INFLUENCE OF CHrysanthemum Stunt VIRUS ON RNA AND NUCLEOTIDES OF CHrysanthemum LEAVES

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Received December 14, 1973

(Communicated by Dr. L. D. Kapoor, F.A.Sc.)

ABSTRACT

Chrysanthemum stunt virus (CSV) increased the total RNA of the leaves. The total of AMP, ADP, ATP, and UMP, UDP and UTP was higher than the CMP, CDP, CTP plus GMP, GDP and GTP. The maximum changes were found in AMP, UMP, UDP and UTP. The studies suggested that analogue of adenine and uracil might prove useful.

I. INTRODUCTION

Viruses in Chrysanthemum have been reported by Brierley (1953) and Singh and Misra (1968). Chrysanthemum stunt virus (CSV) is highly contagious and rate of its spread in a susceptible variety has been observed in a ten-fold increase per annum, despite intensive roguing. Secondly CSV is only spread by handling. Its transmission by insects is not known. Therefore, the probability of intensification of Virus material and its spread in high rate increases with the crop-like Chrysanthemum which is propagated vegetatively. Chemotherapy has been used successfully to control viruses. Observations in changes in RNA and soluble nucleotides of CSV infected-host will be useful to gain understanding about the infection and help in logic use of chemicals to control CSV.

II. MATERIAL AND METHODS

The plants of the Chrysanthemum CV, 'Evening star' were grown under controlled and uniform conditions. This particular cultivar was selected because of its great susceptibility for CSV. The plants were infected with an inoculum of CSV as suggested by Hollings (1955). Composite samples of 5 g. fresh leaves were collected for the analysis of RNA and soluble nucleotides.
The RNA was isolated from the cleared homogenate and determined by the method suggested by Ogur and Rosen (1950) and modified by West (1962). Soluble nucleotides were separated and identified by a modified procedure explained by Cherry et al. (1961) and Singh (1971) who used ion exchange chromatographic techniques and spectroscopic analysis of the components isolated.

III. RESULTS

Ribonucleic acid.—The total RNV content in leaves increased gradually with the age of leaves. This increase was two to three fold in quantity on fresh weight basis. Table I and Fig. 1 further indicated that there was a significant increase in RNA content of virus-infected leaves.

Table I

Influence of CSV on total RNA content in leaves of Chrysanthemum

<table>
<thead>
<tr>
<th>Days after inoculation</th>
<th>Healthy</th>
<th>Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.57</td>
<td>0.63</td>
</tr>
<tr>
<td>60</td>
<td>0.95</td>
<td>1.14</td>
</tr>
<tr>
<td>90</td>
<td>1.52</td>
<td>1.74</td>
</tr>
<tr>
<td>120</td>
<td>1.50</td>
<td>1.74</td>
</tr>
</tbody>
</table>

Significant at 5% level.
C.D. at 5% 0.046.

Ribonucleotides.—Infected and non-infected tissues of 'Evening Star' Chrysanthemum were analysed on 120-day after treatment. Elution-chromatogram in Fig. 2 shows the nucleotides present in the infected and non-infected samples.

Table II shows the changes in nucleotides on 120th day after infection. There was considerable increase (79) in total adenosine mono- and tri-phosphates. Adenosine mono-phosphate was not observed in the tissues of non-infected leaves. Twenty-seven per cent drop was noted in the ADP
content of infected tissues. The content of ATP and AMP were higher than the control. Especially the amount of AMP in infected plants increased considerably as compared to non-infected plants.

![Graph showing RNA content over days after inoculation.](image)

**Fig. 1**

The infected and non-infected tissues did not differ significantly in the amount of total cytosine contents. However, cytosine tri- and di-phosphates were completely absent in infected tissues. Only cytosine mono-phosphates were completely absent in infected tissues. Cytosine mono-phosphate increased (18) significantly as compared to control.

The content of total guanosine phosphates was lower in infected tissues. The amount of guanosine tri- and di-phosphates was lower than the non-infected tissue. Guanosine di-phosphate was not observed in infected tissues. The amount of guanosine mono-phosphate increased to the extent of 32 per cent in virus-infected leaves.

The total content of uridine mono- di- and tri-phosphates of the infected tissues were lower than the UMP, UDP and UTP of non-infected tissues. This reduction was 48·0, 75·0 and 10·4 per cent in the contents of UTP, UDP and UMP respectively.

