Diallel Analysis of *Wheat streak mosaic virus* Resistance in Winter Wheat

Frederic Hakizimana, Amir M. H. Ibrahim,* Marie A. C. Langham, Scott D. Haley, and Jackie C. Rudd

ABSTRACT

***Wheat streak mosaic virus* (WSMV) (Family: Potyviridae; Genus: *Tritimovirus*), disseminated naturally by the wheat curl mite (*Aceria tosichella* Keifer), is an important disease of wheat (*Triticum aestivum* L. em Thell.) worldwide. Breeding for resistant cultivars remains the best strategy to control the disease. Nine winter wheat genotypes with differential reaction to WSMV were crossed in a complete diallel mating design to determine the combining ability of WSMV resistance. Parents, F_1, and reciprocal crosses were inoculated at the seedling (2–3 leaves) stage with a WSMV-SD isolate and evaluated for reaction under greenhouse conditions. Disease reaction was assessed twice (at 1-wk intervals) by a 1-to-5 scale (1 = no visible symptoms to light green streaks, 5 = severe yellow streaks and necrosis). Data were analyzed according to Griffing's Method 3 and Model 1, where one set of F_1 and reciprocal F_1 are included. Highly significant genotype effects ($P < 0.01$) were observed for WSMV resistance. General combining ability (GCA) and specific combining ability (SCA) effects for WSMV resistance were highly significant ($P < 0.01$), indicating that both additive and nonadditive genetic effects are involved in the inheritance of WSMV resistance. The reciprocal effects were not significant ($P > 0.05$). The ratio of combining ability variance components [$(2\sigma^2_{GCA})/(2\sigma^2_{GCA} + \sigma^2_{SCA})$] was small (0.1), indicating that nonadditive (i.e., dominance and epistasis) gene effects were more important than additive gene effects in controlling WSMV resistance in these crosses; therefore, progeny performance cannot be adequately predicted from GCA effects alone.**

WSMV, DISSEMINATED NATURALLY by the wheat curl mite, is an important disease in Canada, USA, and Europe, especially in areas where winter wheat is regularly grown (Slykhuis, 1955; Shawn and Hill, 1984; Bottacin and Nassuth, 1990). Estimates of crop loss caused by WSMV in the Great Plains during 1987 ranged from 31.9 to 95.3% (Edwards and McMullen, 1988). Recommended cultural practices, such as crop rotations and delayed planting dates, are not always used by farmers. Thus, the development of resistant cultivars remains the most reliable and effective means of control (Stoddard et al., 1987).

The diallel cross is helpful to plant breeders in making decisions regarding the type of breeding system to use and in selecting breeding materials of greatest promise (Gardner and Eberhart, 1966). Diallel mating designs have been used primarily to estimate genetic variances when parents are either random individuals or inbred lines from a random-mating population in linkage equilibrium. They have also been used to estimate general combining ability (GCA) and specific combining ability (SCA) effects from crosses of fixed lines (Gardner and Eberhart, 1966; Zhang and Kang, 1997).

Diallel crosses have been used extensively to study the genetics of resistance to viral diseases in wheat, such as *Wheat soilborne mosaic virus*, *Barley yellow dwarf virus*, and *Wheatspindle streak mosaic virus* (Dubey et al., 1970; Cisar et al., 1982; and Van Koevering et al., 1987). Little information is available, however, regarding the combining ability of WSMV resistance in wheat. Therefore, a diallel mating design of parents with known differential reactions to the WSMV (Hakizimana, 2001) was used to determine the combining ability of resistance in a chosen set of winter wheat germplasm.

MATERIALS AND METHODS

Genetic Materials

On the basis of diversity of origin and the level of resistance to WSMV from previous screening tests, nine winter wheat genotypes were chosen for this diallel study (Table 1). A full diallel, including reciprocals, was made during the winter and fall of 1997-1998 and 1998-1999.

