INHERITANCE OF RESISTANCE TO WHEAT STREAK MOSAIC VIRUS IN WHEAT LINE KS06HW79

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Abstract

Wheat streak mosaic virus (WSMV) is a disease that causes significant yield losses in wheat (*Triticum aestivum* L.). Host resistance is the primary approach for control. KS06HW79 is a wheat line with WSMV resistance up to 21^{o}C. To study the inheritance of resistance in KS06HW79, it was crossed with two WSMV-susceptible wheat genotypes, KS020638-M-5 and Brawl CL Plus. Parental lines, F_1, F_2, and check varieties were mechanically inoculated and evaluated for WSMV resistance at 21°C in growth chambers. The segregation pattern in two F_2 populations fit a one-recessive-gene model (1 resistant : 3 susceptible) and a dominant-suppression-epistasis model (3 resistant : 13 susceptible). To determine which model was a better fit, WSMV resistance was evaluated for $F_{2:3}$ families generated from resistant F_2 plants in both crosses. Approximately two thirds of the $F_{2:3}$ families in each cross showed segregation for WSMV resistance, suggesting that the dominant-suppression epistasis model better explained the WSMV resistance in KS06HW79. This model was also supported by two KS06HW79-derived doubled haploid populations, which had a segregation ratio of 1 resistant : 3 susceptible. Therefore, the WSMV resistance in KS06HW79 is likely controlled by two dominant genes, one of which is a suppressor.

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Chapter 1 - Literature Review

Wheat

Origin

As a staple crop grown worldwide, common wheat (*Triticum aestivum* L.) is one of the most intensively researched due to its ubiquity across cultures and food systems, though its exact origin and taxonomy are still subject to debate. The emergence of *T. aestivum* can be approximately traced back to the beginning of the Neolithic Revolution when hunter gatherers in western Asia transitioned into a form of modern agriculture, and three cereal crops were domesticated: barley, einkorn, and emmer. It is currently thought that emmer was domesticated in Jordan, einkorn in Turkey, and barley in the fertile crescent (Harlan 1966). While this probably occurred approximately 10,000 years ago, it took another one thousand years for the more familiar hexaploid bread wheat to arrive (Feldman 2001) through various ploidy changes. Due to the complex evolution and ploidy nature of wheat, its nomenclature is often confusing. Today's wheat is a hexaploid (2n = 6x = 42), with three distinct genomes: AA, BB, and DD. This is a result of domestication via allopolyploidy, where an organism's genomes are derived from two or more different species. *Triticum urartu* (2n=14, genome AA) and *Aegilops speltoides* (2n=14, genome BB) hybridized and polyploidized approximately 400,000 years ago. The tetraploid wheat progeny, termed *Triticum turgidum* (2n=28, genome AABB) underwent another hybridization with goat grass diploid *Aegilops tauschii* (2n=14, genome DD) approximately 8,000 years ago on Caspian Sea coastal areas to form modern wheat, *Triticum aestivum* (2n=42, genome AABBDD) (Wang et al. 2013).

Distribution

Most wheat grown today is categorized according to the season it is grown in, the grain color, and/or kernel hardness. “Winter wheat” seeds are planted in fall, remain vegetative over winter, and are harvested in early summer. “Spring wheat” seeds are usually planted in spring and allowed to mature in late summer. In the United States, six main wheat classes are grown: durum, hard white, soft white, soft red winter, hard red spring, and hard red winter. The hard red winter wheat class is the most important, comprising approximately 41% of total wheat production in the USA (Curtis et al. 2002). At 732.8 million tons in 2015, wheat is the world’s third highest-produced crop that provides approximately one-fifth of human caloric consumption (USDA 2015). While the hexaploid *T. aestivum* accounts for approximately 95% of wheat grown today, the tetraploid durum wheat, probably derived from domesticated emmer, makes up the last 5% (Feldman and Kislev 2007, Luo et al. 2007) and is mainly used in for semolina flour and macaroni pasta production. The other hulled wheats, such as einkorn, emmer, and spelt, have been mainly relegated to historical importance, but may serve as reserves of genetic diversity (Zohary et al. 2012).

Wheat Streak Mosaic Virus

Taxonomy

Wheat streak mosaic virus (WSMV) was first described in Nebraska in 1922 as “yellow mosaic” (Hunger 2010), and reports of infection have been increasing since then (Hadi et al. 2011). WSMV is well-described and known for infecting plants in the *Poaceae* family, primarily wheat (*Triticum aestivum*). Named for the discoloring yellow streaks on the leaves of its host, WSMV has been under heavy investigation since its discovery due to its global distribution and systematic reduction of yield. The entire nucleotide sequence of WSMV was published by

Stenger et al. (1998), categorized in family *Potyviridae* with other known mite-transferred viruses, and noted to be a 9,384 nucleotide-long RNA. Phylogenetic analyses further revealed that it is closer related to a whitefly-transmitted virus, leading to WSMV's removal from the *Rymovirus* genus and becoming the type member, or defining example, of the new genus *Tritimovirus* within the family *Potyviridae* (Stenger et al. 1998).

Population evolution and distribution

Research on the origin and evolution of WSMV is an ongoing process, as comprehensive analysis of plant virus populations is a relatively new field. Current literature available suggests that WSMV populations in North America diverged from a common ancestor introduced not long after native grasslands in the Great Plains were removed and wheat monoculture instated. Resultant reductions of genetic diversity in WSMV field populations ("bottlenecks") due to wheat harvest can be severe, though mutations affecting virus population fitness are neutral (Stenger et al. 2002, French and Stenger 2003, 2005). A survey attempting to characterize the global extent of genetic diversity in WSMV populations found that two distinct clades existed in populations present in the Pacific Northwest, along with a significant amount of genetic diversity. Additionally, many of the specimens collected may have contained multiple WSMV isolates, since recombination was found to have taken place within the isolates (Robinson and Murray 2013). More phylogenetic analyses are needed to fully understand the origins and implications of WSMV populations.

