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Tomato yellow leaf curl virus (TYLCV) alters the phytochemical constituents in tomato fruits

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Abstract

An investigation was conducted in order to evaluate the responses of field grown tomato varieties (*Marglove* and *Roma* VF) to tomato yellow leaf curl virus (TYLCV). Fruit samples from the virus-infected and uninfected plants were collected at 20 (early stage), 40 (intermediate stage) and 60 days (ripening stage) after anthesis. Results showed higher virus RNA content in fruits of infected plants at early (42.48 to 38.24%) and intermediate stages (34.35 to 19.57%). There was a substantial decrease in DNA content (27.27 and 21.05%) at early and (23.08 and 43.75%) at intermediate stages of both *Marglove* and *Roma* VF, compared to the control, respectively. Similarly, indole acetic acid content was also decreased (27.08 and 24.29%) in fruits of virus-infected *Marglove* and *Roma* VF, respectively. The free ascorbic acid content was found lower (35.29 to 51.52%), while combined ascorbic acid was higher (13.91 to 33.33%) in both varieties. Neither the responses of individual organic acids nor their concentrations in fruits of infected and control plants were identical. Fumaric acid was not detected either in fruits of infected plants of *Marglove* or in healthy and infected *Roma* VF. Individual fruit weight and fruit numbers per plant were lower in the virus-infected plants. This study indicates that the yield of infected tomato plants could be reduced by the infection of TYLCV due to the changes in the concentrations of phytochemical constituents. This suggests that monitoring and management of TYLCV incidence is crucial for yield and quality optimization of field grown tomato.

Keywords*:* Anthesis; ascorbic acids; auxin; nucleic acids; organic acids; phytochemical constituents; virus infection; tomato yield.
Abbreviations: AUX- auxin, DNA- deoxyribonucleic acid, HPLC- high performance liquid chromatography, IAA- indole acetic acid, MP- murate of potash, TMV- tobacco mosaic virus; RNA- ribonucleic acid, TSP- triple super phosphate, TYLCV- tomato yellow leaf curl virus.

Introduction

Tomato (*Lycopersicon esculentum* Miller.) is the world's second most extensively grown horticultural crop after potato. Present world production is about 100 million tons fresh fruit produced on 3.7 million hectares. The production has been reported for 144 countries (FAOSTAT Database, 2004). Unfortunately, plants in nature are constantly challenged by a diverse array of plant pathogenic microorganisms. Many viruses like tomato yellow leaf curl virus (TYLCV), tomato leaf curl virus, tobacco mosaic virus (TMV), potato virus X, potato virus Y, cucumber mosaic virus, beet curly top, etc. are known to infect tomato plants (Navas-Castillo, 1999; Moriones and Navas-Castillo, 2000). Among them, TYLCV is prevalent and widely distributed in many Mediterranean, Middle Eastern, African, and Asian countries including Bangladesh, causes a dramatic yield loss to the crop and poses a great threat to commercial tomato cultivation. Infection levels range from 5 to 100% of plants (Rybicki et al., 2000). Losses from plant diseases can have a significant economic impact, causing a reduction in income for crop producers, distributors, and higher prices for consumers. As TYLCV continues to spread, many isolates have been described in different parts of the world. However, this disease has not been properly investigated in Bangladesh. The biochemical alterations of cellular constituents are reported to be directly related to morphological deviation of virus infected plants and the extent of crop loss is largely determined by visible symptoms (Levy and Marco, 1982). The symptoms specificity and its severity are concerned with the changes of specific cellular components due to virus infection (Sreenivasulu et al., 1989). A dramatic biochemical changes in virus infected plants result in decrease of both quality and quantity of infected crops (Al-Musa, 1982). Various reports suggest that virus multiplication inside the plant cell alters different biochemical constituents of plants and disrupt the physiological process like photosynthesis, transpiration and respiration of the infected plants which affect the growth and yield (Fraser, 1987). They also reported that the determination of cellular constituents in virus infected plant is very important to understand the activities of the host cell and the nature and extent of damage caused by the virus. TYLCV is transmitted exclusively by a single insect species, the whitefly *Bemisia tabaci* in a circulative manner (Ghanim and Czosnek, 2000). The virus differs in nature of survival, host range, transmission and field epidemiology. Although not systematically studied, it is assumed that the disease may be one of the major causes of poor yield to tomato in Bangladesh. Scientists in recent years showed keen interests to find out the biochemical impact of

