Environment & Ecology 35 (2) : 768—773, April—June 2017
Website: environmentandecology.com ISSN 0970-0420

Repercussion of Elevated CO_2 Concentration on Wheat Susceptibility to *Bipolaris Sorokiniana*

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Received 11 June 2016 ; Accepted 13 July 2016 ; Published online 5 August 2016

Abstract Incidence of spot blotch disease on *Triticum aestivum* was studied by imposing three different treatments of CO_2 concentrations (350 ppm, 400 ppm, 500 ppm) to investigate there effect on some of the plant physiological parameters i.e. length and density of stomata, leaf length and protein expression. Experimental results revealed a negative correlation of 0.98 that the stomata density decreased (65.80 / mm^2 to 49.00 / mm^2) with increase in CO_2 concentration from 350 ppm to 500 ppm whereas a positive correlation of 0.85 was observed between the length of stomata and CO_2 concentration (54.20 µm at 350 ppm to 65.20 µm at 500 ppm). Increase in mean leaf length was also found positively correlated (0.972) with CO2 concentration. The mean leaf length increased from 18.95 cm at 350 ppm to 40.68 cm at 500 ppm. It was also inferred from the results that the length and density of stomata were mutually negatively correlated (0.75). SDS-PAGE analysis of five different protein bands (22 kDa, 26 kDa, 32 kDa, 36 kDa and 43 kDa) having different antifungal activities (Thaumatin like properties, β -1, 3-glucanase activity, chitinase activity) revealed that host plant resistance was attributable to expression of PR-protein.

Keywords CO_2 concentration, Stomata length, Stomata density, PR-protein, SDS-PAGE.

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Introduction

Wheat (*Triticum aestivum*) is one of the major staple crop which contributes 30% of crop production in India. According to an estimate, in 2030 the increased demand of wheat production will be 100 million metric ton [1]. However, the crop production is affected due to disease dynamicity, geographic distribution of the pathogen, physiology of the plant and its resistance against the pathogen. Recent changes in the environmental conditions such as soil fertility, variation in rainfall pattern, hot and dry climates and extreme weather events also affect crop production. These changes have resulted in the increase in frequency of disease occurrence, pest epidemics and appearance of odd symptoms.

Among the occurrence of various diseases, spot

blotch, caused by *Bipolaris sorokiniana*, is one of the most destructive disease prevalent under warm climate in rice-wheat cropping system. The conidia of *B. sorokiniana* present on soil surface are dispersed by wind, may enter through the stomata [2], therefore, the length and density of stomata has critical role on the host plant resistance. Also, the climate change increases the susceptibility to spot blotch because of necrotrophic lifestyle of fungus [3]. Oxidative stress, changes in photosynthetic rate, photorespiration, leaf senescence and yield have been resulted due to reactive oxygen species (ROS) induced due to changes in the climatic condition [4].

In this view, the present investigation has been carried out to understand the impact of increased CO_2 concentration on length and density of stomata, leaf length and protein expression determining the resistance against the spot blotch.

Materials and Methods

In vitro infection and sample preparation

The test plant (wheat) seeds and the spores of *B. sorokiniana* were collected from the Division of plant pathology, IARI, Pusa. Seeds were surface sterlized with 5% Sodium Hypochlorite (NaOCl) for 5 min, rinsed in water and air dried. Ten sterilized seeds were then sown in small pots containing perlite (sand : clay in 2 : 1 ratio). These pots were kept at the National Phytotron Facility, IARI under three different CO_2 concentrations viz. 350 ppm (control) 400 ppm and 500 ppm 25°C which were sprayed with a spore suspension (10^4 spores / ml) of *B. sorokiniana* at 30 DAG. Inoculated plants were covered with wet poly bags for 24 h to maintain the moist condition.

Sample collection

Leaf samples were collected just before and three days after the spore inoculation. These leaves were kept in sterile poly bags and preserved with liquid nitrogen.

Length and density of stomata

Counting of stomata was done according to the procedure with some modifications [5]. Leaf samples were taken from the different set of treatments and cut into 1 cm^2 size from the middle of the leaves. The samples were kept in ethanol for overnight, washed with distilled water and stained with cotton blue dye. The excess stain was removed by washing again with distilled water. Density of stomata was counted in the available lens area (2.376 mm^2) and length of the stomata was measured using oculometer at 10X magnification.