Symptoms on wheat

WSMV symptoms begin as tiny chlorotic lines on infected host leaves, which grow longer and form yellowish green streaks, and eventually forming a mosaic pattern. Stunted plant growth is a common feature of WSMV, and serious cases of infection show large chlorotic areas

of merged stripes on leaves, eventually leading to necrotized tissue and death of the plant. At the field level, margins are usually the first to show infection since they are closest to areas with other grasses and crops that serve as shelters for the mite vectors of WSMV (Hunger 2010). Infected fields may show stunted and yellowed plants in a seemingly non-uniform pattern, though weed or volunteer plant hosts are often nearby, and infection often proceeds further within the field as the season progresses. On the microscale, multiple cellular phenomenon can be used to diagnose WSMV according to Gao and Nassuth, including amorphous and cylindrical inclusion bodies accumulating (1992), deformed chloroplasts and nuclei (1993), and membranes growing on the walls of bundle sheath and mesophyll cells (1994).

Yield loss and damage

WSMV has been shown to significantly reduce yields in wheat plants, and losses can be compounded by other biotic and abiotic factors. Water use efficiency and root biomass both have been shown to be reduced by WSMV infections, which exacerbates problems in drought-stricken areas (Price et al. 2010). Drought environments in the Great Plains have been documented to dramatically increase the synergy between WSMV and *Triticum mosaic virus* (TriMV) in several wheat cultivars, leading to unexpected levels of high yield loss (Byamukama et al. 2012, Tatineni et al. 2010). The ability to photosynthesize is reduced by leaf chlorosis and necrosis due to WSMV, and it has also been linked to plant stunting (Langham et al. 2001), lowering the test weight of grain (Atkinson and Grant 1967, Langham et al. 2001), and reducing seed set (Atkinson and Grant 1967). Wheat plants infected by WSMV have shown grains with increased protein content but produce flour with decreased water absorption in comparison with control wheat (Atkinson and Grant 1967). Time of infection by WSMV has been correlated with yield loss due to infection, and the earlier infection occurs in the stages of plant growth the more

severe the yield loss (Hunger et al. 1992). While WSMV has been reported in spring wheat, it is most commonly found on winter wheat. Both types of wheat have shown decreased yield and test weight along with stunting of plants (Langham and Glover 2005). Yield losses due to WSMV have been reported in wheat-growing regions worldwide, including Canada, Europe, Russia, the USA, and most recently Australia (Fahim et al. 2012a). The greatest annual yield losses recorded have occurred in Kansas, USA at 13% and in Alberta, Canada at 18% (Atkinson and Grant 1967, Sim et al. 1988). Artificial inoculation experiments have shown yield losses due to WSMV can be as high as 74% (Rahman et al. 1974, Sharp et al. 2002), and naturally infected field losses of 100% have been reported (McNeil et al. 1996).

Wheat Curl Mite, Vector of WSMV

Origin/Taxonomy

The wheat curl mite (WCM, *Aceria tosichella* Keifer) has a long taxonomic history. First described as a pest of tulips, it was initially termed *Aceria tulipae* Keifer (Keifer 1938), though it was observed to feed on wheat as well as plants in the onion and lily genera. Subsequently, many publications (del Rosario and Sill 1965, Slykhuis 1956, Nault and Styer 1969) used *A. tulipae* when describing its vectoring of WSMV after the first such documentation by Slykhuis (1955). The first publication on *A. tosichella* was not until 1969 by its eponymous author, Keifer, who described it on wheat and barley in Yugoslavia (Keifer 1969). Additionally, the *Aceria* genus was at first merged with the *Eriophyes* genus (Newkirk and Keifer 1971) due to morphological similarities, but was reestablished as its own genus in 1989 (Amrine and Stasny 1994). While most eriophyoid mites restrict themselves to specific hosts within one plant genus (Skoracka et al. 2010), *A. tosichella* appears to be the exception with approximately 90 documented *Poaceae* hosts (Amrine 2003), thereby classifying it as a wide-ranging plant host generalist (Sabelis and

Bruin 1996). Two genetically distinct lineages of WCM were identified by Hein et al. (2012) and designated “Type 1” and “Type 2”. The main differences between the Type 1 and Type 2 genotypes are their responses to wheat resistance genes (Harvey et al. 1999) and vectoring of different viruses. Type 1 transmits the High Plains and TriMV at a much lower rate than does Type 2 (Seifers et al. 2002, McMechan et al. 2012). Additionally, only Type 2 have been shown to transmit WSMV in Australia (Schiffer et al. 2009), while it is transmitted by both types in the U.S. (Seifers et al. 2002).

Description

Wheat curl mites are white, cigar-shaped, and usually less than 0.3 mm long (Jeppson et al. 1975), and therefore not visible to the naked eye. Wheat curl mite life cycles are comprised of an egg, two nymph instars, and an adult stage. On average, this cycle is completed within one week at 25°C. In addition to high humidity, green leaves on a plant host are critical if the mite is to survive, as they provide a source of food and shelter. At any stage of the life cycle, wheat curl mites can live on the crown of perennial grasses or winter wheat to survive near-freezing temperatures for several months. Two to three days of 5°C are often deadly to the nymph and adults, while eggs have been observed to be much more hardy in the same conditions. WSMV is transmitted by both the nymph and adult stages of wheat curl mite, but the nymph is the only stage where virus acquisition occurs (Slykhuis 1955).

