 2.2. Photosynthesis may be increased through increase in Rubisco carboxylation and the decrease in photorespiration under elevated CO<sub>2</sub> (Long *et al.*, 2004; Ainsworth and Rogers, 2007). However, higher temperatures above the optimum may inhibit carboxylation (Yu *et al.*, 2012). Dark respiration sensitivity may depend on

acclimation to high temperature, despite the plant functional group (Campbell *et al.*, 2007; Smith and Dukes, 2013). Water use efficiency may be increased by CO<sub>2</sub> enrichment through decreased stomatal conductance (Long *et al.*, 2004) and density (Teng *et al.*, 2006), which would be negated by warming through enhanced temperature (Warren *et al.*, 2011; Maestre *et al.*, 2012). It is hypothesized that elevated CO<sub>2</sub> may alleviate the damage done to photosynthesis by higher temperatures because with higher CO<sub>2</sub> concentrations there is a synergistic interaction between improved plant water status and protection of photosynthesis against high temperature damage (Yu *et al.*, 2012; Bauweraerts *et al.*, 2013). Zhao *et al.* (2012) reported that high temperature increased the photosynthetic capacity of *P. cathayana* under CO<sub>2</sub> enrichment. CO<sub>2</sub> enrichment decreased the physiological limitation induced by heat in tall fescue plants by reducing stomatal conductance by 20 % and the dark respiration rate by 7%, leading to high water use efficiency and a more positive carbon balance (Yu *et al.*, 2012).



**Figure 2.2: Impact of elevated CO<sub>2</sub> and temperature interaction on crops**

- (1) Increasing carbon dioxide levels in the atmosphere can lead to a rise in the mean global surface temperature
- (2) In crops increased CO<sub>2</sub> concentration enhances Rubisco carboxylation and
- (3) weakens its photorespiration
- (4) Increased temperature above the optimum to specific crops may inhibit carboxylation and in contrast
- (5) promote the oxygenation reaction, photorespiration, and respiration sensitivity may depend on high temperature acclimation
- (6) Plant water status may be adversely affected by high temperature
- (7) CO<sub>2</sub> enrichment modifies the leaf surface, such as stomatal size and its density,
- (8) decreases stomatal conductance and transpiration and consequently
- (9) improves plant water status, (10) the leaf area and other plant tissue growth may be promoted by elevated CO<sub>2</sub> but may not be confirmed with some FACE experiments
- (11) The leaf sugar concentration increases under elevated CO<sub>2</sub> and decreases as the temperature rises. In contrast, an opposite nitrogen reallocation pattern may occur in response to CO<sub>2</sub> enrichment or high temperature as well as their interaction
- (12) Finally, carbon and nitrogen allocation is associated with organs
- (13) Plant growth, phenology, and production, under the combined stresses (Xu *et al.*, 2013).

## 2.4 Effect of elevated CO<sub>2</sub> on crop – pathogen interaction

Plant disease expression results from a three-way interaction of a susceptible host plant, a virulent pathogen and an environment suitable for disease development; referred to as the disease triangle. Changes in environmental conditions are known to exacerbate plant disease symptoms (Boyer, 1995; McElrone *et al.*, 2001) and are implicated in 44% of new disease emergence (Anderson *et al.*, 2004). Thus, the altered environmental conditions associated with climatic change (e.g. temperature regimes, atmospheric chemistry and drought) have the potential to alter the incidence and severity of plant disease epidemics and disease pressures on natural and crop plant systems, as well as to reshape the co-evolutionary relationships between crops and pathogens (Chakraborty, 2005; Burdon *et al.*, 2006; Fargette *et al.*, 2006; Ziska and Runion, 2007; Crowl *et al.*, 2008). Recent free air CO<sub>2</sub> enrichment (FACE) and open-top-chamber (OTC) studies involving plant diseases continue to support earlier findings that show responses to elevated CO<sub>2</sub> levels vary with the host-pathogen system. In some cases predictions of higher disease levels resulting from increased plant growth have been verified, especially for necrotrophic pathogens. However, there are examples of both necrotrophic and biotrophic pathogens showing lower disease levels, increased disease levels, or no effect of increased concentrations of CO<sub>2</sub> (Table 2.3, 2.4 and 2.5). Studies have been reported related to Downy mildew of soybean, Late blight of potato, Brown spot of soybean, Rice blast and Crown rot of wheat but studies related to maydis leaf blight was not found.

**Table 2.3: Effects of elevated CO<sub>2</sub> on crop pathosystems**

Effect on disease severity/disease system	Effects on host	References
<b>Lower disease severity or incidence</b>		
Downy mildew of soybean	No changes in stomatal densities; some changes in cuticular wax structure	Eastburn <i>et al.</i> , 2010
Late blight of potato	No effect on plant growth or canopy structure; increased levels of $\beta$ -1-3 glucanases	Plessl <i>et al.</i> , 2007
<b>Increased disease severity or incidence</b>		
Powdery mildew of Arabidopsis	More stomata on resistant varieties and fewer on susceptible varieties; resistant varieties become more susceptible	Lake & Wade, 2009
Brown spot of soybean	Increased plant height and canopy density	Eastburn <i>et al.</i> , 2010
Rice blast	Lower silicon concentrations in leaf tissues	Kobayashi <i>et al.</i> , 2006
Sheath blight of rice	Increased number of tillers per plant under elevated CO <sub>2</sub>	Kobayashi <i>et al.</i> , 2006
Crown rot of wheat	Increased plant biomass under elevated CO <sub>2</sub>	Melloy <i>et al.</i> , 2010

**Table 2.4: Response of pathogens to elevated CO<sub>2</sub>**

Pathogen	Host	Effect of elevated CO <sub>2</sub>	References
<i>Cladosporium fulvum</i>	tomato	enhances sporulation	Manning & Tiedemann, 1995
<i>Blumeria graminis</i>	Barley	reduced germination and penetration; increased growth rate and fecundity	Hibberd <i>et al.</i> , 1996
<i>Phytophthora parasitica</i>	Tomato	increased pathogen biomass in host tissue	Jwa & Walling, 2001
<i>Puccinia striiformis</i>	Wheat	did not influence fecundity in either resistant or susceptible variety	Chakraborty <i>et al.</i> , 2010
<i>Fusarium pseudograminearum</i>	Wheat	increased pathogen biomass in host tissue	Melloy <i>et al.</i> , 2010
Barley yellow dwarf virus	Oats	increased persistence of infected crops to alter epidemiology	Malstrom and Field, 1997

Table 2.5: Effects of elevated temperature / CO<sub>2</sub> on diseases of field crops

Crop	Disease/pathogen	Pathogen mode of nutrition	Summary of influence	Overall assessment	Reference
Barley	Powdery mildew – <i>Blumeria graminis</i>	Biotrophic	Decreased at high CO <sub>2</sub>	Decrease	Hibberd <i>et al.</i> , 1996
Barley	Smut – <i>Ustilago hordei</i>	Biotrophic	Increased smutted ears at high CO <sub>2</sub>	increase	Manning & Tiedemann, 1995
Maize	Smut – <i>Ustilago maydis</i>	Biotrophic	Inhibited at high CO <sub>2</sub>	Decreases	Manning & Tiedemann, 1995
Potato	Early blight – <i>Alternaria solani</i>	Necrotrophic	No effect of high CO <sub>2</sub>	No change	Manning & Tiedemann, 1995
Rice	Sheath blight – <i>Rhizoctonia solani</i>	Necrotrophic	Increased at high CO <sub>2</sub>	increase	Kobayashi <i>et al.</i> , 2006
Wheat	Stripe rust – <i>Puccinia striiformis</i>	Biotrophic	Increased with pathogen adapting to higher temperature	Increase	Milus <i>et al.</i> , 2006
Wheat	Leaf rust – <i>Puccinia triticina</i>	Biotrophic	Inhibition at higher CO <sub>2</sub> concentration	Increase	Manning & Tiedemann, 1995
Soybean	Stem canker – <i>Phytophthora rasojae</i>	Necrotrophic	<i>Phytoalexin glyceollin</i> production at high CO <sub>2</sub>	Increase or Decrease	Braga <i>et al.</i> , 2006

During literature review it had been observed that different crops (barley, maize, potato, rice, soybean, wheat etc.) have been studied either for the effect of elevated temperature or elevated CO<sub>2</sub> on their various diseases, but not simultaneously at a time. With this background in the present study effect of simultaneous exposure of elevated CO<sub>2</sub> and elevated temperature to maize crop genotypes on maydis leaf blight disease development as well as biotic stress resistance potential has been done.

## **2.5 Maize: crop geography and ecology**

The centre of origin for *Zea mays* L. has been thought to be the Mesoamerican region, now Mexico and Central America (Watson and Dallwitz, 1992). Domestication of maize began around 6000 years ago (Mangelsdorf, 1974). Christopher Columbus during his journey found that corn was cultivated on Haiti (North America) where it was called “Mahiz”. From this the name ‘Maize’ was derived. So, Christopher Columbus was probably the first person to see and describe maize. According to Vavilov, the high lands of Peru, Bolivia and Ecuador are the starting point of maize as these regions are having great diversity of native forms of maize (De Wit *et al.*, 1972). But according to another belief, maize was originated in Mexico or Central America as this area is considered to be the home of teosinte grass (*Euchlaena Mexicana* Schrad. or *Zea mays* ssp. *parviglumis*), a relative of maize, which shows a large variety in this area. De Condolle (1886) assumed that corn must have originated in New Granada, now Colombia. Mangelsdorf and Reeves (1939) postulated that maize had its origin in the lowlands of South America primarily because of historical reference to pod corn in that area. It is now considered that corn had perhaps more than one origin. Several workers say that it was originated in both Mexico and South America. Maize is believed to have been introduced into Asia from some part of America (Tollenaar and Dwyer, 1999).

Maize (*Zea mays* L.) is an essential cereal food crop of the world with highest production and productivity as compared to rice and wheat. It is the most multifaceted crop which is being grown in more than 166 countries across the globe including tropical, sub-tropical and temperate regions from sea level to 3000 m above mean sea level. A crop of maize is sown and harvested somewhere in the world in every month of the year. It is grown from 60°N in Canada and Russia to 40°S latitude in Argentina, from below sea-level to an altitude of > 4000 m in the

Peruvian Andes, and in regions with < 25 cm rainfall in semi-arid plains of Russia to regions of > 1000 cm rainfall in north-east India (Sharma and Dass, 2014).

Maize is relatively a warm weather crop and requires considerable moisture and warmth from germination to flowering and requires 120–140 days to mature. The most suitable temperature for germination is 21°C and for growth 32°C. Extreme high temperature and low humidity during flowering may damage the foliage, desiccate the pollen and interfere with proper pollination, resulting in poor grain filling. In India, it is cultivated in all the states, except Kerala. It can be grown successfully on a wide range of soils, ranging from loamy sand to clay loam. The soil pH should be almost neutral (6.5–7.0). It is normally planted at a depth of 5 to 8 cm, provided moisture is adequate at that depth. It absorbs water and begins to swell. This takes place at a faster rate at high temperatures, which are prevalent in most tropical environments in the summer season. Under these conditions, the seed starts to germinate in 2–3 days. In the winter season and in other low soil temperature conditions such as highlands, the process is delayed and radical emergence may take as many as 6 to 8 days (Onderdonk and Ketcheson, 1972).

In India, its production has increased more than 12 times from a mere 1.73 million tons in 1950–51 to 21.76 million tons in 2011–12 and it is estimated to be 22.23 million tons in 2012–13 (Anon., 2014a) and presently it occupies 9.3 million hectare area with the mean yield of 2.60 tons/hectare. Together with rice and wheat, maize provides at least 30% of the food calories of more than 4.5 billion people in 94 developing countries. They include 900 million consumers whose income is less than US \$ 2 per day and for whom maize is the preferred staple, including 120–140 million poor farm families. Between now and 2050, the demand for maize in the developing world will be doubled (Anon., 2014b). By 2050, global maize consumption is expected to increase from 32 to 52 kilograms per person per year. Changing diets, population growth and the demand for maize as a source of fuel and animal feed will also increase consumer prices (Anon., 2014c). So, maize yields need to increase by 60% by 2050 to meet demand (Anon., 2014d).

Like other crops, maize also encounters various diseases caused by fungal, bacterial and viral pathogens. Maize is affected by many fungal diseases such as rust, stalk rot, leaf spot, downy mildews, turcicum leaf blight, maydis leaf blight etc. Of these,

maydis leaf blight (MLB), also known as southern corn leaf blight is caused by the ascomycete fungi *Bipolaris maydis* (Nisikado and Miyake) Shoemaker and is also a major disease of maize. It has presence in almost all maize growing areas of our country.

## **2.6 Effect of elevated temperature on maize**

Transitory or constant high temperature can cause an array of morpho-anatomical, physiological and biochemical changes in maize crop. High temperature (>30°C) greatly affects the germination percentage and seedling growth of maize. At temperatures above 38°C maize crops are unable to maintain adequate moisture in its system, evaporation from the soil and transpiration from plant surface also increases, further compounding the drought effect (Pingali and Pandey, 2001).

Flowering and grain filling stages are the most sensitive stages of maize for heat stress (Dass *et al.*, 2010). High temperatures can delay anthesis and damage most of the tassels, resulting in little or no pollen production and increasing the occurrence of male sterile plants in field. Pingali and Pandey (2001) reported that pollen desiccation and silk death can occur at 45°C (Dass *et al.*, 2010). Temperatures above 35°C can result in reduction of leaf area by up to 33% (Thiagarajah and Hunt. 1982). Tollenaar *et al.* (1979) reported influence of high temperature on the duration from planting to silking through their effect on number of leaves per plant. Further, high temperatures have been reported to impede maize plant height by 15%, reduce kernel number per plant, number of leaves, stem diameter, ear characteristics and final dry matter yield (Traore *et al.*, 2000).

High temperatures are closely associated with certain key physiological processes of plant growth in maize such as delay in appearance of new leaf by up to 6 days, and tasselling up to 3 days, poor ear growth rates and leaf elongation rate, reduced radiation use efficiency, water use efficiency, harvest index and net photosynthesis (Traore *et al.*, 2000). Suwa *et al.* (2010) reported reduction in cob growth rate and biomass partitioning to ear hence substantial effect on cob architecture. In addition, high temperatures from tassel initiation to tassel emergence delayed silking more than anthesis, thus increasing the de-synchronization and reducing chances of proper fertilization (Struik *et al.*, 1986; Cicchino *et al.*, 2010). Viability of pollen is as

crucial as anthesis for efficient sexual plant reproduction and ensuring maximum kernel set. Heat stress is one of the most important factors influencing pollen viability; it not only affects pollen viability after dehiscence, when the pollen is exposed to the environment, but also during its development inside the anther (Bots and Mariani, 2005). Fonseca *et al.* (2005) found that the pollen shed from anthers remains viable longer at low temperatures as compared to high temperatures and seed set can drastically get reduced from 51 to 80%. It was also observed that pollen desiccation and subsequent loss of viability is a function of three major environmental components:- air temperature, relative humidity and time of exposure. Pollen water potential have been reported to be the major component affected by high temperatures resulting in damaged pollen and reduced pollen shed (Schoper *et al.*, 1986). High temperature were also found to result in reduced silk solute potential, however this parameter was not found to affect seed set or seed production (Schoper *et al.*, 1987). Hence, it is sensitivity of mature pollen to heat stress which is responsible for failure for fertilization at high temperatures (Dupuis and Dumas, 1990). Further, the genotypes with high temperature resistant pollen are characterized not only by high germination percentage, but also by a better ability to develop normal pollen tubes at the high temperatures (Herrero *et al.*, 1980). A number of factors are responsible for kernel loss under heat stress. In addition to pollination failure floret differentiation and kernel abortion is a source of kernel loss (Rattalino Edreira *et al.*, 2014).

## **2.7 Effect of elevated CO<sub>2</sub> on maize**

Maize is a C<sub>4</sub> crop. In C<sub>4</sub> crops Rubisco is located in the bundle sheath cell chloroplasts, where concentration of CO<sub>2</sub> is 3 to 6 times higher than the atmosphere (von Caemmerer and Furbank, 2003). Recently Erbs *et al.* (2015) found that when maize plants were grown in 380 ppm and 550 ppm CO<sub>2</sub> concentration level using free-air CO<sub>2</sub> enrichment, then the maize plant was not found to respond to increased CO<sub>2</sub> level as long as no drought stress was prominent, but elevated CO<sub>2</sub> concentration was found to mitigate the drought stress impact on elemental composition and quality traits of maize.

In another study, Wang *et al.* (2015) grown maize plants under four level of CO<sub>2</sub> concentration (500, 1000, 3000 and 5000 ppm) to find out the effect on

photosynthesis. They found that maize plant had the highest photosynthetic electron transport and photochemistry quenching under 500–3000 ppm CO<sub>2</sub> concentration, but then decreased substantially at 5000 ppm CO<sub>2</sub>. So, photosynthetic down-regulation and a decrease in photosynthetic electron transport occurred in maize in response to super-elevated concentration of CO<sub>2</sub> at 3000 and 5000 ppm.

## **2.8 Combined effects of elevated temperature and CO<sub>2</sub> on maize**

Recently Qu *et al.* (2014) studied the effect of high temperature and elevated CO<sub>2</sub> concentrations on maize seedlings. They grew plants at 380 ppm CO<sub>2</sub> and 560 ppm CO<sub>2</sub> levels. During high temperature treatment at 45°C for 15 min. they found that high temperature reduced photosynthetic rate by 25 to 38% in different CO<sub>2</sub> levels, but on stomatal conductance and transpiration there was no significant effect. Thus elevated CO<sub>2</sub> failed in protecting photosynthesis rate from 45°C in maize.

## **2.9 Maydis leaf blight**

The maydis leaf blight was first reported by Drechsler (1925) on maize in Florida and teosinte in the Philippines. In India, the disease was first reported by Munjal and Kapoor (1960) from the specimens collected from Malda region (West Bengal) by Butler in 1905. Maydis leaf blight is also known as southern corn leaf blight due to its prevalence in the southern region of United States of America. Although it does not cause epidemic in India, but yield reduction up to 30.3% have been reported in susceptible cultivars (Payak and Sharma, 1978).

### **2.9.1 Pathogen**

Identity of *Bipolaris maydis* (syn. *Helminthosporium maydis*, Teliomorph: *Cochliobolus heterostrophus*) can be confirmed by microscopic examination of conidia that are produced within lesions and in culture. Conidia are also produced profusely within lesions when diseased leaves are incubated in a damp chamber for 24 to 48 hours. Conidia are 15–20 × 70–160 μm in dimension. Conidia are olivaceous brown, spindle-shaped, and taper to round ends. Conidia are 5 to 11 septate and are characterized by bipolar germination. There are 3 physiological races of *B. maydis*: Race T, Race O and Race C. Race T and Race C are pathogenic only

to maize germplasm with cytoplasm male-sterile T and cytoplasm male-sterile C, respectively. Of these races, 'O' is prevalent in India (Misra, 1979).

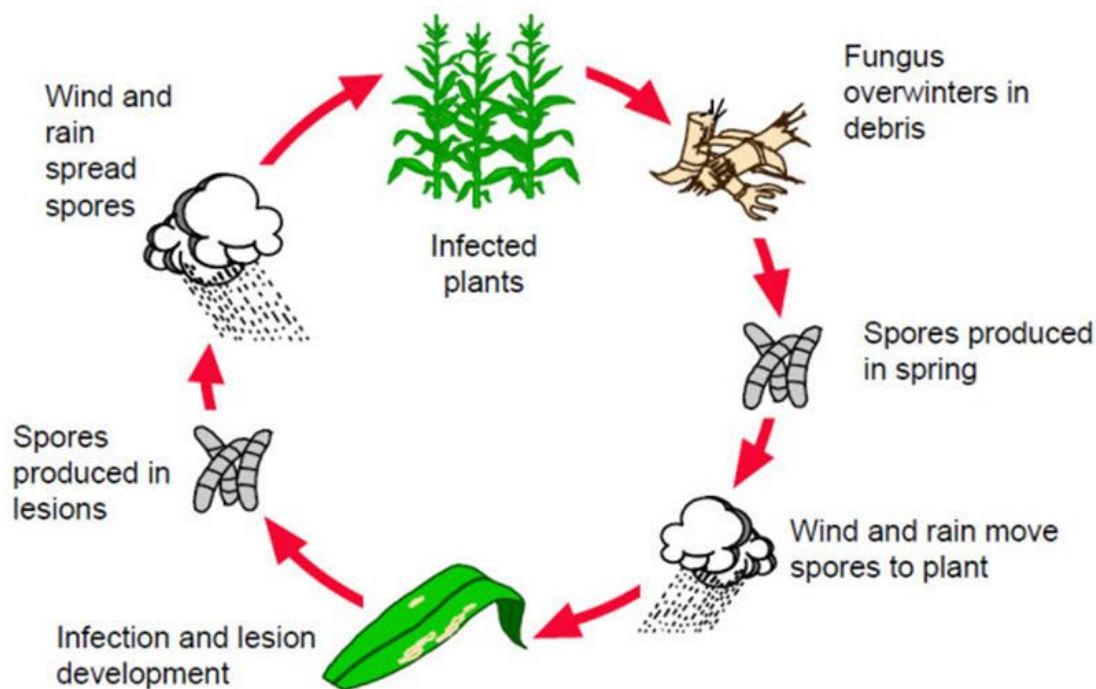
### **2.9.2 Life cycle**

The fungi overwinter as mycelium in infected crop debris that remains on the soil surface between growing seasons (Figure 2.3). At the onset of the subsequent growing season, in response to favourable temperatures and humidity, mycelia within the crop debris begin sporulating (producing conidia). Conidia are then disseminated to freshly planted maize in the vicinity by wind and rain splash. Conidia germinate and infect plants through stomata, giving rise to characteristic lesions within which conidia are produced, which leads to secondary disease cycles. In favourable environmental conditions, the disease cycle can be completed in as little as 72 hours.

*Bipolaris maydis* is a member of the ascomycetes, the sac fungi. Mycelium and spores can overwinter in soil and crop debris. Hence, plough down the infected crop debris can reduce the disease inoculum. Spores are blown off by wind or in water droplets onto the surface of leaves and after they have germinated they enter the plant through the stomata. The fungus produces a toxin that attacks the mitochondria and destroys the plant's ability to capture energy from metabolism. There are very important differences in the type of toxins produced by different races of the agent. Under optimal conditions (20–30°C and moist weather), the fungus can complete its life cycle in 2–3 days.

### **2.9.3 Crop losses due to maydis leaf blight**

Out of the many biotic and abiotic stresses, diseases are playing a direct role in affecting production of maize and earnings. Payak and Sharma (1985) reported 61 diseases of both tropical and temperate origin in maize and determined the major maize diseases causing average yield loss of 13.2% of which foliar diseases alone cause 5% loss. The MLB disease has the potential to reduce the grain yield up to extent of 41 percent in susceptible cultivar (Sharma and Rai, 2000).



**Figure 2.3: Stages in life cycle of *Bipolaris maydis*- maydis leaf blight pathogen**

#### **2.9.4 Geographical distribution of maydis leaf blight in India**

Regions with a warm (20 to 32°C) and damp growing season are most at risk from maydis leaf blight. Long, dry, sunny periods during the growing season are unfavourable for the disease. The fungi overwinter in infected crop debris and therefore regions where infected crop debris overwinters and where maize is cultivated continuously, are at risk. The disease is also known as southern blight of maize. MLB disease is distributed all over the world in warm temperate to tropical (20–30°C) maize producing areas. In India, MLB disease occurs in *kharif* (June to September) maize when the climate is hot and humid and is prevalent in J&K, HP, Sikkim, Meghalaya, Assam, Punjab, Haryana, Rajasthan, Delhi, UP, Bihar, MP, Gujarat, Maharashtra, AP, Karnataka and TN. MLB is found in all tropical and temperate maize growing regions where the growing season is characterized by warm and wet conditions.

#### **2.9.5 Symptoms**

The exact symptoms depend upon the race of the pathogen and the stage of corn affected. As a general rule, tan lesions are seen on leaves with the number and size depending upon the fungal race and the strain of corn. In the worst case the lesions

are numerous and can be several centimetres long and have dark red or purple edges. Ears are also infected with a black substance that is actually masses of conidia (asexual spores) on kernels that can lead to ear and cob rot.

The disease is caused by *Bipolaris maydis* [= *Cochliobolus heterostrophus* (Drechs.)]. Diseased lesions are small, assume a rectangular shape and are parallel to each other. The lesions are numerous (Plate 2.1). Their width is restricted by the veins. So far, three races of *B. maydis* viz. Race O, Race T and Race C have been reported. Lesions produced by the 'T' strain are oval and larger than those produced by the 'O' strain. A major difference is that the 'T' strain affects husks and leaf sheaths, while the lesions of 'O' strain are restricted on the leaves. Race O and T differ in symptoms produced, cytoplasmic specificity, production of toxins, optimum growth temperature, reproductive rate and plant parts attacked (Hooker, 1972).

Race O produces lesions that are initially small and diamond-shaped. These lesions elongate as they mature, although growth of lesions is restricted by leaf veins. Final lesions are rectangular (2–6 × 3–22 mm), restricted by leaf veins, and tan in colour. Lesions caused by isolates of Race O are restricted to leaves. Symptoms of MLB caused by Race T are oval and slightly larger (6–12 × 6–27 mm) than those caused by Race O. Lesion borders are usually characterized by dark, brown borders. Race T causes lesions on all above ground parts of the plant (including stems, sheaths and ears) and can also cause ear rots. Seedlings from seeds infected with Race T often wilt and die within 3 to 4 weeks. Race T causes lesions on all above ground parts of the plant (including stems, sheaths and ears) and can also cause ear rots.

## **2.10 Factors which affects host-pathogen interaction**

### **2.10.1 Response of pest to inoculum level and stage of growth**

Raziq and Ahmad (1992) studied the effect of different inoculum levels of *Bipolaris maydis* (Nisik.) Shoemaker, viz.  $2 \times 10^4$ ,  $3 \times 10^4$  and  $4 \times 10^4$  spores/ml applied at tasseling, silking and milking stages. They recorded disease severity, yield and yield components of maize variety Azam. The disease severity was highest when plants were inoculated with  $4 \times 10^4$  spores/ml at tasseling stage. Grain yield, fresh ear weight, number of kernels/ear and 200 kernel weight were 28.3, 30.9, 32.5 and

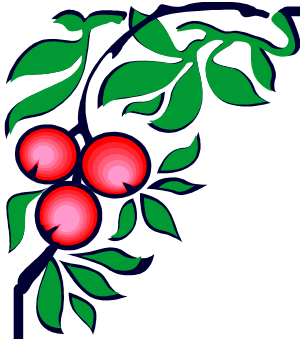
11.8% respectively in this treatment. Plots, where no inoculation was made, showed the lowest disease attack and the highest grain yield and yield component.



**Plate 2.1: Typical symptom of maydis leaf blight disease**

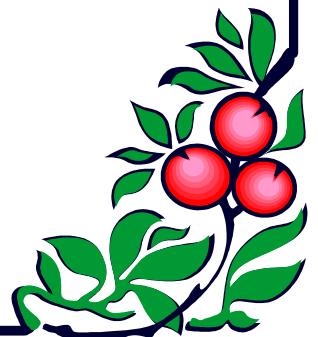
### **2.10.2 Response of pest to agronomic practices and applied nutrient level**

Pal and Kaiser (2001) studied the *kharif* (summer/monsoon) maize, which is popular in the eastern part of the country, may be predisposed to maydis leaf blight, if proper agronomic practices are not followed. Under the artificial epiphytotic conditions showed that the disease incidence was favoured by planting in July, while early planting in May or June or late planting in August reduced the incidence. Disease incidence gradually increased with the increase in plant density and it was maximum at a population of 70000 ha<sup>-1</sup>, while it was minimum at 40000 ha<sup>-1</sup>. Nitrogen alone or in combination with phosphorus and potassium, or with the increase in the dose of nitrogen and the maximum infection occurred at the highest dose of nitrogen at 160 kg ha<sup>-1</sup>. *In vitro* study, however, showed that nitrogen significantly increased the linear growth of the pathogen, while both Phosphorus and Potassium individually or in combination with nitrogen reduced it. Nitrogen also significantly increased the percentage of conidial germination, while both phosphorus and potassium individually or in combination with nitrogen reduced it.



## **Chapter-3**

# **MATERIALS AND METHODS**



## Materials and Methods

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### 3.1 Location of the experimental site

The study was carried out on the experimental farm of Indian Agricultural Research Institute, New Delhi. New Delhi is situated at 28°35' N latitude, 77°12' E longitude and at an altitude of 228.16 m above mean sea level. The location lies in the subtropical belt, has continental monsoon climate exhibiting a masked seasonal rhythm, hot summer, cool winter, un-reliable rainfall and great variation in temperature. In summer, the maximum temperature may reach up to 46°C and in winter, the temperature may be as low as 1°C. It receives an annual average rainfall of 75 cm, out of which about 91 % occurs during June-August. Under average climatic conditions, the area receives 750 mm annual rainfall, about 80% of which occurs from June to September. The mean annual maximum temperature is 35°C while the mean annual minimum temperature is 18°C.

### 3.2 Plant material

Certified seeds of two genotypes of maize PEHM 5 and CM 119 were used in the study. For 1<sup>st</sup> objective seeds of both genotypes were sown in pots (15 L capacity with 30 cm diameter filled with 14 kg of soil and FYM in 3:1 ratio) during the *kharif* season. Plants were maintained in pots throughout experimental crop growth period from seedling to maturity. Pots containing plants of both genotypes were exposed to temperature and carbon dioxide (CO<sub>2</sub>) treatments in different combinations in open top chambers (OTCs) (for 25 days) starting from three leaf stage (10 days old plants). Plants were irrigated regularly and fertilizers were applied as per requirements. For 2<sup>nd</sup> objective plants of PEHM 5 variety were grown in OTCs following standard agronomic practices at 60 cm × 20 cm plant to plant and row to row spacing and exposed to temperature and CO<sub>2</sub> treatments throughout the crop growth period.

### 3.3 Open Top Chambers (OTCs)

The OTCs used in present study were of cylindrical shape with 3 m diameter, 2.5 m height and a door. The basic cylindrical shape of the OTCs was fabricated with galvanized iron (GI) pipe and fixed in the field (Plate 3.1). The fabricated structure was covered with polyvinyl chloride (PVC) sheet which have more than 90% light transmittance. The top portion of one set of the OTC with all intact side walls was kept

open to maintain the temperature level of 1.5°C higher than ambient temperature (1.5T) and elevated CO<sub>2</sub> (550 ppm) concentration (ECO<sub>2</sub>) (Plate 3.2). For maintaining the level of 1.5T + ambient CO<sub>2</sub> (ACO<sub>2</sub>) concentration treatment, similar type of OTC was used (as Plate 3.2), without external supply of CO<sub>2</sub>. For maintaining the temperature level of 3°C higher than ambient temperature (3T) + ECO<sub>2</sub> in OTC, top of the OTC was partially covered with PVC shelter and side walls were kept intact (Plate 3a and 3b). For (3T) + ACO<sub>2</sub> treatment, OTCs as in Plate 3.3a and 3.3b were used, without external supply of CO<sub>2</sub>. Likewise to maintain the ambient temperature (AT) + ECO<sub>2</sub> level, top portion of the OTC was kept opened and side walls were kept partially covered (Plate 3.4) and elevated CO<sub>2</sub> levels were maintained through gas supply. OTC (as in Plate 3.4) for AT + ACO<sub>2</sub> treatment, were used without external supply of CO<sub>2</sub>. The plenum at the base (at height of 0.3 m) was constructed to support perforated pipes for releasing carbon CO<sub>2</sub> in the OTCs.

### **3.3.1 CO<sub>2</sub> supply to OTCs**

The elevated CO<sub>2</sub> concentrations were maintained inside the OTCs using high pressurized CO<sub>2</sub> cylinders (of commercial grade 100% CO<sub>2</sub> gas cylinders of 30 kg capacity) with the help of dual stage regulators and gas flow meter. CO<sub>2</sub> was supplied from the cylinders to the OTCs through 6 mm polyurethane tubing and mixed with the ambient air at the outlet of the air blowers and subsequently distributed evenly inside the OTCs. CO<sub>2</sub> gas was released to the chambers through a manifold fitted with copper tubing. Within each chamber the copper tubing was again fitted with solenoid valve and rotameters to regulate the gas supply. The uniformity of the CO<sub>2</sub> was maintained by pumping CO<sub>2</sub> gas diluted with air by air compressor.

### **3.3.2 Monitoring system of CO<sub>2</sub> and temperature control**

CO<sub>2</sub> levels build up inside the chambers were monitored using an online CO<sub>2</sub> analyser (Model- Industrial Scientific, USA CDU-440) connected with pump to draw the air sample from OTCs (Plate 3.5a and 3.5b).



**Plate 3.1: Cylindrical shape OTCs fabricated with galvanized iron (GI) pipe**



**Plate 3.2: OTC with intact side walls and open upper top to maintain temperature 1.5°C higher than ambient temperature with and without external CO<sub>2</sub> supply**



3.3a

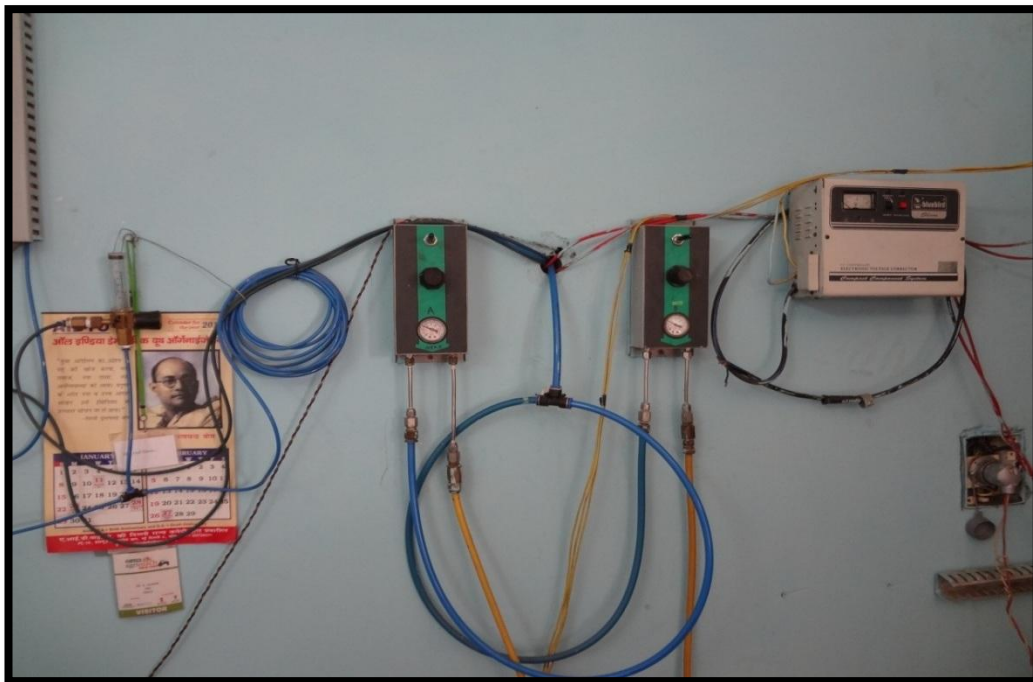


3.3b

**Plate 3.3a and 3.3b: OTC with intact side walls and partially covered upper top to maintain temperature 3°C higher than ambient temperature with and without external CO<sub>2</sub> supply**



**Plate 3.4: OTC with partially opened side walls and open upper top to maintain ambient with and without external CO<sub>2</sub> supply**



3.5a



3.5b

Plate 3.5a and 3.5b: CO<sub>2</sub> supply and monitoring system

### 3.4 Temperature and CO<sub>2</sub> Treatments

In the present study two levels of CO<sub>2</sub> and three levels of temperature were used. The different combinations of CO<sub>2</sub> and temperature levels were as follows:

**T0** – Ambient CO<sub>2</sub> (395 ± 10 ppm) (ACO<sub>2</sub>) + Ambient temperature (AT)/ ACO<sub>2</sub> + AT

**T1** - Ambient CO<sub>2</sub> (395 ppm ± 10 ppm) + 1.5°C (1.5T) higher temperature than ambient level / ACO<sub>2</sub> + 1.5T

**T2** - Ambient CO<sub>2</sub> (395 ppm ± 10 ppm) + 3°C (3T) higher temperature than ambient level / ACO<sub>2</sub> + 3T

**T3** - 550 ± 20 ppm CO<sub>2</sub> (ECO<sub>2</sub>) + ambient temperature (AT) / ECO<sub>2</sub> + AT

**T4** - 550 ± 20 ppm CO<sub>2</sub> level (ECO<sub>2</sub>) + 1.5°C (1.5T) higher temperatures than ambient level / ECO<sub>2</sub> + 1.5T

**T5** - 550 ± 20 ppm CO<sub>2</sub> level (ECO<sub>2</sub>) + 3°C (3T) higher temperature than ambient level / ECO<sub>2</sub> + 3T

### 3.5 Preparation of inoculum of *Bipolaris maydis* causing Maydis leaf Blight

#### 3.5.1 Isolation of pathogen

Maydis leaf Blight (MLB) diseased leaf samples were cut into 1 to 1.5 cm long bits. The bits were cleaned in sterilized distilled water and surface sterilized with 0.1 percent mercuric chloride for 30–60 seconds, washed thoroughly three times with sterilized distilled water and blot dried. These bits were then placed on PDA medium in Petri plates and were incubated at 28 ± 2°C for 2–3 days. After three days of incubation fine radiating mycelial growth was observed from edge of the infected bits. A small bit of actively growing mycelium was then transferred into the PDA slants and thus the pure culture was obtained. These slants were periodically transferred at one month interval to new slants. Cultures were maintained on sterile PDA slants in refrigerator at 4°C.