Experimental Design

A randomized complete block with four replications, in which one of the replications served as a control, was used. Five seeds per genotype were planted in a plastic pot (13 cm in diameter) containing a sieved mixture of topsoil, peat moss, vermiculite, and sand in a 3:1:1:1 ratio. Each pot represented one experimental unit. Two weeks after emergence, plants were thinned to two per pot. Each pot was fertilized with 6 g of Osmocote (14-14-14 NPK) slow release granule (Sierra Chemical Co., Milpitas, CA), and all pots were regularly watered as needed.

This experiment was repeated in time with a first planting in February 2000, and a second planting in April 2000. With each planting, three replications of infected plants and one control set for comparison were used. Pots containing plants were placed on greenhouse benches with a 16-h photoperiod and a light intensity of 200 μmol m^{-2} s^{-1}. Temperature regimes for the first and second planting environments averaged 24/16°C and 29/18°C day/night, respectively.

Inoculum Production and Plant Inoculation

Inoculum was produced by mechanically infecting seedlings of susceptible greenhouse-grown 'Arapahoe' winter wheat with a wheat streak mosaic virus isolate (WSMV-SD) collected in South Dakota by Dr. M. A. C. Langham. WSMV-SD is

F. Hakizimana, A.M.H. Ibrahim, and M.A.C. Langham, Plant Science Dep., South Dakota State Univ., Brookings, SD 57007; S.D. Haley, Soil and Crop Sciences Dep., Colorado State Univ., Fort Collins, CO 80523; J.C. Rudd, Texas Agric. Exp. Stn., 6500 Amarillo Blvd. W., Amarillo, TX 79106. Joint contribution of Dep. of Plant Science, South Dakota State Univ., Texas Agric. Exp. Stn., and Soil and Crop Sciences Dep., Colorado State Univ. Research partially supported through funding from the South Dakota Wheat Commission. South Dakota Agric. Exp. Stn. Journal Series no. 3330. Manuscript submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree in Agronomy at South Dakota State Univ. Received 7 Aug. 2002. *Corresponding author (amir_ibrahim@sdstate.edu).

Published in Crop Sci. 44:89–92 (2004).

677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: GCA, general combining ability; SCA, specific combining ability; WSMV, *Wheat streak mosaic virus*.

Table 1. Origin, pedigree, and previous *Wheat streak mosaic virus* (WSMV) reaction of nine winter wheat genotypes used as parents in the diallel.

Genotype	Origin or source†	Pedigree	WSMV symptom rating‡	Reaction to WSMV
NE91648	NE	NE82761/Trapper/2/CO652363	2	Moderately resistant
Jagger	KS	KS82W418/Stephens	2	Moderately resistant
2137	KS	W2440/W9488A//2163	2	Moderately resistant
Dawn	SD	II21031/Trapper/2/CO652363	1	Resistant
SD93267	SD	Shield/Roughrider//SD76598-7/Agassiz	1	Resistant
Harding	SD	Brule//Bennett/Chisholm/3/Arapahoe	2	Moderately resistant
KS93WGRC27	KS	Karl*3/*E. intermedia*	1	Resistant
Roughrider	ND	SeuSeun/CI12500 /2/RedChief/Pawnee /3/Cheyenne/4/Hume/5/Yogo/Frontana/2/2*Minter	5	Susceptible
Sage	KS	Agent/4*Scout	5	Susceptible

† CO = Colorado, KS = Kansas, NE = Nebraska, ND = North Dakota, SD = South Dakota.
‡ Symptom rating on a scale of 1–5: 1–2 = resistant, 2–3 = moderately resistant, 3–4 = susceptible, 4–5 = very susceptible.

serologically reactive with antisera to several known WSMV isolates and has been well characterized in field studies. A comparison of WSMV-SD with other WSMV isolates is incomplete at this time. Seedling plants of Arapahoe were inoculated 7 d after emergence by an air-blast inoculation technique (Wu and Langham, 1996). Two weeks after inoculation, foliage was cut approximately 2.5 cm above the soil surface to make inoculum. Inoculum was prepared by blending infected Arapahoe wheat with 0.02 *M* potassium phosphate buffer [1:3 tissue (g): buffer (mL)] at pH 7.0 in a 1:6 ratio at high speed in a kitchen blender. The resulting extract was filtered through cheesecloth, and 1% (w/v) silica carbide was added (Bottacin and Nassuth, 1990; Wu and Langham, 1996). All entries were inoculated by rubbing plants with cheesecloth saturated with the sap extract of WSMV-SD isolate. Virus-free inoculation buffer with 1% silica carbide was used on control plants.

