EFFECT OF ELEVATED CO₂ ON INFECTION OF THREE FOLIAR DISEASES IN OILSEED *BRASSICA JUNCEA*

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SUMMARY

The present investigation was carried out in a free-air CO₂ enrichment (FACE) facility to assess the effect of elevated CO₂ on the incidence and severity of three foliar diseases of *Brassica juncea* cv. Pusa Tarak. Exposure of plants to elevated concentration of CO₂ (550 ppm) revealed lower incidence and severity of Alternaria blight caused by *Alternaria brassicae* and downy mildew caused by *Hyaloperonospora brassicae*, while white rust infection caused by *Albugo candida* increased. Leaves of mustard plants grown under elevated CO₂ had a higher amount of epicuticular wax which, together with higher concentration of total phenols and phenylalanine ammonia lyase activity, may have increased the ability of mustard plants to resist infection by *A. brassicae* and *H. brassicae*. Mustard plants grown under elevated CO₂ showed a decrease in stomatal density and pore size, and consequently also in stomatal conductance. This might explain the decrease in disease index of downy mildew caused by the stomata-invading pathogen *H. brassicae*. There was no change in leaf protein concentration, whereas sugars were three times as concentrated in plants grown under high vs. ambient CO₂. There was an increase in the concentration of total glucosinolates (GSs) under FACE in plants grown under elevated CO₂, but a decrease in their diversity. Namely, the aliphatic GS gluconapin was more abundant in plants grown under high CO₂, while three out of eight different GSs found in the leaves of plants grown under ambient CO₂ could not be detected. Higher sugar availability and lower GSs diversity may account for the higher incidence and severity of white rust caused by the obligate biotroph *A. candida*.

Key words: Alternaria blight, downy mildew, free-air CO₂ enrichment (FACE), glucosinolates, white rust.

INTRODUCTION

Carbon dioxide concentration in the atmosphere has risen from pre-industrial levels of about 275 ppm to the current 390 ppm, and is projected to continue to rise to at least 550 ppm by the year 2050 (Intergovernmental Panel on Climate Change, 2007). In comparison to studies on the effect of elevated CO₂ on plant growth, physiology and productivity (Ainsworth et al., 2002; Dermody et al., 2008), very few attempts have been made to assess the impact on disease expression in plants. The influence of elevated CO₂ on the incidence of crop diseases can have long term implications for food security and ecological balance. However, most of the crop models that predict the performance of crop species in future projected scenarios fail to take into account the impact of pathogens under elevated CO₂ regimes (Chakraborty et al., 2008).

The shift in the profile of defensive compounds and/or other changes in plant morphology, physiology or anatomy under elevated CO₂ has been observed to alter incidence or severity of disease caused by a particular pathogen (Hartley et al., 2000; Matros et al., 2006). In the past few years, there is an upsurge in the number of studies on the effect of free-air CO₂ enrichment (FACE) on plant-pathogen interaction (Kobayashi et al., 2006; Plessl et al., 2007; Riikonen et al., 2008; Eastburn et al., 2010; McElrone et al., 2010). However, these reports are not consistent because of the complex interactions occurring between plants and pathogens at various levels (Jeger and Pautasso, 2008). The diverse conclusions from these studies cannot be extrapolated to other economically important agricultural crops and their pathogens. This implies that predicting effects of elevated CO₂ for unstudied pathosystems will be quite challenging. Therefore, the need for more realistic studies under projected environmental conditions to gain better understanding of plant-pathogen interactions has been emphasized by many scientists (Chakraborty et al., 2008; Melloy et al., 2010).

Oilseeds play an important role in the agricultural and industrial sectors of India. *Brassica juncea* (mustard) is an important oilseed crop cultivated on about six million hectares (Mondal et al., 2007). Since mustard is cul-
CO$_2$ alters susceptibility of *Brassica juncea*

MATERIALS AND METHODS

Experimental design. The experimental site is located at the Division of Environmental Science, Indian Agricultural Research Institute (IARI), New Delhi (India). The experiment consisted of two treatments, control (plants grown in open field under natural conditions) and elevated CO$_2$ (plants grown under FACE). CO$_2$ in the field was measured periodically using an infra-red gas analyzer (IRGA) (Licor 6200, USA). The concentration of CO$_2$ in the field ranged between 387 ppm and 391 ppm, with a mean of 390 ppm.

