Original Research Article

Alternations in biochemical components in chilli (*Capsicum annuum* L.) plants infected with chilli leaf curl viral disease

Abstract

Chilli (*Capsicum annuum* L.) is an important spice crop grown all over the world. Chilli leaf curl, a viral disease outbreak in almost every chilli grown area causing significant yield losses. An experiment was conducted to analyze the biochemical changes in infected and healthy plant of chilli. The chlorophyll a, b and total were highest in healthy leaves then infected leaves. Moreover, severely infected leaves have lower amounts of chlorophyll contents. Out of nine cultivars total phenol and tannin contents were found maximum in Gucchedar, POL-75 and Byddagi Kaddi in comparison to highly susceptible cultivars Musa Badi, Phuley Joyti and NPKT-2.

Key words: *Capsicum annuum L.***,** bio-chemical, Chilli leaf curl virus, yield losses

Introduction

India is the largest producer of chillies in the world with the production of about 8.5 lakh tones, followed by China, Pakistan and Maxico. Chilli (*Capsicum annuum* L.) belonging to order solanales of family solanaceae is one of the widely cultivated crops grown for the value of its green and ripe fruits in India. India is rich in maximum diversity of chilli varieties with differing habit, size, shape, colour and pungency of fruit. Besides, traditional use of chilli as vegetables, spices, condiments, souces and pickles, it is also used in pharmaceuticals, cosmetics and beverages (Tiwari *et al.*, 2005). India produces approximately 12.60 thousands metric tonnes from an area of 792.1 thousands hectare (Anon., 2014). Uttar Pradesh occupies an area of 13.47 thousand hectares with production of 10.30 metric tonnes (Anon., 2014). The yield of chilli is much lower than potential yield of improved varieties, it is due to several factors- lack of improved varieties against incidence of different pest and diseases are the main constraints for getting low productivity (Dey *et al.*, 2001).

Viral diseases of plants cause economic losses to the tune of 15 billion dollars per annum on global basis (Van Fanbing, 1999), particularly in tropical and semitropical regions. These regions provide ideal conditions for the perpetuation and transmissions of viruses through vectors. Natural occurrence of several viruses have been reported on chilli (*Capsicum annuum* L.) by various workers (Martelli and Quacquarelli, 1983), among them leaf curl virus has been reported most destructive for chilli, tomato, potato, okra, cotton and cucumber cultivation in terms of incidence and yield losses (Green, 1992). First incidence of leaf curl virus affecting chilli in India was first reported from Jodhpur during December, 2004 and emerged as a serious problem in major chilli growing area of Rajasthan with very high disease incidence upto 100 per cent plants infection in farmers fields (Senanayake *et al.,* 2007). Leaf curl virus is a member of genus *Begomoviruses,* family Geminiviridae. The genome of *Begomoviruses* have a single or double stranded circular DNA of C. 2.7kb, which is encapsulated in a quasi-isometric geminate particles of C.20-30nm (Harrison, 1995). During the past two decades, agricultural intensification has resulted *Begomoviruses* inciting disease out breaks in tropical and subtropical regions, causing 80 per cent yield losses of many crops (Singh *et al.,* 1979, Morales and Anderson, 2001). Vasudeva and Samraj (1948) first reported leaf curl disease on tomato from India. The virus is transmitted in nature by the white fly (*Bemisia tabaci*) in a semi-persistant (Circulative) manner. The minimum acquisition and inoculation feeding periods are 15-30 minutes, the latent period in the vector is more than 20 hours and virus is retained by the vector upto 20 days (Cohen and Nitzany, 1966). Single white fly can carry a finite number of virions in the range of 600 million (Zeidan and Czoosnek, 1991).

Elicitors stimulate the contact between plants and phytopathogens and thereby trigger defensive mechanisms that constrain the invasion of pathogenic viruses. The phenol and tannins are more studied biochemicals, which regulate the expression of resistance genes and induced its synthesis in plant (Thind *et al.,* 1996). These techniques for the detection of chlorophyll, phenol and tannin have provided an opportunity to develop methods for the diagnosis of plant resistance. Analysis of bio-chemical provides very useful information on the defense mechanism of plant against pathogen infection.

