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Regional Distribution of Barley Yellow Dwarf Virus Strains in Korea and Identification of Resistant Wheat

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ABSTRACT: Barley Yellow Dwarf Virus (BYDV) has been a major disease causing a severe loss of yield in winter cereals worldwide. It has been recently reported that BYDV occurs frequently in wheat field and also causes serious yield reduction in Korea. This study was performed to investigate the regional distributions of BYDV strains in Korea and to identify the resistant cultivars or lines of wheat to the predominant BYDV strains, providing basic information for the breeding of BYDV-resistant wheat varieties. Using RT-PCR and *Eco*RI digestion methods, the regional distribution of BYDV strains in Korea from 1999 to 2000 showed that PAV strain was mainly detected about 65% (Vic-PAV 52.6%; CN-PAV 47.4%) and MAV strain about 3%. Using ELISA test for the examination of BYDV resistance with 17 cultivars and 4 lines among Korean wheat, three cultivars, Gurumil, Topdongmil, and Olgurumil, were susceptible to BYDV and the others were resistant. In plant growth and yield component responses to BYDV infection, Gurumil showed significant difference between the uninfected and the infected, suggesting the most susceptible to BYDV among Korean wheat, but Eunpamil and Seohae118 did no difference, an indication that they have the highest resistance.

Keywords : Wheat, BYDV resistance, Regional distribution, PAV, MAV, ELISA

Barley Yellow Dwarf Virus (BYDV) is the important aphid-borne and phloem-limited luteovirus that infects all major cereal crops including barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), oat (*Avena sativa* L.), and other wild grasses as well. BYDV is found worldwide in 54 countries from seven continents. Strains related to BYDV-PAV, which are typically more damaging among BYDV strains, have been found to be the most prevalent in more than 50% of the countries surveyed (Lister and Ranieri, 1995). The virus interferes with physiological processes within the plant and in turn causes the symptoms of chlorosis, stunting and yield loss (Jensen and D'Arcy, 1995). Common effects of the virus on agronomic characteristics include reductions in yield, yield components, height, aboveground dry weight, and root growth (Baltenberger *et al.*, 1987; Burnett and Gill, 1976; Carrigan *et al.*, 1981; Hoffman and Kolb, 1997)

Present virus control strategies include cultural practices such as varying the sowing date to avoid immigrations of viruliferous aphid vectors and applying insecticides to reduce the spread of aphids within crops (Gourmet *et al.*, 1996; Wangai *et al.*, 2000). However, neither of these methods is very satisfactory. Currently, the development and use of cereal varieties exhibiting resistance is the preferred approach for control (Plumb and Johnstone, 1995; Burnett *et al.*, 1995).

In barley, a recessive resistance gene, *yd1*, was identified in the cultivar 'Rojo' (Suneson, 1955), but it was rarely used in plant breeding programs because it confers a low level of resistance. A semidominant resistance gene, *Yd2*, was identified in Ethiopian barley and mapped to the long arm of chromosome 3, based first on morphological markers and more recently on restriction fragment length polymorphism (RFLP) markers (Collins *et al.*, 1996; Schaller *et al.*, 1964). The *Yd2* gene confers a high level of resistance and has been widely used in plant breeding programs (Delogu *et al.*, 1995). Although major genes for resistance were found in barley soon after the discovery of BYDV, it was not until recently that major genes were reported in wheat. In bread wheat, tolerance exhibited in a cultivar 'Anza' and other wheat lines is conditioned by a partially dominant gene, *Bdv1*, that has been identified in wide crosses with 'Agropyron' (Singh *et al.*, 1993). Other tolerance genes also exist, but the variations at tolerance level found in wheat are not as high as that found in barley. Most of the tolerance in wheat appears to be quantitative in nature (Cisar *et al.*, 1982). Researchers have suggested that tolerance to BYDV in wheat should be characterized in terms of yield and components of yield (Hoffman and Kolb, 1998). However, it is also important to measure virus concentration within the plant in the aspect of true resistance (i.e. relatively low virus production). For measuring BYDV concentration, virion purification (Jedlinski *et al.*, 1977) and serological assays (ELISA) (Skaria *et al.*, 1985) have been used to estimate BYDV

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capsid protein antigen titer as an index of resistance. ELISA test is relatively quick and simple, especially when a large number of genotypes need to be evaluated.

