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Relationships Between Soil-Borne Virus Infection and Root Growth Damage in Korean Hulless Barley Cultivars

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Viral infections and root growth were examined to elucidate the relationship between viral resistance and root growth in 26 Korean hulless barley cultivars. Viral resistance was estimated in experimental filed of Honam agricultural research institute for 3 years. Length and number of seminal and adventitious roots were examined for evaluation of root growth in both field and greenhouse conditions 30 days after seeding. Dominant viral infection occurred in Korean hulless barley by *Barley yellow mosaic virus* (BaYMV) in fields; however, susceptible cultivars were infected by either BaYMV, *Barley mild mosaic virus* (BaMMV) or both. Only four cultivars, including Donghanchalssalbori, Kwangwhalssalbori, Namhossalbori and Naehanssalbori, presented stable resistance to viral infections. Susceptible cultivars to viral infection in fields showed shorter seminal root length and fewer adventitious root number than resistant cultivars. Resistant cultivars showed better root growth and significant difference in adventitious root length in green house conditions. Increase in the number of seminal roots in resistant cultivars was derived from decreased damage of roots by the viral infection compared to the susceptible cultivars.

Keywords : barley root damage, *Barley yellow mosaic virus*, viral resistance

Viral diseases of barley became more prevalent around the southern malting barley regions in Korea from late 1990's (So et al., 1997). Among viral diseases, *Barley yellow mosaic virus* (BaYMV) and *Barley mild mosaic virus* (BaMMV) are dominant in barley with either single or dual infection (Park et al., 2004a; So et al., 1990, 1993). BaYMV and BaMMV are soil borne diseases transmitted by root infecting fungus, *Polymyxa graminis*, which has a wide host range and can survive in various soil conditions (Adams et al., 1988; Legreve et al., 2000; So et al., 1993). Therefore, barley roots may be essential as a site for entry, establishment and propagation of soil-borne pathogens followed by movement to other plant parts, resulting in poor plant growth and development.

Barley has two types of root systems such as seminal (primary or seedling) root and adventitious (nodal or crown) root and there is no difference in their ability for the absorption of water and nutritious substances (Hackett, 1968). Many studies have been done for assessing the effects of biotic (Leone et al., 2001) or abiotic (Pasternak et al., 2005) stresses on the plant growth and development based on the damage of plant root system. These aspects in relation to barley root damage and viral disease were reported only in *Barley yellow dwarf virus*, an aphid transmittable disease in winter cereals (Haver and Comeau, 1992; Kolb et al., 1991). Other reports were focused on the effect of viral infection on the plant growth, cell damage and yield reduction in barley (Frahm, 1989; Kim et al., 2003; Park et al., 2006). However, little is known about the relationships between soil-borne virus infection and damage or growth of barley roots. Hulless barley has been one of the major food sources together with rice in Korea. Hulless barley is more susceptible than covered barley to viral diseases, showing various resistance degrees depending on the cultivar. The improvement of hulless barley in resistance to viral infection is one of the major subjects in Korean barley breeding program. Therefore, this study was conducted to elucidate the effect of viral infection on root growth in Korean hulless barley cultivars and to evaluate the resistance of Korean hulless barley cultivars to soil-borne viruses in fields.

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Materials and Methods

Viral infection in fields. To investigate the degree of viral infection in the field, twenty-six Korean hulless barley cultivars were planted in BaYMV and BaMMV infested fields of Department of Rice and Winter Cereal Crop, National Institute of Crop Science, Rural Development Administration, growing them in the same fields for 3 years from 2004 to 2006. The seeding date every year was the tenth day of October, which is 10 days earlier than recommended sawing time, to induce a higher disease incidence. Fertilization and other cultivation managements were done according to the RDA Manual (RDA, 2004). The field test was conducted in three replications.

Diseased index and evaluation of viral infection. Disease symptoms on leaves caused by viral infection were referred as disease degree index (DI) in this study. Determination of DI was according to So et al. (1997), in which no infection was recorded as 0, below 10% as 1, 11-30% as 3, 31-50% as 5, 51-70% as 7 and over 70% as 9. DI was investigated in mid March every year from 2005 through 2007. Barley leaves collected from the fields were stored at –20°C until the evaluation of viral infection. Detection of viral infection was performed using ELISA according to the procedure described by So et al. (1997). Antisera of the three major viruses of Korean barley, BaYMV, BaMMV and *Soil-borne wheat mosaic virus* (SBWMV) (Park et al., 2004) were used to determine viral infections.

