

# OCCURRENCE AND ALTERNATION OF CYTORHABDOVIRUSES ON WHEAT IN NORTHERN CHINA

## OCORRÊNCIA E ALTERNÂNCIA DE CYTORHABDOVIRUS EM TRIGO NO NORTE DA CHINA

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**ABSTRACT:** *Northern cereal mosaic cytorhabdovirus* (NCMV) and *Barley yellow striate mosaic cytorhabdovirus* (BYSMV) are two of the most important viral pathogens of wheat. Northern China is the main wheat-producing region in the country. Wheat growing regions pertaining to four provinces, located in northern China, were surveyed for occurrence of NCMV and BYSMV during the growing seasons of the years 2010 and 2016. Wheat leaf samples were collected randomly from symptomatic plants displaying stunting, chlorotic stripes or mosaic. Roughly 73 samples were collected in the year 2010 from 13 fields, and 154 samples were collected in 2016 from 41 fields. Samples were tested for the presence of NCMV or BYSMV using multiplex reverse transcription-polymerase chain reaction (mRT-PCR). The results suggested that BYSMV (49.32% in 2010, 82.47% in 2016) is gradually replacing NCMV (87.67% in 2010, 13.64% in 2016) and becoming the main cytorhabdovirus in different wheat growing regions in northern China.

**KEYWORDS:** Cytorhabdovirus. Wheat. mRT-PCR. Survey.

### INTRODUCTION

Wheat (*Triticum aestivum*) is the most widely grown food crop in the world. The production of wheat in China is the largest globally (<http://www.fao.org/faostat/en/#home>). Henan, Shandong, Hebei, and Shanxi provinces contribute to nearly 60% of the production in China.

More than 50 plant viruses are known to infect wheat in nature. Nearly 80% of these viruses are transmitted by vectors (insects or fungi) (WANG et al., 2010). Wheat viral diseases present a serious hazard that threatens wheat production globally. *Northern cereal mosaic cytorhabdovirus* (NCMV) is one of the most important viruses of wheat. NCMV occurs mainly in China, Japan and Korea (TORIYAMA, 1986). NCMV has economic implications in wheat by causing wheat rosette stunt disease (WRSD). This virus affected large areas of the Chinese provinces of Shandong and Hebei from 1965 to 1977 (CHUI et al., 1979). *Barley yellow striate mosaic cytorhabdovirus* (BYSMV) was first isolated from small brown planthoppers (SBPH, *Laodelphax striatellus*) in Italy (CONTI 1969), and has also been found in Australia, Africa, Asia and South America (GREBER 1982; LOCKHART et al., 1986; IZADPANAHA et al., 1991; MAKKOUK et al., 1996; DUMÓN et al., 2011). In Iran, the virus drastically affected the yield in wheat and millet plants (IZADPANAHA et al., 1991). In recent years,

this virus has been emerging in northern China, posing a threat to the security of wheat production (DI et al., 2016).

NCMV and BYSMV are members of the genus *Cytorhabdovirus* sharing high similarity in amino acid sequences with each other (YAN et al., 2015). Both viruses are transmitted by SBPH in a persistent manner (SHINKAI 1963; MILNE et al., 1986). They also share a similar host range with 13 species of Poaceae, including wheat, oat (*Avena sativa*), foxtail millet (*Setaria italica*), sorghum (*Sorghum bicolor*) and maize (*Zea mays*) (CONTI 1980; ZHANG et al., 1981; LIU et al., 1982; RUAN et al., 1982). Wheat plants infected by NCMV or BYSMV show typical stunting, chlorotic stripes or mosaic symptoms on their leaves. No obvious differences can be observed in early symptoms. Wheat is generally infected with one or both viruses in the field.

According to our previous investigations, NCMV was rarely detected in Hebei province; however, BYSMV could be detected at a high frequency (DI et al., 2016). In this study we tested field wheat samples with typical symptoms of virus disease collected from the years 2010 and 2016 in northern China. We contrasted the investigation results in order to clarify the occurrence and alternation of these two cytorhabdoviruses on wheat in northern China.

