Response of Tall Fescue Genotypes to a New Strain of *Brome mosaic virus*

M. A. Rouf Mian, J. C. Zwonitzer, A. A. Hopkins, X. S. Ding, and R. S. Nelson, The Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73402

ABSTRACT

Mian, M. A. R., Zwonitzer, J. C., Hopkins, A. A., Ding, X. S., and Nelson, R. S. 2005. Response of tall fescue genotypes to a new strain of *Brome mosaic virus*. Plant Dis. 89:224-227.

Brome mosaic virus (BMV) infects many different species within the *Poaceae* family. A new strain of BMV, named F-BMV, was identified in a tall fescue (*Festuca arundinacea* Schreb.) plant. Here, we report the identification and characterization of tall fescue plants resistant to F-BMV, and the effects of F-BMV infection on their growth and development. Susceptible plants infected with F-BMV produced 40% fewer tillers and 42% less dry matter compared with virus-resistant plants in a greenhouse study. In the field, susceptible plants infected with F-BMV produced 25% fewer tillers, 36% less dry matter, 10% less plant height, and 40% lower seed yield compared with virus-resistant plants. In a field evaluation of a tall fescue mapping population, the virus symptom scores were negatively correlated with production of dry matter ($r = -0.55$), plant height ($r = -0.55$), and seed yield ($r = -0.33$). Thus, F-BMV has the potential to cause significant economic damage to susceptible tall fescue plants. These results indicate that the virus can present a serious challenge for long-term maintenance of valuable plant materials. A survey of tall fescue plants from Arkansas, Oklahoma, and Oregon indicated that the prevalence F-BMV in the field was very low.

Brome mosaic virus (BMV) was first reported and described in 1944 (6). Although it is not generally considered to be an economically damaging plant pathogen, severe reductions in forage yields of susceptible smooth brome (90%) and orchard grass (80%) due to BMV infections have been reported (7). The virus has also killed young sweet corn seedlings of cv. Golden Giant (7).

BMV is a widespread virus infecting grasses in Africa, North America, Europe, and Asia (4,5). BMV has one of the broadest host ranges among viruses naturally infecting many different species of the *Poaceae* family (5,12). The virus produces typical mosaic, mottling, or streaking symptoms in wheat, barley, oat, rye, sorghum, Johnson grass, sudan grass, and many other grasses (6,7). Tall fescue (*Festuca arundinacea* Schreb.) was reported as a natural host of BMV in 1973 (8). BMV also is common in smooth brome grass (*Bromus inermis*) and persists in clones in many areas of the American Great Plains.

The virus often is spread from infected smooth brome grass to previously uninfected areas through animal and machinery traffic. Mechanical transmission by humans also can become significant in certain areas (5). Beetles (*Chaetocnema aridula* and *Oulema melanopus*) are reported to be the main natural vector for transmission of BMV (9). In general, BMV is not transmitted through seed (3,7) or by the aphids *Aceria tosichella*, *Myzus persicae*, *Schizaphis graminum*, and *Rhopalosiphon maydis* (11). Nematode species in the genus *Xiphenema* have transmitted BMV in laboratory settings (10).

In 1999, we received plant clones of a tall fescue breeding population from the University of Missouri, Columbia. In the course of growing these plants in the greenhouse and in a field in Ardmore, OK, we observed typical mosaic symptoms on the leaves of some plants in this population. Plants with mosaic symptoms exhibited stunted growth and low vigor. The causal agent was identified and characterized as a new strain of BMV and was named F-BMV (X. S. Ding, M. A. R. Mian, and R. S. Nelson, *unpublished*). F-BMV has approximately 99% nucleotide identity with the well-characterized R-BMV strain, with differences in 114 nucleotides compared with the nucleotide sequence of R-BMV published by Ahlquist et al. (1). It also induces milder symptoms on barley, maize, and fescue than the R-BMV strain (X. S. Ding, M. A. R. Mian, and R. S. Nelson, *unpublished*). The objectives of the present study were to (i) identify F-BMV-resistant and -susceptible tall fescue genotypes and (ii) evaluate the ef-

fect of F-BMV on the growth and development of tall fescue.