Evaluation of root growth damage. Barley root growth damage was investigated by root growth difference between resistant and susceptible barley cultivars after inoculation with soil-borne viruses such as BaYMV and BaMMV. Four resistant and nine susceptible barley cultivars selected from the above field test were planted in pots with soils with or without the viral infections in greenhouse conditions. Both cultivars were planted in two soil types simultaneously. Ten plants each from resistant or susceptible cultivars were collected from the pots after 20 days of planting. Root growth difference was evaluated by the number and length of seminal and adventitious-roots. The root damages were investigated in two sides: First, the root damages of susceptible cultivars were compared to those of the resistant ones; second, the difference in root growth between the plants were measured manually.

Results and Discussion

Disease degrees in Korean hulless barley cultivars. In the field test for three years, four and nine cultivars out of the total 26 Korean hulless barley cultivars examined showed stable resistant (DI, 0-1) and susceptible (DI, >7) disease degree indices, respectively (Table 1). So et al. (1998) reported that viral disease resistance is variable depending on kind of barley varieties such as hulled, hulless and malting barley. Hulless barley generally showed moderate disease degrees and relied on resistant properties of each cultivar. Similar results were also obtained in our study with somewhat weakened resistance of the hulless barley cultivars. These selected resistant and susceptible cultivars were used as materials for examining the root growth damage by

Table 1. Annual viral disease indices (DI) for three years (2005-2007) on the hulless barley cultivars used in this study

Cultivar	DI*			cultivars	DI		
	2005	2006	2007		2005	2006	2007
Jaegangssalbori	3	3	3	Hobanchalssalbori	5	7	5
Jaeanssalbori	3	3	3	Baegdong	9	9	9
Saehanchalssalbori	7	7	7	Songhagbori	9	7	7
Saechalssalbori	7	5	7	Daehossalbori	1	3	1
Kinssalbori	9	7	9	Olssalbori	7	7	7
Hinchalssalbori	5	7	7	Namhossalbori	1	1	1
Poongsanchalssalbori	5	7	7	Neulssalbori	7	7	9
Jinmichalssalbori	7	7	7	Saenulssalbori	3	5	1
Donghossalbori	1	3	7	Hinssalbori	7	7	7
Saessalbori	7	7	7	Naehanssalbori	1	1	1
Ganghossalbori	5	7	7	Cheonghossalbori	–	3	1
Donghanchalssalbori	1	1	1	Chunchussalbori	–	7	5
Kwangwhalssalbori	1	1	1	Dasong	–	3	1

*DI were measured by symptom-experessed areas relative to the total leaf area; 0=no symptom, 1=<10%, 3=11~30%, 5=31~50%, 7=51~70%, and 9=>70% (So et al., 1997) This test was conducted in virus-infested fields continuously planted with the same hulless barley cultivars in every September for three years from 2005 through 2007.

Table 2. Infections of *Barley yellow mosaic virus* (BaYMV), *Barley mild mosaic virus* (BaMMV) and *Soil-borne wheat mosaic virus* (SBWMV) on selected resistant and susceptible cultivars

Cultivar	Viral infection			Cultivar	Viral infection		
	BaYMV	BaMMV	SBWMV		BaYMV	BaMMV	SBWMV
Donghanchalssalbori	–	–	–	Saehanchalssalbori	+	–	–
Kwangwhalssalbori	–	–	–	Jinmichapssalbori	+	+	–
Namhossalbori	–	–	–	Kinssalbori	+	+	–
Naehanssalbori	–	+	–	Saessalbori	+	+	–
				Baegdong	+	+	–
				Songhagbori	+	–	–
				Olssalbori	–	+	–
				Neulssalbori	+	–	–
				Hinssalbori	+	–	–

Viral infections were diagnosed by ELISA to major three viral diseases in barley, BaYMV, BaMMV and SBWMV, in Korean.

the viral infection.