viruses. Al-Musa and Monsour (1983) reported that TYLCV cause the prevalent disease characterized by stunting, chlorosis, curling, rosette, enations, and distortion on leaves, flowers and fruits. Alam et al. (1996) noticed biochemical alterations only in leaves of tomato caused by different viruses. Makkouk et al. (1979) and Al-Musa (1982) reported quantitative and qualitative reductions in yield in infected plants compared to healthy. So far, there is no information regarding biochemical status of the fruits which may cause yield reduction in tomato by TYLCV. Physiological and genetic base for the organic acid, nucleic acid and auxin accumulation in fruits of infected tomato plants have not properly been investigated by researchers. In view of its importance, the present study was undertaken to follow the changes in ascorbic acids, nucleic acids, auxin, and individual organic acids in fruits of tomato plants after TYLCV infection. The nutritional status and yield in fruits of infected tomato caused by TYLCV were compared to healthy plants.

Materials and methods

Soil and crop

The study was carried out at the experimental farm and in the laboratory during *rabi* season (October to February) at BSMR Agricultural University, Gazipur, Bangladesh. The soil of the experimental area had silty-clay loam texture with pH=5.5. The soil contained 8.1% total nitrogen and 1.9% organic matters. A small piece of medium and low land (3 x 1 m) was selected for raising seedlings for the field experiments. Well-decomposed farmyard manure of 10 t/ha was applied during the final land preparation. The crop was fertilized with 200 kg urea, 80 kg triple super phosphate (TSP) and 70 kg murate of potash (MP) per hectare, respectively (Michel et al*.,* 1997). Weeding and irrigation were done when necessary. Each plot was surrounded by 20-25 cm high and mud plastered levee to protect entering the irrigation water. Clean and healthy matured seeds (germination rate >85%) were pre-germinated in a moist-plastic tray in the dark at 28^0C for 24 h before sowing in the seedbed. Thirty-two-day old tomato seedlings of *Marglove* and *Roma* VF were transplanted in 1.5 cm depth at the spacing of 60 x 40 cm using sterilized forceps. No insecticide or pesticide was used during the whole period of plant growth in the field to allow the pathogen to cause infection naturally in the tomato plants. M*arglove* and *Roma* VF varieties were selected based on their popularity as vegetable crop in Bangladesh, and are susceptible to TYLCV.

Disease identification

The severity of the TYLCV infestation on the tomato plants was visually evaluated on individual leaves of each plant. Leaf symptoms include chlorotic margins, small leaves which are cupped, thick and rubbery. Top of infected plants may look like the head of broccoli. The majority (up to 90%) of flowers abscise after infection, thus few fruits are produced according to Al-Musa (1982). Infected young leaves were collected from identical position on the main stem for laboratory test. Modified "dotimmunobinding assay" (DIBA) (Akanda et al., 1991) method was performed for the presence of TYLCV virus. Plants infected naturally at flowering stage were selected for the analyses.

Experimental design and data collection

Randomized complete block design (RCBD) with 3 replications was followed for the field experiments. Data were recorded regarding individual fruit weight (g), fruit per plant, yield/plant (kg), and nutritive quality such as organic acids (total and free), ascorbic acids (total and free), nucleic acids (DNA and RNA) content, individual organic acids (oxalic, malic, tartaric, fumaric and malonic) as well as auxin/indole-3-acetic acid (Aux/IAA) content. The data presented are the means of 3 replicates.

