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Influence Of Tomato Yellow Leaf Curl Viral Infection On Antioxidant Content In Tomato (*Lycopersicon Esculentum*)

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Abstract

Tomato (*Lycopersicum esculentum*) is one of the most significant and extensively grown vegetable crop all over the world. Tomato Leaf Curl Viral disease is one of the key viral diseases leading to loss in productivity across the country. Antioxidants existent in plants play a vital role in this defence. In order to understand the basis of the tolerance study of the variation in the level of antioxidants in tomato against viral infections was necessary. Our study therefore focuses on analysing the influence of TLCV infection on variation in antioxidant content in tomato plants. Various antioxidants including alkaloids, ascorbic acid, total phenol, saponin and flavanoids were compared in healthy controls and TLCV infected tomato cultivars for a period of 30 days post inoculation. Previous reports suggest an increase in the antioxidant content in tomato plants in response to bacterial and fungal infection. The mechanism of host plant tolerance to various infections consists of a series of changes in biochemical events. This study reveals significant increase in the amounts of antioxidants post infection with ascorbic acid showing maximum rise.

1. INTRODUCTION:

Tomato (*Lycopersicon esculentum*) is the second most important vegetable crop next to potato. Tomato belongs to the family Solanaceae and ranks 2nd in importance among vegetables. India ranks 3rd in the production of tomato worldwide followed by USA and China[1].
Most plants have protective or defence mechanisms to protect themselves against disease and infections. Antioxidants are one major type of naturally occurring substances playing a vital role during defence in plants. Two types of natural antioxidant defence mechanisms have been found; enzymatic & peptide defence mechanism and non-enzymatic mechanisms including phenolic defence compounds, nitrogenous compounds and chlorophyll derivatives.

Antioxidants like ascorbic acid, reduced glutathione, flavanoids, alkaloids, saponins and total phenol protects the plant against various infections or diseases[2]. The quantities of antioxidants in the plant pre and post infections vary.

The present study therefore focuses on observing the influence of the production of various antioxidant compounds on the growth of tomato plants and its tolerance to Tomato Leaf Curl Virus.

2. MATERIALS AND METHODS:

2.1 ESTABLISHMENT OF INFECTED PLANTS:

Ten plants of sand, soil and compost containing different concentrations of fertilizer were used. After 30 days from the date of seeds sowing the developed tomato seedlings were transplanted in black nursery polypropylene bags.

2.1.1 EXTRACTION OF VIRUS

Hundred grams of TLCV infected young leaves of tomato plants were collected, washed and kept at –40 ºC overnight. Frozen leaves were homogenized using pestle and mortar in 0.1M phosphate buffer (1:1 w/v) containing 1% mercaptoethanol, 10mM EDTA, 1mM cysteine hydrochloride. The suspension was filtered through two layered muslin cloth. The filtrate was collected, mixed with half volume of chloroform and stirred at 4ºC for 30 minutes. This was followed by centrifugation at 10,000 rpm for 15 minutes. Aqueous phase was collected and 15% poly ethylene glycol (PEG: Mol. Wt. 8000) along with 1M sodium chloride (NaCl) was added. After stirring at 4ºC for 2 hours; the obtained mixture was further centrifuged at 16,000 rpm for 50 minutes. The precipitates were dissolved in 0.1M potassium phosphate buffer, pH 7.8 and filtered using a 0.45 micron syringe filter. The concentration of the obtained viral sample was estimated by reading the absorbance at 240 nm.

2.1.2 INOCULATION OF PLANT WITH TLCV

After one month of sowing, 2-3 young leaves, of seven out of the ten plants were bruised using silicon carbide and infected by rubbing a cotton wrapped stick dipped in TLCV sap to a final concentration of 35 µg of virus per plant. Remaining three plants were retained as controls. Ascorbic acid, total phenols, flavonoids, saponins and alkaloids were determined at 15 days intervals after infection.

2.2 ESTIMATION OF ASCORBIC ACID

2.2.1 EXTRACTION OF ASCORBIC ACID

Ascorbic acid was extracted from 1gm of the plant sample using 4% TCA and the volume was made up to 10 ml with the same. The supernatant obtained after centrifugation at 2000 rpm for 10 minutes was treated with a pinch of activated charcoal, shaken vigorously using a cyclomixer and kept for 5 minutes. The charcoal particles were removed by centrifugation and aliquots were used for the assay.

2.2.2 ASSAY OF ASCORBIC ACID

Two aliquots of samples obtained above were made up to 2 ml with 4% TCA. 0.5 ml of DNPH reagent was added to each tube. This was followed by addition of 2 drops of 10% thiourea solution. The contents were mixed and incubated at 37°C for 3 hours resulting in the formation of osazone crystals. The crystals were dissolved in 2.5 ml of cold 85% sulphuric acid. For blank solutions, DNPH reagents and thiourea were added after the addition of sulphuric acid. Control tubes were made using a standard solution of 0.1 mg/ml. The tubes were cooled in ice and the absorbance was read at 540 nm in a spectrophotometer.

2.2.3 ESTIMATION OF TOTAL PHENOLS

2.2.3.1 EXTRACTION OF TOTAL PHENOLS

0.5 gm of sample was homogenized in 10X volume of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The extraction was repeated with 80% ethanol. The supernatants were pooled and evaporated to dryness. The residue was then dissolved in a known volume of distilled water.

2.2.3.2 ASSAY OF TOTAL PHENOLS

0.5 ml and 1 ml of the samples obtained above were made up to 3 ml with distilled water. After addition of 0.5 ml of F-C reagent the tubes were placed in a boiling water bath for exactly one minute. Control tubes were made using a standard solution of 100 µg/ml. The tubes were cooled and the absorbance was read at 650 nm in a spectrophotometer against a reagent blank.

