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SHORT COMMUNICATION

CHANGES IN PHOTOSYNTHESIS RELATED PARAMETERS IN ISABGOL (*PLANTAGO OVATA*) UNDER DOWNY MILDEW INFECTION

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Sequential changes in leaf chlorophyll content and associated gas exchange parameters in isabgol (*Plantago ovata*) were recorded over a period of nine days starting from initial sporulation to severe chlorosis in a highly downy mildew susceptible cultivar (Niharika). The Downy mildew caused significant reduction in chlorophyll content. The loss of chlorophyll was more severe in case of chlorophyll 'a' compared to chlorophyll 'b'. Net photosynthetic rate and stomatal conductance in the diseased leaves also reduced compared to healthy ones. However, internal CO_2 concentration (C*i*) increased in diseased leaves. Difference between healthy and infected leaves in terms of stomatal conductance and C*i* became significant at ninth day of study. In the complex situation of variable disease severity and leaf physiological stage, we found net photosynthetic rate was highly correlated (r = ~0.8) with the disease severity.

***Keywords*: Carbon assimilation, isabgol, obligate pathogen, peronosporales**

Plants, by virtue of their green leaf pigments – chlorophylls, have unique ability to synthesise complex carbohydrates from simple carbon dioxide and water utilising light energy. Because of this unique autotrophy, many micro- and macro-scopic organisms (heterotrophs) depend on green plants for their survival. As the pathogens deprive the host plant of the nutrients, many far reaching implications are expected from such interactions. Reduction of chlorophyll content due to microbial pathogen infection is almost universal in majority of plants. However, in diseases caused by obligates – where survival of the pathogen is dependent on the longevity of the host tissues – the reduction in chlorophyll and development of necrosis is slow. Reduction in chlorophyll content in chloroplast usually has direct effect on the physiological functions performed by this organelle. As a result, photosynthesis is affected in the infected cells. Reduction in net photosynthetic rate due to infection by various pathogens (Bassanezi *et al*. 2002, Habermann *et al*. 2003, Maust *et al.* 2003, Ryšlavá *et al*. 2003, Shtienberg 1992) is well documented. Loss in anabolic mechanism has direct influence on productivity of the crop plants. Hence, these diseases results in significant yield loss.

Isabgol (*Plantago ovata*) is an important medicinal plant commercially cultivated in India. The epidermal layer of seed coat, 'isabgol husk' of commerce, is well known remedy of constipation, intestinal irritation, etc. Downy mildew caused by *Peronospora plantaginis* is

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one of the major constraints in successful cultivation of this crop. The disease causes serious yield reduction (Mandal *et al*. 2007). In isabgol-downy mildew interaction, changes in host leaf pigments and transpiration rate are also reported (Rathore *et al*. 2001, Mandal *et al.* 2009). However, quantitative data on impact of disease on chlorophyll content and photosynthetic performance is not available. Hence, the present field study was conducted with an aim to monitor changes in chlorophyll content and gas exchange parameters of downy mildew infected isabgol leaves and to work out the relationship between disease severity and CO_2 assimilation parameters.

The experiments were conducted at the research farm of Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand, Gujarat, India (73° E longitude, 22.5° N latitude). The observations were taken on a susceptible cultivar (cv Niharika) of isabgol at 50-days after sowing (DAS) under field conditions. For monitoring changes in chlorophyll content and gas exchange parameters of downy mildew infected isabgol leaves, the plants were randomly selected and single leaf from a plant was tagged. Fully expanded leaves, well exposed to sunlight, free from any abnormalities and of similar age (4^{th} to 5^{th} leaf from top) were taken as healthy. Similar leaves having initiation of downy mildew infection showing mild chlorosis were selected for diseased category. For each sampling, three replicates were maintained and five leaves were selected per treatment and the observations were taken at alternate days for consecutive nine days.