Dispersion

Mites are wingless, so their main dispersal method is via wind (Slykhuis 1955), though they have been documented to travel longer distances on winged insects (Gibson and Painter 1957). When host plants desiccate, wheat curl mites gather in large numbers on the tip of leaves or flowers, occasionally forming chains that are carried away by the next wind (Jeppson et al.

1975). Typically, wheat curl mites have two annual peak dispersal times: during July, and during late August through early October. Subsequently, the rate of dispersion to winter wheat has been observed to slowly decrease until November (Nault and Styer 1969), though it increases in the summer months due to mature, desiccating host plants (Liu et al. 2005).

Transmission of WSMV

Nonviruliferous WCM infestations can reduce wheat yields by up to 17% (Harvey et al. 2002), but the greatest economic impact caused by WCM is as the primary vector of multiple viruses, especially WSMV (Navia et al. 2013). Though characterized, the transmission of WSMV by WCM needs further study. Four primary modes of transmission have been described in *Hemipteran*-virus relationships: non-persistent, semi-persistent, persistent circulative, and persistent propagative. These classes are defined according to virus acquisition by the vector, periods of latency and retention inside it, transstadial/transovarial (via life stage or parental infection, respectively) passage through it, circulation of the virus throughout its hemolymph (the mite analog of blood), and replication of the virus inside it (Ng and Falk 2006). WSMV transmittance by the WCM has been suggested to be semi-persistent since the virus has been documented in both the salivary glands and hemocoel, or "veins", of the mite. WSMV has also been documented to survive up to five days in the mite's midgut (Paliwal 1980). WSMV can be taken up by the mite from an infected host plant after just fifteen minutes of feeding. Transmission efficiency is lowered with shorter acquisition times, therefore longer feeding times by the mite results in increased transmission ability (Orlob 1966). Viability of WSMV inside the vector is a function of environmental temperature. Slykhuis (1955) found WSMV at room temperature to be retained in mites for up to six days, while Orlob (1966) found that viruliferous mites maintained under 3°C were still able to infect plants after two months. A possible

explanation for this is that mites develop slower under near-freezing temperatures and therefore promote longer internal virus maintenance (Orlob 1966). WSMV has also been documented to be spread by seed, but such occurrences are considered a low risk in virus-free areas, and an insignificant risk in regions already affected by WCM and WSMV (Lanoiselet et al. 2008).

Management of WCM and WSMV

Disease cycle

The disease triangle, first alluded to by Duggar (1909) and conceptualized by Stevens (1960), describes the interaction between a pathogen, a host, and an environment that can result in a disease. This system can be expanded to include plant-virus-vector interactions, where an arthropod vector transmits a virus to the host plant (Gray and Banerjee 1999). In this case, the host plant can refer to weeds, but is more often wheat or corn. For winter wheat, WSMV infections may occur in fall when mites carrying the virus migrate from previously infected plants, which can be annual grass weeds or crops, onto new seedlings (Connin 1956, Christian and Willis 1993). Infection at such an early life stage of winter wheat plants has been shown to be associated with the highest yield losses (Slykhuis et al. 1957, Hunger et al. 1992). Subsequently, these young infected plants become a source of further infection in their neighbors. While WSMV can infect winter wheat in the springtime, resultant yield losses are usually insignificant (Somsen and Sill 1970). However, winter wheat and other perennial grasses infected with WSMV in the spring may function as reserves of mite and virus sources that infect spring wheat plants. Depending on weather, mite field dispersal is highest between May and June when winter wheat matures. Any spring wheat fields planted near infected winter wheat plants run a much higher risk of WSMV infection (Langham and Glover 2005). Volunteer wheat seedlings, which can be derived from spikes shattered by hail, may come from mite-infested

kernels. These seedlings have been shown to be instantly colonized by wheat curl mites (Gibson and Painter 1956). These infested seedlings may serve as a "green bridge" that allow both mite and virus to survive in between crop plantings (Gibson and Painter 1957, Thomas et al. 2004). Additionally, volunteer wheat grows particularly well with summer rains, which in turn allows higher mite growth and survival rates and greater chance for WSMV infection (Connin 1956, Christian and Willis 1993, Thomas et al. 2004).

Chemical control

While many agricultural pests and diseases can be readily curbed by herbicides, pesticides, or fungicides, any attempts at chemical control of WSMV have been shown to be ineffective. Several pesticide application tests in the 1950s showed no effect on wheat curl mites, with some actually showing a dramatic increase in mite population post-application (Kantack and Knutson 1958). This is most likely due to the mites staying near their food source, protected in the leaf whorls or spike recesses (Hein 2010). Studies of systemic insecticides on wheat curl mite have found that a granular carbamate pesticide was able to lower mite populations and WSMV infection when applied in fall (Harvey et al. 1979), but a spring application did not show such success. Additionally, a study on imidacloprid seed treatment found no reduction in mite or WSMV occurrence when compared to untreated controls (Harvey et al. 1998).

Cultural control

A crucial source of mites and WSMV infection early in season are volunteer wheat plants in wheat fields (Thomas et al. 2004). Early pre-planting steps such as removing possible mite and WSMV sources can reduce the risk of WSMV infection (Slykhuis 1955). Success has also been found by sowing winter wheat in a field after the wheat plants in close proximity to the sown seeds desiccate (Slykhuis et al. 1957). Thomas et al. (2004) showed that migrating mite

populations can be suppressed by destroying volunteer wheat and wild grasses via herbicide or tillage two weeks before planting. However, a more effective approach appears to be destroying weeds and volunteer wheat plants three weeks in advance of the planting date, using tillage in dry conditions and either tillage or glyphosate in wet conditions (Thomas et al. 2004, Jiang et al. 2005). Additionally, spring wheat should not be planted near or in infected fields of winter wheat in order to prevent the spread of mites and virus (Langham and Glover 2005). Wosula et al. (2015) documented the temperature and humidity levels that contribute to the survival of WCM off-host, and reiterated the crucial practice of removing volunteer wheat before planting.

