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Identification of resistant sources against chilli leaf curl virus disease through field and molecular screening in chilli

Rajesh Kumar*, Indivar Prasad, Achuit Kumar Singh, Ashutosh Rai, K Nagendran, PM Singh and Jagdish Singh

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Abstract

A study was conducted to discover resistant sources in 109 F_6 families of a Recombinant Inbreed Lines (RIL) population against Chilli Leaf Curl Virus (ChiLCV) disease, a serious menace to chilli crop in India. The Population was characterized for yield and disease related traits that revealed presence of significant variability in the population. Fruit yield followed by number of fruits per plant, fruit weight and PDI (at 120 days after transplanting) were key traits contributing significant portion of this variation as explained by principal component analysis. Twenty virus-free plants were identified under natural field condition at 60 and 120 days after transplanting along with molecular screenings using universal primers. These potent plants having ChiLCV resistance and superior plant and fruit morphology will be utilized in breeding programme for development of resistant varieties/hybrids against chilli leaf curl virus disease.

Keywords: Chilli, Virus, Screening, Primer, PDI

Introduction

Chilli (*Capsicum spp.)* is an important vegetable and spice crop for farmers globally. Having originated in Mexico (Villalon 1981), chilli moved widely and is grown in almost all agro-ecological condition in the world in one form or the other. It is extensively used for cuisines, nutrient, colour and flavour. Chillies are rich in vitamins A, C and E and also good source of potassium and folic acid. Fresh red chilli has more vitamin A than carrots, more vitamin C than oranges (Osuna-Garcia et al. 1998, Marin et al. 2004, Hornero-Mendez et al. 2002). Out of 30 species of chilli (*Capsicum* spp.) five are cultivated *viz. C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens* and C. *pubescens*. Among them *C. annuum* is the most commonly cultivated worldwide (Tong and Bosland 1999, Bosland and Votava, 2003). In India, *C. annuum* is widely cultivated for its pungent (hot pepper) and non-pungent (sweet pepper, bell pepper) fruits. Cultivation of *C. frutescens*, *C. chinense*, and *C. baccatum* is very limited in India for kitchen gardening/aesthetic use (Kumar and Rai 2005). India is the largest producer, consumer and exporter of chilli in the world and it is exported to USA, Canada, UK, Saudi Arabia, Singapore, Malaysia, Germany and many countries across the world. Other prominent exporting countries besides India include Spain, Japan and Thailand. Indian hot pepper is mainly exported in the form of curry powder and curry paste along with dry chillies. As per latest estimate, in India, chillies (dry-red and fresh-green fruits) is being cultivated on 848,041 ha with a total production of 2.1 million tonnes of dry fruits and 67892 tonnes of fresh fruits (FAOSTAT 2018). However, as per the first advanced estimate of NHB for 2018-19, green chillies were grown in an area of 366 thousand ha with the production of 3737 thousand tonnes and 2172 thousand tonnes of dry chilli was produced from an area of 739 thousand ha. Andhra Pradesh, Karnataka, Maharashtra, Orissa and Tamil Nadu account for more than 75% of the area and production of chilli in India (Source: Spice Board, India). Sustainable production of chilli is essential for meeting both domestic and export demand.

Production of chillies is highly vulnerable to by many biotic factors, both insect-pests & diseases. Among them, chilli leaf curl virus disease (ChiLCV) is considered to be the important yield limiting factors of chilli cultivations in India. The disease was reported for the first time in India by Senanayake et al. (2007) to be caused by begomoviruses (DNA virus) belonging to family Geminiviride. Most Begomoviruses have a bipartite genome consisting of a DNA-A and DNA-B components. DNA-A encodes for genes responsible for viral replication, regulation of gene expression, suppression of gene silencing and particle encapsidation, while DNA-B encodes for proteins involved in viral movement, host range determination and symptom development (Lazarowitz 1992). So far, more than 100 viruses have

ICAR- Indian Institute of Vegetable Research, Varanasi, UP
*Corresponding author, Email: rajes74@gmail.com

been reported to be associated with leaf curl disease of chilli globally. Begomoviruses, transmitted by whitefly is one of the most destructive viruses in terms of incidence and yield loss. In severe cases, 100 per cent losses of marketable fruit have been reported. The typical symptoms consist of leaf curling, rolling and puckering, blistering of interveinous areas, thickening and swelling of the veins, shortening of internodes and petioles, crowding of leaves and stunting of the whole plant. ChiLCV belongs to genus Begomovirus, family Geminiviridae characteristically has circular single-stranded DNA genomes encapsidated in twin particles.