In these days, it has been reported that BYDV occurs frequently in Korea and causes severe growth retardation and yield loss in wheat field. Thus the breeding of wheat cultivars resistant to BYDV is needed to limit economic losses from this disease. The objectives of this study were to investigate the regional distribution of BYDV strains in Korea and then evaluate the resistance to a Korean prevalent BYDV strain, PAV, in wheat. Resistance to BYDV was screened with ELISA tests, and then evaluated by the plant growth and yield component responses to BYDV infection. Results of this study will be useful as basic information for the breeding of wheat varieties resistant to BYDV.

MATERIALS AND METHODS

Plant materials and growth condition

To investigate the regional distribution of BYDV strains in Korea, wheat, barley and oats plant showing yellowing or reddening in leaves were collected at 23 regions from 1999 to 2000. Plant samples were stored at 70°C until analysis.

Seventeen wheat cultivars and 4 wheat lines among Korean wheat were screened to evaluate BYDV resistance. BYDV resistant cultivars, Frontana and Anza, and a BYDV susceptible cultivar, Bobwhite, provided from CIMMYT, Mexico, were used as the check cultivars. The kernels were germinated in a plastic box contained with the culture soil for 30 days at 4°C in the dark. After vernalization, seedlings showing uniform coleoptile and seminal root growth were transplanted to 1/50 ha pots (two seedlings per pot, 6 pots per variety) and placed into the glasshouse at National Experiment Station, Suwon, Korea. The 144 pots (24 varieties including check cultivars by two treatments by three replications) were arranged in a completely randomized block design on the glasshouse bench. Plants were allowed to grow under glasshouse conditions at 18 ± 5°C air temperature with 45 ± 10% relative humidity. Seven days later, when the second leaf emerged from the leaf roll, experimental treatments were started. Treatments included a control (no aphids) and a BYDV infection treatment.

RT-PCR amplification and *Eco*RI restriction enzyme digestion

Total RNA extraction from leaf samples, BYDV-specific primers, RT-PCR reaction, *Eco*RI digestion, and agarose gel electrophoresis were conducted as previously described by Woo *et al.* (2001, in press).

BYDV inoculation into wheat plants

Plants were infected with Vic-PAV found to be the most prevalent strain by investigation of regional distribution in Korea. *Rhopalosiphum padi* L., a prevalent vector in Korea, was used as a vector of Vic-PAV. Non-viruliferous colonies of aphids used in this experiment originated from nymphs deposited on parafilm membranes by adult aphids collected in the field. Non-viruliferous nymphs were transferred to 'Olquiri' (*Avena sativa* L.) plants infected with Vic-PAV, and allowed to feed and reproduce for a month.

Virus inoculation was accomplished by placing 20 viruliferous aphids per cage over the plants in the second leaf stage. The number of aphids on each plant was monitored daily to maintain about 25 to 30 individual aphids per plant. Plants were sprayed with Konido (Imidacloprid, Dongbu Hannong Chemical Co, Ltd., Korea) to remove aphids at 10 days after inoculation. Control plants also were caged for the same length of time and received an insecticide treatment.

ELISA test

ELISA test to measure virus titers was performed by an indirect triple antibody sandwich (TAS) ELISA method using ELISA kit (Sanofi, France) at 50 days after inoculation. Each well of microplates was coated with antibodies and incubated at 37°C for 2 hours. After rinsing 3 times with PBS-Tween buffer (pH 7.4), sample homogenates (200 μl/well) were added to each well. After the plates were incubated at 4°C for 15 hours, plates were washed 2 times by PBS-Tween. The plates were incubated for 1.5 hours at 37°C following an addition of second-level antibodies. After rinsing 3 times with PBS-Tween, conjugated antibodies (GAM-PAL) were added and incubated for 1.5 hours at 37°C. Following washing 3 times with PBS-Tween, substrate solution was added to each well and incubated at room temperature for 1 hour. After reaction optical densities at 405 nm were measured by the Immuno Reader (TECAN, UK). Tested samples were assessed to be resistant to BYDV if the A405 (Absorbance at 405 nm) value were less than the resistant check cultivars.

