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POTATO APICAL LEAF CURL DISEASE SCALE FOR SCREENING OF ADVANCED POTATO HYBRIDS USING NOVEL AGRO-INOCULATION TECHNIQUE

Salej Sood[1*], A. Jeevalatha[2], Vinay Bhardwaj[1#], S. Sundaresha[1], Ashwani Kumar[1], G. Vanishree[1], Sanjeev Sharma[1] and SK Chakrabarti[1]

ABSTRACT: Potato apical leaf curl disease (PALCD) caused by ToLCNDV has emerged as a serious threat to potato cultivation in India. Host resistance to the virus is the potent strategy to curb the menace of this disease. Identification of potato germplasm free from ToLCNDV is a major challenge for healthy seed production and also for an effective resistance breeding programme. Novel and simple agro-inoculation of ToLCNDV was done to select resistant genotypes of potatoes. A set of 9 advanced hybrids and 4 control varieties were evaluated for resistance to ToLCNDV under controlled conditions at ICAR-Central Potato Research Institute, Shimla. Clear symptoms were visible in susceptible genotypes within 30 days after the first inoculation. Based on symptoms of the disease, a scale (0-5) was developed for ToLCNDV resistance screening. Phenotyping results showed the resistant reaction of SM/00-42, SM/00-120 and VMT5-1 to ToLCNDV. However, virus load quantification of inoculated plant samples revealed low virus load in LBY 18, VMT5-1 and SM/00-42. All the four control varieties showed moderately susceptible to susceptible reaction with Kufri Pukhraj showing the highest virus load among all genotypes. The hybrid, SM/00-42 was found to promising due to its resistant reaction and very low virus load. This can be effectively utilized in ToLCNDV resistance breeding or direct deployment after agronomic evaluation. Moreover, the scale developed in the present study could be used in preliminary screening for the disease.

Keywords: Potato hybrids, Screening, ToLCNDV- [potato], Potato apical leaf curl disease scale, Virus load.

INTRODUCTION

Potato has been supporting food security globally for over 400 years since its spread in new continents. It is a vital industrial crop for many nations including India however, there are many hurdles and challenges in potato cultivation. The crop is attacked by many diseases, which are widespread and affect crop growth and production. Viruses are of particular importance in the Indian context due to the cultivation of potatoes in warmer areas. The begomoviruses are the most destructive and are particularly important in this climate change scenario. Particularly, the recombinants of tomato leaf curl viruses are being reported to affect new hosts and cause severe economic losses (Ashwathappa *et al*., 2020; Hanamasagar *et al*., 2021; Venkataravanappa *et al*., 2021). Garg *et al*. (1996) reported a new virus disease in potatoes named potato apical leaf curl disease and the virus was earlier named as Potato Apical Leaf Curl Virus (Garg *et al*., 2001). This whitefly transmitted begomovirus of the geminiviridae family has unique twin particle morphology and bipartite genomes, i.e. DNA-A and DNA-B. Usharani *et al*. (2004) cloned the genomic components of this virus and based on the nucleotide sequence, identified it to be a strain of Tomato leaf curl New Delhi virus (ToLCNDV) in tomato and designated it as Tomato leaf curl New Delhi virus- potato (ToLCNDV - Pot). Symptoms appear as curling of apical leaves with distinct mosaic due to primary infection (Usharani *et al*., 2004) and in secondary infection, the entire plant shows severe leaf

[1]ICAR-Central Potato Research Institute, Shimla-171 001, Himachal Pradesh,
[2]ICAR-Indian Institute of Spices Research, Kozhikode- 673 012
[*]-salej1plp@gmail.com; [#]-vinaycpri@gmail.com

curling and stunting symptoms (Sohrab et al., 2013). Initially, the sporadic incidence of the disease was reported by Lakra (2003) at Hisar, Haryana, while the severe infection was later observed in Western UP and other areas of North India (Chandel *et al*., 2010; Saha *et al*., 2014). At present, it is one of the important viral diseases of potatoes as severe infection (40-100%) results in heavy yield losses up to 50% in susceptible varieties (Jeevalatha *et al*., 2017a; Kumar *et al*., 2021). Keeping the above in view, it is important to screen the potatoes for ToLCNDV incidence for healthy seed production and an effective resistance breeding program. In the present study, 9 advanced hybrids and 4 varieties as control were evaluated for resistance to ToLCNDV.

