RESEARCH PAPER

Responses of wild *Vigna* species/sub-species to yellow mosaic disease viruses, detected by a PCR-based method

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Summary. Forty-eight accessions of wild *Vigna* species/sub-species were grown to verify their reactions to yellow mosaic disease (YMD), under field conditions in New Delhi (India) during 2012 and 2013. Symptoms of YMD that developed on wild *Vigna* were similar to those observed on cultivated species. Symptomatic plants produced few flowers and pods with reduced seed size. The infection coefficient was in the range of 0–71%. The causal virus was identified by PCR using species-specific primers to detect all the four viruses responsible for YMD in pulse crops. All the YMD-affected wild *Vigna* species/sub-species accessions were infected by *Mungbean yellow mosaic India virus* (MYMIV), with positive amplification of the targeted DNA fragment, except one accession of *V. hainiana* (IC331450) which was infected wild species/sub-species of *Vigna* at New Delhi. Eight accessions belonging to *V. synthetic* allotetraploid, *V. umbellata*, *V. mungo* var. *mungo*, *V. trilobata*, *V. trinervia* var. *bourneae*, *V. radiata* var. *sublobata* and *V. dalzelliana* were completely free from YMD and gave negative PCR results with primers specific to all the four viruses. This confirms resistance to YMD in these wild *Vigna* species.

Key words: Mungbean yellow mosaic virus, Mungbean yellow mosaic India virus.

Introduction

The genus *Vigna* is important among food legumes as a source of dietary proteins, minerals and vitamins, and is grown in many countries. This genus constitutes a large heterogeneous group of plants distributed among seven subgenera (Verdcourt, 1970; Marechal *et al.*, 1978), including *Vigna*, *Haydonia*, *Lasiospron*, *Sigmoidotropis*, *Plectotropis*, *Ceratotropis* and *Macrorhyncha*. The subgenus *Ceratotropis* has been differentiated as a homogeneous group with highly specialized and complex floral morphology (Marechal *et al.*, 1978; Tateishi and Ohashi, 1990). It includes 16–17 species distributed across Asia, out of which five (*Vigna radiata*, *V. mungo*, *V. aconitifolia*, *V. umbellata* and *V. angularis*) are cultivated in different parts of the world depending upon climatic conditions and nutritional importance of the plant products.

Diversity in legume crops and their wild relatives is abundant in India (Arora and Nayar, 1984). The major geographic areas in India of distribution of wild related taxa of legume crops are Western and Eastern Ghats, North eastern hills, North western plains, Peninsular region and Northern Himalayas, where the greatest number of species occurs, including V. bourneae, V. capensis, V. dalzelliana, V. khandalensis, V. grandis, V. hainiana, V. minima, V. mungo var. sylvestris, V. radiata var. sublobata, V. aconitifolia var. silvestris, V. radiata var. setulosa, V. trilobata and V. vexillata (Arora, 1988).

Crop wild relatives (CWR), which include the progenitors of crops and other species more or less

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closely related to them, offer a broad pool of potentially useful genetic resources. However, their contribution to the development of new cultivars depends upon: i) improved procedures for inter crossing species from different gene pools; ii) advances in molecular methods for managing backcrossing programs; iii) increased numbers of wild species accessions in the gene banks; and iv) extensive information available on beneficial traits associated with wild relatives (Hajjar and Hodgkin, 2007). Wild relatives in the primary and secondary gene pool are not difficult to cross, compared with the species belonging to the tertiary and quaternary gene pools; nevertheless, it is still possible to cross wild species from the tertiary gene pool (Rick and Chetelat, 1995; Brar and Kush, 1997; Mallikarjuna et al., 2006).

