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Virus Specific Screening of Leaf Curl Resistant *Capsicum annuum* Genotype

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Abstract—*Chilli is a major crop of India and is cultivated in most of the states of the country. It is affected by various biotic and abiotic stresses. Leaf curl disease of chilli has now emerged as a major threat for chilli cultivation in the tropical countries of the world. It is characterized by severe puckering of leaves, stunting and no or malformed fruit formation. This disease is caused by members of the genus begomovirus of family geminiviridae, which infect chilli via whitefly transmission vectors. Leaf curl is said to be a complex disease as it is not a single virus which causes the disease rather several viruses are involved in disease symptom production. To breed for begomovirus resistance, identification of resistant sources is the foremost step. Just as concept of race specific resistance breeding in fungal and bacterial disease, resistance against viral diseases should also be virus specific. Following this strategy DLS-sel-10 a resistant source to leaf curl disease was screened against pre-dominant begomoviruses infecting chilli in India. Chilli leaf curl virus (ChiLCV) and Tomato leaf curl New Delhi virus (ToLCNDV) were the viruses which were used for testing the resistance of DLS-Sel-10 and the susceptible line Phule Mukta. Virulifeorus whiteflies and agro-inoculation were used as screening methods. DLS-Sel-10 showed resistance to ChiLCV and ToLCNDV while the susceptible source used showed susceptibility to ChiLCV but resistance to ToLCNDV. Screening of DLS-Sel-10 with a mixture of ChiLCV and ToLCNDV again showed resistance response but Phule Mukta exhibited increase severity of symptom upon infection with mixture of virus in comparison to infection with ChiLCV alone.*

1. INTRODUCTION

Leaf curl disease is one of the major threat to chilli cultivation in different parts of India (Dhanraj et al. 1968; Chattopadhyay et al. 2008). Disease is a result of infestation by begomoviruses which are spread by a complex of whitefly (*Bemisia tabaci) (*Brown *et al*. 2012). The viruses of the genus *Begomovirus* consists of genome made up of either two genomic components commonly known as bipartite viruses (consisting of DNA-A and DNA-B) or a single component known as monopartite. Chilli cultivation in India is usually taken in the *Kharif* season which is the cropping season from July –October when the country receives south-west monsoon. The disease generally appears in the end of June about 45-55 days after sowing and spreads rapidly in July. Occurrence of leaf curl disease has become so devastating that the farmers have abandoned taking the crop in *Kharif* which is the main growing season. The disease progress becomes slow in August and almost comes to a halt by mid October. The disease symptoms include vein thickening type symptoms on young upper leaves of plants followed by leaf puckering and severe stunting of plants. There is no or malformed fruit formation thereby impacting overall yield and quality of fruits.

In India, a very high disease incidence (up to 100% of plants during December 2004) in farmer's fields in Narwa and Tinwari villages at Jodhpur District Rajasthan was also observed (Senanayake et al. 2007). A disease incidence up to 100% during December 2008 in Vellanad region of Kerala was reported. Severe upward curling, stunted plant growth, leaf thickening and vein clearing were observed at Jodhpur (Rajasthan) (Senanayake et al. 2012). The severely affected plants were stunted bearing hardly any fruits. The incidence of the disease varied from field to field and village to village (14–100%). The incidence was greater in Tinwari where 100% of the plants of chilli variety Haripur Raipur showed severe leaf curl, whereas, at Narwan, the incidence of the disease in the same cultivar varied from 14 to 44%. A survey was carried out in major pepper growing areas in Punjab, and a maximum leaf curl incidence was observed in Ludhiana (79.4%) followed by Tarn Taran (77%), Sangrur (72.2%), Patiala (68.6%) and Ferozepur (57.5%) (Kaur et al. 2016).

