



## Genetics and gene action for resistance to leaf curl disease in chilli (*Capsicum annuum*)

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Received: 09 May 2018; Accepted: 29 November 2018

**Key words:** Artificial challenging, Chilli leaf curl virus, Inheritance, Viruliferous whiteflies

Chilli (*Capsicum annuum* L.) is the most widely cultivated commercial spice and vegetable crop in Asia, Africa and South America. It has become an important crop all over India, cultivated for green vegetable, spice and for oleoresin and capsaicin extraction in industries. In India, chilli is cultivated in all the states including Andhra Pradesh, Maharashtra, Karnataka, Telangana, Odisha, Tamil Nadu, West Bengal and Punjab over an area of 8,31,000 ha with total production of 18,72,000 metric tonnes of dry chilli with productivity of 2 metric tonnes/ha (NHB Data 2016-17). While consumption of chilli is highest in India, maximum export is also from India. Tremendous foreign exchange is being earned by exporting chilli powder, oleoresin of low, medium or high pungency and paste to foreign markets like Sri Lanka, Kuwait, Iran and the UK.

Among the several pathological diseases, viruses are known to cause heavy loss contributing to low yield and reduced fruit quality (Kang *et al.* 1973). In severe condition, 100% losses of marketable fruit have been reported (Marte and Wetter 1986). Natural occurrence of more than 45 viruses including pepper leaf curl virus, pepper veinal mottle virus and pepper vein bending virus have been reported to infect chilli pepper worldwide (Green and Kim 1991). In India, leaf curl disease in chilli is emerging as the most destructive disease caused by a complex of viruses belonging to begomoviruses (monopartite or bipartite genomes) that are transmitted by whitefly, *Bemisia tabaci*. Losses of up to 80% have been reported in many parts of northern India (Singh *et al.* 1979). The characteristic field symptoms are upward curling, puckering and reduced size of leaves whereas severely affected plants remain stunted and produce no fruit. Many indirect approaches like systemic insecticides,

nylon net coverings on the nursery beds or use of mulches, barrier crops like maize etc. may reduce chilli leaf curl virus (ChiLCV) infection marginally but it is not possible to ensure complete control with chemical, agronomic and cultural interventions. The available cultivars of chilli pepper under cultivation in India lack inbuilt resistance to the pathogen. It is, therefore, imperative to lay emphasis on the development of cultivars that are resistant/tolerant to chilli leaf curl virus (ChiLCV) with acceptable horticultural traits. Little efforts have been made to identify the gene(s) responsible for conferring resistance against this disease, which is of utmost importance to transfer resistant gene(s) into susceptible commercial varieties and/or developing hybrids. Detail and systematic study on genetics involved in resistance to leaf curl disease caused by begomoviruses still remains incomplete. In view of the above facts, the present study was undertaken to study the genetics of chilli leaf curl disease resistance.

The experimental material included one resistant line (WBC-Sel-5) and one susceptible line (PhuleMukta) which were maintained at the Division of Vegetable Science, Indian Agricultural Research Institute, New Delhi. WBC-Sel-5 was identified as a resistant source to leaf curl disease after four seasons of consecutive screening in the earlier studies (Srivastava *et al.* 2017). WBC-Sel-5 is cluster bearing, erect fruit type chilli with purple leaves and purple fruits showing field resistance to leaf curl disease, while PhuleMukta (PM) is single and drooping fruit type chilli showing susceptibility to leaf curl disease. Both these genotypes and their crosses and subsequent generations were used for natural and artificial screening using whitefly inoculation with ChiLCV isolate. The crosses between PhuleMukta (susceptible parent, P<sub>1</sub>) and WBC-SEL-5 (resistant parent, P<sub>2</sub>) were made to develop the first filial generation (F<sub>1</sub>) and subsequently selfing was done to get F<sub>2</sub> generation. Plants of 5 generations were exposed to challenge (artificial) inoculation under the insect proof cages inside a polyhouse structure. The non-viruliferous culture of whiteflies (*Bemisia tabaci*) were reared on brinjal plants kept in insect rearing cages to generate

