

Screening of Wheat Varieties for Resistance to Head Scab Caused by *Fusarium graminearum* Schwabe

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Abstract : In this study, variability in pathogenicity among *Fusarium graminearum* isolates from different locations in Ohio was determined. The most virulent isolates from these locations were chosen and used in segregation studies to evaluate the resistance of some resistant by resistant winter wheat crosses to *Fusarium* head scab.

Differences in pathogenicity levels were significant among isolates even from the same location. Several generalizations can be made about the segregating generations in this study. The results indicated that Ning 7840 carries multiple genes for resistance. In addition, we found that Freedom and Ning 7840 have at least one resistance gene different. Although the level of resistance observed in the resistant by resistant cross appear to be adequate to protect the cultivar against head scab damage, higher levels of resistance would be useful in the future. As more resistant cultivars are identified, some popular but *Fusarium* scab susceptible cultivars, may be replaced.

Key Words: wheat varieties, *Fusarium graminearum*, head scab, resistance

***Fusarium graminearum* Schwabe'un Neden Olduğu Başak Uyuzuna Karşı Dayanıklı Buğday Varyetelerinin Belirlenmesi**

Özet : Bu çalışmada Ohio'da çeşitli bölgelerden elde edilen *Fusarium graminearum* Schwabe izolatlarının patojenisiteleri arasındaki farklılıklar saptandı. Bu izolatlardan en virulent olanlar seçildi ve bazı dayanıklı X dayanıklı kışlık buğday varyetelerinin *Fusarium* başak uyuzuna karşı dayanıklılıklarını değerlendirmek için yapılan segregasyon çalışmalarında kullanıldı.

Patojenisite düzeylerinde, aynı bölgeden alınan izolatlar arasında bile önemli derecede farklılıklar görüldü. Bu çalışmada segregasyon yapan hatlar hakkında bir dizi genelleme yapılabilir. Sonuçlar Ning 7840'in dayanıklılık için multigen taşıdığını gösterdi. Ayrıca Freedom ve Ning 7840'in dayanıklılık genlerinden en az bir tanesinin birbirinden farklı olduğu saptandı. Dayanıklı X dayanıklı buğday melezinde gözlemlenen dayanıklılık seviyesi, buğday çeşidini *Fusarium* başak uyuzuna karşı korumada yeterli görünse de, gelecekte daha yüksek dayanıklılık seviyelerinin ortaya konulması faydalı olacaktır. Daha dayanıklı çeşitler tanımlandıkça bunlar, daha popüler ama duyarlı olan çeşitlerle yer değiştirebilecektir.

Anahtar Sözcükler: buğday çeşitleri, *Fusarium graminearum*, başak uyuzu, dayanıklılık

Introduction

One of the most important yield reducing diseases of winter wheat (*Triticum aestivum* L.) is *Fusarium* head blight or scab caused by *Fusarium graminearum* (teleomorph *Gibberella zeae* (Schw.) Petch). Scab is a destructive disease in the humid and semihumid wheat-growing regions of the world when the weather is favorable for infection (1). Since 1993, *Fusarium* head blight has been a serious problem in the Northern plains states of the US. It has been the most prevalent disease and one of the major yield reducing hazards in Ohio in recent years. Losses to farm income were estimated at \$68 million and \$107 million during 1995 and 1996,

respectively, in Ohio (2, 3). Damage includes yield reduction, loss of grain quality, mycotoxin contamination and seedling blight (4). Scab is best recognized on heads where one or more spikelets or the entire head appears prematurely bleached (5). Bleached spikelets are usually sterile or contain only partially filled seed (6).

It is well known that variability in pathogenicity exists in *Fusarium graminearum*. Variation in the virulence of *F. graminearum* might affect successful selection of *Fusarium* head blight resistant wheats (7).

Reaction to *F. graminearum* is cultivar dependent (7). Reduction in quality is more severe on more susceptible

cultivars. Many investigators have stated that the nature of the scab resistance of wheat is complex. Wheat scab resistance consists of at least two components: resistance to initial infection, and resistance to the spread of the fungus within a spike (8). Resistance to the spread of the fungus within a plant (resistance to hyphal development in the tissues) may vary with stage of maturity (flowering, milk and soft dough stage) and variety (8).

