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BIOCHEMICAL CHANGES TO *CAPSICUM ANNUUM* LEAVES INFECTED WITH GEMINI VIRUS

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Abstract

Capsicum annuum a member of Solanaceae or 'nightshade' family forms a familiar condiment obtained from the fruits. In present investigation, the biochemical changes in diseased leaf of *Capsicum annuum* (infected with Gemini virus) were observed and estimated. Chlorophyll a, b and total chlorophyll were more in healthy leaf as compared to diseased leaf, while total soluble sugar contents, starch contents and protein contents were more in the diseased leaf. The reducing sugar was moderately less in diseased leaf *Capsicum annuum* as compared to healthy.

Key words : Chlorophyll a, chlorophyll b, total soluble sugar, gemini virus, *Capsicum annuum*.

Introduction

The genus *Capsicum* is a member of Solanaceae or 'nightshade' family and it is sources of familiar condiment, which obtained from their fruits. *Capsicum annuum* L. is extensively used in culinary as spice in food and also in ayurvedic system of medicine.

In recent years, leaf curl disease has emerged as one of the most important and destructive diseases of chilli in India and causing severe losses in yield. The virus induced leaf curl symptoms are characterized by vein clearing, upward curling, deformation of leaves stunting of plants and abscission of flower buds. Ansari *et al*. (2006) and Meena *et al*. (2007) detected the chilli leaf curl virus by using AVF_9 and AVR_{10} primers for the amplification of coat protein gene of geminivirus.

Change in normal metabolism of host following infection is a wide spread phenomenon in a plant. Any symptom due to infection, brought by any pathological organism in plants is always associated with some biochemical changes in the tissues. Several workers have studied the biochemical changes in various plants after infection with pathological organism (Jain and Yadav, 2003; Parashar and Lodha, 2007; Sharma *et al*., 2011; Meena *et al*., 2014). The pathogen induces cell response by releasing its secretion, which comprises of auxin, enzymes and toxins to alter the host cell metabolism. With the development of disease a complex series of biochemical reactions proceed in an orderly and highly integrated manner. Equilibrium in metabolism is established between host and the parasite in localized phase of infection (Sharma, 2004).

As with microbial infections the progression of viral diseases is the result of complex host pathogen interaction. Alteration of metabolic pathways in plants infected with virus bacteria and fungi are quite expected. In foliar disease each of the principal process governing carbon flow (photosynthesis, respiration and translocation) can be affected producing profound imbalance, which reduces productivity even in uninfected parts of the plants (Daly, 1976).

For a proper understanding of host pathogen interactions it becomes obligatory to estimate quantitatively the proteins, carbohydrates, enzymes, etc. present in a particular host plant, so as to draw meaningful conclusions on host pathogen interaction. Hence, an attempt has been made to study the changes in the biochemical profile of healthy and diseased leaf of *Capsicum annuum*.

Materials and Methods

Plant materials

Healthy and diseased leaves of chilli were collected for the estimation of chlorophyll a, b and total chlorophyll, total soluble sugar, reducing sugar, starch and protein contents.

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Estimation of chlorophyll a, b and total chlorophyll

Fresh healthy and diseased leaves were collected from the field and washed. One gram of each sample was taken and ground with 20-40 ml of 80% acetone. The homogenate was centrifuged. The procedure was repeated till the residue was completely devoid of chlorophyll; the extract was made up to 50 ml.

The absorbance of the solution was recorded at 645nm and 663nm against the solvent (acetone) blank and concentrations of chlorophyll a, chlorophyll b and total chlorophyll were calculated using the following equation (Anonymous, 1949); total chlorophyll: 20.2(A645) + 8.02(A663); Chlorophyll a: 12.7(A663) – 2.69(A645); chlorophyll b: 22.9(A645) – 4.68(A663).

Estimation of total soluble sugar contents and reducing sugars contents

To extract total soluble sugar, 500 mg each of healthy and diseased leaves were ground with 10.0 ml of 80% ethanol. It was centrifuged at 2000 rpm for 20 minutes and supernatants of each sample were collected separately and were used for estimation of total soluble sugar and reducing sugars contents. The phenol sulphuric acid reagent method of Dubois *et al*. (1951) was used for the estimation of total soluble sugar while the dinitrosalicylic acid (DNSA) method of Miller (1972) was used for the estimation of reducing sugar. The quantity of total sugar was expressed as mg/g fresh weight of tissue.

Estimation of reducing sugar

1.0 ml extract of each sample was collected separately in a test tube. To this 1.0 ml DNSA reagent was added. The mixture was heated for 5 minutes in boiling water bath. After the colour had developed, 1.0 ml of 40% sodium-potassium tartrate was added, while it was still warm. After cooling the tubes in running tap water, the absorbance was measured at 575 nm against blank. Blank was prepared by adding all reagents used in sample preparation except plant material. The quantity of reducing sugars was expressed as mg/g fresh weight of tissue.

Estimation of starch contents

500 mg each of healthy and diseased leaves were homogenized with 10.0 ml of 80% ethanol. Each sample was centrifuged at 2000 rpm for 20 minutes, supernatant of each sample was discarded and the residue was collected.

The residual mass obtained after the extraction of total soluble sugars of normal and gall tissues were suspended in 5.0 ml of distilled water and subsequently 6.5 ml of 52% perchloric acid was added to the residue. After stirring the content for 15 minutes, the mixture was centrifuged for 20 minutes at 2000 rpm. The supernatant was decanted and collected. The whole procedure was repeated thrice. Supernatants of each step were then pooled and total volume was made up to 100.0 ml with distilled water. The mixture was then filtered through Whatman no. 42 filter paper. 1.0 ml aliquot of this filtrate was analyzed for starch content following the same procedure as that of total soluble sugars. Quantity of starch was calculated in terms of glucose equivalent and factor 0.9 was used to convert the value of glucose to starch. Quantity of starch was expressed in terms of mg/g fresh weight of tissue.