## MATERIAL AND METHODS

A survey of viral diseases affecting wheat was conducted during the growing season in 13 and 41 locations of northern China in 2010 and 2016, respectively. Leaf samples were taken from three to seventeen locations from each province (Hebei, Shandong, Henan and Shanxi), with a minimum of three samples per location. A total of 73 and 154 wheat plants were collected in 2010 and 2016, respectively. Wheat leaf samples were collected from symptomatic plants showing stunting, chlorotic stripes or mosaic patterns. The samples were labeled, brought to the laboratory, and were kept at -70°C until use.

Total RNAs were extracted from samples using the RNA isolation system (Cwbio, Beijing, China) according to the manufacturer's instruction. The presence of NCMV and BYSMV were tested using multiplex reverse transcription-polymerase

chain reaction (mRT-PCR) with specific primers (Table 1) synthesized by Sangon (Shanghai, China). The mRT-PCR reaction was performed in a 12.5 µl mixture containing 6.25 µl of 2× OneStep RT-PCR Buffer, 0.25 µl of HiFi-MMLV OneStep RT-PCR Enzyme Mix, 0.125 µl (0.1 µM) of each primer, 1 µl of total RNA template and 4.5 µl of RNase-free ddH<sub>2</sub>O. The mRT-PCR condition was set at one cycle at 45°C for 30 min and 95°C for 5 min, followed by 30 cycles of 94°C for 30 s, 54°C for 30 s, and 65°C for 1 min 20s, and a final extension step at 65°C for 10 min. The mRT-PCR products were electrophoresed on a 1.8% TBE agarose gel with Gold View (5 µl/100 mL) and observed under UV illumination.

The results of mRT-PCR were recorded and statistical differences among the mean values from the different sampling years and different viruses were analyzed using the statistical package SPSS version 19 (SPSS Inc., Chicago, IL, USA).

**Table 1.** Primers used in mRT-PCR for simultaneous detection of NCMV and BYSMV.

Primer	Nucleotide sequence (5'-3')	Expected size (bp)	Virus species	GenBank
NCMVF	AAACAGAGCTTCACGGAGACTTGG	509	NCMV	GU985153.1
NCMVR	AACACTAACCCCTCCCACGTCTGTA			
BYSMVF	TCCGCAGGTAGACGCCAAGAAG	254	BYSMV	FJ665628.1
BYSMVR	CGCAGTCCCAGTCAGAAAGGTG			

## RESULTS AND DISCUSSION

A total of 13 fields were surveyed in 2010, and 73 leaf samples were collected. As indicated in Table 2, the mRT-PCR results revealed that 64 out of the 73 samples (88%) contained NCMV, and 36 out of the 73 samples (49%) contained BYSMV.

A total of 41 fields were surveyed in 2016, and 154 leaf samples were collected. As indicated in Table 2, the mRT-PCR results revealed that 21 out of the 154 samples (14%) contained NCMV, and 127 out of 154 samples (82%) contained BYSMV. Henan and Shanxi provinces had the highest incidences of NCMV in 2010 (100%), but in 2016 the incidences declined to 57% (Henan) and 0% (Shanxi). In contrast, Henan and Shanxi provinces had the lowest incidences of BYSMV in 2010 (0%), but in 2016 these incidences increased to 54% (Henan) and 80% (Shanxi) (Table 2).

Between 2010 and 2016, the incidences of BYSMV in Hebei (100% in 2010, 90% in 2016) and Shandong (72% in 2010, 93% in 2016) provinces did not change significantly, but the incidences of NCMV were both dramatically decreased (61% to 5% in Hebei, 100% to 5% in Shandong; Table 2).

Statistically, we observed an extremely significant difference ( $P < 0.01$ ) in the rate of NCMV-infected between 2010 (88%) and 2016 (14%), indicating the reduction of this virus on wheat in northern China. There was a statistically significant difference ( $P < 0.05$ ) between the rates of BYSMV-infected (82%) and NCMV-infected (14%) in 2016, indicating a replacement of viral disease on wheat in northern China.

In the present study, the occurrence of NCMV and BYSMV on wheat in northern China was investigated. Symptoms of stunting, chlorotic stripes or mosaic patterns were observed in wheat plants in the surveyed areas in both 2010 and 2016. These symptoms were similar to those previously reported from virus-infected wheat plants in other countries (LEE et al., 1977; LOCKHART et al., 1986; IZADPANAH et al., 1991).