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sufficient whitefly population. A strain of chilli leaf curl virus (ChiLCV) was isolated, and maintained on a susceptible chilli genotype under the controlled conditions. From the brinjal plants, healthy adult whiteflies were collected and released on ChiLCV infected chilli plants placed in the cages for 24 h. After the acquisition period, whiteflies were considered to be viruliferous and were released on the healthy seedlings at 4–5 leaf stage of  $P_1$ ,  $P_2$ ,  $F_1$ , and  $F_2$  generations of *Capsicum annuum*, which were kept in screening cages under challenged condition. Five viruliferous whiteflies were released per plant in each cage. After 15 days of inoculation, plants were sprayed with Dimethoate (2 ml/l) to exterminate the whiteflies. The plants were kept in the glasshouse for symptom expression, and symptom severity was scored on individual plant basis according to disease scoring scale (Kumar *et al.* 2006).

To study the genetics of resistance to ChiLCV under the challenged conditions, 15 seedlings of both parents and  $F_1$ , and 128 seedlings of  $F_2$  were grown in the screening cages and viruliferous whiteflies were released through aspirator. Under the natural screening condition, 20 seedlings of parents and  $F_1$ , and 125 seedlings of  $F_2$  were grown. Natural screening was undertaken inside a low tunnel covered with net. The experiment was undertaken in *kharif* season when the whitefly pressure is highest under north Indian condition. The seedlings were transplanted in June inside the net and after one month the net was opened to expose the seedlings to whiteflies naturally present in the environment. After two days, the net was closed again to allow the whiteflies sitting on the test plants to multiply and continue their lifecycle inside the net only.

Under challenged conditions, disease scoring was done 7 days after inoculation till 28 days at weekly intervals as per the disease scoring scale ranging from 0–5 (Kumar *et al.* 2006) where (0) signified no symptoms; (1) up to 5% curling and clearing of upper leaves; (2) 6–25% curling, clearing of leaves and swelling of veins; (3) 26 to 50% curling puckering and yellowing of leaves and swelling of veins; (4) 51 to 75% leaf curling and stunted plant growth and blistering of internodes; (5) More than 75% curling and deformed small leaves, stunted plant growth with small flowers and no or small fruit. The percentage of leaf curl data was recorded on weekly basis and after five weeks of inoculation, the genotypes were assigned a specific severity grade. Under natural condition, disease scoring was done once on 28<sup>th</sup> day after the inoculation. To understand the genetic inheritance of the resistance for ChiLCV in the  $P_1$ ,  $P_2$ ,  $F_1$  and  $F_2$  of the cross, all individual plants were classified into resistant and susceptible categories as per the disease scale. A Chi square test was performed to test the goodness of fit from observed segregations for resistance to leaf curl disease.

The plants of susceptible parental line Phule Mukta showed 100% disease incidence, while all the plants of resistant parent WBC-SEL-5 were disease free indicating 0% disease incidence (Table 1). The  $F_1$  plants of Phule Mukta showed 100% disease incidence and  $F_2$  plants gave 80%

disease incidence. All the  $F_1$  plants exhibited susceptible response under both the conditions of study indicating that the gene for resistance is recessive in nature. Disease incidence was high under natural as well as challenged inoculation, indicating that disease pressure for screening under natural condition and challenged inoculation was almost similar. This result rules out any chances of escape that is usually said to occur when screening is undertaken under natural conditions. This may be attributed to the fact that natural screening was undertaken inside net house where the naturally occurring whiteflies were introduced. The whiteflies were allowed to multiply inside the net house itself which helped to build sufficient whitefly population for disease occurrence.

On the basis of disease score card, only 3 plants had high resistance while 33 plants were highly susceptible under natural condition. Under challenged condition, the disease score range was 4 plants with high resistant, 6 plants were resistance, 16 plants were moderately resistant, 37 plants were moderately susceptible, 39 were susceptible and 26 plants were highly susceptible. The susceptible parent showed susceptibility and resistant one manifested resistance under both the conditions of screening. Some individuals of  $F_2$  population showed susceptibility scores higher than susceptible parent. This probably indicates role of some modifier genes (genes that have small quantitative

Table 1 Leaf curl disease incidence in chilli plants under natural and artificial condition