Transgenic control

Public acceptance of transgenes is a matter of controversy, but genetic modification is still a useful analytical tool for virus-plant interactions. The first attempt at transgenic control of WSMV in wheat was use of the RNAi (RNA interference) method by Sivamani et al. (2000), who transformed *T. aestivum* embryos with the replicase gene (*NIb*) of WSMV. Li et al. (2005) performed experiments with the WSMV coat protein (CP) gene in wheat, and Fahim et al. documented resistance to WSMV by designing a hairpin construct of its *NIa* protease (2010), and a construct that concurrently targeted five different WSMV genes (2011). Most recently, Cruz et al. (2014) demonstrated stable resistance to WSMV after selection in multiple generations by generating a hairpin construct using part of WSMV's CP. While transgenic applications can provide answers in non-production settings, they are still constrained by current consumer hesitation, leaving conventional methods as the primary venue for conferring WSMV resistance.

Breeding for host resistance to WCM and WSMV

Breeding efforts to reduce the effects of WSMV in wheat have been comprised of two main approaches: vector control and virus resistance. The first approach attempts to prevent the reproduction, movement, and feeding of the mite vectors, while the second focuses on increasing or conferring virus resistance genes into host plants.

Host resistance to WCM

Conventional breeding has shown some success in controlling the wheat curl mite (WCM), beginning with varieties identified by Martin et al. (1984) that displayed resistance to the wheat curl mite. This led to the release and widespread use of the resistant cultivar 'TAM 107' which derived its WCM resistance from a rye (*Secale cereale*) translocation. However, WCM was observed to have the ability to colonize TAM 107 in Kansas (Harvey et al. 1999). Since then, sources have been documented that confer plant resistance to wheat curl mite colonization ("*Cmc*"). *Cmc1* was transferred from the D-genome donor of modern wheat, *Aegilops squarrosa* (Thomas and Conner 1986). The *Cmc2* gene was transferred from *Thinopyrum ponticum* (Whelan and Hart 1988). *Cmc3* is a single dominant gene derived from a rye translocation, and *Cmc4* was derived from *Aegilops tauschii* (Malik et al. 2003). Current efforts to identify sources of WCM resistance have found wheat relatives *Thinopyrum intermedium*, *Thinoypyrum ponticum*, and *Hordeum marinum* to be promising, especially two chromosomes contained in *Th. intermedium* addition lines (Richardson et al. 2014).

Host resistance to WSMV

Breeding for host resistance to WSMV has shown some progress since resistance sources have been identified in both common wheat and its wild relatives, though it can be difficult to use genes from wild sources due to poor agronomic traits associated with alien fragments. The

first identified gene conditioning WSMV resistance from a wheat relative was in a translocation from the short arm of chromosome 4D in wheatgrass (*Thinopyrum intermedium*) and designated *Wsm1* (Friebe et al. 1991, Gill et al. 1995, Wells et al. 1973, 1982). Several markers are diagnostic of *Wsm1*: a PCR (polymerase chain reaction) fragment that is 241 base pairs long (Seifers et al. 2007, Talbert et al. 1996), some expressed sequence tag (EST)-based markers from the 4DS wheat chromosome (Qi et al. 2007), and the sequence tagged site (STS) marker *XBG263898* (Liu et al. 2014). *Wsm1* has been associated with lower field yield losses due to WSMV, though yield penalties were reported in un-inoculated lines carrying the *Wsm1*-bearing translocation (Sharp et al. 2002). To remedy this, Friebe et al. (2009) generated recombinants of the chromosome's translocation to shorten the fragment carrying *Wsm1* and reduce the yield penalties. Graybosch et al. (2009) subsequently released 'Mace', a hard red winter wheat that was the first to carry the shortened fragment with *Wsm1*. A WSMV resistance source was identified from a wheat-*Th. intermedium* ditelosomic addition line, which had a telechromosome from the long arm of a group-7 chromosome from *Th. intermedium*'s S genome, designated 7S#3L. A compensating Robertsonian translocation was generated where 7S#3L was translocated onto the short arm of wheat chromosome 7B, which resulted in the translocation chromosome T7BS·7S#3L that carries the WSMV resistance gene *Wsm3* (Friebe et al. 2011). Fahim et al. (2012a) reported resistance sources derived from amphiploids of wheat with *Th. scirpeum* and *Th. intermedium*. Cultivated wheat has also been discovered to show WSMV resistance. Genetic studies on experimental winter wheat line CO960293-2 found a single dominant WSMV resistance gene on the short arm of chromosome 3B (Lu et al. 2011), although the exact origin of the resistance is uncertain due to the WSMV susceptibility found in both parents of CO960293-2 (Haley et al. 2002, Seifers et al. 2006). The gene was designated *Wsm2*,