The extent of genetic variation and relationships among important pulse yielding Asiatic Vigna species, particularly V. mungo and V. radiata, with wild taxa such as V. radiata var. sublobata, V. radiata var. setulosa, V. mungo var. sylvestris and V. hainiana, as well as other cultivated taxa occurring in India, have been previously studied (Bisht et al., 2005a,b). Nevertheless, there are very few published reports on pathogens affecting wild Vigna species. Among biotic stresses of Vigna, yellow mosaic disease (YMD) has been emerging as the most widespread and destructive, causing very significant crop yield losses. This disease was first reported by Nariani (1960). The whitefly-transmitted bipartite begomoviruses Mungbean yellow mosaic India virus (MYMIV), Mungbean yellow mosaic virus (MYMV), Horsegram yellow mosaic virus (HgYMV) and Dolichus yellow mosaic virus (DoYMV) are four distinct etiological agents of this disease in legume crops in India and other South Asian countries (Pant et al., 2001; Kundagrami et al., 2009; Naimuddin et al., 2011a; Akhtar et al., 2013). Disease incidence and severity as high as 100% in farmers' fields is common in those areas, often resulting in considerable yield losses both in India (Pant et al., 2001) and in Pakistan (Hussain et al., 2004). Some previous reports have referred to resistant genotypes of V. radiata and V. mungo against YMD (Singh, 2004; Bisht et al., 2005a,b) without considering the etiological agent, but there are only a few reports pertaining to YMD viruses, especially on wild Vigna species (Naimuddin et al., 2011b). Since four distinct viruses are reported to cause YMD in legumes (Malathi and John, 2008), their identification in each infected plant becomes imperative to

determine strategies for conserving and utilizing the wild host taxa as a part of the biodiversity. Therefore, the present study was conducted on wild *Vigna* spp. with the aim of identifying genetic resources resistant to YMD and to the causal virus, which is a prerequisite for a breeding programme aimed at achieving effective management of YMD.

Materials and methods

In order to identify resistant sources against YMD and the causal virus, a field trial was conducted in randomized block design with plot size of 2.5×2.4 m² giving a row to row space 30 cm and plant to plant 10 cm, with three replications. Sowing was carried out on 10th June during the crop seasons of 2012 and 2013 at new area experimental farm of NBPGR, New Delhi, which is situated at 28°08' North latitude and 77°12' East longitude, at altitude of 229 m above mean sea level. This location represents the trans Gangetic Plain agro-climatic region of India. In this area the occurrence of YMD is consistent especially on various legume crops including soybean, greengram, blackgram and cowpea. The experiment contained 48 accessions of wild Vigna species/sub-species collected from different regions of India (Table 2), as well as Barabanki Local as a disease spreader and IPU02-43 as a resistant control. Each plot consisted of eight lines representing six different germplasm accessions and two checks (susceptible and resistant), making a total of 200 plants in each plot. In addition, two rows of susceptible check were also grown all around the experimental area. Since the virus spread is by insect vectors, the crop was not sprayed with pesticide. Disease was scored using the 0 to 9 scale of Mayee and Datar (1986) after 4 and 8 weeks of sowing. Disease severity was calculated and the data recorded were statistically analyzed using Co-variance of Randomized Block Design.

In order to identify the virus, total nucleic acid was extracted from leaves of representative plants of all the wild *Vigna* accessions, and from checks with or without characteristic yellow mosaic symptoms using a modified CTAB protocol. Leaf tissue from each plant (100 mg) was ground using a pre-chilled (-20°C) sterile mortar and pestle in 500 mL CTAB buffer (CTAB 2%; 100 mM Tris, pH 8.0; 20 mL EDTA; 1.4M NaCI) with 2% β -mercaptoethanol. Macerated samples were collected in sterilized 1.5 mL eppendorf tubes and incubated for 15 min at 65°C shaking

two to three times. An aliquot of 500 µL of chloroform + isoamyl alcohol (24:1) was added to each macerate and the mixture was centrifuged at 15,000 g for 10 min. The liquid phase was carefully transferred into a fresh 1.5 mL tube and remaining solution was again extracted as described above, with the liquid phase again removed and added to the extract. Sodium acetate (3M; pH 5.2) and ethanol, respectively 1/10 and 2.5 volume of liquid phase were mixed well and centrifuged at 15,000 g for 10 min. Each resulting pellet was washed with 70% ethanol, air dried and dissolved in 100 µL nuclease free water. Total nucleic acid was used as template to amplify the target DNA fragments using specific primer pairs (Table 1). Polymerase chain reaction was performed in a T1 Thermocycler (Biometra®) Eppendorf PRO programmed for one step of initial denaturation for 2.5 min and 35 cycles of denaturation at 94°C for 45 sec, annealing temperature as given in Table 1 for 1 min, and extension at 72°C for 1 min, followed by one step of final extension at 72°C for 10 min. PCR was carried

out in total reaction mixture volumes of 25 μ L that each contained 12.5 μ L of Dream *Taq* Green Master mix (Fermentas); 2 μ L of DNA template (total nucleic acid -50 ng μ L⁻¹), primers (50 mole μ L⁻¹) each and 8.5 μ L of distilled water. PCR products were subjected to electrophoresis in 1% agarose at 50 V for 45 min in Tris-acetate-EDTA buffer containing ethidium bromide 0.1%. Gels were viewed under a UV transilluminator, and photographed using a Sony Cybershot camera (model HS-3).