#### 3.5.2 Culture of pathogen

The culture was freshly multiplied in PDA medium and incubated in BOD incubators at 28 ± 2°C. The isolate was passed once through the host by inoculating a highly susceptible maize genotype CM 119 grown in the net house.

### 3.5.1.3 Mass multiplication of inoculum

For mass multiplication of *Bipolaris maydis*, sorghum seeds were soaked in water for 24 hours and excess water was removed and 40g of seeds were dispensed in 500 ml conical flask. The flasks containing sorghum seeds were autoclaved twice at 15 lb pressure for 30 min (consecutively for two days). Little quantity of 8–10 days old fungal culture (grown on PDA slants) was placed in the sterilized seeds and incubated at  $27 \pm 1^\circ\text{C}$  for 10–15 days. For uniform growth of the fungus, shaking of the seed at 3 to 4 days interval was done. The mass culture was taken out from the conical flasks, broken the bigger lumps and shade dried for 2 to 3 days. The shade dried sorghum seeds (culture of *B. maydis*) were grinded in a mixture-cum-grinder into powder form and stored the ground inoculum at  $6-9^\circ\text{C}$  till its use. At the time of inoculation, inoculum was diluted by adding desired level of freshly ground sorghum seeds in 2:1 ratio (Ahuja and Payak, 1978).



**Plate 3.6a:** Inoculum of *Bipolaris maydis* multiplied in sorghum grains



**Plate 3.6b:** Powder form of the inoculum of *Bipolaris maydis*

### 3.6 Inoculation method

For inoculation two pinch of the inoculum (approx. 3–4 g) in powder form was taken in between fingers and placed it into the central whorl of 35 days old maize plants (Payak and Sharma, 1983) after 25 days of exposure to elevated temperature and  $\text{CO}_2$  levels and after that light spraying of water was done with the help of sprayer to maintain adequate moisture for longer period to permit spore germination. Inoculation was done in evening hours (4–4:30 PM) to avoid the maximum day temperature.

Equal number of plants of both genotypes PEHM 5 and CM 119 in each treatment were kept uninoculated for quantification of disease related attributes.



**Plate 3.7: Maize plant inoculation with maydis leaf blight pathogen**

### **3.7 Methodology to assess the impact of elevated CO<sub>2</sub> and temperature on maize**

In the present study the impact of the elevated CO<sub>2</sub> and temperature treatments on maize and subsequently its susceptibility to disease development on exposure to the MLB pathogen was assessed for PEHM 5 and CM 119 genotypes and the key growth stages of PEHM 5 variety. The baseline data was generated on different growth, physiological and biochemical parameters for maize genotypes and their key growth stages response to the treatments. The data obtained on the parameters were subjected to 3 way analysis of variance (ANOVA) using a Completely Randomized Design (CRD) for testing their significance level of impact of treatments. ANOVA data analysis was followed by principal component analysis (PCA) technique (Andrews *et al.*, 2002) using SPSS (Version 16.0). PCA is a mathematical procedure that transforms several (possibly) correlated variables into a (smaller) number of uncorrelated variables (PC). The objective of PCA is to reduce the dimensionality of the parameter dataset and to identify new meaningful underlying variables, knowing that the PC are dependent on the units used to measure the original variables as well as on the range of values they assume. Principal components (PC) for a dataset are defined as linear combinations of variables that account for maximum variance within the set by describing vectors of closet fit to the  $n$  observation in  $p$ -dimensional space, subject to being orthogonal to one another. The first PC accounts for as much of the

variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible. PCs with eigen value  $\geq 0.6$  and explained at least 5% of the variation of the data were examined. Under a particular PC, only the variables with high factor loading were retained. High factor loading were defined as having absolute value within 10% of the highest factor loading (Sharma *et al.*, 2005). When more than one variable was retained under a single PC, multivariate correlation were employed to determine if the variables could be considered redundant or not (Andrews and Carroll, 2001). If the highly loaded factors were not correlated then each was considered important for the crop response to elevated CO<sub>2</sub> and temperature.

After identifying the key parameters for crop response to elevated CO<sub>2</sub> and temperature, observed data of each key parameter was transformed using a linear scoring method as suggested by Andrews *et al.* (2002). To assign the scores, indicators were arranged in order depending on whether a higher value was considered 'good' or 'bad' for crop response to stress conditions. In case, if 'more is better' indicator, each observation was divided by the highest observed value such that the highest observed value received a score of 1. If 'less is better' indicator, the lowest observed value (in the numerator) was divided by each observation (in the denominator) such that the lowest observed value received a score of 1.

After transformation using a linear scoring method, a radar plot was prepared to indicate contribution of the key crop parameter scores towards their response to elevated CO<sub>2</sub> and temperature for the PEHM 5 and CM 119 genotypes as well as the key growth stages of PEHM 5 variety. The methodology followed for the observation of growth, biochemical and physiological parameters were as follows:

### **3.8 Growth Parameters**

#### **3.8.1 Plant height**

Plant height was measured with the help of a meter scale starting from soil surface to a point where two developing leaves at the top of the plant forms a "V" shaped structure and represented in cm.

#### **3.8.2 Stem girth**

Measured with the help of vernier calipers and represented in cm.

### 3.8.3 Leaf area

Leaf area was measured with the help of Leaf area meter (Model LI-3100, LI-COR, Inc., USA).

### 3.8.4 Biomass

Destructive harvesting was done for the estimation of above-ground and below-ground biomass. Fresh weight of above- and below ground parts plant was also observed separately for leaves, stems and cobs. For dry weight estimation the same samples were kept in dry oven for 48 hours at 70°C temperatures

## 3.9 Biochemical parameters

### 3.9.1 Relative Leaf Water Content (RLWC)

Relative Leaf Water Content was determined according to the method of Singh (1997), and calculated by the formula:

$$\text{RLWC (\%)} = \frac{(FW - DW)}{(TW - DW)} \times 100$$

FW = Fresh weight, DW = dry weight, and TW = turgid weight.

Fresh weight was obtained by weighing 30 discs of 5 mm diameter cut from the fresh leaves for every sample. The discs were then immersed in distilled water overnight till they got saturated, blotted dry and then weighed to get the turgid weight. The same leaf discs were oven dried at 70 °C for 4–5 hrs. and reweighed to obtain the dry weight.

### 3.9.2 Total chlorophyll

Total chlorophyll estimation was done according to the method described by Arnon (1949). 2 gram of fresh leaf sample from each treatment was blended and then extracted with 15 ml of 80% acetone and left for 15 minutes for thorough extraction. The liquid portion was decanted into another test-tube and centrifuged at 2,500 rpm for 3 minutes. The supernatant was then collected and the absorbance taken at 645 nm and 663 nm using a spectrophotometer.

Calculations were done using the formula below:

$$\text{Chlorophyll a} = 12.7_{DX663} - 2.69_{DX645} \times V/1000W \text{ mg/g}$$

$$\text{Chlorophyll b} = 22.9_{DX645} - 4.68_{DX663} \times V/1000W \text{ mg/g}$$

$$\text{Total Chlorophyll} = (\text{Chlorophyll a} + \text{Chlorophyll b}) \text{ mg/g}$$

Dx = Absorbance of the extract at the wavelength X nm

V = total volume of the chlorophyll solution (ml),

and  $W$  = weight of the tissue extract (g).

### 3.9.3 Leaf extract pH

Five g of the fresh leaves was homogenized in 50 ml deionized water. This was then filtered and the pH of leaf extracted determined after calibrating pH meter with buffer solution of pH 4 and pH 9.

### 3.9.4 Ascorbic Acid

Ascorbic acid content was determined by the method as described by Sadasivam and Balasubraminan (1987). Stock standard was made by dissolving 100 mg ascorbic acid in 100 ml of 4% oxalic acid solution in standard flask (1mg/ml). Working standard was made by diluting 10 ml of the stock solution to 100 ml with 4% oxalic acid. The concentration of working standard was 100 $\mu$ g/ml. Dye solution used for the titration was made by weighing 42 mg sodium bicarbonate into small volume of distilled water and 52 mg 2,6 dichlorophenol indophenol was dissolved to make volume up to 200 ml with distilled water. 1 g leaf sample was extracted in 4% oxalic acid (10 ml) and volume was made up to 50 ml with 4% oxalic acid and was centrifuged at 5000 rpm for 20 minutes at 4°C. 5 ml of the supernatant was collected through pipette, 10 ml of 4% oxalic acid was added to it and titrated against the dye. Amount of Ascorbic acid in the sample was calculated in mg/g sample as per the following formula:

$$\begin{aligned} &\text{Ascorbic acid (mg/g sample)} \\ &= (0.5 \text{ mg/V1 ml}) \times (V2/15 \text{ ml}) \times (100 \text{ ml/wt. of sample in g}) \times 100 \end{aligned}$$

### 3.9.5 Lipid Peroxidation

Leaf lipid peroxidation was estimated by following the method of Heath and Packer (1968). 2 g plant sample was homogenized in a pestle and mortar with 10 mL of 0.1% Tri chloro acetic acid (TCA) and incubated for 30 minutes at room temperature. It was then filtered through Whatman's no. 1 filter paper and to the filtrate TCA (4mL; 20%) containing 0.5% Thio- barbituric acid (TBA) was added and kept in boiling water bath for 30 minutes. After incubation test tubes were quickly cooled using ice jacket to stop the reaction and immediately absorbance reading was taken at 532 and 600 nm using UV-visible spectrophotometer. Blank was prepared with 5 mL of 20% TCA with 0.5M TBA. Lipid peroxidation level was

expressed as Malondialdehyde content in nmol using 155 Mm/cm molar extinction coefficient.

$$\text{Malondialdehyde (MDA) content nmol/g} = \frac{(\text{Abs}_{532} - \text{Abs}_{600}) \times V \times 1000}{\epsilon \times M}$$

V = Grinding volume;  $\epsilon$  = MDA molar extinction coefficient; M = tissue weight

### 3.9.6 Total Phenol

Total Phenol Content was estimated by Folin Ciocalteu (FC) method. 50 mg freeze dried plant material was homogenized with 10 mL of 70% methanol. This solution was then centrifuged for 10 minutes at 3000 rpm. Supernatant was separated and volume make-up up to 25 ml was done with 70% methanol. 1 ml of this extract + 1 ml FC reagent + 2 ml of Na<sub>2</sub>CO<sub>3</sub> (20% w/v) solution was taken in glass tubes. Then tubes were shaken properly and were heated in boiling water bath for 1 minute. Tubes were cooled in running tap water. Transmittance was measured at 650 nm in spectrophotometer. Standard was prepared by dissolving 10 mg caffeic acid in 100 ml 70% methanol to give a stock solution of 100 µg/ml phenol and polyphenolic content was expressed as mg/100g of fresh weight of tissue.

### 3.10 Physiological parameters

Photosynthetic rate, stomatal conductance and transpiration rate were measured by portable photosynthesis system (LI-6400-40 Portable Photosynthesis system Infrared gas analyser (IRGA). Photosynthetic rate, stomatal conductance and transpiration was expressed in the units of µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>, mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> and mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> respectively.

### **3.11 Yield and its attributes**

**3.11.1 Cob weight:** De-husked cobs were weighed by a weighing machine and weight was represented in terms of g.

**3.11.2 Cob girth:** Cob girth was measured with the help of vernier calipers.

**3.11.3 Number of rows per cob:** Number of rows per cob was counted manually.

**3.11.4 Grain weight per plant:** Total weight of grains in cob(s) / plant was calculated.

**3.11.5 Harvest index:** Weight of grains per plant was calculated. Biomass of whole plant was also taken. The ratio of weight of the grains and biomass was expressed as harvest index.

### **3.12 Assessment of disease development**

Maydis leaf blight disease development was observed on artificially inoculated maize cultivars PEHM 5 and CM119 pre exposed to elevated CO<sub>2</sub> and temperature under field and lab/ conditions.

#### **3.12.1 Field experiment**

In the field experiments maize cultivars were grown in pots and exposed for 25 days duration to different treatments. After short term exposure maize cultivars were inoculated with the pathogen and monitored. After 6 days of incubation period maize cultivars were assessed for disease development by image analysis technique and analysed for changes in growth, biochemical, and physiological parameters. The plants were maintained in pots till maturity for monitoring yield parameters. Disease severity was recorded using 1-5 rating scale developed by Payak and Sharma (1983). Data regarding the disease development was recorded 2 times after 20 days and 35 days of inoculation.

**Table 3.1: Maydis leaf blight disease rating scale (Payak and Sharma, 1983)**

Rating scale	Disease severity (%)	PDI*	Disease reaction
1.0	Very slight to slight infection, one or two to few scattered lesions on lower leaves	20.0	Resistant (Score: ≤ 2.0) (PDI: ≤ 40.0)
2.0	Light infection, moderate number of lesions on lower leaves only	40.0	
3.0	Moderate infection, abundant lesions on lower leaves, few on middle leaves	60.0	Moderately resistant (Score: 2.1 – 3.0) (PDI: 40.1 – 60.0)
4.0	Heavy infections abundant on lower and middle leaves, extending to upper leaves	80.0	Moderately susceptible (Score: 3.1 – 4.0) (PDI: 60.1 – 80.0)
5.0	Very heavy infection, lesions abundant on almost all leaves, plants prematurely dry or killed by the disease.	100.0	Susceptible (Score: ≥4.1) (PDI: ≥ 80.0)

\*Percent disease index (PDI)

Percent disease index (PDI) was calculated by using the following formula:

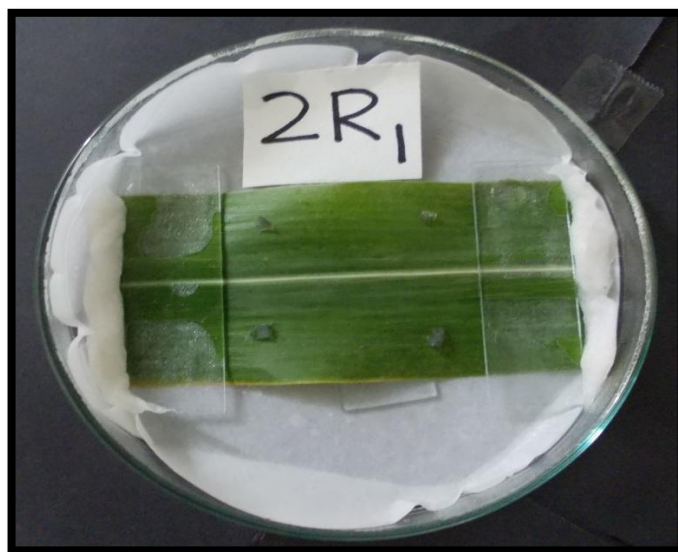
$$\text{PDI (\%)} = \frac{\Sigma \text{ Total of Disease scales in each scale}}{\text{Total observations} \times \text{Highest score/scale}} \times 100$$

Images of leaves of inoculated plants under different treatments were captured by camera (Model–Nikon Coolpix S2800) fixed on tripod after 6 days of inoculation i.e., at appearance of first disease symptoms to next 10 days at 1 day interval by keeping a centimetre scale of 30 cm so that a fixed length of leaf (25 cm) can be measured. Captured images were analysed through ImageJ software to estimate the percentage area of leaves infected and the rate of progress of infection.

### 3.12.2 Disease development assessment under controlled lab conditions

Detached leaf technique developed by Tedford *et al.* (1990) was followed for the assessment of disease development under controlled conditions by *Bipolaris maydis* pathogen on the leaf lamina (14 cm × 7cm) of each plant. In the present study this technique was used to assess the impact of elevated CO<sub>2</sub> and temperature on biotic stress tolerance potential of key growth stages of maize cultivar PEHM 5. PEHM 5 plants were exposed to the different treatments of the study in OTCs throughout the crop growth period. The leaves of plants exposed to different treatments were brought from field to lab at three growth stages - vegetative, tasseling and dent stage.

These leaves were inoculated with the maydis leaf blight pathogen as per detached leaf technique. Before inoculation leaves were soaked for 30 seconds in a 0.05% NaOCl<sub>3</sub> solution for surface-sterilization, rinsed in sterile distilled water and blotted dry on sterilized blotted papers. Leaves by abaxial side were placed down in round glass petridishes measuring 16 cm diameter, each containing one layer of sterile Whatman no.1 filter paper and 10 ml of sterile distilled water. Discs of *B. maydis* mycelium (3 mm in diameter) from PDA were taken from the growing edge of mycelial mats with a sterile cork borer. Four discs from culture were taken and were kept on detached leaves at four equidistant points on both side of mid-rib as shown in the figure (Plate 3.8). Petridishes were kept in incubation chamber at 27°C. The image of rate of development of the lesions per leaf was recorded through the camera at 48, 72 and 96 hours after inoculation and analysed through ImageJ software.



**Plate 3.8: Detached leaf inoculation technique of maydis leaf blight disease**

### 3.12.3 Quantification of rate of disease development through ImageJ software

Images of leaves of maize cultivars captured to assess disease development under field and lab experiment were analysed using the ImageJ software. Precautions taken while capturing the images to minimise the manual error were:

- i. Images were taken from a fixed distance by using tripod stand.
- ii. Images were taken in flesh-off mode of camera in the evening time (5 to 6 PM) to minimise the error due to reflection from sunlight.

Following steps were followed for analysis of images:

- i. Image was opened using “File” as shown in Plate 3.9a, 3.9b and 3.9c.
- ii. “Free hand selection” tool was used to make margins around leaf to cut the leaf from outside image (Plate 3.9d).
- iii. After that steps followed were:
  - Image → Crop image
  - Edit → Clear outside
- iv. Then image was converted into 8 bit by (Plate 3.9e, 3.9f) :
  - Image → Type → 8 bit
- v. Setting scale: - Images of leaves of 25 cm in length were taken by using scale while taking images. For scaling, a straight line was made across the leaves at an angle of 0° by selecting the tool of “Straight, segmented or freehand lines, or arrows” (Plate 3.9g).
- vi. Then, Analyse → Set scale.  
Known distance was put as 25 and unit as ‘cm’ and then “Global” was selected.
- vii. Then, following steps were followed:
  - Process → Binary → Make binary (Plate 3.9h)
  - Image → Adjust → Threshold (Plate 3.9i)
  - Analyse → Analyse particles (Plate 3.9j, 3.9k, 3.9l)
    - a. 0.02 was entered as minimum particle size, toggled show outline and “Display results, Clear results and Summarize” was selected. Then clicked ‘OK’.
- viii. In this way drawing of picture was obtained showing different green areas with different serial numbers (3.9m).
- ix. For calculating total leaf area again ‘Freehand selection tool’ was selected and margin of the leaf was demarcated. Then
  - Analyse → Measure
 In this way total leaf area will be calculated.  
Thus, infected area = Total leaf area – Total green area of drawing.

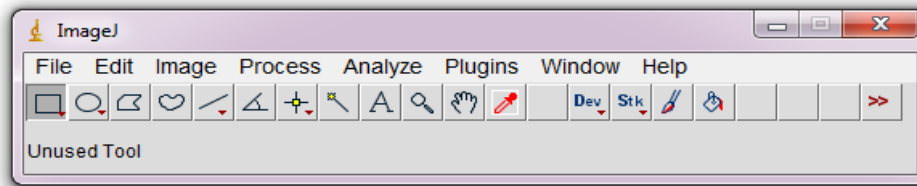


Plate 3.9a

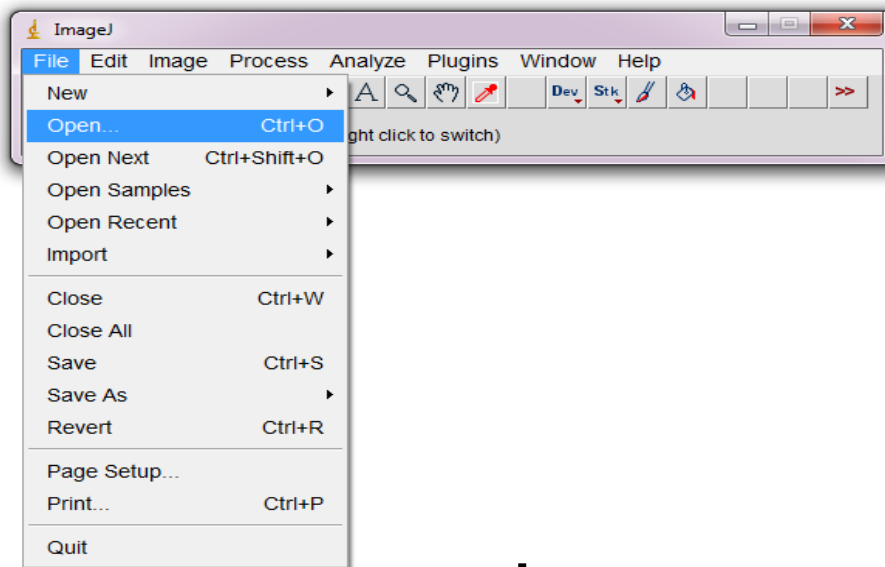


Plate 3.9b

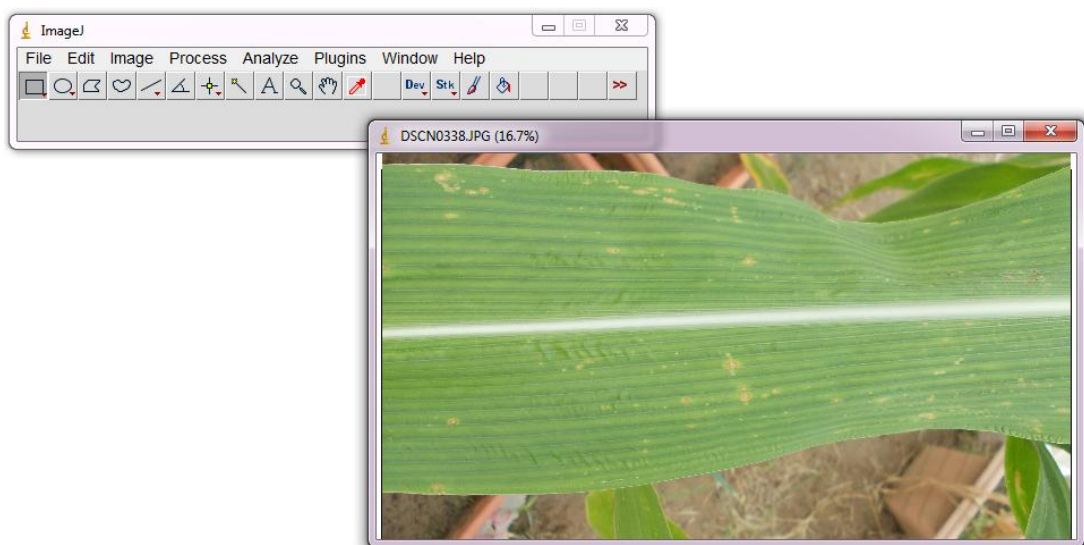


Plate 3.9c



Plate 3.9d

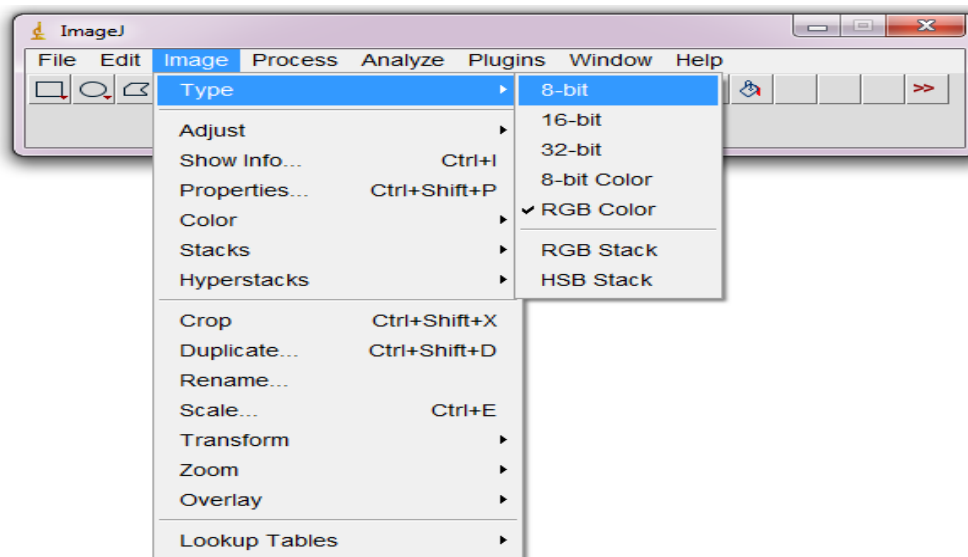


Plate 3.9e

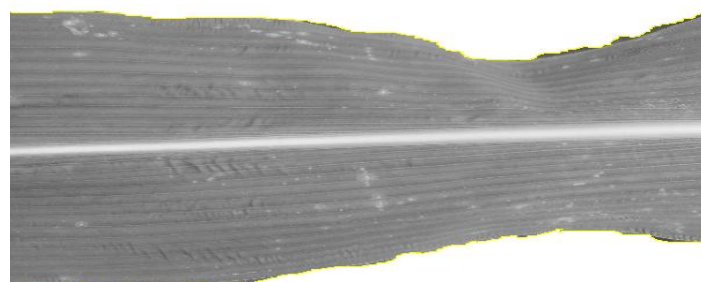


Plate 3.9f

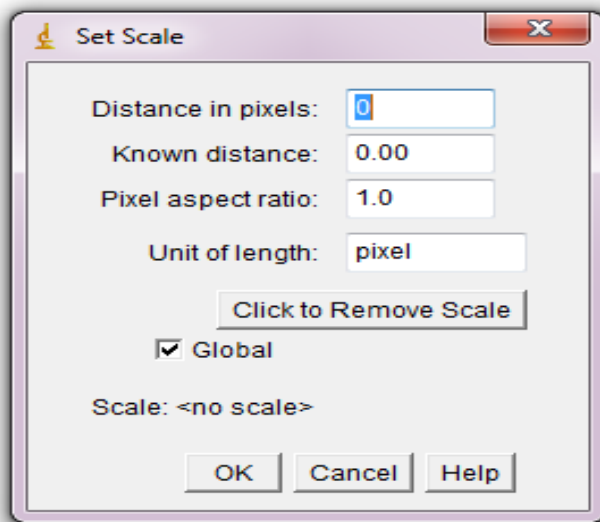


Plate 3.9g

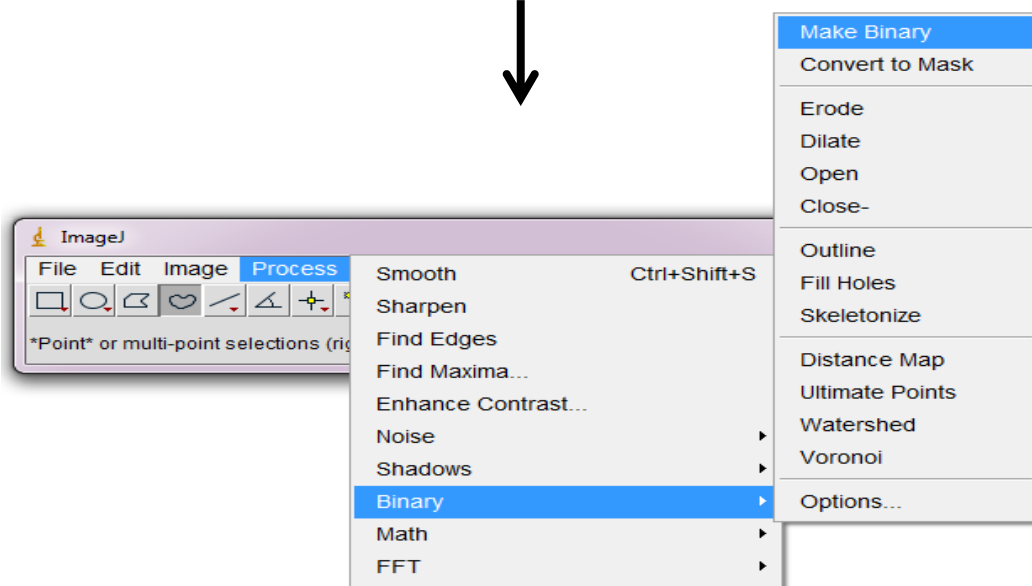


Plate 3.9h



Plate 3.9i

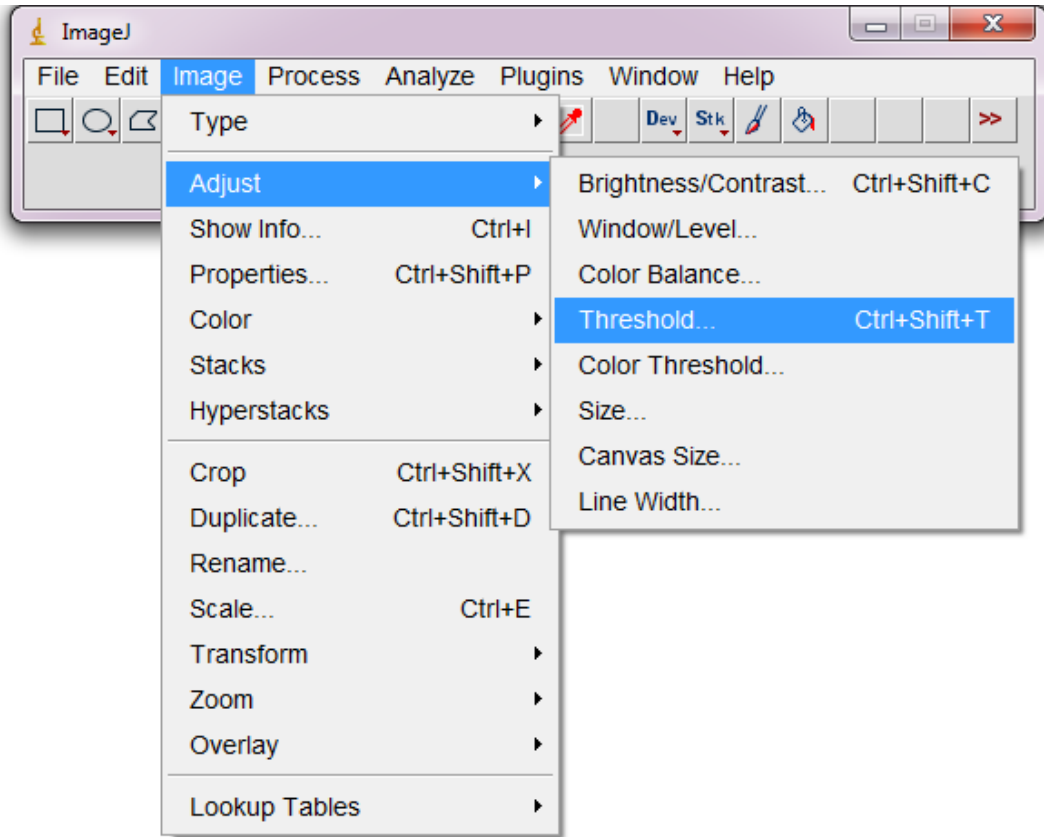
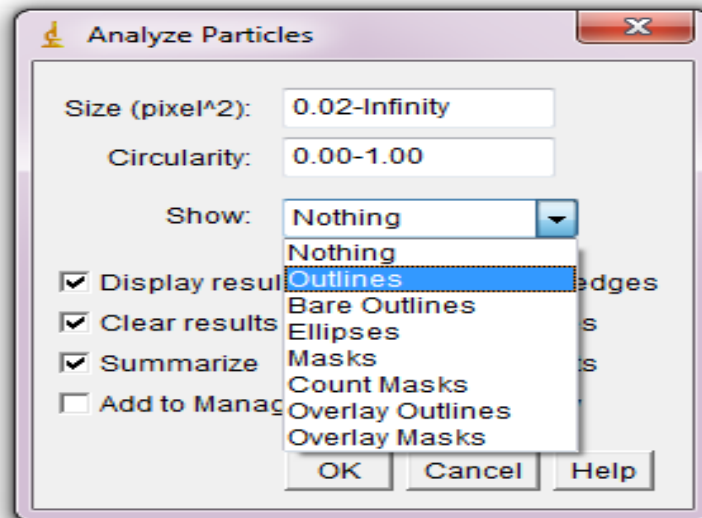


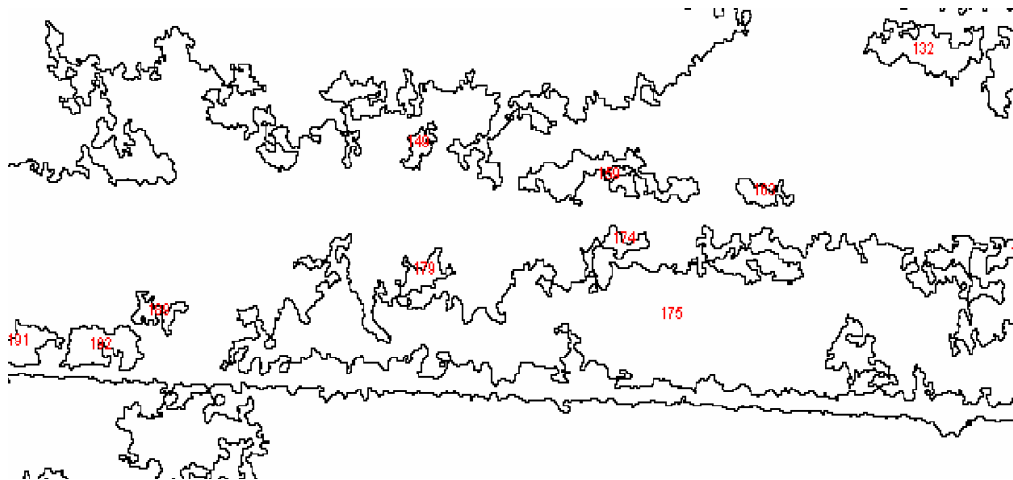
Plate 3.9j



Plate 3.9k



**Plate 3.9l**



**Plate 3.9m**

**Plate 3.9 (a-m): Steps for quantification of rate of disease development through ImageJ software**

### 3.13 Diseases tolerance attributes

Yield observed PEHM 5 and CM119 under different treatments was used to generate value of the following disease indices (Gupta *et al.*, 2002). For calculating disease indices following notations have been used:

$Yp$  – Yield of a genotype without disease under different treatments

$Ys$  – The yield of a genotype with disease under different treatments

$Yp-$  – Mean yield without disease in all treatments

$Ys-$  – Mean yield with disease in all treatments

#### 3.13.1 Disease stress intensity (DSI)

Value of DSI ranges from 0 to 1, and the larger the value of DSI, the more severe is the impact of the treatment.

$$(DSI) = 1 - \left( \frac{Ys-}{Yp-} \right)$$

#### 3.13.2 Disease tolerance (TOL)

A large and small value of TOL under different treatment represents relatively more and less sensitivity to disease respectively.

$$TOL) = Yp - Ys$$

#### 3.13.3 Disease stress susceptibility index (DSSI)

The smaller the value of DSSI, the greater is the disease stress tolerance capacity.

$$DSSI = \frac{1 - \frac{Ys}{Yp}}{DSI}$$

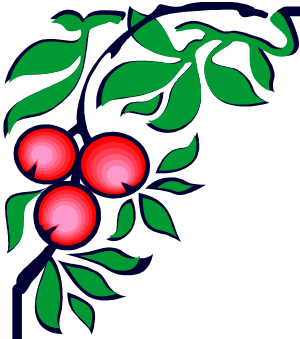
#### 3.13.4 Disease stress tolerance index (DSTI)

A higher DSTI value for a genotype suggests the higher level of its disease tolerance capacity and yield potential.

$$DSTI = \frac{(Yp)(Ys)}{(Yp-)^2}$$

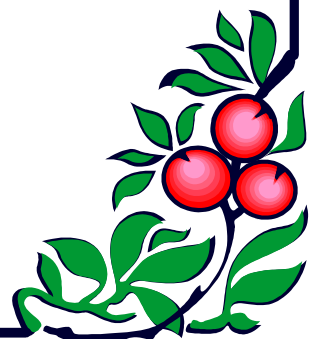
### 3.14 Data analysis

Statistical analysis of the data was done using SPSS 16.0 (SPSS Inc., Chicago, USA) for windows. Analysis of variance (ANOVA) was done to test whether the differences were statistically significant using completely randomized design for testing their level of significance.