Biochemical analyses

Fruits samples were collected from the healthy and virus infected tomato plants at early (20 days after anthesis), intermediate (40 days after anthesis) and at ripening stages (60 days after anthesis) from the similar positions (based on blooming time of flower) of both diseased and control plants (uninfected). The juice was extracted by pressing and immediately centrifuging extracts at 5,000 rpm for 10 min. The supernatant was frozen and stored at -20^0C in a sealed polypropylene tube until analysis. Content of nucleic acids (RNA and DNA) was determined following the method proposed by Spirin (1958). The free and total organic acid content were performed as described by Shiraishi (1995). The combined organic acid was calculated by deduction of free organic acid from the total organic acid (total organic acid - free organic acid) = combined organic acid. The free and total ascorbic acid were measured according to the procedure described by Pleshkov (1976). The combined ascorbic acid was calculated by deduction of free ascorbic acid from total ascorbic acid (total ascorbic acid - free ascorbic acid) = combined ascorbic acid. The procedure followed for the analysis of individual organic acids was essentially according to Shiraishi et al. (1986), however certain modifications suggested by Hossain and Quadir (1992). Auxin/IAA content was quantified by indolo-pyrone method described by Stoessl and Venis (1970) and Blaskesley et al. (1983) with some modifications proposed by Hossain et al. (1995) using HPLC. The amount was calculated by comparing with standard IAA.

Results and discussion

Content of organic acids

The effect of virus infection on organic acid composition is remarkable. Table 1 demonstrated the free, combined and total organic acid content in fruit of infected plants of both varieties. The content of free organic acid was found to be declined in fruits of infected plants in both varieties. In *Marglove* variety, the content of organic acids was 43.33, 45.95 and 38.98% in early, intermediate and ripening stages, respectively. Similar results were also found for the same parameters for *Roma* VF. On the other hand, higher content (13.91 to 33.33%) of combined organic acid was detected in fruits of infected plants in both varieties. Among the three stages, higher combined acid content was observed at ripening stage in fruits of infected plants for *Marglove*. Similar trend was pbserved in *Roma* VF. In fruits of infected plants, the total organic acid was always found to be lower compared to healthy plants at all stages. Reduction of total organic acid in fruits of infected plants of variety *Marglove* ranged from 6.41 to 15.73% at different stages, whereas the same parameter in the *Roma* VF ranged from 8.45 to 12.83%.

Table 1. Changes in organic acids (mg/100 g) in fruits of the tomato plants after infection with TYLCV at three stages.

Varieties	Plant status	Growth stages								
		Early			Intermediate			Ripening		
		Free	Combined	Total	Free	Combined	Total	Free	Combined	Total
Marglove	Healthy	0.30	0.48	0.78	0.37	0.34	0.71	0.59	0.30	0.89
	Infected	0.17 (-43.33)	0.56 (+16.67)	0.73 (-6.41)	0.20 (45.95)	0.42 (+23.53)	0.62 (-12.68)	0.36 (-38.98)	0.39 (+30.01)	0.75 (-15.73)
Roma VF	Healthy	0.27	0.44	0.71	0.33	0.30	0.63	0.51	0.27	0.78
	Infected	0.14 (-48.15)	0.51 (+13.91)	0.65 (-8.45)	0.17 (-51.52)	0.37 (+25.67)	0.54 (-14.29)	0.33 (-35.29)	0.35 (+33.33)	0.68 (-12.83)

Values (+ or -) in the parentheses indicate percent changes over healthy fruits

Table 2. Changes in ascorbic acids (mg/100 g) in fruits of the tomato plants after infection with TYLCV at three stages

Varieties	Plant status	Growth stages								
		Early			Intermediate			Ripening		
		Free	Combined	Total	Free	Combined	Total	Free	Combined	Total
Marglove	Healthy	13.24	3.13	16.37	18.25	5.88	24.13	22.35	8.91	31.26
	Infected	10.11 (-23.64)	5.81 (+85.62)	15.92 (-2.70)	14.55 (-20.27)	9.58 (+63.61)	23.19 (-3.90)	18.67 (-16.47)	11.64 (+30.64)	30.31 (-4.01)
Roma VF	Healthy	12.59	3.16	15.85	16.92	5.81	22.73	21.03	9.11	30.14
	Infected	10.06 (-20.91)	5.64 (+78.48)	15.21 (-3.85)	13.88 (-17.97)	7.97 (+37.31)	21.85 (-4.92)	17.91 (-14.84)	11.37 (+26.81)	29.28 (-3.84)