Methods and Materials

1. Estimation of chlorophyll

1.1 Collection of samples

The healthy and infected leaves of chilli cultivars *viz.*,Bydagi Kaddi, Musa Badi, Phuley Joyti, CA-960, Gucchedar, POL-75 , NPKT-2, PDC-54 and Pusa Sadabhar were collected from field at the time of flowering and fruiting stage for the estimation of chlorophyll. Collected leaves were washed with distilled water and excess water of the leaves was soaked with filter paper. Then 100mg leaves were grind into 80 per cent acetone with the help of mortar and pestle The grind solutions were taken into test tubes and final volume made to 10 ml by adding 80 per cent acetone. The solutions were centrifuged at 5000rpm for 10 minutes. The supernatant was taken in clean test tubes separately and repeated the step for two times until the pellet is clear. The absorbance was recorded at 663 and 645 nm in a spectrophotometer (SL 159 UV VIS spectrophotometer) against 80 per cent acetone blank. The amount of chlorophyll a, b, and total chlorophyll (mg/g fresh weight) were calculated according to Arnons formula, (1949).

$$\text{Chlorophyll a (mg/g tissue)} = 12.7\,(A_{663}) - 2.69\,(A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll b (mg/g tissue)} = 12.9\,(A_{645}) - 4.68\,(A_{663}) \times \frac{V}{1000 \times W}$$

$$\text{Total chlorophyll (mg/g tissue)} = 20.2\,(A_{645}) - 8.02\,(A_{663}) \times \frac{V}{1000 \times W}$$

Where,

A= absorbance at specific wavelength,

V= final volume of chlorophyll extract in 80% acetone

W= fresh weight of tissue extracted

The values of chlorophyll a and b were added to obtain the total chlorophyll content in mg /g fresh weight of leaf.

2.1 Estimation of total phenol

2.1.1 Procedure

The healthy and infected leaves of chilli cultivars *viz.*, Bydagi Kaddi, Musa Badi, Phuley Joyti, CA-960, Gucchedar, POL-75, NPKT-2, PDC-54 and Pusa Sadabhar were collected from field at the time of flowering and fruiting stage for the estimation of total phenol. A quantity of 1.0 g of fresh leaves sample was weighted and cut into small pieces then placed in smearing methanol until the green colour was extracted. Leaf tissues were homogenized after decanting the methanol. These homogenized tissues were again boiled in methanol for further 5

minutes and then filtered. Residual material was washed with 80% acidified (0.1% HCL Conc.) methanol. Methanol was evaporated using a rotavapor and the aqueous layer was collected to adjust the final volume as ml/g of weight with distilled water. The aqueous portion of the extract was then washed with n-hexane to remove the green colour. Total phenols were estimated using Folin-Ciolcalteu reagent, according to the modified method of Bray and Thorpe (1954). Tubes were kept in boiling water for one minute and allowed to cool for few minutes then measured absorbance at 650nm against a reagent blank. Standard curve was prepared using different concentration of catechol. The absorbance was measured in a spectrophotometer at 650nm against blank reagent.

3.1.1 Calculation

The amount of total phenols was calculated with the help of standard curve by the following formula.

$$\text{Total phenol (mg/100g fresh leaves)} = \frac{\text{G.F. x O. D. x Total volume}}{\text{Aliquot taken x Weig ht of sample x 10000}}$$

Where,

G.F. = graph factors

O.D. = Observance value

3.2 Estimation of tannin content

Sodium tungstate (100) and phosphormolibic acid (20g) were dissolved in 750ml distilled water and later 50ml phosphoric acid was added into the solution. Mixture was relaxed for two hours and volume was made to one liter with distilled water.

3.2.1 Procedure

The healthy and infected leaves of chilli cultivars *viz.*,Bydagi Kaddi, Musa Badi, Phuley Joyti, CA-960, Gucchedar, POL-75 , NPKT-2, PDC-54 and Pusa Sadabhar were collected from field at the time of flowering and fruiting stage for the estimation of total tannin. The crude powders of the leaves were prepared for photometric determination of tannins. The standard procedure was followed by Folin-Denis method. Powdered material of leaves (0.5g) was transferred into 250ml conical flask and 75ml of water added to it and heated for 30 minutes. The solution was centrifuged at 2000rpm for 20 minutes supernatant was collected and collects the supernatant in 100ml volumetric flask and make up the volume. Then transfer 1.0ml of extract sample into 100ml volumetric flask containg 75ml distilled water, added 5.0ml of Folin-Denish reagent and 10ml sodium carbonate

solution (sodium carbonate 350g was dissolved in 1000ml water at 70^0C temperature). Solution was allowed to stand overnight and then it was filtered through glass-wool and diluted it with 100ml of distilled water and shaken well. Observation were recorded at 700nm after 30 minutes against blank.

3.2.2 Preparation of standard curve

Standard tannic acid solution of 0.1 to 1.0 per cent (100g of tannic acid was dissolved in 100 ml distilled water) were taken in separate clean test tubes, 0.5ml Folin-Denish reagent and 1ml of sodium carbonate solution were added to each test tube. Volume of each test tube was made upto 10.0ml with adding distilled water and kept undisturbed for about 30 minutes and read the absorbance.