Total protein contents

Total protein contents were estimated by using the methods of Lowry *et al*. (1951). For this 500 mg each of fresh normal and diseased leaf tissues were extracted with 5.0 ml of 5% trichloroacetic acid (TCA). The homogenate was centrifuged at 2000 rpm for 20 minutes and the supernatant was discarded. The residue was dissolved in 5.0 ml of 0.1 N NaOH. The 0.1 ml of this solution was making up to 1.0 ml by adding distilled water. The dissolved residue was subsequently treated with 5.0 ml of alkaline copper reagent (for 10 minutes) and 0.5 ml of folin-ciocalteu reagent (for 10 minutes). The optical density was measured at 750 nm in a spectrophotometer (UV-VIS-Systronics-118). The amount of protein in the sample was calculated with a standard curve prepared from bovine serum albumin (BSA) and expressed as mg/g fresh weight of the tissue.

Results

Chlorophyll a, b and total chlorophyll content in healthy leaf were 0.2834 mg/g, 0.1650 mg/g and 0.4932 mg/g of fresh weight of tissue, respectively, while in diseased leaf chlorophyll a, b and total chlorophyll content were decreased and reached up to 0.0489 mg/g, 0.0779 mg/g and 0.1425 mg/g of fresh weight of tissue, respectively (table 1).

Similar to chlorophyll content, reducing sugar content was also decreased in diseased leaf (0.34 mg/g of fresh weight of tissue) in compare to healthy leaf (0.50mg/g of fresh weight of tissue) (table 1). Apart from this the total soluble sugar; starch and protein content were significantly increased in diseased leaf in compare to healthy leaf and reached up to 3.90 mg/g, 1.8mg/g and 1.20 mg/g of fresh weight of tissue, respectively, while in healthy leaf these were 3.60 mg/g, 1.3 mg/g and 0.90 mg/g of fresh weight of tissue, respectively (table 1).

Table 1 : Changes in biochemical profile of *Capsicum annuum* leaves infected with gemini virus.

Parameter	Concentration of various biochemical compound (mg/g of fresh weight of tissue) in healthy and diseased leaf	
	Healthy leaf	Diseased leaf
Chlorophyll a	0.2834 ± 0.021	0.0489 ± 0.019
Chlorophyll b	0.1650 ± 0.011	0.0779 ± 0.017
Total chlorophyll	0.4932 ± 0.005	0.1425 ± 0.009
Reducing sugar	0.50 ± 0.020	0.34 ± 0.013
Total soluble sugar	3.60 ± 0.008	3.90 ± 0.012
Starch	1.3 ± 0.015	1.8 ± 0.018
Protein	0.90 ± 0.007	1.20 ± 0.022

Experiments were performed in triplicate.

Discussion

Chlorophyll pigments are necessary in the plants for the manufacture of carbohydrates which form the basis of all foods for both plants and animals, which enter into the composition of all plant tissues. It is for this reason that chlorophyll pigments are regarded among the most important chemical substances in nature.

Decrease in chlorophyll content in infected leaves was due to chlorosis and necrosis of diseased plant parts especially leaves. Similar observations were recorded in rice varieties by Emanual *et al*. (2002) and urd bean by Malik *et al*. (2002). Parthasarathi *et al*. (1976) and Sethi (1989) observed destruction of both chlorophyll a and b in case of spike affected sandal plant. Marcos *et al*. (2005) described that *Sunflower chlorotic mottle virus* (SUCMOV) caused chlorotic mottling symptoms and important growth reduction and yield losses in sunflower. After symptoms became evident CO_2 fixation rate decreased, nevertheless soluble sugars and starch increased but chlorophyll contents decreased in infected leaves.

Muqit *et al*. (2007) observed decreasement in chlorophyll a, b and total chlorophyll contents in ash gourd infected with three different viruses.

Several authors have reported increase in starch content as consequence of viral and yellows type of diseases (Quanjer, 1931 and Diener, 1963). Increase in sugar content due to viral disease in plant was also reported by many workers (True *et al*., 1918; Rosa, 1927; Iyengar and Varadaraja, 1928; Mandahar and Garg, 1973; and Sethi, 1989). A significant increase in the contents of reducing and non reducing sugar was observed in leaves and fruits of chilli infected with chilli mosaic virus (Patel, 2004).

Virus induced disturbance of carbohydrate metabolism is supposed to be responsible for the so called starch lesions. After a period of active photosynthesis, the site of local infection contained less starch than the neighbouring non infected tissues, whereas after period of darkness infected tissue contained more starch than the non-infected ones thus infection decreased the rate of starch synthesis and translocation. Tecsi *et al*. (1994) observed starch accumulation and low sucrose content during the infection of cotyledons of *Cucurbita pepo* L. with cucumber mosaic virus (CMV). Sampol *et al*. (2003) observed that virus infection resulted in decreased photosynthesis by 50% as observed in masley grapevines. A significant increase in the contents of reducing and non reducing sugar was observed in leaves and fruits of chilli infected with chilli mosaic virus (Patel, 2004).

The proteins are complex polymer of amino acid with high molecular weight. They are most important chemical constituents of living organisms. Protein contents were higher in diseased leaf as compared to healthy leaf. Similar results were observed by Prakash *et al*. (1995) in leaves and seeds of *Amaranthus* and *Chenopodium* infected with cucumber mosaic virus. Increase in protein contents was also observed in chilli varieties infected with chilli mosaic virus (Patel, 2004).

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