## **Chapter-4**

# **RESULTS**

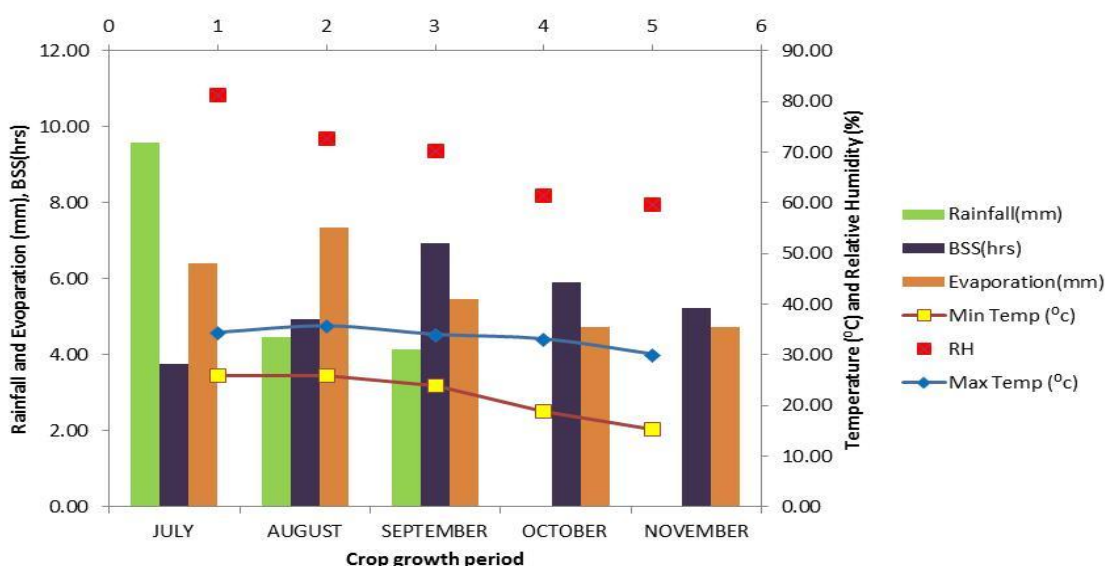


## Results

In the present study experiments were carried out to assess – (i) the impact of elevated temperature and CO<sub>2</sub> exposure on maydis leaf blight (MLB) disease tolerance potential of maize genotypes PEHM 5 and CM 119 and (ii) The impact of elevated temperature and CO<sub>2</sub> on disease tolerance potential of key growth stages of maize variety PEHM 5. In both the experiments crop plants were also monitored for the changes in growth, physiological, biochemical, and yield parameters due to elevated temperature and CO<sub>2</sub> exposure to demarcate the key parameter which can be used as an indicator for assessing the impact of CO<sub>2</sub> and temperature (abiotic stress) on biotic stress tolerance potential of maize crop.

### Meteorological conditions during the crop growth period

During cropping season, meteorological data were also recorded. During the crop growth period mean maximum temperature and mean minimum temperature was  $34.1 \pm 2.5^{\circ}\text{C}$  and  $22.7 \pm 4.0^{\circ}\text{C}$ , respectively. Mean relative humidity was  $69 \pm 8.9\%$  during the crop growth period, which was above threshold optimum value of maize crop at flowering and grain filling stage (Figure 4.1).



**Figure 4.1: Meteorological conditions of experimental site during the crop growth period**

## **Impact of elevated temperature and CO<sub>2</sub> on tolerance potential of maize genotypes**

Impact of elevated temperature and CO<sub>2</sub> on tolerance potential of maize genotypes was assessed through the data generated on response of growth, biochemical and physiological parameters of PEHM 5 and CM 119 plants to different treatment.

### **Response of growth parameters to elevated temperature and CO<sub>2</sub> on PEHM 5 and CM 119 genotypes**

#### **Plant height**

In the present study enhancement in mean height of both PEHM 5 and CM 119 plants was observed due to elevated CO<sub>2</sub> levels, whereas with elevated temperature decrease in height was observed; but due to interactive effect of CO<sub>2</sub> and temperature maximum height was observed in T3 treatment in plants of both PEHM 5 and CM 119 genotypes. Plants of PEHM 5 genotype were taller as compared to CM 119. Decrease in plant height of PEHM 5 under different treatments was in the order of **T3 > T4 > T0 > T1 > T5 > T2**; and for CM 119 it was in the order of **T3 > T4 > T0 > T1 > T2 > T5**.

Both at ambient CO<sub>2</sub> and elevated CO<sub>2</sub> level with increase in temperature plant height was found to decrease significantly, but the impact was high when the temperature was increased up to 3°C as compared when the temperature was increased only up to 1.5°C (Table 4.1).

#### **Stem girth**

Result of the study shows that average stem girth of PEHM 5 plants were more as compared to CM 119 plants. Enhancement in stem girth was observed in both PEHM 5 and CM 119 genotypes in ECO<sub>2</sub> + 1.5T (550 ppm CO<sub>2</sub> + 1.5°C elevated treatment) treatment but in ECO<sub>2</sub> + 3T (550 ppm CO<sub>2</sub> + 3°C elevated temperature) treatment decrease in stem girth was observed for both genotypes. Under interactive effect of CO<sub>2</sub> and temperature maximum stem girth in both PEHM 5 and CM 119 plants were observed in T3 treatment.

Stem girth of PEHM 5 under different treatments was in the order of **T3 > T4 > T1 > T0 > T2 > T5**; whereas, stem girth of CM 119 under different treatment was **T3 > T1 > T4 > T5 > T0 > T2** (Table 4.2).

**Table 4.1: Effect of elevated temperature and CO<sub>2</sub> on plant height of maize genotypes**

Plant height (cm)						
Temperature	PEHM 5			CM 119		
	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	159.4	168	<b>163.7</b>	110	117	<b>113.5</b>
1.5T	151.4	166.6	<b>162.5</b>	107	111.5	<b>109.25</b>
3T	143.2	144	<b>143.6</b>	100	99.5	<b>99.75</b>
Mean	<b>151.3</b>	<b>159.5</b>		<b>105.6</b>	<b>109.3</b>	

Factors	C.D.	SE(d)	SE(m)
Genotype (G)	1.62	0.80	0.57
CO <sub>2</sub> level (C)	NS	0.80	0.57
G × C	2.29	1.13	0.80
Temperature (T)	1.98	0.98	0.69
G × T	2.80	1.39	0.98
C × T	2.12	1.39	0.98
G × T × C	3.96	1.97	1.39

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature +1.5°C temperature; 3T, ambient temperature + 3°C temperature

**Table 4.2: Effect of elevated temperature and CO<sub>2</sub> on stem girth of maize genotypes**

Stem girth (cm)						
	PEHM 5			CM 119		
Temperature	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	6.40	7.00	<b>6.70</b>	4.08	4.64	<b>4.36</b>
1.5T	6.64	6.52	<b>6.58</b>	4.50	4.46	<b>4.48</b>
3T	6.12	5.78	<b>5.95</b>	4.06	4.18	<b>4.12</b>
Mean	<b>6.39</b>	<b>6.43</b>		<b>4.21</b>	<b>4.43</b>	

Factors	C.D.	SE(d)	SE(m)
Genotype (G)	0.15	0.07	0.05
CO <sub>2</sub> level (C)	NS	0.07	0.05
G × C	NS	0.11	0.07
Temperature (T)	0.19	0.09	0.06
G × T	0.27	0.13	0.09
C × T	0.27	0.13	0.09
G × T × C	NS	0.19	0.13

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature

### Leaf area

Average leaf area was more in PEHM 5 plants as compared to CM 119 plants. In both the genotypes decrease in leaf area was observed with increase in temperature stress. Impact of enhanced CO<sub>2</sub> level on leaf area of both genotypes was positive. The average of leaf area in both PEHM 5 and CM 119 was maximum under T3 treatment. The effect of treatments on leaf area of PEHM 5 was in the order of **T3 > T4 > T0 > T1 > T5 > T2**; whereas, leaf area of CM 119 under different treatment was in the order of **T3 > T4 > T0 > T1 > T2 > T5** (Table 4.3). In both PEHM 5 and CM 119 detrimental effect of elevated temperature was found at both ambient and elevated CO<sub>2</sub> levels on growth parameters.

**Table 4.3: Effect of elevated temperature and CO<sub>2</sub> on leaf area of maize genotypes**

Leaf area (cm <sup>2</sup> )						
Temperature	PEHM 5			CM 119		
	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	452.50	465.90	<b>459.20</b>	308.63	422.28	<b>365.45</b>
1.5T	378.04	463.82	<b>420.93</b>	307.24	383.82	<b>345.53</b>
3T	330.30	352.04	<b>341.17</b>	289.53	282.63	<b>286.08</b>
Mean	<b>386.94</b>	<b>427.25</b>		<b>301.80</b>	<b>362.91</b>	

Factors	C.D.	SE(d)	SE(m)
Genotype (G)	9.98	4.96	3.50
CO <sub>2</sub> level (C)	9.98	4.96	3.50
G × C	14.11	7.01	4.96
Temperature (T)	12.22	6.07	4.29
G × T	NS	8.59	6.07
C × T	17.28	8.59	6.07
G × T × C	24.44	12.15	8.59

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature

### **Response of biochemical parameters of maize genotypes to elevated temperature and CO<sub>2</sub>**

#### **Total chlorophyll**

Result shows that at ambient CO<sub>2</sub> level when temperature was increased up to 1.5°C, then there was enhancement in total chlorophyll content in both PEHM 5 and CM 119 plants, but when temperature was increased to the level of 3°C, the total chlorophyll content was decreased significantly. Under elevated CO<sub>2</sub> level enhancement in chlorophyll content was observed, but it was nullified with increase in temperature (Table 4.4).

Chlorophyll content of PEHM 5 under different treatments was in the order of **T3 > T4 > T1 > T0 > T2 > T5**; whereas, chlorophyll content of CM 119 under different treatments were in the order of **T3 > T1 = T4 > T0 > T2 > T5**.

**Table 4.4: Effect of elevated temperature and CO<sub>2</sub> on total chlorophyll content of maize genotypes**

Total chlorophyll content (mg/g)						
	PEHM 5			CM 119		
Temperature	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	0.571	0.604	<b>0.588</b>	0.360	0.389	<b>0.375</b>
1.5T	0.583	0.589	<b>0.586</b>	0.379	0.379	<b>0.379</b>
3T	0.552	0.546	<b>0.549</b>	0.322	0.282	<b>0.302</b>
Mean	<b>0.569</b>	<b>0.580</b>		<b>0.354</b>	<b>0.350</b>	

Factors	C.D.	SE(d)	SE(m)
Genotype (G)	0.03	0.01	0.01
CO <sub>2</sub> level (C)	0.03	0.01	0.01
G × C	0.04	0.02	0.01
Temperature (T)	NS	0.02	0.01
G × T	0.05	0.02	0.02
C × T	0.05	0.02	0.02
G × T × C	0.08	0.03	0.02

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature

### Relative leaf water content (RLWC)

Result shows that at ambient CO<sub>2</sub> level, when temperature was increased up to 1.5°C, then RLWC was found to increase significantly in both PEHM 5 and CM 119, but when temperature was increased up to 3°C, there was significant decrease in RLWC (Table 4.5). Also, RLWC at elevated CO<sub>2</sub> was found more in both PEHM 5 and CM 119 as compared to ambient CO<sub>2</sub> level. Under different treatments RLWC for both PEHM 5 and CM 119 was in the order of **T3 > T4 > T1 > T0 > T5 > T2**.

**Table 4.5: Effect of elevated temperature and CO<sub>2</sub> on relative leaf water content (RLWC) of maize genotypes**

Temperature	RLWC (%)					
	PEHM 5			CM 119		
	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	37.08	49.91	<b>43.50</b>	35.59	45.73	<b>40.66</b>
1.5T	38.19	42.44	<b>40.31</b>	37.55	45.15	<b>41.35</b>
3T	23.91	35.84	<b>29.88</b>	20.98	27.99	<b>24.49</b>
Mean	<b>33.06</b>	<b>42.73</b>		<b>31.37</b>	<b>39.62</b>	

Factors	C.D.	SE(d)	SE(m)
Genotype (G)	1.32	0.65	0.46
CO <sub>2</sub> level (C)	1.32	0.65	0.46
G × C	1.87	0.93	0.65
Temperature (T)	1.62	0.80	0.57
G × T	2.29	1.14	0.80
C × T	2.29	1.14	0.80
G × T × C	3.24	1.61	1.14

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature

### Leaf extract pH

Leaf extract pH of both PEHM 5 and CM 119 was acidic. The variation in leaf extract pH at maturity in two genotypes under different treatments was found in the range of 3.55 to 4.88 (Table 4.6). Leaf extract pH of PEHM 5 under different treatments was found in the range of 4.04 to 4.88 and was in the order of **T1 > T5 > T3 > T4 > T0 > T2**; and leaf extract pH of CM 119 under different treatment was found in the range of 3.5 to 4.8 and was in the order of **T2 > T5 > T3 > T0 > T4 > T1**. Leaf extract pH was found to increase when temperature was increased up to 3°C at elevated CO<sub>2</sub> level in both genotypes but, there was slight decrease at ambient CO<sub>2</sub> in PEHM 5. Also at elevated CO<sub>2</sub> level there was decrease in leaf extract pH when temperature was increased upto 1.5°C level in both genotypes.

### Ascorbic acid content

Under control treatment ascorbic acid content was more in PEHM 5 as compared to CM 119. Ascorbic acid content of PEHM 5 under different treatments was in the order of **T3 > T5 > T2 > T4 > T1 > T0**. The Ascorbic acid content of PEHM 5 plants was 31.67% and 14.02% more under T3 and T5 treatments, respectively as compared to T0. Ascorbic acid content of CM 119 under different treatment was in the order of **T4 > T1 > T2 > T3 > T5 > T0**; and this was 98.91% and 55.67% more under T4 and T1 treatments, respectively as compared to T0. Effect of CO<sub>2</sub> level in interaction with temperature on ascorbic acid content of both the genotypes was significant (Table 4.7).

### Total phenol content

At ACO<sub>2</sub> level total phenol content of PEHM 5 was higher in all treatments as compared to CM 119. Also, at ACO<sub>2</sub> level exposure to elevated temperature level enhanced total phenol content in both PEHM 5 and CM 119. Total phenol content of PEHM 5 under different treatments was in the order of **T3 > T4 > T0 > T1 > T5 > T2**; and it was 3.90% and 2.60% more under T3 and T4 treatments, respectively as compared to T0 treatment. Total phenol content of CM 119 under different treatment was in the order of **T3 > T4 > T5 > T0 > T1 > T2** and it was 10.1%, 9.3% and 8.2% more under T3, T4 and T5, respectively as compared to T0 treatment (Table 4.8).

**Table 4.6: Effect of elevated temperature and CO<sub>2</sub> on leaf extract pH of maize genotypes**

Leaf extract pH						
	PEHM 5			CM 119		
Temperature	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	4.18	4.45	<b>4.32</b>	3.79	4.05	<b>3.92</b>
1.5T	4.88	4.38	<b>4.63</b>	3.55	3.72	<b>3.63</b>
3T	4.04	4.48	<b>4.26</b>	4.80	4.07	<b>4.43</b>
Mean	<b>4.37</b>	<b>4.44</b>		<b>4.04</b>	<b>3.94</b>	

Factors	C.D.	SE(d)	SE(m)
Genotype (G)	0.23	0.11	0.08
CO <sub>2</sub> level (C)	0.23	0.11	0.08
G × C	NS	0.16	0.11
Temperature (T)	0.04	0.14	0.09
G × T	0.39	0.19	0.14
C × T	0.32	0.19	0.14
G × T × C	0.55	0.28	0.19

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature

**Table 4.7: Effect of elevated temperature and CO<sub>2</sub> on ascorbic acid content of maize genotypes**

Temperature	Ascorbic acid (mg/g)					
	PEHM 5			CM 119		
	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	2.216	2.914	<b>2.565</b>	1.850	2.775	<b>2.313</b>
1.5T	2.270	2.304	<b>2.287</b>	2.885	3.685	<b>3.285</b>
3T	2.402	2.520	<b>2.461</b>	2.780	2.430	<b>2.605</b>
Mean	<b>2.296</b>	<b>2.579</b>		<b>2.505</b>	<b>2.963</b>	

Factors	C.D.	SE(d)	SE(m)
Genotype (G)	NS	0.14	0.10
CO <sub>2</sub> level (C)	0.29	0.14	0.10
G × C	NS	0.21	0.14
Temperature (T)	0.23	0.18	0.12
G × T	0.51	0.25	0.18
C × T	0.51	0.25	0.18
G × T × C	NS	0.36	0.25

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature

**Table 4.8: Effect of elevated temperature and CO<sub>2</sub> on total phenol content of maize genotypes**

Total Phenol content (mg/100g fresh weight)						
	PEHM 5			CM 119		
Temperature	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	57.90	60.16	<b>59.03</b>	50.070	53.00	<b>53.78</b>
1.5T	57.61	59.41	<b>58.51</b>	46.96	52.50	<b>49.70</b>
3T	49.21	56.66	<b>52.93</b>	40.97	51.33	<b>41.15</b>
Mean	<b>54.90</b>	<b>58.74</b>		<b>45.93</b>	<b>48.21</b>	

Factors	C.D.	SE(d)	SE(m)
Genotype (G)	1.87	0.93	0.65
CO <sub>2</sub> level (C)	1.87	0.93	0.65
G × C	2.64	1.31	0.93
Temperature (T)	1.11	1.13	0.80
G × T	3.23	1.61	1.13
C × T	NS	1.61	1.13
G × T × C	NS	2.27	1.61

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature

### Lipid peroxidation

Lipid peroxidation was assessed by estimating Malondialdehyde (MDA) content which reflects the lipid peroxidation of plant samples. Result shows that MDA content was higher in CM 119 as compared to PEHM 5 in all treatments. Significant variations observed in MDA content in plants of PEHM 5 and CM 119 under different treatments indicates that lipid peroxidation will be significantly affected by elevated temperature and CO<sub>2</sub> levels. MDA content of PEHM 5 under different

treatments was in the order of **T2 > T5 > T3 > T4 > T0 > T1**; and it was 8.81%, 5.62% and 3.56% more under T2, T5 and T3 treatment as compared to T0 treatment; whereas, MDA content of CM 119 under different treatment was in the order of **T5 > T2 > T3 > T4 > T1 > T0**; and it was 34.42%, 33.82%, 31.75% and 11.27% more in T5, T2, T3 and T4, respectively (Table 4.9).

**Table 4.9: Effect of elevated temperature and CO<sub>2</sub> on Malondialdehyde (MDA) content of maize genotypes**

Malondialdehyde content ( nmol/g)						
Temperature	PEHM 5			CM 119		
	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	10.66	11.04	<b>10.85</b>	16.85	22.20	<b>19.53</b>
1.5T	10.54	10.88	<b>10.71</b>	17.95	18.75	<b>18.35</b>
3T	11.60	11.26	<b>11.43</b>	22.55	22.65	<b>22.60</b>
Mean	<b>10.93</b>	<b>11.06</b>		<b>19.12</b>	<b>21.20</b>	

Factors	C.D.	SE(d)	SE(m)
Genotype (G)	0.30	0.15	0.10
CO <sub>2</sub> level (C)	0.30	0.15	0.10
G × C	0.42	0.21	0.15
Temperature (T)	0.37	0.18	0.13
G × T	0.52	0.26	0.18
C × T	0.52	0.26	0.18
G × T × C	0.74	0.36	0.26

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature

## Response of physiological parameters of maize genotypes to elevated temperature and CO<sub>2</sub>

### Photosynthetic rate

Rate of photosynthesis was found to increase significantly at ACO<sub>2</sub> level when temperature was increased to 1.5°C level in both PEHM 5 and CM 119 plants and decrease when temperature was increased to 3°C, but at ECO<sub>2</sub> level with increase in temperature photosynthetic rate was found to decrease significantly at both levels of elevated temperature (Table 4.10).

Photosynthetic rate of both PEHM 5 and CM 119 under different treatments was in the order of **T3 > T4 > T1 > T0 > T5 > T2**.

**Table 4.10: Effect of elevated temperature and CO<sub>2</sub> on photosynthetic rate of maize genotypes**

Temperature	Photosynthetic rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )					
	PEHM 5			CM 119		
	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	14.11	21.83	<b>17.97</b>	10.09	19.67	<b>14.88</b>
1.5T	14.21	19.23	<b>16.72</b>	10.47	18.21	<b>14.34</b>
3T	12.46	12.94	<b>12.70</b>	6.35	6.61	<b>6.48</b>
Mean	<b>13.59</b>	<b>18.00</b>		<b>8.97</b>	<b>14.83</b>	

Factors	C.D.	SE(d)	SE(m)
Genotype (G)	1.28	0.63	0.45
CO <sub>2</sub> level (C)	1.28	0.63	0.45
G × C	1.81	0.90	0.63
Temperature (T)	1.56	0.77	0.51
G × T	2.21	1.10	0.77
C × T	2.21	1.10	0.77
G × T × C	3.13	1.55	1.10

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature

### Stomatal conductance

Stomatal conductance in PEHM 5 and CM 119 at ambient CO<sub>2</sub> level was found to increase when temperature was increased by 1.5°C over the ambient temperature, but it was appreciably decreased at 3°C elevated temperature treatment than control treatment. With elevated CO<sub>2</sub> treatments stomatal conductance was reduced appreciably at both 1.5°C and 3°C elevated temperature than control treatment (Table 4.11). Stomatal conductance of PEHM 5 and CM 119 under different treatments was in the order of **T1 > T0 > T3 > T4 > T5 > T2**

**Table 4.11: Effect of elevated temperature and CO<sub>2</sub> on stomatal conductance of maize genotypes**

Stomatal conductance (mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )						
	PEHM 5			CM 119		
Temperature	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	0.026	0.015	<b>0.021</b>	0.035	0.029	<b>0.032</b>
1.5T	0.046	0.011	<b>0.029</b>	0.042	0.024	<b>0.033</b>
3T	0.007	0.010	<b>0.009</b>	0.012	0.022	<b>0.017</b>
Mean	<b>0.026</b>	<b>0.012</b>		<b>0.030</b>	<b>0.025</b>	

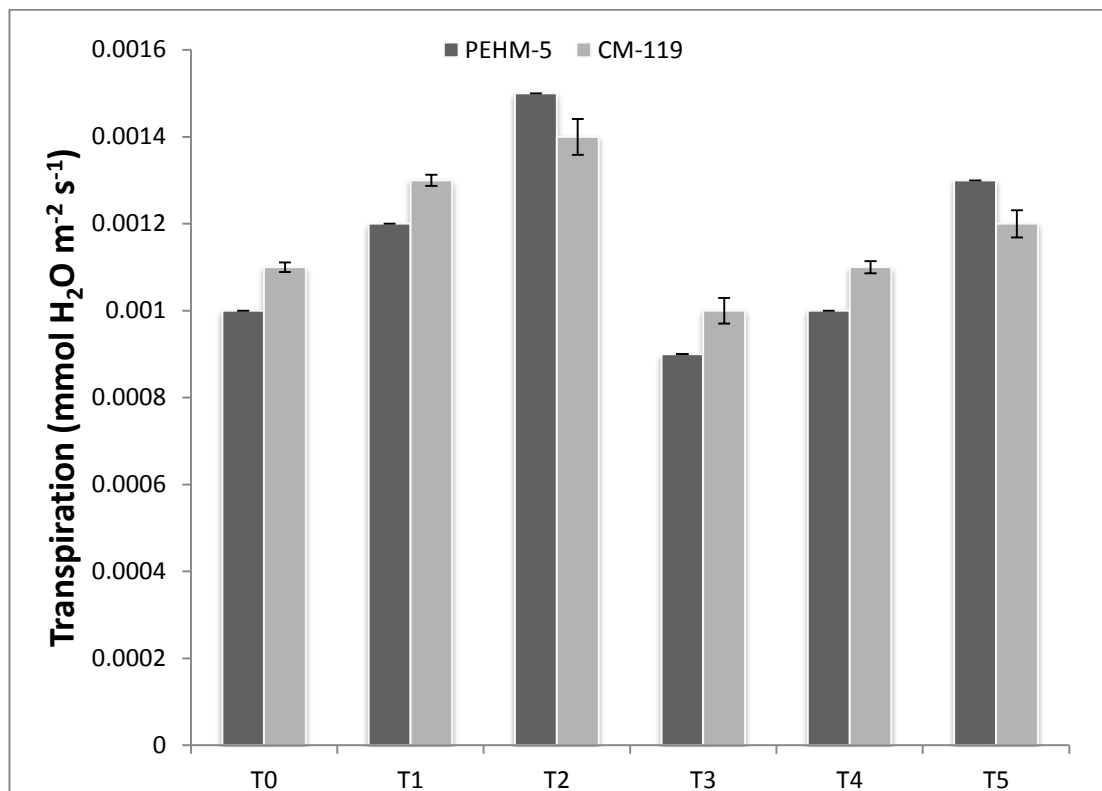
  

Factors	C.D.	SE(d)	SE(m)
Genotype (G)	NS	0.003	0.002
CO <sub>2</sub> level (C)	0.005	0.003	0.002
G × C	0.008	0.004	0.003
Temperature (T)	0.007	0.003	0.002
G × T	0.009	0.005	0.003
C × T	0.009	0.005	0.003
G × T × C	0.013	0.007	0.005

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature

### Transpiration rate

Transpiration rate in PEHM 5 was more than CM 119. Transpiration rate in PEHM 5 and CM 119 was low in elevated CO<sub>2</sub> treatments compared to ambient CO<sub>2</sub> treatments. Rate of transpiration was found to increase with increase in temperature. In the present study transpiration rate under different treatments for PEHM 5 was in the order of **T2 > T5 > T1 > T0 = T4 > T3**; and for CM 119 it was in the order of **T2 > T1 > T5 > T0 = T4 > T3**. In PEHM 5, it was 20%, 50% and 30% higher under T1, T2 and T5 treatments and 10% less under T3 treatment, respectively as compared to control; whereas, in CM 119, it was 18.18%, 27.27% and 9.09% higher under T1, T2 and T5 and 9.09% lower under T3 treatment, respectively as compared to control.



**Figure 4.2: Effect of elevated temperature and CO<sub>2</sub> on transpiration of maize genotypes**

T0, Ambient CO<sub>2</sub> level + Ambient temperature level; T1, Ambient CO<sub>2</sub> level + 1.5°C elevated temperature than ambient level; T2, Ambient CO<sub>2</sub> level + 3°C elevated temperature than ambient level; T3, 550 ppm CO<sub>2</sub> level + Ambient temperature level; T4, 550 ppm CO<sub>2</sub> level + 1.5°C elevated temperature than ambient level; T5, 550 ppm CO<sub>2</sub> level + 1.5°C elevated temperature than ambient level

## **Response of yield parameters of maize genotypes to elevated CO<sub>2</sub> and temperature**

### **Cob weight**

Cob weight in PEHM 5 plants under different treatments was in the order of **T3 > T4 > T1 > T0 > T5 > T2**. Cob weight increased 8.11% and 4.60% in T3 and T4 treatments, respectively and decreased 9.05% and 4.84% in T1 and T5, respectively as compared to control.

Whereas for cob weight in CM 119 plants it was in the order of **T0 > T3 > T4 > T1 > T5 > T2**, and it was found to decrease in all treatments as compared to control but decrease was minimum in case of T3 and T4 treatments (9.41% and 9.60%, respectively) and maximum in T2 and T5 treatments (26.27% and 24.31%, respectively) as compared to control (Table 4.12).

### **Cob girth**

Cob girth in PEHM 5 plants under different treatments was in the order of **T3 > T5 > T2 > T4 > T0 > T1**. Cob girth increased 19.43%, 18.59% and 5.07% in T3, T5 and T2 treatments, respectively and decreased 0.84% in T1 as compared to control.

Whereas, for CM 119 plants it was in the order of **T0 > T4 > T1 > T5 > T2 > T3**; and it was found to decrease in all treatments as compared to control but decrease was minimum in case of T1 and T4 treatments (10.48% and 5.59%, respectively) and maximum in T2, T3 and T5 treatments (18.18%, 18.88% and 17.48%, respectively) as compared to control.

### **Number of rows per cob**

Number of rows per cob in PEHM 5 plants under different treatments was in the order of **T3 > T4 = T5 > T1 > T0 = T2**. Number of rows increased in all treatments except T2 and the increase was 1.81%, 7.27%, 3.63% and 3.63%, respectively in T1, T3, T4 and T5 treatments, respectively as compared to control; whereas, for CM 119 plants it was in the order of **T0 > T5 > T4 > T3 > T2 > T1**. Number of rows per cob decreased in all treatments and maximum decrease was in T1, T2 and T3 treatments (46.66%, 37.33% and 26.66%, respectively as compared to control).

### **Grain weight per plant**

Grain weight per plant in PEHM 5 under different treatments was in the order of **T3 > T4 > T1 > T0 > T5 > T2**. Grain weight per plant increased in T1, T3 and T4 treatments and decreased in T2 and T5 treatments. The increase was 0.66%, 34.14% and 9.3% in T1, T3 and T4 treatments, respectively and decrease was 26.43% and 4.29% in T2 and T5 treatments, respectively as compared to control; whereas, for CM 119 plants it was in the order of **T3 > T4 > T0 > T1 > T5 > T2**. Grain weight per plant decreased in all treatments except T3 and T4. Decrease in grain weight per plant was 4.89%, 40.79% and 28.67% in T1, T2, and T5 treatments, respectively and increase was 27.97% and 18.88% in T3 and T4 treatments, respectively as compared to control.

### **Harvest index**

Harvest index in PEHM 5 under different treatments was in the order of **T3 > T1 > T4 > T2 > T5 > T0**. Harvest index increased in all treatments. The increase was 52.36%, 20.60%, 66.52%, 21.88% and 5.57% in T1, T2, T3, T4 and T5 treatments, respectively as compared to control; whereas, for CM 119 plants it was in the order of **T3 > T4 > T2 > T0 > T5 > T1**.

Harvest index increased in all treatments except T1 and T5. Harvest index increased 1.88%, 20.28% and 8.49% in T2, T3 and T4 treatments, respectively and decreased 2.83% and 0.47% in T1 and T5 treatments, respectively as compared to control.

**Table 4.12: Effect of elevated temperature and CO<sub>2</sub> on yield attributes of maize genotypes**

Treatment	Cob weight (g)		Cob girth (cm)		Number of rows per cob		Grain weight per plant (g)		Harvest index	
	PEHM 5	CM 119	PEHM 5	CM 119	PEHM 5	CM 119	PEHM 5	CM 119	PEHM 5	CM 119
T0	25.62 ± 0.44	22.50 ± 0.34	7.10 ± 0.18	7.15 ± 0.01	11.00 ± 0.44	7.50 ± 0.31	27.24 ± 0.75	4.29 ± 0.27*	0.223 ± 0.002	0.212 ± 0.002
T1	25.88 ± 0.69	18.75 ± 0.30*	7.04 ± 0.08	6.40 ± 0.06*	11.20 ± 0.80	4.00 ± 0.31*	27.42 ± 0.21*	4.08 ± 0.19*	0.345 ± 0.012*	0.206 ± 0.006
T2	23.30 ± 0.23*	15.80 ± 0.19*	7.46 ± 0.37	5.85 ± 0.01*	11.00 ± 0.70	5.00 ± 0.31*	20.04 ± 0.44	2.54 ± 0.16*	0.271 ± 0.002*	0.216 ± 0.007
T3	27.70 ± 0.57*	20.10 ± 0.50*	8.48 ± 0.17*	5.80 ± 0.03*	11.80 ± 0.37*	5.50 ± 0.15*	36.54 ± 0.97*	5.49 ± 0.20	0.378 ± 0.001*	0.255 ± 0.005*
T4	26.80 ± 1.12	20.05 ± 0.23*	7.20 ± 0.37	6.75 ± 0.04*	11.40 ± 0.51	6.50 ± 0.15*	29.80 ± 0.59*	5.10 ± 0.18	0.274 ± 0.004*	0.230 ± 0.004*
T5	24.38 ± 0.56	16.30 ± 0.47*	8.42 ± 0.29*	5.90 ± 0.28*	11.40 ± 0.51	7.00 ± 0.31	26.10 ± 1.09	3.06 ± 0.33*	0.236 ± 0.006*	0.211 ± 0.003
C.D. (P = 0.05)	1.195	1.062	0.792	0.357	0.722	0.804	2.178	0.686	0.017	0.014

\*Significant at  $P < 0.05$ ; T0, Ambient CO<sub>2</sub> + Ambient temperature level; T1, Ambient CO<sub>2</sub> + 1.5°C elevated temperature level; T2, Ambient CO<sub>2</sub> + 3°C elevated temperature level; T3, 550 ppm CO<sub>2</sub> + Ambient temperature level; T4, 550 ppm CO<sub>2</sub> + 1.5°C elevated temperature level; T5, 550 ppm CO<sub>2</sub> + 3°C elevated temperature level

### **Principal component analysis of growth, physiological, and biochemical response of maize genotypes under elevated carbon dioxide and temperature**

Data generated for different growth, physiological and biochemical parameters were subjected to PCA to identify the key parameters and linear scoring of key parameters was done to know their contribution in the response of plant. After subjected to PCA, the amount of variability explained by PC1, PC2, PC3 PC4, and PC5 was 40.58%, 16.95%, 11.93%, 8.45% and 6.10%, respectively. However, the cumulative variance was 84.05% (Table 4.13). Within each PC, only highly weighted factors (having absolute values within 10% of the highest factor loading) were retained for minimum data set (MDS). In PC1 three variables quantified for the next step were plant height, stem girth and chlorophyll content. These three variables were found well correlated ( $r > 0.70$ ) in the intercorrelation study (Table 4.14). So, only total chlorophyll content was retained for minimum data set (MDS) as it was having the highest factor loading as compared to plant height and stem girth. Likewise, in PC2, PC3 PC4 and PC5; MDA content, total phenol content, RLWC and ascorbic acid content was retained, respectively for the MDS. For different principal components % variance explained and eigen values and factor loadings are shown in table 4.13.

After that by doing linear scoring for each replication in different parameters retained for MDS in both PEHM 5 and CM 119 average linear scoring was calculated. In order to know the contribution of each of the indicator towards crop response the average linear scores under each treatment have been depicted in Figure 4.3. Considering the average linear scores the order of importance of key parameters in influencing crop health for PEHM 5 was total phenol content (0.82) > ascorbic acid content (0.75) > relative leaf water content (0.71) > total chlorophyll content (0.58) > MDA content (0.51), with a corresponding contribution of 24.23%, 22.15%, 21.15%, 17.31% and 15.16%, respectively. Similarly, for CM 119 the order of importance of key parameters in influencing crop health was MDA content (0.83) > relative leaf water content (0.67) > total chlorophyll content (0.57) > total phenol content (0.52) > ascorbic acid content (0.51), with a corresponding contribution of 36.6%, 21.57%, 18.51%, 11.74% and 11.57%, respectively.