and a simple-sequence repeat marker, *Xbarc102*, was found to be 3.9 cM from it (Lu et al. 2011, 2012, Tan et al. 2016). *Wsm2* has been successfully introgressed into cultivars 'RonL' (Seifers et al. 2006), 'Snowmass' (Haley et al. 2011), 'Clara CL' (Martin et al. 2014), and 'Oakley CL' (Zhang et al. 2015), though several other wheat lines with WSMV resistance have been documented. Both KS03HW12 (Seifers et al. 2007) and CO960333 (Seifers et al. 2013b) are from the U.S., and several lines have been identified in Iran (Masumi et al. 1999, Hassani and Assad 2004a, b). Seifers et al. (2013a) screened 2,429 USDA-ARS National Small Grains Collection wheat accessions, and identified 20 lines that, depending on WSMV isolate used, did not show WSMV symptom development for up to 21 days when kept at 18°C. Thus far, all documented sources of WSMV resistance are effective only up to a specific temperature, known as temperature-sensitive resistance (TSR). *Wsm2* provides TSR up to 18°C, *Wsm1* up to 20°C, *Wsm3* up to 24°C (Lu et al. 2011, Gill et al. 1995, Seifers et al. 2013b, Liu et al. 2011), and Fahim et al. (2012b) documented a doubled haploid line (c2652) with WSMV resistance up to 28°C. However, wheat lines carrying *Wsm2* have been documented to show susceptibility to WSMV in the field under warm conditions. Therefore, further sources of resistance that are efficacious at higher temperatures are needed as global temperatures increase. Seifers et al. (2013b) documented one such source in wheat line CO960333 that was effective up to 21°C, and derived line KS06HW79 via the cross CO960333-1/KS99HW41//KS99HW37. However, the genetic basis of the resistance in CO960333 and KS06HW79 are unknown, and determining its control will allow the incorporation of this new source into elite cultivars. This thesis examines the genetic inheritance of the resistance to WSMV in KS06HW79.

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Chapter 2 - Inheritance of resistance to wheat streak mosaic virus in wheat line KS06HW79

Introduction

Wheat streak mosaic virus (WSMV) is a globally distributed plant pathogenic virus from the family *Potyviridae*, genus *Tritimovirus* (Stenger et al. 1998). WSMV is vectored by the wheat curl mite (WCM: *Aceria tosichella* Keifer) (Navia et al. 2013). WSMV-infected plants typically display yellow streaks on leaves, stunted growth, and yield reduction, as well as decreased root development and water use efficiency (Price et al. 2010). WSMV has caused great economic impacts in regions where its primary host, wheat, is the major crop. Wheat yield losses due to WSMV have been reported in Canada, Europe, Russia, Australia, and the USA (Fahim et al. 2012a), with the most severe yield losses reported in Kansas, USA at 13% and Alberta, Canada at 18% (Atkinson and Grant 1967, Sim et al. 1988). Tests using artificial inoculation of WSMV have shown yield losses up to 74% (Rahman et al. 1974, Sharp et al. 2002). Field losses of up to 100% have also been reported (McNeil et al. 1996).

There are no effective chemicals to control WSMV or its vector, leaving host genetic resistance as the primary control method. Two *Aegilops*-derived genes, *Cmc1* and *Cmc4* (Thomas and Conner 1986, Malik et al. 2003), one *Thinopyrum*-derived gene (*Cmc2*), and one rye-derived gene, *Cmc3* (Whelan and Hart 1988, Malik et al. 2003), have been identified that confer resistance to WCM plant colonization. However, the ability of WCM to rapidly overcome such resistance genes (Harvey et al. 1995, 1997, 1999) has made this type of host resistance less attractive. Alternative efforts have investigated host resistance to the virus itself. WSMV resistance genes discovered in both wheat and its wild relatives have been shown to be effective. The initial source of WSMV resistance was derived from a translocated chromosome fragment

of an intermediate wheatgrass, *Thinopyrum intermedium*, which provided the first described gene to confer WSMV resistance and designated *Wsm1* (Friebe et al. 1991, Gill et al. 1995, Wells et al. 1973, 1982). WSMV-inoculated lines carrying *Wsm1* showed reduced yield losses in field trials, but yield penalties from 11% to 28% were also reported due to the translocation (Sharp et al. 2002). To reduce the yield penalty, the *Wsm1*-carrying fragment was shortened through backcrossing and selection (Friebe et al. 2009). 'Mace' was released as the first cultivar containing *Wsm1* on the shortened fragment (Graybosch et al. 2009). Another resistance gene from *Th. intermedium* termed *Wsm3* has been recently identified and transferred into wheat backgrounds (Friebe et al. 2011). A resistance source from another wild species, *Th. scirpeum*, has also been reported (Fahim et al. 2012a). WSMV resistance from wild wheat relatives is potentially useful, but possible yield penalties due to alien fragments have limited its application in breeding programs. Fortunately, WSMV resistance has also been discovered in cultivated wheat.

The first WSMV resistance source from common wheat was discovered in breeding line CO960293-2, though its exact genetic origin is unclear since its parents were both susceptible to WSMV in growth chamber and greenhouse experiments (Haley et al. 2002, Seifers et al. 2006). A genetic study revealed that the WSMV resistance in CO960293-2 was controlled by a single dominant gene (Lu et al. 2011). Genetic mapping via simple sequence repeat (SSR) markers further located this gene on the short arm of chromosome 3B. This gene was designated as *Wsm2*, and the closest marker, *Xbarc102,* was located 3.9 cM proximal to it (Lu et al. 2011, 2012, Tan et al. 2016). *Wsm2* has been successfully incorporated into a number of wheat varieties, including 'RonL' (Seifers et al. 2006), 'Snowmass' (Haley et al. 2011), 'Clara CL' (Martin et al. 2014), and 'Oakley CL' (Zhang et al. 2015). Several other WSMV-resistant wheat

germplasm lines have been identified, including wheat lines KS03HW12 (Seifers et al. 2007) and CO960333 (Seifers et al. 2013b) from the U.S., several wheat lines from Iran (Masumi et al. 1999, Hassani and Assad 2004a, b), and a source from a doubled haploid wheat line (Fahim et al. 2012b). Most recently, Seifers et al. (2013a) identified 20 WSMV-resistant wheat lines out of the USDA-ARS National Small Grains Collection.