Leaf curl of chilli is a complex disease as it is not a single virus which causes the disease but there have been reports that several viruses are involved in disease symptom production. To date, in India, *chilli leaf curl virus* (ChiLCV), *chilli leaf curl India virus* (ChiLCINV), *chilli leaf curl Vellanad virus* (ChiLCVV), *tomato leaf curl Joydebpur virus* and *tomato leaf curl New Delhi virus* (ToLCNDV) are known to be associated with chilli leaf curl disease (Khan et al., 2006 ; Kumar et al., 2011, 2012 ; Senanayake et al., 2007 ; Shih et al., 2007). Studies of Kumar *et al* 2015 revealed the association of distinct begomovirus species with six different groups of betasatellites [ChiLCV, *Pepper leaf curl bangaladesh virus*

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(PepLCBV), *Tomato leaf curl virus* (ToLCV), ToLCNDV, *Papaya leaf curl virus* (PaLCuV) and beta satellites like ToLCBDB, , ChiLCB, ToLRnB, ToLCJoB, CroYVMB, RaLC].

Resistance breeding is the only solution to leaf curl disease in chilli. Resistance breeding program starts with identification of resistance lines. Till date screening of breeding material under natural conditions at hot spots having ample virus inoculum and vector population with a conducive environment has been the most easy approach to first identify genotypes having resistance. Using this strategy, we had identified the genotypes DLS-SEL-10 as resistant line (Srivastava *et al*. 2017). Resistance in these lines needs to be further confirmed by artificial inoculation techniques. Similar to the concept of race specific resistance breeding program against fungi and bacteria similar approach need to be followed for breeding against begomoviruses *viz.*, virus specific breeding. The present experiment was therefore undertaken to test the level of resistance in one of the identified line DLS-Sel-10 against the pre-dominant begomoviruses causing leaf curl disease in chilli.

2. MATERIAL & METHOD

2.1 Identification of major pre-dominant viruses infecting chilli

Infected leaf samples were collected from the different states of India growing chilli like Karnataka, Madhya Pradesh, West Bengal, Rajasthan, Andhra Pradesh and New Delhi. Generic primers detecting presence of begomoviruses were first used to screen for confirmation of presence of the virus. The infested samples were then screened with virus specific primers like ChiLCV, ChiLCINV, ChiLCVV, *tomato leaf curl Joydebpur virus* (ToLCJV), ToLCNDV and *tomato leaf curl Palampur virus* (ToLCPaLCV). The samples were then observed for different virus specific bands.

2.2 Artificial challenging of DLS-Sel-10 using viruliferous whiteflies

Resistant genotype DLS-Sel-10 and a susceptible variety cultivated in India Phule Mukta was used as our test genotypes. Challenge infection with viruliferous white fly fed with teo the pre-dominant viruses identified in the first experiment.

Whitefly colonies were maintained in a controlled condition. Control of relative humidity, temperature and light intensity is essential for optimal colony growth. A temperature of 28-35°C, 30-50% relative humidity, and a 14 hr photoperiod was maintained which yielded a colony that developed from egg to adult in 21 days. Relative humidity was kept below 70% to discourage the growth of insect and plant fungal pathogens. Cleanliness was maintained as it is essential in a whitefly colony to maintain optimal rearing conditions.

Whiteflies were maintained on brinjal plants in cages (Plate 1). Cages were constructed of acrylic sheet materials was used for screening. All this was constructed keeping in mind to prevent whitefly escape or infiltration and also of sufficient size to maintain enough plants to generate the whitefly population needed.

Plate 1: Rearing of whiteflies in insect proof cage

For the acquisition of whiteflies, chilli leaf curl infected plants of hot pepper were raised in small (4”) pots and they were covered with the cages mentioned above. Healthy whiteflies were released in to the cage placed over the young seedling. After 24 hours of feeding on the infected chilli plants, whiteflies were considered to be viruliferous and were used for challenge inoculation of the healthy seedlings of test genotype.

Screening was done against two predominant begomoviruses. Pure isolates of chilli plants carrying pre-dominant viruses was maintained separately. These isolates served as our stock source for respective viruses. These stock source were used for feeding healthy whiteflies to convert them into viruliferous. Resistant genotype and susceptible genotype were inoculated first with the most pre-dominant virus and was observed for symptom development. Similarly another set of resistant and susceptible lines were inoculated with secong pre-dominant virus. After two days of whitefly feeding on healthy seedlings, the whiteflies were killed by spraying spiromefisin@0.5ml/ltr. The plants were then observed for symptom development. At one week interval. We also had one set experiment where the genotypes were exposed to healthy whiteflies carrying no virus. This served as our mock sample.

