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Distribution and Isolation of Soil borne Wheat Mosaic Virus in Korea

Kui-Jae Lee, Hyun-Suk Lim, Hyung-Moo Kim, Wang Hyu Lee
Faculty of Biological Resources Science, College of Agriculture, Chonbuk National University, Chonju 561-756, Korea

ABSTRACT

This study was conducted to investigate the occurrence of Soil borne wheat mosaic virus(SbWMV) in barley fields in Korea and to examine the host pathogenicity of SbWMV. By using the ELISA test, SbWMV was detected in the six regions : Suwon, Milyang, Jinju, Youngkwang, Iksan, and Chonju. SbWMV was isolated from the two strains, Albori strain from Jinju and Eunpamil strain from Milyang. SbWMV was collected from leaves showing mosaic, yellowing and necrosis stripes. SbWMV was inoculated mechanically on 1～1.5 leaf stages with leaf-rubbing to identify the host pathogenicity of 36 Korean barley cultivars, a wheat cultivar, two rye cultivars, three Japanese barley cultivars and *Chenopodium amaranticola.* Viral sympoms of inoculated leaves appeared on inoulted leaves about 4 to 6 weeks of inoculation. Baegdong and Tapgolbori, infected from Albori strain and Eunpamil strain infected from Samdobori showed much higher susceptibility than *C. amaranticola* and *C. quinoa* which showed ring spots and chlorotic spots respectively. Virus particles were observed by the electron microscope. They were rod-shapes, which are bipartite, of 142 nm or 281 nm in length with 20 nm diameter on infected leaves. Specific detection and identification of SbWMV was set up using the RT-PCR. PCR fragments of SbWMV(0.5kb) were obtained by using the designed primers for SbWMV RNA 2.

Key Words : SbWMV, soil borne virus, pathogenicity, wheat, barley, RT-PCR.

INTRODUCTION

Virus diseases with various isolates occur in all barley fields in Korea. Virus diseases in barley are transmitted by and classified in terms of soil, insect, and seed infection. There have been reports on viruses for barley as a host plant in Korea, such as northern cereal mosaic virus (NCMV), barley yellow mosaic virus (BaYMV), barley mild mosaic virus (BaMMV), soilborne wheat mosaic virus (SbWMV), barley stripe mosaic virus, barley yellow dwarf virus (BYDV), and wheat spindle strike mosaic virus (WSSMV) (Lee, 1981 ; So *et al.*, 1997, 1998). The properties of BaYMV and BaMMV are filamentous particles of bipartite. They are 250-300 nm and 500-600 nm in length respectively, and 13 nm in diameter (Huth *et al.*, 1984 ; Lee *et al*, 1998 ; So *et al.*, 1998). These are ssRNA viruses, RNA 1 with 7.6kb and RNA 2 with 3.5kb in length (Kashiwazaki, 1996, Shirako and Brakke, 1984), and are classified as

Corresponding author : **Kui Jae Lee,** Tel: +82- 063-2702525, E-mail: kuijael@moak.chonbuk.ac.kr

bymovirus by potyviridae (Barnett, 1991). Meanwhile, soilborne wheat mosaic viruses are two virus particles (Shirako, and Brakke, 1984) with 142 nm and 281 nm in length, and of 20 nm in diameter (Brakke and Hsu, 1985, Tsuzuzaki *et al.*, 1973). In addition, SbWMVs are rod-shaped particles and belong to the furovirus group. These viruses are transmitted by the soilborne fungus, *Polymyxa graminis* (Rao & Brakke, 1969). *P. graminis* survives many years in the soil, therefore it is difficult to control the SbWMV at once. Diseased symptoms become milder and tend to disappear as the weather becomes warmer. BaYMV was first reported by Lee (1981) in Korea and occurred in barley field in Chonnam, Chonbuk, Kyungnam, and Kyungbuk provinces (So *et al*, 1998). On the other hand, there are reports on BaMMV strains, such as BaMMV (Huth & Adams, 1990) from Germany, BaMMV-MK from England, BaMMV-Nal, -Kal from Japan and BaMMV-Kor (So *et al.* 1997) from Korea. After confirmation of the virus gene structure (Kashiwazaki, 1996), the reverse transcription polymerase chain reaction (RT-PCR) is used increasingly for the identification and classification of viruses. As SbWMV is occurring in barley and wheat fields, this study was carried out to establish the isolation of SbWMV and to test host pathogenicity with inoculation and diagnosis by RT-PCR.