All known WSMV resistance sources are temperature sensitive, regardless of being derived from wheat relatives or common wheat. *Wsm1* is effective up to 20°C, while *Wsm3* provides effective resistance up to 24°C (Friebe et al. 2011, Seifers et al. 2013b). So far, most of the resistant sources from common wheat have shown WSMV resistance up to 18°C (Seifers et al. 2006, 2007, 2013a). However, CO960333 and its derived line, KS06HW79, through selection from a cross of CO960333-1/KS99HW41//KS99HW37, provide resistance up to 21°C (Seifers et al. 2013b). Wheat varieties with *Wsm2* have been found to be susceptible under warm field conditions in western Kansas. Therefore, incorporation of WSMV resistance that is effective at higher temperatures, such as the resistance in KS06HW79, is needed. However, little is known about the genetic basis of WSMV resistance in KS06HW79 or CO960333. With a full understanding of its genetic control and identification of the linked molecular markers, breeding programs could efficiently incorporate this resistance into superior varieties. The objective of this study was to determine the inheritance pattern of WSMV resistance in KS06HW79.

Materials and Methods

Two WSMV-susceptible wheat accessions, KS020638-M-5 and 'Brawl CL Plus', were each crossed with KS06HW79. KS020638-M-5 (KS940786-17-2/Jagalene//Trego) is a breeding line developed by the wheat breeding program at Kansas State University, Manhattan, KS. Brawl CL Plus (PI 664255, Haley et al. 2012) is a Tazamox-tolerant wheat variety released by the

Colorado Agricultural Experimental Station. F_1 seeds were obtained from the two crosses and some F_1 seeds from each cross were grown to produce the F_2. F_1 and F_2 plants from each cross were then evaluated for WSMV resistance together with their parental lines and check varieties, RonL (PI 648020), KS96HW10-3, and 'Tomahawk' (PI 552814). RonL is a wheat variety carrying *Wsm2* and was developed by the Kansas State University wheat breeding program at Hays, KS. KS96HW10-3 is a *Wsm1*-carrying breeding line developed by the Kansas State University wheat breeding program at Hays, KS. Tomahawk is a WSMV-susceptible variety released by AgriPro in 1991.

Seeds were planted in short rows in 30 by 50 cm metal flats filled with potting mix (Sungro®, Canada). Eleven seeds were planted in each row. According to the number of F_2 seeds, the experiment for the cross of KS06HW79/ KS020638-M-5 was planted in one metal flat while the experiment for the cross of KS06HW79/Brawl CL Plus was planted in two metal flats. In each flat, parental lines and check varieties were each planted in one row. F_1 seeds from each cross were planted in only one row due to the limited number available. The rest of the rows in the metal flats were planted with F_2 seeds. The experiment was conducted in a growth chamber (Percival Model, PGC-15WC) under a 12 h photoperiod at 21°C. Each plant was mechanically inoculated with the Sidney 81 isolate as described by Seifers et al. (2006) at the two-leaf stage. Plants were visually assessed four weeks after inoculation using a 1 to 5 scale: 1= no symptoms, 2 = a few streaks, 3 = moderate mosaic, 4 = severe mosaic, and 5 = severe mosaic with yellowed leaves. Chi-square analysis was used to determine if they fit the hypothesized segregation ratios of 1 resistant : 3 susceptible (one-recessive-gene model), and 3 resistant : 13 susceptible (dominant suppression epistasis model). Any plants with unclear symptoms (not typical streaks caused by WSMV) were analyzed with Enzyme Linked Immunosorbent Assay (ELISA) as

described by Seifers et al. (2006) for confirmation, where plants were considered as uninfected if their values were less than 200% of the value of healthy control plants. For further confirmation of the F_2 results, symptomless plants (scored as 1) were selfed to produce $F_{2:3}$ families and evaluated for WSMV resistance as described above for F_2 plants. $F_{2:3}$ families were planted together with corresponding parental lines and check varieties (Tomahawk and RonL). Each line was planted in one row with 11 seeds. Each parental line had three replications and each check variety had two replications. After phenotyping, ELISA was conducted for plants with unclear symptoms or those scored as 2 (minor symptoms) for confirmation. Chi-square analysis was used to test a hypothesized segregation ratio of 1 no segregation (a family with all resistant plants) : 2 segregating (a family with mixed resistant and susceptible plants).

Two accessions susceptible to WSMV that were developed by the wheat breeding program at Kansas State University, KS13-6126 and KS12HM57, were each crossed with KS06HW79 to produce F_1 seeds, from which two doubled haploid (DH) populations were generated by Heartland Plant Innovations (Manhattan, KS). The DH plants were evaluated for WSMV resistance together with their corresponding parental lines and check varieties Tomahawk and RonL. DH lines were planted in short rows in 30 by 50 cm metal flats filled with potting mix (Sungro®, Canada), with each row filled with four to eight seeds according to seed availability. Each parental line had three replications and each check variety had two replications. After phenotyping, ELISA was conducted for plants with unclear symptoms or those scored as 2 (minor symptoms) for confirmation. Chi-square analysis was used to test a hypothesized segregation ratio of 1 resistant : 3 susceptible.