Healthy whiteflies (*Bemisia tabaci*) were multiplied on brinjal plants covered with insect proof net cages. The wild *Vigna* accessions found resistant under natural conditions were grown in plastic pots. Ten plants of each accession were maintained in two pots (five per pot) kept in a greenhouse, and these pots were covered with insect proof net cages. Healthy whiteflies were released on MYMIV-infected plants (cv. Barabanki Local) for acquisition access feeding for 24 h. Viruliferous whiteflies (20–30 per pot) were released for 48 h on to 15–20 d old plants of

Primer ID	Virus/component identified	Expected amplicon size	Annealing conditions		
NM1			F49C		
NM2	MYMIV/DINA A	~950bp	54 C		
MYMIV-MPF		1000bp	53°C		
MYMIV-MPR		~10000p	55 C		
MYMV-CPF	MYMV/DNA A	~1000bp	54°C		
MYMV-CPR	WITWIV / DINAA	-1000bp	54 C		
MYMV-MPF	MYMV/DNA B	~900hp	58°C		
MYMV-MPR		9000p	00 0		
HgYMV-CPF	H@YMV/DNA A	~900bp	56°C		
HgYMV-CPR	1191111, 21,111	Joop	50 C		
HgYMV-MPF	H9YMV/DNA B	~900bp	64°C		
HgYMV -MPR	1.91111,21112	Jooop			
DoYMV-CPF	DoYMV/DNA A	~900bp	54°C		
DoYMV-CPR	201111,210111	Jooop			
Beta01	β satellite DNA	1300bp	61°C		
Beta02	1	r			

Table 1. Details of the primers used for the detection of viruses causing yellow mosaic disease (Briddon *et al.*, 2002; Naimuddin and Akram, 2010). test accessions to inoculate with the virus (MYMIV). After inoculation feeding, the test plants were sprayed with the insecticide imidacloprid 17.8SL at 0.01% concentration, to kill the whiteflies. Plants were observed for 60 d for symptom development. One plant from each pot was randomly selected and used for PCR detection of MYMIV.

Results and discussion

Symptoms observed on the wild *Vigna* accessions were similar to the YMD visible in greengram and blackgram (Nene, 1972). The characteristic symptoms caused by the infection were yellow and golden mosaic in the leaves. Symptoms started as scattered small specks or yellow spots in the leaf laminae which enlarged to irregular yellow and green patches alternating with each other on mature leaves. The yellow areas increased, and coalesced resulting in complete yellowing of the leaves (Figure 1). Disease-affected plants produced fewer flowers and pods than healthy plants, with reduced pod and seed size. Though most of the germplasm accessions showed YMD symptoms, the disease severity differed ranging between 0–71% (Table 3). All the yellow mosaic-affected leaf

samples gave positive results with MYMIV specific primer pairs NMI/NM2 and MYMIV-MPF/MYMIV-MPR, and yielded amplifications of fragments of 950 bp (Figure 2A) and 900 bp respectively. This indicated that YMD in wild species/sub-species of Vigna grown at Delhi was caused by MYMIV. PCR results with primers specific to MYMV, HgYMV and DoYMV were negative. The accession IC331450 of V. haniana gave a negative result with primers specific to MYMIV, HgYMV and DoYMV but gave positive amplification with the primers specific to MYMV (Figure 2B). The accessions with no symptoms were also tested and most of them were either negative or showed very faint amplification of targeted DNA fragments with all the primers used for detection of viruses involved in YMD in pulses.

The results presented here show that *Vigna* accessions varied greatly in their reaction to YMD in both the years. The differences in disease severity between both the years were statistically not significant except that disease severity index was slightly greater during the year 2012 than 2013. Therefore, the data of both the years were pooled. Among 48 accessions, only eight were found free from YMD, indicating the presence of resistance. These lines



Figure 1. Wild *Vigna* species showing yellow mosaic disease symptoms on a) *Vigna trilobata*; b and c) *V. trinervia* var. *bourneae* and resistant reaction on d) *Vigna synthetic* allotetraploid; e) *V. trilobata*; and f) *V. mungo* var. *sylvestris*.