MATERIALS AND METHODS

Identification of F-BMV-resistant and -susceptible genotypes in two tall fescue cultivars. Virus screening was performed by infecting randomly selected seedlings (genotypes) from two tall fescue cultivars, Kentucky 31 (Ky31) and Jesup. As an out-crossing crop, each individual plant in a tall fescue cultivar was a different genotype, and the cultivar was a collection of genotypes with certain traits of interest in common. Resistant and susceptible plants were identified by inoculating 2-week-old seedlings in a growth chamber with F-BMV. For each tall fescue seedling, the second and third leaves were inoculated with a crude extract prepared from F-BMV-infected barley leaves (1:5, wt/vol, in 0.1 M phosphate buffer, pH 6.0) as described previously (2). Carborundum was added to the crude extract prior to mechanical inoculation. All plants were inoculated a second time 1 week after the first inoculation to prevent escapes. The inoculated plants were maintained in growth chambers with a cycle of 24°C for 16 h of light and 20°C for 8 h of dark. The photosynthetic photon flux density (400 to 700 nm) was maintained at 150 $\mu\text{mol s}^{-1} \text{m}^{-2}$. The virus-inoculated plants were scored for disease severity 3 weeks after the second inoculation based on visual virus symptoms and enzyme-linked immunosorbent assay (ELISA).

Double-antibody sandwich (DAS)-ELISA using an antibody against BMV virions was conducted as described previously (2). Briefly, 2-cm-long leaf sections were collected from each plant and placed in 2.0-ml tubes, weighed, and ground to fine powder using a Mixer Mill (Type MM 300; RETSCH, Hann, Germany). Sodium phosphate buffer (0.1 M, pH 7.0) was added into each tube (200 μl per 0.1 g of fresh weight tissue). Crude extract (2 μl) was loaded into each well containing 150 μl of ELISA buffer (phosphate buffer containing 0.05% Tween 20 and 0.2% ovalbumin). The plates were read 30 min after adding substrate. Samples which resulted in optical density (OD) values three times greater than healthy controls were considered positive. Plants that showed virus-like symptoms and gave positive ELISA OD values were considered to be susceptible to BMV infection. Plants that did not develop virus-like symptoms and gave negative OD values were considered resistant.

Corresponding author: M. A. R. Mian
E-mail: mian.3@osu.edu

Current address of M. A. R. Mian: United States Department of Agriculture-Agricultural Research Service, 1680 Madison Avenue, Wooster, OH 44691.

This research work was funded by The Samuel Roberts Noble Foundation.

Accepted for publication 26 October 2004.

DOI: 10.1094/PD-89-0224

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For additional analysis, the resistant and infected susceptible plants from the initial screening were moved from growth chambers to a greenhouse where they were grown in 15.2-cm round plastic pots filled with Metro Mix 350 (Scotts Co., Marysville, OH) for an additional 3 months. The susceptible and resistant plants were placed next to each other in an alternating order on a greenhouse bench. Plants were clipped with scissors once every 2 to 3 weeks (a total of 6 times prior to the start of the greenhouse experiment and 10 times prior to the start of the field experiment). Each clipping session was begun by first clipping a plant with virus symptoms and then using the contaminated scissors to clip a resistant plant, so that clipping of a virus-infected plant would be followed by the clipping of a resistant plant. This was done as an extra precaution to ensure that none of the plants initially identified as resistant escaped F-BMV inoculation.

Greenhouse evaluation of selected plants. In all, 11 F-BMV-resistant and 11 F-BMV-susceptible plants from each of the two tall fescue cultivars, Ky31 and Jesup, were randomly selected for this experiment. Twelve replicates of each of the 44 plants were started by planting two tillers of each plant in 15.2-cm plastic pots filled with Metro Mix 350. Tillers in each of the pots were developmentally matched. After 2 weeks of growth, when plants were well

established, the four pots containing the most uniform-sized plants were selected to represent the four replicates of each genotype. Selected plants were transferred into 3.8-liter pots and then placed on a greenhouse bench in a randomized complete block design using a 2-by-2-by-11 factorial arrangement (2 cultivars $\times$ 2 virus response groups $\times$ 11 genotypes) with four replicates. Pots were rotated within each replicate twice weekly in an orderly fashion to minimize border and micro-environment effects on plants. Plants were fertilized with N at 100 ppm (15-16-17 Peter's brand (Scotts Co., Marysville, OH) as a constant feed and grown for 8 weeks. At the end of this period, tillers of each plant were counted. Only tillers with at least one fully expanded leaf were included in the count. Each plant was also given a virus symptom (VS) score using a scale of 1 to 5, where 1 = no VS, 2 = VS on <15% of leaf surfaces, 3 = VS on >15 and <30% of leaf surfaces, 4 = VS on >30 and <50% of leaf surfaces, and 5 = VS on >50% of leaf surfaces. Each plant then was harvested by cutting at the soil surface for aboveground dry matter determination. Plant biomass was dried in a drying oven for 10 days at 68°C before recording the dry matter weight.