Evasive measures, such as pesticide sprays to control vectors, removal of diseased plants and agronomic interventions, have been tried with little success. The exploitation of host plant resistance is effective, economical, ecologically safe and durable approach to viral disease management. Many workers have reported resistant sources from different parts of India like BS-35, GKC-29 and Bhut Jhalokia from IIVR, Varanasi (Rai et al. 2014), Saurian 2010, Perennial and Japani Loungi (Ahmad et al. 2016) and S-343, SL 475 and SL 476 (Jindal 2014, Thakur et al. 2019). Field screening under natural conditions for identifying resistant sources and their inheritance pattern has been made in the past (Bal et al. 1995, Kumar et al. 2009, Anandhi and Khader 2011). There is no strong evidence of high-level resistance against ChiLCVD in cultivated genotypes of chilli. Till date no resistance cultivar is available and there is urgent need of resistant varieties to overcome this problem. Considering the extensive damage caused by leaf curl disease caused by viruses in chilli, the present study was undertaken to identify the resistant source in F_6 families of interspecific RIL population of resistant line, BS-35 and susceptible line, Kashi Sinduri, through field screening under natural conditions supplemented with molecular screening.

Materials and Methods

Plant materials: Germplasm of chilli were screened for ChiLCV tolerance and good sources of tolerance were found in BS 35, GKC 29 and EC-497636 in previously conducted study at ICAR-IIVR (Kumar et al. 2006). These tolerant lines were later characterized and utilized in breeding programme. A set of recombinant inbred lines (RILs) population was developed using resistant line BS-35, a natural interspecific derivative of *C. frutescens* and *C. chinense* and susceptible paprika variety Kashi Sinduri with the target to evolve chilli lines having variabilities in resistance, pungency and fruit morphology. In order to explore the resistance, the population of 109 families in F_6 generation were planted and screened for the reaction of ChiLCVD caused by whitefly transmitted begomovirus during cropping season of kharif, 2018-19. The RIL population was planted in augmented block design with three repetitive checks namely BS-35 (resistant parent), Kashi Sinduri (susceptible parent) and Pusa Jwala (susceptible check). The population was characterized morphologically for yield contributing traits and ChiLCV disease reactions. Data was analyzed statistically employing multivariate technique (PCA) to find out superior plants/families.

Screening for ChiLCVD reactions under natural field condition: The cropping season of 2018-19 witnessed heavy incidence of chilli leaf curl virus disease in the field couple with large population of whitefly population. It provided an ideal situation for screening of tolerant material through natural screening in native environment. Spraying of insecticide was avoided in the experimental plot right from transplanting till final harvesting to avoid chance of disease escape. The disease scoring was done as per the standard procedure mentioned in Table 1 and 0 – 4 grades were given based on the symptom appearance on the individual plants under field condition (Kumar et al. 2006). Percent disease index (PDI) of ChiLCVD was determined for all the families using the formula [sum of all score x 100/ (Total number of plants observed x maximum grade)], at an interval of 60 days and 120 days after transplanting. RIL population was screened for disease incidence at 60 and 120 DAT (days after transplanting). A total of 2208 individual plants of all 109 F_6 families were screened for disease on 0-4 score (assigned based on the symptom appearance on the plants under field condition (Fig 1).