Results

The WSM ratings for both F_1 and F_2 plants derived from the crosses of KS06HW79/Brawl CL Plus and KS06HW79/KS020638-M-5 are shown in Table 1. There were a total of 26 and 33 plants with unclear symptoms in the experiments for the crosses of KS06HW79/Brawl CL Plus and KS06HW79/KS020638-M-5, respectively. The phenotypic scores of those plants were adjusted based on ELISA results. ELISA scores for the cross of KS06HW79/Brawl CL plus are shown in Table 6 and the scores for the cross of KS06HW79/KS020638-M-5 are shown in Table 7. In both experiments, all plants of susceptible check varieties Tomahawk and RonL, and the susceptible parents Brawl CL Plus and KS020638-M-5 showed moderate to severe symptoms with scores ranging from 3 to 5, while all plants of the resistant parent KS06HW79 and the resistant check line KS96HW10-3 were symptomless. All the F_1 plants from both crosses showed moderate to severe symptoms, indicating that WSMV resistance in KS06HW79 might be recessive. In each experiment, F_2 plants showed a range of scores from 1 to 5 while most of the symptomatic plants showed moderate to severe symptoms. Based on the performance of parental lines, asymptomatic plants were classified as resistant, while a score of 2 or greater was classified as susceptible. The segregation ratio in the F_2 from both crosses fit both 1 resistant : 3 susceptible (one-recessive-gene model, $\chi^2 = 0.15$ and $P = 0.70$ in the cross of KS06HW79/Brawl CL Plus, and $\chi^2 = 1.10$ and $P = 0.29$ in the cross of KS06HW79/KS020638-M-5) and 3 resistant : 13 susceptible (dominant suppression epistasis model, $\chi^2 = 5.85$ and $P = 0.02$ in the cross of KS06HW79/Brawl CL Plus, and $\chi^2 = 2.12$ and $P = 0.15$ in the cross of KS06HW79/KS020638-M-5).

To validate the segregation ratio in the F_2, resistant F_2 plants from both crosses were advanced to generate $F_{2:3}$ families. The WSMV reactions of the check varieties and parental lines

grown with the $F_{2:3}$ families are shown in Table 2 and the WSMV reactions of the $F_{2:3}$ families are shown in Table 3. In this experiment, the plants that scored as 2 and a few plants with unclear scores of 3 were tested with ELISA and their phenotypic scores were adjusted based on ELISA values. ELISA scores for the cross of KS06HW79/Brawl CL Plus are shown in Table 8 and scores for the cross of KS06HW79/KS020638-M-5 are shown in Table 9. All plants of the susceptible check variety Tomahawk and the susceptible parents KS020638-M-5 and Brawl CL Plus showed moderate to severe mosaic symptoms, whereas all plants of KS06HW79 were symptomless except one with a score of 3. Several F_2 plants died during generation advancement. Therefore, we had fewer $F_{2:3}$ families than resistant F_2 plants. As shown in Table 3, there were two types of $F_{2:3}$ families: one with all resistant plants (R, no segregation) and the other with a mixture of both resistant and susceptible plants (M, segregating). This indicates that the dominant suppression epistasis model is a better fit, since complete resistance in all these $F_{2:3}$ families would be expected in the one-recessive-gene model. In the dominant suppression epistasis model, the hypothesized genotype of KS06HW79 is *AAbb* while the genotype of susceptible lines is *aaBB*, with *A* being the WSMV resistance gene and *B* being the suppressor gene. According to Mendelian genetics, the resistant F_2 genotype should be either *AAbb* or *Aabb* with an expected ratio of 1 : 2. F_2 plants with the genotype *AAbb* should not segregate in the F_3 generation, while F_2 plants with the genotype *Aabb* should segregate and have both resistant and susceptible plants in the F_3 generation. Therefore, a segregation ratio of 1 R : 2 M was expected among the $F_{2:3}$ families derived from resistant F_2 plants. Chi-square tests validated this segregation ratio ($\chi^2 = 0.13$ and $P = 0.71$ in the cross of KS06HW79/Brawl CL Plus, and $\chi^2 = 3.53$ and $P = 0.06$ in the cross of KS06HW79/KS020638-M-5), which further confirmed the dominant suppression epistasis model for WSMV resistance in KS06HW79.

The WSMV reactions of the check and parental lines grown with DH populations KS06HW79/KS12HM57 and KS06HW79/KS13-6126 are shown in Table 4, and the WSMV reactions of the DH lines in Table 5. In the test for the KS06HW79/KS12HM57 population, 23 individual plants with unclear symptoms were tested with ELISA and had their phenotypic scores adjusted based on the results (Table 10). Susceptible checks Tomahawk and RonL showed minor to severe symptoms, and all plants of susceptible check KS12HM57 were moderately susceptible. All plants of resistant parent KS06HW79 were resistant except one showing slight symptoms. Based on performance of parental lines, a DH line comprised of asymptomatic plants was classified as resistant, while a DH line comprised of plants with a score of 2 or above was classified as susceptible. There were 71 susceptible lines and 12 resistant lines. The segregation ratio fit an expected 1 R : 3 S segregation ratio ($\chi^2 = 6.00$ and $P = 0.13$). This further confirms the dominant suppression epistasis model, with the DH populations being comprised of one hypothesized resistant genotype *AAbb* and three hypothesized susceptible genotypes *AABB*, *aaBB*, and *aabb*. In the test for the KS06HW79/KS13-6126 population, 23 individual plants with unclear symptoms had their phenotypic scores adjusted based on ELISA results (Table 11). Susceptible checks Tomahawk and 2013-6126 showed moderate to severe symptoms, while RonL showed mostly moderate symptoms and two asymptomatic plants. Resistant parent KS06HW79 showed complete resistance. Among the 71 DH lines, there were 52 susceptible lines and 19 resistant lines. The segregation ratio also fit an expected 1 R : 3 S segregation ratio ($\chi^2 = 0.43$ and $P = 0.73$).