Values (+ or -) in the parentheses indicate percent changes over healthy fruits

Table 3. Changes in nucleic acids (mg/g, dry-wt. basis) in fruits of the tomato plants after infection with TYLCV at two stages

Growth stages	Marglove				Roma VF			
	Early		Intermediate		Early		Intermediate	
	RNA	DNA	RNA	DNA	RNA	DNA	RNA	DNA
Healthy	1.53	0.22	0.99	0.13	1.36	0.19	0.92	0.16
Infected	2.18 (+42.48)	0.16 (-27.27)	1.33 (+34.35)	0.10 (-23.08)	1.88 (+38.24)	0.15 (-21.05)	1.10 (+19.57)	0.09 (-43.75)

Values (+ or -) in the parentheses indicate percent changes over healthy fruit

Lower free acid content was observed at early stage of both varieties and higher content at ripening stages. This trend in general agrees with earlier investigations on leaves of TMV-infected plants (Ryzhkov, 1957; Schlegel, 1958). They observed that organic acids cause a significant increase in the amount of virus formed. This effect may be indication of changes in the organic acid metabolism of the plant imposed by virus infection. Such a change would presumably result in a corresponding change in the organic acid composition of diseased plants. Therefore, it can be concluded that lower free form contents of organic acid in diseased fruits is due to either a greater rate of consumption of the acid or a decrease in the rate of free form production.

Content of ascorbic acids

Table 2 displays the results of analyses of free, combined and total ascorbic acid contents in infected tomato fruits at three different stages. Results clearly demonstrated that at all stages free ascorbic content was substantially reduced in infected fruits than healthy ones. Accumulation of free ascorbic acid in fruits of infected plants at early, intermediate and ripening stages was 23.64, 20.27 and 16.47%, respectively, lower than healthy counterparts in *Marglove* variety, whereas it was 20.91, 17.97 and 14.84% lower in the case of variety *Roma* VF. Destruction rate of free ascorbic acid in fruits of infected plants variety *Marglove* was higher than *Roma* VF variety. For both varieties at early, intermediate and ripening stages, the accumulation of combined ascorbic acid in fruits of virus infected tomato plants was found higher compared to their healthy counterparts. Changing rate of combined ascorbic acid at early stage for both varieties was higher (85.62 to 78.48 to) followed by intermediate (63.21 to 37.31%) and ripening stages (30.64 to 26.81%). It is known that combined ascorbic acid does not take part in biochemical process. Instead it serves as reserve form in plant. In this study, the reason for the increase in the combined ascorbic acid in fruits of infected plants of both varieties could not be ascertained, and could be subjected to further investigation. Total ascorbic acid is a combination of free and combined ascorbic acids. The accumulation of total ascorbic acid contents in fruits of virus infected plants was observed slightly lower (4.01 to 2.70%) than fruits of healthy plants, at all stages, for both varieties which might be attributed to lesser degree of metabolism. This slightly lower content of total ascorbic acid in infected plant may be due to higher accumulation of combined ascorbic acid. Our results corroborated the results of Milo and Santilli, (1967) which observed the rapid decrease in ascorbic acid in TMV-infected leaves of pinto bean was approximately stoichiometrically equivalent to the increase in dehydroascorbate concentration. In accordance with these reports, inactivation of ascorbic acid oxidation system by infection was suggested (Fodor et al., 2001; Chen et al., 2003). Our results imply that reduction responsible for virus localization precede those associated with the virus-induced ascorbic acid oxidation and lesion appearance. It is known that ascorbic acid plays vital roles in different biochemical and physiological processes in living organisms. Deviation from normal shows different anomalies, and may make plant more susceptible to virus infection as observed in this study.

Table 4. Auxin/ IAA content (ng/g, dry-wt. basis) in fruits of tomato plants after infection with TYLCV at early stage.