Disease Assessment

Diseased plants were rated for WSMV visual symptoms on a scale of 1 to 5 (1 = no visible symptoms to light green streaks; 2 = broken light green and a few yellow streaks; 3 = mixed green and yellow streaks; 4 = yellow streaks; and 5 = severe yellow streaks and necrosis) (Slykhuis, 1955; Wu and Langham, 1996). The inoculated and control plants were maintained in the greenhouse until all the visual ratings were completed.

Statistical Analysis

This experiment was conducted twice in the greenhouse in two different planting environments (winter and spring). Data were analyzed with the DIALLEL-SAS program of Zhang and Kang (1997). Estimates of general combining ability (GCA), specific combining ability (SCA), and reciprocals were obtained according to Griffing's Method 3 (Griffing, 1956), where one set of F_1 plants and reciprocals were included [$p(p-1)$ entries]. The experimental materials were regarded as the population about which inferences were made. The general linear model for Griffing's Method 3 and Model 1 is:

$$X_{ijkt} = \mu + t_i + b_{ki} + v_{ij} + (tv)_{ijt} + e_{ijkt},$$

where X_{ijk} = observed disease reaction (i and j, parents; t, environment; k, replication), μ = population mean, t_i = environment effect, b_{ki} = block or replication within environment effect, v_{ij} = genotype effect = $g_i + g_j + s_{ij} + r_{ij}$ [where g_i = general combining ability (GCA) effect for the ith parent, g_j = GCA effect for the jth parent, s_{ij} = specific combining ability (SCA) effect for the ijth F_1 hybrid, and r_{ij} = reciprocal effect for the ijth or jith F_1 hybrid (Zhang and Kang, 1997)], $(tv)_{ijt}$ = interaction between genotypes and environments, and e_{ijkt} = residual effect.

Significance of GCA, SCA, and reciprocal effects was determined by a t test (Griffing, 1956).

RESULTS AND DISCUSSION

The statistical analysis across environments revealed highly significant differences ($P < 0.01$) among genotypes. Highly significant differences were also observed between environments and among replicates within environments (Table 2). The second planting environment gave the highest expression of WSMV symptoms as indicated by the higher mean value (Table 3), probably due to high temperature.

Ranking of parents for WSMV rating across the two environments was similar, with a positive Spearman's rank correlation coefficient (Steel and Torrie, 1997) between the two environments ($r_s = 0.69$; $P < 0.05$). The most resistant parents were 'Dawn', SD93267, and 'Harding', whereas the most susceptible ones were 'KS93WGRC27', 'Roughrider', and 'Sage'. NE91648, 'Jagger', and '2137' were considered moderately susceptible parents (Hakizimana, 2001).

The ANOVA revealed that F_1 crosses mean square

Table 2. Combined analysis of variance for *Wheat streak mosaic virus* (WSMV) symptom rating† in a 9-by-9 complete diallel winter wheat cross.

Source‡	df	Mean square
Environment (E)	1	5.52**
Reps within E	4	0.89**
Genotypes (G)	71	0.82**
GCA	8	2.1 **
SCA	27	1.27**
Reciprocal	36	0.19NS
G × E	71	0.24**
GCA × E	8	0.60**
SCA × E	27	0.18NS
Reciprocal × E	36	0.22*
M × E	8	0.15NS
N × E	28	0.23*
Error	284	0.14

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
NS = Not significant.
† 1–5 scale (1 = no visible symptoms to light green streaks, 5 = severe yellow streaks and necrosis).
‡ GCA = general combining ability; SCA = specific combining ability; M = maternal; N = nonmaternal.

for symptom rating was significant (Table 2), indicating that WSMV resistance is genetically controlled in the germplasm tested in this study. Highly significant differences ($P < 0.01$) were observed for GCA, indicating that there were differences in performance of genotypes as parents in hybrid combinations. Highly significant differences ($P < 0.01$) were also observed for SCA, suggesting the importance of non-additive (i.e., dominance and epistasis) gene effects.