Leaf curl disease screening	Line	Total plants	Resistant	Susceptible	Disease incidence (DI%)
Natural condition	Phule Mukta	20	1	19	95
	WBC-Sel-5	20	20	0	0
	$F_1$	20	0	20	100
	$F_2$	125	24	101	80
Artificial condition	Phule Mukta	15	0	15	100
	WBC-Sel-5	15	15	0	0
	$F_1$	15	0	15	100
	$F_2$	128	26	102	80

Table 2 Disease score range of  $F_2$  individuals

Score range (Phenotypic score)	Number of $F_2$ individuals	
	Natural (125)	Artificial (128)
0 (HR)	3	4
1 (R)	6	6
2 (MR)	15	16
3 (MS)	34	37
4 (S)	33	39
5 (HS)	34	26

HR, Highly resistant; R, resistant; MR, Moderately resistant; MS, Moderately susceptible; S, Susceptible; HS, Highly susceptible.

Table 3. Chi-square ( $\chi^2$ ) analysis

Generation	No. of plants tested	Resistant plants	Susceptible plants	Expected ratio in $F_2$ (R:S)	$\chi^2$	Table $\chi^2$ value
<i>Challenged Inoculation</i>						
$P_1$ (Phule Mukta)	15	0	15	1:3	1.125	3.841
$P_2$ (WBC-Sel-5)	15	15	0			
$F_2$	128	26	102			
<i>Natural inoculation</i>						
$P_1$ (Phule Mukta)	20	1	19	1:3	1.682	3.841
$P_2$ (WBC-SEL-5)	20	20	0			
$F_2$	125	24	101			

effects on the level of expression of another gene) which when comes in certain combinations increases susceptibility scores of the  $F_2$  individuals. But this needs to be further validated and confirmed through more extensive genetic studies.

The scoring data for highly resistant, resistant and moderate resistance were pooled as resistance ones and similarly susceptible ones for moderately susceptible, susceptible and highly susceptible. The test shows significant results indicating the genetics of resistance to be governed by a single recessive gene.

The  $F_1$  plants of WBC-Sel-5 (resistant) and Phule Mukta (susceptible) were susceptible indicating the recessive nature of resistance genes in resistant sources. Under natural condition, 125  $F_2$  progenies segregated into 24 resistant and 101 susceptible plants while under challenge inoculation, 128  $F_2$  progenies gave 26 resistant and 102 susceptible plants (Table 3). The inheritance studies using  $F_2$  population under both challenge and natural conditions showed resistance to leaf curl disease was governed by single gene with recessive expression. This is evidenced by the non-significance of the  $\chi^2$  test holding the hypothesis that the genes governing resistance to leaf curl disease segregates in the ratio of 3:1. Bal *et al.* (1995) first reported inheritance of resistance to leaf curl disease under field condition to be recessive in nature. This result is also consistent with the findings of Rai *et al.* (2014), who also reported monogenic recessive control of resistance against pepper leaf curl virus, another strain of begomovirus causing leaf curl in chilli. In contrast, tremendous progress has been made in crops like tomato, where at least five non allelic resistant genes to tomato yellow leaf curl viruses (TYLCV) have been identified and mapped on tomato genome (Kadirvel *et al.* 2013).

#### SUMMARY

Leaf curl disease of chilli has become a big menace

and destroys the crop every year during *kharif* season because of which the farmers have abandoned growing the crop in *kharif* which is the main season of cultivation. Resistance breeding is the only safe and economical solution for this problem. WBC-Sel-5 was identified as a resistant genotype to ChiLCV after 4 seasons of rigorous field evaluation. This resistant line was used to generate  $F_1$  and  $F_2$  generations with susceptible recipient parent (Phule Mukta).  $F_2$  population raised from a cross of susceptible parent Phule Mukta and resistant parent WBC-Sel-5 was screened against chilli leaf curl virus along with parents and  $F_1$  in both natural and challenged inoculation using viruliferous white flies carrying chilli leaf curl virus. All the  $F_1$  plants were susceptible to leaf curl disease and the  $F_2$  population showed susceptible and resistant individuals segregating in 3:1 ratio under both natural and artificial epiphytotic conditions indicating monogenic recessive genetic control of resistance to chilli leaf curl virus disease.

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