Plant status	Marglove	Roma VF
Healthy	26.15	31.65
Diseased	19.08	23.98
Percent reduction	(-27.08)	(-24.29)
Mean	22.62	26.81

Values (+ or -) in the parentheses indicate percent changes over healthy fruits

Table 5. Changes in the concentration (%) of individual organic acids in fruits of the tomato plants after infection TYLCV at early stage

Organic acids	Name of variety			
	Marglove		Roma VF	
	Healthy	Diseased	Healthy	Diseased
Oxalic	60	34	51	29
Malic	25	53	32	63
Tartaric	11	4	16	nd
Fumaric	2.5	nd	nd	nd
Malonic	1.5	09	01	08

nd: could not be detected

Content of nucleic acids (DNA and RNA)

Changes of nucleic acids (DNA and RNA) in fruits of TYLCV infected plants at early and intermediate stages were presented in Table 3. The present study showed higher content of RNA (38.23 to 42.48%) in fruits of virus infected plant at early and intermediate stages of *Marglove* and *Roma* VF compared to control (healthy). Similar results were also reported in yellow vein mosaic virus (YVMV) infected leaves of country bean by Hossain and Haider (1993). This may be due to enhanced activity of RNA synthesis and RNA polymerase after virus infection. These two enzymes are suggested to be responsible for higher RNA content. In fruits of virus infected plants a lower content of DNA (ranging from 21.05 to 43.75%) observed at two stages. Lower content of DNA in infected leaves of country bean (by YVMV) and papaya (by papaya ring spot virus- papaya strain) was also demonstrated by Hossain and Haider (1993) and Rahman et al. (2008). This also has been reported that DNA synthesis was reduced to half in the root tips of French bean (*Phaseolus vulgaris* L.) infected by tobacco ring spot virus about three days after inoculation of the leaves (Atchinson, 1973). Therefore, we concluded that reduction in DNA content in fruits of diseased samples might be due to the breakdown or denaturation of nucleotides- the sub-structures of DNA (deoxyribose sugar molecules, nitrogenous bases and phosphate groups).

Indole acetic acid (IAA) content

Auxin/IAA content determined at early stage in the fruits of infected plants of both varieties compared to their healthy counterparts is presented in Table 4. Results showed a substantial reduction of 24.29 and 27.08% in fruits of infected plants in *Marglove* and *Roma* VF varieties, respectively.

Table 6. Yield performance of the tomato plants after infection with TYLCV

Parameters	Marglove			Roma VF		
	Healthy	Infected	Mean	Healthy	Infected	Mean
Individual fruit wt (g)	49.72	40.01 (-19.52)	44.87	41.65	32.71 9 (-21.46)	37.22
Fruit/plant	55.15	39.24 (-28.58)	47.19	39.13	28.25 (-30.69)	33.69
Yield/plant (kg)	2.74	1.57 (-42.70)	2.15	1.65	0.92 (-44.24)	1.29

Values (+ or -) in the parentheses indicate percent changes over healthy fruits

Degrading tendency of auxin in both varieties in diseased plants was more or less similar. Differences in IAA content between *Marglove* and *Roma* VF were small and not substantial. Similar results were made by Narayanasamy et al. (1972) who observed that cotton plants infected by stenosis disease have growth abnormalities which might possibly be due to an abnormal reduction in the IAA-content of the infected plants. Silvestri et al. (2008) reported that TMV decreased IAA/auxin levels in tobacco tissues, which has been proposed to influence TMV susceptibility in tobacco. Therefore, the lower content of auxin in fruits of infected plants may be due to either its biochemical or enzymatic inhibition in its biosynthesis in shoot tip of the fruit via basipetal movement. Alternatively, IAA in plant may be oxidized in presence of IAA-oxidase (Tomaszewski and Thimann, 1996). Therefore, it is assumed that the activity of IAA-oxidase in the infected plants and fruits are enhanced, which is responsible for the degradation of auxin content. This decrease may have been associated with the stunted exhibition in infected plants (Padmanabhan et al., 2005). Virus infection is known to interfere with the auxin levels of the host plant. Virus infection which causes necrosis of the phloem can disturb the accumulation balance of auxin in infected plant tissues responsible for proper growth and development. Therefore, it can be concluded that auxins may be of far greater importance than other substances affecting the response of host cells.