Evaluation of plant growth and yield component responses to BYDV infection

Stem length, 1st internode length, 2nd internode length, flag leaf width and length, productive tillers, total tillers, dry weight of aerial part, panicle length, grains per plant, and 1000-grain weight were measured at 90 days after inoculation.

RESULTS

Regional distribution of BYDV strains in Korea

The regional distribution of BYDV strains in Korea was investigated with wheat, barley, and oat plants showing BYDV symptoms collected at various regions from 1999 to 2000. Total 88 plants were collected at 23 regions such as Gurye, Asan, Gochang, Jeongeup, Youngcheon, etc., (Table 1) and the detection and classification of BYDV strains were performed by RT-PCR and *EcoR*I restriction analysis as previously reported (Woo *et al.*, 2001). PAV strains were mainly detected 64.8% of total 88 samples and classified into Vic-PAV (52.6%) and CN-PAV (47.4%). However MAV strains were detected only 3.4% in Goryong, Iksan, and Suwon (Table 1). The results show that PAV strains, the most prevalent BYDV worldwide, are also the most predominant ones in Korea. This supports the report consistently that vector aphids *Rhopalosiphum padi* L. and *Macrosiphum avenae* L. have been frequently occurred in wheat field of Korea (Im *et al.*, 2000).

Table 1. Regional distribution of Barley Yellow Dwarf Virus strains in Korea.

Regions	Collected samples†	PAV			MAV
		Total	Vic-PAV	CN-PAV	
Uljin	7	4	3	1	
Chilgok	4	3	1	2	
Imsil	6	3		3	
Songju	2	1	1		
Asan	10	7	6	1	
Goryong	2	2	1	1	1
Iksan	4	3	2	1	1
Jeongeup	6	5		5	
Suwon	4	3	3		1
Youngcheon	4	4	2	2	
Pohang	2	2	1	1	
Gyongju	1	1		1	
Cheongwon	1	1		1	
Gochang	5	3	3		
Younggwang	3	1		1	
Hampyong	2	1		1	
Youngam	3	1		1	
Gurye	10	5	3	2	
Gangjin	2	1		1	
Boseong	1	1	1		
Gwangju	5	3	2	1	
Hamyang	3	1		1	
Hadong	1	1	1		
Total	88	57	30(52.6)‡	27(47.4)‡	3
%	100.0	64.8	-	-	3.4

†Collected from 1999 to 2000.
‡% of Vic-PAV and CN-PAV occupied in the total PAV strains.

Evaluation of BYDV resistance in wheat using ELISA test

The result of evaluation of BYDV resistance in 17 wheat

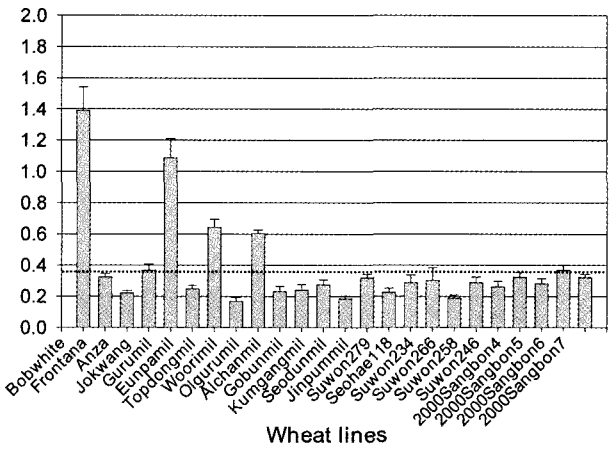


Fig. 1. ELISA values at 405 nm for leaf extracts of wheat infected with Barley Yellow Dwarf Virus.

