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Natural Epiphytotic Screening of Chilli Germplasm Lines Against Leaf Curl Virus Complex

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ABSTRACT: *Leaf curl complex in chilli is a major biotic stress of tropical and sub tropical regions which causes heavy losses to the chilli crop. Till date, resistance breeding through conventional methods results from sexual hybridization followed by selection of best genetic recombinant with resistance. The entire program relies on identification of stable resistant sources. In the present study, 62 germplasm lines of Capsicum annuum L. were assessed for resistance to the disease under natural disease epiphytotic condition in trans gangetic plains of northern India. As screening was carried out under natural disease conditions, chances of escape cannot be rule out. To ascertain the resistance ability of the identified chilli lines, the experiment was undertaken including the identified resistant lines for three more consecutive seasons with susceptible checks at regular intervals to confirm the resistance reaction. In addition to field screening, resistant source of chilli were subjected to virus indexing against the major leaf curl causing begomoviruses viz. Tomato leaf curl New Delhi virus and Chilli leaf curl India virus. In the first season of evaluation, five lines (namely DLS-Sel-10, WBC-Sel-5, PBC 142, PBC-345 and Tiwari) showing resistance reaction were identified. Evaluation of identified lines for disease resistance for the next three continuous seasons (Kharif, 2013, summer, 2014 and Kharif, 2014) resulted in breakdown of resistance of 2 lines namely Tiwari and PBC-345 with the remaining three showing resistance (DLS-Sel-10-resistance, WBC-Sel-5-resistance, PBC 142-moderate resistance).*

INTRODUCTION

Hot pepper commonly known as chilli in India is one of the most important commercial crop which is grown almost throughout the country. The world's hottest chilli "Naga Jolokia" is cultivated in hilly terrain of Assam in a small town Tezpur, India. Different varieties are grown for vegetables, spices, condiments, sauces and pickles. Chilli occupies an important place in Indian diet. It is an indispensable item in the kitchen, as it is consumed daily as a condiment in one form or the other. Among the spices consumed per head, dried chilli fruits constitute a major share. Currently, chillies are used throughout the world as a spice and also in the making of beverages and medicines. If some varieties of chillies are famous for red colour because of the pigment 'capsanthin,' others are known for biting pungency attributed to 'capsaicin.' India is the only country which is rich in many varieties with different quality factors. Chillies are rich in vitamins, especially in vitamin A and C. They are also packed with potassium, magnesium and iron. Chillies have long been used for pain relief as they are known to inhibit pain messengers, extracts of chilli peppers are used for alleviating the pain of arthritis, headaches, burns and neuralgia (Bosland and Votava, 2000).

Hot pepper production is severely affected by various biotic stresses which includes infestation by all the major group of pathogens (bacterial, fungal and viral). Leaf curl disease of chilli has emerged as a serious problem in all the major chilli growing area of the country. Leaf curl in chilli is caused by begomoviruses which is the most devastating virus of the chilli plant (Senanayake, 2006). The genus *Begomovirus* includes species with monopartite or bipartite genomes that are transmitted by whitefly, *Bemisia tabaci*. The characteristic field symptoms are upward curling, puckering and reduced size of leaves. Severely affected plants are stunted and produced no fruit. In India, Tomato leaf curl New Delhi virus (ToLCNDV) was recently shown to be associated with chilli leaf curl disease occurring in Lucknow (Khan *et*

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al., 2006). Reports of Khan *et al*. (2006) also show that chilli leaf curl in India is caused by more than one begomovirus.

There were many factors affecting the population of whitefly such as climate (temperature, rainfall and relative humidity), natural enemies, surrounding area and host plants. Raj *et al*. (2005), found that the population dynamic of *B. tabaci* in cotton fields were mainly due to climatic factors such as humidity and temperature. Previous study Senanayak *et al* (2012) found that if host plants were cultivated continuously in time and space, the population of *B. tabaci* would increase and cause greater damage to host plants grown later in the planting season. In fact, outbreaks of *B. tabaci* in Brazil occurred under such circumstances (Mishra *et al*., 1963). The distribution of immature whiteflies varied significantly among the leaf strata. The upper stratum harboured lower number of whitefly larvae. The population on the upper stratum was highly exposed to the rain which might have a direct effect on the whitefly larvae, which could be brushed off from the under surfaces of the leaves.