Detection of viral infection. The selected materials were inspected against three major viruses, BaYMV, BaMMV and SBWMV, in domestic barley fields. All of suspectible cultivars were infected by BaYMV and BaMMV, either singly or in combination, and dominant infections occurring on Korean barley cultivars were caused by BaYMV (Table 2), which is in good agreement with the previous report (Park et al., 2004a). BaYMV was dominant viral disease in Korean barley field as mixed with BaMMV. Although Naehanssalbori was infected by BaMMV, it was selected as resistant because of weakened symptoms and normal-looking growth in the field. This disease has a general potential to be recovered with masking of symptoms, especially in conditions of temperature increase from April. Increase of the resistance degree may be also related to this recover potential of the barley cultivar. It was assumed that Naehanssalbori has bigger a recover potential related to climatic changes especially temperature in April. So this recovery has been used as a criterion to resistance decision of the lines in Korean barley breeding program (So et al., 1997). Naehanssalbori has been known as a resistant cultivar in several reports (Gilda et al., 2006; Park et al., 2006; So et al., 1997) as same as in this result. Also, it was assumed that the resistant gene's operation in this cultivar considering to some reports mentioned that the *rym*4, one of the BaYMV and BaYMV resistant genes, works degradation of infected virus and fewer virus transmission by the vector, *P. graminis* (Adams et al., 1987; Schenk et al., 1995).

Root damage degrees with different resistance. Examination of root damages in resistant and susceptible cultivars showed that the susceptible cultivars tended to have shorter and less seminal and adventitious root, although there were no significant differences between the two cultivar groups in the field test (data not shown). Many factors such as varietal properties, moisture content and nutrient status in soil and other micro-environmental field conditions affect on the plant growth (Briggs, 1978), which may alleviate or compensate root growth damages caused by the viral infection. To identify the root damage more clearly, we tested same plant materials in green house conditions. In this experiment, resistant cultivars showed better root growth

Table 3. Root growth statuses of selected resistant and susceptible barley cultivars under green-house conditions infested with soil-borne viruses

Disease response	Cultivar	Seminal root		Adventitious root	
		Number	Length	Number	Length
R	Donghanchalbori	3.9	20.3	1.2	7.2
	Kwangwhalssalbori	3.9	17.0	2.2	4.5
	Namhossalbori	3.9	16.9	1.2	4.7
	Naehanssalbori	4.3	16.5	1.7	4.5
	Mean	**4.0**	**17.7**	**1.6**	**5.2**
S	Saehanchalssalbori	4.0	7.8	2.0	4.0
	Jinmichapssalbori	3.3	18.9	1.2	3.5
	Kinssalbori	3.0	10.9	1.0	1.4
	Saessalbori	3.5	15.9	1.5	2.0
	Baegdong	3.7	20.0	1.7	5.3
	Songhagbori	2.9	8.6	0.6	3.2
	Olssalbori	3.6	11.7	1.6	4.7
	Neulssalbori	4.4	17.4	1.1	2.8
	Hinssalbori	4.0	21.8	1.0	2.0
	Mean	**3.6^{ns}**	**14.8^{ns}**	**1.3^{ns}**	**3.2***

Root growth statuses (length and number of seminal and adventitious roots) were examined 20 days after planting barley seeds in pots containing virus-infested soils.
^{ns,*}: Non-significant and significant at 0.05 probability levels, respectively.

Table 4. Comparison of root growth status between resistant and susceptible barley cultivars grown in virus-infested and non-infested soil conditions

