Introduction

At present, chilly is produced in India about 1260.1 thousands metric ton from an area of 792.1 thousands hectare [1]. India is the largest producer, consumer and exporter of chilli, exporting to USA, Canada, UK, Saudi Arabia, Singapore, Malaysia, Germany and many countries across the world and contributes 25% of total world production. Andhra Pradesh ranks first in area, production and productivity of chilli contributing 30% of the total area and 51% of total production and West Bengal share is 5% only. Agricultural crops of India, like other countries, are also threatened by several biotic and abiotic factors [2]. Chilli leaf curl complex disease is one of the major limiting factors in chilli production & the reasons behind this complex disease are whitefly (Bemisia tabaci) transmitted begomoviruses, thrips & mites, causing significant reductions in yield and quality of chilli. In our present investigation attempt was taken to study the incidence and distribution of the Chilli Leaf Curl Complex Disease predominantly occurring & infecting chilli crops in different locations of West Bengal & detection of begomoviruses through PCR based method using one pair of degenerate primer SPG1/SPG2. The survey was carried out in 18 villages and total 54 fields in six districts randomly selected in West Bengal during July-August, 2015 when the crop was at 2-3 month old. The highest leaf curl incidence was noticed in Cooch Behar district (30-90%) followed by Burdwan (20-85%) and North 24 parganas (45-75%) in 2015. While the lowest incidence was observed at Purulia (18-43%) district. Among six districts Cooch Behar, Jalpaiguri (Madarihat), Burdwan, North 24 parganas, Nadia chilli leaf curl samples showed positive reactions in PCR amplification of a target sequence (920 nt long) specific to begomovirus confirmed the presence of begomovirus in the samples tested. Moreover, these studies supply precise information about the frequency and distribution of distinct begomoviruses in chilli in West Bengal.

Keywords: Survey; Chilli leaf curl; Begomovirus; PCR; West Bengal

Abstract

Chilli (Capsicum annum L) is popularly known as ‘wonder spice’ and is an important cash crop and remunerative vegetable crop of India and is grown both for home market and export. Chilli leaf curl complex disease is one of the major limiting factors in chilli production & the reasons behind this complex disease are whitefly (Bemisia tabaci) transmitted begomoviruses, thrips & mites, causing significant reductions in yield and quality of chilli. In our present investigation attempt was taken to study the incidence and distribution of the Chilli Leaf Curl Complex Disease predominantly occurring & infecting chilli crops in different locations of West Bengal & detection of begomoviruses through PCR based method using one pair of degenerate primer SPG1/SPG2. The survey was carried out in 18 villages and total 54 fields in six districts randomly selected in West Bengal during July-August, 2015 when the crop was at 2-3 month old. The highest leaf curl incidence was noticed in Cooch Behar district (30-90%) followed by Burdwan (20-85%) and North 24 parganas (45-75%) in 2015. While the lowest incidence was observed at Purulia (18-43%) district. Among six districts Cooch Behar, Jalpaiguri (Madarihat), Burdwan, North 24 parganas, Nadia chilli leaf curl samples showed positive reactions in PCR amplification of a target sequence (920 nt long) specific to begomovirus confirmed the presence of begomovirus in the samples tested. Moreover, these studies supply precise information about the frequency and distribution of distinct begomoviruses in chilli in West Bengal.

Keywords: Survey; Chilli leaf curl; Begomovirus; PCR; West Bengal

Abbreviations: CLCV: Chilli Leaf Curl Virus; CTAB: Cetyl Trimethyl Ammonium Bromide; PCR: Polymerase Chain Reaction; NB: North Bengal; SB: South Bengal

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Results and Discussion

Survey, Disease Incidence and Symptomatology

Field survey were conducted during July-August, 2015 in 18 villages and total 54 fields in major chilli growing areas of six districts namely Coochbehar, Jalpaiguri, Nadia, Purulia, North 24 Parganas and Burdwan. The results of survey revealed that most of the fields showed mixed infection of virus, mites and thrips. Different kinds of disease symptoms like mild to severe leaf curling (downward and upward), leaf blistering, leaf crinkling and leaf narrowing of leaves with stunted growth and bushy appearance were observed in the field conditions (Figure 1). The severely infected plants produced no fruit; however, less infected plants produced fruits of significantly reduced sizes. The disease incidence was found to be varied from location to location and region to region. Disease incidence of about 20-90% was found in North Bengal (NB) and it was recorded as 18-85% in South Bengal (SB) (Table 2).

The highest leaf curl incidence was noticed in Bararangrash (90%) followed by Hosenabad (85%) and Polta (75%) whereas the lowest leaf curl incidence was noticed in Dhanera (18%) (Table 1). When the leaf curl disease incidence data was analyzed at district wise the average maximum incidence was found 60% at Cooch Behar, while the least incidence was observed at Jalpaiguri (30%) may be due to favorable environmental condition was found for the growth and transmission of white fly (Bemisia tabaci), mites and thrips (Table 1) and (Figure 2). Infection of leaf curling was found moderate to severe in all blocks. Whereas downward curling and crinkling of leaves giving an inverted boat shaped appearance of mite infection was severe in Alipurdura-l and upward leaf curling with elongated petiole indicated thrips abundance in Kashipur.
Figure 2: Minimum & Maximum Chilli Leaf Curl Incidence in Six Districts of West Bengal.

Table 1: Survey of Chilli Leaf Curl Complex Disease Incidence during the Growing Period of 2015 in Different Villages of West Bengal (WB).