Field evaluation of selected plants. Eight additional clonal replicates from the same 44 plants (described in the green-

house evaluation) were established in pots in the greenhouse. Four uniform size clones of each genotype at approximately the six-tiller stage were space planted in a field in Ardmore during fall 2000. The experimental design was a randomized complete block in a 2-by-2-by-11 factorial arrangement (2 cultivars $\times$ 2 virus response groups $\times$ 11 genotypes) with four replicates. In the spring of 2001, the VS scores were recorded. Agronomic data were collected for heading date (days from 30 April), plant height, tiller number, seed yield, and plant dry weight. The data from all experiments were analyzed using the GLM procedures of SAS (SAS Institute, Cary, NC). Replicates were considered random effects and the genotypes were considered fixed effects.

Field evaluation of the mapping population. In this trial, the effect of F-BMV on field plantings of the University of Missouri mapping population (13) was evaluated. Four replicates of clonally propagated plants of 86 F₂ progeny lines and the two parents were planted in a randomized complete block design in a field in Ardmore, OK in fall, 1999. In spring 2000, plants were scored for VS (using the same 1-to-5 scale as above), and the heading date (days from 30 April), plant height, seed yield, and plant dry weight data were recorded.

Field survey of BMV. Plant samples were collected from four regions of the

Fig. 1. Leaves from a *Brome mosaic virus*-susceptible tall fescue plant with the virus symptoms (left) and leaves from a virus-resistant tall fescue plant (right).

United States. These were south-central Oklahoma, northeastern Oklahoma, north-western Arkansas, and the state of Oregon. In each region, four well-distributed tall fescue plantings (each at least 25 acres in size) were selected for sample collection between April and June 2001. From each field, 25 tall fescue plants were selected randomly for collection of leaf samples. The youngest fully expanded leaf from five vegetative tillers of each plant was collected in a zip-lock plastic bag and immediately placed on dry ice. Leaf samples then were shipped to the Noble Foundation for analysis. A 2-cm leaf section from the middle of each of three randomly selected leaves from each zip-lock bag (representing a tall fescue plant) was transferred to a 2.0-ml micro-centrifuge tube. Samples in the micro-centrifuge tubes were frozen in liquid nitrogen and ground to a fine powder using a Mixer Mill (Type MM 300; RETSCH). ELISA was performed according to the protocol mentioned above.

RESULTS

Identification of F-BMV-resistant and -susceptible genotypes from two tall fescue cultivars. Both resistant and susceptible seedlings (genotypes) were recovered from each of the two cultivars. For example, in cv. Ky31, there were 19 resistant and 17 susceptible plants. The proportions of resistant and susceptible plants were similar in both cultivars, with an approximate ratio of 1 resistant to 1 susceptible seedling. Symptoms on susceptible plants included mosaic on leaves and stunting of the plants (Fig. 1). Resistant

plants did not show virus symptoms and no virus was detectable by ELISA. For the greenhouse and field experiments, an inoculated plant was considered resistant if it had no visible virus symptoms and also was found to be virus free by ELISA at the start of each experiment.

Greenhouse evaluation of selected plants. The single degree of freedom (single-df) comparison between the virus-resistant plant group and the virus-susceptible plant group indicated significant differences for all parameters evaluated (Table 1). None of the plants in the resistant group showed virus symptoms, whereas those in the susceptible group had virus symptoms over at least 15% of the leaf area. Averaged over the cultivars, the susceptible plant group produced 40% fewer tillers than the resistant plant group. Similarly, the susceptible plant group produced 42% less dry matter than the resistant plant group. Averaged over plant groups (F-BMV-resistant and -susceptible), the cultivars differed in dry matter ($P < 0.05$) but not in tiller numbers (Table 1). The plant group-cultivar interactions were significant for both tiller numbers ($P < 0.05$) and dry matter ($P < 0.01$). Magnitudes of reductions in tiller numbers and dry matter of the susceptible plant group compared with the resistant plant group were greater in Ky31 compared with the difference between the two groups in Jesup (Table 1). For example, the susceptible plant group of Ky31 produced 55% less dry matter compared with the resistant group of the same cultivar, while the susceptible plant group of Jesup produced

17% less dry matter compared with the resistant plant group of Jesup.