MATERIALS AND METHODS

SbWMV survey and host plant

SbWMV was investigated in barley fields as the following 11 areas : Suwon, Yaesan, Youngkwang, Naju, Kochang, Jinju, Milyang, Chonju, Hwangdeung, Youngam, and Iksan. The experiment was Conducted from February 1999 to May 2000. A total of 36 Korean barley cultivars were used as follows : 15 covered barley cultivars, 14 naked barley cultivars and seven malting barley cultivars from National Honam Agriculture Experiment Station. In addition, a Korean wheat cultivar, two Korean rye cultivars and three Japanese barley cultivars were used. *C. amaranticola* and *C. quinoa* were used to obtain pure SbWMV.

Virus isolation and sap inoculation test

Virus infected leaves were obtained from the selected plants of Albori in Jinju area and Eunpamil in Milyang area. They showed symptoms of mosaic, yellowing, necrosis and short or long yellow stripes. In all experiments, 10～15 seeds of both barley and wheat cultivar were sown in small pots and 5～7 seedlings were established. Inoculum sap was prepared in 0.1M phosphate buffer, pH 7.0, containing 1mM KCN by adding 0.3g of infected leaves and celite as an abrasive. Host plants were inoculated by leaf-rubbing with the inoculum sap at 1.5～2 leaf stages. After inoculation, the inoculated plants were sprayed with distilled water and were grown in a growth chamber which maintained the temperature at 13～15℃.

ELISA test

The ELISA test was performed according to Clark and Adams' method (Clark and Adams, 1977). ELISA samples were collected from leaves of the infected plants showing obvious virus symptoms in the 11 areas. Leaf samples (0.3g) were ground in 3㎖ of 0.1M PBS-T which contains the SbWMV antisera obtained from Japan National Agricultural Research Center.

Electron microscopic observation

The Dip method (Hitchborn and Hills, 1965) was used to measure the size of SbWMV particles. Briefly, diseased leaves were cut into 3㎜ sections and a drop of 2% phospho-tungstic acid was added. Ground leaves were soaked in a 200-mesh grid covered with formvar film for 3 seconds, and dried for 1 min after blotting PTA-liquid remaining on grid. Virus particles were reacted by using Protein-A-gold and observed by

electron microscopy.

RT-PCR

Total RNA was extracted from infected leaves of Albori and Eunpamil. Extracted total RNA 1 μl was reacted 20pmol reverse primer (HS-4 : 5'-CGAAAGTCTTAGTAAGATAT-3', HS-2 : 5'-CTCGAACCTTCCCATTTCAA-3') at 65℃ for 10min, and then reverse trnascription was carried out at 42℃ for 30min in was 5 x Expand RT buffer, 0.1M DTT, 5mM dNTPs, 10 unit Expand RT, 40unit RNase inhibitor and synthesized the first strand cDNA. The PCR mixture contained 10 x Expand long-term buffer 3, 2.5mM $MgCl_2$, 5mM dNTPs, Expand long-term enzyme, Expand first cDNA, 20pmol of reverse primer and 20pmol of forward primer (HS-3: 5'-AACGCGGCACACATAGTTTT-3', HS-1 : 5'-TAAATAAAGGTTTACACTGG-3'). The reaction was carried for 30sec at 90℃ for 1 cycle and was denatured for 30sec at 90℃, annealed for 45sec at 55℃and 45sec at 70℃ for total 40 cycles and then extended for 3min at 70℃.

Fig. 1. Map showing the survey sites in Korea.

Table 1. Occurrence of three fungus-transmitted viruses in barley at 11 regions in Korea

Location	ELISA detection		
	SbWMV	BaYMV	BaMMV
Suwon	+*	+	+
Yaesan	−	+	+
Hwangdung	−	+	+
Iksan	+	+	+
Chonju	+	+	+
Kochang	−	+	+
Yeongkwang	+	+	+
Naju	−	+	+
Yeongam	−	+	+
Jinju	+	+	+
Milyang	+	+	+

* Detected (+) and not detected (-) by ELISA.

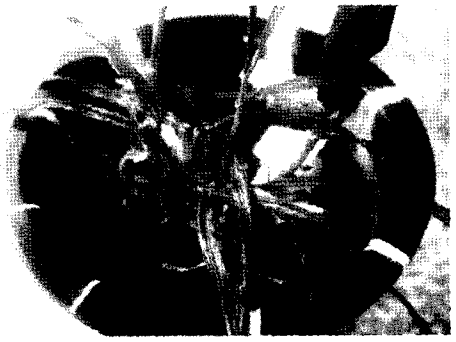

Fig. 2. Symptoms on barley cv. Albori after 6 weeks of artificial inoculation with soilborne wheat mosaic virus.

Fig. 3. Symptoms on *Chenopodium amaranticola* after 3 weeks of artificial inoculation with soilborne wheat mosaic virus.

