Electrolyte leakage and carotenoid content in chickpea leaves in response to infection with *Ascochyta rabiei*

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**ABSTRACT:** The electrolyte leakage and levels of carotenoid content in leaves of chickpea *Ascochyta* blight resistant E100Y and susceptible H-208 cultivar were determined 2, 4, 6, 8 and 10 days after challenge inoculation with two isolates of blight pathogen. There was significant increase in the activity of electrolytes after inoculation with both the isolates in resistant and susceptible genotypes as compared to uninoculated control. However, increase was more pronounced in 6 as well as 10 days after inoculation as compared to control in resistant genotype while it was more from 8 to 10 days in susceptible genotypes. The carotenoid content was also significantly increased in resistant as well as susceptible genotype after inoculation with both the isolates of the pathogen. However, increase was more pronounced in susceptible genotype with both the isolates after 10 days of inoculation.

**Key words:** Electrolyte leakage, carotenoid content, *Ascochyta rabiei*, chickpea.

Chickpea blight caused by *Ascochyta rabiei* (Pass.) Labrousse is one of the economically important diseases of chickpea. One of the germplasm types E 100Y showed high resistance to this disease (Jalali and Khirbat, 1984). Annually, 25-50% of the crop is being destroyed in Punjab province alone due to this disease (Sattar, 1933). Similar losses are encountered in Haryana as well (Jalali, 1981).

Biochemical basis for resistance to *Ascochyta rabiei* has not yet been studied. Among several factors, electrolyte leakage appears to be an important phenomenon involved in pathogenesis. Changes in membrane permeability are the first detectable events in the onset of diseases caused by different pathogens (Thatchere, 1943; Wheeler and Hanchen, 1968; Vidhyasekaran et al, 1986; Vidhyasekaran, 1997). Disruption of cell membranes has been observed in many infected tissues (Aist, 1974; Leestadelmann et al., 1991; Park et al. 1992). Toxin produced by pathogens are known to induce electrolyte leakage (Vidhyasekaran, et al., 1986; Kohimoto and Otani, 1991). Electrolyte leakage has also been observed in resistant interactions particularly in hypersensitively reacting cells (Brisset and Paulin, 1991; Whalen et al., 1993).

Carotenoids have been shown to be related to carotenoids inactivate toxin produced by fungus *Cercospora oryzae* to *Cercospora* disease resistance in rice (Batchvarova et al., 1992). Carotenoids may also be involved in susceptible reactions. Active oxygen species are involved in disease resistance (Vidhyasekaran, 1997) and carotenoids quench such species (Goodwin, 1980) particularly singlet oxygen (Daub and Hangarter, 1983). Active oxygen species induce phenolics and phytoalexin synthesis and lignification (Vera-Estrella, et al., 1993; Rogers et al., 1988; Van Huytsee, 1987) and carotenoids induce susceptibility by suppressing one active oxygen species. Thus, increased electrolyte leakage and carotenoids are involved in resistant or susceptible reaction depending upon the host pathogen interaction. The present studies were undertaken to assess their role in chickpea - *Ascochyta rabiei* interactions.

**MATERIALS AND METHODS**

*Ascochyta* blight pathogen was isolated from two chickpea varieties having, blight infection at Research Farm, CCS Haryana Agricultural University, Hisar. Isolates used were isolated from variety H-208 (Isolate I) and PB-7 (Isolate II). The seeds of contrasting genotypes were planted in pots containing sterilized soil. Spore suspension (3 x 10^4 spores/ml) prepared from 8-10 day old cultures were used to inoculate 30 day old plants in the month of February. Plants sprayed with water served as control. The leaf samples were collected from inoculated as well as uninoculated plants from both the genotypes at 2, 4, 6, 8 and 10 days after inoculation. For electrolyte leakage, four chickpea leaves of inoculated and uninoculated genotypes (E 100Y and H 208) were taken and each leaf was cut into two nearly equal pieces. These were immersed in
50 ml of de-ionised water contained in 100 ml conical flasks. There were three replications for each treatment. The flasks were placed in shaker cum water bath at 37± 1°C and shaken for 8 h at 70 strokes per minute. Leaf material was then filtered and the conductance of leachates was determined with a digital conductivity meter. The conductivity of electrolytes was expressed as μ mhos.

For carotenoid estimation, 50 mg of leaf material was homogenised in 5ml of 80 percent ethanol with pestle and mortar under diffused light and centrifuged at 5000 rpm for 15 minutes. The supernatant was kept aside and residue was extracted again with 80 percent ethanol till supernatant became colourless. All supernatant samples were pooled together and total volume made to 15 ml with 80 percent ethanol. The absorbance was measured at 663, 645 and 440 nm against 80 percent ethanol as blank using spectronic 1001. Chlorophylls were calculated according to the equation given below:

Chlorophyll a = 12.7 $A_{663}$ - 2.69 $A_{645}$ mg/l.