To study the influence of disease severity on photosynthetic capacity, twenty five downy mildew affected leaves (showing wide variations in disease affected area and stage of disease development) from randomly selected plants were marked for observations. Leaf gas exchange parameters (net photosynthetic rate, stomatal conductance and intercellular CO_2 concentration) were measured using a portable infrared gas analyser (LI-6400, LI-COR Inc., Lincoln, USA). Measurements were made using a standard leaf chamber (2×3 cm) having transparent top. For taking readings from infected leaves, care was taken to keep affected portions inside the chamber. Measurement was done during active photosynthetic period (10:00–11:30 and 14:00–15:00 hrs) under clear sky at ambient CO_2 and relative humidity. A length of 3 cm of the lamina, from where the gas exchange data was recorded, was excised and brought to the laboratory in an ice box. Individual leaves were scanned at 1200 dpi (digits per inch) resolutions with an imaging scanner (HP ScanJet 5590, HP India, New Delhi, India). After this, chlorophylls from the leaf samples were extracted in dimethyl sulphoxide (DMSO) following the method of Hiscox and Israelstam (1979). Leaf samples were extracted in 8 ml of DMSO for 1 hr at 65°C under darkness. At the end, the green solution was decanted, volume was made to 10 ml with DMSO and its absorbance was taken at 649 and 665 nm in a spectrophotometer (Biomate 3, Thermo Electron Corporation, Madison, WI, USA). Total, diseased and healthy areas in the digitised image were calculated manually using image analysis software Image J (Ver.1.42q, NIH, USA). Data on healthy and infected leaves were subjected to paired t test at p= 0.05. Simple linear correlation between different parameters from the second experiment was calculated. Significance of the correlation coefficients was tested at p= 0.05 and 0.01.

At the beginning of the study, the locally infected leaves showed mild chlorosis. Sporulation was initiated on such sites as evident from sparse external growth of the pathogen. With the passage of time, affected portions of the leaf turned more chlorotic. During this period, sporulation of the pathogen became dense and turned dark in colour. Towards the end of the experiment (on

Table 1. Simple correlation coefficients between net photosynthetic rate and other factors

Independent factors	Dependent factor	
	Net Photosynthetic rate (mmol $CO_2/m^2/sec$)	
	Set 1	Set 2
Healthy area (cm^2)	0.49*	0.90**
Diseased area (cm^2)	–0.50*	–0.56**
% Healthy area	0.77**	0.84**
Chlorophyll (mg/g)	0.41*	0.27

** and ** indicate significance of correlations at p=0.05 and p=0.01, respectively*

ninth day of observation), the infected leaves turned bronzed blighted. However, the control leaves remained healthy during the period of study. Quantitative analysis of chlorophyll contents from healthy and infected leaves clearly indicated significant difference (Fig. 1). At the beginning of the experiment (on first day of observation), chlorophyll *a* (Chl*a*) content of healthy leaves was 0.82 mg g^{-1} FW (Fig. 1A) whereas, diseased leaves had significantly lower Chl*a* (0.60 mg g^{-1} FW). During next two days of observations (on third and fifth days of experiment) healthy leaves showed an upward trend in Chl*a* content from 1.09 to 1.30 mg g^{-1} FW. In the infected leaves, however, Chl*a* content increased on third day only to reach the maximum level (0.78 mg g^{-1} FW). Thereafter, in the affected leaves, Chl*a* content gradually reduced to 0.59, 0.43 and 0.36 mg g^{-1} FW respectively at fifth, seventh and ninth day of experiment. During seventh and ninth day of the study, Chl*a* contents in healthy leaf also showed a downward trend with 1.22 and 1.06 mg g^{-1} FW, respectively. Chlorophyll *b* (Chl*b*) content also followed the same trend as that of Chl*a* in healthy leaves with 0.15, 0.20 and 0.24 mg g^{-1} FW at first, third and fifth days of study, respectively. Chl*b* content in infected leaves also increased till fifth day with values of 0.13, 0.18 and 0.20 mg g^{-1} FW during first, third and fifth day respectively. Chl*b* content was stationary at 0.25 mg g^{-1} FW in healthy leaves at seventh and ninth days. However, in infected leaves, it was significantly reduced to 0.14 mg g^{-1} FW at seventh and ninth days of observations. Hence, reduction in total chlorophyll content in the diseased leaves was 25.70% compared to healthy leaves at first day of experiment. The reduction became severe with a loss of 61.97% compared to healthy leaves at ninth day of observation. Ratio of Chl*a* and Chl*b* showed a decreasing trend with the increase in age of leaf. On the first day of observation, the difference was 0.89 which reached to 1.74 on the ninth day. The differences were significant at all days of observations.