Seeds of *Brassica juncea* cv. Pusa Tarak were sown in the month of October in clay loam soil (pH 7.0; N 10 g m$^{-2}$; P 30 g m$^{-2}$; K 6 g m$^{-2}$) in rows with spacing of 40 cm between the rows and 20 cm between the plants. Seed rate was kept uniform and thinning was done after one month. No fertilizer or pesticide was used during the course of the experiment. Weeding was done mechanically at regular intervals and plots were irrigated with tap water.

The Mid-FACE facility consisted of an octagonal plenum of 6 m diameter, made of irrigation pipes of 20 cm diameter with circular vents for CO$_2$ injection. The height of the plenum was adjusted according to crop height for uniform distribution of CO$_2$ levels inside the FACE ring. CO$_2$ was injected through gas cylinders and maintained at 550 ppm above the canopy level. To regulate the volume of CO$_2$ released into the plot and facilitate its uniform distribution, the FACE facility was equipped with IRGA. The IRGA collects information from multiple sensors and transmits control signals to electrically stimulated relays and proportional integrative differential (PID) valves. In addition, wind speed and direction were monitored through a specially developed PID algorithm (Uprety et al., 2006). One of the
The greatest advantages of FACE is that it does not affect the plot's microenvironment. This enables researchers to investigate the effect of elevated CO₂ as a single altered factor, with other environmental conditions left unchanged (Leadley et al., 1997).

**Disease assessment.** Disease severity of Alternaria blight, downy mildew and white rust was recorded in mustard plants through visual inspection beginning one week after seedling emergence and until inception of leaf senescence. Natural incidence of these diseases was recorded once in randomly selected sixty plants when the plant attained full maturity in March. On the other hand, disease severity was recorded every 10 days from the month of January to March in both ambient and treated plots. Lesion area (pustule area for white rust) and percent leaf area infection for all the three pathogens were recorded manually by tracing twenty diseased leaves from twenty different plants. Disease incidence was calculated by dividing the number of infected leaves by the total number of leaves. Disease severity was determined using an arbitrary 0-5 scale based on the percentage of leaf area covered with lesions/pustules, where 0 = 0%, 1 = 5%, 2 = 5.1-10%, 3 = 10.1-25%, 4 = 25.1-50%, 5 = >50%. Many small coalescing pustules were regarded as a single pustule. The percentage disease index (PDI) was calculated using the Wheeler’s (1969) formula:

\[
\text{PDI} = \left( \frac{\text{Sum of numerical grading} \times 100}{\text{No. of leaves examined} \times \text{Maximum disease grade}} \right)
\]

**Leaf epidermal characteristics.** Concentration of epicuticular wax was determined using the method of Barnes et al. (1996) with slight modification. A total of twenty leaves from twenty different plants were sampled for each treatment. Fifty leaf discs 1 cm in diameter punched randomly from twenty leaves were immersed in 10 ml chloroform (HPLC grade) for 2-3 sec at room temperature with gentle agitation. The chloroform extract was filtered through Whatman filter paper No.1 in evaporating flask and the filtrate was evaporated under vacuum. The wax was collected and weight per unit leaf area was calculated. The analysis was carried out with six replicates.