Chlorophyll b = 22.9 $A_{645}$ - 4.68 $A_{663}$ mg/l.

Total Chlorophyll = 8.02 $A_{663}$ + 20.2 $A_{645}$ mg/l.

where $A_{663}$ and $A_{645}$ are absorbance value at respective wavelengths. Carotenoids were calculated by the method of Kalar (1960):

Carotenoids = 4.695 $A_{440}$ - 0.268 [Chlorophyll (a+b)] mg/l.

where $A_{440}$ is the absorbance value at 440 nm.

RESULTS AND DISCUSSION

The electrolyte leakage was higher in resistant genotype as compared to susceptible genotype after inoculation. There was significant increase in the activity of electrolytes after inoculation with both the isolates of the pathogen when compared with uninoculated control. Increase was more pronounced in 6 as well as 10 days after inoculation in resistant genotype while it was more from 8-10 days in susceptible genotype (Table 1).

Electrolyte leakage has been implicated in governing resistance/susceptibility in many host plants (Brisset and Paulin 1991; Whalen et al., 1993). Several investigators have reported permeability changes during pathogenesis in various diseases (Chile and Vyas, 1985; Sujithamma and Reddy, 1986; Vidhysekharan et al. 1986; Vidhysekharan, 1997). In the present investigation the increase was more pronounced in resistant genotype as compared to susceptible genotype at 10 days of inoculation. Roy (1977) reported that increase in electrolyte leakage in pathogen infected leaves was maximum after 2 days and then dropped considerably. It is known that alongwith the electrolyte, leakage of phenol also takes place. One of the probable reasons may be that phenol compounds get oxidized by oxidases and converted to quinone or higher molecular weight compounds which block the cell membrane, pores and thus decreasing the outward flow of electrolytes. The electrolytes themselves get depleted as a result of outward flow from the cells.

It is evident from Table 2 that there was significant increase in carotenoid content activity after inoculation only in susceptible genotype. However, increase was more pronounced in susceptible genotype with both the isolates after 10 days of inoculation.

Carotenoid content also plays role in the susceptibility of the host plants. In the present investigation, increase was more pronounced in susceptible genotype with both the isolates after 10 days of inoculation. Similarly, several workers reported that the carotenoid content induces susceptibility by suppressing one active oxygen species (Goodwin, 1980; Daub and

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### Table 1. Electrolytic leakage (μ mho) in the leaves of resistant and susceptible genotypes of chickpea in response to *Ascochyta rabiei* inoculation at different intervals

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Days after inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H-208 (S)</td>
</tr>
<tr>
<td></td>
<td>E 100 Y(R)</td>
</tr>
<tr>
<td>Inoculated with</td>
<td></td>
</tr>
<tr>
<td>Isolate-I</td>
<td>15 10 21 158 1074 18 23 412 417 1604</td>
</tr>
<tr>
<td>Isolate-II</td>
<td>8 11 24 334 757 16 23 28 361 1939</td>
</tr>
<tr>
<td>Uninoculated</td>
<td>8 11 11 21 12 14 15 17</td>
</tr>
</tbody>
</table>

Least significant difference to compare any two means=48

### Table 2. Carotenoid content (mg/g) in the leaves of resistant (R) and susceptible (S) genotypes of chickpea in response to *Ascochyta rabiei* inoculation at different intervals

<table>
<thead>
<tr>
<th>Treatments</th>
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</tr>
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<tr>
<td></td>
<td>H-208(S)</td>
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<tr>
<td></td>
<td>E 100 Y(R)</td>
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<tr>
<td>Inoculated with</td>
<td></td>
</tr>
<tr>
<td>Isolate-I</td>
<td>1.7 2.3 2.5 2.5 3.3 2.0 2.3 2.0 2.3 2.1</td>
</tr>
<tr>
<td>Isolate-II</td>
<td>1.6 2.1 2.4 2.5 3.2 2.0 2.9 2.2 2.0 2.3</td>
</tr>
<tr>
<td>Uninoculated</td>
<td>1.0 1.9 2.0 2.2 2.2 1.9 1.9 1.9 1.9 2.4</td>
</tr>
</tbody>
</table>

Least significant difference to compare any two means=0.4
Hangarter, 1983). Due to this reason, active oxygen species can not induce phenolics, phytoalexin synthesis and lignification in host plants.

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