Field evaluation of the selected plants. The average VS score for the resistant plant group was 1.0, meaning that none of the plants in this group had visible virus symptoms in the field. The average VS score for the susceptible plant group was 3.5 (Table 2). Single-df comparison revealed significant differences between the resistant plant group and the susceptible plant group for all parameters (Table 2). On average, the virus delayed the heading date by 2 days. The susceptible plant group had 25% less tillers, produced shorter plants (10%), and had 36% less above-ground dry matter and 40% less seed yield than the resistant plant group. Averaged over plant groups (F-BMV-resistant and F-BMV-susceptible), cultivars did not differ in VS scores, plant height, and seed yield; and there was no significant plant group-cultivar interaction for VS and plant height. However, Jesup headed 6 days earlier, and produced 25% more tillers and 11% less dry matter than Ky31 (Table 2). The plant group-cultivar interactions were significant ($P < 0.01$) for heading date, tiller numbers, dry matter, and seed yield.

Field evaluation of the mapping population. Significant differences ($P < 0.01$) were observed among the progeny of the mapping population for VS. Of the 86 F_2 progeny, 50 progeny had VS scores of ≤ 2.0 or lower, 23 progeny had scores > 2.0 and < 3.0 , 10 progeny had scores > 3.0 and < 4.0 , two progeny had scores > 4.0 and < 5.0 , and one progeny had a score of 5.0. The female parent, HD28-56, had a score

Table 1. Virus symptom (VS) score, tiller number, and aboveground dry matter for virus-resistant and virus-infected susceptible tall fescue genotypes after 8 weeks of growth in a greenhouse^a

Plant group ^b	VS score ^c	Tiller number			Dry matter (g)		
		Average	Jesup	Ky31	Average	Jesup	Ky31
Resistant	1.0**	22.5**	22.6**	22.3**	3.8**	3.0*	4.4**
Susceptible	3.5	13.4 (40)	15.2 (33)	11.8 (47)	2.2 (42)	2.5 (17)	2.0 (55)
Cultivar average	...	...	18.9	17.1	...	2.8	3.2

^a Numbers in the parenthesis indicate the percent reductions in the trait for the virus-susceptible plant group due to virus infection compared with the virus-resistant plant group; ** and * indicate significant differences between the two plant groups at $P \leq 0.01$ and $P \leq 0.05$, respectively; ... = data not relevant.

^b Resistant plant group included a total of 22 virus-resistant plants, 11 plants from each of the two tall fescue cvs. Jesup and Ky31. Similarly, the susceptible plant group includes 22 virus-infected susceptible plants, 11 plants from each of the two tall fescue cultivars.

^c Disease severity rating scale: 1 = no VS, 2 = $< 15\%$ leaf area with VS, 3 = > 15 and $< 30\%$ leaf area with VS, 4 = > 30 and $< 50\%$ leaf area with VS, and 5 = $> 50\%$ leaf area with VS.

Table 2. Virus symptom (VS) score, heading date, tiller number, plant height, aboveground dry matter, and seed yield for selected virus-resistant and virus-susceptible tall fescue genotypes grown in a field in Ardmore, OK^a

Group ^b	Score ^c	Heading date			Tiller number			Ht (cm) ^d	Dry matter (g)			Seed yield (g)		
		Avg	Jesup	KY31	Avg	Jesup	KY31		Avg	Jesup	KY31	Avg	Jesup	KY31
Resistant	1.0**	26.7*	24.2 ^{NS}	29.2*	57**	60**	53**	37.2**	244**	223**	266**	31.6**	29.7**	33.4**
Susceptible	3.5	28.7	24.8	32.2	43 (25)	50 (17)	35 (34)	33.6 (10)	157 (36)	157 (30)	158 (41)	19.0 (40)	21.2 (29)	16.9 (51)
Average	...	...	24.5	30.7	...	55	44	...	...	190	212	...	25.4	25.2

^a Numbers in the parenthesis indicate the percent reductions in the trait for the virus-susceptible plant group due to virus infection compared with the virus-resistant plant group; Avg = average; ** and * indicate significant differences between the two plant groups at $P \leq 0.01$ and $P \leq 0.05$, respectively, and NS = not significant; ... = data not relevant.

^b Plant groups. Resistant plant group includes a total of 22 virus-resistant plants, 11 plants from each of the two tall fescue cultivars Jesup and Ky31. Similarly, the susceptible plant group includes 22 virus-infected susceptible plants, 11 plants from each of the two tall fescue cultivars. Average = cultivar average.