Table 2. Pathogeneicity of SbWMV strain of Albori and Eunpamil on various host plants

Host plant	Inoculation Source		Host plant	Inoculation Source		Host plant	Inoculation Source	
	A*	E**		A*	E**		A*	E**
Covered barley cultivar			**Naked barley culativar**			Doosan 29	2/6	1/11
Albori	39/109***	7/46	Baegdong	43/94	11/48	Jejubori	2/3	1/1
Alchanbori	2/6	5/35	Chalssalbori	2/7	1/11	Jinkwangbori	0/3	1/4
Daebaegbori	5/6	3/6	Chunchussalbori	2/6	0/25	Jinyangbori	4/7	2/7
Gangbori	4/34	2/7	Duwonchapssalbori	2/5	1/19	Namhyangbori	0/6	0/6
Keunalbori	6/6	3/6	Ganghossalbori	4/5	2/5	Sacheon 6	2/6	3/3
Milyangketbori	3/19	0/4	Kwanghwalaalbori	3/26	5/37	Samdobori	1/6	2/6
Miragbori	2/6	3/5	Hinchalssalbori	5/14	3/7	**Japanese barley cultivar**		
Nakyeongbori	4/5	3/5	Hinssalbori	3/3	2/5	Ishukushirazu	23/88	7/44
Olbori	3/32	0/7	Mudeungssalbori	0/5	2/6	Mokkseiko	8/26	4/5
Oweolbori	4/7	0/22	Naehanssalbori	5/5	2/3	New golden	11/29	8/28
Saealbori	2/6	2/7	Nuelssalbori	5/15	3/4	**Wheat cultivar**		
Saegangbori	4/7	3/7	Olssalbori	1/14	2/6	Eunpamil	0/4	0/4
Saeolboi	2/24	0/7	Saeaalbori	2/10	0/15	Rye cultivar		
Seodunchalbori	6/7	3/7	Songhagbori	2/6	3/6	Chilbohomil	0/26	2/24
Tapgolbori	2/6	2/7	**Malted barley cultivar**			Chochunhomil	0/31	0/52

*A : Albori isolate, **E : Eunpamil isolate.

***Total number of plants with symptoms/ inoculation from experiments.

RESULTS

Occurrence of SbWMV

Occurrence of SbWMV in Korean barley fields was investigated in 11 areas (Table 1). ELASA positive (+) reactions were detected in only six areas Suwon, Jinju, Milyang, Chonju, Youngkwang, and Iksan by using the serological test (Fig. 1). However, BaYMVs and BaMMVs were positively detected in all 11 areas.

Host pathogenicity on the inoculation test

Different levels of host pathogenicity for SbWMV strains were observed by the inoculation test after 4 weeks of inoculation. Initial symptoms of light green mosaic appeared in inoculated leaves, and more severe symptoms appeared in newly emerging leaves. After 4 to 6 weeks of inoculation, symptoms showed narrow, short or slightly long yellow stripes and necrosis (Fig. 2). With Albori strain inoculum, virus did not infect Eunpamil, Chochunhomil, and Chilbohomil. In covered barley cultivars, Albori, Daebaegbori, Keunalbori, Nagyeongbori, Seodunchalbori and in naked barley cultivars, Baegdong, Ganghossalbori, Hinssalbori, Naehanssalbori and Neulssalbori had higher susceptibility. And the exception of these barley cultivars and Jejubori of malting barley had generally low susceptibility but Mudeungssalbori in naked barley and Namhyangbori in malting barley had resistance (Table 2). With Eunpamil strain inoculum, Daebaegbori, Miragbori and Nagyeongbori had high susceptibility. While in, Alchanbori, Gangbori, Keunalbori, Saealbori, Saegangbori, Seodunchalbori and Tapgolbori had low susceptibility in covered barley. In naked barley cultivars, Baegdong, Hinchalssalbori, Naehanssalbori, Neulssalbori, and Songhagbori had higher susceptibility, and

Chalssalbori, Duwonchapssalbori, Ganghossalbori, Kwanghwalssalbori, Hinssalbori, Mudeungssalbori and Olssalbori had lower susceptibility. But, Milyangketbori, Olbori and Saealbori of covered barley, Chunchussalbori and Saessalbori of naked barley and Namhyangbori in malting barley had resistance to virus strains. Sacheon 6 and Doosan 29 had higher susceptibility, but Eunpamil, Chilbohomil and Chochunhomil were not infected. Eunpamil strain had low susceptibility like Albori strain inoculation. Meanwhile, Japanese cultivars had susceptibility to both Albori strain and Eunpamil strain. *C. amarantivolar* and *C. quinoa* were inoculated to obtain pure-isolated SbWMV. *C. amaranticilar* showed ring spot and *C. quinoa* showed chlorotic spot (Fig. 3).