**Table 4.13: Performance of crop health indicators in terms of factor loading /eigen vector values in principal component analysis**

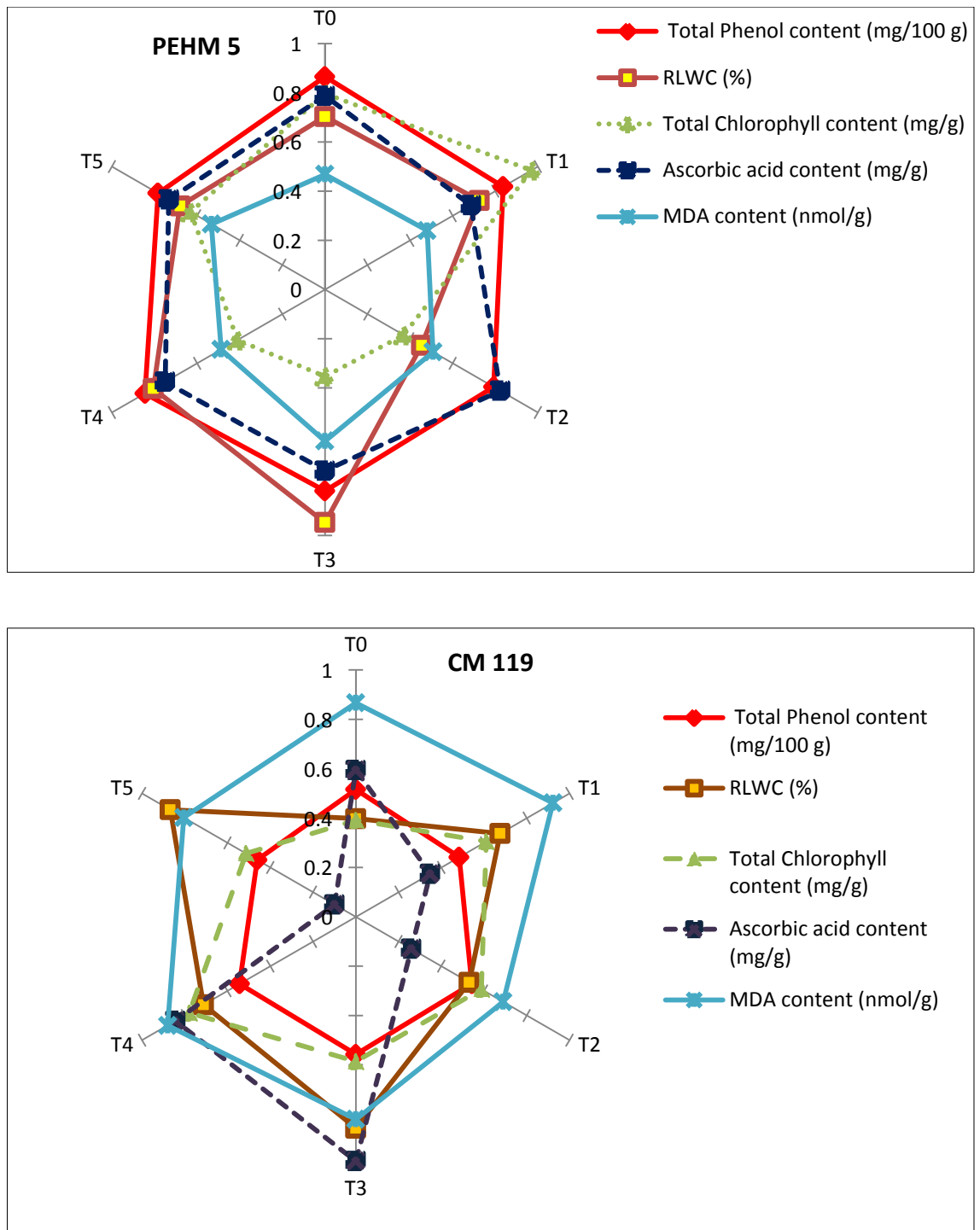
PCs	PC1	PC2	PC3	PC4	PC5
Eigen value	4.871	2.035	1.432	1.015	0.733
% Variance	40.589	16.958	11.937	8.459	6.106
Cumulative %	40.589	57.547	69.484	77.943	84.050
Factor loading/eigen vector					
Plant height	<b>0.867</b>	0.090	0.186	0.091	-0.111
Stem girth	<b>0.900</b>	0.098	0.233	0.159	-0.103
Leaf area	0.508	0.064	0.699	-0.136	0.187
Chlorophyll content	<b>0.940</b>	0.149	0.115	0.195	-0.050
RLWC	0.048	0.047	0.105	<b>0.947</b>	0.086
Photosynthetic rate	0.283	0.763	0.377	0.197	0.024
Stomatal conductance	-0.016	0.679	-0.026	-0.467	0.232
Transpiration rate	0.093	-0.053	-0.058	0.058	0.000
Leaf extract pH	0.221	0.045	0.034	0.060	0.104
Ascorbic acid content	-0.201	0.082	0.166	0.070	<b>0.894</b>
Total phenol content	0.524	0.404	<b>0.896</b>	0.009	0.390
MDA content	-0.969	<b>0.894</b>	0.040	-0.018	0.051

\*Bold values indicate the eigen vectors within 10% of the highest factor loadings

**Table 4.14: Inter-correlations between highly weighted variables under different principal components**

PC 1 variables	Plant height	Stem girth	Total Chlorophyll content
Plant height	1	0.940 <sup>**</sup>	0.854 <sup>**</sup>
Stem girth	0.940 <sup>**</sup>	1	0.811 <sup>**</sup>
Total Chlorophyll content	0.854 <sup>**</sup>	0.811 <sup>**</sup>	1

<sup>\*\*</sup> $P < 0.01$



**Figure 4.3: Radar graphs for PEHM 5 and CM 119 depicting the average linear score of key indicators which are most influenced by disease under different treatments**

T0, Ambient CO<sub>2</sub> level + Ambient temperature level; T1, Ambient CO<sub>2</sub> level + 1.5°C elevated temperature than ambient level; T2, Ambient CO<sub>2</sub> level + 3°C elevated temperature than ambient level; T3, 550 ppm CO<sub>2</sub> level + Ambient temperature level; T4, 550 ppm CO<sub>2</sub> level + 1.5°C elevated temperature than ambient level; T5, 550 ppm CO<sub>2</sub> level + 1.5°C elevated temperature than ambient level

### **Impact of elevated temperature and CO<sub>2</sub> on maydis leaf blight (MLB) disease development in maize genotypes**

MLB disease severity in PEHM 5 and CM 119 exposed to different treatments under field conditions with artificial inoculation was assessed as per the rating scale of Payak and Sharma (1983), which was converted in terms of Percentage disease index (PDI). PDI value was high in CM 119 plants in all treatments as compared to PEHM 5 plants. Maximum and minimum disease severity was recorded in plants of PEHM 5 and CM 119 exposed to T2 and T3 treatment, respectively (Table 4.15).

**Table 4.15: Percentage disease index value for MLB disease in maize genotypes**

Treatment	Percentage disease index	
	PEHM 5	CM 119
T0	68.41	83.28
T1	71.22	88.33
T2	76.45	95.43
T3	61.74	78.71
T4	65.25	79.57
T5	74.86	90.14

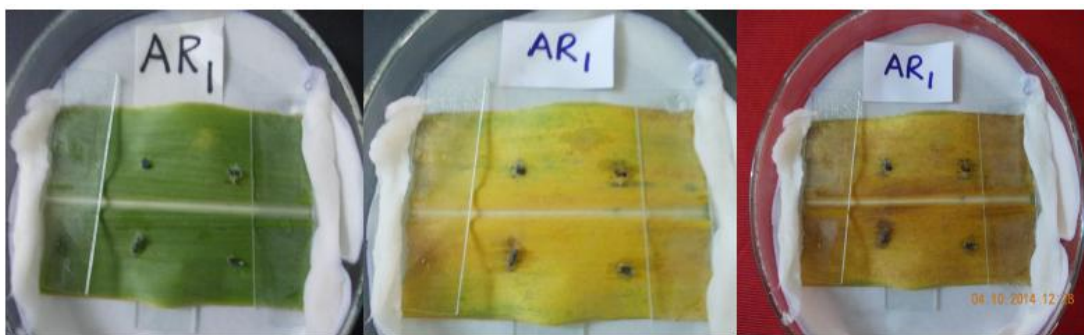
In PEHM 5 and CM 119 variety disease severity and PDI value was in the order of **T2 > T5 > T1 > T0 > T4 > T3**. Elevated temperature exposure caused more disease incidence in both genotypes as compared to elevated CO<sub>2</sub> exposure.

### **Disease development assessment in maize genotypes under controlled lab conditions**

It was done by Detached leaf technique.

#### **Detached leaf technique**

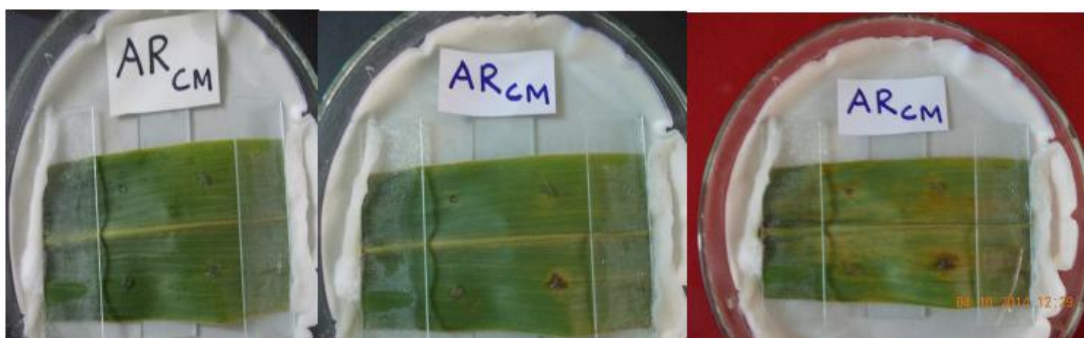
Under lab condition healthy leaves from PEHM 5 and CM 119 plants grown under different treatments were inoculated with MLB pathogen and observed for disease progress. Observations taken after of 48, 72 and 96 h of inoculation showed that the infection was in the order of **T2 > T5 > T1 > T0 > T4 > T3**.



Day 2 (after 48 h)

Day 3 (after 72 h)

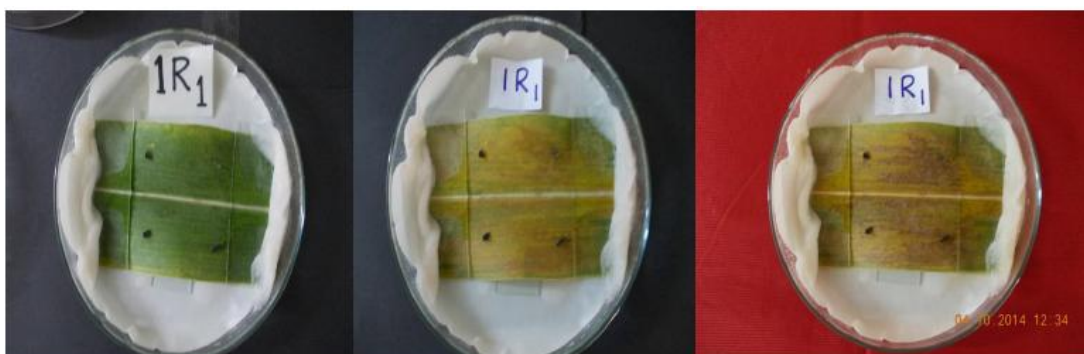
Day 4 (after 96 h)



Day 2 (after 48 h)

Day 3 (after 72 h)

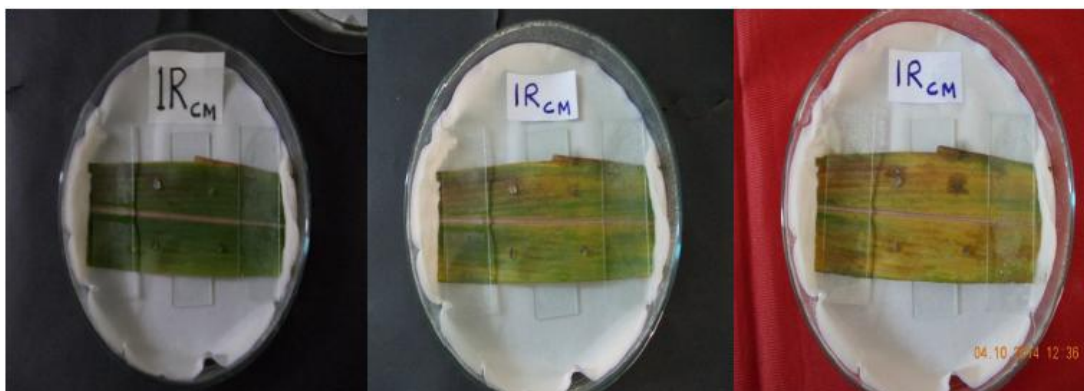
Day 4 (after 96 h)



Day 2 (after 48 h)

Day 3 (after 72 h)

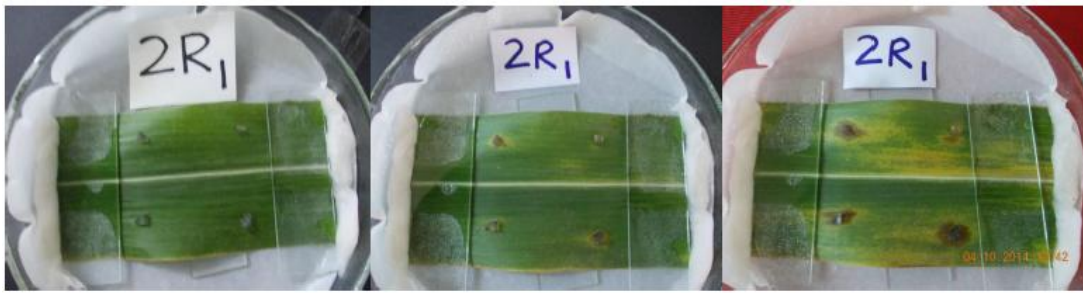
Day 4 (after 96 h)



Day 2 (after 48 h)

Day 3 (after 72 h)

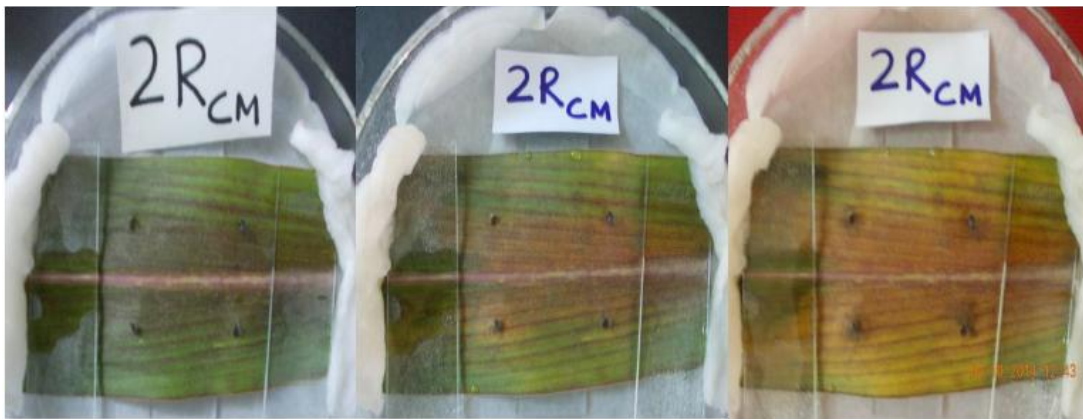
Day 4 (after 96 h)



Day 2 (after 48 h)

Day 3 (after 72 h)

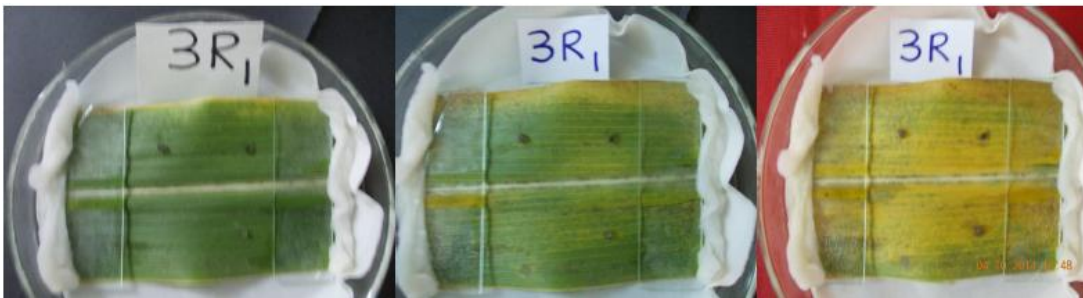
Day 4 (after 96 h)



Day 2 (after 48 h)

Day 3 (after 72 h)

Day 4 (after 96 h)



Day 2 (after 48 h)

Day 3 (after 72 h)

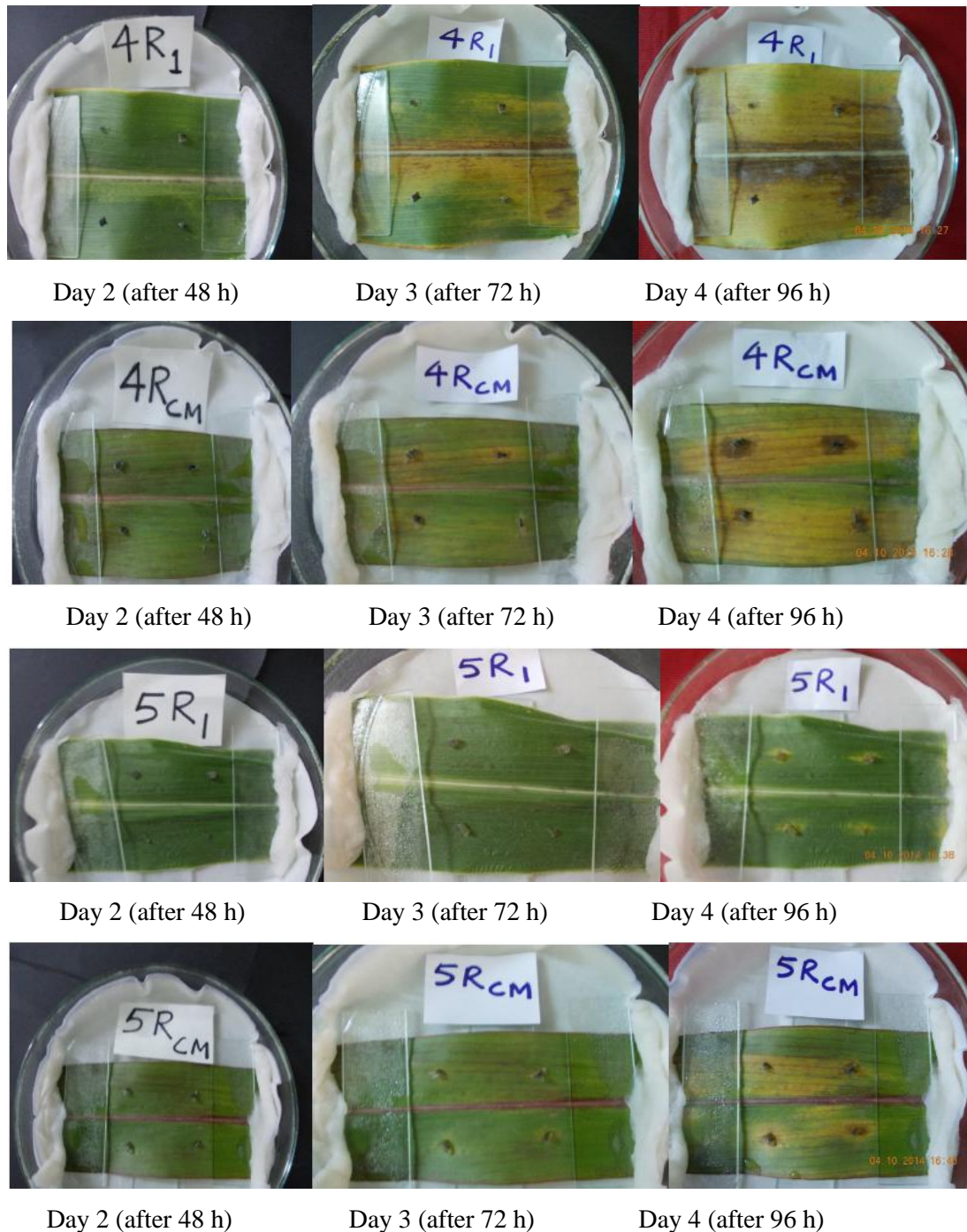
Day 4 (after 96 h)



Day 2 (after 48 h)

Day 3 (after 72 h)

Day 4 (after 96 h)



**Plate 4.1: MLB disease development in leaves of both genotypes under different treatments inoculated with MLB pathogen after 48, 72 and 96 hours of inoculation**

A, Ambient temperature and carbon dioxide level; R<sub>1</sub>, Leaf sample of PEHM 5 plant; R<sub>CM</sub>, Leaf sample of CM 119 plants; 1, 550ppm elevated CO<sub>2</sub> than ambient level + 1.5<sup>0</sup>C elevated temperature than ambient level; 2, Ambient CO<sub>2</sub> level + 1.5<sup>0</sup>C elevated temperature than ambient level; 3, 550 ppm elevated CO<sub>2</sub> than ambient level + 3<sup>0</sup>C elevated temperature than ambient level; 4, 550ppm elevated CO<sub>2</sub> than ambient level + Ambient temperature; 5, Ambient CO<sub>2</sub> level + 3<sup>0</sup>C elevated temperature than ambient level

### **Analysis of disease progress using ImageJ software**

Images of leaves of inoculated plants captured after 6 days of inoculation i.e., at appearance of first disease symptoms to next 19 days at 1 day interval under different treatments were analysed to estimate the rate of progress of infection (Figure 4.3 and 4.4).

**PEHM 5:** On 19<sup>th</sup> day of observation maximum infection was recorded in T2 treatment ( $44.3 \pm 6.0\%$ ) and minimum infection was recorded in T3 ( $30.6 \pm 1.0\%$ ) treatment (Table 4.16). Rate of increase in infection was maximum in T2 treatment (increased from  $15.6 \pm 2.4\%$  on 1<sup>st</sup> day to  $44.3 \pm 6.0\%$  on 19<sup>th</sup> day) and minimum in T3 treatment (increased from  $13.4 \pm 0.7\%$  on 1<sup>st</sup> day to  $30.6 \pm 1.0\%$  on 19<sup>th</sup> day). After 15<sup>th</sup> day of observation infection was almost stabilised and very little increase was found till 19<sup>th</sup> day. It indicated that PEHM 5 was developing resistance towards MLB. The disease severity under different treatments followed the order of **T2 > T5 > T1 > T0 > T4 > T3**.

**CM 119:** In image of last day of observation (i.e., of 19<sup>th</sup> day) maximum infection was recorded in T2 treatment ( $65.6 \pm 3.4\%$ ) and minimum infection was recorded in T3 ( $46.3 \pm 0.2\%$ ) treatment (Table 4.16, Figure 4.4). Rate of increase in infection was highest in T1 treatment (increased from  $12.9 \pm 1.9\%$  on 11<sup>th</sup> day to  $62.0 \pm 2.1\%$  on 19<sup>th</sup> day) and lowest in T3 treatment (increased from  $13.8 \pm 0.9\%$  on 11<sup>th</sup> day to  $46.3 \pm 0.2\%$  on 19<sup>th</sup> day). The disease severity was in the order of **T2 > T5 > T1 > T0 > T4 > T3**.

When all three methods of disease assessment (rating scale, detached leaf and imageJ) were compared disease severity was less with elevated CO<sub>2</sub> levels in both the genotypes (Figure 4.4 and 4.5). Under control treatment disease severity was 57% more in CM 119 as compared to PEHM 5. At ambient as well as elevated CO<sub>2</sub> levels increase in temperature stress enhanced disease severity significantly. The increase in disease severity in PEHM 5 under ambient CO<sub>2</sub> level with 1.5°C and 3°C increased temperature stress was 9% and 26% more, respectively. Increase in disease severity in PEHM 5 under elevated CO<sub>2</sub> level with 1.5°C and 3°C increased temperature stress was 6.5% and 33% more, respectively. Similarly in CM 119 at ambient CO<sub>2</sub> level with 1.5°C and 3°C increased temperature stress caused 6% and

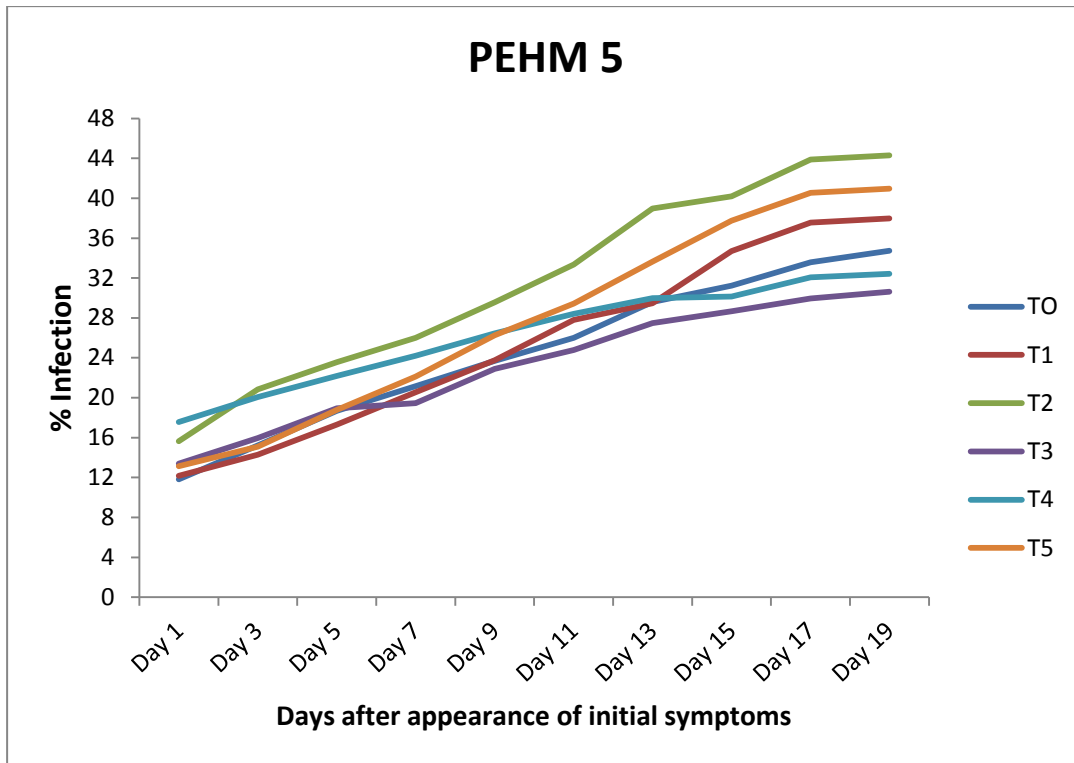
12% enhancement in disease severity and at elevated CO<sub>2</sub> level with 1.5°C and 3°C increased temperature stress caused 6.74% and 35.95% increase in disease severity (table 4.16).

**Table 4.16: Effect of elevated temperature and CO<sub>2</sub> on percentage infection in maize genotypes (recorded on last day of observation)**

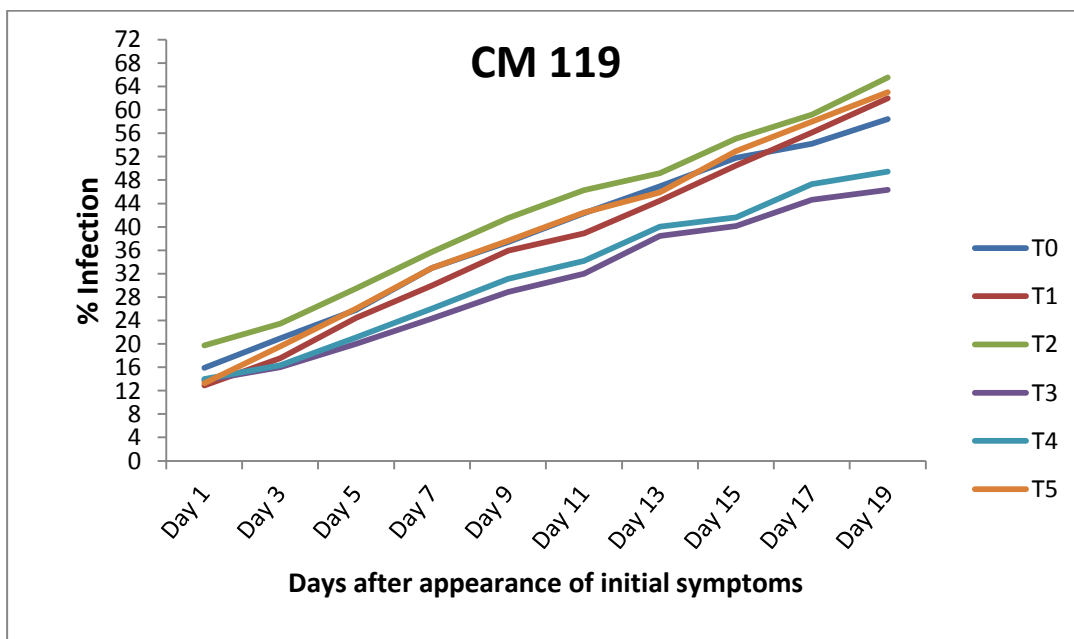
Percentage infection on last day of observation						
Temperature	PEHM 5			CM 119		
	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	34.72	30.62	<b>32.67</b>	58.40	46.34	<b>52.37</b>
1.5T	37.96	32.43	<b>35.20</b>	61.98	49.47	<b>55.72</b>
3T	44.30	40.96	<b>42.63</b>	65.55	63.00	<b>64.28</b>
Mean	<b>38.99</b>	<b>34.67</b>		<b>61.98</b>	<b>52.94</b>	

Factors	C.D.	SE(d)	SE(m)
Genotype (G)	0.007	0.003	0.002
CO <sub>2</sub> level (C)	0.007	0.003	0.002
G × C	0.01	0.005	0.003
Temperature (T)	0.008	0.004	0.003
G × T	0.012	0.006	0.004
C × T	0.012	0.006	0.004
G × T × C	0.017	0.008	0.006

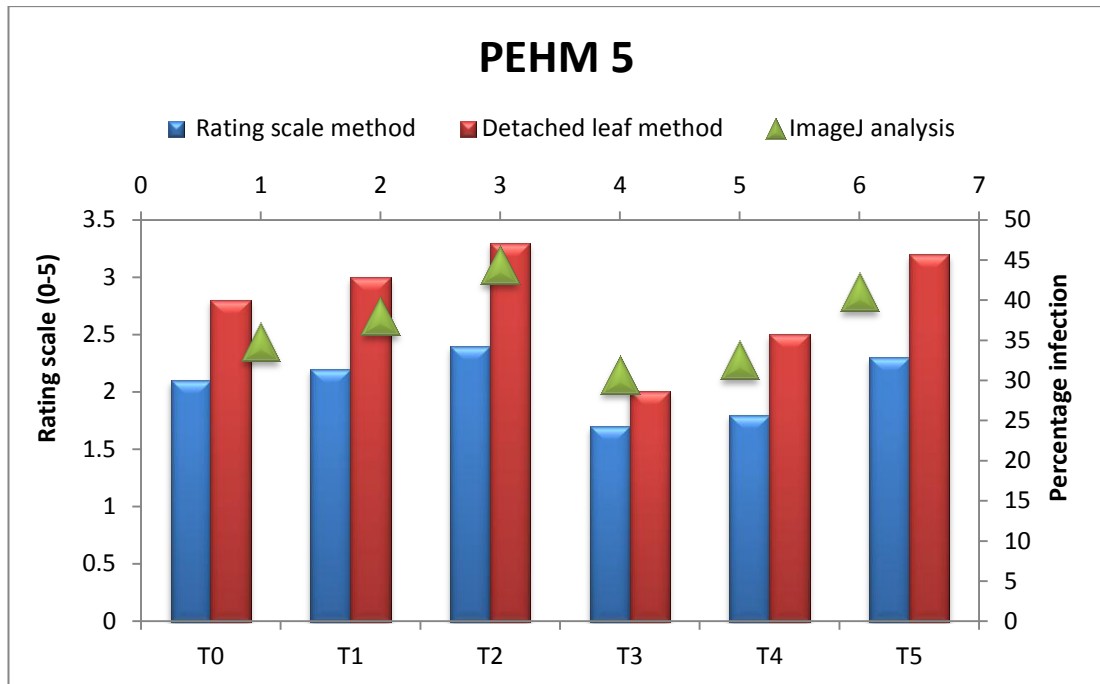
Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature



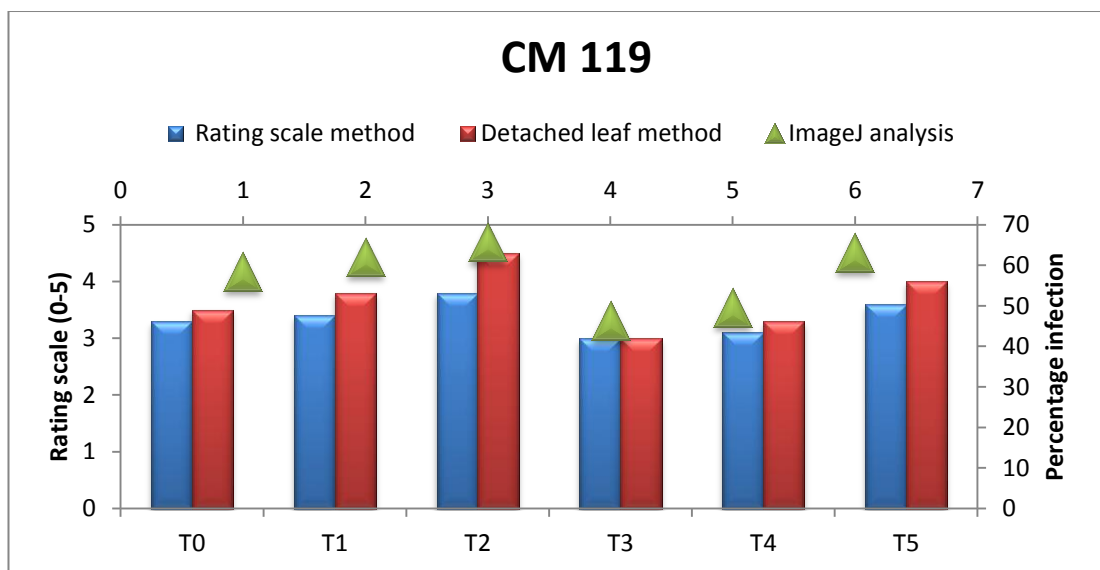
**Figure 4.4: MLB disease development under different treatments in PEHM 5**



**Figure 4.5: MLB disease development under different treatments in CM 119**



**Figure 4.6: Comparison of different methods of disease assessment (Rating scale, ImageJ analysis and Detached leaf method) for PEHM 5 under elevated levels of temperature and carbon dioxide level**



**Figure 4.7: Comparison of disease assessment (Rating scale, ImageJ analysis and Detached leaf method) in CM 119 under elevated levels of temperature and carbon dioxide level**

T0, Ambient CO<sub>2</sub> level + Ambient temperature level; T1, Ambient CO<sub>2</sub> level + 1.5°C elevated temperature than ambient level; T2, Ambient CO<sub>2</sub> level + 3°C elevated temperature than ambient level; T3, 550 ppm CO<sub>2</sub> level + Ambient temperature level; T4, 550 ppm CO<sub>2</sub> level + 1.5°C elevated temperature than ambient level; T5, 550 ppm CO<sub>2</sub> level + 1.5°C elevated temperature than ambient level

### **Impact of elevated temperature and CO<sub>2</sub> on disease stress tolerance attributes**

Disease stress tolerance attributes were calculated for both genotypes. For calculating attributes yield was recorded in g/plant unit in healthy and diseased plants of both genotypes under different treatments (Table 4.17).

#### **Disease tolerance (TOL)**

Larger value of the index represents relatively more sensitivity to disease stress. Average disease tolerance value was found higher in CM 119 as compared to PEHM 5. For PEHM 5 TOL value was in the order of **T4 > T2 > T5 > T3 > T1 > T0**, and for CM 119 it was **T3 > T5 > T1 > T0 > T4 > T3**.

#### **Disease stress susceptibility index (DSSI)**

Smaller the value of index, greater is the disease stress tolerance capacity. The average DSSI value was higher in CM 119 as compared to PEHM 5. For PEHM 5, DSSI value was in the order of **T2 > T5 > T4 > T1 > T0 > T3** and for CM 119, it was in the order of **T2 > T5 > T1 > T3 = T4 > T0**. T2 treatment was found more sensitive towards disease stress in both the genotypes.

#### **Disease stress tolerance index (DSTI)**

The higher value of the index indicates higher disease tolerance and yield potential. DSTI value was higher in PEHM 5 in all treatments as compared to CM 119. For PEHM 5, DSTI value was in the order of **T3 > T4 > T1 = T5 > T0 > T2**; and for CM 119 it was in the order of **T3 > T4 > T1 > T0 > T4 = T5**.

**Table 4.17: Disease stress tolerance attributes for maize genotypes under different treatments**

Treatment	Yield (g/plant) without disease (Yp)		Yield (g/plant) with disease (Ys)		TOL		DSSI		DSTI	
	PEHM 5	CM 119	PEHM 5	CM 119	PEHM 5	CM 119	PEHM 5	CM 119	PEHM 5	CM 119
T0	36.08	19.22	27.24	4.29	8.84	14.93	0.68	1.00	0.00051	0.000079
T1	38.4	19.36	27.42	4.08	10.98	15.28	0.79	1.01	0.00055	0.000075
T2	35.08	14.29	20.04	2.54	15.04	11.75	1.19	1.05	0.00036	0.000035
T3	47.62	22.06	36.54	5.49	11.08	16.57	0.65	0.96	0.00090	0.000116
T4	45.22	20.44	29.80	5.10	15.42	15.34	0.95	0.96	0.00070	0.000100
T5	40.46	16.26	26.10	3.06	14.36	13.20	0.98	1.04	0.00055	0.000048
Mean	<b>40.47</b>	<b>18.60</b>	<b>27.86</b>	<b>4.09</b>	<b>12.62</b>	<b>14.51</b>	<b>0.87</b>	<b>1.01</b>	<b>0.00060</b>	<b>0.000075</b>

Yp = Yield (g/plant) of genotypes without disease

Ys = Yield (g/plant) of genotypes under diseased condition

Yp<sup>-</sup> = Mean yield of genotypes without disease

Ys<sup>-</sup> = Mean yield of genotypes with disease

TOL = Disease tolerance (larger value represents relatively more sensitivity to disease stress)

DSSI = Disease stress susceptibility index (smaller the value greater is the disease stress tolerance)

DSTI = Disease stress tolerance index (higher the value, higher is its disease tolerance and yield potential)

T0, Ambient CO<sub>2</sub> + Ambient temperature level; T1, Ambient CO<sub>2</sub> + 1.5°C elevated temperature level; T2, Ambient CO<sub>2</sub> + 3°C elevated temperature level; T3, 550 ppm CO<sub>2</sub> + Ambient temperature level; T4, 550 ppm CO<sub>2</sub> + 1.5°C elevated temperature level; T5, 550 ppm CO<sub>2</sub> + 3°C elevated temperature level

## Objective 2

### To assess impact of elevated temperature and CO<sub>2</sub> on maydis leaf blight tolerance potential of maize

For the assessment of impact of elevated temperature and CO<sub>2</sub> on maydis leaf blight tolerance potential of maize (PEHM 5), three key growth stages – vegetative, tasseling and dent stage were selected. At selected growth stages growth, physiological and bio-chemical parameters were evaluated to correlate them with maydis leaf blight disease tolerance. Disease assessment was done through detached leaf method under controlled lab conditions.