The most effective and economical strategy for disease control is to incorporate genetic resistance into wheat cultivars (9). Characterization of inheritance of resistance to scab in wheat is important for development of resistant cultivars (10). During the past 15 years, hundreds of varieties and thousands of hybrids and selections of *Triticum* spp. have been tested for resistance to Fusarial head blight. No complete resistance or immunity to scab has been observed in wheat. Some progress has been made in determining the number of genetic factors involved in scab resistance.

Some sources from Chinese and Ohio breeding lines exhibit scab resistance (11). In 1994, crosses were made between resistant Chinese lines and adapted Midwestern soft red winter wheats (11). Parents of these crosses were evaluated for resistance in the greenhouse in 1995 (11). Progeny were produced as $P_1 \times P_2 = F_2$ populations in 1995 (11). Seeds from the heads were saved for evaluation as F2 plants. We have just begun evaluating the resistance of some resistant by resistant winter wheat crosses to *Fusarium* head scab. Our overall objective is to investigate the inheritance of resistance to *Fusarium* head blight in these cultivars using a quantitative genetic approach and to estimate the number of effective genes by evaluating resistant:susceptible segregation ratios. Our specific objectives are to determine the variability in pathogenicity (virulence) among *Fusarium graminearum* isolates from different locations in Ohio and to determine if genes for resistance in certain cultivars are the same, by evaluating the reaction of resistant by resistant wheat crosses to *F. graminearum*.

Materials and Methods

Variability in pathogenicity among *Fusarium graminearum* isolates. The isolates of *G. zeae* used for inoculation were obtained from scab infected winter wheats collected at Trumbull Co., Wood Co., Crawford Co. and Seneca Co. during 1996. Heads with spikelets

that were bleached and contained shriveled, discolored seed were collected. Frequently a pink to orange discoloration caused by sporodochia containing conidia, conidiophores and mycelium was observed during high humidity conditions. *Fusarium graminearum* was isolated from the spikelets and seeds displaying these symptoms.

For isolation purposes, water agar and Nash-Snyder medium (12) were used.

Isolate identification was based on growth on carnation leaf agar (CLA) (13). Young carnation leaves (*Dianthus caryophyllus*) were harvested from actively growing plants. The leaves were cut into pieces 5X5 mm and dried in an oven at 45-55°C for 2 h. CLA was prepared by placing several sterile leaf pieces in a petri dish and floating them on 1.5-2.0% water agar cooled to 45°C. The cultures were incubated under fluorescent lights with a photoperiod of 12 h and a day temperature of 25°C and a night temperature of 20°C. Seeds from sampled heads were surface sterilized (in 10% Et OH for 10 sec), rinsed with sterilized water and incubated on CLA in petri dishes under cool-white fluorescent light for 12 h per day from a 40-watt lamp placed 25 cm above the plates for 5-7 days (13). Each isolate was then checked for bacterial contamination. Cultures were examined after 1 and 2 weeks and identified according to Nelson et al. (12). Sixteen isolates were identified as *Fusarium graminearum* (Table 1).

Cultures were initiated from single spores seeded on water agar (12). A suspension of conidia was prepared from each isolate in a 10 ml sterile water blank and the tubes were shaken for 30 with a Vortex-Genie. The suspension was diluted to obtain 1-10 conidia/ low power microscope field when a drop from a 3 mm diameter loop was examined on a slide. This suspension was poured over solidified water agar so as to cover the agar surface, and the excess moisture was drained off. The dishes, thus

Table 1. The list of sixteen *Fusarium graminearum* isolates from five different locations.

Isolate Locations:	Isolate Code Numbers:
Trumbull Co. Variety Clark, OARDC NW Branch,	1-1, 1-2, 1-4, 1-5, 1-6
Wood Co.	2-2, 2-3, 2-6
OARDC NW Branch, Wood Co.	3-1, 3-2
Crawford Co.	4-2, 4-4, 4-5
Seneca Co.	5-5, 5-7, 5-8

seeded, were left half-open on a clean air bench until dry and again covered. The dishes were incubated at room temperature for 16 to 24 h and examined under a dissecting microscope. Small squares of agar containing single germinating conidia were cut out with a needle and transferred to potato dextrose agar (PDA). Cultures were selected based primarily on ability to produce spores.