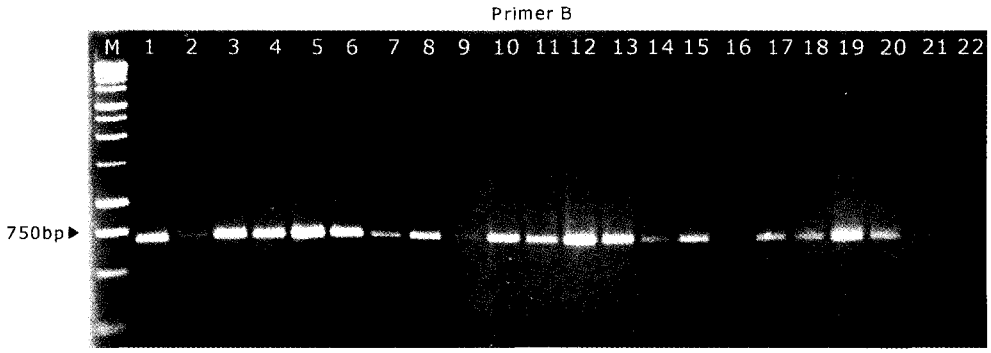


Fig. 2. Detection of Barley Yellow Dwarf Virus from the infected wheat by RT-PCR.

cultivars and 4 wheat lines using ELISA test is shown in Fig. 1. Three cultivars, Gurumil, Topdongmil, and Olgurumil, were susceptible to BYDV and the others were resistant, based on the A405 values of the susceptible and resistant check cultivars.

While high yielding lines have been developed in wheat, the resistant lines to BYDV may have been unintentionally selected simultaneously, because this assumption may be explained in the results, showing that most of Korean wheat lines were evaluated as having BYDV-resistance. As shown in Fig. 2, the infection of BYDV inoculated wheat plants was confirmed using RT-PCR reactions with primer B (Woo *et al.*, 2001).

Evaluation of plant growth and yield component responses to BYDV infection

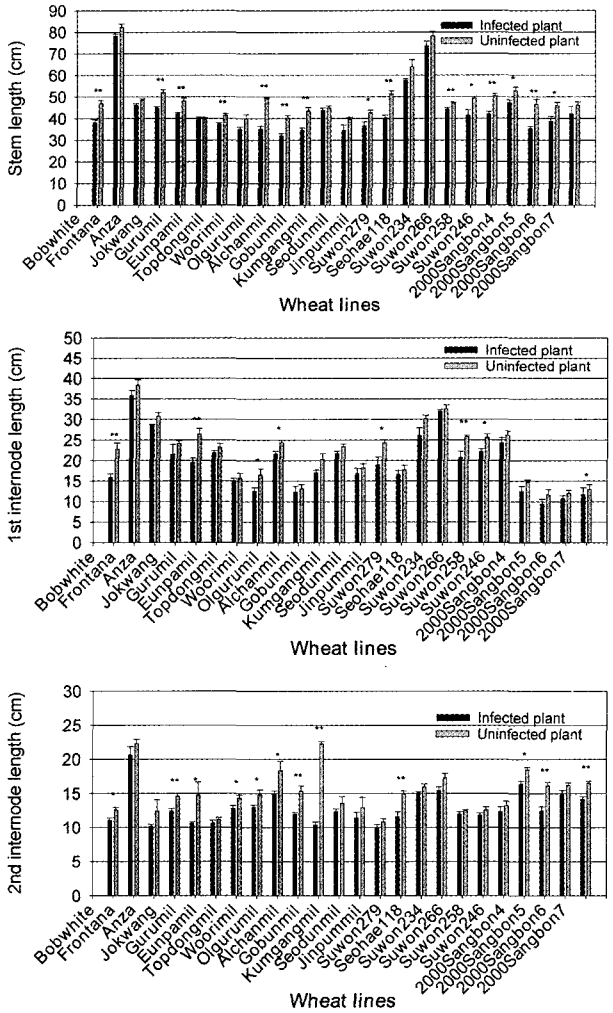
The major characteristics of plant growth and grain yield were examined to evaluate the plant responses to BYDV infection. Responses of plant growth to BYDV infection were expressed as ratio of the infected to the uninfected in Table 2. Gurumil showed significant difference in all characteristics between the infected and the uninfected, but Eunpamil, Kumgangmil, and Seohae118 did no difference. Carrigan *et al.* (1980) have reported that BYDV infection has a great effect on stem length, but Fig. 3 indicates that flag leaf development is also influenced by BYDV infection.