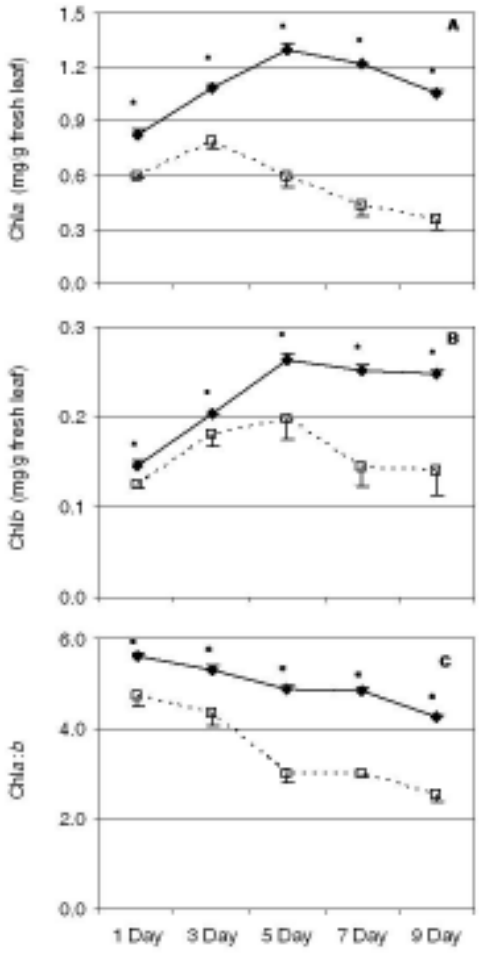

Fig. 1. Sequential changes of Chl*a* (A) and Chl*b* (B) contents and ratio of these two (C) in healthy and diseased leaves over a period of nine days. The bars represent the standard deviation (s.d.) of the mean. * indicates significant difference between healthy and diseased leaves by paired *t* test at *P*=0.05

Leaf infection by *P. plantaginis* adversely affected the photosynthetic capacity which was evinced from the reduced gas exchange parameters of the leaves (Fig. 2). Net photosynthesis rate in the healthy leaves was 16.26 μ mol CO_2 m^{-2} s^{-1} at first day of observation. During third day of observation net photosynthesis rate in the healthy leaves increased to 21.07 μ mol CO_2 m^{-2} s^{-1} and diseased ones recorded 42.67% lower than the healthy leaves. Net photosynthesis rate showed a decreasing trend during fifth (20.43 μ mol CO_2 m^{-2} s^{-1}) to ninth (16.35 μ mol CO_2 m^{-2} s^{-1}) day of study in the healthy leaves. During the same time period, net CO_2 assimilation rate in downy mildew affected leaves drastically reduced from 8.84 to 3.48 μ mol CO_2 m^{-2} s^{-1}. The diseased leaf samples showed net photosynthesis rate of 1.85 to 17.23 μ mol CO_2 m^{-2} s^{-1}, depending upon the status of the leaf discs. Stomatal conductance in healthy leaves showed an overall increasing trend during the course of the study. However, it decreased in the diseased leaves. Significant difference between the healthy and diseased leaves in

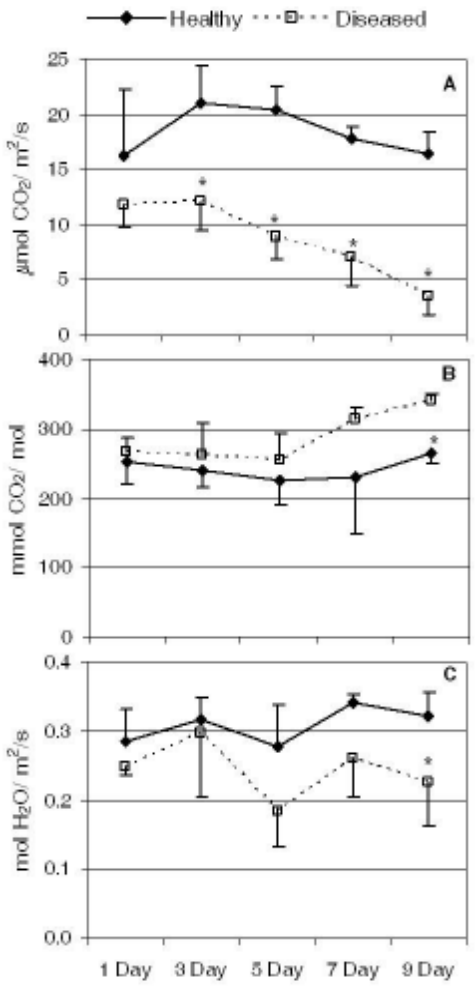

Fig. 2. Sequential changes of net photosynthesis (A), internal CO_2 concentration (B) and stomatal conductance (C) in healthy and downy mildew infected leaves. The bars represent the standard deviation (s.d.) of the mean. Absence of s.d. bars means it is not graphically evident.* indicates significant difference between healthy and diseased leaves by paired *t* test at *P*=0.05