Although spores produced on CLA normally maintained the original sporodochial morphology, long-term preservation and storage of these cultures were maintained on PDA slant tubes. The possibility of rapid cultural variation on this carbohydrate-rich medium necessitated frequent culture renewal. Colors produced by isolates on PDA slants helped in identification.

Sixteen isolates of the fungus were grown on malt extract agar (MEA) in petri dishes for 1-2 weeks. Conidia were harvested from MEA plates by flooding plates with sterile water and blending the spore suspension from three cultures of each isolate together with sterile distilled water to form a suspension of spores, hyphae and some agar. The conidial concentration was adjusted to 10^5 per ml (11).

The spring wheat variety Bob white was used for pathogenicity tests. The plants grown in pots under greenhouse conditions were inoculated using the single spikelet inoculation technique in which a drop of spore suspension was introduced into the medial spikelets of the head at the time of flowering (14). A conidial suspension was injected into the spikelets at anthesis using a hypodermic syringe with a fine (0.7 mm) hypodermic needle without damaging the epidermis and causing excessive leakage of the fluid being injected. As each plant reached anthesis, one spikelet near the middle of each head of the main tiller was inoculated with approximately 10 μ l of conidial suspension (14). The plants were incubated in a moist chamber on the greenhouse bench at 23-25°C and a relative humidity of 100% for two consecutive nights, and then left on the greenhouse bench where day temperatures range from 19 to 30°C and night temperatures range from 17 to 21°C. Spikelets with obvious symptoms were assessed for disease severity 12-15 days after inoculation. Disease evaluations were based on the proportion of scabbed spikelets per head according to the Horsfall-Barrett scale (15). Two groups of tests were conducted using a randomized block design with four replicate plants per test.

Evaluation of resistant by resistant wheat crosses.

One resistant by resistant winter wheat population was evaluated to determine segregation for resistance to *F. graminearum*. The germ plasm tested included the crosses of a resistant germ plasm line (Ning 7840) with a commercial resistant cultivar (Freedom) (RXR) and with a susceptible line (OH 542) (RXS), plus the parental lines (Table 2). Seeds of 60-75 $F_{2,3}$ families per cross were tested to obtain segregation ratios (Table 2), and 20-30 plants of the parent lines were tested to ensure a good estimation of environmental variation (Table 2).

Table 2. Parents and $F_{2,3}$ families used in tests and number of inoculated plants.

Parents:	Ning 7840	25 plants
	Freedom	25 plants
	OH 542	25 plants
$F_{2,3}$ families:	Ning 7840 * Freedom	60-75 plants
	Ning 7840 * OH542	60-75 plants

Seeds were treated with a suspension of Gustafson LSP Flowable Fungicide (5 ml in 32 ml H_2O) before planting to control seed-borne *Fusarium* contamination.

The experiments were arranged in a completely randomized block design. Each winter wheat genotype was tested by inoculating five or six plants. This resulted in tests of 300-375 plants per population including the two parents and the susceptible check line.

Seeds of the first group of lines and $F_{2,3}$ families were planted in trays containing a greenhouse soil mix on 5 August 1996. Seedlings were put in a cold room to be vernalized at 40°F for seven weeks beginning on 12 August 1996. Seedlings were transplanted into pots 7 weeks later and a second group of the same populations was planted in trays on 21 August 1996. These populations were put into the cold room on 28 August 1996. Each seedling was transplanted into a 15.4 cm pot and maintained in the greenhouse at 18.5-21.1°C. The first group of cultivars was transplanted on 20-25 October 1996 and the second group of cultivars was transplanted on 11-15 November 1996. Pots were kept in the greenhouse until flowering. Artificial inoculation with two highly virulent isolates was used to select for *Fusarium* head blight resistance. Progeny and parents were inoculated as they reached anthesis by placing 10 μ l of conidial suspension of *F. graminearum* in a single

spikelet near the middle of the head using the same inoculation procedure as in the pathogenicity tests (11). One to two heads of each plant were inoculated with five to six plants being inoculated per line. At 17-21 days postinoculation, severity of head blight was measured. Mean percentage of affected spikes/head of the lines were placed in reaction classes and graphed to evaluate progeny reaction distribution. Means represent averages from two greenhouse trials.

