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DETECTION OF CHILLI LEAF CURL SRI LANKA VIRUS IN CHILLI PLANTS SHOWING DIFFERENT VIRUS LIKE SYMPTOMS AND IN ALTERNATIVE WEED HOSTS

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ABSTRACT

Chilli (*Capsicum annuum* L) is a commonly grown spice crop in Sri Lanka. Average chilli yield in Sri Lanka is 1 mt/ha though the potential yield is 3 mt/ha. Low yield of chilli is mainly due to abiotic and biotic stresses including diseases. Among the diseases chilli leaf curling is known as the most devastating disease in chilli. Almost all of the cultivating chilli varieties in Sri Lanka are found to be susceptible to the Leaf Curl Disease of Chilli (LCDC). Chilli leaf curl Sri Lanka virus has been reported in association with LCDC in Sri Lanka. In this study, the possible association of Chilli leaf curl Sri Lanka virus with seven different symptom types in chilli plants and 18 commonly growing weeds were tested using Chilli leaf curl Sri Lanka virus specific primers in Polymerase Chain Reaction (PCR). The results revealed the presence of Chilli leaf curl Sri Lanka virus in association with four different symptom groups reported from commonly cultivating chilli varieties. The results also revealed the presence of Chilli leaf curl Sri Lanka virus in nine weeds, out of 18 weeds tested namely, Pitasudupala (*Boerhavia diffusa* of family Nyctaginaceae), Gandapana (*Lantana camara* of family Verbenaceae), Maduruthala (*Ocimum tenuiflorum* of family Ocimum), Wathupalu (*Mikania micrantha* of family Asteraceae), Balunaguta (*Stachytarpheta indica* of family Verbinaceae), Wal Aba (*Cleome viscosa* of family Capparidaceae) Wal rubber (*Euphorbia heterophylla* of family Euphorbiraceae), Walkoththamalli (*Scoparia dulcis* of family Scrophulariaceae) and Pethithora (*Cassia tora* of family Fabaceae). The findings of this study revealed that the virus can be in association with chilli plants having different symptoms and the alternative weed hosts of Chilli leaf curl Sri Lanka virus. This information can be applied to manage the LCDC in Sri Lanka.

Key words: Begomovirus, Chilli, Chilli leaf curl Sri Lanka virus, Common weeds

INTRODUCTION

Chilli (*Capsicum annuum* L.), of the family Solanaceae originated in the Latin American regions of the New Mexico and Guatemala as a wild crop around 7500 BC and has been consumed by humans since 7200 BC (MacNeish 1964). In Sri Lanka chilli is grown in diverse climate regions during both *Maha* and *Yala* seasons. Average chilli yield in Sri Lanka is 1 mt/ha though the potential yield is 3 mt/ha. Low yields are contributed by many factors including diseases (Weerarathne and Yapa, 2002). Most popular chilli varieties recommended for cultivation by the Department of Agriculture, Sri Lanka are MI-1, MI-2, KA-2, Arunalu and MI- Hot (Kannangara *et al*., 2013). All of these chilli varieties were found to be susceptible to Leaf Curl Disease of Chilli (LCDC) (Senanayake *et al*., 2014). LCDC is known as the most damaging disease reported from all the chilli cultivating areas in Sri Lanka and it causes serious yield losses (Rajapakse *et al*., 2003). Major symptoms of LCDC include severe upward curling, stunted plant growth, leaf thickening, and vein clearing. The severely affected plants may grow stunted and bear less number of fruits (Joshi and Dubey, 1976; Peiris 1953). Association of begomoviruses with chilli leaf curl symptoms was confirmed recently (Senanayake *et al*., 2007). Major reasons for LCDC are known as thrips and mite attacks and involvement of viruses transmitted by whitefly (*Bemisia tabaci* Genn Family: Aleyrodidae) (Johnpulle, 1939; Peiris 1953). In Sri Lanka LCDC was observed as early as in 1938 (Park and Fernando, 1938; Paul and Fernando, 1939) and the associated virus was named as vein clearing virus at that time (Peiris, 1953; Fernando and Peiris, 1957). Association of several begomoviruses with LCDC has been reported by now (Chattopadhyay *et al*., 2008; Senanayake *et al*., 2006, 2012). Senanayake *et al*. (2012) reported that Chilli leaf curl virus can be transmitted to *Solanum lycopersicum* L, *Nicotiana benthamiana* and N. *tabacum*. Nehra and Gaur (2015) reported the natural occurrence of Chilli leaf curl virus in *Petunia hybrida* in India.