MATERIALS AND METHODS

Nine advanced multiple resistant hybrids and four varieties viz., LBY-18, SM/00-120, SM/08-12, VMT 5-1, SM/08-11, SM/00-42, SM/03-23, SM/09-161, SM/05-75, Kufri Jyoti, Kufri Pukhraj, Kufri Surya and Kufri Himalini were used in the present study. The advanced hybrids used in the present study have been developed from the hybridization of elite parental lines involving one of the parents with high resistance to late blight. The hybrids have many desirable tuber traits apart from good tuber yield & other agronomic traits.

Infection through agro-inoculation

The Agro-inoculation method was used to create disease in the potato hybrids by following previously standardized methods (Jeevalatha *et al*., 2017b). The procedure for inoculation involves gentle pricking of seed potato sprouts with fine 5ml syringe needles, followed by incubation in a mixture of agrobacterium cells carrying infectious clones of ToLCNDV isolate MOD-21 (DNA A and DNA B components in 1:1 ratio) for 4-5 hrs in a rotary shaker maintained at 28°C. The treated tubers were planted in medium-size plastic pots at ICAR-Central Potato Research Institute, Shimla in the year 2017. The 2-3 leaf stage plants were again twice inoculated with the same agrobacterium mixture through stem injection and leaf infiltration methods after 15 and 20 days of planting/first inoculation. The pots were kept in the glasshouse, where the temperature was congenial (>25°C) for the virus to multiply. For each genotype five plants were raised, which included three inoculated plants and two un-inoculated controls. The plants were observed for the appearance of ToLCNDV symptoms in the hybrids after 30 days of the first inoculation. The observations in each genotype were recorded in triplicate at 15 days intervals i.e. 30, 45 and 60 days after planting/first inoculation.

Viral load quantification

Leaf samples were collected randomly from the top and middle part of the plants at 30 days after planting/first inoculation and were immediately frozen in liquid nitrogen and stored at -80°C. Total genomic DNA was extracted from the leaf samples using the GenElute™ Plant Genomic DNA Miniprep kit (Sigma-Aldrich, Missouri, USA) following the manufacturer's instructions. The total DNA was quantified and diluted to 20 ng/µl before using in real-time PCR quantification. Real-time PCR assay was carried out to quantify the viral load using coat protein gene-specific primers (LCV-CPFP- 5′-ACCGTCGTCCTACAGGATCTC-3′ and LCV-CPRP-5′-GCTCGGTTCATTGTCAAACATGT-3′) and an increase in viral load was compared between control and inoculated plants as well as susceptible and resistant potato hybrids by plotting C_T values. Absolute quantification of the viral load was performed using serially diluted (10^1 to 10^9 copies) plasmid carrying coat protein gene of ToLCNDV. Leaf samples of three biological replicates were pooled and used in the real-time PCR assay. Three technical replicates were maintained for each sample.

ToLCNDV scale

The disease symptoms on plants were assessed categorically to propose a scale for ToLCNDV resistance screening.

RESULTS AND DISCUSSION

Apical leaf curl disease in potatoes is caused by a variant of ToLCNDV (ToLCNDV) and is one of the most important diseases of potatoes in India. Due to the planting of potato, tomato and cucurbits at the same time and exposure of early sown potato to high temperature, the higher population of whiteflies transmit the virus to potato from tomato and cucurbits (Saha *et al*., 2014; Sohrab *et al*., 2013). The disease is now widely spread in all potato-growing regions of India (Jeevalatha *et al*., 2012, 2013, 2017a; Saha *et al*., 2014). In Indo-Gangetic plains, higher disease incidence with 40–100% infection has been observed resulting in heavy yield losses in susceptible varieties (Venkatasalam *et al*., 2011). It is important to identify resistant hybrids for this virus to sustain potato productivity in the country.

