First Report of Chinese Wheat Mosaic Virus that Infects Barley in Japan

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Chinese wheat mosaic virus (CWMV), a member of the genus *Furovirus* in the family *Virgaviridae* (Adams et al. 2017), has a positive-sense RNA genome and is transmitted by *Polymyxa graminis*. CWMV is a causal agent of yellow mosaic disease in winter wheat in China (Guo et al. 2019). CWMV has also been detected in wheat plants in limited areas of northern Japan (Nagano and Iwate Prefectures) (Fuji et al. 2022; Maejima et al. 2010; Shirako and Maejima 2008). In preliminary tests using Western blotting with an antiserum raised against CWMV capsid protein (by Dr. Y. Shirako, Tokyo University), we detected a furovirus in a breeding line of barley ('Tozan Kawa 111' [*Hordeum vulgare* L.], collected in April 2012) grown in an experimental field infested with CWMV and wheat yellow mosaic virus (genus *Bymovirus*) in Nagano Prefecture. To investigate the infection of barley plants with cereal plant-associated soil-borne viruses, we collected leaf samples of Tozan Kawa 111 plants (10 plants) showing yellow mosaic symptoms but with no apparent wilting or stunting in April 2016 (2015/16 growing season). Total RNA was extracted from symptomatic leaf samples with TaKaRa RNAiso reagent (TaKaRa Bio) and subjected to reverse transcription polymerase chain reaction (RT-PCR) to detect virus agents. After cDNA synthesis using Moloney murine leukemia virus reverse transcription (Thermo Fisher Scientific) with random hexamers, PCR amplification with QuickTaq

HS Dye Mix (Toyobo Co.) was conducted using primer sets specific to two furoviruses and three bymoviruses known to infect wheat and barley plants in Japan (Tamada and Kondo 2013). RT-PCR analysis detected infection with CWMV in the leaf samples of Tozan Kawa 111 plants, but not with the other soil-borne viruses tested. The amplified PCR products (752 and 718 bp for CWMV RNA1 and RNA2, respectively) were purified by Wizard SV Gel and PCR Clean-Up System (Promega) and subjected to Sanger sequencing to confirm their nucleotide sequences. The virus sequences from PCR amplicons were deposited in GenBank/DBJ/ENA with accession numbers LC657081 (RNA1) and LC657082 (RNA2). Nucleotide Basic Local Alignment Search Tool (BLASTn) analysis showed that the sequences have 98.7 and 98.8% nucleotide sequence identity with RNA1 of CWMV Japanese northern isolate (accession no. AB299271) and RNA2 of CWMV Nagano-A isolate (AB935554), respectively. Identical sequences were also found in symptomatic wheat leaf samples (cultivar Fukuho Komugi) obtained from the same field. Rod-shaped particles were observed by transmission electron microscopy (Hitachi H-7650) in symptomatic Tozan Kawa 111 leaf samples. Using reverse transcription loop-mediated isothermal amplification (Fukuta et al. 2013), CWMV was detected in Tozan Kawa 111 plants in the same field in the 2015/16 to 2017/18 growing seasons but not in the 2018/19 to 2020/21 growing seasons. In the 2015/16 to 2017/18 growing seasons, CWMV was detected in the barley plants (pooled five plant samples) cultivar Kashimamugi, which showed similar yellow mosaic symptoms but not in most of the other barley variants planted in the field. To our knowledge, this is the first report of CWMV field infection in plants other than wheat (Kühne 2009). Further extensive virus screening in fields and virus inoculation experiments are necessary to understand the pathology of CWMV in barley and possibly in other cereal crops.

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