LCDC start to appear from the nursery stage onwards. Presently, application of insecticides to control the vector of the virus, whitefly is used as the main management strategy for LCDC. Senanayake *et al*. (2014) reported the field tolerance of some chilli accessions to LCDC. Despite insecticide applications and the availability of field tolerant varieties LCDC is still present in an enormous scale in the country. Different symptoms

can be observed in chilli fields in association with LCDC and many weeds are also found in those fields. Those weeds may act as alternative hosts for leaf curl viruses. Therefore, this study was carried out at the Field Crops Research and Development Institute (FCRDI), Mahailluppallama to find out whether Chilli leaf curl Sri Lanka virus present with different symptoms in chilli and in common weeds (as alternative hosts) that are found in and around chilli fields.

MATERIALS AND METHODS

The study was carried out as two experiments. In the first experiment Chilli leaf curl associated symptoms were observed in chilli fields of the FCRDI, Mahailluppallama during *Maha* 2014/15 and chilli plants with conspicuous different symptoms were selected. Tender leaf samples were collected from those plants separately. DNA was isolated from 100 mg of leaf samples using CTAB method (Ausubel *et al*., 1987).

In the second experiment 18 weeds commonly found in and around chilli fields, which were showing leaf curling associated symptoms and which were not showing any symptom were selected. Presence and absence of symptoms were recorded from them and DNA isolation was done from younger leaf samples of those weeds as in the first experiment.

Polymerase chain reaction (PCR) was performed using Chilli leaf curl Sri Lanka virus specific primers, JS 35F; TGCCAGAGCGGCATCAGCGG, JS 36R; GTCCCCATTGTCCCCCATTCC (Senanayake *et al*., 2013) for both chilli and weed DNA. The PCR mix consisted of 100 ng of DNA, 5 µl of 5X reaction buffer (Promega, Medison, USA), 1 µl of 10 mM dNTP mix (Promega, Medison, USA), 1 µl each of forward and reverse primers (10 picomole), 0.30 U of Go Taq Flexi DNA polymerase (5U/ µl, Promega, Medison, USA) and sterilized distil water to make 25 µl of total volume. The PCR cycle consisted of initial denaturation at 94°C for 1 min followed by 30 PCR cycles, each consisting of denaturation at 94°C for 30 sec, primer annealing at 58°C for 1 min and extension at 72°C for 1 min. The final extension was carried out at 72°C for 7 min. The amplified products were run on 1% Agarose gel.

RESULTS AND DISCUSSION

Detection of Chilli leaf curl Sri Lanka virus with different symptom groups

Plants with seven different symptom groups, which were distributed in a more or less similar manner were observed in chilli fields of the FCRDI during *Maha* 2014/15 (Table 1). The symptoms varied from mild puckering to a mixture of severer leaf curl associated symptoms. The primers used in the study are based on the DNA- A of a monopartite begomovirus and are capable of detecting the presence of Chilli leaf curl Sri Lanka virus in plant samples. In this study, except three samples, which showed the symptoms; a) Reduced leaf size in younger leaves and boat shaped leaves with bushy appearance (symptom group 2), b). Margins rolled upwards, necrosis at tip and reduced leaf size (symptom group 4), and c). Mild puckering (symptom group 5), all the others samples showed positive reaction in PCR (Figure 1) with Chilli leaf curl Sri Lanka virus specific primers giving the correct amplicon size (501 bp). It confirms the presence of Chilli leaf curl Sri Lanka Virus in those samples. The sample with reduced leaf size in younger leaves and boat shaped leaves with bushy appearance (symptom group 2) did not show any amplification with the specific primers. The samples with Margins rolled upwards, necrosis at tip and reduced leaf size (symptom group 4) and Mild puckering (symptom group 5) showed PCR band sizes in PCR, which were different from expected band size. These results may be due to the presence of a different virus or a variation in the viral genome.

