LETTER TO THE EDITOR

MOLECULAR CHARACTERIZATION OF AN IRIS SEVERE MOSAIC VIRUS ISOLATE FROM INDIA

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Most vegetatively propagated crops, without rigorous selection for health, are becoming increasingly prone to viral infection. Iris (*Iris hollandica*) is a perennial herbaceous plant that yields beautiful flowers of various colors and types. It has been used both as an ornamental flower and a source of perfumes and drugs and is among the top ten flowers in the UK and USA. Iris is susceptible to a number of viruses including Iris mild mosaic virus (IMMV, the species *Iris mild mosaic virus*, the genus *Potyvirus*) and Bean yellow mosaic virus (BYMV, the species *Bean yellow mosaic virus*, the genus *Potyvirus*), Narcissus latent virus (NLV, the species *narcissus latent virus*, the genus *Macluravirus*), and Iris severe mosaic virus (ISMV, the species *Iris severe mosaic virus*, the genus *Potyvirus*, the family *Potyviridae*) (*1*). ISMV is serologically distinct from other iris-infecting potyviruses, such as IMMV and BYMV (*2*). This virus has been found to occur naturally in bulbous and rhizomatous iris and in *Crocus vernus*. In iris, ISMV causes distinct chlorotic stripes or mosaic patterns.

The detection of ISMV by ELISA and immuno-electron microscopy (IEM) has already been reported from different parts of the world (*2, 5*). Nucleotide sequence of ISMV coat protein (CP) gene is also available for The Dutch (*6*) and the Korean isolate (GeneBank Acc. No. AF034839). However, no systematic effort has been made to detect and characterize the Indian isolate of ISMV, which is very important in the light of strict quarantine rules imposed by several countries.

The present study was directed towards detection and characterization of the ISMV isolate infecting iris in India by bioassay, ELISA, RT-PCR and immunocapture RT-PCR (IC-RT-PCR). Results of the experiments were confirmed by sequencing of the PCR product. This study will be helpful for detection of the Indian isolate of the virus in iris and its complete characterization.

A number of iris plants were inoculated with the sap of ISMV-infected iris plants for host range and bioassay studies at 3–4 leaf stage in triplicate and were maintained at 20–25^{0}C for 3–4 weeks. The inoculated plants were observed for symptoms periodically and analyzed by ELISA for confirmation of bioassay results.

Two iris cultivars (Prof. Blaauw and Bluemagic) growing for several years in the Kangra Valley, H.P., India were

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Abbreviations: BYMV = Bean yellow mosaic virus; CP = coat protein; EM = electron microscopy; IC-RT-PCR = immunocapture RT-PCR; IEM = immuno-electron microscopy; IMMV = Iris mild mosaic virus; ISMV = Iris severe mosaic virus; NLV = Narcissus latent virus; IHBT Communication No. 0329

	ISMV Ind	ISMV Neth	ISMV USA	JYMV	NYSV	OYDV	PPV	PPA	SYSV	TurMV	WOYDV
ISMV Ind	X	99.310	98.504	65.906	64.387	64.478	65.638	62.931	64.234	64.907	64.181
ISMV Neth	98.958	X	99.084	66.193	63.623	64.909	65.782	62.947	63.228	63.034	62.513
ISMV USA	97.222	98.276	X	65.957	64.539	64.061	65.581	62.804	65.160	64.489	64.338
JYMV	70.513	67.677	70.638	X	71.757	67.853	74.828	67.175	65.423	70.292	66.430
NYSV	64.336	65.944	64.583	79.128	X	62.761	70.455	67.586	63.879	71.265	63.520
OYDV	71.127	72.113	69.792	70.358	70.450	X	66.479	65.351	71.126	65.945	71.835
PPV	69.328	69.444	69.038	73.021	74.793	73.077	X	68.724	65.287	71.100	67.370
PVA	60.351	65.363	60.627	69.497	69.729	66.738	63.100	X	65.875	70.353	62.832
SYSV	68.531	69.414	67.708	70.455	70.644	79.487	70.513	67.527	X	64.064	89.724
TurMV	70.124	66.667	71.186	75.298	77.662	72.138	70.325	70.190	71.861	X	64.307
WOYSV	67.483	68.519	66.667	70.455	70.783	78.248	72.121	65.653	97.898	73.333	X