It is remarkable that the inhibition of flag leaf development usually accompanies with chlorosis, considering that it can cause yield loss by photosynthetic rate reduction of flag leaf. Responses of yield components to BYDV infection were shown in Table 3, expressed as ratio of the infected to the uninfected. Gurumil showed significant difference of yield components between the infected and the uninfected

Table 2. Responses of plant growth to Barley Yellow Dwarf Virus infection in wheat.

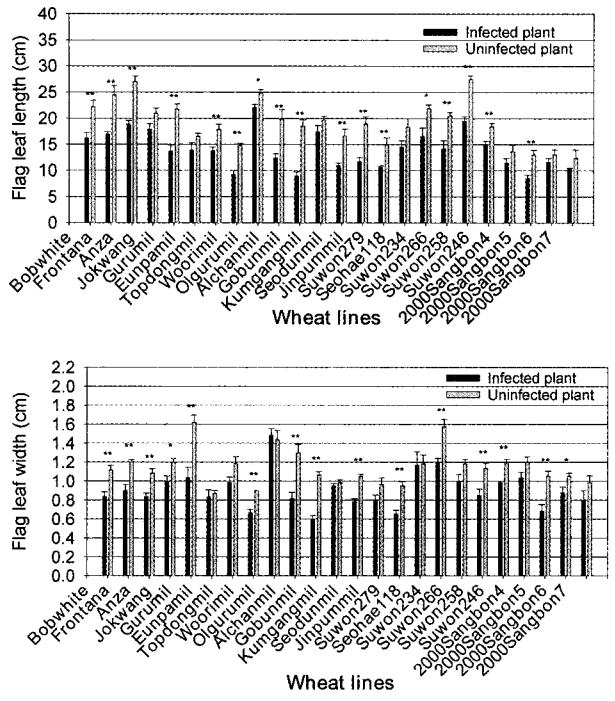
Wheat lines	% to the control				
	Stem length	1st internode length	2nd internode length	Flag leaf length	Flag leaf width
Bobwhite	81.1**	69.7**	88.2**	73.0**	74.6**
Frontana	95.0	93.0	92.6	69.3**	75.0**
Anza	95.1	93.0	81.7	69.9**	76.9**
Jokwang	86.3**	89.5	85.2**	85.4	83.3*
Gurumil	87.7**	73.8**	72.0*	62.8**	63.9**
Eunpamil	98.5	94.2	95.2	83.7	96.2
Topdongmil	89.9**	94.3	89.3*	77.4**	83.1
Woorimil	87.9	75.7*	88.1*	62.4**	74.1**
Olgurumil	71.6**	88.7*	81.2*	88.9*	103.5
Alchanmil	79.9**	94.4	77.8**	62.4**	62.8**
Gobunmil	79.9**	83.9	46.6**	48.5**	56.3**
Kumgangmil	97.4	93.1	91.0	88.6	96.6
Seodunmil	87.4	92.4	88.4	65.7**	74.6**
Jinpummil	85.4*	78.0*	91.0	61.8**	82.8
Suwon279	78.3**	94.0	77.5**	70.6**	68.4**
Suhae118	90.2	86.6	93.2	79.1	98.6
Suwon234	94.0	98.3	89.2	75.5*	76.2**
Suwon266	94.0**	80.5**	96.1	69.2**	84.5
Suwon258	84.1*	86.7*	94.0	71.0**	75.0**
Suwon246	83.5**	92.4	93.1	81.7**	83.1**
Sangbon4	89.3*	84.3	88.6*	83.9	86.1
Sangbon5	75.9**	81.6	77.2**	65.2**	65.1**
Sangbon6	84.1*	89.3	91.4	88.8	83.8*
Sangbon7	91.7	90.3*	85.7**	84.3	81.4

*, **Significant at the 0.05 and 0.01 probability levels, respectively.