^c Disease severity rating scales: 1 = no VS, 2 = $< 15\%$ leaf area with VS, 3 = > 15 and $< 30\%$ leaf area with VS, 4 = > 30 and $< 50\%$ leaf area with VS, and 5 = $> 50\%$ leaf area with VS.

^d Plant height in centimeters.

of 1.0 whereas the male parent, Ky31, had a score of 2.0. Thus, there were large transgressive segregations among the progeny toward susceptibility to F-BMV. One reason for the higher level of susceptibility among the progeny could be that F_2 lines are subject to inbreeding depression in tall fescue and the self-pollinated progeny were considerably more susceptible to the virus compared with their parental lines. This hypothesis was also supported by the low VS score (1.8) of the F_1 parent of the F_2 population. The VS scores of the plants had significant ($P < 0.01$) negative (Pearson's) correlations with plant dry weight ($r = -0.55$), seed yield ($r = -0.33$), and plant height ($r = -0.55$), whereas the virus significantly ($P < 0.05$) delayed the time to heading ($r = 0.29$).

Field survey of BMV. Of the 400 plant samples that were collected and analyzed by ELISA, only 2 individuals (1 from South Central Oklahoma and 1 from Northwestern Arkansas) were positive for BMV. The remaining plants were determined to be virus free.

DISCUSSION

BMV is a widespread virus infecting grasses in Africa, North America, Europe, and Asia, but it is not considered an economically damaging plant pathogen (4). However, the results from this study indicate that F-BMV can adversely affect susceptible tall fescue genotypes. The virus can present a formidable challenge in long-term maintenance of otherwise valuable plant materials (e.g., mapping populations and unique sources of germ plasm that need to be maintained as clones). This is particularly true for out-crossing plants, including tall fescue, because of difficulties in seed production from self-pollinated plants due to self-incompatibility.

The genotypes initially characterized as resistant through the seedling inoculation procedure remained virus free throughout the course of this study. We have been maintaining the clones of the resistant genotypes in a greenhouse along with F-BMV-infected (susceptible) plants since their identification during the summer of 2000. Both resistant and F-BMV-infected plants are clipped frequently (once or twice a month) with scissors immediately

after clipping virus-infected plants as described above. From our experience, virus can spread from plant to plant in the HD28-56 $\times$ Ky31 tall fescue mapping population via contaminated scissors. For more than 4 years after the initial characterization by artificial inoculation of seedlings, resistant genotypes have remained virus free. Accordingly, we are convinced that the potential of escapes using this inoculation process are rare. Clones of these resistant genotypes can be requested from the Samuel Roberts Noble Foundation for research purposes.

In this study, only a small proportion (<10%) of susceptible tall fescue genotypes infected with F-BMV eventually died due to virus infection. Most susceptible plants continued to survive through generation of new tillers. The cumulative growth and development of susceptible plants, however, was less than that of resistant plants. The VS scores for most of the susceptible plants were 3 to 4 with an average of 3.5 using the 1-to-5 VS score scale. Tall fescue is an indeterminate perennial grass with profuse tillering ability. Such a growth habit may be the reason for its survival while carrying the virus, and this also may explain why we did not observe the most severe symptoms (score 5) more often. It also is possible that the resistance of tall fescue to F-BMV is controlled by multiple genes rather than a single gene. This also would explain why we did not see a uniform VS score among the susceptible plants. The determination of the number of genes controlling F-BMV infection in tall fescue (an out-crossing hexaploid species) can be a daunting task and, thus, was beyond the scope of the current study.

Resistance to the virus is highly desirable in parental materials of mapping populations and breeding stocks that need to be maintained as clones. Our research indicates that resistant cultivars could be developed effectively in grass-breeding programs and would provide the most economically feasible means for preventing a BMV outbreak in tall fescue. According to the findings from our survey of a limited number of plants from several tall fescue-growing regions of the United States in 2001, the prevalence of BMV in

tall fescue fields is very low. Thus, BMV does not appear to be an immediate problem to the production of tall fescue in the surveyed regions at this time.

ACKNOWLEDGMENTS

We thank R. J. Tyrl (Oklahoma State University, Stillwater), C. P. West (University of Arkansas, Fayetteville), and L. Brilman (Seed Research of Oregon Inc., Corvallis) for collection of the leaf samples.

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