Electron microscopic Observation

SbWMV particles were observed in diseased leaves of Baegdong barley cultivar infected with Albori strain. The SbWMV particles were rod-shaped, 142 nm and 281 nm in length and 20 nm in diameter (Fig. 4). The Protein-A-gold method to observe virus paraticle revealed that many gold particles attached around SbWMVs (Fig. 5).

DISCUSSION

ELISA test, was used to detect SbWMV in six previously wheat and barley regions, Suwon, Milyang, Jinju, Iksan, Chonju, and Youngkwang. Lee (1981) found SbWMV only present result in Iksan area of Chonbuk province, while the revealed that SbWMV was developed in six regions. Using *C. amaranticolar*, pure-isolation and propagation were performed and aly found that SbWMV particles were rod-shaped ones of 142 nm and 281 nm in length and 20 nm in diameter. These corresponde with the findings of Sherwood *et al.* (1990). After 4 weeks of inoculation, initial symptoms showed lighter green mosaic than original leaves, and with the lapse of 4~6 weeks, symptoms showed short or long yellow stripes, stunt, necrosis with mosaic. Meanwhile, according to Usigi and Saito 1970) results, when Albori isolate inoculated on *C. amaranticolar* and *C. quinoa*, ring spots and chlorotic spots were observed. Albori and Eunpamil strain were inoculated onto covered barley, naked barley, malt barley, wheat, rye and Japanese cultivars to identify the host-pathogenecity. As a result, pathogenecity of SbWMV had a difference between Albori and Eunpamil strain. These results could possibly showed that vavious types of SbWMV occurre in Korea. Lee (1981) reported that SbWMV occurred in Iksan but there is no report on

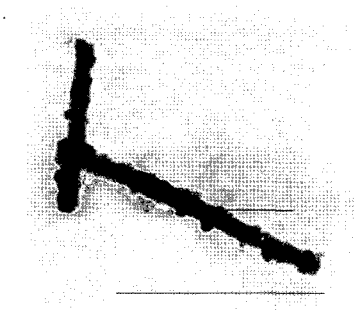

Fig. 4. Electron micrography of soilborne wheat mosaic virus particle. The particles are rod-shaped. Bar. 1㎛ and arrow indicates SbWMV particles.

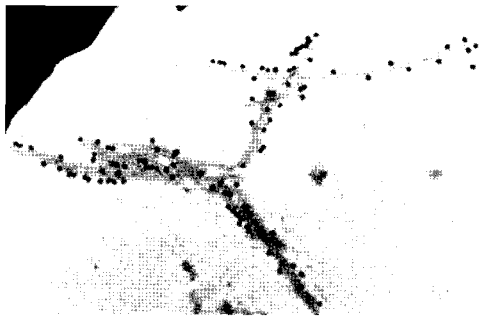

Fig. 5. Immuno-electron microscopy of soilborne wheat mosaic virus.

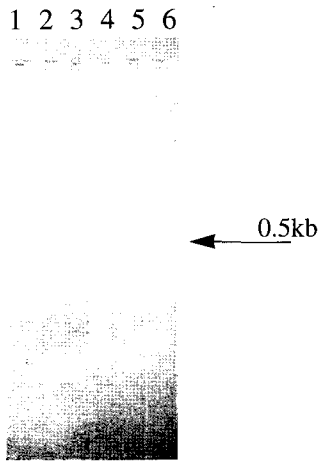

Fig. 6. RT-PCR products from total RNA by using specific primers on 2% agarose gel. Lane 1 : DRI gest Ⅲ marker asa standard size, lane 2 : RNA 1 of Eunpamil strain, lane 3: RNA 1 of Albori strain, lane 4 : RNA 2 of Eunpamil strain, lane 5 : RNA 2 of Albori strain, lane 6 : 100bp ladder.

methods of inoculation for Korean and Japanese barley and wheat cultivars. RT-PCR, was used to examine the diagnosis-possibility of RNA 1 and RNA 2 for SbWMV from total RNA extraction of infected barley. As a result, RNA 1 did not amplify, but obtained 0.6kb products of RNA 2 (Fig. 6). These results support the pindings of Pennington et al., (1993) However RNA 1 could not amplify in our experiments. Which contlast the Pennington et al.,(1993) report on SbWMV. Therefore, this report suggests to use diagnnose an isolation method that properly the occurrence of SbWMV in barley and wheat cultivating areas.

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