Baker (1978) and Cisar et al. (1982) suggested that the progeny performances could be predicted by the use of the ratio of combining ability variance components $[(2\sigma^2_{GCA})/(2\sigma^2_{GCA} + \sigma^2_{SCA})]$. The closer this ratio is to unity, the greater the predictability based on GCA alone. This ratio was 0.1 in our study, indicating that nonadditive gene effects were more important than additive gene effects in controlling the inheritance of WSMV resistance in the germplasm we used. Therefore, the best WSMV resistant progeny cannot simply be produced by crossing the two parents with the lowest symptom rating GCA effects alone. The genotype × environment interaction (GE) was significant (Table 2); thus, we partitioned it into GCA × E and SCA × E interaction effects (Table 2). Only GCA × E was found to be significant, indicating that WSMV symptom rating was sensitive to environmental conditions and that data from additional environments or seasons would make GCA effects more precise. The SCA effects, on the other hand, would be stable across environments as indicated by the nonsignificant SCA × E interaction.

Assessing contribution of individual lines to hybrid resistance was accomplished by comparing the GCA effects among the parents (Table 3). The GCA effects were highly significant ($P < 0.01$) for all the parents except for Jagger, 2137, and KS93WGRC27. In this study, the GCA effect of a parent was consistent with that parent's level of resistance, except for KS93-WGRC27. A parent with a significant negative GCA value would contribute a high level of WSMV resistance, whereas a parent with a positive value would contribute a high level of susceptibility. Resistant genotypes such as Harding, SD93267, and Dawn showed highly significant negative GCA effects, indicating that they contributed a high level of resistance in hybrid combinations (Table 3). While the source of resistance in these three genotypes is not known, pedigrees for Dawn and Harding clearly indicate the absence of chromosomal translocations conferring WSMV resistance. The presence of a chromosomal translocation in SD93267 is possible on the basis of pedigree, as one of its parents, SD76598-7 (CI15322//Agent/4*Scout/3/Centurk pedigree), may have carried a chromosomal translocation from CI15322 that carries an *Agropyron elongatum* (Host) P. Beauv. segment for WSMV resistance. C-banding studies, however, have failed to confirm the presence of alien chromatin in SD93267. The presence of alien chromatin in SD93267 might be confirmed by a technique such as in situ hybridization (Hohmann et al., 1996).

The susceptible parents Roughrider and Sage exhibited highly significant positive GCA effects, indicating that they would contribute a high level of susceptibility to their F_1 progenies. Another parent, KS93WGRC27, which carries an *Elytrigia intermedia* (Host) Nevski [syn. *Agropyron intermedium* (Host) P. Beauv.] chromosome segment, was previously described by Gill et al. (1995) to provide an effective level of resistance to WSMV. It was the most susceptible parent in our study and it did not exhibit significant negative GCA effect. The observation of a susceptible WSMV reaction of this parent could be attributed to temperature sensitivity of the resistance caused by high light intensity or sporadic high temperature events during the greenhouse-growing season. Seifers et al. (1995) reported that the resistance to WSMV from *E. intermedia* translocated wheat lines was effective at 20°C but not at 25°C in growth chamber tests. Among the three moderately susceptible parents, two showed no significant GCA effects. The third one, NE91648, exhibited a large, positive, highly significant

Table 3. Estimates of specific combining ability (SCA) effects, general combining ability (GCA) effects, means of two environments (Env.), and overall means for wheat streak mosaic virus symptom rating (rating scale: 1 = no visible symptoms to light green streaks; 2 = broken light green and a few yellow streaks; 3 = mixed green and yellow streaks; 4 = yellow streaks; and 5 = severe yellow streaks and necrosis) for nine winter wheat genotypes.