Results

Pathogenicity tests. Virulence of sixteen *F. graminearum* isolates from Trumbull Co., Wood Co., Crawford Co. and Seneca Co. was compared. Isolates from these locations exhibited variation in virulence (Figure 1). Isolate differences in virulence were significant (p=0.05). Isolates from Seneca Co. and Trumbull Co. were the most virulent.

The most virulent isolates from these two different locations (1-2 and 5-7) were chosen and used in the segregation studies. In addition, differences in virulence levels were significant among isolates from the same location (i.e., NW Branch and Crawford Co.).

Evaluation of RXR and RXS crosses. Freedom had the highest level of resistance; Ning 7840 and most progeny of the R X R cross were moderately resistant. Progeny of the R X S cross segregated into resistant to susceptible type reactions, whereas OH542 was susceptible (Figure 2). The mean scab severity of F_{2,3} families representing the crosses of Ning 7840 X Freedom (R X R) and Ning 7840 X OH542 (R X S) was close to the midparental value (Figure 3). However, the mean percent scab for F_{2,3} families from Ning 7840 X Freedom was somewhat lower than the midparental value. The R X S cross produced segregating progeny with near-normal distributions, indicating polygenic inheritance. This distribution indicated that the Ning 7840 carries multiple genes for resistance (Figure 2). Progeny of the R X R cross did not have a normal distribution. This indicated that Freedom and Ning 7840 have different genes for resistance because the mean disease level in some of the F_{2,3} families was lower than that of the Freedom and Ning 7840 parents and the variation in the F_{2,3} families was greater than that of the parents. Segregation for resistance was evident between crosses involving resistant parents, suggesting the presence of different alleles for disease reaction between the resistant parents. Segregation distribution of the F_{2,3}