Results discussion

4.1 Chlorophyll content

Results presented in Table (1) showed that average chlorophyll a was significantly higher in healthy leaves (0.684 mg g^{-1}) in comparision to infected leaves (0.633 mg g^{-1}). In case of variety, it was found maximum in NPKT-2 and Musa Badi (0.73 mg g^{-1}) followed by Phuley Joyti (0.70 mg g^{-1}), Pusa Sadabhar (0.69 mg g^{-1}), CA-960 (0.69 mg g-^{1}), Pol-75 (0.68 mg g^{-1}) and Gucchedar (0.66 mg g^{-1}) in healthy leaves. NPKT-2 and Musa Badi have similar amount of chlorophyll a. Whereas infected leaves, the highest chlorophyll a contents was found in Phuley Joyti and Gucchedar (0.74 mg g^{-1}) and lowest in Pusa Sadabahar (0.60 mg g^{-1}) and NPKT-2 (0.60 mg g^{-1}). The mean chlorophyll b content was significantly highest in healthy leaves (0.984 mg g^{-1}) and lowest in infected leaves (0.832 mg g^{-1}). However, the average chlorophyll b content was found highest in Pusa Sadabhar (1.20 mg g^{-1}) followed by CA-960 (1.03 mg g^{-1}), Gucchedar (1.06 mg g^{-1}) and Phuley Joyti (1.16 mg g^{-1}) in healthy leaves. In case of infected leaves, the maximum chlorophyll b content was found CA-960 (1.08 mg g^{-1}) as compared with Pusa Sadabhar (1.06 mg g^{-1}), Gucchedar (1.02 mg g^{-1}) and Pol-75 (0.80 mg g^{-1}). The total chlorophyll content was also found highest in healthy leaves (1.678 mg g^{-1}) as compared with infected leaves (1.508 mg g^{-1}). The average total chlorophyll content was recorded maximum in Pusa Sadabhar (1.90 mg g^{-1}) followed by Gucchedar (1.78 mg g^{-1}), CA-960 (1.73 mg g^{-1}) and Phuley Joyti (1.86 mg g^{-1}) in healthy leaves. Whereas infected leaves, it was highest in Gucchedar (1.77 mg g^{-1}) and lowest in NPKT-2 (1.27 mg g^{-1}). Chlorophyll a, and b contents were found either similar or at par between most of the cultivars.

4.2 Total Phenol

Data presented in Table (2) revealed that average total phenol content was significantly lowest in leaf curl infected leaves (5.582 mg g^{-1}) as compared to healthy leaves (7.48 mg g^{-1}). Whereas in variety, the average total phenolic content was found maximum in Gucchedar (9.46 mg g^{-1}) followed by POL-75 (8.90 mg g^{-1}), Byddagi Kaddi (6.94 mg g^{-1}) and Pusa Sadabhar (5.91 mg g^{-1}). While in healthy leaves, it was found highest in Gucchedar (10.35 mg g^{-1}) followed by POL-75 (10.06 mg g^{-1}), Bydagi Kaddi (9.72 mg g^{-1}), Pusa Sadabhar (7.96 mg g^{-1}), NPKT-2 (7.91 mg g^{-1}), PDC-54 (6.88 mg g^{-1}), CA-960 (6.17 mg g^{-1}), Phuley Jyoti (4.51mg g^{-1}) and Musa Badi (3.80 mg g^{-1}). But in infected leaves, total phenolic content was significantly lowest in Musa Badi (1.74 mg g^{-1}) in comparison to Phuley Joyti (2.84 mg g^{-1}), NPKT-2 (5.444 mg g^{-1}) and CA-960 (4.67 mg g^{-1}).

4.3 Total Tannin

Results are described after the critical examination of data presented in Table (3). The average total tannin content was observed significantly higher in healthy leaves (4.028 mg g^{-1}) as compared with infected leaves (3.128 mg g^{-1}). Whereas in variety, the average total tannin content was highest in Gucchedar (5.59 mg g^{-1}) followed by CA-960 (4.50 mg g^{-1}), Phuley Joyti (4.56 mg g^{-1}), Pusa Sadabhar (4.59 mg g^{-1}) and Bydagi Kaddi (4.30 mg g^{-1}). In Infected leaves it was highest in Gucchedar (4.74 mg g^{-1}) and lowest in Musa Badi (1.18 mg g^{-1}). While in healthy leaves of individual variety showed maximum 5.80 mg g^{-1} tannin content in Gucchedar and minimum 1.57 mg g-^{1} in Musa Badi. The total tannin content was found same in Pusa Sadabhar (4.59 mg g^{-1}) and Phuley Joyti (4.56 mg g^{-1}).