Under these circumstances resistance breeding appears to be the most promising and environment friendly approach. Screening of different varieties of chilli lines against leaf curl complex disease would help in the recognition of available resistant germplasm against the disease, and will be utilized for chilli improvement program in India. Identification of resistant source to control the disease is an economical way and will prevent hazards caused due to indiscriminate spraying of pesticides. Several previous reports are there supporting screening studies to identify resistant lines (Kumar *et al*., 2006, Kumar, 2008, Kumar *et al*., 2009, Kumar *et al*, 2011). This experiment was undertaken to identify sources of resistance to leaf curl disease from within the *C. annuum* complex. Screening was concentrated to *C. annuum* lines with a thought to first explore if any source of resistance is available within the most cultivated species of *Capsicum*. This will definitely help to reduce the crossing incompatibilities issues arising when we undertake interspecific hybridization programs.

MATERIAL AND METHODS

Experimental Site and epiphytotic condition:

The entire experiment was conducted at experimental farms of Division of Vegetable Science, IARI, New Delhi which comes under trans gangetic ecological region of northern India. There are two cropping periods for chilli at Delhi (i) summer season where transplanting is done in February month and *Kharif* season where transplanting is done in the month of August. Generally, in summer cropping periods, whitefly population starts to increase and reaches the highest peak during fruiting stages while in *Kharif* it is maximum during the vegetative and flowering stages. The population decreases when the chilli plants reach the end of economic life. These trends are mainly due to climatic factors where high temperature coupled with humidity results in sharp increase in whitefly population. Summer in northern India lasts from April to July while in the rest of India from March to June. By the first week of July, the entire country experiences monsoon rain; on average, South India receives more rainfall than North India. So, high temperature along with high humidity resulting from incoming monsoon results in fast multiplication of whiteflies.

Plant material and experimental design

In the first season i.e., summer, 2013, we screened a collection of sixty two *Capsicum annuum* lines which included popular varieties of chilli being cultivated in different parts of the country, breeding lines recovered from various hybridization programs and some AVRDC collections. The list of the various lines is given in Table 1. All these lines were grown in three replications following RCBD design. Each replication had around 20 plants with a spacing of 0.6 m between rows and 0.45m among the plants. Identified resistant lines (Italicized in Table 1) identified from the first experiment were screened for next three consecutive seasons (*Kharif*, 2013; summer, 2014; *Kharif*, 2014) along with susceptible controls at regular intervals. Two replication of each resistant line was grown in three rows with five rows of susceptible check before and after it subsequently. Planting spacings were same as followed in the first season.

OBSERVATIONS AND MEASUREMENTS

The variables measured were disease incidence and severity for the different lines tested in the first season. Scales for classifying the lines tested for leaf curl disease reactions were adopted as developed by Banerjee (1987) and used by Kumar *et al*. (2006).

0 No symptom
1 0 to 5% curling and clearing of upper leaves.
2 6 to 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 set.

Scoring of the lines was done at one month interval for four months from the date of transplanting.

Incidence

The incidence of the disease, is the number or proportion of plant units that are diseased (the number or proportion of plants, leaves, stems and fruit that show any symptoms) in relation to the total number of the units examined. Incidence calculation will be done all the populations under study using the following formula:

$$Incidence(I) = \frac{Number\ of\ plants * (or\ parts) \times 100}{Total\ number\ of\ plants\ (or\ parts)\ observed}$$

In this case, 10 plants will be scored in each line and in each replication

Severity

The formula to calculate severity using an evaluation scale is as follows:

$$A = \frac{(Number\ of\ plants \times each\ degree) \times 100}{Number\ of\ plants\ evaluated \times Highest\ degree}$$

DATA ANALYSIS

First experiment (Summer, 2013)

The data recorded for each of the lines were used to detect the resistant, tolerant or susceptible genotypes based on the score chart detailed in the section observation and measurement. Mean values of resistance reaction for each line under study were subjected to Analysis of Variance (Panse and Sukhatme, 1967).

RESULTS AND DISCUSSIONS

First experiment

On the basis of disease incidence and severity values obtained under field conditions on screening of sixty two genotypes the maximum number of genotypes were susceptible (45) followed by moderately susceptible (5) and resistant (12). None of the genotypes were found to show high resistance or immune reaction (Table 1). The resistant reactions of the twelve lines namely DLS-Sel-10, PBC-142, PBC-345, WBC-Sel-5, Phule Jyoti, Pant Chilli 3, DCL-2, DKC-8, Chilli Japani Longi, Uttkal yellow, DCL-1 and Punjab Gucchedar were further confirmed through natural screening for consecutive three seasons.

Critical information in the assessment of disease is the amount of disease that is present. This can be measured as the proportion of a plant community that is diseased (disease incidence) or as the proportion of plant area that is affected (disease severity).