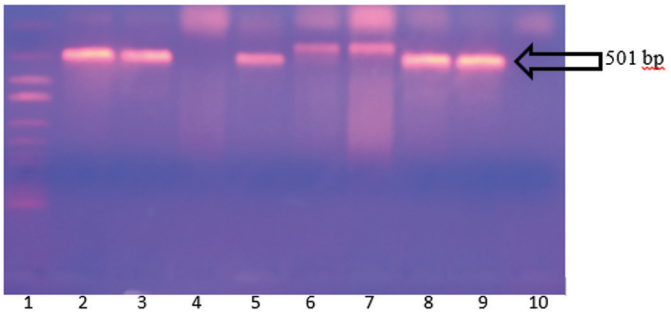

Figure 1. Response of chilli plants with different symptoms groups to Chilli leaf curl Sri Lanka virus specific primers

1 = I kb ladder, 2 = +ve Sample , 3 = Symptom group 1, 4 = Symptom group 2, 5 = Symptom group 3, 6 = Symptom group 4, 7 = Symptom group 5, 8 = Symptom group 6, 9 = Symptom group 7,10= -ve sample

Table 1. Different symptoms groups observed in chilli fields during *Maha* 2014/15 and their reaction to PCR with Chilli leaf curl Sri Lanka virus specific primers

Symptom group	Symptoms associated with the plant	Variety from which symptoms were recorded	PCR Reaction to Chilli leaf curl Sri Lanka virus specific primers
1	Puckering, reduced leaf size, resetting like appearance, tips rolled upward	2006.selection	+ve
2	Reduced leaf size - younger leaves, boat shaped leaves, Bushy appearance	CAH 36	-ve
3	Puckering , severe vein banding, reduced leaf sizes, rosette type	Arunalu	+ve
4	Margins rolled upwards, Necrosis at tip, small leaves	Arunalu	-ve
5	Mild puckering	Waraniya Purple	-ve
6	Puckering	MI Waraniya-I	+ve
7	Tip rolled upward, Bushy like appearance	MICH3	+ve

Detection of Chilli leaf curl Sri Lanka virus in common weeds

Out of 18 weeds tested only four weeds showed visual symptoms. A type of Bevila (*Malvastrum coromandelianum*) showed yellow mosaic like symptoms while Podisinnomaran (*Chromalaena odorata*), Wathupalu (*Mikania micrantha*) and Girithilla (*Argyreia populifolia*) showed curling like symptom. However, only Wathupalu was positive to PCR reaction with Chilli leaf curl specific primers. All the other weeds tested in this study did not show any visual symptom associated with LCDC in Sri Lanka (Table 2).

The PCR reaction with Chilli leaf curl Sri Lanka virus specific primers to common weeds showed that nine weeds, out of 18 tested, were positive to Chilli leaf curl Sri Lanka virus. The positive weeds were Pitusudupala (*Boerhavia diffusa* of family Nyctaginaceae), Gandapana (*Lantana camara* of family Verbenaceae), Maduruthala (*Ocimum tenuiflorum* of family Ocimum), Wathupalu (*Mikania micrantha* of family Asteraceae), Balunaguta (*Stachytarpheta indica* of family Verbinaceae), Wal Aba (*Cleome viscosa* of family Capparidaceae), Wal rubber (*Euphorbia heterophylla* of

family Euphorbiraceae), Walkoththamalli (*Scoparia dulcis* of family Scrophulariaceae) and Pethithora (*Cassia tora* of family Fabaceae) (Table 2 and Fig. 2).