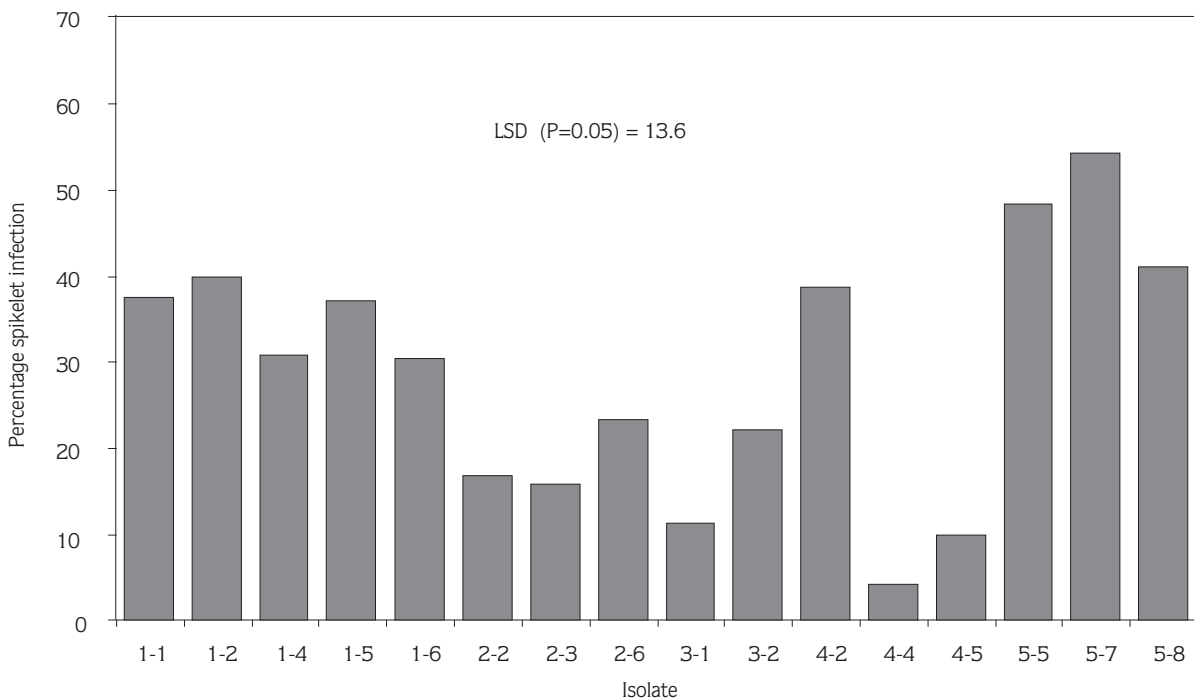


Figure 1. Isolate differences in virulence levels.

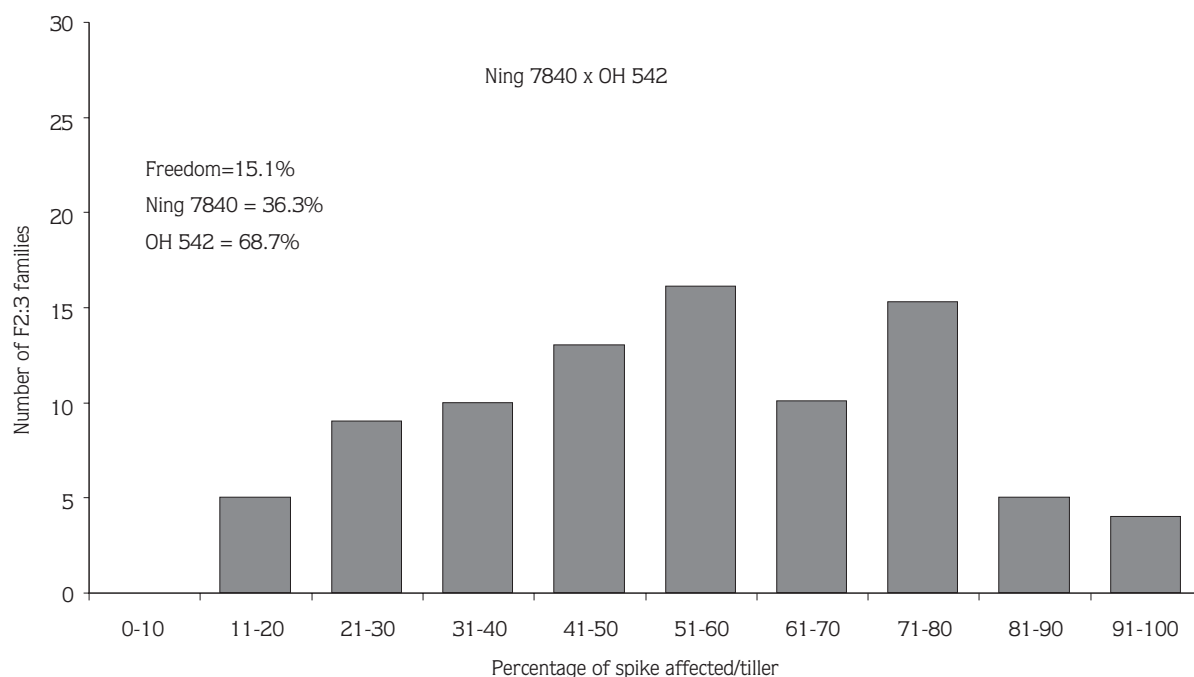


Figure 2. The mean percentages of the affected spikes/head of the $F_{2:3}$ families representing the crosses of Ning 7840 X OH 542 (R X S).