### Effect of elevated temperature and CO<sub>2</sub> on growth parameters at three growth stages of maize

#### Plant height

At vegetative stage maximum and minimum plant height was recorded in T3 treatment (109.4 cm) and T0 treatment (86.4 cm), respectively. Variation in plant height at vegetative was in the order of **T3 > T4 > T5 > T1 > T2 > T0** (Table 4.18). The increase in plant height between the period of vegetative stage and tasseling stage, i.e. at tasseling stage was maximum and minimum in T3 treatment (35.97%), and T2 treatment (23.63%), respectively. Variation in plant height at tasseling stage was in the order of **T3 > T4 > T1 > T5 > T0 > T2**.

The maximum increase in plant height from tasseling to dent stage was found in T0 treatment (43.08%), and lowest increase was found in T3 treatment (23.38%). Thus, at tasseling stage T0 treatment was found least sensitive and T3 treatment was found most sensitive. Variation in plant height at dent stage was in the order of **T3 > T4 > T0 > T1 > T5 > T2**. The effect on plant height due to interaction of CO<sub>2</sub> and temperature was significant at all the three stages but effect due to interaction between growth stage and CO<sub>2</sub> level was non-significant.

#### Stem girth

At vegetative stage maximum and minimum stem girth was recorded in T3 treatment (5.44 cm) and T2 treatment (4.26 cm), respectively. Variation in stem girth at vegetative stage was in the order of **T3 > T4 = T1 > T0 > T5 > T2**.

The increase in stem girth between the period of vegetative stage and tasseling stage, i.e. at tasseling stage was maximum and minimum in T3 treatment (40.44%), and T5

treatment (32.87%), respectively. Variation in stem girth at tasseling stage was in the order of **T3 >T4 = T1 >T0 > T5 > T2**, which was same as that of vegetative stage.

The maximum increase in stem girth from tasseling to dent stage was found in T5 treatment (13.40%), and lowest increase was found in T3 treatment (4.45%). Thus, at tasseling stage T5 treatment was found least sensitive and T3 treatment was found most sensitive. Variation in stem girth at dent stage was in the order of **T3 >T4 > T1 >T0 > T5 > T2** (Table 4.19). The effect of interaction between temperature and CO<sub>2</sub> was significant, but the effect due to interaction between growth stage and CO<sub>2</sub> and growth stage and temperature was non-significant.

**Table 4.18: Effect of elevated temperature and CO<sub>2</sub> on plant height of PEHM 5 at different growth stages**

	Plant height (cm)								
	Vegetative stage			Tasseling stage			Dent stage		
	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	86.4	109.4	<b>97.9</b>	114.2	148.8	<b>131.5</b>	163.4	183.6	<b>173.5</b>
AT +1.5 <sup>0</sup> C	94.4	102.2	<b>98.3</b>	124.8	136	<b>130.4</b>	161.2	171.8	<b>166.5</b>
AT +3 <sup>0</sup> C	91.4	96	<b>93.7</b>	113	121.8	<b>117.4</b>	145.8	158.2	<b>152</b>
Mean	<b>90.7</b>	<b>102.5</b>		<b>117.3</b>	<b>135.5</b>		<b>156.8</b>	<b>171.2</b>	

Factors	C.D	SE(d)	SE(m)
Growth stage (S)	2.85	1.43	1.01
CO <sub>2</sub> level (C)	2.33	1.17	0.82
S × C	NS	2.02	1.43
Temperature (T)	2.85	1.43	1.01
S × T	4.95	2.48	1.75
T × C	4.04	2.02	1.43
S × T × C	NS	3.51	2.48

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature

**Table 4.19: Effect of elevated temperature and CO<sub>2</sub> on stem girth of PEHM 5 at different growth stages**

Temperature	Stem girth (cm)								
	Vegetative stage			Tasseling stage			Dent stage		
	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	4.86	5.44	<b>5.15</b>	6.62	7.64	<b>7.13</b>	6.94	7.98	<b>7.46</b>
1.5T	5	5	<b>5</b>	7	7	<b>7</b>	7.42	7.54	<b>7.48</b>
3T	4.26	4.38	<b>4.32</b>	5.68	5.82	<b>5.75</b>	6.04	6.6	<b>6.32</b>
Mean	<b>4.70</b>	<b>4.94</b>		<b>6.43</b>	<b>6.82</b>		<b>6.8</b>	<b>7.37</b>	

Factors	C.D.	SE(d)	SE(m)
Growth stage (S)	0.18	0.09	0.06
CO <sub>2</sub> level (C)	0.14	0.07	0.05
S × C	NS	0.12	0.09
Temperature (T)	0.18	0.09	0.06
S × T	NS	0.15	0.11
T × C	0.25	0.12	0.09
S × T × C	NS	0.22	0.15

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature

### Leaf area

At vegetative stage maximum and minimum leaf area was recorded in T3 treatment (428.55 cm<sup>2</sup>) and T2 treatment (251.01 cm<sup>2</sup>), respectively. Variation in leaf area at vegetative was in the order of **T3 > T4 > T1 > T5 > T0 > T2**. The increase in leaf area between the period of vegetative stage and tasseling stage, i.e. at tasseling stage was maximum and minimum in T2 treatment (78.39%) and T1 treatment (7.41%), respectively. Variation in leaf area at tasseling stage was in the order of **T3 > T2 > T0 > T4 > T5 > T1**. The maximum increase in leaf area from tasseling to dent stage was found in T1 treatment (41.64%), and minimum increase was found in T3 treatment (10.04%). Variation in leaf area at dent stage was in the order of **T3 > T0 > T4 > T2 > T5 > T1** (Table 4.20).

**Table 4.20: Effect of elevated temperature and CO<sub>2</sub> on leaf area at different growth stages in PEHM 5**

Temperature	Leaf area (cm <sup>2</sup> )								
	Vegetative stage			Tasseling stage			Dent stage		
	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	278.69	428.56	<b>353.62</b>	431.92	495.69	<b>463.81</b>	526.97	545.45	<b>536.21</b>
1.5T	305.10	362.83	<b>333.96</b>	327.72	422.49	<b>375.10</b>	464.18	498.39	<b>481.29</b>
3T	251.02	288.06	<b>269.54</b>	447.81	363.62	<b>405.71</b>	496.87	455.72	<b>476.29</b>
Mean	<b>278.27</b>	<b>359.82</b>		<b>402.48</b>	<b>427.26</b>		<b>496.00</b>	<b>499.85</b>	

Factors	C.D.	SE(d)	SE(m)
Growth stage (S)	11.01	5.52	3.90
CO <sub>2</sub> level (C)	8.99	4.51	3.19
S × C	15.58	7.81	5.52
Temperature (T)	11.01	5.52	3.90
S × T	19.08	9.57	6.76
T × C	15.58	7.81	5.52
S × T × C	26.99	13.53	9.57

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature

### Effect of elevated temperature and CO<sub>2</sub> on growth parameters at three growth stages of maize

#### Chlorophyll content

At vegetative stage maximum and minimum chlorophyll content was recorded in T0 treatment ( $1.330 \pm 0.03$  mg/g) and T1 treatment ( $1.281 \pm 0.02$  mg/g), respectively. Variation in chlorophyll content at vegetative stage was in the order of **T0 > T1 > T3 > T2 > T4 > T5**. The decrease in chlorophyll content between the period of vegetative stage and tasseling stage, i.e. at tasseling stage was maximum and minimum in T5 treatment (30.66%), and T1 treatment (19.52%), respectively. Variation in chlorophyll content at tasseling stage was in the order of **T1 > T0 > T4**

>**T3** > **T2** > **T5**. The maximum decrease in chlorophyll content from tasseling to dent stage was found in T4 treatment (26.14%), and minimum decrease was found in T5 treatment (16.06%). Variation in chlorophyll content at dent stage was in the order of **T1** > **T0** > **T3** > **T4** > **T2** > **T5** (Table 4.21).

**Table 4.21: Effect of elevated temperature and CO<sub>2</sub> on total chlorophyll content at different growth stages in PEHM 5**

Temperature	Total chlorophyll content (mg/g)								
	Vegetative stage			Tasseling stage			Dent stage		
	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	1.33	1.26	<b>1.29</b>	0.99	0.91	<b>0.95</b>	0.78	0.75	<b>0.77</b>
1.5T	1.28	1.20	<b>1.24</b>	1.03	0.97	<b>1.00</b>	0.79	0.71	<b>0.75</b>
3T	1.25	1.13	<b>1.19</b>	0.87	0.79	<b>0.83</b>	0.69	0.66	<b>0.67</b>
Mean	<b>1.28</b>	<b>1.20</b>		<b>0.96</b>	<b>0.89</b>		<b>0.75</b>	<b>0.71</b>	

Factors	C.D.	SE(d)	SE(m)
Growth stage (S)	0.036	0.018	0.013
CO <sub>2</sub> level (C)	0.03	0.015	0.011
S × C	NS	0.026	0.018
Temperature (T)	0.036	0.018	0.013
S × T	NS	0.032	0.022
T × C	NS	0.026	0.018
S × T × C	NS	0.045	0.032

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature

### Relative leaf water content (RLWC)

At vegetative stage maximum and minimum RLWC was recorded in T0 treatment (22.80 ± 1.01 %) and T4 treatment (12.60 ± 1.42 %), respectively. Variation in RLWC at vegetative stage was in the order of **T0** > **T2** > **T3** > **T1** > **T5** > **T4**.

RLWC first increased from vegetative to tasseling and then decreased to some extent. The increase in RLWC between the period of vegetative stage and tasseling stage, i.e. at tasseling stage was maximum and minimum in T4 treatment (311.06%), and T2 treatment (56.57%), respectively. Variation in chlorophyll content at tasseling stage was in the order of **T0 > T4 > T1 > T5 > T3 > T2**.

The maximum decrease in RLWC from tasseling to dent stage was found in T0 treatment (15.36 %), and minimum decrease was found in T3 treatment (3.23 %). Variation in chlorophyll content at dent stage was in the order of **T4 > T0 > T1 > T5 > T3 > T2** (Table 4.22).

**Table 4.22: Effect of elevated temperature and CO<sub>2</sub> on RLWC at different growth stages in PEHM 5**

Relative leaf water content (%)									
	Vegetative stage			Tasseling stage			Dent stage		
Temperature	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	22.80	18.25	<b>20.52</b>	53.86	39.85	<b>46.86</b>	45.592	38.566	<b>42.07</b>
1.5T	13.30	12.60	<b>12.95</b>	50.51	51.80	<b>51.10</b>	44.892	47.444	<b>46.16</b>
3T	21.51	13.11	<b>17.31</b>	33.69	41.27	<b>37.48</b>	32.380	38.862	<b>35.62</b>
Mean	<b>19.20</b>	<b>14.65</b>		<b>46.02</b>	<b>44.31</b>		<b>40.95</b>	<b>41.62</b>	

Factors	C.D.	SE(d)	SE(m)
Growth stage (S)	1.812	0.909	0.643
CO <sub>2</sub> level (C)	1.48	0.742	0.525
S × C	2.563	1.285	0.909
Temperature (T)	1.812	0.909	0.643
S × T	3.139	1.574	1.113
T × C	2.563	1.285	0.909
S × T × C	4.439	2.226	1.574

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature

### Leaf extract pH

At vegetative stage maximum and minimum leaf extract pH was recorded in T3 treatment ( $7.02 \pm 0.11$ ) and T1 treatment ( $6.21 \pm 0.30$ ), respectively. Variation in leaf extract pH at vegetative stage was in the order of **T3 > T5 > T4 > T0 > T2 > T1**. Leaf extract pH was almost decreased from vegetative to tasseling and then dent stage. Variation in leaf extract pH at tasseling stage was in the order of **T3 > T5 > T4 > T2 > T0 > T1**. From tasseling to dent stage there was slight increase in the pH of T0 and T2 treatment and decrease in all other treatments. The maximum decrease in pH from tasseling to dent stage was found in T5 treatment (4.97 %), and minimum decrease was found in T3 treatment (1.25 %). The increase in T0 and T2 treatment was 0.60 % and 0.78 %, respectively. Variation in pH at dent stage was in the order of **T3 > T2 > T4 > T5 > T0 > T1** (Table 4.23).

**Table 4.23: Effect of elevated temperature and CO<sub>2</sub> on leaf extract pH at different growth stages in PEHM 5**

Temperature	Leaf extract pH								
	Vegetative stage			Tasseling stage			Dent stage		
	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	6.22	7.02	<b>6.62</b>	5.90	6.67	<b>6.29</b>	5.94	6.59	<b>6.26</b>
1.5T	6.21	6.33	<b>6.27</b>	5.81	6.25	<b>6.03</b>	5.59	6.08	<b>5.84</b>
3T	6.22	6.48	<b>6.35</b>	6.10	6.30	<b>6.20</b>	6.15	5.99	<b>6.07</b>
Mean	<b>6.22</b>	<b>6.61</b>		<b>5.94</b>	<b>6.41</b>		<b>5.89</b>	<b>6.22</b>	

Factors	C.D.	SE(d)	SE(m)
Growth stage (S)	0.21	0.10	0.07
CO <sub>2</sub> level (C)	0.17	0.08	0.06
S × C	NS	0.15	0.10
Temperature (T)	0.21	0.10	0.07
S × T	NS	0.18	0.13
T × C	0.30	0.15	0.10
S × T × C	NS	0.26	0.18

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature

### Ascorbic acid content

At vegetative stage maximum and minimum ascorbic acid content was recorded in T3 treatment ( $3.07 \pm 0.29$  mg/g) and T2 treatment ( $2.11 \pm 0.13$  mg/g), respectively. Variation in ascorbic acid content at vegetative stage was in the order of **T3 > T0 > T4 > T5 > T2 > T1**. Ascorbic acid content increased 13-14% from vegetative to tasseling stage in all the treatments.

Variation in ascorbic acid content at tasseling and dent stage was in the same order as of vegetative stage i.e., **T3 > T0 > T5 > T4 > T1 > T2**. From tasseling to dent stage there was 9-10% decrease in ascorbic acid content in T0, T3 and T5 treatments and 5-7% increase in T1, T2, and T4 treatments (Table 4.24).

**Table 4.24: Effect of elevated temperature and CO<sub>2</sub> on ascorbic acid content at different growth stages in PEHM 5**

Temperature	Ascorbic acid (mg/g)								
	Vegetative stage			Tasseling stage			Dent stage		
	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	2.89	3.07	<b>2.98</b>	3.95	4.30	<b>4.12</b>	3.79	4.12	<b>3.95</b>
1.5T	2.12	2.62	<b>2.37</b>	3.10	3.33	<b>3.21</b>	3.43	3.75	<b>3.59</b>
3T	2.11	2.54	<b>2.32</b>	3.03	3.82	<b>3.42</b>	3.35	3.60	<b>3.47</b>
Mean	<b>2.37</b>	<b>2.74</b>		<b>3.36</b>	<b>3.81</b>		<b>3.52</b>	<b>3.82</b>	

Factors	C.D.	SE(d)	SE(m)
Growth stage (S)	0.29	0.14	0.10
CO <sub>2</sub> level (C)	0.22	0.11	0.08
S × C	0.14	0.20	0.14
Temperature (T)	NS	0.14	0.10
S × T	0.50	0.25	0.17
T × C	NS	0.20	0.14
S × T × C	0.71	0.35	0.25

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature

### Total phenol content

At vegetative stage maximum and minimum total phenol content was found in T3 treatment ( $53.81 \pm 1.01$  mg/g) and T2 treatment ( $39.30 \pm 1.78$  mg/g), respectively. Variation in total phenol content at vegetative stage was found in the order of **T3 > T0 > T4 > T5 > T1 > T2**.

On an average total phenol content increased from vegetative to tasseling stage in plants under all treatments. The increase in total phenol content between the period of vegetative stage and tasseling stage, i.e. at tasseling stage was maximum and minimum in T0 treatment (18.1%), and T1 treatment (1.1%), respectively. Variation in total phenol content at tasseling stage was in the order of **T3 > T0 > T4 > T5 > T1 > T2**. From tasseling to dent stage there was slight decrease in the total phenol content of T0 (6.3%), T3 (2.2%) and T4 (0.7%) treatment and it was found to increase in all other treatments. Increase in T1, T2 and T5 was 8.3%, 3.5%, and 6.6%, respectively from tasseling to dent stage. Variation in total phenol content at dent stage was in the order of **T3 > T0 > T4 > T5 > T1 > T2** (Table 4.25).

### Lipid peroxidation

Lipid peroxidation was assessed by estimating **Malondialdehyde** (MDA) content which reflects the lipid peroxidation of plant samples. At vegetative stage maximum and minimum Malondialdehyde content was found in T2 treatment ( $4.94 \pm 0.05$  nmol/g) and T3 treatment ( $2.39 \pm 0.13$  nmol/g), respectively. Variation in Malondialdehyde content at vegetative stage was found in the order of **T2 > T1 > T5 > T4 > T0 > T3**.

Malondialdehyde content decreased from vegetative to tasseling in T0, T2 and T5 treatment and increased in T1, T3 and T4 treatment. Decrease in T0, T2 and T3 treatment was 5.1%, 8.1% and 22%, respectively and increased in T1, T3 and T4 treatment was 2.2%, 4% and 5.4%, respectively. Variation in Malondialdehyde content at tasseling stage was in the order of **T2 > T1 > T5 > T4 > T0 > T3**.

From tasseling to dent stage there was decrease in the Malondialdehyde content in T0, T1, T2 and T5 treatment (11%, 9%, 8% and 3%, respectively) and it was found to increase in T3 and T4 treatment (13.7% and 5.1%, respectively). Variation in Malondialdehyde content at dent stage was in the order of **T2 > T1 > T5 > T4 > T0 > T3** (Table 4.26).

**Table 4.25: Effect of elevated temperature and CO<sub>2</sub> on total phenol content at different growth stages in PEHM 5**

Total phenol content (mg/100g)									
	Vegetative stage			Tasseling stage			Dent stage		
Temperature	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	45.4	55.8	<b>50.6</b>	55.5	58.1	<b>56.8</b>	54.4	56.8	<b>55.6</b>
1.5T	44.6	43.5	<b>44.1</b>	45.2	53.3	<b>49.3</b>	48.8	53.0	<b>50.9</b>
3T	39.6	46.9	<b>43.2</b>	41.2	46.2	<b>43.7</b>	42.7	49.5	<b>46.1</b>
Mean	<b>43.2</b>	<b>48.7</b>		<b>47.3</b>	<b>52.5</b>		<b>48.6</b>	<b>53.1</b>	

Factors	C.D.	SE(d)	SE(m)
Growth stage (S)	1.94	0.97	0.69
CO <sub>2</sub> level (C)	1.58	0.79	0.56
S × C	2.75	1.37	0.97
Temperature (T)	1.94	0.97	0.69
S × T	3.36	1.68	1.19
T × C	2.75	1.37	0.97
S × T × C	NS	2.38	1.68

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature

**Table 4.26: Effect of elevated temperature and CO<sub>2</sub> on MDA content at different growth stages in PEHM 5**

Malondialdehyde content (nmol/g)									
	Vegetative stage			Tasseling stage			Dent stage		
Temperature	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	3.8	2.4	<b>3.1</b>	3.6	2.5	<b>3.1</b>	3.2	2.9	<b>3.1</b>
1.5T	4.3	3.5	<b>3.9</b>	4.4	3.7	<b>4.1</b>	4.0	3.9	<b>4.0</b>
3T	4.9	4.5	<b>4.7</b>	4.5	3.5	<b>4.0</b>	4.1	3.4	<b>3.8</b>
Mean	<b>4.3</b>	<b>3.4</b>		<b>4.2</b>	<b>3.2</b>		<b>3.8</b>	<b>3.4</b>	

Factors	C.D.	SE(d)	SE(m)
Growth stage (S)	0.19	0.09	0.06
CO <sub>2</sub> level (C)	0.15	0.07	0.05
S × C	0.26	0.13	0.09
Temperature (T)	0.19	0.09	0.06
S × T	NS	0.16	0.11
T × C	0.26	0.13	0.09
S × T × C	0.46	0.23	0.16

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature

### Effect of elevated temperature and CO<sub>2</sub> on physiological parameters at three growth stages of maize

#### Photosynthetic rate

Both at ambient and elevated CO<sub>2</sub> level from vegetative to tasseling, and from tasseling to dent stage photosynthetic rate was found to decrease. At vegetative stage maximum photosynthetic rate was found in T4 treatment and minimum photosynthetic rate was found in T5 treatment. Similarly, at tasseling stage maximum and minimum photosynthetic rate was found in T0 and T5 treatment, respectively. At dent stage, maximum and minimum photosynthetic rate was observed in T3 and T4 treatment, respectively. Effect of temperature was significant on photosynthetic

rate but effect due to interaction between temperature and CO<sub>2</sub> was not significant (Table 4.27).

**Table 4.27: Effect of elevated temperature and CO<sub>2</sub> on photosynthetic rate at different growth stages in PEHM 5**

Photosynthetic rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )									
	Vegetative stage			Tasseling stage			Dent stage		
Temperature	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	30.45	30.50	<b>30.47</b>	32.50	20.10	<b>26.30</b>	13.80	19.60	<b>16.7</b>
1.5T	28.77	32.10	<b>30.43</b>	30.80	26.80	<b>28.80</b>	6.40	6.30	<b>6.35</b>
3T	31.05	27.10	<b>29.07</b>	19.40	11.20	<b>15.30</b>	5.70	10.40	<b>8.05</b>
Mean	<b>30.09</b>	<b>29.90</b>		<b>27.56</b>	<b>19.36</b>		<b>8.63</b>	<b>12.1</b>	

Factors	C.D.	SE(d)	SE(m)
Growth stage (S)	3.761	1.886	1.334
CO <sub>2</sub> level (C)	NS	1.54	1.089
S × C	NS	2.667	1.886
Temperature (T)	3.761	1.886	1.334
S × T	NS	3.267	2.31
T × C	NS	2.667	1.886
S × T × C	NS	4.62	3.267

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature

### Stomatal conductance

At all the three stages stomatal conductance was found to decrease both by increasing temperature and CO<sub>2</sub> level. At all the stages maximum stomatal conductance was found in T1 treatment and minimum was found in T5 treatment. The effect of temperature and CO<sub>2</sub> was significant, but their interaction was not significant (table 4.28).

**Table 4.28: Effect of elevated temperature and CO<sub>2</sub> on stomatal conductance at different growth stages in PEHM 5**

Stomatal conductance (mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )									
	Vegetative stage			Tasseling stage			Dent stage		
Temperature	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	0.290	0.167	<b>0.229</b>	0.181	0.161	<b>0.171</b>	0.073	0.053	<b>0.063</b>
1.5T	0.198	0.189	<b>0.194</b>	0.121	0.133	<b>0.127</b>	0.023	0.030	<b>0.027</b>
3T	0.158	0.118	<b>0.138</b>	0.910	0.063	<b>0.487</b>	0.017	0.024	<b>0.021</b>
Mean	<b>0.215</b>	<b>0.158</b>		<b>0.404</b>	<b>0.119</b>		<b>0.038</b>	<b>0.036</b>	

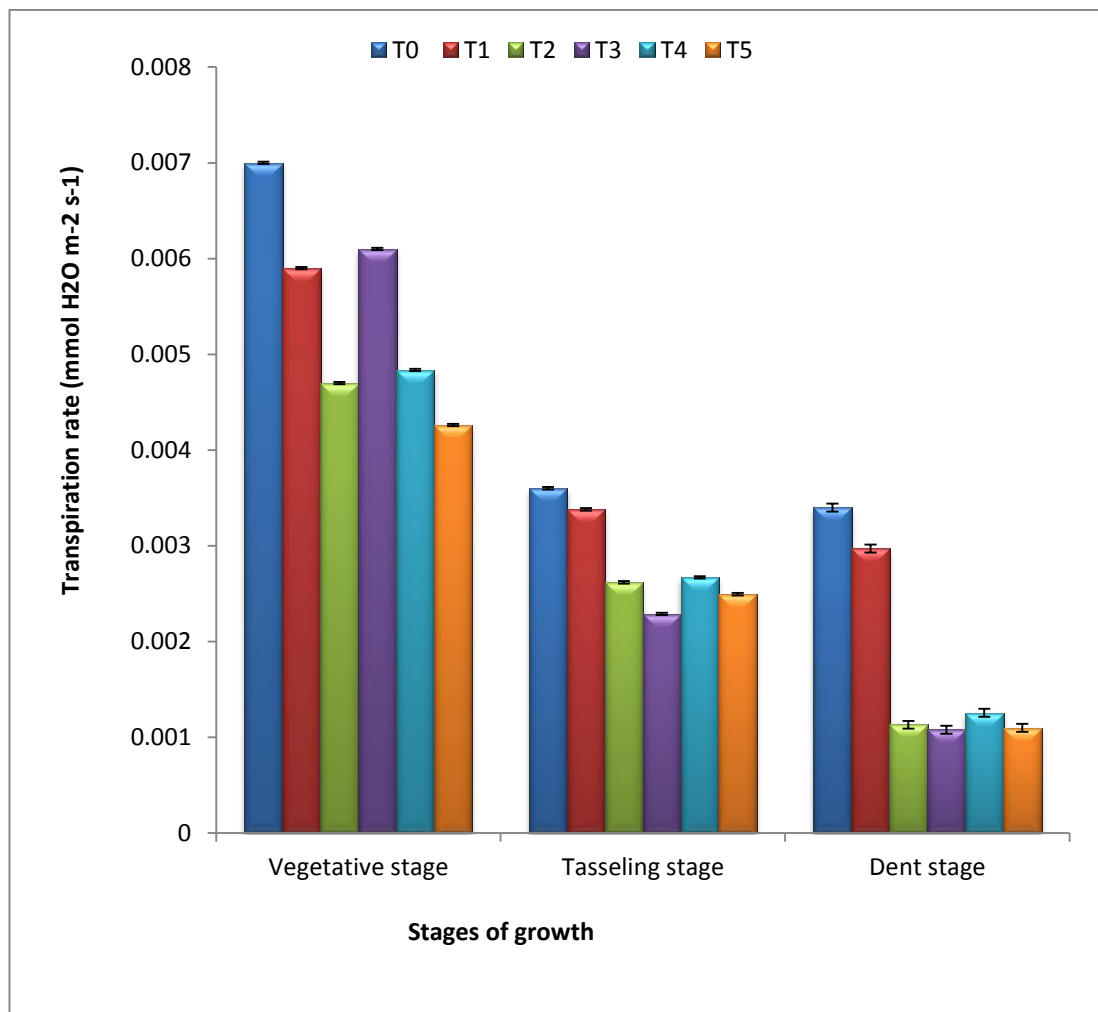
  

Factors	C.D.	SE(d)	SE(m)
Growth stage (S)	0.013	0.006	0.005
CO <sub>2</sub> level (C)	0.011	0.005	0.004
S × C	0.018	0.009	0.006
Temperature (T)	0.013	0.006	0.005
S × T	0.022	0.011	0.008
T × C	NS	0.009	0.006
S × T × C	0.031	0.016	0.011

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature

### Transpiration rate

Result shows that transpiration rate was found to decrease substantially from vegetative to tasseling, and then from tasseling to dent stages. At all the stages transpiration rate was found to be highest in T0 treatment. At vegetative, tasseling and dent stages rate of transpiration was lowest in T5, T3 and T3, respectively. The difference due to temperature and CO<sub>2</sub> and their interaction was significant (Figure 4.8).



**Figure 4.8: Effect of elevated temperature and CO<sub>2</sub> on transpiration of PEHM 5**

T0, Ambient CO<sub>2</sub> level + Ambient temperature level; T1, Ambient CO<sub>2</sub> level + 1.5°C elevated temperature than ambient level; T2, Ambient CO<sub>2</sub> level + 3°C elevated temperature than ambient level; T3, 550 ppm CO<sub>2</sub> level + Ambient temperature level; T4, 550 ppm CO<sub>2</sub> level + 1.5°C elevated temperature than ambient level; T5, 550 ppm CO<sub>2</sub> level + 1.5°C elevated temperature than ambient level

## **Principal component analysis response of growth, physiological, and biochemical parameters at different growth stages of maizes under elevated temperature and CO<sub>2</sub>**

Principal component analysis was done to determine the contribution of growth, biochemical and physiological parameters of different growth stages towards response and tolerance of maize to maydis leaf blight disease under elevated temperature and CO<sub>2</sub>.

### **Vegetative stage**

After subjected to PCA, the amount of variability explained by PC1, PC2, PC3, PC4, and PC5 was 34.68%, 18.79%, 15.04%, 8.51% and 6.20%, respectively. However, the cumulative variance was 83.24% (Table 4.29). In PC1 two variables quantified for the next step were leaf area and stomatal conductance. These two variables were found well correlated ( $r > 0.70$ ) in the intercorrelation study (Table 4.30). So, only leaf area was retained for MDS as it was having the higher factor loading (0.926) as compared to stomatal conductance. Likewise, in PC2 total phenol content and MDA content were quantified for the next step. These two variables were not well correlated ( $r < 0.70$ ) in the intercorrelation study, so both the variables were retained for MDS. Similarly, in PC3, PC4 and PC5, RLWC, ascorbic acid content and photosynthetic rate was retained, respectively for MDS. After that average linear scores under each treatment were computed and have been depicted in Figure 4.9. Considering the average linear scores the order of importance of key parameters in influencing crop health for PEHM 5 was total leaf area (0.72) > total phenol content (0.70) > relative leaf water content (0.62) > photosynthetic rate (0.58) > ascorbic acid content (0.57) > MDA content (0.50), with a corresponding contribution of 19.51%, 18.92%, 16.77%, 15.71%, 15.52% and 13.53%, respectively.

**Table 4.29: Performance of crop health indicators in terms of factor loading / eigen vector values in principal component analysis (for PEHM 5 at vegetative stage)**

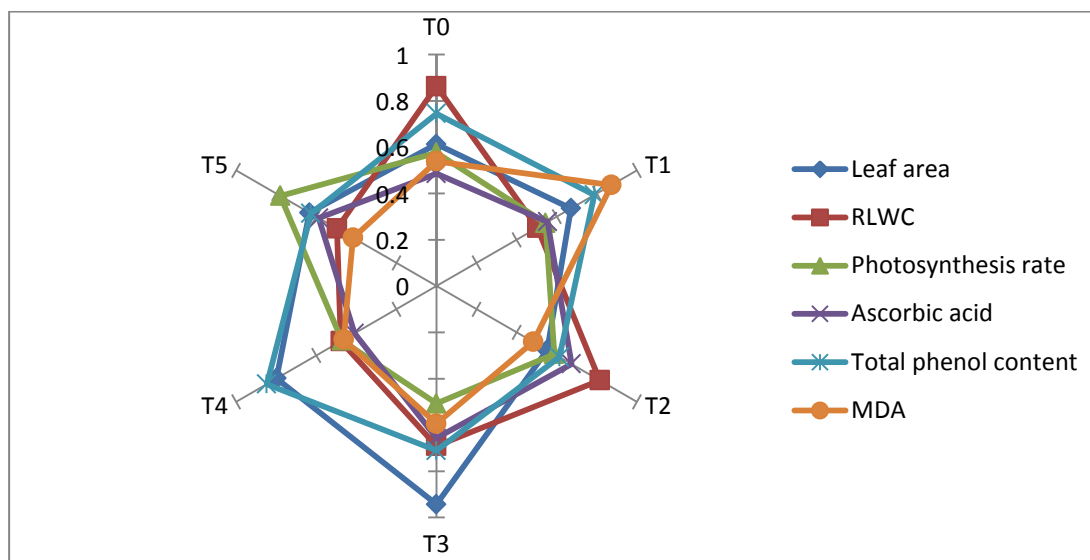
PCs	PC1	PC2	PC3	PC4	PC5
Eigen value	4.162	2.256	1.805	1.022	0.745
% Variance	34.687	18.796	15.042	8.518	6.205
Cumulative %	34.687	53.483	68.526	77.044	83.249
Factor loading/eigen vector					
Plant height	0.727	0.313	- 0.259	- 0.200	- 0.166
Stem girth	0.777	- 0.394	0.042	- 0.126	- 0.045
Leaf area	<b>0.926</b>	0.036	- 0.206	- 0.095	- 0.052
Chlorophyll content	0.109	- 0.550	0.632	- 0.041	0.140
RLWC	- 0.085	0.083	<b>0.750</b>	0.503	0.042
Photosynthetic Rate	- 0.368	0.549	- 0.154	- 0.203	<b>0.547</b>
Stomatal conductance	<b>0.845</b>	0.234	0.295	0.243	0.090
Transpiration rate	0.826	0.410	0.116	0.203	- 0.065
Leaf extract pH	0.629	0.378	0.064	- 0.055	0.409
Ascorbic acid content	0.009	- 0.293	- 0.623	<b>0.617</b>	0.307
Total phenol content	- 0.383	<b>0.715</b>	0.411	- 0.177	- 0.039
MDA content	- 0.282	<b>0.653</b>	- 0.218	0.384	- 0.340

\*Bold values indicate the eigen vectors within 10% of the highest factor loadings

**Table 4.30: Inter-correlations between highly weighted variables under different principal components**

PC 1 variables	Leaf area	Stomatal conductance
Leaf area	1	0.708**
Stomatal conductance	0.708**	1
PC 2 variables	Total phenol content	MDA content
Total phenol content	1	0.378*
MDA content	0.378*	1

\*\* $P < 0.01$ , \*  $P < 0.05$



**Figure 4.9: Radar graphs for PEHM 5 at vegetative stage depicting the average linear score of key indicators which were found important under different treatments**

T0, Ambient CO<sub>2</sub> level + Ambient temperature level; T1, Ambient CO<sub>2</sub> level + 1.5°C elevated temperature than ambient level; T2, Ambient CO<sub>2</sub> level + 3°C elevated temperature than ambient level; T3, 550 ppm CO<sub>2</sub> level + Ambient temperature level; T4, 550 ppm CO<sub>2</sub> level + 1.5°C elevated temperature than ambient level; T5, 550 ppm CO<sub>2</sub> level + 1.5°C elevated temperature than ambient level

### Tasseling stage

At tasseling stage key parameters identified through PCA were stem girth, leaf area, stomatal conductance, transpiration rate, leaf extract pH and total phenol content.

Order of importance of key parameters in influencing crop health for PEHM 5 was stem girth (0.83) > leaf extract pH (0.81) > leaf area (0.78) > total phenol content (0.69) > stomatal conductance (0.58) > transpiration rate (0.48), with a corresponding contribution of 19.99%, 19.40%, 18.64%, 16.57%, 13.88% and 11.48%, respectively.