Table 1. Infection type classification given by Kalloo, 1987 and modified by Kumar et al, 2006)

Class	Grade	Description of symptoms
Immune	0	No symptom
Highly resistant	1	0 to 5% curling and clearing of upper leaves
Resistant	2	6 to 25 curling, clearing of leaves and swelling of veins
Moderately susceptible	3	26 to 50% curling, puckering and yellowing of leaves and swelling of veins
Susceptible	4	51 to 75% leaf curling and stunted plant growth and blistering of internodes
Highly susceptible	5	More than 75% curling and deformed small leaves, stunted plant growth with small flowers and no or small fruit set

Molecular screening for detection of virus: Individual plants with 0 or 1 score within families were marked and DNA was isolated for molecular screening with universal begomovirus primers (Rojas et al., 1993) for

Fig 1. Screening* of chilli progenies for ChiLCV disease using Roja's universal primer

* Scoring for the leaf curl disease symptoms was done as per the standard procedure as the score of 0 was given for no symptom, 1 for curling of leaves up to 25%, no stunted growth, with normal flowering and fruiting, 2 for curling of leaves 26 to 50%, mild stunted growth, reduced flowering with normal sized fruits, 3 for curling of leaves 51 to 75%, stunted growth, distortion of leaves, reduction and malformation of fruit size, plant continue to grow and 4 for curling of leaves more than 75%, severe stunting of plants, no flowering and fruit formation, malformation of entire plants, plant growth stops.

detection of the virus particle. DNA was extracted by CTAB method from the leaves of all the test plants at 60 and 120 DAT. A pair of degenerate primer PAL1v1978 & PAR1c715 were used for DNA-A (Rojas *et al*., 1993). 200 ng genomic DNA was used as a template for PCR reaction. PCR reaction master mix contained 25 ìl total volumes with 2.5 ìl of PCR buffer (10X), 1 unit of *Taq* polymerase (5U/ìl), 1 ìl dNTPs (10 mM) and both primers 0.2 ìM each and the total volume was maintained by SDW. PCR condition was set for initial denaturation at 95 ^{0}C for 4 min, 30 cycles each of at 95 ^{0}C for 1min for denaturation, 56 ^{0}C 1m for annealing, 72 ^{0}C 1.5 min for extension and the final cycle was followed by 15min at 72^{0}C. Amplified PCR product was run on 1% agarose gel with 1kb ladder and bands were analyzed on trans-illuminator. Further, total DNA was used to amplify betasatellite molecule (nearly ~1.3 kb) by using a specific pair of betasatellite primer Beta F and Beta R (Briddon et al., 2002). The PCR conditions were set for initial denaturation at 95 °C for 4 min and subsequently 30 cycles of denaturation at 95 °C for 1min, annealing at 60 °C for 1min, and extension at 72 °C for 1:30 min and the final extension period of 15 min at 72 °C. Master mix reaction contained 200 ng DNA template, 2.5 µl PCR (10X) buffer, 1 µl dNTPs (10 mM), primers (0.2 mM) each, 0.3 µl (1 Unit) of *Taq* polymerase and the volume was adjusted to 25 µl with SDW. PCR products were run on 1% agarose gel for 3 h at 60 V and gel was stained with ethidium bromide (10 mg/mL), in the adjacent well load 1 kb DNA marker (Thermo Scientific) as a control and the tank was filled with 1X TAE (Tris-acetate-EDTA buffer, pH 8). The gel image was viewed on UV trans-illuminator..