stomatal conductance was observed at ninth day of the study. At this time, stomatal conductance in diseased leaves was 31.25% lower than that of healthy leaves. Interestingly, internal CO_2 concentration (C*i*) showed a reverse trend. C*i* was always lower in the healthy leaves compared to infected ones. At first day of observation, healthy leaves had 252.00 mol CO_2 mol^{-1} of C*i*, lower than the diseased leaves (268.61 µ mol CO_2 mol^{-1}). Both healthy and diseased leaves showed increasing trend in C*i* with initial decrease till fifth day. However, the difference between healthy and diseased was non-significant till seventh day of experiment. At the ninth day C*i* was 341.22 µ mol CO_2 mol^{-1} in diseased leaves while 266.25 µ mol CO_2 mol^{-1} in healthy leaves. Net photosynthetic rate was highly correlated (r= 0.86) with total leaf chlorophyll content of healthy leaves. However, such relation was not established in the diseased leaves. Among the different factors studied, total healthy leaf area and leaf chlorophyll content had positive correlations with photosynthesis in healthy plants. On the other hand, diseased leaf area had negative correlation with the photosynthetic rate. Proportion of healthy leaf area showed significantly high correlation with net photosynthetic rate which was more or less stable (r = 0.77 and 0.84) between the observations.

The present study indicated that downy mildew induced gradual reduction of leaf chlorophyll content in isabgol. Mild chlorosis during initiation of sporulation to development of blighted symptoms in the infected leaves took nine days under the field conditions. However, during the period under study, diseased portion of the leaf always had significantly lower chlorophyll contents (Fig. 1). In case of diseases caused by necrotrophic pathogens, several enzymes and toxins have been reported to be responsible in loss of chlorophyll and cell death. Rathore *et al*. (2001) have reported lower chlorophyll content in the downy mildew affected leaves of isabgol as compared to healthy leaves. They have observed that disease had more drastic effect on Chl*a* than Chl*b* content. The present study also established that *P. plantaginis* reduced Chl*a* content more severely compared to Chl*b* in isabgol leaf (Fig. 1). Studies in other host-pathogen systems show similar decrease in Chl*a*:Chl*b* ratio after infection (Wilhelmová, 2005). We presume the degradation of reaction centre is one of the reasons for the decrease in Chl a/b ratio. Mandal *et al*. (2009) earlier reported the reduction in PSII activity concomitant with decrease in chlorophyll content in downy mildew affected leaves of isabgol. Study of gas exchange parameters revealed that disease caused extreme reduction in CO_2 assimilation and stomatal conductance. Alteration in stomatal conductance and C*i* are indicated as major reasons for reduced photosynthetic activity in some host-pathogen systems (Bassanezi *et al.* 2002, Moriondo *et al*. 2005). However, in the present study, difference between infected and

healthy leaves in terms of stomatal conductance and C*i* were nonsignificant at the initial period of study (Fig. 2B, 2C). Hence, lower CO_2 assimilation was due to non-stomatal interference. Higher C_i could have been partially due to the respiration of the active intercellular pathogen. Similar results were obtained by Bassanezi *et al*. (2002) in bean-rust interaction and by Ribeiro *et al*. (2003) in fastidious bacteria infected sweet orange plants. Net photosynthesis and total cholorphyll content followed the similar trend at all days of observations. Chlorophyll content and photosynthetic rate shared significantly high correlation (r = 0.86) in the healthy leaves. Moriondo *et al.* (2005) observed that in downy mildew infected grape leaf, green tissues away from the lesion were not significantly affected in terms of photosynthetic activity, while Shtienberg (1992) found reduction in photosynthetic activity was proportional to the disease severity in downy mildew infected leaves of cucumber. In the present study, leaf chlorophyll and net photosynthetic rate did not follow significant correlation in the second experiment. Unlike earlier, where similar types of diseased leaves (in terms of leaf age and infection status) were selected, samples varied widely between themselves in the second experiment. Such sampling was intentionally followed to simulate the complex disease situation encountered under field conditions. The variations in physiological states (age) of the leaves along with the source-sink relationship are possibly responsible for the apparent disparity in the correlation between chlorophyll content and net photosynthesis.

Form the study it is concluded that under the influence of downy mildew, the chlorophyll content and photosynthetic rate of isabgol gets reduced and it is significantly correlated with proportion of healthy/ diseased area. These parameters could be considered as factors in assessing the loss of productivity and yield of isabgol.

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