Disease response	Cultivar	Seminal Root				Adventitious Root			
		Number		Length		Number		Length	
		IF	NIF	IF	NIF	IF	NIF	IF	NIF
R	Donghanchalbori	3.9	4.3	20.3	13.8	1.2	2.3	7.2	9.2
	Kwangwhalssalbori	3.9	4.7	17.0	17.8	2.2	2.5	4.5	5.5
	Namhossalbori	3.9	4.7	16.9	19.2	1.2	1.3	4.7	5.8
	Naehanssalbori	4.3	4.4	16.5	17.2	1.7	2.1	4.5	5.5
	Mean	**4.0**	**4.5****	**17.7**	**17.0**^{ns}	**1.6**	**2.1**^{ns}	**5.2**	**6.5**^{ns}
S	Saehanchalbori	4.0	4.5	7.8	15.3	2.0	2.3	4.0	9.0
	Jinmichapssalbori	3.3	5.0	18.9	22.1	1.2	3.3	3.5	13.8
	Kinssalbori	3.0	5.0	10.9	17.9	1.0	3.0	1.4	5.4
	Saessalbori	3.5	3.9	15.9	12.2	1.5	2.3	2.0	5.2
	Baegdong	3.7	4.7	20.0	20.3	1.7	2.0	5.3	7.2
	Songhagbori	2.9	4.7	8.6	17.7	0.6	1.3	3.2	3.5
	Olssalbori	3.6	5.3	11.7	15.4	1.6	3.0	4.7	6.3
	Neulssalbori	4.4	4.7	17.4	25.5	1.1	2.7	2.8	17.4
	Hinssalbori	4.0	5.8	21.8	19.9	1.0	2.5	2.0	10.9
	Mean	**3.6**	**4.8****	**14.8**	**18.5**^{ns}	**1.3**	**2.5****	**3.2**	**8.7****

IF and NIF mean virus-infested soil and non-infested soil, respectively. Root growth statuses (length and number of seminal and adventitious root) were examined 20 days after planing barley seeds in pots containing virus-infested soils. R: resistant, S: susceptible.
^{ns,**}: Non-significant and significant at 0.01 probability levels, respectively.

than susceptible cultivars like in the field experiment (Table 3). The adventitious root length was significantly different between the susceptible and resistant cultivars, which indicates that the viral infection may affect the whole root growth, especially adventitious root length. These results coincide with those of the previous report that *Barley yellow dwarf virus* (BYDV) infection decreased root length more than root number (Kainz, 1980).

In another experiment in which growths of seminal and adventitious roots were compared between susceptible and resistant cultivars, adventitious root number and length of the susceptible cultivars were affected in addition to seminal root number, of which resistant cultivars was affected by the viral infection (Table 4). Hoffman et al. (1997) reported that BYDV infection retards the root growth more than shoot growth, even though BYDV infection occurs on leaves through aphid transmission. In our experiments also the soil-borne viruses BaYMV and BaMMV may inhibit primarily the root growth (elongation), especially of the adventitious roots, suggesting that the adventitious root growth may be more related to the disease resistant degree. All of the European barley cultivars are susceptible to *P. graminis* infection, implying that searching for resistance to the vector infection can be used as an important key for developing the viral resistant barley (Ward et al., 2005). In our study also, it may be considered that the primary damages on the seminal root in the resistant cultivars may be due to the initial establishment of the vector on the root system, providing the initial viral infection, but that continuous secondary infection and invasion to the adventitious root system may be blocked by the resistance activities of the cultivars. In the susceptible cultivars, however, the root growth damage might occur continuously from the first seminal root system to the secondary adventitious root system, decreasing its elongation and proliferation in root number. As the viruses infecting plants move from the underground roots to aboveground plant part such as stems and leaves through the phloem, damages of the resistant cultivars only on the seminal roots but no on the adventitious roots are probably derived from inhibited viral movement through the phloem after the initial infection because the adventitious root system generally originates from stem tissues. By the BaYMV infection, barley yield reduced from 40 to 100% depending on the climatic factors and varietal resistant degrees (Frahm, 1989; Park et al., 2003 and 2006).

In our study, it is shown that the viral infection affects initially the primary root growth (seminal root) for both susceptible and resistant barley cultivars, but that the secondary root growth (adventitious root) was inhibited only in the susceptible cultivars probably unblocked viral movement to aboveground plant part through the phloem as

shown in previous studies (Park et al., 2004b; Schenk et al., 1995), finally resulting in resulted plant growth inhibition and yield reduction (Park et al., 2006). In case of BYDV, the viral resistance is related to the potential of rapid root growth of barley and wheat (Comeau and Jedlinski, 1990), which was also found similar to our examinations. In this sense, the features of root growths and damages can be used as a morphological marker in determining resistance or susceptibility of barley cultivars against the two major viruses BaYMV and BaMMV.

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