Mature leaves from three-month-old mustard plants grown under ambient or elevated CO₂ were used for scanning electron microscopy. The segments were prepared by vacuum-infiltrating small pieces (<15 mm²) of tissue in 2 ml of Karnovsky’s fixative (Karnovsky, 1965). The tissue was then dehydrated in a graded ethanol series and held in 95% ethanol for 2 h. The tissue segments were mounted on aluminum stub with silver pane or double adhesive tape and dried to the critical point. The dried samples were coated with gold (24 karat) of thickness 125/22 nm and photographed with a Leo 435 VP Scanning Electron Microscope (SEM Cambridge, UK). Stomatal density and stomatal aperture size were measured using the bar scale on the scanning electron micrographs. The uppermost, fully expanded leaf from ten three-month-old plants was selected, and 10 estimates of stomatal conductance were measured using a portable photosynthesis system, Licor 6400 XT (Licor, USA) during a clear day.

**Leaf chemistry.** Chemical analyses were done in six replicates. Each replicate consisted of five leaves pooled from five randomly selected plants. For sugars and glucosinolates, the sampled leaves were frozen in liquid nitrogen ground to a fine powder with pestle and mortar and stored at -80°C till analysis. The concentration of protein, phenols and the activity of phenylalanine ammonia lyase were determined in freshly harvested leaf samples. For carbon, hydrogen, nitrogen and sulfur (CHNS) analysis, the plant tissue was sampled as above and oven-dried at 70°C for 72 h.

The concentration of total sugars was assessed in leaves according to Yemm and Willis (1954), using the anthrone reagent. The absorbance of the solution was determined using a UV-visible spectrophotometer (Beckman Coulter DU 730, USA) at 630 nm. The amount of total sugars was determined from a standard curve of glucose and calculated on a fresh weight basis.

Reducing sugars were estimated following Miller (1959) using 3,5-dinitrosalicylic acid (DNSA). DNSA reacts with reducing sugars to form 3-amino 5-nitrosalicylic acid, which was measured at 510 nm by a UV-visible spectrophotometer. The amount of reducing sugars was determined from a standard curve of glucose and calculated on a fresh weight basis. Non-reducing sugars were calculated by subtracting the amount of reducing sugars from the amount of total sugars.

The protein concentration was determined by the method of Bradford (1976). Freshly harvested leaves (1 g) were ground in liquid nitrogen and homogenized in 0.1 M phosphate buffer, pH 7.0. The homogenate was centrifuged at 12,000 g for 10 min at 4°C. The supernatant was collected and used for protein estimation. The amount of protein in the sample was estimated from a standard curve of bovine serum albumin (BSA, Sigma-Aldrich, USA).

Carbon, nitrogen and sulphur were determined in a CHNS analyzer (Vario Elementar III, Germany). Oven-dried leaf tissue (3 mg) was oxidized in the combustion tubes in the presence of oxygen at 1,150°C using tungsten (IV) oxide as catalyst. In order to remove residual oxygen not consumed in the initial combustion and to convert oxides of these elements into their gaseous forms, helium gas was used to swap out the combustion product and passed over copper to reduce nitrogen oxides and sulfuric anhydride to nitrogen and sulfurous anhydride. All elements were separated by gas chromatography and were detected by thermal conductivity.
CO₂ alters susceptibility of *Brassica juncea*

detectors. Sulphanilic acid (Merck, Germany) was used as a standard.

The activity of phenylalanine ammonia lyase (PAL) (E.C. 4.1.1.5) was assayed according to Dunn et al. (1998) with a slight modification. The enzyme activity was tested in extracts of leaves from three-month-old plants, on which specks of disease were visible. PAL enzyme catalyzes conversion of phenylalanine to trans-cinnamic acid which can be read spectrophotometrically at 290 nm. The activity was expressed as µg of trans-cinnamic acid h⁻¹ mg⁻¹ protein. The standard curve of enzyme activity was prepared using L-phenylalanine (SRL) as substrate.

The estimation of total phenols was carried out according to Bray and Thorpe (1954). Leaf samples (1 g fresh weight each) were homogenized in 70% ethanol and centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was collected and assayed for phenol concentration by spectrophotometric reading of absorbance at 650 nm. A standard curve was prepared using pyro-catechol (Loba Chemie, India).