**Table 2.** Occurrence of NCMV and BYSMV on wheat plants collected from four provinces of northern China in 2010 and 2016.

Province	Sample Year	Number of locations	NCMV	BYSMV	Co-infections	NCMV incidence (%)	BYSMV incidence (%)
Hebei	2010	3	23/14*	23/23	14	60.87	100.00
	2016	17	61/3	61/55	1	4.92	90.16
Shandong	2010	4	18/18	18/13	13	100.00	72.22
	2016	7	40/2	40/37	0	5.00	92.50
Henan	2010	3	22/22	22/0	0	100.00	0.00
	2016	8	28/16	28/15	3	57.14	53.57
Shanxi	2010	3	10/10	10/0	0	100.00	0.00
	2016	9	25/0	25/20	0	0.00	80.00
<b>Total</b>	<b>2010</b>	<b>13</b>	<b>73/64</b>	<b>73/36</b>	<b>27</b>	<b>87.67</b>	<b>49.32</b>
	<b>2016</b>	<b>41</b>	<b>154/21</b>	<b>154/127</b>	<b>4</b>	<b>13.64</b>	<b>82.47</b>

\*: Number of plants tested / infected.

NCMV is one of the most important pathogens of wheat. In China, NCMV was first noted in the Hebei province in 1949 showing a sporadic occurrence. The first epiphytotic of this disease occurred in 1965 in Shandong province, and henceforth it had been frequently reported by several other districts located in the north and northwest parts of China (CHIU et al., 1979). In the 1980s, NCMV occurred in the Lingbei district of Inner Mongolia (YANG et al., 1987). Crop losses ranged from 30,000 to 40,000 tons per year. BYSMV was first reported in China during 2014 (DI et al., 2014). According to our investigation, the infection area of BYSMV gradually expanded (DI et al., 2016).

Previous studies showed that 335 out of the 367 (91%) wheat samples collected from the north of China in 2010 were infected by NCMV (DUAN et al., 2013). Similarly, in the present study, the average infection rate was 88% in 2010; however, the average infection rate of NCMV decreased to 14% in 2016; in Hebei, Shandong and Shanxi provinces the rate was less than or equal to 5%. Conversely, BYSMV showed an increase in the

average infection rate between 2010 and 2016. This indicates that BYSMV has replaced NCMV as the major virus impacting field wheat in northern China. However, the reason for this replacement is not very clear. This demands further study.

## CONCLUSION

The presence of NCMV and BYSMV in wheat plants of the studied area were confirmed by mRT-PCR methods. The results demonstrate that BYSMV is replacing NCMV as the main cytorhabdovirus in wheat growing areas of northern China.

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**RESUMO:** O cytorhabdovirus do mosaico do cereal do norte (NCMV) e o cytorhabdovirus do mosaico estriado amarelo da cevada (BYSMV) são dois dos mais importantes patógenos virais do trigo. O norte da China é a principal região produtora de trigo do país. As regiões produtoras de trigo pertencentes a quatro províncias do norte da China foram pesquisadas quanto à ocorrência de NCMV e BYSMV durante as safras dos anos de 2010 e 2016. Amostras de folhas de trigo foram coletadas aleatoriamente de plantas sintomáticas, exibindo listras ou mosaico clorótico com baixo crescimento. Cerca de 73 amostras foram coletadas no ano de 2010 a partir de 13 campos, e 154 amostras foram coletadas em 2016 de 41 campos. As amostras foram testadas quanto à presença de NCMV ou BYSMV usando reação em cadeia da polimerase

de transcrição reversa multiplex (mRT-PCR). Os resultados sugerem que o BYSMV (49,32% em 2010, 82,47% em 2016) está gradualmente substituindo o NCMV (87,67% em 2010, 13,64% em 2016) e se tornando o principal cytorhabdovirus em diferentes regiões produtoras de trigo no norte da China.

**PALAVRAS-CHAVE:** Cytorhabdovirus. Trigo. mRT-PCR. Pesquisa.

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