*, **Significant at the 0.05 and 0.01 probability levels, respectively.

Fig. 3. Responses of plant growth to Barley Yellow Dwarf Virus infection in wheat.



*, **Significant at the 0.05 and 0.01 probability levels, respectively.

Fig. 3. Continued.

except for tiller number, while Jokwangmil, Eunpamil, Sangbon 4, and Sangbon 6 did no difference of all yield components. Tiller number, productive tillers and total tillers were not influenced by BYDV infection (Fig. 4) which is contradictory to Baltenberger *et al.* (1987). Therefore, it is needed to compare tiller number between them at various growth stages, since tiller number changes at a growth stage. Panicle length was also inhibited by BYDV infection (Fig. 4), which suggests that BYDV infection at an early stage gives an effect on the plant growth until the late growth stage.

DISCUSSION

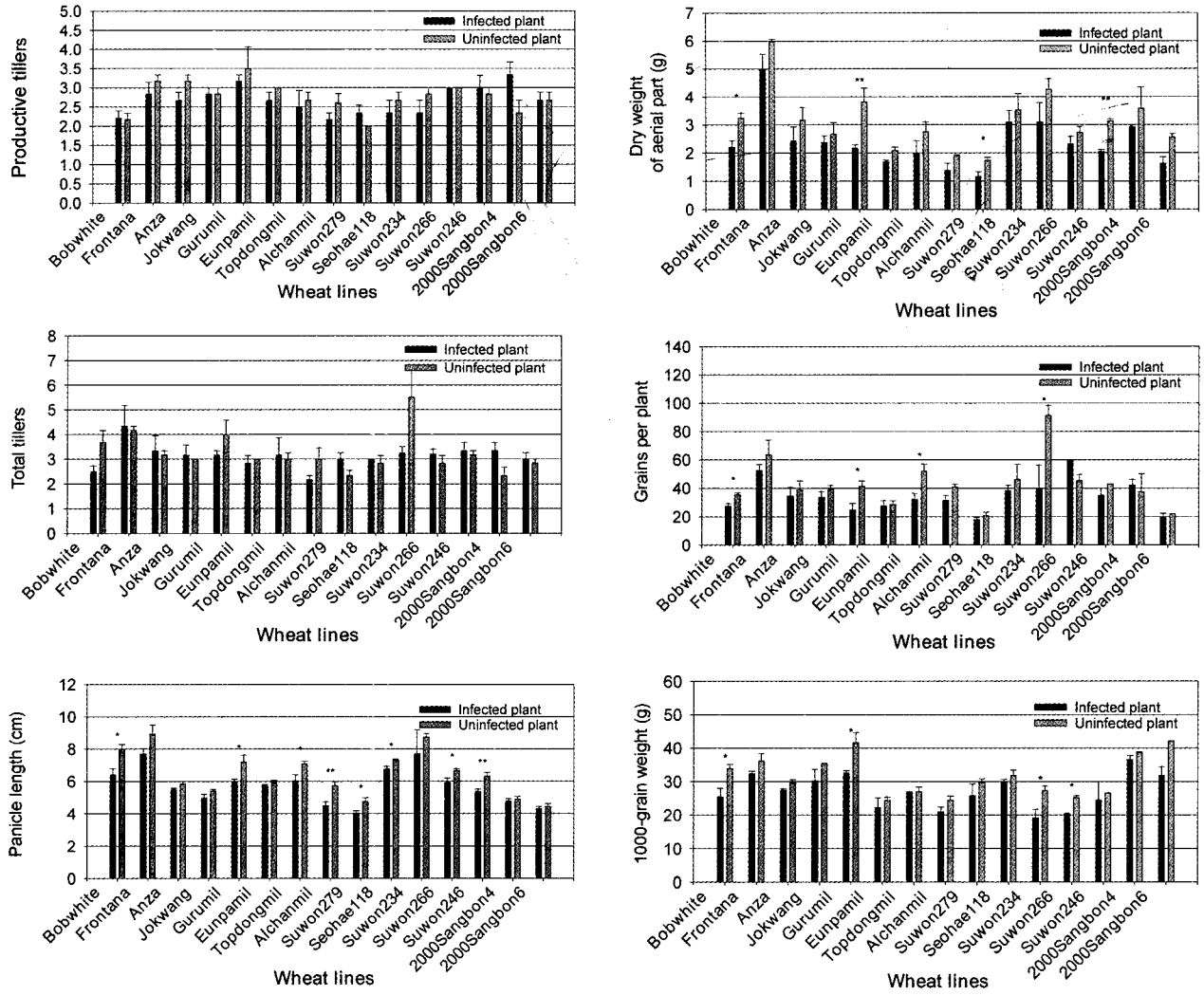
Investigation of regional distribution of BYDV in Korea from 1999 to 2000 showed that PAV strains were mainly detected 64.8% of total 88 samples and MAV strains only 3.4%. In addition, PAV strains were classified into Vic-PAV (52.6%) and CN-PAV (47.4%). The results indicate that PAV strains are the most prevalent BYDV strain in Korea. MAV strains were detected from samples collected at Goryong, Iksan, and Suwon. The RPV strains transmitted by an aphid vector, *Rhopalosiphum padi* L., have been frequently occurred in the central Indiana, USA (K. L. Perry, unpublished report). We suggest that further investigation of MAV strain distribution should be accomplished centering on the regions, considering possible expansion of MAV strains. Since a vector *Rhopalosiphum padi* L. is also prevalent in Korea, it is required to identify RPV strains continuously.