Figure 2. Gel photograph of some of the yellow mosaic disease samples of wild *Vigna* processed for MYMIV and MYMV detection with specific primers NM1/NM2 (A) and MYMV-CPF/MYMV-CPR (B), respectively. M=DNA ladder (1 kb), Lane 1=Positive control (DNA was extracted from symptomatic leaves, cv. Barabanki local), Lane 2=Negative control (No DNA was added in PCR mix), Lane 3=IC277019, Lane 4=IC337486, Lane 5=IC331450, Lane 6=IC372406, lane 7=IC583605, Lane 10=IC583666. Out of eight accessions seven showed positive reaction with MYMIV (A) and one with MYMV (B).

were: IC251454 (*V. synthetic* allotetraploid), IC251447 (*V. umbellata*), IC251387 and IC251383 (*V. mungo* var. *mungo*), IC331454 (*V. trilobata*), IC583655 (*V. trinervia* var. *bourneae*), IC583672 (*V. radiata* var. *sublobata*) and IC541388 (*V. dalzelliana*) (Table 2). One germplasm accession, IC251454, remained completely free from YMD, leaf crinkle and powdery mildew diseases, and it also had broader leaves, late maturity and remained green at the time of harvest (Figure 1 D).

Four viruses causing YMD in legume crops were targeted for detection in the above samples, and PCR-based positive identification of MYMIV and MYMV as causing YMD on wild accessions of *Vigna* was achieved.

Four species of begomoviruses *viz.*, MYMIV, MYMV, HgYMV and DoYMV, are reported to cause yellow mosaic disease in cultivated species of *Vigna* and are transmitted by the whitefly *Bemisia tabaci* (Malathi and John, 2008). MYMIV is distributed in northern, central and eastern India (Usharani *et al.*, 2004; Akhtar *et al.*, 2013), whereas, MYMV is dominant in southern parts of India (Karthikayan *et al.*, 2004). It is important to study the YMD status on wild *Vigna* species in natural habitats or ecosystems in the context of changing climatic situations, emergence of new pests/pathogens and incidence of new disease(s), especially for vector-borne pathogens. PCR-based reports on detection and identification of

Table 2. Vigna species assessed in this study for resistance to yellow mosaic disease, with information on where they were obtained.

Accession	Botanical name	Village	District	State	Latitude	Longitude
IC-247406	V. radiata var. sublobata	-	Trissur	Kerala	10.5166	76.2166
IC-250192	Vigna mungo	-	-	-	-	-
IC-250210	Vigna mungo	-	-	-	-	-
IC-250214	Vigna mungo	-	-	-	-	-
IC-250219	Vigna mungo	-	-	-	-	-
IC-250229	Vigna mungo	-	-	-	-	-
IC-250233	Vigna mungo	-	-	-	-	-
IC-250245	Vigna mungo	-	-	-	-	-
IC-250252	Vigna mungo	-	-	-	-	-
IC-251383	V. mungo var. mungo	-	-	-	-	-
IC-251385	V. mungo var. mungo	-	-	-	-	-
IC-251387	V. mungo var. mungo	Balurghat	Balurghat	W.B	-	-
IC-251447	V. umbellata (cultivated)	P.I.Div., IARI	New Delhi	New Delhi	-	-
IC-251454	V. synthetic allotetraploid	-	-	-	-	-
IC-277014	V. mungo var. sylvestris	Kahrusi	Pune	Maharashtra	18.5333	73.8666
IC-277019	V. radiata var. sublobata	Khandale ghat	Pune	Maharashtra	18.7500	73.3833
IC-277021	V. mungo var. sylvestris	Khopoli	Raigarh	Maharashtra	18.7833	73.3333
IC-277031	V. mungo var. sylvestris	Murud	Ratnagiri	Maharashtra	18.3258	72.9650
IC-281165	V. radiata var. sublobata	Rayiranellur Pattambi	Palghat	Kerala	-	-
IC-331436	V. trilobata	Jiban Deipur (Banpur)	Khurda	Orissa	20.1833	85.6166
IC-331450	V. hainiana	Jaypuriaguda	Malkangiri	Orissa	-	-
IC-331454	V. trilobata	Ghumia	Raipur	Chhatisgarh	21.2666	82.5166
IC-331456	V. trilobata	Sariah	Bilaspur	Chhatisgarh	21.9833	82.7333
IC-331610	V. trilobata	Pantnagar Univ. Farm	US Nagar	Uttaranchal	-	-
IC-485638	Vigna mungo	-	-	-	-	-
IC-530558	Vigna mungo	-	-	-	-	-
IC-337486	V. radiata var. sublobata	PWLS	Palghat	Kerala	10.3833	76.8166
IC-372406	V. trinervia var. bourneae	Thadipadam	Malapuram	Kerala	11.0666	76.0666
IC-539792	V. trinervia var. bourneae	Kundapura	Udupi	Karnataka	13.6333	74.7000
IC-539795	V. dalzelliana	Chiranecode	Udupi	Karnataka	13.3500	74.7500
IC-539798	V. mungo var. sylvestris	Bommanahalli	Uttar Kannad	Karnataka	15.3000	74.2666
IC-541388	V. dalzelliana	Janakpur, Rangat	Andaman	A & N Islands	12.5000	92.9000
IC-582740	V. trinervia var. bourneae	Upper sholayar	Coimbatore	Kerala	11.2700	76.8200