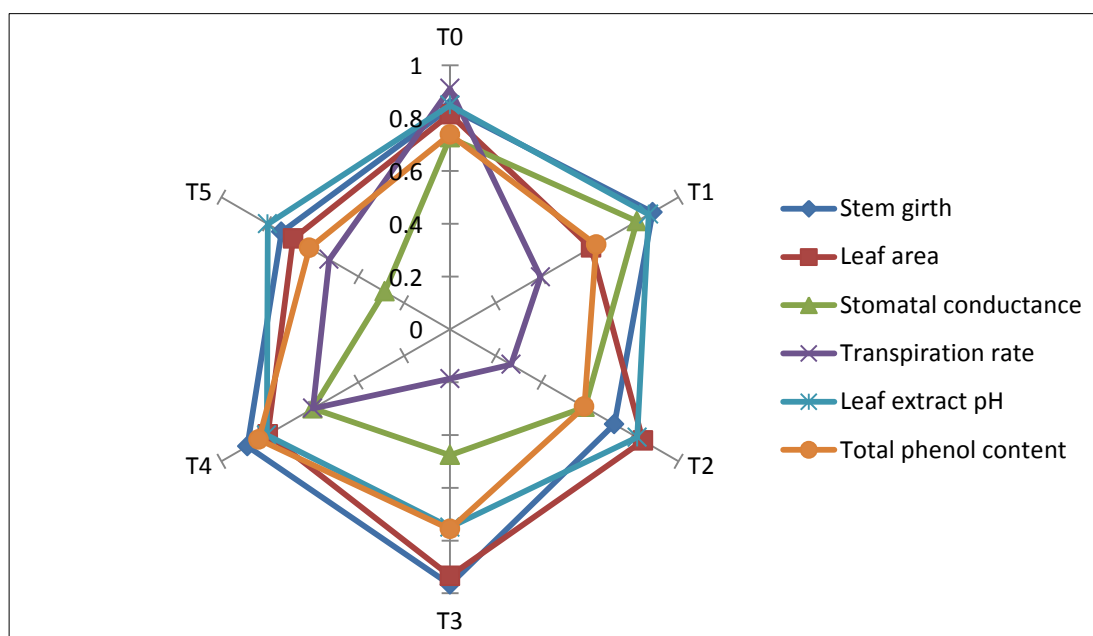
**Table 4.31: Performance of crop health indicators in terms of factor loading /eigen vector values in principal component analysis (at tasseling stage)**

PCs	PC1	PC2	PC3	PC4	PC5	PC6
Eigen value	3.567	2.539	1.527	1.038	0.917	0.717
% Variance	29.728	21.157	12.726	8.647	7.641	5.974
Cumulative %	29.728	50.885	63.611	72.258	79.899	85.873
Factor loading/eigen vector						
Plant height	<b>0.780</b>	-0.088	-0.194	0.167	0.323	0.157
Stem girth	<b>0.844</b>	0.292	-0.101	0.025	0.038	<b>0.268</b>
Leaf area	0.268	-0.072	-0.055	<b>0.910</b>	0.098	-0.181
Chlorophyll content	0.144	<b>0.825</b>	0.236	-0.106	0.083	0.070
RLWC	0.337	0.431	0.628	-0.328	-0.260	0.208
Photosynthetic rate	-0.115	-0.036	0.032	-0.020	0.056	0.057
Stomatal conductance	-0.043	<b>0.892</b>	0.047	0.044	-0.251	0.148
Transpiration rate	-0.174	0.074	<b>0.910</b>	0.020	0.000	0.156
Leaf extract pH	0.205	-0.153	-0.038	0.117	<b>0.928</b>	-0.097
Ascorbic acid content	-0.186	-0.183	-0.204	0.184	0.104	-0.897
Total phenol content	-0.743	-0.100	-0.522	-0.145	-0.068	<b>0.247</b>
MDA content	-0.324	-0.631	0.118	0.507	0.170	0.015

\*Bold values indicate the eigen vectors within 10% of the highest factor loading

**Table 4.32: Inter-correlations between highly weighted variables under different PCs**

PC 1 variables	Plant height	Stem girth
Plant height	1	0.660**
Stem girth	0.660**	1
PC 2 variables	Chlorophyll content	Stomatal conductance
Chlorophyll content	1	0.604**
Stomatal conductance	0.604**	1
PC 6 variables	Stem girth	Total phenol content
Stem girth	1	-0.493**
Total phenol content	-0.493**	1

\*\* $P < 0.01$ 

**Figure 4.10: Radar graphs for PEHM 5 at tasseling stage depicting the average linear score of key indicators which were found important under different treatments**

T0, Ambient CO<sub>2</sub> level + Ambient temperature level; T1, Ambient CO<sub>2</sub> level + 1.5°C elevated temperature than ambient level; T2, Ambient CO<sub>2</sub> level + 3°C elevated temperature than ambient level; T3, 550 ppm CO<sub>2</sub> level + Ambient temperature level; T4, 550 ppm CO<sub>2</sub> level + 1.5°C elevated temperature than ambient level; T5, 550 ppm CO<sub>2</sub> level + 1.5°C elevated temperature than ambient level

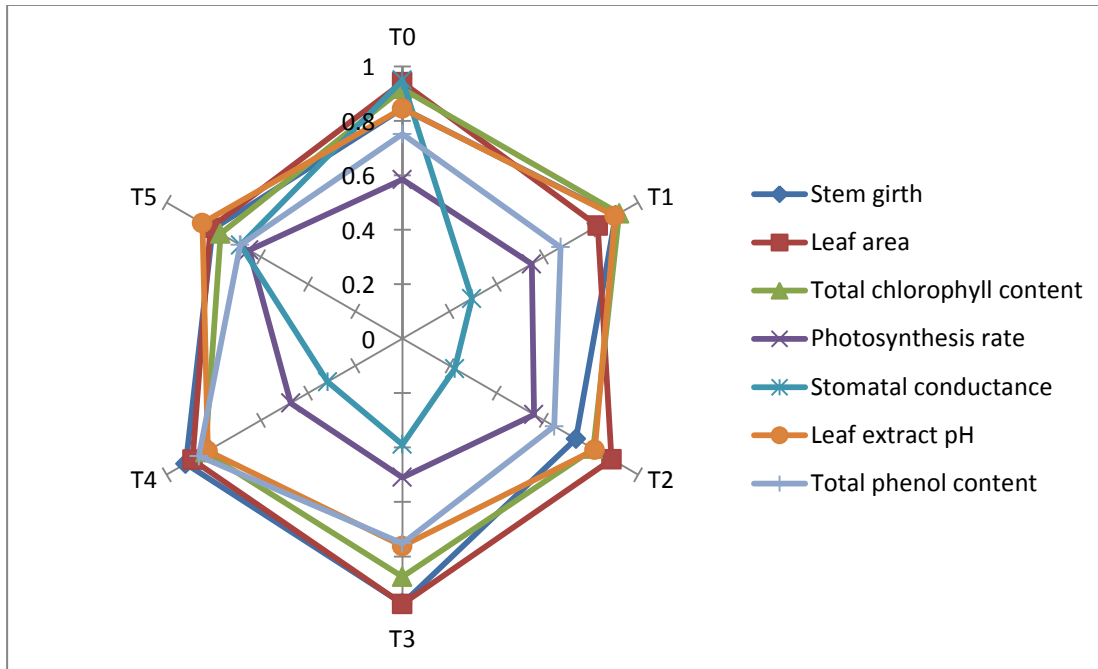
### Dent stage

At dent stage key parameters identified were stem girth, leaf area, stomatal conductance, leaf extract pH, total chlorophyll content total phenol content. Order of importance of key parameters was stem girth (0.83) > leaf extract pH (0.81) > leaf area (0.78) > total phenol content (0.69) > stomatal conductance (0.58) > transpiration rate (0.48), with a corresponding contribution of 19.99%, 19.40%, 18.64%, 16.57%, 13.88% and 11.48%, respectively.

**Table 4.33: Performance of crop health indicators in terms of factor loading /eigen vector values in principal component analysis (Dent stage)**

PCs	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigen value	3.253	2.409	1.520	1.078	0.952	0.810	0.741
% Variance	27.111	20.072	12.664	8.986	7.930	6.754	6.171
Cumulative %	27.111	47.183	59.846	68.833	76.762	83.517	89.688
	Factor loading/eigen vector						
Plant height	0.516	0.008	0.635	-0.315	0.291	0.174	0.120
Stem girth	<b>0.772</b>	-0.010	0.406	-0.239	0.236	0.001	-0.004
Leaf area	-0.028	0.050	<b>0.937</b>	0.006	0.033	0.086	-0.140
Chlorophyll content	0.263	0.370	0.051	0.420	<b>0.611</b>	0.152	-0.152
RLWC	0.567	0.610	0.070	-0.224	-0.014	-0.413	-0.076
Photosynthetic rate	-0.089	0.161	-0.096	0.148	-0.049	-0.010	<b>0.935</b>
Stomatal conductance	-0.227	<b>0.883</b>	0.115	-0.017	0.110	0.080	0.149
Transpiration rate	-0.181	0.637	-0.253	0.435	0.220	-0.253	0.275
Leaf extract pH	0.024	-0.033	0.135	-0.105	-0.078	<b>0.964</b>	-0.019
Ascorbic acid content	-0.055	-0.060	-0.109	0.246	-0.909	0.154	-0.002
Total phenol content	-0.133	-0.009	-0.069	<b>0.888</b>	-0.152	-0.093	0.166
MDA content	-0.861	0.285	0.161	-0.061	0.000	-0.045	0.148

\*Bold values indicate the eigen vectors within 10% of the highest factor loadings

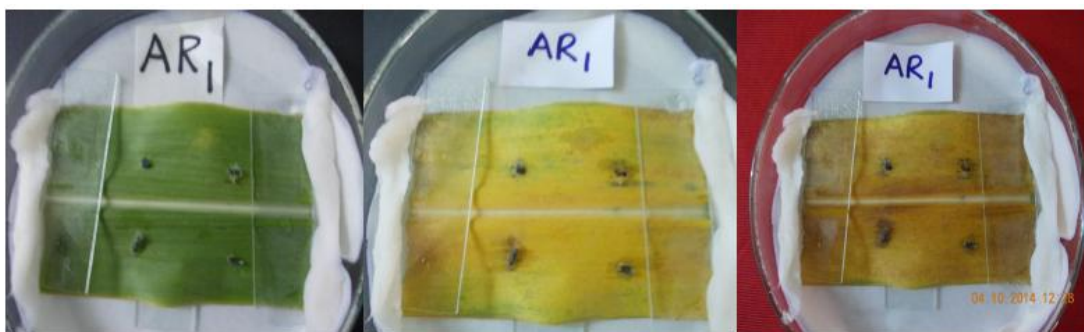


**Figure 4.11: Radar graphs for PEHM 5 at dent stage depicting the average linear score of key indicators which were found important under different treatments**

T0, Ambient CO<sub>2</sub> level + Ambient temperature level; T1, Ambient CO<sub>2</sub> level + 1.5°C elevated temperature than ambient level; T2, Ambient CO<sub>2</sub> level + 3°C elevated temperature than ambient level; T3, 550 ppm CO<sub>2</sub> level + Ambient temperature level; T4, 550 ppm CO<sub>2</sub> level + 1.5°C elevated temperature than ambient level; T5, 550 ppm CO<sub>2</sub> level + 1.5°C elevated temperature than ambient level

### Maydis leaf blight (MLB) Disease tolerance potential in maize

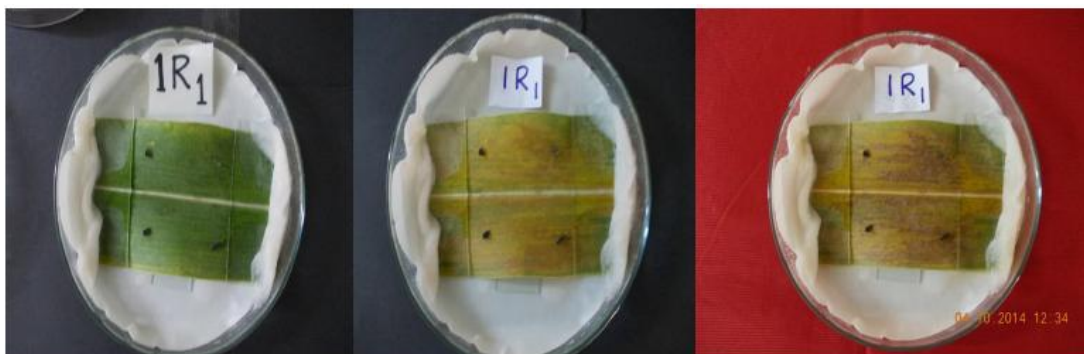
MLB disease tolerance potential of maize under elevated temperature and CO<sub>2</sub> was assessed at vegetative, tasseling and dent growth stages under controlled lab condition through detached leaf method. Under lab condition healthy leaves from plants of different growth stages grown under different treatments were inoculated with MLB pathogen and observed for disease progress. Observations taken after 48, 72 and 96 h of inoculation showed that the infection was in the order of **T2 > T1 > T5 > T4 > T0 > T3**.



Day 2 (after 48 h)

Day 3 (after 72 h)

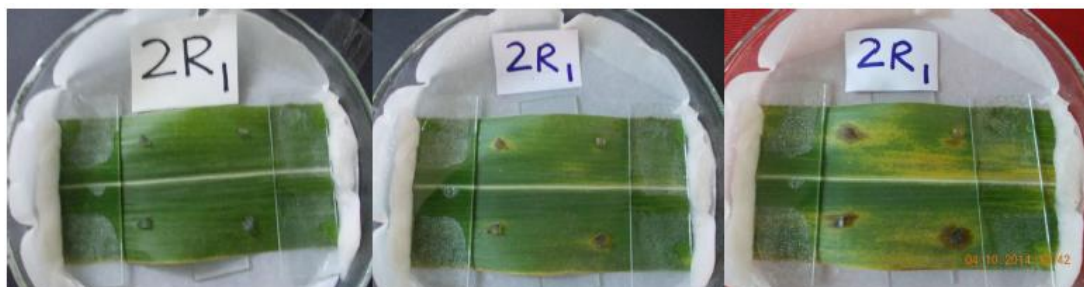
Day 4 (after 96 h)



Day 2 (after 48 h)

Day 3 (after 72 h)

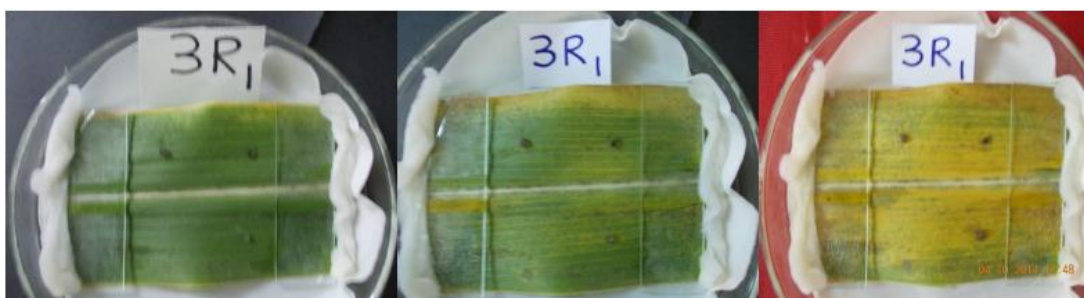
Day 4 (after 96 h)



Day 2 (after 48 h)

Day 3 (after 72 h)

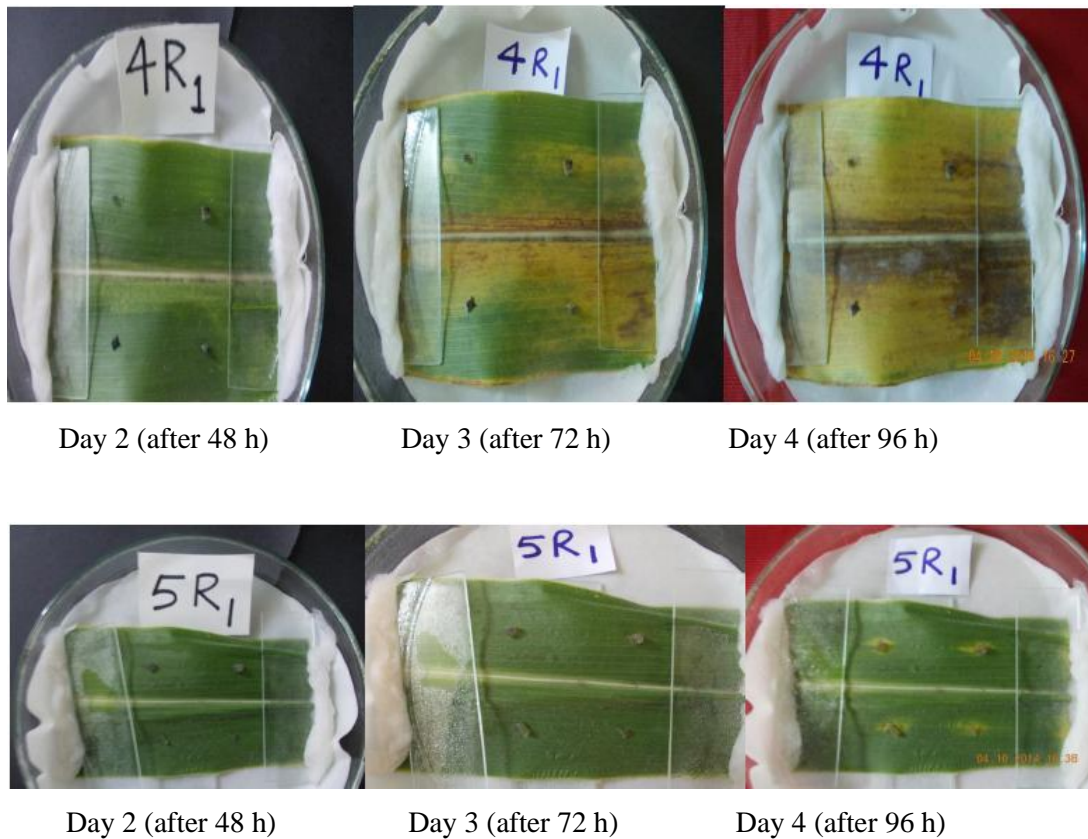
Day 4 (after 96 h)



Day 2 (after 48 h)

Day 3 (after 72 h)

Day 4 (after 96 h)



**Plate 4.2: MLB disease development in leaves of vegetative stage plants under different treatments inoculated with MLB pathogen after 48, 72 and 96 hours of inoculation**

A, Ambient temperature and carbon dioxide level; R<sub>1</sub>, Leaf sample of vegetative growth stages PEHM 5 plant; 1, 550ppm elevated CO<sub>2</sub> than ambient level + 1.5<sup>0</sup>C elevated temperature than ambient level; 2, Ambient CO<sub>2</sub> level + 1.5<sup>0</sup>C elevated temperature than ambient level; 3, 550ppm elevated CO<sub>2</sub> than ambient level + 3<sup>0</sup>C elevated temperature than ambient level; 4, 550ppm elevated CO<sub>2</sub> than ambient level + Ambient temperature; 5, Ambient CO<sub>2</sub> level + 3<sup>0</sup>C elevated temperature than ambient level

On tasseling and dent stage leaves of PEHM 5 from plants under different treatments, establishment of MLB pathogen was not successful. Therefore tolerance potential of tasseling and dent stage to disease was assessed through correlation with content of biochemical defence compounds i.e., total phenol, MDA and ascorbic acid content (Table 4.35). Disease severity observed under different treatment at vegetative stage in PEHM 5 was correlated with the, total phenol, MDA and ascorbic acid content. It was observed that phenol and ascorbic acid content were negatively correlated and MDA content was positively correlated with disease severity (Figure 4.12, 4.13 and 4.14).

**Table 4.34: Effect of elevated temperature and CO<sub>2</sub> on MLB disease development on vegetative growth stage in PEHM 5**

Percentage disease infection									
Temperature	48 h after inoculation			72 h after inoculation			96 h after inoculation		
	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean	ACO <sub>2</sub>	ECO <sub>2</sub>	Mean
AT	6.22	7.42	<b>6.82</b>	21.37	25.02	<b>23.20</b>	92.56	85.05	<b>88.81</b>
1.5T	7.11	6.29	<b>6.70</b>	23.93	28.58	<b>26.26</b>	89.25	87.47	<b>88.36</b>
3T	5.77	6.19	<b>5.98</b>	23.17	24.59	<b>23.88</b>	83.98	83.47	<b>83.72</b>
Mean	<b>6.36</b>	<b>6.63</b>		<b>22.82</b>	<b>26.06</b>		<b>88.60</b>	<b>85.33</b>	

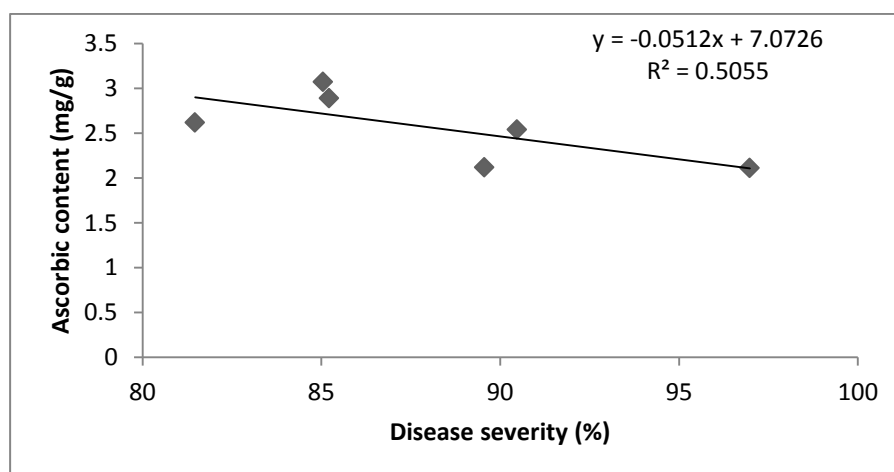
Factors	C.D.	SE(d)	SE(m)
Period of observation (P)	0.007	0.004	0.003
CO <sub>2</sub> level (C)	0.006	0.003	0.002
P × C	0.011	0.005	0.004
Temperature level (T)	0.007	0.004	0.003
P × T	0.013	0.006	0.005
T × C	0.011	0.005	0.004
P × T × C	0.018	0.009	0.006

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature

**Table 4.35: Effect of elevated temperature and CO<sub>2</sub> on defense compounds content in PEHM 5 at different growth stages**

Treatment	Vegetative stage			Tasseling stage			Dent stage		
	*AA	TP	MDA	AA	TP	MDA	AA	TP	MDA
T0	2.89	45.4	3.8	3.95	55.5	3.6	3.79	54.4	4.04
T1	2.12	44.6	4.3	3.10	45.2	4.4	3.43	48.8	2.92
T2	2.11	39.6	4.9	3.03	41.2	4.5	3.35	42.7	3.86
T3	3.07	55.8	2.4	4.30	58.1	2.5	4.12	56.8	3.18
T4	2.62	43.5	3.5	3.33	53.3	3.7	3.75	53.0	3.35
T5	2.54	46.9	4.7	3.82	46.2	3.5	3.60	49.5	4.05
<b>Average</b>	<b>2.56</b>	<b>45.9</b>	<b>3.9</b>	<b>3.58</b>	<b>49.9</b>	<b>3.8</b>	<b>3.70</b>	<b>50.9</b>	<b>3.6</b>

\*AA, Ascorbic acid content (mg/g); TP, Total phenol content (mg/100g); MDA, Malondialdehyde content (nmol/g)



**Figure 4.12: Corelation between disease severity and ascorbic acid content of PEHM 5**

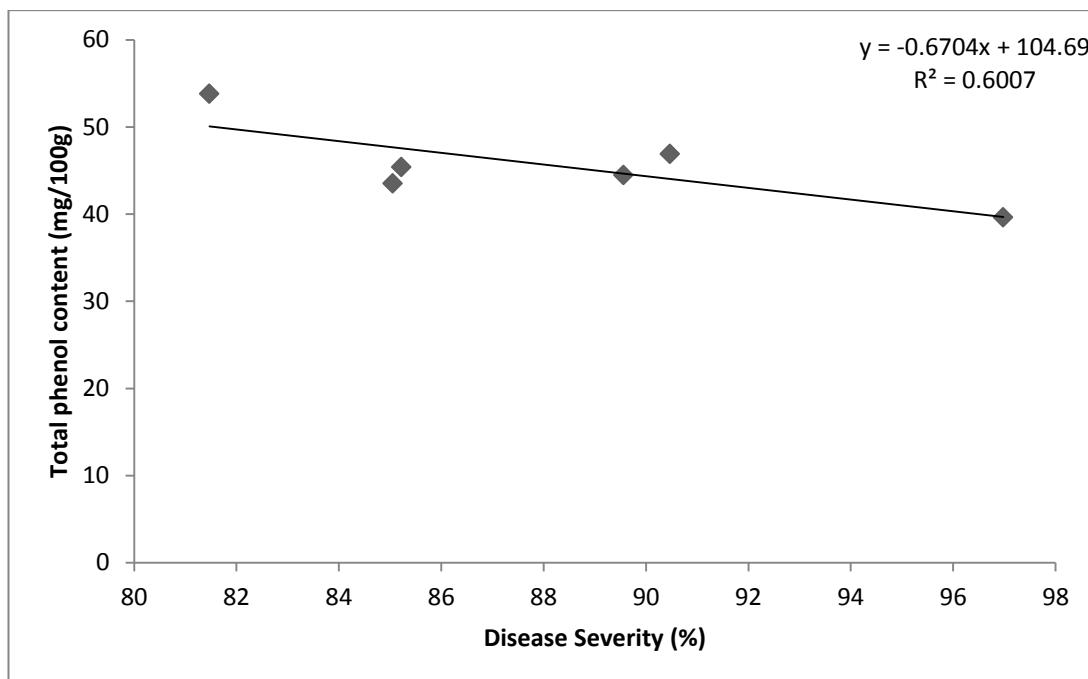


Figure 4.13: Corealtion between disease severity and total phenol content of PEHM 5

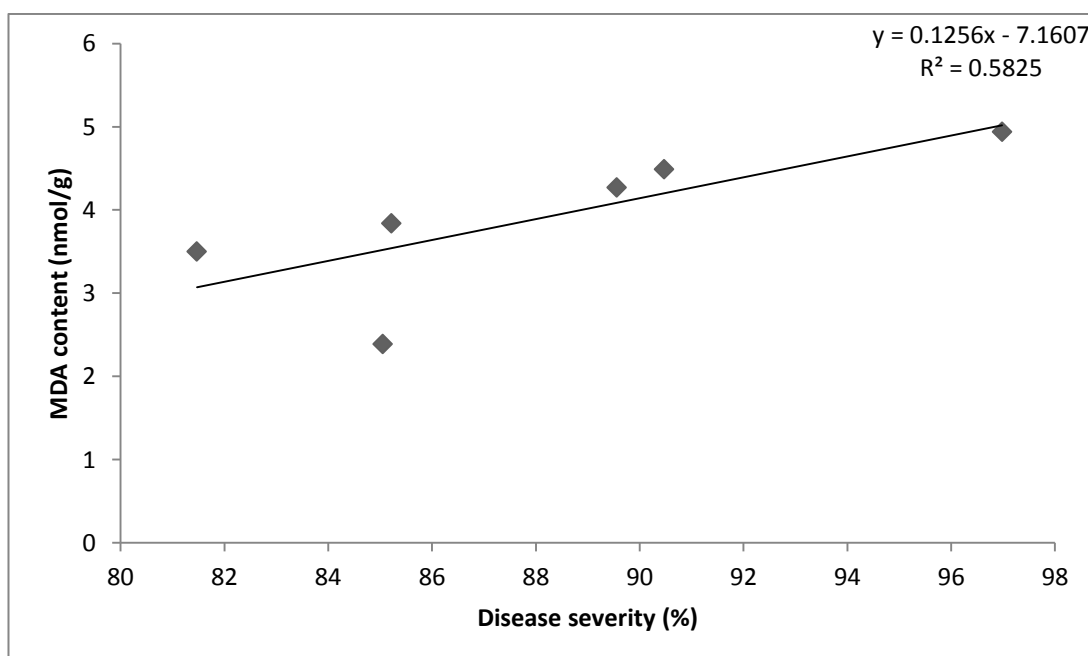


Figure 4.14: Corealtion between disease severity and MDA content of PEHM 5

## Yield

The yield was found highest in T3 treatment and was lowest in T2 treatment. At ambient temperature yield was found to increase significantly at elevated CO<sub>2</sub> levels. Positive response of yield was also found when temperature was increased to 1.5°C. at elevated CO<sub>2</sub> levels. Though interactive effect of CO<sub>2</sub> and temperature on yield was nonsignificant. In present study average yield under different treatment was in the order T3>T4>T5>T1>T0>T2 (Table 4.36).

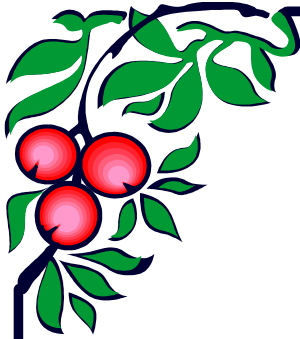
**Table 4.36: Effect of elevated temperature and CO<sub>2</sub> on grain yield per plant for PEHM 5**

	AT	1.5T	3T	Mean
ACO <sub>2</sub>	36.08	38.4	35.08	<b>36.52</b>
ECO <sub>2</sub>	47.62	45.22	40.46	<b>44.43</b>
Mean	<b>41.85</b>	<b>41.81</b>	<b>37.77</b>	

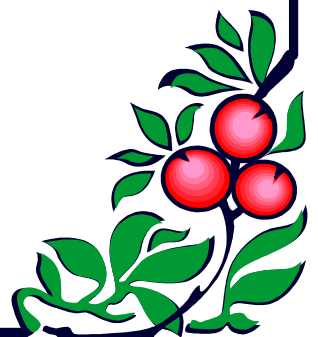
Factors	C.D.	SE(d)	SE(m)
CO <sub>2</sub> level (C)	2.301	1.108	0.784
Temperature (T)	2.818	1.357	0.96
C × T	N/A	1.919	1.357

Significant at  $P < 0.05$ ; ACO<sub>2</sub>, ambient CO<sub>2</sub> concentration; ECO<sub>2</sub>, 550 ppm CO<sub>2</sub> concentration; AT, ambient temperature; 1.5T, ambient temperature + 1.5°C temperature; 3T, ambient temperature + 3°C temperature



## **Chapter-5**

# **DISCUSSION**



## Discussion

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Recent IPCC assessment reports have reported that at global scale climate change has occurred, and will be continued in near future at different rates. Due to climate change, global atmospheric carbon dioxide (CO<sub>2</sub>) concentration is rising and projected to reach 560 ppmv by 2050 and global surface temperature increase by the end of 21<sup>st</sup> century is likely to exceed 1.5°C relative to 1850 (IPCC, 2013). Climate change will influence growth, productivity and distribution of crops with variable magnitude. According to the universally accepted concept of “plant disease triangle” the three legs of the triangle – host, pathogen, and environment – must be present and interact appropriately for plant disease to result. CO<sub>2</sub> and temperature are among the most important environmental variables that may affect the plant disease triangle in various ways. Plant-pathogen interactions are determined by complex changes in crops and agricultural practices that may result from climate change thereby uncertainties about disease occurrence prevails. Despite the studies on how CO<sub>2</sub> and temperature together will influence plant diseases, the impact on primary production in agricultural systems specifically with respect to India is very limited. It is important to develop understanding on this aspect. With this hypothesis in the present study, experiments were carried out to observe the response of maize varieties and its different growth stages pre exposed with elevated levels of CO<sub>2</sub> and temperature to maydis leaf blight disease tolerance.

The meteorological conditions during the crop growth period at the experimental site were as per the optimum requirement of the maize crop. At the experimental site the mean maximum temperature and mean minimum temperature was 34.1°C ± 2.5 and 22.7 ± 4.0°C respectively. The optimum temperature for maize plant in day and night times ranges from 22 to 32°C and 16.7 to 23.3°C, respectively.

Results of the study showed that elevated levels of temperature and CO<sub>2</sub> (abiotic stress) exposure to maize crop altered its response (tolerance or susceptibility) as well as interaction with the MLB disease causing pathogen (biotic stresses) in terms of disease development. It was observed that between the two maize genotypes, functional traits (growth, physiological and biochemical processes, yield and disease tolerance potential)

were more adversely affected in CM 119 as compared to PEHM 5 from the elevated levels of CO<sub>2</sub> and temperature exposure. The response of growth parameters reflect the growth behavior of a crop, besides genetic characteristics, availability of essential nutrients, space, water and environmental condition under which it is grown. It might be due to genetic difference among different maize hybrids (Rahman *et al.*, 2013). In literature also variability in response of other maize genotypes to abiotic (Karim *et al.*, 2000) and biotic stress (Sharma *et al.*, 2000) has been reported.

The principal component analysis done for the response of the growth, physiology and biochemical parameters of the genotypes shows that out of all the growth, physiology and biochemical parameters observed in the present study, the contribution of some of the parameters was more detrimental as compared to other parameters. The key parameters with respect to both PEHM 5 and CM 119 were chlorophyll content, RLWC, ascorbic acid, total phenol content and MDA content, but the percentage contribution of each was variable.

Maize being a C<sub>4</sub> crop has higher temperature optima for growth and physiological processes, thus better adapted to warmer climate. On these temperatures the photosynthetic rate become rapid than respiration resulting in increased plant growth. Plant growth is affected badly when the temperature decreases below 5°C or rises above 32°C (Naveed *et al.*, 2014).

Results of our study also shows that the growth, physiology, yield and synthesis of biochemical defense compounds against the pathogen were more adversely affected under +3°C than the +1.5°C temperature stress under ambient as well as elevated CO<sub>2</sub> levels as compared to control. In previous studies also it has been reported that maize crops response is significantly affected by temperature and it is not altered by CO<sub>2</sub> enrichment (Kim *et al.*, 2007).

Effect of elevated levels of CO<sub>2</sub> in C<sub>4</sub> species, was less positive than C<sub>3</sub> plants, but certainly not nil (Ghannoum *et al.*, 1997). Maize like other C<sub>4</sub> crops do not show response to doubling of the ambient CO<sub>2</sub> concentration, but it is not as much to C<sub>3</sub> crops, where growth stimulating response is almost invariably found (Poorter, 1993). In our study also under elevated CO<sub>2</sub> impact on some of the functional traits (growth, biochemical, photosynthesis w.r.t physiological and yield parameters) was positive on

both the maize genotypes. But this enhancement was reversed when plants are exposed to elevated temperature with elevated CO<sub>2</sub>, though it is slightly more than individual elevated temperature treatments. Increase in plant height, stem girth and leaf area was observed in both the genotypes with elevated CO<sub>2</sub> and ambient temperature levels, whereas with increase in temperature levels enhancement effect of elevated CO<sub>2</sub> on growth parameters was not exhibited in both genotypes. Though temperature stress due to +1.5°C with ambient CO<sub>2</sub> levels enhanced stem girth (4 and 10%) of both genotypes, but in plant height (3–5%) and leaf area (up to 16%) decline was observed. Similar to our observation Karim *et al.* (2000) also reported decrease in growth parameters of six tropical maize genotypes with temperature stress. In the present study it was found that at ACO<sub>2</sub> due to +1.5°C and +3°C temperature treatment leaf area was decreased by 16.45% and 27%, respectively. Similar findings were also reported by Thiagarajah and Hunt (1982), where temperature above 35°C resulted in reduction of leaf area by 33%. In literature it has been reported that at high temperature demand of growing parts of plant (sinks) is high and assimilate declines, with elevated CO<sub>2</sub> levels the response will be positive (Morison and Lawlor, 1999).

On comparing response of growth parameters at three growth stages to the treatments, significant ( $P < 0.05$ ) effect was observed due to interactive effect of CO<sub>2</sub> and temperature. At all growth stages growth parameters response was positive under ECO<sub>2</sub> and negative under elevated temperature treatment plants as compared to control. In literature no study indicated the response of growth stages to the elevated CO<sub>2</sub> and temperature.

Similar to growth parameters, biochemical response of genotypes as well as of three growth stages was altered due to elevated CO<sub>2</sub> and temperature. Biochemical defense is a powerful mechanism in plants which helps in resistance against disease. Accumulation of these substances provides workable protection against diseases.

Chlorophyll is one of the key components for photosynthesis, rate of photosynthesis increases with increase in chlorophyll content (Taiz and Zeiger, 2010). In the present study also variation in average total chlorophyll content of both the genotypes was observed. Under control treatment it was low in CM 119 than PEHM 5. Slight increase and then decrease in chlorophyll content of both genotypes with low temperature stress

(+1.5°C) and at high temperature stress (+3°C), respectively was observed. The present study results show positive and negative effect of +1.5°C and +3°C temperature stress, respectively on chlorophyll content. The reduction in chlorophyll content found was may be due to more rapid leaf senescence under higher temperature. The enhancement effect of elevated CO<sub>2</sub> as it was with the growth parameters was not observed with the chlorophyll content. Chlorophyll content was highest in control treatment at all the three growth stage, except at tasseling stage when it was more for T1 treatment plants. Chlorophyll content varies with plant species, leaf age and biotic and abiotic condition (Katiyar and Dubey, 2001).

RLWC (relative leaf water content) of a leaf represents its water content as compared to its saturation state. Optimum level of RLWC helps plant to maintain its physiological processes under stress condition. RLWC of leaves of PEHM 5 was higher than CM 119. The effect of treatments on RLWC of genotypes was statistically significant ( $P < 0.05$ ). RLWC was highest for both the genotypes under T3 treatment and lowest in T2 treatment, indicates that higher temperature stress adversely affect the water status in maize genotypes. RLWC was found to increase in maize with ageing; it was maximum at dent stage and minimum at vegetative stage. Similar to chlorophyll content, RLWC was also high for control treatment at all the three growth stage, except at dent stage when it was more for T4 treatment plants. Many investigations have shown that when leaves are subjected to stress, leaves exhibit large reductions in relative water content and water potential (Nayyar and Gupta, 2006).

Results shows that leaf extract pH of maize plants become acidic with abiotic and biotic stress exposure in both maize genotypes. It was more acidic in CM 119 as compared to PEHM 5. Leaf extract pH of maize plant was decreasing with increasing crop age. The acidic pH found was may be due to synthesis of secondary metabolites defense compounds in response to abiotic and biotic stress exposure. Leaf extract pH integrates the effects of many compounds and processes in the leaf that affect its exchange capacity for H<sup>+</sup> ions. However, some substances are particularly strong determinants of leaf pH. High concentrations of metal cations (calcium, magnesium, potassium) will give high pH, whereas high concentrations of organic acids and of C-rich secondary metabolites (chemical defense compounds) such as phenol, tannins, will tend to give a

lower pH (Pérez-Harguindeguy *et al.*, 2013). Susceptibility and tolerance of plants increases with low and high pH respectively (Singh and Verma, 2007; Kumar and Nandini, 2013). Felle *et al.* (2004) also reported transiently decreased in apoplastic pH of barley leaves following infection by the powdery mildew fungus *Blumeria graminis* f. sp. *hordei*.

Reactive oxygen species (ROS) are generated as natural products during the stress exposure in plants. The accumulation of ROS could break the balance between ROS production and the capacity of plants to scavenge for them. Ascorbic acid acts as an ROS scavenger and protects the cell membrane from its toxic action (Smirnoff, 2005; Szarka *et al.*, 2007). It activates many physiological and defense mechanism in the plants. Its reducing power increases as the concentration increases (Raza and Murthy, 1988; Sairam and Saxena, 2000; Agbaire and Esiefarienrhe, 2009); but its' reducing activity is dependent on pH, so more reducing activity is found at higher pH because high pH increases the efficiency of conversion of hexose sugar to ascorbic acid (Lui and Ding, 2008; Chouhan *et al.*, 2012). In the present study ascorbic acid content was high in PHEM 5 as compared to CM 119, indicates that it is relatively more resistant to stress induced by the pathogen after exposure to elevated CO<sub>2</sub> and temperature. Ascorbic acid concentration was increasing with increase in age under control treatment. The increase in ascorbic acid concentration was more in plants grown under elevated CO<sub>2</sub> than elevated temperature at all the three growth stages. Temperature stress negatively affected the ascorbic acid synthesis.