	Parent											
Parent	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**GCA Effect†**	**Mean Env.1**	**Mean Env.2**	**Overall mean**
	SCA effect†											
1. NE91648									**0.22****	**2.90**	**2.92**	**2.91**
2. Jagger	**1.32****								**0.08**	**2.90**	**2.84**	**2.87**
3. 2137	**−0.09**	**−0.25****							**−0.05**	**2.67**	**2.77**	**2.72**
4. Dawn	**−0.18**	**−0.09**	**−0.13**						**−0.14**	**2.52**	**2.79**	**2.66**
5. SD93267	**−0.48****	**−0.29****	**0.17**	**0.16**					**−0.18***	**1.83**	**3.08**	**2.46**
6. Harding	**−0.25****	**−0.26****	**0.16**	**0.07**	**0.16**				**−0.19***	**1.77**	**2.63**	**2.20**
7. KS93WGRC27	**−0.18**	**−0.05**	**−0.14**	**−0.01**	**0.06**	**0.15**			**−0.04**	**3.67**	**3.42**	**3.54**
8. Roughrider	**0.03**	**−0.28****	**0.05**	**0.13**	**0.23***	**0.04**	**−0.14**		**0.14**	**3.33**	**3.00**	**3.17**
9. Sage	**−0.18**	**−0.20***	**0.24***	**0.06**	**−0.00**	**−0.07**	**0.16**	**0.02**	**0.18***	**3.17**	**3.09**	**3.13**
Mean										**2.75**	**2.95**	**2.85**
LSD (0.05)				**0.19‡**					**0.16‡**	**0.67**	**0.58**	**0.46**
SE				**0.14**					**0.12**	**0.26**	**0.18**	**0.20**

*** Significant at the 0.05 probability level.**
**** Significant at the 0.01 probability level.**
† LSD (0.05) for testing differences between GCA effects = 0.23; LSD (0.05) for testing differences between SCA effects with a common parent = 0.28; LSD (0.05) for testing differences between SCA effects with no common parent = 0.25.
‡ LSD (0.05) for testing significance of effects (s_{ij} and g_i, respectively).

GCA effect, indicating that progenies from crosses with this parent would be susceptible.

The SCA effects in each parental combination are shown in Table 3. Gardner and Eberhart (1966), Baker (1978), and Cisar et al. (1982) reported that highly significant SCA mean squares indicated that certain progeny had higher or lower levels of resistance than expected on the basis of the GCA of the two parents involved. Therefore, a complex type of inheritance of resistance to the disease under study may be involved in some parents.

Significant SCA effects were detected in 10 of the 36 possible combinations, indicating the presence of nonadditive effects. Significant negative SCA effects were observed for the combinations Roughrider/Jagger (susceptible and moderately resistant parents), Sage/Jagger (susceptible and moderately susceptible parents), 2137/Jagger (moderately susceptible parents), and SD93267/NE91648, SD93267/Jagger, Harding/NE91648, and Harding/Jagger (resistant and moderately susceptible parents). These results indicate that resistance of these progenies was higher than would be expected from the average resistance of their respective inbred parents based on WSMV symptom rating, suggesting that WSMV-resistant genotypes could be produced from susceptible parents possibly due to transgressive segregation or inter- and intra-locus gene interactions. The largest positive SCA effects corresponded especially to the hybrid Jagger/NE91648. This combination was more susceptible than predicted from average parental performance, indicating the importance of nonadditive gene effects in this particular cross.

There were no significant differences for the reciprocals, indicating the absence of significant maternal (M) and nonmaternal (N) effects for WSMV resistance (Table 2). The reciprocal $\times$ E interaction was significant, however. This significance was found to be due to N $\times$ E interaction effect, indicating that the interactions between extranuclear factors for WSMV resistance were affected by the environment (Borges, 1987).