Table 2. Weed type, nature of symptoms and PCR response of common weeds found in and around chilli fields of FCRDI, Mahailluppallama to Chilli leaf curl Sri Lanka virus specific primers

Common name of the weed	Scientific Name	Family	Symptoms	Response to chilli leaf curl specific primers
Balunaguta	*Stachytarpheta indica*	Verbinaceae	No observable symptoms	+ve
Type of Kochchi	*Capsicum frutescens*	Solanaceae	No observable symptoms	-ve
Kalanduru	*Cyperus rotundus*	Cyperaceae	No observable symptoms	-ve
Kathurupila	*Tephrosia purpurea*	Fabaceae	No observable symptoms	-ve
Podisinnomaran	*Chromalaena odorata*	Asteraceae	No observable symptoms	-ve
Pitasuda-pala	*Boerhavia diffusa L.*	Nyctaginaceae	No observable symptoms	+ve
Type of Bevila	*Malvastrum coromandelianum*	Malvaceae	Yellow mosaic like symptoms	-ve
Maduruthala	*Ocimum tenuiflorum*	Ocimum	No observable symptoms	+ ve
Gandapana	*Lantana camara*	Verbenaceae	No observable symptoms	+ ve
Wathupalu	*Mikania micrantha*	Asteraceae	Leaf curling	+ ve
Nidikumba	*Mimosa pudica*	Fabaceae	No observable symptoms	- ve
Girithilla	*Argyreia populifolia*	Convolvulaceae	Curling, Puckering	- ve
Wal Aba	*Cleome viscosa*	Capparidaceae	No observable symptoms	+ve
Wal Koththamalli	*Scoparia dulcis*	Scrophulariaceae	No observable symptoms	+ve
Kurunegala desi	*Tridax procumbens*	Asteraceae	No observable symptoms	-ve
Wal Rubber	*Euphorbia heterophylla*	Euphorbiraceae	No observable symptoms	+ve
Kuppamenia	*Acalypha indica*	Euphorbiacea	No observable symptoms	-ve
Pethi Thora	*Cassia tora*	Fabaceae	No observable symptoms	+ve

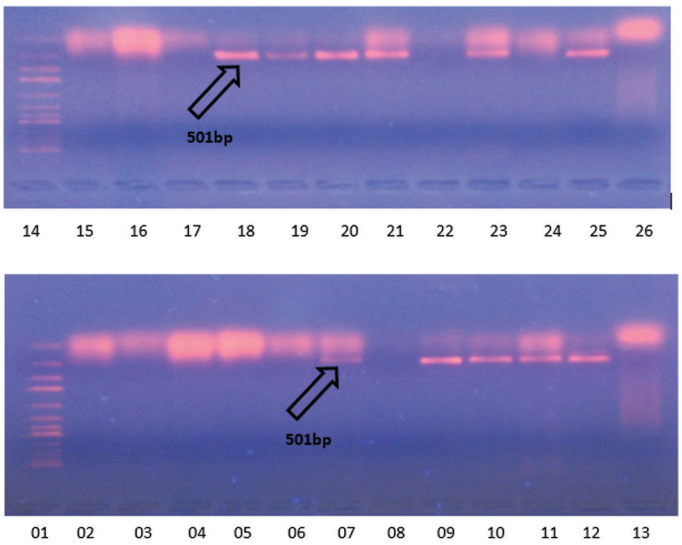

Figure 2 . PCR amplifications in weed DNA samples tested with Chilli leaf curl Sri Lanka virus specific primers (JS35F and JS36R)

01= 1 Kb ladder, 02 = Balunaguta, 03 = Type of Kochchi, 04 = Kalnduru, 05 = Kathurupila, 06 = Podisinnomaran, 07 = Pitasuda-pala, 08 = Type of Bevila, 09 = Positive sample , 10 = Maduruthala, 11 = Gandapana , 12 = Wathupalu , 13 = Negative sample, 14 = 1 Kb ladder, 15 = Nidikumba, 16 = Podisinnomaran, 17 = Ginithilla, 18 = Balunaguta, 19 = Positive sample, 20 = Wal Aba, 21 = Wal Koththamalli, 22 = Kurunegala desi, 23 = Wal Rubber, 24 = Kuppamenia, 25 = Pethi Thora, 26= Negative sample.