Results and Discussion

Morphological characterization: A total of 109 recombinant inbred lines (RILs) derived from the cross of BS-35 and Kashi Sinduri in F_6 advanced generation were characterized for their morphological traits and resistance to ChiLCVD. Analyzed data revealed significant variation among families for fruit length, PDI at 60 DAT, PDI at 120 DAT and yield (q/ha). Results revealed that 4% (95) plants scored 0, 39% (864) plants scored 1, 33% (726) plants scored 2, 16% (358) plants scored 3 and 7% (165) plants scored 4 for the disease depicting a typical normal distribution for the disease reaction. The population also exhibited wide variability for various traits like fruit length (1.8-12.9 cm), fruits per plant (3 - 83) and ten fruit weight (16 - 115 g) and disease reaction (highly resistant to highly susceptible). The fruit colour varied from light green to dark green with erect or pendant orientation of fruits on the plant (Table 2 and 5). Similar results were reported by Rai et al. (2013) and Sarmah et al. (2018) while estimating genetic diversity in chilli germplasm. The RILs *viz*. IIVRC-GT-113-3-3-3-3, IIVRC-GT-46-5-2-4-1, IIVRC-GT-183-2-1-4-2, IIVRC-GT-191-1-5-1-1, IIVRC-GT-197-1-8-4-1, IIVRC-GT-191-2-2-4-2, IIVRC-GT-109-2-4-9-4, IIVRC-GT-144-1-2-7-2, IIVRC-GT-137-3-3-3-4, IIVRC-GT- 115-2-5-2-2 were found promising for resistance to ChiLCV disease under field screening (Fig 2).

Table 2: Analysis of Variance and variability for various traits recorded in RIL population

Trait	MSS families	Mean	Range	C.V.
Fruit Length (cm)	2.61	6.83	1.8-12.9	11.9
Fruit Diameter (cm)	0.04	1.10	0.45-1.80	13.3
No of fruits/plant	279.87	36.77	3-83	23.8
10 Fruits weight (g)	242.75	45.39	16-115	22.5
Plant Height (cm)	129.66	51.78	25-85	16.4
PDI at 60 DAT (%)	127.01**	42.74	12.0-72.2	4.5
PDI at 120 DAT (%)	187.46**	46.91	11.0-80.0	7.3
Fruit Yield (q/ha)	854.74**	56.89	3.3-126.9	9.0

**Significant at 1%; *Significant at 5%

IIVRC-GT-191-2-2-4-2 IIVRC-GT-183-2-1-4-2

Fig 2. Highly resistant families against ChiLCV derived from Kashi Sinduri x BS-35

Principal component analysis: Principal component analysis (PCA) is a widely used multivariate data analysis technique in plant sciences for data reduction and grouping of genotypes (Khavari et al. 2011, Prasad et al. 2015). In the present investigation, covariance matrix was used for PCA. As per analyzed data, seven PCA

had Eigen value >1, out of which three PCs accounted for 90% of the total variance in the data which was sufficient to capture the whole variability present (Table 3). The proportions of total variance attributable to the first three PCs were 61, 15 and 13%, respectively as shown in scree plot (Fig 3). The results showed that yield followed by number of fruit per plant, fruit weight and PDI (120 DAT) had the highest loadings in PC1, PC2 and PC3, which indicated significant importance of these traits conferring variability in the RIL population. PC2 and PC3 were mainly associated with fruit weight followed by PDI (120 DAT). Based on the above discussion, it is suggested that these traits played important roles in generating variability in the RIL population under heavy ChiLCV infestation. Similarly, Sarma et al (2018) also reported that the principal component explained 34.93% of the total variation contributed by mostly leaf and fruit characteristics in chilli germplasm.

Table 3: Principal component analysis (PCA) of RIL population for various recorded traits

	PC1	PC2	PC3
Eigen Value	1152.87	281.16	258.91
Difference	871.71	22.25	145.01
Proportion of variance	0.61	0.15	0.13
Cumulative Variance	0.61	0.76	0.90
Traits			
Fruit Length (cm)	0.023	-0.045	0.036
Fruit Diameter (cm)	0.001	-0.005	0.005
No of fruits/plant	0.402	0.462	-0.335
10 Fruits weight (g)	0.245	-0.658	0.486
Plant Height (cm)	0.098	0.122	0.228
PDI at 60 DAT (%)	-0.071	0.349	0.451
PDI at 120 DAT (%)	-0.051	0.463	0.626
Fruit Yield (q/ha)	0.872	0.015	0.064