ISMV Ind = ISMV Indian isolate, Acc. No. AJ549755.
ISMV Neth = ISMV Dutch isolate, Acc. No. X75939.
ISMV USA = ISMV Korean isolate, Acc. No. AF034839.
JYMV = Japanese yam mosaic virus, Acc. No. AB029404
NYSV = Narcissus yellow stripe virus, Acc. No. AJ311372.
OYDV = Onion yellow dwarf virus, Acc. No. AJ409311.
PPV = Plum pox virus, Acc. No. AF401296.
PVA = Potato virus A, Acc. No. AF543709.
SYSV = Shallot yellow stripe virus, Acc. No. AJ311370.
TurMV = Turnip mosaic virus, Acc. No. AF185963.
WOYSV = Welsh onion yellow stripe virus, Acc. No. AB000844.
For other abbreviations see their list at the front page.

screened for the presence of ISMV by ELISA with virus-specific antibodies (Adgen, USA) and by electron microscopy (EM) and IEM with the same antibodies as those used in ELISA according to already published protocols.

Total RNA was isolated using a RNeasy Plant Mini Kit (Qiagen, Germeny) and RT-PCR was performed using ISMV-specific primers ISMV P1 and P2 (GeneBank Acc No. AJ001234 and AJ001233) designed for the coat protein region. The RT reaction was carried out using Moloney murine leukemia virus reverse transcriptase (Amersham Biosciences) as described by the manufacturer. The amplification was done with Taq polymerase (Genei, India) initially for 10 cycles with a denaturation step at 94°C for 1min, an annealing step at 50°C for 2 mins, an elongation step at 72°C for 3 mins followed by 25 cycles of denaturation at 94°C for 1min, annealing at 55°C for 2 mins, and elongation at 72°C for 3 mins. Final elongation was done at 72°C for 10 mins. The IC-RT-PCR was performed as described (*3*). The sequencing was performed in an automated sequencer (ABI Prism, 310) with an universal primer using the Sanger's dideoxy chain termination method. The sequence was compared with known sequences of ISMV and other potyviruses.

None of the inoculated herbaceous host plants showed any symptoms even 3 weeks after inoculation. The virus could only be transmitted to healthy Iris plants in which it showed characteristic symptoms of the ISMV infection indicating that the virus has a very limited host range and infects only *Iris* spp. in accord with earlier reports (*1*) which showed that ISMV infects only *Iris* and *Crocus* spp.

Both the tested cvs. (Prof. Blaauw and Bluemagic) were found to be positive for ISMV. The cv. Bluemagic showed a more severe infection than the cv. Prof. Blaauw. Since ISMV is serologically distinct from other iris-infecting potyviruses, the reaction in ELISA was very specific with no cross-reaction. The detection of ISMV in iris has been reported earlier (*2, 5*).

EM examination of clarified virus concentrate prepared from infected iris leaf tissues showed the presence of flexuous virus particles of about 760 x 12 nm in size, typical of potyvirus infection (*4*). In addition, the IEM employing trapping, clumping and decoration with ISMV-specific antiserum indicated that the virus is an isolate of ISMV. The detection of ISMV using EM and IEM has also been reported earlier (*2*).

RT-PCR and IC-RT-PCR revealed an amplification product of about 870 bp using virus-specific primers and antibodies. Due to the IC step, IC-RT-PCR is considered more sensitive and specific than RT-PCR.

The nucleotide sequencing of the Indian isolate revealed a fragment of 869 bp, whose translation product was found to have 99% and 97% homology with the isolates from The Netherlands and Korea, respectively (the table). These results suggest that the Indian isolate under study is similar to the isolates mentioned above. However, its deduced amino acid sequence differed at two positions from the Dutch isolate and at five positions from the Korean isolate. Also, a three amino acid stretch (211–213) differed from the corresponding sequences of the two isolates. One asparagine (211) was missing and two amino acids (212–213) were replaced by similar amino acids, namely leucine by isoleucine (212) and threonine by tyrosine (213).

When compared to similar portion of CP of other potyviruses the Indian isolate of ISMV showed a 60–90% homology in the gene and a 60–97% homology in the deduced amino acid sequence (*1*).

The present study describes the effort to detect and to characterize the Indian isolate of ISMV by bioassay, ELISA, RT-PCR, IC-RT-PCR and nucleotide sequencing of the PCR product.

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