CONCLUSION

The estimated small ratio of combining ability variance components $[(2\sigma^2_{GCA})/(2\sigma^2_{GCA} + \sigma^2_{SCA})]$ indicated that nonadditive gene effects were more important than additive gene effects in determining WSMV resistance in the winter wheat germplasm evaluated in this study. The reciprocal effects were absent, indicating that the direction of the cross was not important during the process of pollination. The nonmaternal $\times$ E interaction effect was significant, however, indicating that the interactions between extranuclear factors for WSMV resistance were affected by the environment. KS93WGRC27, which carries an *E. intermedia* chromosome segment for WSMV resistance, showed low general combining abilities and was susceptible to WSMV in our study, likely due to temperature sensitivity. Harding, Dawn, and SD93267 were found to have good general combining abilities for WSMV resistance. Therefore, they should be deployed in wheat breeding programs to improve the levels of resistance even though the source that conferred resistance in these lines is unknown.

REFERENCES

Baker, R.J. 1978. Issues in diallel analysis. Crop Sci. 18:533–536.

Borges, O.L. 1987. Diallel analysis of maize resistance to sorghum downy mildew. Crop Sci. 27:178–180.

Bottacin, A., and A. Nassuth. 1990. Evaluation of Ontario-grown cereals for susceptibility to wheat streak mosaic virus. Can. J. Plant Pathol. 12:267–272.

Cisar, C., C.M. Brown, and H. Jedlinski. 1982. Diallel analyses for tolerance in winter wheat to the barley yellow dwarf virus. Crop Sci. 22:328–333.

Dubey, S.N., C.M. Brown, and A.L. Hooker. 1970. Inheritance of field reaction to soil-borne wheat mosaic virus. Crop Sci. 10:93–95.

Edwards, M.C., and M.P. McMullen. 1988. Variation in tolerance to wheat streak mosaic virus among cultivars of hard red spring wheat. Plant Dis. 72:705–707.

Gardner, C.O., and S.A. Eberhart. 1966. Analysis and interpretation of the variety cross diallel and related populations. Biometrics 22:439–452.

Gill, B.S., B. Friebe, D.L. Wilson, T.J. Martin, and T.S. Cox. 1995. Registration of KS93WGRC27 wheat streak mosaic virus resistant T 4DL. 4Ai#2s wheat germplasm. Crop Sci. 35:1236–1237.

Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. Aust. J. Biol. Sci. 9:463–493.

Hakizimana, F. 2001. Wheat streak mosaic virus resistance studies in winter wheat (*Triticum aestivum* L.). Ph.D. Dissertation (AA-13032775). South Dakota State Univ., Brookings.

Hohmann, U., K. Badaeva, W. Busch, B. Friebe, and B.S. Gill. 1996. Molecular cytogenetic analysis of *Agropyron* chromatin specifying resistance to barley yellow dwarf virus in wheat. Genome 39:336–347.

Seifers, D.L., T.J. Martin, T.L. Harvey, and B.S. Gill. 1995. Temperature sensitivity and efficacy of wheat streak mosaic virus resistance derived from *Agropyron intermedium*. Plant Dis. 79:1104–1106.

Shawn, I.M., and J.P. Hill. 1984. Identification and occurrence of wheat streak mosaic virus in winter wheat in Colorado and its effects on several wheat cultivars. Plant Dis. 68:579–581.

Slykhuis, J.T. 1955. *Aceria tulipae* Keiffer (Aceria: Eriophyidae) in relation to the spread of wheat streak mosaic virus. Phytopathology 41:116–128.

Steel, R.G.D., and J.H. Torrie. 1997. Principles and procedures of statistics, a biometrical approach. 3rd ed. McGraw-Hill, Inc, New York.

Stoddard, S.L., B.S. Gill, and S.A. Lommel. 1987. Genetic expression of wheat streak mosaic virus resistance in two wheat-wheatgrass hybrids. Crop Sci. 27:514–519.

Van Koevering, M., K.Z. Hauffer, D.W. Fulbright, T.G. Isleib, and E.H. Everson. 1987. Heritability of resistance in winter wheat to wheat spindle streak mosaic virus. Phytopathology 77:742–744.

Wu, Z., and M.A.C. Langham. 1996. Variation of wheat streak mosaic virus concentration in early growth of winter wheat. Proc. South Dakota Acad. Sci. 75:127–137.

Zhang, Y., and M.S. Kang. 1997. Diallel-SAS: A SAS program for Griffing's diallel analyses. Agron. J. 89:176–182.