The resistant and susceptible genotype was then screened with a mixture of the two pre-dominant viruses together. The response of the genotypes was then noted. Scoring of test pla

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3. RESULTS AND DISCUSSION

Screening of infested samples from different parts of the country revealed ChiLCV and ToLCNDV as the most pre-dominant viruses infesting chilli in India. Samples from all the states surveyed showed presence of ChiLCV. Some samples also showed presence ToLCNDV. Other viruses were region specific and not cosmopolitan in nature. Hence ChiLCV and ToLCNDV viruses were identified as the most pre-dominant virus causing leaf curl in India.

Artificial challenging of DLS-Sel-10 and Phule Mukta with ChiLCV produced symptom free response in DLS-Sel-10 while produced symptoms within 14 days of inoculation in Phule Mukta (Plate 2a & Plate 2b). In contrast to this, both DLS-Sel-10 and Phule Mukta was free of infection with ToLCNDV. On challenging the two genotypes with a mixture of the two viruses failed to produce any symptom in DLS-Sel-10 but Phule Mukta produces much more severe symptom on infestation with ChiLCV + ToLCNDV (Table 1, 2 & 3).

Table 1: Response of test genotype to ChiLCV

Test Genotype	Symptom after 7 days	Symptom after 14 days	Symptom after 21 days	Symptom after 28 days
DLS-Sel-10	No symptom	No symptom	No symptom	No symptom
Phule Mukta	No symptom	Symptom visible	Score 3	Score 4

Table 2: Response of test genotype to ToLCNDV

Test Genotype	Symptom after 7 days	Symptom after 14 days	Symptom after 21 days	Symptom after 28 days
DLS-Sel-10	No symptom	No symptom	No symptom	No symptom
Phule Mukta	No symptom	No symptom	No symptom	No symptom

Table 3: Response of test genotype to ChiLCV+ToLCNDV

Test Genotype	Symptom after 7 days	Symptom after 14 days	Symptom after 21 days	Symptom after 28 days
DLS-Sel-10	No symptom	No symptom	No symptom	No symptom
Phule Mukta	No symptom	Score 3	Score 5	Score 5

The experiment definitely confirms the resistance of DLS-Sel-10 but shows that Phule Mukta was susceptible to ChiLCV but resistant to ToLCNDV. The mixture of ChiLCV along with ToLCNDV increased susceptibility response of Phule Mukta. The present study reveals that a mixture of two viruses attacks the plant defense machinery and completely disrupts it thereby increasing susceptibility. This may the possible reason for the havoc created due to chilli leaf curl disease. Two or more viruses are able to disrupt plant's defense strategy more efficiently than alone. The susceptible line Phule Mukta though resistant to ToLCNDV in isolation but in when the two viruses are together Phulle Mukta fails to resist.

Different response to different virus also gives an indication that genetics of resistance against different viruses may also be different. This necessitates the importance of studies on inheritance of resistance to different viruses to be done separately. Hence genetics of resistance to ChiLCV and ToLCNDV should be studied separately. Apart from these two viruses genetics of resistance against other begomoviruses causing leaf curl disease should also be studied. All the genes so identified can later on be pyramided in a single line through gene pyramiding. This line will mimic horizontal resistance and this resistance will be difficult for the viruses to break resistance so easily.

After identification of resistant genotype, identification of genes for resistance to these viruses becomes our next strategy for leaf curl resistance breeding.

Plate 1a: Susceptile Response in Phule Mukta

Plate 2a: Resistant response in DLS-Sel-10

4. CONCLUSION

DLS-Sel-10 shows resistance to both ChiLCV and ToLCNDV in isolation as well as in mixture. Phule Mukta shows susceptibility to ChiLCV but resistance to TOLCNDV.

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Mixture of two viruses produces increased severity of leaf curl in Phule Mukta.

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