The genotypes under evaluation showed variable reactions to ToLCNDV infection. Initially, the symptoms appeared as chlorotic spots on leaves with light yellowing in susceptible genotypes. Later on curling, crinkling of apical leaves with distinct mosaic symptoms were observed. In severe cases, whole plant leaf curling and stunting was seen. Similar symptoms have been reported earlier in susceptible cultivar Kufri Pukhraj through agro-inoculation (Jeevalatha *et al*., 2017b). To screen the genotypes, it was necessary to first devise a scale based on the symptoms on the plant canopy. We observed the symptoms on inoculated plants in comparison to control plants and also the highly susceptible control plants maintained under controlled conditions. Based on the symptoms recorded throughout the season, a 0-5 scale was devised for the categorization of plant reaction to virus infection (Fig.1). This is the first report of the

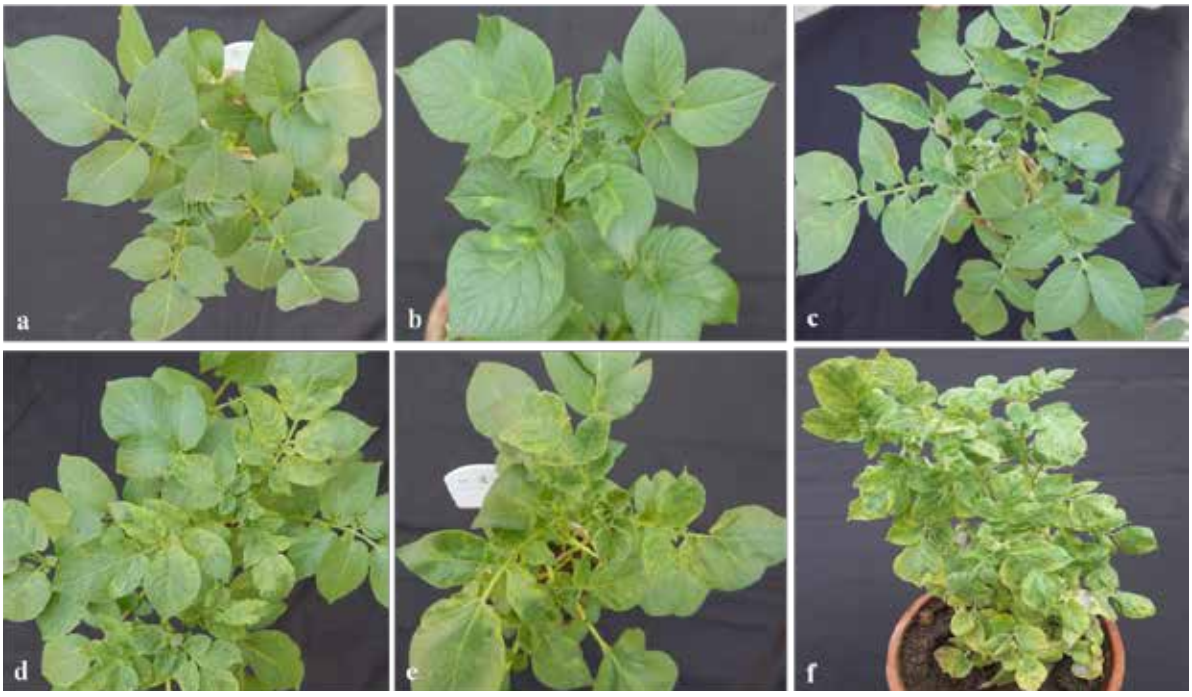


Fig. 1. Scale for apical leaf curl disease based on symptoms in potato plants

development of a scale for ToLCNDV resistance screening in potato genotypes. The symptoms and percentage of infection for classification have been provided in Fig. 1 & Table 1.

a) Highly resistant (Score 0) – No symptoms; b) Resistant (Score 1: 1- 10 % infection) - Yellow spots in few leaves; c) Moderately resistant (Score 2: 11- 25 % infection) - Mild mosaic in most of the leaves and small leaves d) Moderately susceptible (Score 3: 26-50 % infection) - Severe mosaic and crinkling e) Susceptible (Score 4: 51-75 % infection) - Severe mosaic, crinkling, small leaves & rosetting) Highly susceptible (Score 5: >75 % infection) - Severe mosaic, crinkling, small leaves & rosetting, shortening of internodal length (stunted growth).

On the 30th day after the first inoculation, no symptoms on advanced hybrids, SM/00-42, SM/00-120 (0 score) and yellow spots on a few leaves in VMT 5-1 (1 score) were observed. Advanced hybrid, SM/08-11 showed mild mosaic symptoms (2 scores), whereas remaining advanced hybrids and control varieties showed mosaic and puckering symptoms with a score of 3. At 45 days after the first inoculation, the score remained the same for advanced hybrids, SM/00-42, SM/00-120 and VMT 5-1, and there was no progression of symptoms indicating that these hybrids could resist the incidence of ToLCNDV. Hybrid, SM/08-11 showed mild mosaic symptoms, while the severity of symptoms increased in most of the advanced hybrids and control varieties. At 60 days after the first inoculation, yellow spots started appearing in SM/00-42 and SM/00-120 (1 score) also and the symptoms on SM/08-11 remained the same (mild mosaic).