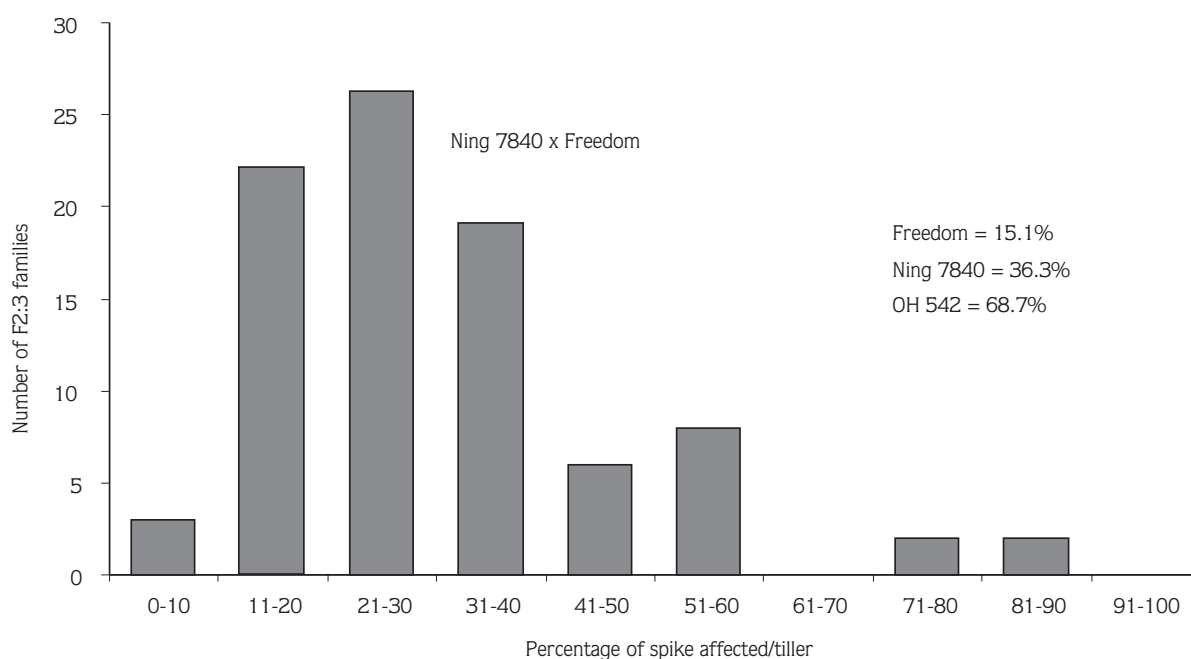


Figure 3. The mean percentages of the affected spikes/head of the $F_{2:3}$ families representing the crosses of Ning 7840 X Freedom (R X R).

families of the cross between the two resistant parents indicates a difference in at least one resistance gene.

Discussion

The results we obtained in the pathogenicity tests confirmed previous studies (14) that there were

differences in the abilities of different isolates of *F. graminearum* to colonize wheat heads. A more virulent isolate of *F. graminearum* occurred in one location than in the other. In addition, differences in virulence levels were significant among isolates from the same location (i.e., NW Branch and Crawford Co.). The most virulent isolates were used to screen for scab resistance. The reason for evaluating resistance with highly virulent isolates was to ensure the identification of high levels of resistance and to reduce experimental error (7).

The Horsfall-Barrett scale with 10 categories of infection was used in this study (15). Schroeder and Christensen (1) found that the number of infected spikelets could be directly correlated with the number of infected kernels, which substantiates the usefulness of a visual assay. Wilcoxson et al. (7) reported that in spring wheat cultivars the percentage of infected spikelets provided a better visual estimation of resistance than the percentage of infected heads, as it reflected the product of disease incidence and severity. Since we screened large numbers of populations consisting of unique individuals (F_2 populations), this rate scale precluded precise counting of the total number of spikelets and the number of scabbed spikelets.