In this study the association of Chilli leaf curl Sri Lanka virus with different symptoms was confirmed through molecular tools. However, presence of other begomoviruses in association with tested chilli samples is also possible. Information on the association of the virus with different symptoms is useful to identify the viral involvement in LCDC and to apply management practices in controlling LCDC.

Suguira *et al*. (1975) described that LCDC is due to a mixed infection of mosaic and leaf curl viruses. The symptoms LCDC are expressed as vein clearing, vein banding , vein yellowing, systematic cholorotic spots and mottling. *Tobacco Mosaic Virus* (TMV), *Potato Virus Y* and *Cucumber Mosaic Virus* (CMV) account for those symptoms. He also stated that leaf curl symptoms are found to be more severe than mosaic symptoms in a mixed infection. However, these facts have not been confirmed at molecular level.

Suguira *et al*. (1975) reported that chilli leaf curl virus can occur in Hulanthala (*Ageratum conyzoides*) of family Asteraceae, Mottu (*Physalis floridana*) of family Amaranthaceae, tomato (*Lycopersicom esculentum* L) of family Solanaceae, *P.hybrida* of family Solanaceae, *Nicotiana tabacum* of family Solanaceae, *Datura stramonium* of family Solanaceae in Sri Lanka. Most of these weeds that did not show leaf curl or related symptoms may act as hidden carriers. The results of this study show that plants belong to different families can harbor Chilli leaf curl Sri Lanka virus. Chilli leaf curl Sri Lanka virus is a monopartite begomovirus and transmitted by the whitefly (*Bemisia tabaci* Genn.) (Senanayake *et al*., 2013). Being a member of the genus begomovirus this virus is not getting transmitted through mechanical means. Therefore, the host plant reported for the presence of the virus in this study must be hosts for the whitefly too.

The specific primers used in this study are capable of detecting chilli leaf curl virus separately from *Tomato leaf curl Sri Lanka virus*, which is widely occurring in Sri Lanka (Senanayake *et al*., 2013). Also these primers are capable of detecting, two other viruses namely Chilli leaf curl Salem virus (Genebank accession no.: HM007119) and Chilli leaf curl Vellanad virus (Genebank accession no.:007121), which are almost similar to Chilli leaf curl Sri Lanka virus according to sequence data. All the weeds, which showed the positive reaction to Chilli leaf curl virus specific primers in this study in Sri Lanka, have been reported to harbor other known begomoviruses in other countries. A species related to Balunaguta (*Stachytarpheta indica*), *Stachytarpheta jamaicensis*, has been reported to be showing leaf curl and chlorosis symptoms in the Hainan province of China (Xiong *et al*., 2005) Association of *Datura leaf distortion virus* has been reported from *Boerhavia diffusa* (Pitasudapala) in Venezuela (Fiallo-Olivé *et al*., 2013). *Ocimum tenuiflorum* (Maduruthala) has been reported to be infected with Tomato leaf curl viruses in India (Gaur, 2012). Marwal *et al*, 2013 showed that *Lantana camara* (Gandapana) hosts for begomoviruses in India. Wang *et al*. (2008) reported the presence of a begomovirus, Mikania micrantha wilt virus (MMWV) in *Mikania micrantha* (Wathupalu). Presence of a begomovirus in association with *Euphorbia heterophylla* (Wal Rubber) has been reported by Zambrano *et al*.(2008) in Venezuela and the virus was named as Euphorbia mosaic Venezuela virus (EuMVV). Interestingly they also showed that the EuMVV can transmit to *Solanum lycopersicum* and *Capsicum annuum* by biolistics of infectious cloned DNA-A. Deighton (1938) reported that leaf curl of tobacco in Sierra Leone can