The scale was used to screen the hybrids for their reaction to ToLCNDV. Among the nine hybrids, SM/00-42, SM/00-120 and VMT 5-1 showed a resistant reaction, SM/08-11 showed a moderately resistant reaction, while all others including control varieties were moderately susceptible to susceptible. None of the hybrids was highly resistant to ToLCNDV (Table 1). In an earlier study of field screening of potato genotypes at Hisar,

Table 1. Reaction of genotypes to ToLCNDV

S. No.	Genotype (s)	Symptoms	Mean score*	Reaction
	Hybrids			
1	LBY-18	Severe mosaic, puckering, small leaves, bunchyness	4.3	HS
2	SM/00-120	Few yellow spots	0.3	R
3	SM/08-12	Severe mosaic, small leaves and puckering	3.7	S
4	VMT 5-1	Few yellow spots	1.0	R
5	SM/08-11	Mild mosaic	2.0	MR
6	SM/00-42	Few yellow spots	0.3	R
7	SM/03-23	Sever mosaic, puckering, small leaves	3.7	S
8	SM/09-161	Severe mossaic	3.3	S
9	SM/05-75	Small leaves, bunchyness, mosaic	3.3	S
	Controls			
10	Kufri Jyoti	Severe mosaic, puckering	3.7	S
11	Kufri Pukhraj	Severe mosaic, puckering	3.7	S
12	Kufri Surya	Severe mosaic	3.0	MS
13	Kufri Himalini	Sever mosaic	3.0	MS

*Mean scores recorded after 30, 45 and 60 days after planting /first inoculation

eleven genotypes were found resistant while Kufri Pukhraj showed a highly susceptible reaction with a high white flies population (Mann *et al*., 2017). Several field studies conducted earlier under natural conditions for ToLCNDV resistance have resulted in the identification of resistant genotypes (Kumar and Gupta, 2015; Kumar *et al*., 2015; Lakhra, 2008), however, the plants were artificially inoculated and exposed to congenital conditions in the present study for evaluation and validation.

To support and validate the symptom expression in all genotypes the real-time PCR was performed to quantify the virus load. Among all the hybrids, VMT 5-1 (1.9×10^4), LBY 18 (3.7×10^4) and SM/00-42 (7.0×10^4) had the least virus load in inoculated plants. The highest virus load of $1.03 \times E^{12}$ was observed in Kufri Pukhraj while the other three controls varieties viz., Kufri Himalini, Kufri Surya and Kufri Jyoti showed values in the decreasing order i.e. $2.83\times E^{10}$, $2.56\times E^{10}$ and $8.15\times E^6$, respectively. Except for Kufri Jyoti, all other control varieties had higher virus load in comparison to advanced hybrids under evaluation. Three hybrids namely, SM/03-23, SM/09-161 and SM/08-12 recorded high virus load greater than $1.0\times E^{10}$ and their mean phenotype score reading was also >3.3. It reveals that these three hybrids do not have inherent resistance to stop the virus multiplication. In addition, the hybrids SM/08-11, SM/05-75 and SM/00-120 also observed lesser virus load in comparison to susceptible controls i.e. Kufri Pukhraj, Kufri Himalini, Kufri Surya and the three afore-mentioned advanced hybrids (Fig. 2). High viral load in susceptible variety, Kufri Pukhraj and a very low viral load in resistant cultivar, Kufri Bahar have been reported by Jeevalatha *et al*. (2017c). This indicates that although these three hybrids have the genetic resistance to restrict the virus multiplication but is not fully functional. The results observed through quantification using real-time PCR were almost similar to those of phenotypic evaluation based on disease symptoms in different genotypes. The only exception was LBY 18, where the symptoms were clear and showed high susceptibility but virus load was less in comparison to other susceptible genotypes.

Among the hybrids, SM/00-42 and VMT 5-1 showed resistance reaction both in the appearance of disease symptoms as well as quantification of virus load through real-time PCR of inoculated as well as uninoculated plants. Both of these advanced hybrids, SM/00-42 and VMT 5-1 appear ideal candidates for deployment in resistant breeding to ToLCNDV or as the direct release for cultivation based on other agronomic traits and adaptability traits. Quantification of virus load and disease scale corroborates the reliable selection of resistant genotypes. Moreover, the high similarity between symptom expression and virus quantification results indicate that the genetic resistance of potato genotypes can be easily identified using the proposed scale and the same can be effectively utilized for preliminary screening of hybrids for resistance to ToLCNDV in potato.