The extraction and purification of glucosinolates (GSs) was done according to Kraling et al. (1990) with a slight modification. Crude GSs were extracted from dried powdered samples (200 mg) with 3 ml of 70% warm methanol for 5 min in a water bath at 70°C. The mixture was centrifuged at 3,405 rpm for 4 min and the supernatant was decanted in another tube. The extraction was repeated twice and the supernatant obtained from both extractions were pooled. Around 500 µl of extract was added to a mini column filled with diethylamine ethanol sephadex A-25 activated with 500 µl of imidazole formide. The desulfated GSs were eluted with 500 µl of HPLC grade water, filtered through 0.22 µm filters and stored at -20°C until HPLC analysis. Gluco-trapoelin was used as an internal standard for GSs quantitative analysis. The GSs concentration was determined by HPLC (Shimadzu UFLC, Japan). The desulfated extract (10 µl) was injected into a C-18 silica column. The mobile phase constituting a mixture of deionized water and acetonitrile was run at a flow rate of 0.4 ml min⁻¹. The peaks of the GSs were detected at 229 nm.

**Statistical analysis.** Results for ambient and elevated CO₂ treatments are given as mean ± standard deviation of six replicates. The significance of differences between means of the two treatments was assessed using the Student’s t-test. An asterisk (*) indicates that means are statistically different at P<0.05. The data were analyzed using the Statistical Package for Social Sciences version 16 (SPSS, USA) for Windows.

**RESULTS**

As mentioned, field surveys conducted during the mustard-growing season revealed the occurrence of three diseases: Alternaria blight, downy mildew and white rust. White pustules of *A. candida* were evident from second week of January, while lesions of Alternaria blight and downy mildew were observed from first week of February (Fig. 1). In ambient as well as elevated CO₂ plots, the size of lesions caused by *A. brassicae* and *H. brassicae* increased with time (Fig. 1a, b). Lesions were largest at the fruiting stage of the plants.

Fig. 1. Effect of elevated CO₂ on lesion size of (a) Alternaria blight; (b) Downy mildew; and (c) pustule size of White rust in *B. juncea* recorded every ten days from the month of January to March. Asterisk indicates significant differences at P<0.05.
(March) beyond which there was no further increase in severity of the three diseases. Lesions caused by *A. brassicae* and *H. brassicae* were smaller in plants under elevated CO₂ compared to those grown at ambient CO₂, while the size of pustules caused by *A. candida* were bigger in plants under elevated CO₂ compared to those grown in ambient CO₂. At the final stage of sampling (fruiting stage of plants) the severity of Alternaria blight and downy mildew was significantly reduced by 32.13 and 65.79%, respectively, for plants grown under elevated CO₂ as compared to plants in ambient CO₂ (Fig. 2a). The incidence of these two diseases was reduced by 33.4 and 19.26%, respectively, under elevated CO₂ relative to ambient CO₂ conditions (Fig. 2b). However, for downy mildew the decrease in incidence was not statistically significant. The pustule size (Fig. 1c) and severity of white rust increased with plant maturity. Percent disease index and the number of plants infected with *A. candida* were higher under elevated CO₂ as compared to plants grown in ambient conditions of CO₂.

**Leaf epidermal characteristics.** Significant changes were observed in epidermal characteristics of mustard leaves grown under elevated CO₂. Concentration of leaf epicuticular wax was 92% higher in plants exposed to elevated CO₂ as compared to control plants (Table 1). Scanning electron micrographs of mustard leaf surface showed reduction in stomatal density (Fig. 3 and Table 1). Length and width of stomatal aperture were also significantly reduced under elevated CO₂ (Table 1). Stomatal conductance was reduced in plots with elevated CO₂. The differences observed were statistically significant (Table 1).