infect many other hosts including *Scoparia dulcis* (Wal koththamalli). EFSA Panel on Plant Health (2013) reported that *Scoparia dulcis* is a host plant for whitefly (*Bemisia tabaci* Genn), which transmits a large number of begomoviruses. Macroptilium golden mosaic virus (MaGMV), a begomovirus was reported from *Cassia tora* (Pethi thora) in Brazil. Salati *et al*. (2002) reported the presence of *Tomato yellow leaf curl virus* in *Cleome viscosa* (Wal Aba) in Dominican Republic. These previous findings support the findings of this study, the presence of Chilli leaf curl Sri Lanka virus, a begomovirus in some weeds.

Weeds, which gave negative PCR results for chilli leaf curl Sri Lanka virus in this study, have been reported for the presence of some other begomoviruses in different countries. *Cassava mosaic virus* has been reported in *Chromalaena odorata* (Podisinnomaran) in Nigeria (Eni and Fasasi, 2013). Jiang and Zhou (2005) reported *Malvastrum yellow vein Yunnan virus* (MYVYNV) in *Malvastrum coromandelianum* in China. *Ageratum leaf curl virus* and *Malvastrum leaf curl virus* have also been reported from *Malvastrum coromandelianum* (Type of Bevila) in China (Huang and Zhou, 2006). *Mimosa pudica* (Nidikumba) has been reported to get infected with *Ageratum yellow vein virus*-Guangxi in India (Gaur *et al*., 2011). Presence of begomoviruses in *Acalypha indica* (Kuppamania) has been confirmed in India by Saha *et al*. (2013). There is a possibility that these weeds can also act as alternative host for the Chilli leaf curl Sri Lanka virus. Kalanduru (*Cyperus rotundus* of family Cyperaceae), which is found as one of the commonest weeds in Sri Lanka did not give positive results to Chilli Leaf curl virus specific primers confirming the non-presence of begomoviruses in monocotyledonous plants.

Chilli leaf curl is considered the most devastating disease in Sri Lanka. Leaf curl symptoms appear in chilli plants from the seedling stage in Sri Lanka even without observing leaf curl infected chilli plants around or nearby a new cultivation. Findings of this study give a major clue why the management of chilli leaf curl in Sri Lanka has become difficult. Presences of the virus in many weeds, which grow throughout the year, help whiteflies to acquire the virus from them and spread to healthy plants. This situation may be aggravated by the long distance movement of whiteflies (Zhou *et al*., 2007).

CONCLUSION

In this study it was able to confirm the presence of Chilli leaf curl Sri Lanka virus with different symptoms in chilli plants and this information is valuable for disease management. This is the first study in Sri Lanka that determined a number of hosts for a begomovirus using molecular tools. In this study the presence of Chilli leaf curl Sri Lanka virus was reported from nine weeds belonging to different families, Pitasudupala (*Boerhavia diffusa* of family Nyctaginaceae), Gandapana (*Lantana camara* of family Verbenaceae), Maduruthala (*Ocimum tenuiflorum* of family Ocimum), Wathupalu (*Mikania micrantha* of family Asteraceae), Balunaguta (*Stachytarpheta indica* of family Verbinaceae), Wal Aba (*Cleome viscosa* of family Capparidaceae), Wal rubber (*Euphorbia heterophylla* of family Euphorbiraceae), Walkoththamalli (*Scoparia dulcis* of family Scrophulariaceae) and Pethithora (*Cassia tora* of family Fabaceae). This information can be used to reduce the leaf curl disease incidence in chilli cultivation in Sri Lanka through efficient management of alternative weed hosts of the virus.

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