Leaf length

Leaf growth was assessed in a nondestructive manner by measuring leaf length to the nearest millimeter using a ruler made of graph paper, preventing damage of young leaf tissue.

Extraction of PR Protein

Protein was extracted using the standard method followed by precipitation with Tri-chloro acetic acid (TCA). One gram of leaf sample was taken (Shimazdu AUW 320) and crushed in liquid nitrogen. Four ml of extraction buffer (Tris buffer and QB buffer) was added to it followed by repeated centrifugation (Eppendorf 5418R) at 12,000 rpm (4°C) for 15 min. Supernatant was collected into another micro centrifuge tube and pellet was discarded. The supernatant so obtained was further used for protein precipitation. Extracted protein was quantified using nanodrop spectrophotometer (ND 2000) at 280 nm. Extracted protein was also subjected to the SDS PAGE for the band observation of the PR–proteins.

Statistical analysis

All the statistical analysis was carried out using SAS software.

Results and Discussion

During the investigation, spot blotch was induced *in vitro* by exposing the wheat plant to the fungal pathogen *Bipolaris sorokiniana*. Symptoms of spot blotch

Figure-1: Symptoms of Spot Blotch on wheat leaves

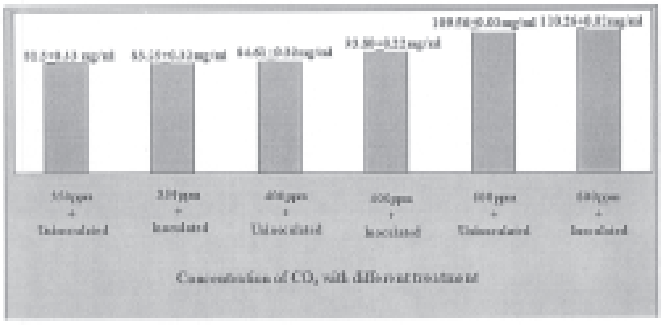

Fig. 1. Symptoms of spot blotch on wheat leaves. **Fig. 2.** Effect of CO_2 concentration and *B. sorokiniana* infection on plant protein concentration.

appeared within three days of inoculation with curling and dark patches on the leaves (Fig. 1). Stomata size and density, as well as leaf length were assessed which is presented in the Table 1. The expression pattern of the isolated protein has been shown in Figure 2.

Table 1. Changes in leaf physiology of plant upon infection at elevated CO_2. **Level of significance 0.05.

Sl. No.	Parameters	Level of treatments	Mean	Standard deviation	Least significant difference	*t*-grouping
1.	Density of Stomata (per mm^2)	350 ppm	65.80	7.50		A**
		400 ppm	58.20	4.27	7.75	B**
		500 ppm	49.00	4.53		B**
2.	Length of stomata (µm)	350 ppm	54.20	6.98		B
		400 ppm	63.00	4.12	8.05	A
		500 ppm	65.20	6.06		A
3.	Leaf length (cm)	350 ppm	18.95	0.90		A**
		400 ppm	30.25	0.74	1.33	B**
		500 ppm	40.69	1.20		C**

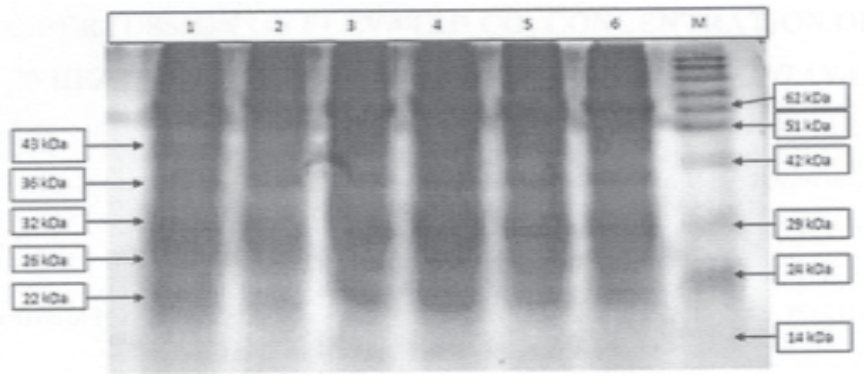

Fig. 3. SDS PAGE of the isolated protein from wheat leaves.