Individual organic acids

Table 5 shows contents and position of important organic acids in fruits of infected and healthy plants in studied varieties. Five organic acids including oxalic, malic, tartaric, fumaric and malonic acid were identified in healthy fruits of *Marglove* at early stage. Substantial differences were found between the organic acids of healthy and detached diseased fruits in which, oxalic acid occupied the first position followed by malic, tartaric, fumaric and malonic acids. In infected plants of both varieties malic acid content was in the first rank followed by oxalic, malic and tartaric acid. Unexpectedly, fumaric acid was not detected in fruits of infected plant of *Marglove* at all. But in the case of *Roma* VF, four organic acids were detected in fruits of healthy plants, whereas only three were detected in infected plants. Oxalic acid was found in first position followed by malic, tartaric and malonic acids in fruits of healthy plants, whereas, malic acid was found in first position followed by oxalic and malonic acids in fruits of infected plants. In *Roma* VF, fumaric acid was not detected in fruits of healthy plants, but surprisingly, in fruits of infected plants fumaric as well as tartaric acids were not detected in *Roma* VF. The position of organic acids shifted based on the available concentrations due to virus infection in both varieties. Similar results were reported by Haider and Hossain (1994) and Alam et al. (1996) in infected leaves of country bean and okra. Our results also corroborate the results of Schlegel (1957) who found more malic and isocitric acid, but only half amount of succinic and fumaric acid in the tobacco mosaic virus-infected plants. In accordance with these reports, our results suggest that infection will alter different metabolic reactions of plants in which, concentration of different organic acids are changed accordingly. It is still unclear why the fumaric acid could not be detected in fruits of diseased plant of *Marglove* variety and healthy and diseased fruits of *Roma* VF. One plausible reason may be the presence of non-detectable quantities of fumaric acid in infected fruits of *Marglove* and healthy and diseased fruits of *Roma* VF. Therefore, they did not show any differences in this case. However, this discrepancy remains to be confirmed by further investigations.

Yield losses due to TYLCV of tomato

Yield performance of tomato collected from infected plants is presented in Table 6. Growth of the emerged tomato seedlings initially was rapid in all the treatments but declined towards the end of experiment, probably because the plants growth was becoming limited by the disease due to TYLCV infection (data not shown). Individual fruit weight and number of fruits per plant in infected plants of *Marglove* and *Roma* VF were found lower than healthy counterparts. Individual fruit weight and fruits per plant were reduced in infected plants over healthy ones which finally affected the yield. The yield in infected plants of *Marglove* and *Roma* VF were found lower than their healthy counterparts. Yield reduction of infected plants of *Marglove* and *Roma* VF was recorded 42.70 and 44.24 kg/plant, respectively. Heavy crop loss is common with most of the virus diseases. The yield loss is, however, related with the severity of the symptoms (Bos, 1967 and 1976). Yield reduction of different crops, even when their vegetative organs are infected by virus, has been reported by Haider and Hossain (1994) and Alam et al. (1996). They reported the metabolic changes in okra (*Abelmoschus esculentus* L.) and yield reduction due to yellow vein mosaic virus. Levy and Marco (1982) reported the alteration of cellular component in potato tuber due to infection of potato virus Y (PVY) and potato leaf roll virus which ultimately affected the growth and yield of potato. Our results are in agreement with these studies. Therefore, it appears that the yield loss is related with the cause of reduced fruit weight and fruit number per plant, which might consequently associated with the severity of the disease.

Conclusion

In conclusion, it appears that the yield reduction is a consequent effect of TYLCV infection in tomato plants. The metabolic processes are changed due to alteration in ascorbic acids, organic acids and nucleic acid contents, position and concentration of individual organic acids and endogenous

auxin content in fruits of TYLCV infected plants, which might be the cause of yield reduction as well as quality deterioration of tomato.

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