**Leaf chemistry.** The carbon concentration increased, while concentration of nitrogen decreased in plants grown under elevated CO₂ thus leading to an increase in the C/N ratio. Plants reared at elevated CO₂ showed no significant effect on uptake of sulphur, resulting in decreased N/S ratio (Table 2). Concentration of total sugars in mustard plants grown at elevated CO₂ was three times higher than those grown in ambient CO₂ levels (Table 2).
times that in plants grown in ambient CO₂. This increase was primarily due to a six-fold increase in concentration of non-reducing sugars in elevated CO₂ plots. No significant change in concentration of reducing sugars was observed (Fig. 4a). There was no change in protein concentration under elevated CO₂ as compared to ambient conditions (Fig. 4b).

Cultivation of mustard plants at elevated CO₂ led to significant increase in PAL activity with a corresponding rise in the concentration of total phenols (Fig. 5a, b). The influence of elevated CO₂ was also studied on the nitrogen- and sulphur-containing antimicrobial compounds, glucosinolates (GSs) in the leaves of three-month-old plants. The tissue was extracted in methanol to study the profile of GSs. The total concentration of GSs increased by 47% under FACE over control (Fig. 5c). This increase was found to be significant at P<0.05. The methanolic extracts were subjected to reverse phase HPLC and GSs were differentiated in the UV chromatograms (Fig. 6). Out of eight GSs identified in the leaves of plants grown under ambient CO₂, only five were detected under FACE. The total area of all peaks monitored in UV chromatograms for high CO₂-raised plants increased in comparison to the control plants. Among all the GSs identified, a major increase in the aliphatic GS gluconapin was observed (about 42% over control). However, the indolic GS glucobrassicin as well as glucosinolin, glucorucin and gluconasturtiin were not detected in tissues of plants grown under elevated CO₂ (Table 3). As glucotrapeolin was added to each extract as an internal standard to calculate concentration of other GSs, its concentration was set to zero, even though the peaks were present in the chromatograms.

**DISCUSSION**

Increased atmospheric concentration of CO₂ led to a modified pattern of disease manifestation in mustard. The effect on the three pathosystems varied considerably. Incidence of Alternaria blight and downy mildew was reduced, while white rust infection increased under elevated CO₂.

Cultivation of plants at elevated CO₂ resulted in a significant increase in concentration of epicuticular wax on mustard leaves. Plant epicuticular waxes are complex mixtures of primarily straight chain aliphatic hydrocarbons with a variety of substituted groups (Walton, 1990). The increase in epicuticular wax under elevated CO₂ is consistent with the carbon-nutrient balance hypothesis, which postulates the accumulation of carbon-based defense metabolites in plants with higher C/N ratio (Hamilton et al., 2001). The amount of epicuticular wax on leaf surface is one of the factors influencing the degree of susceptibility of *Brassica* spp. to *A. brassicaceae* (Conn et al., 1984). Decrease in incidence and severity of Alternaria

**Table 1.** Influence of different concentrations of atmospheric CO₂ on leaf epidermal characteristics of *B. juncea*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Epicuticular wax (mg cm⁻²)</th>
<th>Stomatal characteristics</th>
<th>Stomatal conductance (mol m⁻² s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Stomatal aperture length (µm)</td>
<td>Stomatal aperture width (µm)</td>
</tr>
<tr>
<td>Ambient CO₂</td>
<td>16.8 ± 0.25</td>
<td>13.21 ± 1.00</td>
<td>2.82 ± 0.76</td>
</tr>
<tr>
<td>Elevated CO₂</td>
<td>32* ± 0.08</td>
<td>11.00* ± 0.45</td>
<td>0.69* ± 0.29</td>
</tr>
</tbody>
</table>

Values for epicuticular wax and stomatal characteristics are mean of six replicates (± indicates standard deviation). Values for stomatal conductance are mean of ten replicates. Mean followed by * are significant at P<0.05 between ambient and elevated CO₂ treatment determined by unpaired equal variance t-test.