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Districts</th>
<th>Blocks</th>
<th>Villages</th>
<th>No. of Field Surveyed</th>
<th>Percent Disease Incidence of Chilly Leaf Curl Diseases Complex, July-August, 2015</th>
<th>Average Per Cent Disease Incidence District Wise (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cooch Behar</td>
<td>COB-II</td>
<td>Baranagash</td>
<td>3</td>
<td>90</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Baneswar</td>
<td>3</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Jalpaiguri</td>
<td>Madarihat</td>
<td>Chapaguri</td>
<td>3</td>
<td>20</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lankapara Hat</td>
<td>3</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dakshin Sisubari</td>
<td>3</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Jalpaiguri</td>
<td>Alipurduar-I</td>
<td>Banchukamari</td>
<td>3</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dakshin Sonapur</td>
<td>3</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Patlakawa</td>
<td>3</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>North 24 Parganas</td>
<td>Bongaon</td>
<td>Adityapur</td>
<td>3</td>
<td>45</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Polta</td>
<td>3</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Raghunathpur</td>
<td>3</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Burdwan</td>
<td>Kalna-I</td>
<td>Kalna</td>
<td>3</td>
<td>20</td>
<td>52.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hosenabad</td>
<td>3</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Purulia</td>
<td>Kashipur</td>
<td>Gagnabad</td>
<td>3</td>
<td>43</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dhanera</td>
<td>3</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rambani</td>
<td>3</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Nadia</td>
<td>Santipur</td>
<td>Gayeshpur</td>
<td>3</td>
<td>55</td>
<td>53.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phulia</td>
<td>3</td>
<td>52</td>
<td></td>
</tr>
</tbody>
</table>

Detection of the Virus

Samples which showed distinct visible symptoms of begomovirus infection were subjected to polymerase chain reaction (PCR). One set of universal degenerate primers (SPG1/SPG2) used for the amplification and confirmation of the partial replication associated protein gene (AC1) with expected DNA fragment of 920 bp from the virus infected samples. Positive reactions in PCR amplification of a target sequence (920 nt long) specific to begomovirus confirmed the presence of begomovirus in the samples tested. But Alipurduar-I and Kashipur samples gave negative results on PCR and the symptoms produced on chilli crop indicating towards mite & thrips infestation respectively (Table 2) and (Figures 3 & 4).

Table 2: Types of Chilli Leaf Curl Complex Disease Symptoms and PCR Results of the Samples Collected from Different Blocks of West Bengal during Crop Growing Periods of 2015.

<table>
<thead>
<tr>
<th>State/District</th>
<th>Blocks</th>
<th>Field Symptoms</th>
<th>Percent Disease Incidence</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>West Bengal (NB) /Cooch Behar</td>
<td>COB-II</td>
<td>Severe upward LC, Chl, Bushy plant, Less Fruit</td>
<td>30-90 %</td>
<td>+</td>
</tr>
</tbody>
</table>
WB (NB) / Jalpaiguri
Madarihat
Upward LC, Chl, less fruit
20-45 % +

WB (NB) / Jalpaiguri
Alipurduar-I
Upper & downward leaf curl, ST, B
20-40 % -

West Bengal (SB) / North 24 Parganas
Bongaon
Severe upward LC, ST, Chl, Bushy plant, Less Fruit
45-75 % +

WB (SB) / Nadia
Kalna-I
Mild upward LC, Chl, less fruit
20-85 % +

WB (SB) / Purulia
Kashipur
Mild leaf curl, LR, ST, Leaf crinkle, EP
18-43 % -

WB (SB) / Alipurduar-I
Upper & downward leaf curl, ST, B
20-40 % -

WB (SB) / North 24 Parganas
Bongaon
Severe upward LC, ST, Chl, Bushy plant, Less Fruit
45-75 % +

WB (SB) / Nadia
Kalna-I
Mild upward LC, Chl, less fruit
20-85 % +

WB (SB) / Purulia
Kashipur
Mild leaf curl, LR, ST, Leaf crinkle, EP
18-43 % -

*Randomly One Isolate from each Location was taken for Molecular based Study.

Symptom codes: LC: Leaf Curl; ST: Stunting; Chl: Chlorosis; LR: Leaf Rolling; B: Boat Shaped Leaf; EP: Elongated Petiole; (+) = Presence of the Virus; (-) = Absence of the Virus.

**Figure 3:** PCR Amplification of Begomovirus Samples Collected from North Bengal (NB) using Begomovirus Specific Degenerate primer SPG1/SPG2. Lane M- 1kb DNA Ladder, 1- Madarihat Sample, 2- COB-II Sample, 3- Alipurduar-I Sample.

**Figure 4:** PCR Amplification of Begomovirus Samples Collected from South Bengal (SB) using Degenerate Primer Pair SPG1/SPG2 Sowing the size of Amplicon 920 bp. Lane M- 1kb DNA Ladder, 1- Bongaon, 2- Kalna-I, 3- Santipur, 4- Kashipur.

**Conclusion**

The PCR detection of begomovirus using degenerate primers confirmed the association of begomoviruses with chilli growing in West Bengal; it is due to cultivation of susceptible local cultivars prevailing in the districts. The variation of symptoms between different chilli growing areas indicated mixed infections of begomoviruses, thrips and mites. So the high frequency and distribution of begomoviruses in chilli throughout West Bengal could lead to epidemic in upcoming crop periods if proper management tactics are not taken into view.

**References**

2. AVRDC (2012) the great pyramid: By stacking multiple genes for resistance, AVRDC plant breeders develop tomatoes that are a monument to better pest control. The World Vegetable Centre p. 51.