Results in the present study also show that average phenol content was high in PEHM 5 than CM 119 because of genotypic variation. Total phenol content was lowest in plant exposed to +3°C with ambient CO<sub>2</sub> and maximum under elevated CO<sub>2</sub> conditions. On comparing growth stages increase in phenol content was observed from vegetative stage to dent stage in plants under all treatment. The results also suggested that the accumulation of total phenol was higher in infected genotypes as compared to healthy ones. Statistically ( $P < 0.05$ ), in present study impact of genotype, CO<sub>2</sub> and temperature levels on phenol content is significant (Table 4.8). Literature shows that the concentration of total phenols varies with genotypes, growth stage as well as with CO<sub>2</sub> and temperature treatments. Environmental factors viz. temperature, humidity, light

intensity, the supply of water, minerals and CO<sub>2</sub> influence the growth of a plant and secondary metabolite production (Ramakrishna and Ravishanker, 2011). Similar to our observation, enhancement in phenol content was observed in maize after exposure to elevated CO<sub>2</sub> levels (Li *et al.*, 2008) and post pathogen infection. Some reports that secondary metabolites increase in response to elevated temperature (Jochum *et al.*, 2007), while others report that they decrease (Snow *et al.*, 2003). High phenolic content were also found in strawberry fruits (Wang *et al.*, 2003) when plants were grown under CO<sub>2</sub> enrichment conditions (ambient + 600 µmol/mol). Elevated atmospheric CO<sub>2</sub> under non-limiting nutrient and water supply increased total phenolics in *Pinus eliotti* (Saxon *et al.*, 2004). The same trend was found in perennial grasses (Castells *et al.*, 2002) as well as in several tropical trees (Coley *et al.*, 2002).

ROS synthesis in plants causes oxidation of lipids present in cell membrane. Malonaldehyde (MDA) is a product of lipid peroxidation and usually used as the criterion of stress-induced oxidative damage to membrane (Zhang *et al.*, 2006). In the present study, MDA content was significantly high ( $P < 0.05$ ) in elevated CO<sub>2</sub> and temperature stress exposed and with MLB disease plants (Table 4.9). Increase was more in CM 119 than PEHM 5, indicates more damage and susceptibility in CM 119 than PEHM 5. Similar to our observation Sharma *et al.* (2002) also reported low and high accumulation of MDA in resistant (CHH62) and susceptible (JK2595 and FH3088) maize genotype interaction with *Rhizoctonia solani*. MDA content in control plants was increasing with increase in age. But for other elevated CO<sub>2</sub> and temperature treatments MDA was high at vegetative and tasseling stage and low at dent stage plants.

The basic material of photosynthesis is the atmospheric carbon dioxide content. This gas reaches the place of the biochemical processes through the stomata. For this reason stomatal resistance is a limiting factor for the penetration of CO<sub>2</sub> in the leaf, but also regulates the getting out of water vapors, so the transpiration. Results of the present study show genotypic variation with respect to photosynthesis. Rate of photosynthesis in both the genotypes was enhanced with elevated CO<sub>2</sub> but decline with temperature stress, whereas, stomatal conductance and transpiration is reduced under elevated CO<sub>2</sub> and temperature in both genotypes. The results contradicts the view that in C<sub>4</sub> crops photosynthesis do not increase with elevated levels of CO<sub>2</sub> (Bowes, 1993) and supports

the view that photosynthesis is not saturated at current CO<sub>2</sub> levels but it can increase with elevated CO<sub>2</sub> levels (Wand *et al.*, 1999). The increase in net photosynthesis in C<sub>3</sub> species has been reported as high as 50–100% when CO<sub>2</sub> concentration doubles compared to 10% in C<sub>4</sub> species (Anda and Kocsis, 2008). It has been reported that the warmer temperature increases the ratio of respiration to photosynthesis and elevated CO<sub>2</sub> decreases it (Morison and Lawlor, 1999). Similar to our observation reduced stomatal conductance and transpiration under elevated CO<sub>2</sub> with well water condition has been reported in many literatures. In literature also it has been reported that with elevated CO<sub>2</sub> stomatal conductance reduces at the leaf level by 30–40%, although large differences among species exists (Anda *et al.*, 2002). Decrease in stomatal conductance, causes a decrease in transpiration which leads to an increase in leaf temperature. The temperature of the leaf surface may rise 0.5–1.7°C only due to doubling CO<sub>2</sub> concentration (Idso, 1987; Van de Geijn, 1996) or even up to 3°C, depending on the species and the weather (Rosenberg, 1990). Transpiration is not only affected by the stomatal opening, but also by exchange of the water vapour from the leaf surface to the surrounding atmosphere. It has been reported in the literature that water vapor pressure decreases with the increasing CO<sub>2</sub> level (Rosenberg, 1990).

Grain yield of a crop is the expression of combined effects of various yield components. Among the two genotypes average grain yield/plant was high for PEHM 5 (21.2 g) than CM 119 (4.1 g). Exposure of genotypes to elevated temperature and CO<sub>2</sub> and MLB disease adversely affected their yield. Both genotypes' yield was maximum under elevated CO<sub>2</sub> treatment without MLB disease and minimum under elevated temperature stress with MLB disease. This difference in grain yield among genotypes might be due to their genetic variation and difference in acclimation capacity to high temperature, which reduced the yield drastically due to its detrimental effects on metabolism and duration of phenological phases (Acevedo *et al.*, 1990; Jenner, 1991). High temperature may affect the pollen viability and fertilization which result in large yield reduction in maize (Lobell *et al.*, 2013). It has been estimated that a doubling CO<sub>2</sub> concentration, holding other factors constant, could lead to a 34±6% increase in agricultural yields of C<sub>3</sub> plants and a 14±11% in C<sub>4</sub> plants with a 95% confidence interval (Kimball, 1983).

In the present study exposure of elevated CO<sub>2</sub> and temperature altered the maize genotypes as well as growth stages tolerance to MLB disease. MLB disease severity was more on CM 119 than PEHM 5. It indicates that with elevated levels of CO<sub>2</sub> and temperature the tolerance of CM 119 will be low, so its use as an inbred line for maize breeding programmes should be done cautiously. Previous studies also showed that hybrids are found to perform better than inbred lines for many traits under abiotic and biotic stress (Sharma *et al.*, 2000). It may be because exposure of CO<sub>2</sub> and temperature levels induces changes in plant morphology, physiology and biochemistry and will influence diseases development (Chakraborty, 2005). Undoubtedly the nature of host (e.g. annual vs. perennial, C<sub>3</sub> vs. C<sub>4</sub>) and pathogen (e.g. root-infecting vs. shoot-infecting, biotroph vs. necrotroph) population and climate (e.g. asymmetric temperature shifts will have direct and indirect effects from changes in both maxima and minima) are determining the effect of elevated levels of CO<sub>2</sub> and temperature on disease incidence (Chakraborty, 2005).

Reduced and high disease severity under elevated CO<sub>2</sub> and temperature stressed plants, respectively was likely due to altered leaf chemistry. In our study also we have observed alteration in synthesis of biotic stress related defense compounds (phenol, ascorbic acid and MDA) in plant under different treatment. This indicates that the biochemical defense compounds synthesis was triggered under elevated levels of CO<sub>2</sub> and temperature, however, they were unable to totally inhibit the pathogen may be because of more virulence in pathogen. The presence of a high concentration of phenolic compounds is considered to be one of the major factors for an incompatible host pathogen interaction. After infection by a pathogen, plant cells synthesize phenol oxidising enzymes that oxidise phenols into toxic quinines, which play a crucial role in disease resistance (Jiang *et al.*, 2009; Ashry and Mohamed, 2011). Siqueira *et al.* (1991) discussed the role of phenolic compounds in host pathogen interaction, particularly in disease resistance.

Our results show that MLB development is more influenced by the combination of CO<sub>2</sub> concentration and temperature than by CO<sub>2</sub> level alone. MLB disease severity was less on plants exposed to elevated CO<sub>2</sub> and more on plants exposed to elevated temperature. Different disease indices also indicate that the tolerance of genotypes was highest under elevated CO<sub>2</sub> and minimum under +3°C temperature at ambient level treatment. This

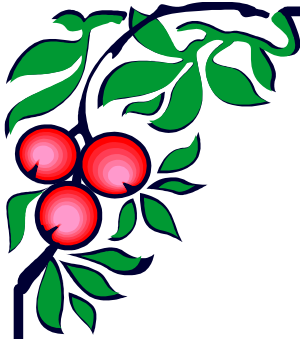
may be increased CO<sub>2</sub> and temperature induces some changes in host physiology and biochemistry, for instance linked to the observed increased assimilation under high concentration of CO<sub>2</sub> that can be involved in the activation of plant defense mechanisms. The similar observation were reported for the rust pathogen *Maravalia cryptostegiae*, used for the biological control of the woody weed rubber vine (*Cryptostegia grandiora*), reduces growth enhancement of the weed under twice-ambient CO<sub>2</sub> from 24% to less than 16% (Chakraborty, 2005). It has been reported that with 3°C increase in the temperature throughout the growing season accelerate senescence of leaves (Morison and Lawlor, 1999). Less disease severity on plants exposed to elevated CO<sub>2</sub> may be due to the healthy vegetative development i.e., plants produced more leaves, which corresponded to greater water content and so, to less susceptibility to MLB pathogen, as it is necrotrophic in nature.

At ambient temperature, elevated CO<sub>2</sub> exposure to plants does not supported growth of MLB pathogen i.e., less disease severity was observed. On the contrary, elevated CO<sub>2</sub> exposure in combination with elevated temperature levels stimulated MLB development on both the genotypes. These results suggest that the increase of CO<sub>2</sub> per se had no positive effect on disease development, while temperature stress per se significantly increased disease development. Similar observation were reported by McElrone *et al.* (2005) that elevated CO<sub>2</sub> significantly reduced disease caused by foliar fungal pathogen, *Phyllosticta minima*, of *Acer rubrum*, with 22%, 27%, and 8% fewer saplings and 14%, 4%, and 5% fewer leaves infected per plant in the three consecutive years, respectively.

In our study, through leaf detached technique maximum infection was observed at the end of 96 h incubation period, when at ambient CO<sub>2</sub> level temperature was increased up to 3°C. Garraway *et al.* (1989) also found similar results in lab condition when they studied exposure of resistant and susceptible isolines of maize (*Zea mays* L.) leaves to high temperature stress, i.e. 42°C for 6 h before inoculation with *Bipolaris maydis* race 'T' followed by incubation in the dark at 28°C for 24 h, resulted in a significant decrease in peroxidase activity (its' high levels indicates tolerance capacity of plant), significant increase in electrolyte leakage as well as in sporulation in both isolines compared with the control (leaves not exposed to high temperature stress before inoculation). Garraway

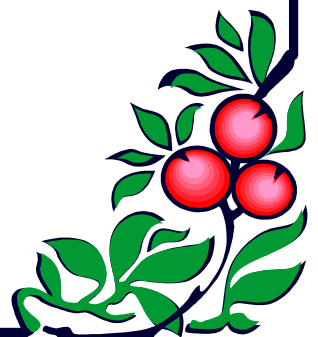
*et al.* (1989) suggested that increased sporulation on maize leaves in response to high temperature stress appeared to be related with increased electrolyte linkage.

On the basis of results of our study we can conclude, with +1.5°C temperature elevation as well with +1.5°C temperature and elevated CO<sub>2</sub>, growth of the MLB pathogen and its incidence of maize crop will be more. Considering that the rising concentrations of CO<sub>2</sub> and temperature in future are likely to indirectly enhance MLB disease severity in susceptible as well as resistant maize genotypes.



## **Chapter-6**

# **SUMMARY AND CONCLUSION**



## Summary and Conclusion

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The impact of elevated temperature and CO<sub>2</sub> on tolerance potential of maize to maydis leaf blight (MLB) disease was assessed at IARI experimental field. Maize plants were exposed to six treatments representing combination of two levels of CO<sub>2</sub> (ambient and elevated) and three levels of temperature (ambient, +1.5°C, +3°C) in open top chambers (OTCs). A pot experiment was carried out to assess the impact of different levels of elevated temperature and CO<sub>2</sub> on response of PEHM 5 (susceptible) and CM 119 (resistant) maize genotypes. A field experiment was carried out to assess the impact different levels of elevated temperature and CO<sub>2</sub> on tolerance potential of maize at different growth stages– vegetative, tasseling and dent stage. After exposure to elevated temperature and CO<sub>2</sub> levels maize plants under both experiments were monitored for the changes with respect to growth, physiological, biochemical, yield and disease severity. The salient findings of the study are presented below:

1. Effect of elevated temperature and CO<sub>2</sub> on morphological growth of PEHM 5 and CM 119 was assessed w.r.t plant height, stem girth and leaf area. All the three growth parameters were adversely affected by +3°C temperature with ambient CO<sub>2</sub> levels as compared to control treatment (ambient CO<sub>2</sub> and ambient temperature). Among the two genotypes the average growth of CM 119 was more adversely affected than PEHM 5 by the treatments.
2. Effect of elevated temperature and CO<sub>2</sub> on biochemical response of PEHM 5 and CM 119 was assessed w.r.t. chlorophyll, relative leaf water content (RLWC), leaf extract pH, ascorbic acid, total phenol and MDA (lipid peroxidation) content. At ACO<sub>2</sub> level when temperature was increased up to 1.5°C, there was enhancement in total chlorophyll and RLWC in both PEHM 5 and CM 119, but when temperature was increased to the level of 3°C, the total chlorophyll content was decreased significantly. Under elevated CO<sub>2</sub> level enhancement in chlorophyll content was observed, but it was nullified with increase in

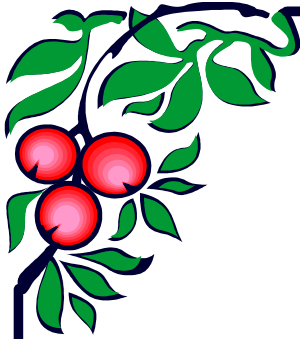
temperature. Leaf extract pH become acidic from near neutral, with increase in temperature stress in both the genotypes.

3. Effect of elevated temperature and CO<sub>2</sub> on biochemical defense compounds (for ascorbic acid, phenol and MDA content of maize genotypes as well as for growth stages was significant at  $P < 0.05$ ). Average content of biochemical defense compounds was more in PEHM 5 than CM 119. Ascorbic acid content was 98.91% and 55.67% more under elevated temperature stress (T4 and T1 treatments), respectively as compared to control in PEHM 5. Total phenol content decline with elevated temperature stress and increase with elevated CO<sub>2</sub> levels. MDA content was high under elevated temperature stress and low with elevated CO<sub>2</sub> levels.
4. Effect of elevated temperature and CO<sub>2</sub> on physiological processes was assessed w.r.t. photosynthesis, stomatal conductance and transpiration for the genotypes as well as growth stages. Effect of treatments on physiological performance was more adverse in CM 119 than PEHM 5. Between growth stages physiological performance of PEHM 5 was best at tasseling stage and least at dent stage. Enhancement in photosynthesis and decrease in stomatal conductance and transpiration rate was observed in elevated CO<sub>2</sub> treated plants as compared to control, whereas decline in all observed physiological processes was observed with elevated temperature stress for both the genotypes as well as growth stages. Under elevated CO<sub>2</sub> and elevated temperature interaction also, physiological performance were adversely affected as compared to control.
5. Principal Component Analysis (PCA) shows that % contribution of the observed functional traits in response to elevated temperature and CO<sub>2</sub> as well as MLB disease development was different for the PEHM 5 and CM 119 as well as the three growth stages. For the vegetative stage of PEHM 5 plants, the order of contribution of the functional traits was – total leaf area (19.5%) > total phenol content (18.9%) > relative leaf water content (16.7%) > photosynthetic rate (15.7%) > ascorbic acid content (15.5%) > MDA content (13.5%), respectively.

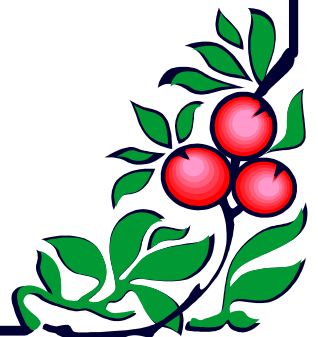
6. Effect of elevated temperature and CO<sub>2</sub> exposure on yield attributes was observed on maize genotypes with and without MLB disease. Average yield under all treatments with and without MLB was more for PEHM 5 than CM 119. Under elevated CO<sub>2</sub> (T3) treatment maximum grain yield/plant of PEHM 5 was 36.54 g/plant and 47.62 g/plant for plants with and without MLB disease, respectively. Under elevated temperature +3°C (T2) treatment minimum grain yield of PEHM 5 was 20.04 g/plant and 35.08 g/plant for plants with and without MLB disease, respectively. Similar trend was observed other yield attributes.
7. In the present study impact of elevated temperature and CO<sub>2</sub> on MLB disease development on PEHM 5 and CM 119 and different growth stages of PEHM 5 was significant under field as well controlled lab conditions. Images captured during disease development were assessed through imageJ software for quantification of disease infected area. Among the two genotypes under control treatment disease severity was more on CM 119 (58.4%) than PEHM 5(34.7%). In elevated temperature +3°C (T2) treatment, maximum (65.55 and 44.3%) disease severity was observed in CM 119 and PEHM 5, respectively. Under controlled condition successful disease establishment was observed on detached leaves of vegetative stage plants only. At that stage the disease severity trend for different treatments was in this order T2 > T5 > T1 > T0 > T4 > T3.
8. Under elevated temperature and CO<sub>2</sub> tolerance potential for MLB disease was high for hybrid variety (PEHM 5) as compared to inbred line (CM 119) of maize. Tolerance potential of maize was lowest in +3°C temperature with ambient and elevated CO<sub>2</sub> levels treatments. Among the growth stages tolerance potential of dent stage to diseases was maximum as compared to vegetative and tasseling stage.

## Conclusion

On the basis of results of our study we can conclude that under elevated temperature and CO<sub>2</sub> levels crops growth, biochemical and physiological performance may be affected, which may favour establishment of MLB pathogen (*Bipolaris maydis*) and disease development on vegetative growth stage of the hybrid as well as inbred lines of maize. Considering that, the rising concentrations of temperature and CO<sub>2</sub> in future are likely to indirectly enhance MLB disease severity in susceptible as well as resistant maize genotypes.



# ABSTRACT



## **Abstract**

### **Effect of Elevated Temperature and Carbon Dioxide on Tolerance Potential of Maize to Maydis Leaf Blight Disease**

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As per recent IPCC (2013) assessment report global scale climate change has occurred and will be continued in near future at different rates. Due to climate change global atmospheric carbon dioxide concentration (CO<sub>2</sub>) are rising and projected to reach between 540 and 970 ppm by the end of 21<sup>st</sup> century and global surface temperature may increase 1.4–5.8°C in association with this doubling of CO<sub>2</sub>. Crop species (both C<sub>3</sub> as well as C<sub>4</sub>) are directly affected by elevated levels of temperature and CO<sub>2</sub> due to changes in the plant physical structures, leaf chemistry and carbon : nitrogen balance etc. which in turn affects yield, tolerance to drought stress and susceptibility to pests and herbivores. Despite abundant evidence about the direct effect of elevated temperature and CO<sub>2</sub> on crop plants, we still need a more thorough understanding of the impact of elevated temperature and CO<sub>2</sub> levels on plant diseases in agricultural ecosystem to predict and plan for disease pressure under future climatic condition. Many studies have reported the effect of elevated temperature and CO<sub>2</sub> on growth, physiological, biochemical and yield of maize crops, but effect of elevated temperature and CO<sub>2</sub> in combination on tolerance potential of maize to diseases is very limited and still needs a systematic study. With this hypothesis the present study was undertaken with the following objectives – (i) To assess impact of elevated temperature and CO<sub>2</sub> on maydis leaf blight (MLB) disease development on resistant and susceptible genotypes of maize and (ii) To assess impact of elevated temperature and carbon dioxide on maydis leaf blight disease tolerance potential of maize. Results of the study showed that elevated levels of temperature and CO<sub>2</sub> exposure to PEHM 5 and CM 119 maize genotypes as well as vegetative, tasseling and dent growth stage of PEHM 5 altered the response of their respective functional traits (growth, physiology and biochemistry) as well as MLB disease development. It was observed that between the two maize genotypes effect of temperature and CO<sub>2</sub> treatments was more adverse on CM 119 as compared to PEHM 5 genotype w.r.t. functional traits as well as MLB disease severity. Among the treatments, at +3°C temperature elevation with ambient CO<sub>2</sub> level effect was most detrimental on functional traits and MLB disease development as compared to control i.e. ambient temperature and CO<sub>2</sub> level. MLB development was more influenced by the combination of temperature and CO<sub>2</sub> than by CO<sub>2</sub> level alone. MLB disease severity was less on plants exposed to elevated CO<sub>2</sub> and more on plants exposed to elevated temperature. Different disease indices also indicate that the tolerance of PEHM 5 was more than CM 119 and it was more adversely affected by temperature stress. Tolerance potential of different growth stages of maize to MLB disease was more at dent stage as compared to vegetative and tasseling stages to elevated CO<sub>2</sub> and temperature treatments.

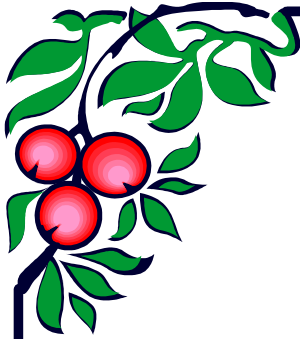
## सारांश

### उच्च तापमान एवम् कार्बन डाइऑक्साइड का मक्का में मेडिस पर्ण झुलसा रोग की संभावित सहनशीलता पर प्रभाव

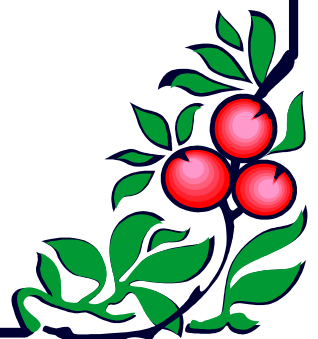
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आई.पी.सी.सी. (2013) की ताज़ा रिपोर्ट के अनुसार विश्वस्तर पर जलवायु परिवर्तन हो रहा है, तथा निकट भविष्य में विभिन्न दरों से जलवायु परिवर्तन होने के आसार हैं | जलवायु परिवर्तन के कारण विश्वस्तर पर वातावरण में कार्बन डाइऑक्साइड की मात्रा बढ़ रही है और ऐसा अनुमान है कि 21वीं सदी के अंत तक इसकी मात्रा 540 पी.पी.एम. से 970 पी.पी.एम. तक बढ़ जाएगी | कार्बन डाइऑक्साइड की मात्रा में दोगुनी बढ़ोतरी के साथ-साथ तापमान में भी 1.4 – 5.8°C बढ़ोतरी की गुंजाईश है | C<sub>3</sub> तथा C<sub>4</sub>, दोनों किस्मों के पौधों में तापमान तथा कार्बन डाइऑक्साइड बढ़ने से प्रत्यक्ष प्रभाव पड़ता है | यद्यपि उच्च तापमान और कार्बन डाइऑक्साइड का फसलों पर पड़ने वाले प्रभाव का हमारे पास काफी प्रमाण उपलब्ध हैं, परन्तु तापमान और कार्बन डाइऑक्साइड के बढ़ने से फसलों में होने वाले विभिन्न रोगों पर क्या प्रभाव पड़ेगा, इसका अंदाज़ लगाना हमारे लिए काफी आवश्यक है, जिसके फलस्वरूप हम भविष्य में होने वाले जलवायु परिवर्तन से फसलों में रोगों के दबाव को न सिर्फ समझ सकें बल्कि उनको कम करने का प्रयास भी कर सकें | इन्हीं बातों को ध्यान में रखते हुए प्रस्तुत अनुसन्धान कार्य दो उद्देश्यों के लिए किया गया :- 1) उच्च तापमान एवम् कार्बन डाइऑक्साइड का मक्के के प्रतिरोधक तथा संवेदनशील अनुवांशिक रूपों पर मेडिस पर्ण झुलसा रोग के संस्थापन का पता लगाने के लिए , तथा 2) उच्च तापमान एवम् कार्बन डाइऑक्साइड का मक्के पर मेडिस पर्ण झुलसा रोग के संभावित सहनशीलता का पता लगाने के लिए | अनुसन्धान कार्य से यह पता चला कि उच्च तापमान एवम् कार्बन डाइऑक्साइड के अनावरण से PEHM 5 तथा CM 119 पर वानस्पतिक, माध्यमिक तथा डेंट अवस्थाओं पर महत्वपूर्ण प्रभाव पड़ा | इसके साथ ही विभिन्न समृद्धि कारकों, कार्यकीय, जैवरासायनिक कारकों तथा मेडिस पर्ण झुलसा के संस्थापन पर भी सार्थक प्रभाव देखा गया | यह पाया गया कि मक्के के दो अनुवांशिक रूपों में CM 119 पर उच्च तापमान एवम् कार्बन डाइऑक्साइड का कार्यात्मक कारकों तथा मेडिस पर्ण झुलसा के सन्दर्भ में नकारात्मक प्रभाव ज्यादा था | विभिन्न उपचारों में नियंत्रण उपचार की तुलना में +3°C उच्च तापमान वर्तमान

CO<sub>2</sub> के साथ, का कार्यात्मक कारकों तथा मेडिस पर्ण झुलसा की तीव्रता पर ज्यादा प्रभाव देखा गया | इसके साथ ही यह पाया गया कि मेडिस पर्ण झुलसा रोग की तीव्रता उच्च CO<sub>2</sub> पर कम थी तथा उच्च तापमान पर ज्यादा थी और संभावित सहनशीलता डेंट स्तर पर ज्यादा थी | अतः हम कह सकते हैं कि भविष्य में तापमान के बढ़ने से मेडिस पर्ण झुलसा रोग की तीव्रता बढ़ सकती है |



# BIBLIOGRAPHY



## Bibliography

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- Aben, S.K., Seneweera, S.P., Ghannoum, O. and Conroy, J.P. (1999). Nitrogen requirements for maximum growth and photosynthesis of rice, *Oryza sativa* L. cv. *Jarrah* grown at 36 and 70 Pa CO<sub>2</sub>. *Australian Journal of Plant Physiology* **26**, 759–766.
- Acevedo, E., Nachit, M. and Ortiz, G. (1990). Effects of heat stress on wheat and possible selection tools for use in breeding for tolerance. In: Saundess, D.A. (ed.). *Wheat for the non-traditional warm areas. A proceeding of International conference*. July 29 – August 3. Maxico. pp. 401–420.
- Agbaire, P.O. and Esiefarienrhe, E. (2009). Air Pollution Tolerance Indices (APTI) of some plants around Otorogun gas plant in Delta State, Nigeria. *Journal of Applied Science and Environment Management* **13**, 11–14.
- Agrios, G. N. (2005). *Plant Pathology*, 5th edn. Elsevier, USA, p. 922.
- Ahuja, S. C. and Payak, M. M. (1978). A field inoculation technique for evaluating maize germplasm to banded leaf & sheath blight. *Indian Phytopathology* **31**, 517–520.
- Aiken, R. (2009). Climate change impacts on crop growth in the central High plains. 21<sup>st</sup> annual central plains irrigation conference, K-State North West Research - Extension Center, Colby, Kansas.
- Ainsworth, E.A. and Long, S.P. (2005). What have we learned from 15 years of free-air CO<sub>2</sub> enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO<sub>2</sub>. *New Phytologist* **165**, 351–371.
- Ainsworth, E.A. and Rogers, A. (2007). The response of photosynthesis and stomatal conductance to rising [CO<sub>2</sub>]: mechanisms and environmental interactions. *Plant, Cell & Environment* **30**, 258–270.
- Ainsworth, E.A., Davey, P.A. and Bernacchi, C.J. (2002). A meta-analysis of elevated [CO<sub>2</sub>] effects on soybean (*Glycine max*) physiology, growth and yield. *Global Change Biology* **8**, 695–709.

- Ainsworth, E.A., Rogers, A., Vodkin, L.O., Walter, A. and Schurr, U. (2006). The effects of elevated CO<sub>2</sub> concentration on soybean gene expression. An analysis of growing and mature leaves. *Plant Physiology* **142**, 135–147.
- Anda, A. and Kocsis, T. (2008). Impact of atmospheric CO<sub>2</sub> enrichment on some elements of microclimate and physiology of locally grown maize. *Applied Ecology and Environmental Research* **6**, 85–94.
- Anda, A., Lőke, Z. and Varga, B. (2002). Processing the standard meteorological data collected in standard meteorological into input of microclimate simulation models. (In Hungarian) – *Légkör* **2**, 28–33.
- Anderson, P.K., Cunningham, A.A., Patel, N.G., Morales, F.J., Epstein, P.R. and Daszak, P. (2004). Emerging infectious diseases of plants: Pathogen pollution, climate change and agro technology drivers. *Trends in Ecology & Evolution* **19**, 535–544.
- Andrews, S.S. and Carroll, C.R. (2001). Designing a soil quality assessment tool for sustainable agro ecosystem management. *Ecological Applications* **11**, 1573–1585.
- Andrews, S.S., Mitchell, J.P., Mancinelli, R., Karlen, D.L., Hartz, T.K., Horwath, W.R., Pettygrove, G.S., Scow, K.M. and Munk, D.S. (2002). On farm assessment of soil quality in California's Central Valley. *Agronomy Journal* **94**, 12–23.
- Anonymous (2014a). Agricultural Statistics at a Glance 2013. Directorate of economics and statistics. Department of agriculture and cooperation, ministry of agriculture, GOI. Available at [www.agricoop.com](http://www.agricoop.com) (visited on September 14, 2014).
- Anonymous (2014b). Maize CRP: extension proposal 2015-2016. CIMMYT publication repository. Available at [www.cimmyt.org/](http://www.cimmyt.org/) (visited on December 07, 2014).
- Anonymous (2014c). Brochure: CGIAR Research Program on Maize. CIMMYT publication repository. Available at [www.cimmyt.org/](http://www.cimmyt.org/) (visited on December 08, 2014).
- Anonymous (2014d). Infographic factsheet: The World of Maize CIMMYT, Int. (CIMMYT IITA CGIAR, 2014). Available at [www.cimmyt.org/](http://www.cimmyt.org/) (visited on December 08, 2014).

- Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts, polyphenoxidase in *Beta vulgaris*. *Plant Physiology* **24**, 1–15.
- Ashraf, M. and Hafeez, M. (2004). Thermotolerance of Pearl Millet and Maize at Early Growth Stages: Growth and Nutrient Relations. *Biologia Plantarum* **48**, 81–86.
- Ashry, N.A. and Mohamed, H.I. (2011). Impact of secondary metabolites and related enzymes in flax resistance and or susceptibility to powdery mildew. *World Journal of Agricultural Sciences* **7**, 78–85.
- Baker, J.T., Allen, L.H. Jr., Boote, K.J. and Jones, J.W. (1989). Response of soybean to air temperature and carbon dioxide concentration. *Crop Science* **29**, 98–105.
- Bauweraerts, I., Wertin, T.M., Ameye, M., McGuire, M.A., Teskey, R.O. and Steppe, K. (2013). The effect of heat waves, elevated [CO<sub>2</sub>] and low soil water availability on northern red oak (*Quercus rubra* L.) seedlings. *Global Change Biology* **19**, 517–528.
- Bots, M. and Mariani, C. (2005). Pollen viability in the field. Research Reports No. CGM 2005-05. University of Nijmegen.
- Bowes, G. (1993). Facing the inevitable: plants and increasing atmospheric CO<sub>2</sub>. *Annual Review of Plant Physiology and Plant Molecular Biology* **44**, 309–332.
- Boyer, J.S. (1995). Biochemical and biophysical aspects of water deficits and the predisposition to disease. *Annual Review of Phytopathology* **33**, 251–274.
- Braga, M.R., Aidar, M.P.M., Marabes, M.A. and de Godoy, R.L. (2006). Effects of elevated CO<sub>2</sub> on the phytoalexin production of two soybean cultivars differing in the resistance to stem canker disease. *Environmental and Experimental Botany* **58**, 85–92.
- Buckley, T. and Schymanski, S. (2014). Stomatal optimisation in relation to atmospheric CO<sub>2</sub>. *New Phytologist* **201**, 372–377.
- Burdon, J.J., Thrall, P.H. and Ericson, A.L. (2006). The current and future dynamics of disease in plant communities. *Annual Review of Phytopathology* **44**, 19–39.
- Cairns, J.E., Sonder, K., Zaidi, P.H., Verhulst, N., Mahuku, G., Babu, R., Nair, S.K., Das, B., Govaerts, B., Vinayan, M.T., Rashid, Z., Noor, J.J., Devi,

- P., San Vicente, F., Prasanna, B.M. and Donald, L.S. (2012). Chapter one - Maize Production in a Changing Climate: Impacts, Adaptation, and Mitigation Strategies. *Advances in Agronomy* **114**, 1–58.
- Campbell, C., Atkinson, L., Zaragoza-Castells, J., Lundmark, M., Atkin, O. and Hurry, V. (2007). Acclimation of photosynthesis and respiration is asynchronous in response to changes in temperature regardless of plant functional group. *New Phytologist* **176**, 375–389.
- Castells, E., Roumet, C., Peñuelas, J. and Roy, J. (2002). Intraspecific variability of phenolic concentrations and their responses to elevated CO<sub>2</sub> in two mediterranean perennial grasses. *Environmental and Experimental Botany* **47**, 205–216.
- Chakraborty, S. (2005). Potential impact of climate change on plant–pathogen interactions. *Australasian Plant Pathology* **34**, 443–448.
- Chakraborty, S. and Newton, A.C. (2011). Climate change, plant diseases and food security: an overview. *Plant Pathology* **60**, 2–14.
- Chakraborty, S., Luck, J., Hollaway, G., Fitzgerald, G. and White, N. (2010). Rust-proofing wheat for a changing climate. BGRI 2010 Technical Workshop, 30-31 May 2010, St Petersburg, Russia.
- Chouhan, A., Iqbal, S., Maheshwari, R. S. and Bafna, A. (2012). Study of air pollution tolerance index of plants growing at Pithampur Industrial area sector 1, 2 and 3. *Research Journal of Recent Sciences* **1**, 172–177.
- Chun, J.A., Wang, Q., Timlin, D., Fleisher, D., and Reddy, V.R. (2011). Effect of elevated carbon dioxide and water stress on gas exchange and water use efficiency in corn. *Agricultural and Forest Meteorology* **151**, 378–384.
- Cicchino, M., Edreira, J.I.R. and Otegui, M.E. (2010). Heat stress during late vegetative growth of maize: Effects on phenology and assessment of optimum temperature. *Crop Science* **50**, 1431–1437.
- CIMMYT (2014). Maize research in Asia, Improving Stress Resilience, Conserving Resources and Empowering Local Communities. International Maize and Wheat Improvement Center (CIMMYT). Mexico, DF (Mexico). CIMMYT. CGIAR. pp. 8. Available at [www.cimmyt.org/](http://www.cimmyt.org/) (visited on February 11, 2015).

- Coley, P.D., Massa, M., Lovelock, C.E. and Winter, K. (2002). Effects of elevated CO<sub>2</sub> on foliar chemistry of saplings of nine species of tropical tree. *Oecologia* **133**, 62–69.
- Crafts-Brandner, S.J. and Salvucci, M.E. (2000). Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO<sub>2</sub>. *Proceedings of the National Academy of Sciences, USA* **97**, 13430–13435.
- Crafts-Brandner, S.J. and Salvucci, M.E. (2002). Sensitivity of photosynthesis in a C<sub>4</sub> plant, maize, to heat stress. *Plant Physiology* **129**, 1773–1780.
- Crowl, T.A., Crist, T.O., Parmenter, R.R., Belovsky, G. and Lugo, A.E. (2008). The spread of invasive species and infectious disease as drivers of ecosystem change. *Frontiers in Ecology and the Environment* **6**, 238–246.
- Cseke, L.J., Tsai, C.J. and Rogers, A. (2009). Transcriptomic comparison in the leaves of two aspen genotypes having similar carbon assimilation rates but different partitioning patterns under elevated [CO<sub>2</sub>]. *New Phytologist* **182**, 891–911.
- Dass, S., Kumar, A., Jat, S.L., Parihar, C.M., Singh, A.K., Chikkappa, G.K. and Jat, M.L. (2012). Maize holds potential for diversification and livelihood security. *Indian Journal of Agronomy (Special Issue)*. **57**, 86–91.
- Dass, S., Singh, I., Chikkappa, G.K., Parihar, C.M., Kual, J., Singode, A., Manivannan, A. and Singh, D.K. (2010). Abiotic Stressess in Maize: Some Issues and Solutions. Directorate of Maize Research, Pusa Campus, New Delhi, pp. 16.
- De Candolle, A. (1886). Origin of Cultivated Plants. 2<sup>nd</sup> edition, reprinted 1959. New York.
- de Graaff, M., van Groenigen, K., Six, J., Hungate, B. and van Kessel, C. (2006). Interactions between plant growth and soil nutrient cycling under elevated CO<sub>2</sub>: a meta-analysis. *Global Change Biology* **12**, 2077–2091.
- De Souza, A.P., Gaspar, M. and Da Silva, E.A. (2008). Elevated CO<sub>2</sub> increases photosynthesis, biomass and productivity, and modifies gene expression in sugarcane. *Plant, Cell & Environment* **31**, 1116–1127.
- De Wit, J., Harlan, J.R., Lambert, R.J. and Eagle, L.M. (1972). Introgression from *Tripsacum* into *Zea* and the origin of corn. *Caryologia* **25**, 25–31.