(Continued)

Table 2. (Continued).

Accession	Botanical name	Village	District	State	Latitude	Longitude
IC-583655	V. trinervia var. bourneae	Venginissery	Thrissur	Kerala	10.4700	76.1900
IC-583659	V. radiata var. sublobata	Konkad	Palakkad	Kerala	10.7900	76.0800
IC-583661	V. trinervia var. bourneae	Pannithadam	Thrissur	Kerala	10.6500	76.1200
IC-583662	V. dalzelliana	Ongalur, Shoranur	Palakkad	Kerala	10.7800	76.2700
IC-583664	V. radiata var. sublobata	Kaippuram	Palakkad	Kerala	10.8800	76.1400
IC-583665	V. trinervia var. bourneae	Kaippuram	Palakkad	Kerala	10.8800	76.1400
IC-583666	V. trinervia var. bourneae	Kottaram	Malappuram	Kerala	10.8800	76.0800
IC-583667	V. trinervia var. bourneae	koppam	Palakkad	Kerala	10.8700	76.2000
IC-583669	V. radiata var. sublobata	Ezhuvanthala Nellayi	Palakkad	Kerala	10.8600	76.2600
IC-583672	V. radiata var. sublobata	keezhoor	Palakkad	Kerala	10.8400	76.3500
IC-583673	V. trinervia var. bourneae	Villankunnu	Thrissur	Kerala	10.5600	76.1700
IC-583674	V. trinervia var. bourneae	Easwaramangalam, Ponnani	Malappuram	Kerala	10.8000	75.9600
IC-583683	V. trinervia var. bourneae	Iringuttiri	Malappuram	Kerala	11.1000	76.3300
IC-583698	V. dalzelliana	Shreekandapuram	Kannur	Kerala	12.0400	75.3400
IC-583700	V. dalzelliana	Edapazhassy	Kannur	Kerala	11.9000	75.4300

viruses causing YMD in wild species/sub species of *Vigna* are very few, except for *V. sylvestris* (Naimuddin *et al.*, 2009). Wild species are known to possess several traits/genes of interest for inducing variability in developing new lines, disease resistance and wider adaptation. Tanksley *et al.*, (1997) highlighted the genetic potential of wild species. It is therefore essential to identify precisely the causal virus of YMD on wild accessions. The results on various *Vigna* species revealed that most of the wild species were infected by the MYMIV prevailing in north India, but one species, *V. hainiana*, was infected by MYMV. This indicate that the virus prevalent in southern India has begun to appear in north India.

Resistant/highly resistant accessions which remained asymptomatic in the field were further tested by experimental inoculations with MYMIV through whiteflies. No visible yellow mosaic symptoms were observed on the plants tested except on the control plants (cv. Barabanki Local). The PCR test showed faint DNA bands indicating the presence of the virus in plants, but its multiplication was restricted very likely due to the resistant nature of these accessions. Further results indicated that there was variation for resistance to YMD within individual species. Different germplasm accessions belonging to same species responded differently to YMD indicating that selection for the resistant parent cannot be simply on the basis of species. Each individual genotype/accession must be examined to verify its susceptibility or resistance response.