**Table 2.** Effect of different concentrations of atmospheric CO₂ on the concentration of carbon, nitrogen, sulphur and carbon-nitrogen ratio in the leaves of *B. juncea*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Carbon (%)</th>
<th>Nitrogen (%)</th>
<th>Sulphur (%)</th>
<th>C/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient CO₂</td>
<td>42.8 ± 0.25</td>
<td>7.05 ± 0.05</td>
<td>0.8± 0.02</td>
<td>6.06 ± 0.04</td>
</tr>
<tr>
<td>Elevated CO₂</td>
<td>44.9* ± 0.28</td>
<td>6.64* ± 0.05</td>
<td>0.88±0.0</td>
<td>6.75*± 0.09</td>
</tr>
</tbody>
</table>

Values are mean of six replicates (± indicates standard deviation). Mean followed by * are significant at P<0.05 between ambient and elevated CO₂ treatment determined by unpaired equal variance t-test.
The blight observed in this study may be due to an increase in leaf epicuticular wax, which can enhance resistance to infection in several ways. Waxy coating on the leaf epidermis decreases the leaching of organic and inorganic substances, thus reducing the germination of conidia. Furthermore, epicuticular wax reduces the wettability and permeability of leaf surface by creating a hydrophobic surface (Conn and Tewari, 1989).

Growth of mustard plants under elevated CO$_2$ induced a decrease in stomatal density and stomatal conductance consistent with previous studies (Royer, 2001; Ainsworth and Rogers, 2007). These epidermal changes restrict the entry of stomata-infecting pathogens, and furthermore alter the microclimate on the leaf surface due to reduced humidity around stomatal pores (McElrone et al., 2005). The downy mildew agent _H. brassicae_ gains entry into the leaves of _B. juncea_ via stomata, so their reduced density, size and conductance in plants grown under elevated CO$_2$ may decrease downy mildew severity.

Plants grown under elevated CO$_2$ show decreased nitrogen concentration (Matros et al., 2006; Plessl et al., 2007). Our results on mustard comply with the earlier reports. Mustard plants grown under elevated CO$_2$ displayed an increase in C/N ratio with a corresponding rise in concentration of total sugars but no change in

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Fig. 4. Effect of elevated CO$_2$ on (a) Concentration of sugars and (b) proteins in leaves of three-month-old plants of _B. juncea_. The mean values were plotted with (±) S.D. of six replicates. Asterisk indicates significant differences at P<0.05.

Fig. 5. Effect of elevated CO$_2$ on (a) phenylalanine ammonia lyase activity; (b) concentration of total phenols; and (C) glucosinolates concentration in leaves of _B. juncea_. The mean values were plotted with (±) S.D. of six replicates. Asterisk indicates significant differences at P<0.05.
protein concentration. Long et al. (2004) also suggested that increased foliar carbohydrate content in plants grown under elevated CO2 indicates a source/sink imbalance and increased C/N ratio. An increase in carbohydrate concentration has been reported to favour infection of sugar-dependent pathogens (Eastburn et al., 2011). The increase in incidence and severity of white rust observed in B. juncea plants grown under elevated CO2 may be due to higher carbohydrate concentration.

Elevated CO2 increased the concentration of total phenols and induced higher PAL activity. PAL activity and phenol concentration have been used as biochemical markers of defence in plants and may be useful for gaining a better understanding of the effect of elevated CO2 on defence mechanism of plants. PAL is a key enzyme of phenylpropanoid metabolism in plants. The trans-cinnamic acid produced as a result of deamination of phenylalanine serves as a precursor of various secondary metabolites including phenols, monomers of lignin and salicylic acid (Creasy et al., 1974). Our observations on PAL activity are in accordance with Matros et al. (2006) where strong and significant increase in the level of PAL activity in tobacco plants was observed under elevated CO2. Increased concentration of total phenols in FACE as compared to ambient air can be attributed to enhanced PAL activity, and is also consistent with the carbon-nutrient balance hypothesis (Hamilton et al., 2001).

In the present study, concentration of GSs in the leaves of mustard plants has been observed to increase significantly under elevated CO2 while their diversity decreased. GSs represent about one-third of total sulphur in plant parts. The concentration of GSs is inversely related to N/S ratio (Falk et al., 2007). Therefore, increase in concentration of GSs may be a consequence of slight decrease in N/S ratio. However, more research is required to elucidate the mechanism involved.