Disease Incidence and severity: In the present experiment, at the end of four months maximum number of genotypes showed 100 per cent disease incidence except for seventeen line of which WBC-Sel-5 (38.33%), DLS-Sel-10 (36.67%), DKC-8 (41.67%), DCL-1 (36.67%) and Chilli Japani long (46.67%) showed least incidence. Similarly, lowest values of disease severity were recorded by DCL-2 (14.47), WBC-Sel-5 (8), DLS-Sel-10 (7.33), DKC-8 (8.67) and DCL-1 (7.67) (Table 1).

Resistant genotype
Susceptible genotype

Table 1
Resistance reaction of genotypes under test to leaf curl complex:

Genotypes	*After 30 days*		*After 60 days*		*After 90 days*		*After 120 days*	
	I	*S*	*I*	*S*	*I*	*S*	*I*	*S*
DCL-2	0.00	0.00	6.67	1.33	25.00	5.00	68.33	14.67
LCA 960	30.00	6.00	81.67	20.33	98.33	41.67	100.00	63.33
Lampang local short	30.00	6.00	90.00	24.33	100.00	44.67	100.00	67.00
LCA 625	50.00	10.00	95.00	27.00	100.00	48.00	100.00	68.33
PBC-142	0.00	0.00	18.33	3.67	40.00	8.67	83.33	23.00
LCA 206	25.00	5.00	86.67	21.67	100.00	43.00	100.00	64.00
LCA 334	63.33	12.67	100.00	32.00	100.00	53.33	100.00	73.33
Gowribidanur	40.00	8.00	100.00	28.33	100.00	49.00	100.00	69.67
Vellayani Athulaya	33.33	6.67	91.67	24.67	100.00	46.00	100.00	63.33
PBC-80	5.00	1.00	56.67	13.00	100.00	32.67	100.00	56.00
Tiwari	0.00	0.00	18.33	3.67	65.00	17.00	93.33	28.67
Arka Suphal	38.33	7.67	95.00	26.67	86.67	47.67	100.00	68.33
Aparna	23.33	4.67	96.67	24.33	100.00	46.67	100.00	67.67
Jwalasakhi	26.67	5.33	86.67	22.00	100.00	42.00	100.00	63.00
Vellayani sambrudhi	99.67	4.67	90.00	21.00	100.00	43.33	100.00	63.00
PBC-345	0.00	0.00	15.00	3.67	40.00	9.67	73.33	21.67
LCA-424	45.00	9.00	96.67	29.67	100.00	50.33	100.00	70.67
WBC-Sel-5	0.00	0.00	3.33	0.67	10.67	5.33	38.33	8.00
Jwalasakhi	41.67	8.33	100.00	27.33	100.00	47.67	100.00	67.67
Phule Jyoti	0.00	0.00	15.00	3.00	52.00	16.33	96.67	32.33
Arka Lohith	45.00	9.00	100.00	28.33	100.00	49.33	100.00	70.33
Kashmir line	18.33	4.00	81.67	19.33	81.67	41.00	100.00	62.00
Palampur local	0.00	0.00	11.67	2.33	78.33	22.67	100.00	41.33
Anugraha	18.33	3.67	68.33	18.67	100.00	41.00	100.00	61.00
Kashi Anmol	23.33	4.67	95.00	23.67	100.00	45.00	100.00	64.33
Swati	23.33	4.67	71.67	17.00	98.33	37.00	100.00	57.33
Ujwala	0.00	0.00	11.67	2.33	66.67	17.33	95.00	34.33
Kullu local	0.00	0.00	16.67	3.33	58.33	20.00	95.00	30.33
Phule Mukta	58.33	11.67	100.00	30.33	100.00	51.67	100.00	71.00
ACS-2000-02	41.67	8.33	100.00	28.00	100.00	48.00	100.00	68.00
KL-1	23.33	4.67	96.67	25.33	100.00	46.00	100.00	66.67
DCL-352	26.67	5.33	72.67	25.33	100.00	46.33	100.00	66.33
Punjab Surkh	25.00	5.00	80.00	20.67	100.00	42.00	100.00	42.00
PC-56	28.33	5.67	100.00	26.50	100.00	45.67	100.00	66.33
JCA-283	31.67	6.33	100.00	27.00	100.00	47.67	100.00	67.67
Pant Chilli 3	0.00	0.00	26.67	5.33	46.67	13.00	76.67	19.00
DLS-Sel-10	0.00	0.00	1.67	0.33	20.00	4.00	36.67	7.33
AVNOC	40.00	8.00	93.33	21.67	98.33	46.00	98.33	68.00
LCA-333	38.33	7.67	100.00	26.33	100.00	47.33	100.00	64.00
SC-25-30	36.67	7.33	100.00	28.00	100.00	48.33	100.00	65.67
Sambrudhi	28.33	5.67	100.00	25.33	100.00	45.67	100.00	66.00
GVC-101	26.67	5.33	96.67	25.00	100.00	46.33	100.00	67.33
GVC-111	25.00	5.00	100.00	25.00	100.00	45.33	100.00	68.00
ACS-2006-02	26.67	5.33	100.00	26.33	100.00	46.33	100.00	65.67
X-235	50.00	10.00	100.00	31.67	100.00	52.00	100.00	73.00
BC-25	36.67	7.67	88.33	23.00	100.00	48.67	91.33	70.00
Ajeet-6	46.67	9.33	100.00	28.67	100.00	49.00	100.00	69.33
DKC-8	0.00	0.00	8.33	1.67	20.00	4.00	41.67	8.67
Chilli Japani Longi	0.00	0.00	18.33	3.67	30.00	7.00	46.67	11.33
Ajeet 3	43.33	8.67	95.00	29.33	100.00	48.67	100.00	68.67
Jayanti	38.33	7.67	88.33	23.33	100.00	43.67	100.00	64.33
Uttkal yellow	0.00	0.00	20.00	4.00	48.33	11.67	83.33	25.67
DCL-524	40.00	8.00	85.00	23.00	100.00	43.67	100.00	67.67
DCL-1	0.00	0.00	13.33	2.67	25.00	5.33	36.67	7.67
PC-2062	40.00	8.00	95.00	27.00	100.00	47.00	100.00	68.33
Ajeet 1	35.00	7.00	100.00	27.00	100.00	46.67	100.00	66.00
Punjab Gucchedar	0.00	0.00	25.00	5.00	36.67	8.67	65.00	16.33
Suryamukhi	8.33	1.67	35.00	7.33	93.33	26.00	100.00	45.33
Hang Jiaon	45.00	9.00	96.67	29.00	100.00	50.33	100.00	70.00
Jawahar mirch	26.67	6.00	100.00	26.00	100.00	47.67	100.00	68.67
G-4	50.00	10.00	100.00	28.67	100.00	49.00	100.00	69.00
Garima	43.33	8.67	96.67	27.67	100.00	48.00	100.00	68.33