Evaluation of BYDV resistance in 17 wheat cultivars and 4 wheat lines using ELISA test showed that Gurumil, Topdongmil, and Olgurumil were susceptible to BYDV and the others were resistant. In plant growth and yield component responses to BYDV infection, Gurumil showed significant

Table 3. Responses of yield components to Barley Yellow Dwarf Virus infection in wheat.

Wheat lines	% to the control					
	Productive tillers	Total tillers	Panicle length	Dry weight	Grains per plant	1000-grain weight
Bobwhite	101.5	68.2	80.3*	68.0*	77.4*	75.0*
Frontana	89.5	104.0	86.4	83.3	82.8	89.6
Anza	84.2	105.3	93.7	76.7	88.7	92.7
Jokwang	100.0	105.6	91.7	88.6	85.5	85.5
Gurumil	90.5	79.2	83.3*	57.1**	59.9*	78.4*
Eunpamil	88.9	94.4	96.6	81.2	97.3	91.5
Topdongmil	93.8	105.6	85.2*	72.2	61.5*	98.7
Alchanmil	83.3	72.2	78.4**	72.6	77.2	85.6
Suwon279	116.7	128.6	85.2*	67.1*	87.1	87.0
Seohae118	87.5	105.9	92.4*	87.8	83.0	93.7
Suwon234	82.4	54.5	88.2	72.5	43.5*	70.2*
Suwon266	100.0	112.9	88.6*	85.1	132.4	81.8*
Suwon246	105.9	105.3	85.0**	65.2**	81.4	92.1
Sangbon4	142.9	142.9	97.6	81.4	112.7	94.6
Sangbon6	100.0	105.9	97.0	63.4	89.4	75.4

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.



*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

Fig. 4. Responses of yield components to Barley Yellow Dwarf Virus infection in wheat.

difference between the uninfected and the infected but Eunpamil and Seohae118 did no difference. Consequently Gurumil were the most susceptible cultivar to BYDV, and Eunpamil and Seohae118 were the most resistant ones. Cooper and Jones (1983) defined 'immune' as the ability of the host to prevent virus from reproduction and movement within the plant, 'resistance' as to reduce virus replication, and 'tolerance' as to exhibit few symptoms even in the presence of high virus titers. According to this definition, since both virus concentration within the plants and inhibition rate of growth and yield components by BYDV infection are low, Eunpamil and Seohae118 are resistant to BYDV infection. However, the absolute grain yield is an important criterion for breeding wheat varieties resistant to BYDV. Therefore, it is needed to evaluate BYDV resistance under more typical of grower's field conditions.

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