Characterization of major resistance components in cultivars with different levels of resistance may provide useful information for the effective use of resistance sources in breeding resistant cultivars. There are several observations that will enable us to work on genetic problems. First, we must determine the number of heterozygous gene pairs involved in the crosses. Before accepting a hypothesis, we should consider the possible gene combinations that control the resistance. Freedom and Ning 7840 are known to have moderate to high levels of resistance to scab. Both are speculated to have multiple genes. If Freedom and Ning 7840 had the same genes, we would have no segregation in F_2 and also no segregation in F_3 and variation in the $F_{2,3}$ families would be equal to that of the Freedom parent and the Ning 7840 parent. If Freedom and Ning 7840 had different genes for resistance, the mean disease level in the $F_{2,3}$ families would be lower than that of the Freedom parent and the Ning 7840 parent (i.e., some susceptible progeny) and the variation in the F_2 derived F_3 lines would be greater than that of the parents. If the two resistant parents each carried two genes for resistance, three possible patterns of segregation would exist. If

Freedom and Ning 7840 had different genes, one gene each (i.e., Freedom=AA, Ning 7840 =BB, F_1 =AaBb), the F_2 would segregate in a 15:1 ratio. If the genes were additive, we would get a 1:4:6:4:1 segregation pattern. If the two genes in Ning 7840 were different from the two genes in Freedom (i.e., Freedom=AABB, Ning7840=CCDD, F_1 = AABbCCDD) the F_2 would segregate in a 255:1 ratio. However, our population was not large enough to detect this ratio. If Freedom and Ning 7840 had one gene in common and one gene different (i.e., Freedom=AABB, Ning 7840=AACC, F_1 =AABbCc) we would expect the F_2 to have no segregation at the A locus and 15:1 segregation at the B and C loci, but with lack of segregation at the A locus and the overall resistance in the population would be higher than in the model where Freedom and Ning 7840 had different genes. If all these genes had additive gene action, we would not be able to detect the difference between a two and four gene model because progeny would segregate in a normal distribution.

Several generalizations can be made about the segregating generations in this study (Figures 2 and 3). The distribution of families of the R X S cross indicates that there are many genes conditioning the resistance in Ning 7840 because we had very normal distribution. In Ning 7840, probably two or more genes are involved in resistance. At least one of these genes, is heterozygous and probably one of them is codominant. Most progeny had mean disease values between Ning 7840 and OH542 (Figure 2). Alleles were homozygous because dominant alleles representing different genes did not segregate. Similarly, recessive alleles from different parents segregated as susceptible progeny. We can conclude that at least one gene in that cross was heterozygous.

Progeny from the R X R cross did not segregate into a normal distribution. In this cross, we had some families with disease levels even lower than those of Freedom and we had some progeny with levels greater than those of the susceptible check OH 542. This indicated that some lines were more resistant than the resistant parent, some lines were more susceptible than the susceptible parent and a range existed between the extreme genotypes. We can conclude from the distribution of the percentage spikelet affected per tiller that there are many genes conditioning disease resistance and these genes was derived from different parents and apparently are different from one another. The resistance allele is

dominant and the susceptible allele is recessive. This study indicated that each cultivar possessed resistance for fungal spread within the wheat head, and Freedom and Ning 7840 have at least one resistance gene different.

Resistance to scab spread within a spike is a quantitative trait. Disease severity was measured in two different greenhouses, and this allowed us some flexibility in carrying out the inoculations.

A set of control cultivars, each with a known degree of resistance or susceptibility, was helpful for assessing experimental error in large-scale experiments. Subtracting the environmental variation, the parents from the total variation found among the F₂ progeny would give an indication of the genetic variation for this trait within the cross, and an indication of the broad sense heritability or the proportion of genetic vs. environmental variation in the greenhouse (Campbell, K., personal communication). There are formulas to determine gene number from this variation as well. Cultivar Freedom and Ning 7840 showed consistently

lower scab severity than OH 542 and F_{2,3} families, indicating that resistance to spread of scab within a spike was relatively stable. OH 542 was consistently, highly susceptible in all trials and was an adequate susceptible control for genetic studies. Overall, resistant parents produced resistant progenies, while susceptible parents yielded susceptible ones. Distribution of reaction classes of Ning 7840 X Freedom F_{2,3} families indicated different genes probably condition the resistance observed in these two cultivars. Fortunately the level of resistance observed in the R X R cross appear to be adequate to protect the cultivar against head scab damage. However, higher levels of resistance would be useful in the future. Different genes from these sources might be pyramided into single lines, and then the lines could serve as parents in wheat improvement programs for head scab resistance. The final goal of a breeding program, however, should be to develop cultivars with the greatest degree of resistance possible.

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