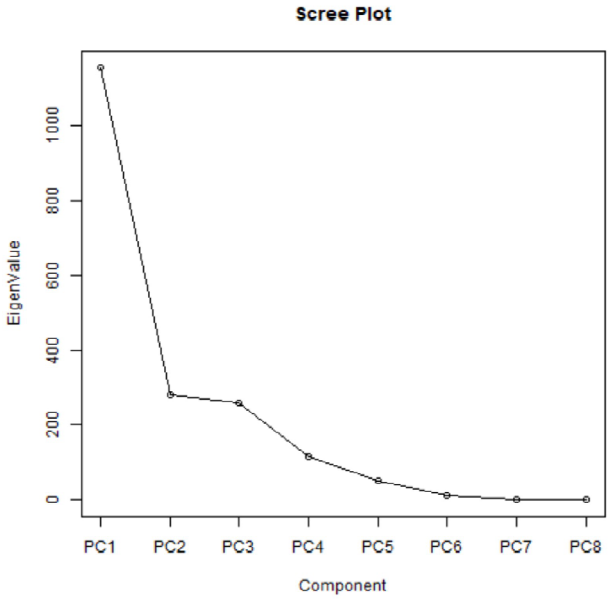

Fig 3. Proportion of variance explained by various PCs

Disease incidence: Out of 109 families screened, it was interesting to note that PDI decreased by 2.5- 20% in 39 families between 60 and 120 DAT indicating that these families had inherent potential to build up resistance to counter ill effect of disease even after initial infection. There was no change in PDI score for 10 families and 60 families had more PDI score (120 DAT) than initial PDI score (60 DAT) indicating susceptibility of these families (Table 4). Since none of the family has zero PDI, it was necessary to look for resistance source at individual plant level within each family (within progeny selection). Molecular screening through virus specific primers was undertaken to confirm resistance in the plants with zero score in field condition. Interestingly, fifteen out of twenty virus resistant plants (later confirmed in the study) belonged to first category of families where the PDI decreased during 60 and 120 DAT. Sharma et al. (2015) and Thakur et al (2019) also reported several mechanisms including RNA silencing conferring resistance response against ChiLCV.

Table 4: Change in PDI values among RILs families between 60 DAT and 120 DAT

S. No.	Nature of Change	No of families	% Change
1	PDI decreases	39	2.5-20
2	No Change in PDI	10	0
3	PDI increases	60	2.5-43.7

Molecular screening: Sixty-five individual plants exhibiting symptomless response under natural field condition (0 scores) at 60 DAT were subjected with the universal begomovirus primer (Rojas et al., 1993) and beta-satellite primer (Briddon et al., 2002) to examine the presence of begomovirus and associated beta-satellite DNA. PCR study revealed that out of a total 65 plants in 55 plants the presence of begomovirus and beta-satellite DNA were observed with either primer

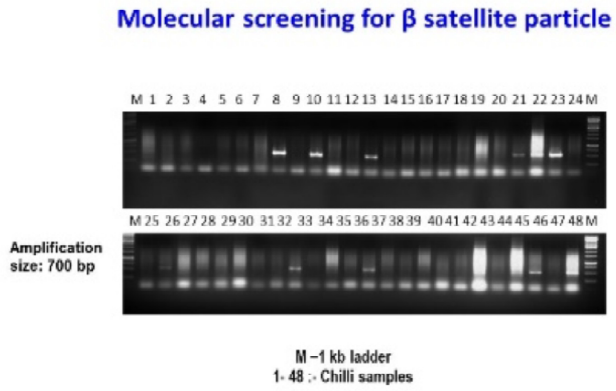

Fig 4. Molecular screening with (a) Universal Rojas primer (b) â Satellite Primer for coinfirmation of resistance.