In short, CTP, CDP and GDP were not detected in infected tissues which were present in non-infected tissues after 120 days of treatment. Adenosine mono-phosphate was detected in very high amount in infected tissues as compared to control, in which it was not detected. Table II further reveals that adenosine and cytosine increased in infected tissues.
TABLE II

Influence of CSV on acid soluble nucleotides of ‘Evening Star’ Chrysanthemum tissues after 120 days of treatment (μg/g fresh weight)

<table>
<thead>
<tr>
<th>Nucleotides</th>
<th>Healthy</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>217</td>
<td>298</td>
</tr>
<tr>
<td>ADP</td>
<td>286</td>
<td>207</td>
</tr>
<tr>
<td>AMP</td>
<td></td>
<td>397</td>
</tr>
<tr>
<td>CTP</td>
<td></td>
<td>258</td>
</tr>
<tr>
<td>CDP</td>
<td></td>
<td>52</td>
</tr>
<tr>
<td>CMP</td>
<td></td>
<td>176</td>
</tr>
<tr>
<td>Total</td>
<td>503</td>
<td>902</td>
</tr>
<tr>
<td>GTP</td>
<td></td>
<td>105</td>
</tr>
<tr>
<td>GDP</td>
<td></td>
<td>88</td>
</tr>
<tr>
<td>GMP</td>
<td></td>
<td>201</td>
</tr>
<tr>
<td>Total</td>
<td>394</td>
<td>351</td>
</tr>
<tr>
<td>UTP</td>
<td></td>
<td>188</td>
</tr>
<tr>
<td>UDP</td>
<td></td>
<td>250</td>
</tr>
<tr>
<td>UMP</td>
<td></td>
<td>211</td>
</tr>
<tr>
<td>Total</td>
<td>649</td>
<td>348</td>
</tr>
</tbody>
</table>

Total nucleotides 2,032 2,097
Amongst these two adenosine increased considerably (79). On the other hand, guanosine and Uracil decreased to the extent of 11 and 46 per cent respectively. However, the total amount of nucleotide slightly increased (3) in infected tissues as compared to non-infected tissues.

IV. DISCUSSION

The increase in ribonucleic acid content was observed with the increase in age of the plant. This increase was found from two to three times in quantity on fresh weight basis, which was in agreement with the results of Monselise et al. (1961) in orange, and Kessler and Monselise (1961) in apple and orange leaves. This increase might be due to decrease in the Ribonuclease activity of leaves with tree age as evidenced by Kessler and Monselise (1961). Alternatively the increased RNA content in CSV-infected leaves might have occurred due to synthesis of viral RNA as suggested by Basler and Commoner (1956).

As shown in the data (Table II), there were marked differences between the soluble nucleotides of virus-infected and non-infected plants. This might be possible since some nucleotides were precursors for the RNA portion of the viral particle and other nucleotides are intimately involved in cellular metabolism. A significant finding could be the observation that nucleotide changes of virus, infected tissues showed some similarities to those reported by Marry and Froti (1958) and Key and Ingle (1968) who reported changes in ATP and AMP respectively in auxin treated tissues.

The total nucleotide content of CSV infected tissue was slightly higher than that of control. Cherry et al. (1961) and West (1962) found an increase in total nucleotide content of tissues during the early stage of treatment. They reported that there was no synthesis of foreign particle to utilize the nucleotides.

The difference in total content of adenosine phosphates was significantly higher between virus-infected and non-infected plants. However, the total content of cytosine phosphate was not significantly higher in diseased tissues than control. The content of guanosine and uridine phosphates was lower in virus-infected than in non-infected plants. This was similar to the results of Singh (1963) in tristeza-infected Key’ lime tissues. He suggested that viral synthesis would use guanosine and uridine phosphate.

The sum of adenine and uracil phosphates was higher than the sum of cytosine and guanine phosphates in virus-infected tissues. This indicates
that the cytosine and guanosine are depleted during the period of infection
to much larger extent than adenosine and uracil. The changes is soluble
nucleotide which could not be discussed here might have involved in such
essential biological processes as oxidative phosphorylation Lehninger et al.
(1958), amino-acid metabolism Clarke (1958) Carbohydrate transformation
Hassid et al. (1959) and lipid synthesis Kennedy (1957).

The significant changes in adenine and uridine phosphates as compared
to cytidine and guanine phosphates might suggest the use of proper analogue
for control. Singh (1973) also advocated the use of analogue of guanine
or cytidine to inhibit the symptoms of tristeza instead of the use of thio-
uracil which increased the symptoms because he found the maximum
changes in cytidine and guanine phosphates and later in other experiments
Singh and Verma proved that 8-azaguanine overcomes the inhibition of
length of laterals caused by tristeza showed that physiological studies mainly
in protein and RNA components may disclose better understanding of
infection and suggest the effective analogue to inhibit the virus.

V. ACKNOWLEDGEMENT

Authors are thankful to Shri U. N. Singh, for helping in the prepara-
tion of manuscript and Dr. Lawson/USDA for supply of the indicator
plants.

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