- DMR (2012). Annual Report 2011-12, Directorate of Maize Research, Pusa Campus, New Delhi-110 012, pp. 103.
- DMR (2013). Annual Report 2012-13, Directorate of Maize Research, Pusa Campus, New Delhi-110012, pp. 82.
- Drechsler, C. (1925). Some graminicolous species of *Helminthosporium*. *Internatinal Journal Agricultural research* **24**, 641–739.
- Driscoll, S.P., Prins, A., Olmos, E., Kunert, K.J. and Foyer, C.H. (2006). Specification of adaxial and abaxial stomata, epidermal structure and photosynthesis to CO<sub>2</sub> enrichment in maize leaves. *Journal of Experimental Botany* **57**, 381–390.
- Dupuis, I. and Dumas, C. (1990). Influence of temperature stress on maize (*in vitro*) fertilization and heat shock protein synthesis in maize (*Zea mays* L.) reproductive tissue. *Plant Physiology* **94**, 665–670.
- Eastburn, D.M., DeGennaro, M.M., DeLucia, E.H., Dermody, O. and McElrone, A.J. (2010). Elevated atmospheric carbon dioxide and ozone alter soybean diseases at SoyFACE. *Global Change Biology* **16**, 320–330.
- Erbs, M., Manderscheid, R., Huther, L., Schenderlein, A., Wieser, H., Danicke, S. and Weigel, H-J. (2015). Free-air CO<sub>2</sub> enrichment modifies maize quality only under drought stress. *Agronomy for Sustainable Development* **35**, 203–212.
- Fargette, D., Konate, G., Fauquet, C., Muller, E., Peterschmitt, M. and Thresh, J.M. (2006). Molecular ecology and emergence of tropical plant viruses. *Annual Review of Phytopathology* **44**, 235–260.
- Felle, H. H., Herrmann, A., Hanstein, S., Huckelhoven, R. and Kogel, K. H. (2004). Apoplastic pH signaling in barley leaves attacked by the powdery mildew fungus *Blumeria graminis* f.sp. *hordei*. *Molecular Plant Microbe Interactions* **17**, 118–123.
- Fonseca, E, A. and Westgate, E, M. (2005). Relationship between desiccation and viability of maize pollen. *Field Crops Research* **94**, 114–125.
- Fukayama, H., Fukuda, T., Masumoto, C., Taniguchi, Y., Sakai, H., Cheng, W., Hasegawa, T. and Miyao, M. (2009). Rice plant response to long term CO<sub>2</sub> enrichment: gene expression profiling. *Plant Science* **177**, 203–210.

- Garraway, M.O., Akhtar, M. and Wokoma, E.C.W.(1989). Effect of high temperature stress on peroxidase activity and electrolyte leakage in maize in relation to sporulation of *Bipolaris maydis* race T. *Phytopathology* (USA) **79**, 800–805.
- Ghannoum, O. (2009). C<sub>4</sub> photosynthesis and water stress. *Annals of Botany* **103**, 635–644.
- Ghannoum, O., von Caemmerer, S., Barlow, E.W.R. and Conroy, J.P. (1997). The effect of CO<sub>2</sub> enrichment and irradiance on the growth, morphology and gas exchange of a C<sub>3</sub> (*Panicum laxum*) and a C<sub>4</sub> (*Panicum antidotale*) grass. *Australian Journal of Plant Physiology* **24**, 227–237.
- Grass, L. and Burris, I. (1995). Effect of heat stress during seed development and maturation on wheat (*Triticum durum*) seed quality. I. Seed germination and seedling vigor. *Canadian Journal of Plant Science* **75**, 821–829.
- Grossman, J. D. and Rice, K. J. (2014). Contemporary evolution of an invasive grass in response to elevated atmospheric CO<sub>2</sub> at a Mojave Desert FACE site. *Ecology Letters* 17: 710–716.
- Gupta, K., Saharan, G.S. and Singh, D. (2002). Effective selection criteria to assess disease tolerance in Indian mustard [*Brassica juncea* (L.) Czern & Coss]. *Journal of Mycology and Plant Pathology* **32**, 72–80.
- Hamilton, J.G., Dermody, O., Aldea, M., Zangerl, A.R., Rogers, A., Berenbaum, M.R. and Delucia, E. H. (2005). Anthropogenic changes in tropospheric composition increase susceptibility of soybean to insect herbivory. *Environmental Entomology* **34**, 479–485.
- Heagle, A.S. (2003). Influence of elevated carbon dioxide on interactions between *Frankliniella occidentalis* and *Trifolium repens*. *Environmental Entomology* **32**, 421–424.
- Heath, R.L. and Packer, L. (1968). Photoperoxidation in isolated chloroplast I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics* **25**, 189–198.
- Herrero, M.P. and Johnson, R.R. (1980). High temperature stress and pollen viability of maize. *Crop Science* **20**, 796–800.

- Hibberd, J., Whitbread, R. and Farrar, J. (1996). Effect of 700  $\mu\text{mol mol}^{-1}$   $\text{CO}_2$  and infection by powdery mildew on the growth and carbon partitioning of barley. *New Phytologist* **134**, 309–315.
- Hooker, A.L. (1972). Southern leaf blight of corn – present status and future prospects. *Journal of Environmental Quality* **1**, 244–249.
- Hussain, M.Z., Vanloocke, A., Siebers, M.H., Ruiz-vera, U.M., Markelz, R.J.C., Leakey, A.D.B., Ort, D.R. and Bernacchi, C.J. (2013). Future carbon dioxide concentration decreases canopy evapotranspiration and soil water depletion by field grown maize. *Global Change Biology* **19**, 1572–1584.
- Idso, S.B. (1987). Effects of atmospheric  $\text{CO}_2$  enrichment on plant growth: the interactive role of air temperature. *Agricultural Ecosystems and Environment* **20**, 1–10.
- IPCC (2013). Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 1535.
- Jablonski, L.M., Wang, X. and Curtis, P.S. (2002). Plant reproduction under elevated  $\text{CO}_2$  conditions: a meta-analysis of reports on 79 crop and wild species. *New Phytologist* **156**, 9–26.
- Jenner, C.F. (1991). Effects of exposure of wheat ears to high temperature on dry matter accumulation and carbohydrate metabolism in grain of two cultivars. I. Immediate response. *Australian Journal of Plant Physiology* **18**, 165–177.
- Jiang, Y.F., Cheng, W.D., Qin, L.Q., Tan, X.J., Zhou, J.G., Xie, H.X. and Chen, L. (2009). Physiological mechanism of exogenous salicylic acid induced resistance to sheath blight of maize (*Zea mays* L.). *Guangxi Academy of Agricultural Sciences* **40**, 242–245.
- Jochum, G.M., Mudge, K.W. and Thomas, R.B. (2007). Elevated temperatures increase leaf senescence and root secondary metabolite concentration in the understory herb *Panax quinquefolius* (Araliaceae). *American Journal of Botany* **94**, 819–826.

- Jwa, N-S. and Walling, L.L. (2001). Influence of elevated CO<sub>2</sub> concentration on disease development in tomato. *New Phytologist* **149**, 509–518.
- Karim, A., Hamid, A. and Rahman, S. (2000). Grain growth and yield performance of wheat under subtropical conditions: II. Effect of water stress at reproductive stage. *Cereal Research Communications* **28**, 101–107.
- Karim, M.A., Fracheboud, Y. and Stamp, P. (1997). Heat tolerance of maize with reference of some physiological characteristics. *Annals of Bangladesh Agriculture* **7**, 27–33.
- Karnosky, D.F., Zak, D.R., Pregitzer, K.S. *et al.* (2003). Tropospheric O<sub>3</sub> moderates responses of temperate hardwood forests to elevated CO<sub>2</sub>: a synthesis of molecular to ecosystem results from the Aspen FACE project. *Functional Ecology* **17**, 289–304.
- Katiyar, V. and Dubey, P. S. (2001). Sulphur dioxide sensitivity on two stage of leaf development in a few tropical tree species. *Indian Journal of Environmental Toxicology* **11**, 78–81.
- Kim, S-H., Dennis, C.G., Richard, C.S., Jeffrey, T.B., Dennis, J.T. and Vangimalla, R.R. (2007). Temperature dependence of growth, development and photosynthesis in maize under elevated CO<sub>2</sub>. *Environmental and Experimental Botany* **61**, 224–236.
- Kimball, B.A. (1983). Carbon dioxide and agricultural yield: an assemblage and analysis of 430 prior observations. *Agronomy Journal* **75**, 779–788.
- Kimball, B.A. (2011). Lessons from FACE: CO<sub>2</sub> effects and interactions with water, nitrogen and temperature. In: Hillel, D. and Rosenzweig, C. (eds.). *Handbook of climate change and agro ecosystems: Impacts, adaptation, and mitigation*. Imperial College Press, Hackensack, NJ. pp. 87–107.
- Kimball, B.A., Kobayashi, K. and Bindi, M. (2002). Responses of agricultural crops to free-air CO<sub>2</sub> enrichment. *Advances in Agronomy* **77**, 293–368.
- Kobayashi, T., Ishiguro, K., Nakajima, T., Kim, H., Okada, M. and Kobayashi, K. (2006). Effects of elevated atmospheric CO<sub>2</sub> concentration on the infection of rice blast and sheath blight. *Phytopathology* **96**, 425–431.
- Körner, C. (2006). Significance of temperature in plant life. In: Morison, J.I.L. and Morecroft, M.D.(eds.). *Plant Growth and Climate Change*. Blackwell Publishing Ltd, Oxford, UK, pp 48–69.

- Kumar, M., and Nandini, N. (2013). Identification and evaluation of air pollution tolerance index of selected avenue tree species of urban Bangalore, India. *International Journal of Emerging Technologies in Computational and Applied Sciences* **13**, 388–390.
- Lake, J.A. and Wade, R.N. (2009). Plant-pathogen interactions and elevated CO<sub>2</sub>: morphological changes in favour of pathogens. *Journal of Experimental Botany* **60**, 3123–3131.
- Lam, S.K., Chen, D., Norton, R. and Armstrong, R. (2013). The effect of elevated atmospheric carbon dioxide concentration on the contribution of residual legume and fertilizer nitrogen to a subsequent wheat crop. *Plant and Soil* **364**, 81–91.
- Law, R.D. and Crafts-Brandner, S.J. (1999). Inhibition and acclimation of photosynthesis to heat stress is closely correlated with activation of ribulose-1, 5-bisphosphate carboxylase/oxygenase. *Plant Physiology* **120**, 173–181.
- Leakey, A.D., Xu, F., Gillespie, K.M., McGrath, J.M., Ainsworth, E.A. and Ort, D.R. (2009). Genomic basis for stimulated respiration by plants growing under elevated carbon dioxide. *Proceedings of the National Academy of Sciences, USA* **106**, 3597– 3602.
- Li, P.H., Ainsworth, E.A., Leakey, A.D.B., Ulanov, A., Lozovaya, V., Ort, D.R. *et al.* (2008). Arabidopsis transcript and metabolite profiles: ecotype-specific responses to open-air elevated [CO<sub>2</sub>]. *Plant, Cell and Environment* **31**, 1673–1687.
- Lobell, D.B., Hammer, G.L., Mclean, G., Messina, C., Roberts, M.J. and Schlenker, W. (2013). The critical role of extreme heat for maize production in the United States. *Nature Climate Change* **3**, 497–501.
- Long, S.P., Ainsworth, E.A., Rogers, A. and Ort, D.R. (2004). Rising atmospheric carbon dioxide: plants FACE the future. *Annual Review of Plant Biology* **55**, 591–628.
- Lui, Y. J. and Ding, H. (2008). Variation in air pollution tolerance index of plants near a steel factory, implication for landscape plants species selection for industrial areas. *WSEAS Trans boundary Environmental Action Project on Environment and Development* **4**, 24–32.

- Machado, S. and Paulsen, G.M. (2001). Combined effects of drought and high temperature on water relations of wheat and sorghum. *Plant and Soil* **233**, 179–187.
- Maestre, F.T., Salguero-Gomez, R. and Quero, J.L. (2012). It is getting hotter in here: determining and projecting the impacts of global environmental change on drylands. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **367**, 3062–3075.
- Malstrom, C.M. and Field, C.B. (1997). Virus-induced differences in the response of oat plants to elevated carbon dioxide. *Plant, Cell and Environment* **20**, 178–188.
- Mangelsdorf, P. C. and Reeves, R. G. (1939). The origin of indian corn and its relatives. *Texas Agricultural Experiment Station Report* 4574.
- Mangelsdorf, P.C. (1974). Corn: its origin, Evolution and Improvement. The Belknap Press of Harvard University Press, Cambridge, Massachusetts.
- Manning, W. and Tiedemann, A. (1995). Climate change: potential effects of increased atmospheric carbon dioxide (CO<sub>2</sub>), ozone (O<sub>3</sub>), and ultraviolet-B (UV-B) radiation on plant diseases. *Environmental Pollution* **88**, 219–245.
- Matros, A., Amme, S., Kettig, B., Buck-Sorlin, G.H., Sonnewald, U. and Mock, H-P. (2006). Growth at elevated CO<sub>2</sub> concentrations leads to modified profiles of secondary metabolites in tobacco cv. SamsunNN and to increased resistance against infection with potato virus Y. *Plant, Cell and Environment*, **29**, 126–137.
- Mazorra, L.M., Nunez, M., Echerarria, E., Coll, F. and Sanchez-Blanco, M.J. (2002). Influence of brassinosteroids and antioxidant enzymes activity in tomato under different temperatures. *Biologia Plantarum* **45**, 593–596.
- McElrone, A.J., Reid, C.D., Hoye, K.A., Hart, E. and Jackson, R.B. (2005). Elevated CO<sub>2</sub> reduces disease incidence and severity of a red maple fungal pathogen via changes in host physiology and leaf chemistry. *Global Change Biology* **11**, 1828–1836.
- McElrone, A.J., Seraldi, J.L. and Forseth, I.N. (2001). Effects of water stress on symptomatology and growth of *Parthenocissus quinquefolia* infected by *Xylella fastidiosa*. *Plant Disease* **85**, 1160–1164.

- Meena, B.L., Meena, R.P., Meena, R.R. and Singh, B. (2014). Popularization of improved maize (*Zea mays* L.) production technology through frontline demonstrations in semi-arid zone IVA of Rajasthan. *Journal of Applied and Natural Science* **6**, 763–769.
- Melloy, P., Hollaway, G., Luck, J., Norton, R., Aitken, E. and Chakraborty, S. (2010). Production and fitness of *Fusarium pseudograminearum* inoculum at elevated carbon dioxide in FACE. *Global Change Biology* **16**, 3363–3373.
- Milcu, A., Lukac, M., Subke, J-A., Manning, P., Heinemeyer, A., Wildman, D., Anderson, R. and Ineson, P. (2012). Biotic carbon feedbacks in a materially closed soil–vegetation–atmosphere system. *Nature Climate Change* **2**, 281–284.
- Milus, E., Seyran, E. and McNew, R. (2006). Aggressiveness of *Puccinia striiformis* f. sp. *tritici* isolates in the south-central United States. *Plant Disease* **90**, 847–852.
- Misra, A.P. (1979). Variability, physiologic specialization and genetics of pathogenicity in *Graminicolus helminthosporia* affecting cereal crops. *Indian Phytopathology* **32**, 1–22.
- Morecroft, M.D. and Paterson, J.P. (2006). Effects of temperature and precipitation changes on plant communities. In: Morison, J. and Morecroft, M.D.(eds.). *Plant Growth and Climate Change*. Blackwell, Oxford. pp. 146–164.
- Morison, J.I.L. and Lawlor, D.W. (1999). Interactions between increasing CO<sub>2</sub> concentration and temperature on plant growth. *Plant, Cell and Environment* **22**, 659–682.
- Munjal, R.L. and Kapoor, J.N. (1960). Some unrecorded diseases of sorghum and maize from India. *Current Science* **29**, 442–443.
- Nasir, A., Singh, V. K., and Singh, A. (2012). Management of maydis leaf blight using fungicides and phytoextracts in maize. *Maize Journal* **1**, 106–109.
- Naveed, S., Aslam, M., Maqbool, M.A., Bano, S., Zaman, Q.U. and Ahmad, R.M. (2014). Physiology of high temperature stress tolerance at reproductive stages in maize. *The Journal of Animal & Plant Sciences* **24**, 1141–1145.

- Nayyar, H. and Gupta, D. (2006). Differential sensitivity of C<sub>3</sub> and C<sub>4</sub> plants to water deficit stress: association with oxidative stress and antioxidants. *Environmental and Experimental Botany* **58**, 106–113.
- Noormets, A., Kull, O., Sober, A., Kubiske, M.E. and Karnosky, D.F. (2010). Elevated CO<sub>2</sub> response of photosynthesis depends on ozone concentration in aspen. *Environmental Pollution* **158**, 992–999.
- Norby, R.J. and Luo, Y. (2004). Evaluating ecosystem responses to rising atmospheric CO<sub>2</sub> and global warming in a multi-factor world. *New Phytologist* **162**, 281–293.
- Onderdonk, J.J. and Ketcheson, J.W. (1972). A standardization of terminology for the morphological description of corn seedlings. *Canadian Journal of Plant Science* **52**, 1003–1006.
- Pal, D. and Kaiser, S. A. K. M. (2001). Effect of agronomic practices on Maydis leaf blight disease of maize. *Journal of Mycopathological Research* **39**, 77–82.
- Payak, M. M. and Sharma, R. C. (1981). Disease and pest situation in high yielding hybrids and composites of maize with special reference to India. A review of pest, diseases and weed complexes in high yielding varieties in Asia and Pacific. F. A. O. Regional office, Bangkok, Thailand. pp. 84–89.
- Payak, M. M. and Sharma, R. C. (1983). Disease rating scales in maize in India. In: techniques of scoring for resistance to diseases of maize in India. All India Co-ordinated Maize Improvement Project, IARI, New Delhi. pp.1–4.
- Payak, M.M. and Sharma, R.C. (1978). Research on diseases of maize. PL-480 Project final technical report (April 1969-March 1975). New Delhi: Indian Council of Agricultural Research. pp. 228.
- Payak, M.M. and Sharma, R.C. (1985). Maize diseases and approaches to their management. *Indian Tropical Pest Management* **31**, 302–310.
- Peacock, J.M. and Heinrich, G.M. (1984). Light and temperature responses in sorghum. In: *Agrometeorology of Sorghum and Millet in the Semi-Arid Tropics: Proceedings of the International Symposium* (ed. ICRISAT), November 1982, ICRISAT, Patancheru, India. pp. 143–158.

- Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S. *et al.* (2013). New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany* **61**, 167–234.
- Pingali, P.L. and Pandey, S. (2001). Participatory Methods in the Development and Dissemination of New Maize Technologies. World Maize Facts and Trends/Overview and Outlook CIMMYT: International Maize and Wheat Improvement Center, Mexico.
- Plessl, M., Elstner, E.F., Rennenberg, H., Habermeyer, J. and Heiser, I. (2007). Influence of elevated CO<sub>2</sub> and ozone concentrations on late blight resistance and growth of potato plants. *Environmental and Experimental Botany* **60**, 447–457.
- Poorter, H. (1993). Interspecific variation in the growth response of plants to an elevated ambient CO<sub>2</sub> concentration. *Vegetatio* **105**, 77–97.
- Prasad, P.V.V., Boote, K.J. and Jr, L.H.A. (2006). Adverse high temperature effects on pollen viability, seed-set, seed yield and harvest index of grain-sorghum [*Sorghum bicolor* (L.) Moench] are more severe at elevated carbon dioxide due to higher tissue temperatures. *Agricultural and Forest Meteorology* **139**, 237–251.
- Qu, M.N., Bunce, J.A. and Shi, Z.S. (2014). Does elevated CO<sub>2</sub> protect photosynthesis from damage by high temperature *via* modifying leaf water status in maize seedlings? *Photosynthetica* **52**, 211–216.
- Rahman, S.U., Arif, M., Hussain, K., Hussain, S., Mukhtar, T., Razaq, A. and Iqbal, R.A. (2013). Evaluation of maize hybrids for tolerance to high temperature stress in Central Punjab. *American Journal of Bioengineering and Biotechnology* **1**, 30–36.
- Ramakrishna, A. and Ravishankar, G.A. (2011). Influence of abiotic stress singles on secondary metabolites in plants. *Plant Signaling and Behavior* **6**, 1720–1731.
- Rattalino Edreira, J.I., Mayer, L.I. and Otegui, M.E. (2014). Heat stress in temperate and tropical maize hybrids: Kernel growth, water relations and assimilate availability for grain filling. *Field Crops Research* **166**, 162–172.

- Raza, S. H. and Murthy, M. S. R. (1988). Air pollution tolerance index of certain plants of Nacharam Industrial Area, Hyderabad. *Indian Journal of Botany* **11**, 91–95.
- Raziq, F. and Ahmad, S. (1992). Comparison of maydis leaf blight epidemics at different stages of maize development. *Sarhad Journal of agriculture* **8**, 569–573.
- Renard, G. (2014). Maize CRP: extension proposal 2015-2016. CIMMYT publication repository. Available at [www.cimmyt.org/](http://www.cimmyt.org/) (visited on February 11, 2015).
- Robredo, A., Pérez-López, U., Sainz de la Maza, H., González-Moro, B., Lacuesta, M., Mena-Petite, A. and Muñoz-Rueda, A. (2007). Elevated CO<sub>2</sub> alleviates the impact of drought on barley improving water status by lowering stomatal conductance and delaying its effects on photosynthesis. *Environmental and Experimental Botany* **59**, 252–263.
- Rosenberg, N.J. (1990). From Climate and CO<sub>2</sub> enrichment to evapotranspiration. In: Waggoner, P.E. (eds.). *Climate change and U.S. water resources*. New York, John Wiley & Sons. pp. 151–175.
- Sadasivam, S. and Balasubramanian, T. (1987). Practical manual in biochemistry, Tamil Nadu Agricultural University, Coimbatore, pp. 14.
- Sairam, R.K. and Saxena, D.C. (2000). Effect of elevated temperature on oxidative stress and antioxidant activity in wheat genotypes. Possible mechanism of heat stress tolerance. *Biologia Plantarum* **43**, 245–251.
- Sakschewski, B., von Bloh, W., Huber, V., Müller, C. and Bondeau, A. (2014). Feeding 10 billion people under climate change: How large is the production gap of current agricultural systems? *Ecological Modelling* **288**, 103–111.
- Salvucci, M.E. and Crafts-Brandner, S.J. (2004). Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. *Physiologia Plantarum* **120**, 179–186.
- Sauer, T.J., Singer, J.W., Prueger, J.H., DeSutter, T.M. and Hatfield, J.L. (2007). Radiation balance and evaporation partitioning in a narrow-row soybean canopy. *Agricultural and Forest Meteorology* **145**, 206–214.

- Saxon, M.E., Davis, M.A., Pritchard, S.G., Runion, G.B., Prior, S.A., Stelzer, H.E. Rogers, H.H. and Dute, R.R. (2004). Influence of elevated CO<sub>2</sub>, nitrogen, and *Pinus elliotti* genotypes on performance of the redheaded pine sawfly *Neodiprion lecontei*. *Canadian Journal of Forest Research* **34**, 1007–1017.
- Schooper, J.B., Lambert, R.J. and Vasilas, B.L. (1986). Maize pollen viability and ear receptivity under water and high temperature stress. *Crop Science* **26**, 1029–1033.
- Schooper, J.B., Lambert, R.J., Vasilas, B.L. and Westgate, M.E. (1987). Pollen viability, pollen shedding, and combining ability for tassel heat tolerance in maize. *Crop Science* **27**, 27–31.
- Shah, N.H. and Paulsen, G.H. (2003). Interaction of drought and high temperature on photosynthesis and grain filling of wheat. *Plant and Soil* **257**, 219–226.
- Sharma, A. R. and Dass, A. (2014). Maize. In: Textbook of field crop production, Volume-1, Prasad, R. (ed.). ICAR, New Delhi. pp. 98–136.
- Sharma, H.C., Sharma, K.K., Seetharama, N. and Ortiz, R. (2000). Prospects for using transgenic resistance to insects in crop improvement. *Electronic Journal of Biotechnology* **3**, 32–47.
- Sharma, K.L., Mandal, U.K., Srinivas, K., Vittal, K.P.R., Mandal, B., Grace, J.K. and Ramesh, V. (2005). Long-term soil management effects on crop yields and soil quality in a dryland Alfisol. *Soil & Tillage Research* **83**, 246–259.
- Sharma, R.C. and Rai, S.N. (2000). Assessment and quantitative analysis of losses due to *Dreschlera maydis* in maize. In: *Proceedings International Conference on Integrated Plant Disease Management for Sustainable Agriculture*, Indian Phytopathological Society, Division of Plant Pathology, IARI, New Delhi, pp. 247–248.
- Sharma, R.C., Vasal, S.K., Gonzalez, F., Batsa, B.K. and Singh, N.N. (2002). Redressal of banded leaf and sheath blight of maize through breeding, chemical and biocontrol agents. In: *Proceedings of the 8th Asian Regional Maize Workshop: New Technologies for the New Millennium*, Bangkok. pp. 391–397.
- Shekhar, M. and Kumar, S. (2013). Maize disease scenario in India and their management through integrated management approach. In: *Maize*

- production systems for improving resource-use efficiency and livelihood security*. Kumar, A., Jat, S.L., Kumar, R. and Yadav, O.P. (eds.). Directorate of Maize Research, Pusa Campus, New Delhi. pp. 123.
- Sicher, R.C. and Bunce, J.A. (2015). The impact of enhanced atmospheric CO<sub>2</sub> concentrations on the responses of maize and soybean to elevated growth temperatures. In: *Combined Stresses in Plants*. Mahalingam, R. (ed.). Springer International Publishing, Switzerland.
- Singh, A. (1997). Air Pollution tolerance indices (APTI) of some plants. [www.bioline.org.br/pdf](http://www.bioline.org.br/pdf). Accessed on 16th September, 2014.
- Singh, B., Chand, D., Maurya, A.K., Sharma, R. and Agarwal, P.C. (2014). Pathogenic fungi intercepted in introduced transgenics during 2006-2013. *Plant Pathology & Quarantine* **4**, 99–107.
- Singh, S.N. and Verma, A. (2007). Phytoremediation of Air Pollutants, A review. In: Singh, S.N. and R.D. Tripathi (eds.). *Environmental Bioremediation Technology*, Springer, Berlin Heidelberg, Vol.1, pp.293–314.
- Sinha, P.G., Kapoor, R., Uprety, D.C. and Bhatnagar, A.K. (2009). Impact of elevated CO<sub>2</sub> concentration on ultrastructure of pericarp and composition of grain in three *Triticum* species of different ploidy levels. *Environmental and Experimental Botany* **66**, 451–456.
- Siqueira, J.O., Nair, M.G., Hammerschmidt, R. and Safir, G.R. (1991). Significance of phenolic compounds in plant–soil–microbial systems. *Critical Reviews in Plant Sciences* **10**, 63–72.
- Smirnoff, N. (2005). Ascorbate, tocopherol and carotenoids: metabolism, pathway engineering and functions. In: Smirnoff, N. (ed.). *Antioxidants and Reactive Oxygen Species in Plants*. Blackwell Publishing Ltd., Oxford, UK. pp. 53–86.
- Smith, N.G. and Dukes, J.S. (2013). Plant respiration and photosynthesis in global-scale models: incorporating acclimation to temperature and CO<sub>2</sub>. *Global Change Biology* **19**, 45–63.
- Snow, M.D., Bard, R.R., Olszyk, D.M., Minster, L.M., Hager, A.N. and Tingey, D.T. (2003). Monoterpene levels in needles of Douglass Fir exposed to elevated CO<sub>2</sub> and temperature. *Physiologia Plantarum* **117**, 352–358.

- Struik, P.C., Doorgeest, M. and Boonman, J.G. (1986). Environmental effects on flowering characteristics and kernel set of maize (*Zea mays* L.). *Netherlands Journal of Agricultural Science* **34**, 469–484.
- Suwa, R., Hakata, H., Hara, H., El-Shemy, H.A., Adu-Gyamfi, J.J., Nguyen, N.T., Kanai, S., Lightfoot, D.A., Mohapatra, P.K. and Fujita, K. (2010). High temperature effects on photosynthate partitioning and sugar metabolism during ear expansion in maize (*Zea mays* L.) genotypes. *Plant Physiology and Biochemistry* **48**, 124–130.
- Szarka, A., Horemans, N., Kovacs, Z., Grof, P., Mayer, M. and Banhegyi, G. (2007). Dehydroascorbate reduction in plant mitochondria is coupled to the respiratory electron transfer chain. *Physiologia Plantarum* **129**, 225–232.
- Taiz, L. and Zeiger, E. (2010). *Plant Physiology*, 5<sup>th</sup> edition. Sinauer, Sunderland, MA.
- Talwar, H.S., Takeda, H., Yashima, S. and Senboku, T. (1999). Growth and photosynthetic responses of groundnut genotypes to high temperature. *Crop Science* **39**, 460–466.
- Taylor, G., Street, N.R., Tricker, P.J., Sjödin, A. *et al.* (2005). The transcriptome of *Populus* in elevated CO<sub>2</sub>. *New Phytologist* **167**, 143–154.
- Tedford, E.C., Miller, T.L. and Nielsen, M.T. (1990). A detached-leaf technique for detecting resistance to *Phytophthora parasitica* var. *nicotianae* in tobacco. *Plant Disease* **74**, 313–316.
- Teng, N., Wang, J., Chen, T., Wu, X., Wang, Y. and Lin, J. (2006). Elevated CO<sub>2</sub> induces physiological, biochemical and structural changes in leaves of *Arabidopsis thaliana*. *New Phytologist* **172**, 92–103.
- Thiagarajah, M.R. and Hunt, L.A. (1982). Effects of temperature on leaf growth in corn (*Zea mays* L.). *Canadian Journal of Botany* **60**, 1647–1652.
- Tiedemann, A.V. and Firsching, K.H. (2000). Interactive effects of elevated ozone and carbon dioxide on growth and yield of leaf rust infected versus non-infected wheat. *Environmental Pollution* **108**, 357–363.
- Todorov, D., Karanov, E., Smith, A. R. and Hall, M. A. (2003). Chlorophyllase activity and chlorophyll content in wild and mutant plants of *Arabidopsis thaliana*. *Biologia Plantarum* **46**, 125–127.

- Tollenaar, M. and Dwyer, L.M. (1999). Physiology of maize. In: Crop Yield-Physiology and Processes. D.L. Smith and C. Hamel. (eds.). Springer-Verlag, Berlin, Heidelberg, Germany.
- Tollenaar, M., Daynard, T.B. and Hunter, R.B. (1979). Effect of temperature on rate of leaf appearance and flowering date in maize. *Crop Science* **19**, 363–366.
- Torbert, H.A., Prior, S.A., Rogers, H.H. and Runion, G.B. (2004). Elevated atmospheric CO<sub>2</sub> effects on N fertilization in grain sorghum and soybean. *Field Crops Research* **88**, 57–67.
- Traore, S.B., Carlson, E.R., Pilcher, D.C. and Rice, E.M. (2000). Bt and non Bt maize growth and development as affected by temperature and drought stress. *Agronomy Journal* **92**, 1027–1035.
- Twine, T.E., Bryant, J.J., Richter, K.T., Bernacchi, C.J., McConaughay, K.D., Morris, S.J. and Leakey, A.D.B. (2013). Impacts of elevated CO<sub>2</sub> concentration on the productivity and surface energy budget of the soybean and maize agro-ecosystem in the Midwest USA. *Global Change Biology* **19**, 2838–2852.
- Van de Geijn, S.C. and Goudriaan, J. (1996). The effects of elevated CO<sub>2</sub> and temperature change on transpiration and crop water use. In: Kor, F. and Sombroek, W. (eds.). *Global climate change and agricultural production*. New York : FAO and John Wiley & Sons. pp. 101–122.
- Vaughan, M.M., Huffaker, A., Schmelz, E.A., Dafoe, N.J., Christensen, S., Sims, J., Martins, V.F., Swerbilow, J., Romero, M., Alborn, H.T., Allen, L.H. and Teal, P.E.A. (2014). Effects of elevated [CO<sub>2</sub>] on maize defence against mycotoxigenic *Fusarium verticillioides*. *Plant, Cell and Environment* **37**, 2691–2706.
- von Caemmerer, S. and Furbank, R.T. (2003). The C<sub>4</sub> pathway: an efficient CO<sub>2</sub> pump. *Photosynthesis Research* **77**, 191–207.
- Wahid, A. (2007). Physiological implications of metabolites biosynthesis in net assimilation and heat stress tolerance of sugarcane (*Saccharum officinarum*) sprouts. *Journal of Plant Research* **120**, 219–228.

- Wahid, A. and Close, T.J. (2007). Expression of dehydrins under heat stress and their relationship with water relations of sugarcane leaves. *Biologia Plantarum* **51**, 104–109.
- Wahid, A., Gelani, S., Ashraf, M. and Foolad, M.R. (2007). Heat tolerance in plants: An overview. *Environmental and Experimental Botany* **61**, 199–223
- Ward, S.J.E., Midgley, G.F., Jones, M.H. and Curtis, P.S. (1999). Responses of wild C<sub>4</sub> and C<sub>3</sub> grass (*Poaceae*) species to elevated atmospheric CO<sub>2</sub> concentration: a meta-analytic test of current theories and perceptions. *Global Change Biology* **5**, 723–741.
- Wang, D., Heckathorn, S.A., Wang, Z. and Philpott, S.M. (2012). A meta-analysis of plant physiological and growth responses to temperature and elevated CO<sub>2</sub>. *Oecologia* **169**, 1–13.
- Wang, M., Xie, B., Fu, Y., Dong, C., Hiu, L., Guanghui, L. and Liu, H. (2015). Effects of different elevated CO<sub>2</sub> concentrations on chlorophyll contents, gas exchange, water use efficiency, and PSII activity on C<sub>3</sub> and C<sub>4</sub> cereal crops in a closed artificial ecosystem. *Photosynthesis Research*. DOI 10.1007/s 11120-015-0134-9.
- Wang, S.Y., Bunce, J.A. and Maas, J.L. (2003). Elevated carbon dioxide increases contents of antioxidant compounds in field-grown strawberries. *Journal of Agricultural and Food Chemistry* **51**, 4315–4320.
- Warren, J.M., Norby, R.J. and Wullschlegel, S.D. (2011). Elevated CO<sub>2</sub> enhances leaf senescence during extreme drought in a temperate forest. *Tree Physiology* **31**, 117–130.
- Watson, L., and Dallwitz, M. J. (1992). The grass genera of the world. C.A.B. International, University of Michigan, Michigan, pp. 1038.
- Wheeler, T.R., Morison, J.I.L., Ellis, R.H. and Hadley, P. (1994). The effects of CO<sub>2</sub>, temperature and their interaction on the growth and yield of carrot (*Daucus carota* L.). *Plant, Cell and Environment* **17**, 1275–1284.
- Wilkes, H.G. (1979). Mexico and Central America as a center for the origin of maize. *Crop Improvement (India)* **6**, 1-18.
- Xu, Z.Z., Shimizu, H., Yagasaki, Y., Ito, S., Zheng, Y.R. and Zhou, G.S. (2013). Interactive effects of elevated CO<sub>2</sub>, drought, and warming on plants. *Journal of Plant Growth Regulation* **32**, 692–707.

- Yu, J., Chen, L., Xu, M. and Huang, B. (2012). Effects of elevated CO<sub>2</sub> on physiological responses of tall fescue to elevated temperature, drought stress, and the combined stresses. *Crop Science* **52**, 1848–1858.
- Zhang, L.L., Han, S.C., Li, Z.G., Liu, N., Li, L.Y., Luo, L.F., Peng, T.X. and Liu, W.H. (2006). Effects of the infestation by *Actinote thalia pyrrha* (Fabricius) on the physiological indexes of *Mikania micrantha* leaves. *Acta Ecologica Sinica* **26**, 1330–1336.
- Zhao, H., Li, Y., Zhang, X., Korpelainen, H. and Li, C. (2012). Sex-related and stage-dependent source-to-sink transition in *Populus cathayana* grown at elevated CO<sub>2</sub> and elevated temperature. *Tree Physiology* **32**, 1325–1338.
- Zinn, K.E., Tunc-Ozdemir, M. and Harper, J.F. (2010). Temperature stress and plant sexual reproduction: uncovering the weakest links. *Journal of Experimental Botany* **61**, 1959–1968.
- Ziska, L.H. and Runion, G.B. (2007). Future weed, pest, and disease problems for plants. In: Newton, P.C.D., Carran, R.A., Edwards, G.R. and Niklaus, P.A. (eds.). *Agroecosystems in a Changing Climate*. Boca Raton, FL, USA: CRC Press, 261–287.