(a) Molecular screening with Universal Primer
(b) Molecular screening with Beta Satellite Primer

Table 5: Performance of 20 virus free plants under field and molecular screening

Pedigree	FL	FD	NF	FW	PH	PDI-I	PDI-II	YLD	SCREEN-I	SCREEN-II	Screening for DNA-A particle	Screening for Beta satellite
IIVRC- GT-46-5-2-4-1	5.8	1.1	12.0	31.0	38.0	37.2 ^{abcdefghijk}	37.6^{ab}	11.0^{fghi}	0	0	-	-
IIVRC-GT-109-2-4-9-4	6.8	1.1	31.0	44.0	49.0	37.2 ^{abcdefghijk}	32.6^{ab}	47.8^{abcdefgh}	0	0	-	-
IIVRC- GT-113-3-3-3-3	4.9	1.3	34.0	38.0	29.0	34.7^{abcdefghijk}	27.6^{ab}	45.1^{abcdefgh}	0	0	-	-
IIVRC-GT-115-2-5-2-2	6.5	1.1	11.0	33.0	73.0	60.4^{abcdefghij}	53.7^{ab}	10.7^{fghi}	0	0	-	-
IIVRC-GT-137-3-3-3-4	6.8	1.3	27.0	55.0	58.0	33.0 ^{abcdefghijk}	37.6^{ab}	52.2^{abcdefgh}	0	0	-	-
IIVRC-GT-144-1-2-7-2	6.7	1.1	48.0	43.0	45.0	52.7 ^{abcdefghij}	52.4^{ab}	81.3^{abcdefgh}	0	0	-	-
IIVRC- GT-183-2-1-4-2	7.2	1.4	28.0	89.0	60.0	46.5 ^{abcdefghij}	39.9^{ab}	97.2^{abcdefgh}	0	0	-	-
IIVRC- GT-191-1-5-1-1	7.5	1.3	17.0	63.0	29.0	17.7 ^{jk}	27.4^{ab}	44.6^{abcdefgh}	0	0	-	-
IIVRC- GT-191-2-2-4-2	6.7	1.1	21.0	44.0	41.0	38.8 ^{abcdefghijk}	35.7^{ab}	39.1 ^{abcdefgh}	0	0	-	-
IIVRC- GT-197-1-8-4-1	5.4	1.1	28.0	35.0	45.0	30.8 ^{efghijkl}	23.8 ^{ab}	41.2^{abcdefgh}	0	0	-	-
IIVRC- GT-148-1-3-2-1	5.7	0.9	38.0	31.0	47.0	36.0 ^{bcdefghijk}	24.6^{ab}	48.5^{abcdefgh}	0	0	-	-
IIVRC- GT-156-3-2-2-3	8.0	1.0	30.0	54.0	49.0	45.2 ^{abcdefghijk}	52.4^{ab}	64.9^{abcdefgh}	0	0	-	-
IIVRC-GT-193-2-1-2-1	11.1	0.9	16.0	74.0	62.0	35.2 ^{bcdefghijk}	39.9^{ab}	48.8^{abcdefgh}	0	0	-	-
IIVRC-GT-193-2-4-4-2	5.9	0.7	34.0	18.0	49.0	40.2^{abcdefghijk}	34.9^{ab}	27.6^{abcdefgh}	0	0	-	-
IIVRC-GT-194-4-4-1-2	7.3	0.8	78.0	38.0	45.0	27.7 ^{fghijk}	27.4^{ab}	114.6 ^{abcef}	0	0	-	-
IIVRC-GT-237-2-3-2-2	9.9	1.7	38.0	86.0	60.0	37.4^{abcdefghijk}	44.9^{ab}	118.5^{adef}	0	0	-	-
IIVRC-GT-260-2-2-1-2	6.8	1.5	40.0	71.0	60.0	22.4 ^{ghijk}	32.4^{ab}	102.7^{abcdefg}	0	0	-	-
IIVRC-GT-275-2-2-2-2	4.3	0.6	68.0	28.0	65.0	30.7 ^{cdefghijk}	25.5^{ab}	68.1^{abcdefgh}	0	0	-	-
IIVRC-GT-124-6-2-2-6	6.8	1.1	48.0	46.0	54.0	30.3 ^{dfghijkl}	27.9^{ab}	53.5^{abcdefgh}	0	0	-	-
IIVRC-GT-126-3-2-2-3	4.6	1.3	38.0	38.0	32.0	42.2 ^{abcdefghijk}	35.1^{ab}	87.6^{abcdefgh}	0	0	-	-
BS 35 (P1)	2.2	0.6	77.3	6.7	75.3	13.6 ^{k}	13.0 ^{b}	13.3 ^{h}	0	0	-	-
Kashi Sinduri (P2)	12.9	1.5	17.6	86.0	51.6	64.3 ^{ab}	75.6 ^{a}	72.3^{abcdefg}	3	3	+	+
Pusa Jwala (Susceptible Check)	10.7	1.0	47.0	43.0	57.0	63.3 ^{abce}	73.6 ^{a}	66.3^{abcdefgh}	4	4	+	+