In conclusion, experimental findings presented here indicate that the accessions of wild *Vigna* species which are resistant to YMD under natural epidemic outbreaks as well as under controlled conditions need to be maintained for further studies. These should include genetic manipulations and breeding aimed to develop useful resistance against MYMIV.

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Accession	Disease severity (%) ^a	Reaction	Virus			
			MYMIV	ΜΥΜν	HgYMV	DoYMV
IC-247406	37.0 (37.4)	S	+	-	-	-
IC-250192	54.3 (47.5)	S	+	-	-	-
IC-250210	31.7 (34.2)	S	+	-	-	-
IC-250214	61.7 (51.8)	S	+	-	-	-
IC-250219	36.7 (37.2)	S	+	-	-	-
IC-250229	70.0 (56.9)	S	+	-	-	-
IC-250233	47.0 (43.1)	S	+	-	-	-
IC-250245	48.3 (43.9)	S	+	-	-	-
IC-250252	46.7 (43.7)	S	+	-	-	-
IC-251383	0.0	R	-	-	-	-
IC-251385	53.3 (46.9)	S	+	-		
IC-251387	0.0	R	-	-	-	-
IC-251447	0.0	R	-	-	-	-
IC-251454	0.0	R	-	-	-	-
IC-277014	43.3 (41.1)	S	+	-	-	-
IC-277019	65.0 (53.7ª)	S	+	-	-	-
IC-277021	50.0	S	-	-	-	-
IC-277031	47.0 (43.3)	S	+	-	-	-
IC-281165	43.3 (41.1)	S	+	-	-	-
IC-331436	42.3 (40.6)	S	+	-	-	-
IC-331450	43.3 (41.1)	S	-	+	-	-
IC-331454	0.0	R	-	-	-	-
IC-331456	34.7 (36.1)	S	+	-	-	-
IC-331610	33.3 (35.2)	S	+	-	-	-
IC-337486	61.7 (51.8)	S	+	-	-	-
IC-372406	35.7 (35.2)	S	+	-	-	-
IC-485638	36.7 (37.2)	S	+	-	-	-
IC-530558	53.3 (46.9)	S	+	-	-	-
IC-539792	32.0 (34.4)	S	+	-	-	-
IC-539795	42.3 (40.6)	S	+	-	-	-
IC-539798	35.7 (35.2)	S	+	-	-	-
IC-541388	0.0	R	+	-	-	-

Table 3. Average disease severity and host reaction of wild *Vigna* species/sub-species accessions against yellow mosaic disease during crop seasons of 2012 and 2013, and PCR detection of viruses.

(Continued)

Table 3. (Continued).

Accession	Disease severity (%) ^a	Reaction	Virus			
Accession			ΜΥΜΙν	ΜΥΜν	HgYMV	DoYMV
IC-582740	35.0 (36.2)	S	+	-	-	-
IC-583655	0.0	R	-	-	-	-
IC-583659	33.3 (35.2)	S	+	-	-	-
IC-583661	33.3 (35.2)	S	+	-	-	-
IC-583662	47.0 (43.1)	S	+	-	-	-
IC-583664	33.3 (35.2)	S	+	-	-	-
IC-583665	33.3 (35.2)	S	+	-	-	-
IC-583666	33.3 (35.2)	S	+	-	-	-
IC-583667	33.3 (35.2)	S	+	-	-	-
IC-583669	37.7 (37.8)	S	+	-	-	-
IC-583672	0.0	R	+	-	-	-
IC-583673	47.0 (43.3)	S	+	-	-	-
IC-583674	35.0 (36.2)	S	+	-	-	-
IC-583683	36.7 (37.2)	S	+	-	-	-
IC-583698	32.0 (34.4)	S	+	-	-	-
IC-583700	35.0 (36.2)	S	-	-	-	-
Barabanki local (S check)	71.0 (57.4)	S	+	-	-	-
IPU 02-43 (R check)	0.0	R	-	-	-	-

^a Figures in parenthesis are angular transformed values; Positive sign indicates the presence of virus; S, susceptible; R, resistant.

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