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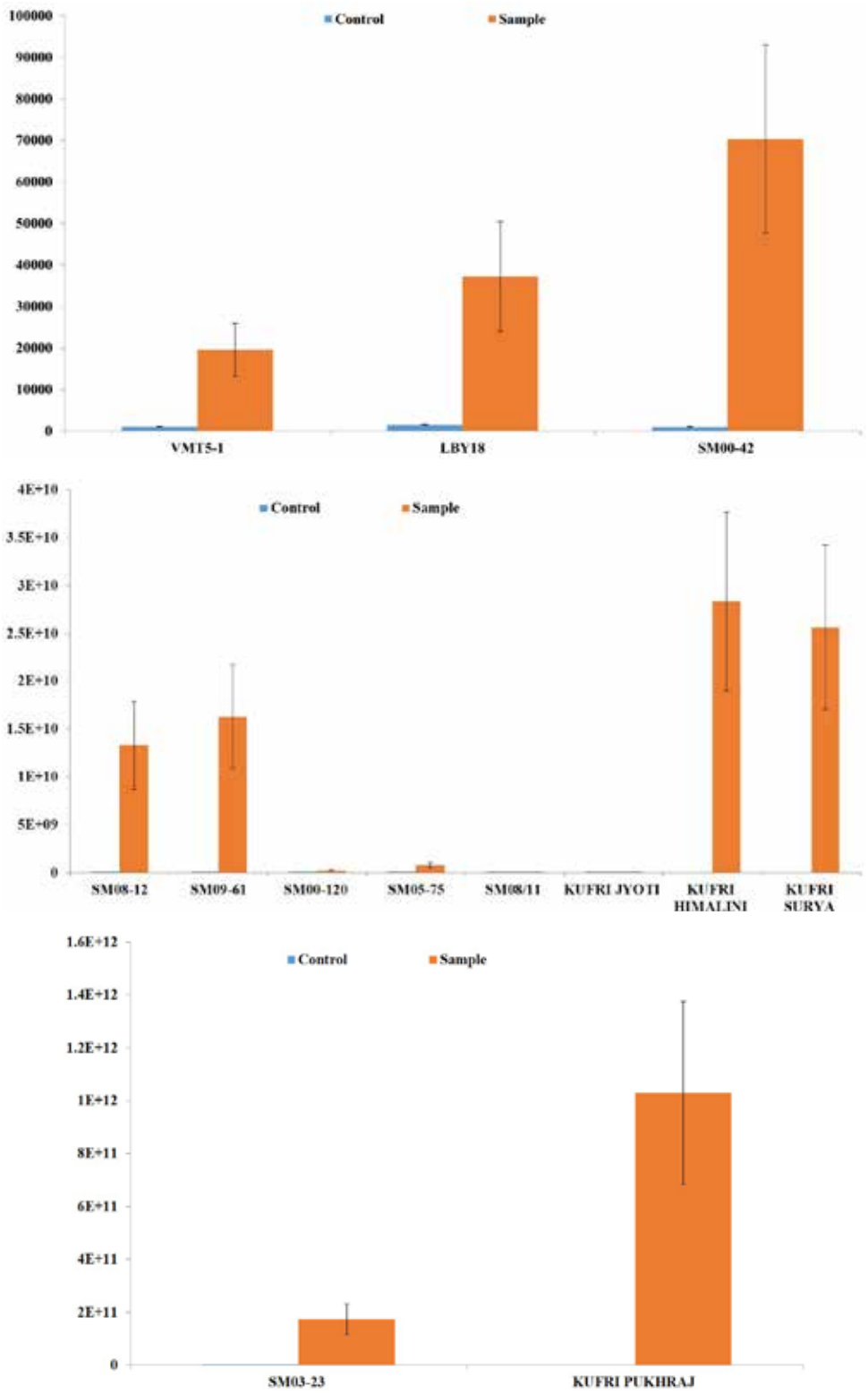


Fig 2. Virus load quantification through real-time PCR in control vs inoculated samples of hybrids.
a) low virus load genotypes; b) medium to high virus load genotypes; c) very high virus load genotypes

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