Bold figures indicate disease scores of resistant lines

Second, third and fourth experiment:

Twelve lines shortlisted as resistant from the first experiment were subjected to three consecutive seasons of natural screening. At the end of second season, three lines namely Phule Jyoti, Pant Chilli-3 and PBC 345 was excluded due its highest susceptibility scores in comparison to other lines. Third season screening helped in discarding of Uttkal Yellow and Punjab Gucchedar which recorded highest susceptibility to leaf curl disease. Finally in the fourth season screening all the lines except PBC-142 showed similar disease score ranging from 1.3 to 1.6 (Table 2). DLS-Sel-10, DCl-1, DCL-1, DKC-8 and chilli japani long had similar phenotypic appearance. Considering these lines to have common ancestral parent, only one line DLS-Sel-10 was selected among them assuming that all of these will be having common resistance gene locus. Hence finally we have ended up with three resistance lines i.e., DLS-Sel-10 (resistant), WBC-Sel-5 (resistant) and PBC-142 (moderately resistant).

Table 2
Reaction of identified resistant genotypes under screening for three consecutive seasons

	Season II		*Season III*		*Season IV*	
	Average Score	*Reaction*	*Score*	*Reaction*	*Score*	*Reaction*
DLS-Sel-10	1.2	R	0.9	R	1.3	R
PBC-142	2.5	MR	2.1	MR	2.8	MR
PBC-345	4.1	S	Excluded		Excluded	
WBC-Sel-5	1.5	R	1.1	R	1.4	R
Phule Jyoti	3.1	MR	Excluded		Excluded	
Pant Chilli 3	3.5	MR	Excluded		Excluded	
DCL-2	1.3	R	1.2	R	1.5	R
DKC-8	1.2	R	1.1	R	1.5	R
Chilli Japani Longi	1.6	R	1.4	R	1.6	R
Uttkal yellow	2.1	MR	3.2	MR	Excluded	
DCL-1	1.3	R	1.5	R	1.3	R
Punjab Gucchedar	2.2	MR	3.5	MR	Excluded	
Susceptible Check	4.7	S	4.7	S	4.7	S

R: Resistant; MR: Moderately resistant

CONCLUSION

Since the identification of resistant source from the germplasm is first step in a resistant breeding programme, the identified symptom-less sources are pre-requisite basic materials to study the inheritance of host plant resistance against PepLCV and execute resistance breeding against this devastating virus. The remaining symptom-less, highly resistant and resistant genotypes with desirable market types identified during this study under field conditions are intended to be selected for confirmation through artificial screening and confirmed resistant genotypes may directly be promoted after seed multiplication.

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