2.2.3.3 ESTIMATION OF ALKALOIDS

5 gm of the dried powder of each sample was weighed into a 25 ml beaker and 200 ml of 10% acetic acid in ethanol was added. The mixture is covered and allowed to stand for 4 hours. This mixture was then filtered and the obtained extract was concentrated to 1/4 of the original volume in a water bath. Concentrated ammonium hydroxide was added drop wise to the extract until the complete precipitation occurred. The entire solution was

allowed to settle and the obtained precipitate was collected and washed with equal volume of dilute ammonium hydroxide. The obtained alkaloid residue was weighed and percentage calculated.

2.2.3.4 ESTIMATION OF SAPONINS

2 gm of each sample was placed into a conical flask and 100 ml of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 hours with continuous stirring at 55°C. The mixture was filtered and the residue re-extracted with another 20 ml of 20% ethanol. The combined extract was reduced to 4 ml over water bath at 90°C. The concentrate was transferred into a 25 ml separating funnel and 2 ml of diethyl ether was added and the mixture was shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated using 6 ml of n-butanol. The combined n-butanol extracts were washed twice with 1 ml of 5% aqueous sodium chloride (NaCl). The remaining solution was heated in a water bath. After evaporation the samples were dried in oven to a constant weight and the percentage saponins content was calculated.

2.2.3.5 ESTIMATION OF FLAVANOIDS

1 gm of each plant sample was extracted repeatedly with 10 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper no. 41. The filtrate was allowed to evaporate until dry in a hot air oven. Weights of the flavonoids were calculated.

3. RESULTS:

3.1 EFFECT ON ASCORBIC ACID

The comparative study of the levels of ascorbic acid between the control and the infected plants initially showed vast variation. At 15 days post infection very large difference between the levels of ascorbic acid in the infected and control plants was observed. It was observed that all the controls showed higher levels of ascorbic acid as compared to the infected plants.

However, the results for the ascorbic acid levels 30 days post infection showed much variation. It was observed that all infected plants had higher ascorbic acids levels as compared to the controls.

3.2 EFFECT ON TOTAL PHENOLS

The comparative study of the levels of phenols between the control and the infected plants showed variation both at 15 days and 30 days post infection. It was observed that all the infected plants showed higher levels of phenolic compounds as compared to the controls.

3.3 EFFECT ON ALKALOIDS

The comparative study of the levels of alkaloids between the control and the infected plants showed variation both at 15 days and 30 days post infection. It was observed that all the infected plants showed higher levels of alkaloids as compared to the controls. Maximum levels of alkaloids were observed 30 days post infection in both controls as well as the infected plants

3.4 EFFECT ON SAPONINS

The comparative study of the levels of saponins between the control and the infected plants did not show much variation both at 15 days and 30 days post infection. Maximum increase in the levels of saponins was seen 30 days post infection in the infected plants.

3.5 EFFECT ON FLAVANOIDS

The comparative study of the levels of flavanoids between the control and the infected plants showed variation both at 15 days and 30 days post infection. It was observed that all the infected plants showed higher levels of flavonoids as compared to the controls.

4. DISCUSSION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most widely grown vegetable crops, popular due to its high nutritive value, taste and versatile use. Total production of tomatoes is very high in India with Gujarat contributing approximately 22% of annual production. Although the total cultivated area and production of tomato in Gujarat has increased gradually over last few years but productivity is still very low compared to the average yield of other states. Among the factors responsible for low yield of tomato, viral diseases are

considered to be the most serious. Tomato is susceptible to more than 200 diseases, out of which 40 are caused by viruses [3]. Among these viral diseases, *Tomato Leaf Curl Virus* (*TLCV*) belonging to family *Geminiviridae* and genus *Begomovirus* is considered most devastating [4].
Most plants have protective or defence mechanisms to protect themselves against disease and infections. Antioxidants are one major type of naturally occurring substances playing a vital role in plants defence. The present study therefore focuses on observing the influence of plant nutrition on the tomato plant growth and its tolerance to Tomato Yellow Leaf Curl Virus in terms of the production of various antioxidant compounds.

It was interesting to find that although levels of ascorbic acid were low initially in plants infected with TLCV, however 30 days post infection, all infected plants had higher ascorbic acids levels as compared to the controls. This could be indicative of an activated defence mechanism in all infected plants 30 days post infection. Previous studies have reported 25.7% increase in ascorbic acid concentration in root knot nematodal infection [6]

Several reports on the levels of alkaloids in plants infected with viral infections shows decrease in the levels of alkaloids. One report suggested potato virus Y infected plants having shown 24.22% decrease in alkaloids concentration post infection [7]. Our study contradicts these reports. It was observed that an increase in levels of alkaloids by 45.84% in TLCV infected plant occurred 15 days post infection. Although, 30 days post infection, decrease in levels of alkaloids was found.
In terms of the levels of the total phenols in the TLCV infected plants although increase in the levels of infected plants was observed our results showed only 32.14% increase in levels of the total phenols as compared to previous reports showing 72% increase in total phenols [7].

Apart from this, both saponins and flavonoids also showed significant increase. Concentration of saponins was seen to increase by 53.58% whereas 27.28% increase in levels of flavonoids was observed. Previous reports have also shown similar rise in levels of saponins and flavonoids [8, 9]. Previous reports suggest an increase in the antioxidant content in tomato plants in response to bacterial and fungal infection. Our study indicates similar mechanism for viral infections as well as with ascorbic acid showing maximum rise.

5. References:

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