Two means with at least one letter common are not statistically significant;

FL= Fruit Length (cm), FD= Fruit Diameter (cm), NF= Number of Fruits per Plant, FW= 10 Fruits weight (g), PH= Plant Height (cm), PDI-I= PDI value at 60 DAT, PDI-II= PDI value at 120 DAT, YLD= Yield (q/ha), SCREEN-I= 1st Field Screening score (60 DAT), SCREEN-II= 2nd Field Screening score (120 DAT)

pairs. Whereas, - in 10 plants viz., IIVRC-GT-113-3-3-3-3, IIVRC-GT-46-5-2-4-1, IIVRC-GT-183-2-1-4-2, IIVRC-GT-191-1-5-1-1, IIVRC-GT-197-1-8-4-1, IIVRC-GT-191-2-2-4-2, IIVRC-GT-109-2-4-9-4, IIVRC-GT-144-1-2-7-2, IIVRC-GT-137-3-3-3-4, IIVRC-GT- 115-2-5-2-2 (Table 5) were found free from both viral DNA and associated betasatellite DNA(Fig 3). Similarly, ninety-five individual plants showing symptomless in field condition (0 scores) at 120 DAT were screened with the help of universal begomovirus primer (Rojas et al., 1993) and betasatellite primer (Briddon et al. 2002). However, among the selected symptomless ninety-five plants, only ten plants were found free from viral DNA and betasatellite DNA particle after the molecular screening with universal begomovirus primer (Rojas et al., 1993) and betasatellite primer (Briddon et al. 2002). Likewise, workers (Sinha et al. 2011 and Kumar et al. 2006) have reported the benefits of molecular markers to screen the germplasm for identification of resistance source in chilli.

Twenty virus-free plants identified in this study are being further confirmed for their resistance through grafting/ alternate grafting technique. Once their resistance is confirmed, they will be very useful in developing virus-free resistant chilli variety/hybrid. Further, this population will be advanced for mapping resistant genes and studying the inheritance pattern of gene(s) conferring resistance to chilli leaf curl virus disease.

lkjk'k

मिर्च की फसल में लगने वाली विनाशकारी रोग ''मिर्च पत्ती मरोड़ विषाणु'' के प्रति प्रतिरोधिता ज्ञात करने के लिए एफ–6 कुलों के कुल 109 पुनर्योजी वंशक्रम लाइनों (आरआईएल) को समाहित कर वर्तमान परीक्षण किया गया। समूह में उपज एवं रोग से सम्बन्धित घटकों के चरित्रीकरण से सार्थक विविधता पाई गयी। पौध रोपण के 120 दिनों उपरान्त, उपज के साथ–साथ प्रति पौध फलों की संख्या, फल भार एवं प्रतिशत रोग सूचकांक (पीडीआई) का प्रमुख घटक विश्लेषण से योगदान देने वाले मुख्य घटक पाये गये। कुल 20 विषाणु मुक्त पौधों की पहचान पौध रोपण के 60 एवं 120 दिनों उपरान्त प्रक्षेत्र एवं अण्विक छंटनी यूनिवर्सल प्राइमर की सहायता से किया गया। इन पौधों का उपयोग प्रजनन कार्यक्रमों एवं ''मिर्च पत्ती मरोड़ विषाणु'' रोग प्रतिरोधी प्रजातियों एवं संकरों के विकास में प्रयोग किया जा सकता है।

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