![Figure 6](image.png)

**Table 3.** Effect of elevated CO2 on individual and total GSs concentration in leaves of B. juncea. Numbering of glucosinolates is based on the elution order of desulfated glucosinolates from HPLC.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name</th>
<th>Trivial Name</th>
<th>Type</th>
<th>Ambient CO2</th>
<th>Elevated CO2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Retention time (min)</td>
<td>Concentration (µ mole g⁻¹)</td>
</tr>
<tr>
<td>1.</td>
<td>SIN</td>
<td>Sinigrin</td>
<td>Aliphatic</td>
<td>4.317</td>
<td>1.754</td>
</tr>
<tr>
<td>2.</td>
<td>ALY</td>
<td>Glucoallysin</td>
<td>Aliphatic</td>
<td>4.869</td>
<td>0.489</td>
</tr>
<tr>
<td>3.</td>
<td>GNA</td>
<td>Gluconapin</td>
<td>Aliphatic</td>
<td>5.826</td>
<td>19.777</td>
</tr>
<tr>
<td>4.</td>
<td>IBV</td>
<td>Glucoiberverin</td>
<td>Aliphatic</td>
<td>7.002</td>
<td>1.424</td>
</tr>
<tr>
<td>5.</td>
<td>GBN</td>
<td>Glucobrassicanapin</td>
<td>Aliphatic</td>
<td>7.461</td>
<td>1.424</td>
</tr>
<tr>
<td>6.</td>
<td>ERU</td>
<td>Glucoerucin</td>
<td>Aliphatic</td>
<td>8.094</td>
<td>1.515</td>
</tr>
<tr>
<td>7.</td>
<td>GBC</td>
<td>Glucobrassicin</td>
<td>Indolic</td>
<td>8.246</td>
<td>0.185</td>
</tr>
<tr>
<td>8.</td>
<td>NAS</td>
<td>Gluconasturtin</td>
<td>Aromatic</td>
<td>8.525</td>
<td>0.937</td>
</tr>
</tbody>
</table>

Total | 27.505 | 40.634
GSs provide protection against herbivores and pathogens. The antifungal activity of the hydrolytic products of GSs varies with the type of pathogen (Falk et al., 2007). Salac et al. (2006) observed enhanced resistance of B. napus against H. brassicaceae with concurrent increase in concentration of GSs. The increase in concentration of GSs under elevated CO2 in Indian mustard may similarly contribute to reduced incidence of downy mildew. On the other hand, specialized pathogens of the crucifers such as A. brassicaceae and A. brassicola can detoxify or tolerate GSs and their hydrolytic products (Giamoustaris and Mithen, 1997). Thus, decrease in incidence of Alternaria blight under elevated CO2 in this study may be primarily due to the observed increase in leaf epicuticular wax, together with other biochemical parameters such as PAL activity and concentration of total phenols. Changes in GSs profile have been reported to modulate plants resistance towards specific pathogens (Brader et al., 2006). The increase in severity of white rust may be due to reduced diversity of GSs in B. juncea leaves grown under high CO2.

Changes in the susceptibility of B. juncea to A. brassicaceae and H. brassicaceae as observed in this study could be largely explained on the basis of modification of leaf epidermal characteristics and leaf chemistry of host plants grown under elevated CO2. However, the increased incidence and severity of white rust could not be explained on the basis of leaf epidermal characteristics, and may be more justifiably attributed to the increased concentration of carbohydrates in leaves of mustard plant grown under elevated CO2 as well as to the adaptive and inherent nature of A. candida. Changes in plant-pathogen interaction under elevated CO2 cannot be always explained on the basis of alterations in plant morphology and physiology, as it may involve several other factors and mechanisms governing the growth of pathogen. Our results reinforce the notion that a complete picture of the specific metabolic and morpho-physiological changes induced by elevated CO2 in each economically important crop is needed to predict and understand the influence of rising CO2 levels on their performances upon interaction with different pathogens.

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