Discussion

It has been noted that the biochemical which suppresses the incidence of whitefly and viruses in plant might be phenol, tannins, flavonoids, saponnins etc. The biochemical study of infected and healthy plants showed the availability of lower amount of total phenol and tannin content in virus infected plants. The accumulation of phenol and tannin were highest in resistant variety and lowest in susceptible variety. Chatterjee and Ghosh (2008) observed lower amounts of phenolics in diseased plants after 110 days of virus inoculation in Mesta plants. Rathi *et al.,* (1986) also found high accumulation of phenolic compounds in resistant varieties and low amount in susceptible varieties of virus infections. Finding are supported with Mishra and Mohanty, (2007), Mishra *et al.*, (2010) and Mahjbeen (2011).Tannin, starch, Polyphenol oxidase and peroxidase were

observed an enhanced quantity in leaf curl infected chilli leaf as compared to healthy leaf, it is due to localization of metabolites and enzymes activity in infected leaves. Singh *et al.,* (2013) observed enhanced amount of tannic acid in healthy roots than root knot infected roots.

Chloropyhll a, b and total chlorophyll content were found higher in healthy leaves as compared to infected leaves. Chia and He (1999) recorded reduced amount of chlorophyll a, b and total chlorophyll content in viral infected leaves. In contrary, chlorophyll contents is greatly increased within four weeks after virus infection and then slowly decreased until the end of cropping period (Funayama *et al.*, 1997). The chlorophyll content was observed maximum in resistant varieties viz., NPKT-2, Musa Badi and Phuley Joyti and minimum in susceptible variety such as Pusa Sadabhar, Gucchedar and Pol-75. The total chlorophyll content, chlorophyll a and chlorophyll b were significantly decreased in turnip mosaic virus infected plants to be reflected by the slightly yellowish colour of leaves (Guoa *et al.*, 2005). The gradual reduction of green pigments like chlorophyll a, b and total at different stage of virus infection in Mesta plant was observed by Chattergee and Ghosh, (2008). The disease development in Mesta also altered by the ratio between chlorophyll a and b, which is probably affecting the photosynthetic efficiency of plants (Endo *et al*., 2000).

Conclusion

Resistant cultivars have higher amount of chlorophyll, tannin and phenol contents as compared to susceptible cultivars.

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Table 1- Estimation of chlorophyll content (mg/g leaf) in healthy and leaf curl viral infected genotypes of chilli.

S.No.	Variety	Chlorophyll a (mg/ g fresh weight)		Chlorophyll b (mg/ g fresh weight)		Total chlorophyll (mg/ g fresh weight)	
		Healthy leaf	Infected leaf	Healthy leaf	Infected leaf	Healthy leaf	Infected leaf
1	Bydagi kaddi	0.64	0.65	0.84	0.74	1.48	1.47
2	Musa badi	0.73	0.66	0.96	0.67	1.70	1.34
3	Phuley joyti	0.70	0.74	1.16	0.79	1.86	1.53
4	CA-960	0.69	0.63	1.03	1.08	1.73	1.71
5	Gucchedar	0.66	0.74	1.06	1.02	1.78	1.77
6	POL-75	0.68	0.70	0.92	0.80	1.58	1.50
7	NPKT-2	0.73	0.60	0.89	0.67	1.63	1.27
8	PDC-54	0.64	0.65	0.80	0.66	1.45	1.32
9	Pusa sadabhar	0.69	0.60	1.20	1.06	1.90	1.67

Table 2- Estimation of total phenol (mg/g leaf) in healthy and leaf curl viral infected genotypes of chilli.

S.No.	Variety	Total phenols	
		Healthy leaf	Infected leaf
1	Bydagi kaddi	9.72	6.94
2	Musa badi	3.80	1.74
3	Phuley joyti	4.51	2.84
4	CA-960	6.17	4.67
5	Gucchedar	10.35	9.46
6	POL-75	10.06	8.90
7	NPKT-2	7.91	5.44
8	PDC-54	6.88	4.34
9	Pusa sadabhar	7.96	5.91

Table 3- Estimation of total tannin (mg/g leaf) in healthy and chilli leaf curl viral infected genotypes of chilli.

S.No.	Variety	Total tannin	
		Healthy leaf	Infected leaf
1	Bydagi kaddi	4.30	3.14
2	Musa badi	1.57	1.18
3	Phuley joyti	4.56	3.67
4	CA-960	4.50	3.86
5	Gucchedar	5.80	4.74
6	POL-75	3.75	2.74
7	NPKT-2	3.47	2.74
8	PDC-54	3.72	2.52
9	Pusa sadabhar	4.59	3.57