Length and density of stomata

Results have shown that the average density of stomata decreased (65/mm^2 to 49/mm^2) at the increasing concentration of CO_2 from 350 ppm to 500 ppm with significant difference at 5% while the average length of stomata (54 µm to 65 µm) increased significantly with the corresponding concentrations of CO_2 (Table 1). However, decrease in density of stomata and increase in length of stomata with increased CO_2 concentration from 400 ppm to 500 ppm was not significant.

Leaf growth

Leaf length is an important parameter to assess the growth of leaf. The present study revealed that as the concentration of CO_2 increases, the average length of a leaf also increases (Table 1). The mean leaf length increased significantly (at 5% level of significance) from 18.95 cm to 40.69 cm and 30.25 cm to 40.69 cm with increase in CO_2 concentration from 350 ppm to 500 ppm and 400 ppm to 500 ppm respectively. This increase in mean leaf length was found to be positively correlated (0.972) with CO_2 concentration.

Table 2. Correlation analysis of CO_2, length of stomata and density of stomata.

	CO_2	Length of stomata	Density of stomata	Leaf length
CO_2	1.00			
Stomata length	0.856	1.00		
Density of stomata	– 0.984	– 0.752	1.00	
Leaf length	0.972	0.609	– 0.791	1.00

Correlation among CO_2 exposure, length of stomata and density of stomata

It was found that CO_2 concentration was positively correlated (0.856) with length of stomata while it was inversely correlated (-0.984) with the density of stomata. Density of stomata and length of stomata were also inversely correlated (-0.752) with each other (Table 2).

Host plant resistant

To study the host plant resistance, leaf samples were subjected for the protein isolation. The concentration of protein was found to be increased with increasing CO_2 concentration, ranging from 81.49 mg/ml to 110.27 mg/ml (Fig. 2). In each case, the mean protein concentration was higher in inoculated plants

than in un-inoculated one.

There was a slight increase in the concentration of protein in infected plants grown in increased CO_2 condition. All the samples were diluted up to 25 mg/ml by adding sterile extraction buffer. Electrophoresis (SDS-PAGE) of the isolated protein from all set of experiments produced five bands with 22 kDa, 26 kDa, 32 kDa, 36 kDa and 43 kDa. Different band intensity were present in all samples observed with significant differences in the protein concentration (Fig. 3). 22 kDa band represents the PR-5 protein. 32 kDab and 36 kDa band represent the PR-2 protein of different class while 26 kDa and kDa band represent the PR-3 protein family.

CO_2 is an important GHG which is markedly increasing with industrial growth and anthropogenic activities. In general, photosynthetic capacity, water - use efficiency, plant growth and yield are positively affected by increased CO_2 concentration [6, 7].

The length and density of stomata are very sensitive to the climatic change. In the present study, increase in stomata length recorded was 11µm and decrease in stomata density was recorded to be 16 / mm^2 while increase in the leaf length upto 21.74 cm was also recorded. The results obtained are in agreement with a study in which elevated CO_2 concentration brought reduction in stomatal conductance and increase in stomatal index, size of stomata guard cell, stroma and epidermal cell [8].

During the present study, an inverse correlation was observed among the length of stomata and density of stomata (-0.752) (Table 2). Reducted density of stomata at elevated CO_2 causes much better retention of water [9], therefore, leads to increased photosynthesis and decreased transpiration.

Changes in stomatal dynamics on elevated CO_2 provide positive feedback mechanism to be infected with pathogens. Upon pathogen attack, plants synthesize specific resistance mechanisms e.g. PR-proteins, phytoalexin. In the present study, isolated protein was subjected to SDS PAGE analysis and based on the molecular weight of bands, activity of PR Protein was found in the plant. It was observed that the concentration of protein increased with increasing CO_2 concentration. The intensity of PR Protein was significant in the SDS-PAGE analysis. This revealed that the infection induces the synthesis of defensive protein but their expression with increasing CO_2 concentration was not able to impart resistance against the disease. Earlier reports have also shown the Chitinase activity, β-1, 3-glucanase activity and peroxidase activity in plant leaves upon induction with the pathogen [10].

Thus, the present study revealed the relationship among the concentration of CO_2, stomata length, stomata density, protein concentration and PR-protein expression. The dynamics of stomata is very sensitive to climate change and is an important parameter for disease infection.

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