Discussion

Previously, Seifers et al. (2013b) observed that WSMV resistance in KS06HW79 was still effective at 21^{o}C while *Wsm2*-bearing RonL was susceptible at that temperature. Therefore,

RonL served as a susceptible check in this study in addition to the susceptible variety Tomahawk. In this study, both susceptible check varieties together with susceptible parental lines were consistently symptomatic and the susceptibility of most plants was scored as moderate to severe, indicating that the inoculum we used was infective and the inoculation was successful. However, the susceptible checks and parents grown with the $F_{2:3}$ lines showed more moderate and less severe symptoms than those in the F_2 and DH experiments. This disparity might be due to minor variations in growth chamber temperature, plant size at inoculation, or inoculum concentration among the three experiments. All plants of the resistant parent KS06HW79 showed no symptoms similar to the resistant check line KS96HW10-3, except for one plant with moderate mosaic symptoms grown with the $F_{2:3}$ experiment and one plant with a few streaks grown with the KS06HW79/KS12HM57 DH experiment. The symptomatic plants of KS06HW79 might have been due to the heterogeneity in the line since it is an $F_{5:6}$ breeding line. It is also possible that the seed source of KS96HW79 used in this study might have been contaminated with a few seeds from other susceptible breeding lines during harvest. Additionally, it is also possible that the resistance in KS96HW79 might have incomplete penetrance. A small percentage of susceptible plants have also been observed in RonL at 18^{o}C, where *Wsm2* was supposed to be effective (Seifers et al. 2013b).

All crosses in this genetic study supported a dominant suppression epistasis model for the WSMV resistance in KS06HW79. In this model, resistance is determined by a resistance gene (*A*) and a suppressor gene (*B*). Both genes have dominant gene action, but the dominant suppressor gene can suppress the expression of the dominant resistant gene. Thus, the F_1 plants (hypothesized genotype *AaBb*) were susceptible in this study and the resistance in KS06HW79 appeared to be controlled by a recessive gene. In the dominant suppression epistasis model, the *P*

value in the Chi-square test for the F_2 segregation in the cross of KS06HW79/Brawl CL Plus was not high, but exceeded the threshold of 0.01. This low *P* value might be due to the small population size, which might lead to some sampling errors. A more powerful test of this model could be obtained by crossing the resistant parent (hypothesized genotype *AAbb*) with a susceptible homozygous recessive parent (hypothesized genotype *aabb*). However, without the assistance of molecular markers, susceptible homozygous recessive genotypes are indistinguishable from other susceptible genotypes, such as *AABB*, *AaBB*, *aaBb*, and *aaBB*. The segregation ratios from the two DH populations, KS06HW79/KS12HM57 and KS06HW79/KS13-6126, further supported the dominant suppression epistasis model. The two DH populations will be used in a genetic mapping study to locate these two genes.

Previous studies have revealed that WSMV resistance is usually controlled by a single dominant gene. The *Wsm1* gene is on a translocation from the short arm of chromosome 4D of *Thinopyrum intermedium*, and has been documented to provide resistance up to 20°C (Gill et al. 1995, Seifers et al. 2013b). Another gene, *Wsm2*, is located on the short arm of chromosome 3B and dominant for resistance up to 18°C (Lu et al. 2011). A translocation from chromosome arm 7S#3L in *T. intermedium* contains *Wsm3* that provides WSMV resistance up to 24°C (Liu et al. 2011). Other genetic studies also revealed one major dominant gene controlled WSMV resistance in eleven wheat lines (Hassani and Assad 2004a, b; Zhang et al. 2015b). In addition to major genes, minor and epistatic gene action has also been observed to contribute to WSMV resistance (Hassani and Assad 2004b, Zhang et al. 2014, 2016). The one-recessive-gene model fit our F_2 data, but it did not sufficiently explain the $F_{2:3}$ data. The current study is the first report of a suppressor gene for WSMV resistance. However, suppressor genes have been documented to control disease resistance in other plant species. In *Arabidopsis*, mutants with genes that

suppressed a hypersensitive reaction were identified by Morel and Dangl (1998). Kwon et al. (2004) reported two suppressor mutations that reactivated defense to *Pseudomonas*. Other investigations have discovered suppressor genes that control disease resistance in several field crops, including ringspot virus resistance in papaya (*Carica papaya* L.) (McPhail-Medina et al. 2012), stripe rust (caused by *Puccinia striiformis* Westend) resistance in Chinese wheat landraces (Wu et al. 2015), and spot blotch (caused by *Cochliobolus sativus*) resistance in barley (*Hordeum vulgare* L.) (Singh et al. 2013).

The inheritance mode of the WSMV resistance revealed for KS06HW79 in this study will be valuable information for breeders to use it in their breeding programs. Further studies are needed to genetically map these two genes and identify closely linked molecular markers, which would facilitate the incorporation of this resistance into new varieties or pyramid them with other resistance genes to provide broader and more durable resistance to WSMV.

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Table 1. Wheat streak mosaic virus resistance rating at four weeks after inoculation and Chi-square tests for F_2 populations derived from the crosses of KS06HW79/Brawl CL Plus and KS06HW79/KS020638-M-5.

Line	Type	Total plants	No. of plants in resistance categories					Expected ratio (R:S)[1]	χ^2	*P* value
			1	2	3	4	5			
Tomahawk	Susceptible check	9					9			
RonL	Susceptible check	10			3	3	4			
KS96HW10-3	Resistant check	9	9							
Brawl CL Plus	Parental line	9				5	4			
KS06HW79	Parental line	8	8							
F_1		4			1	3				
F_2		147	39	11	31	36	30	3:13	5.85	0.02
								1:3	0.15	0.70
Tomahawk	Susceptible check	16				3	13			
RonL	Susceptible check	17		7	4	4	2			
KS96HW10-3	Resistant check	18	18			12				
KS020638-M-5	Parental line	19				12	7			
KS06HW79	Parental line	14	14							
F_1		5				5				
F_2		329	72	31	28	167	31	3:13	2.12	0.15
								1:3	1.10	0.29

[1]Plants with a score of 1 were classified as resistant (R) and plants with a score of 2 or greater were classified as susceptible (S).

Table 2. Wheat streak mosaic virus resistance scores of check and parental lines and varieties grown with $F_{2:3}$ families at four weeks after inoculation.

Lines	Total plants	Number of plants in resistance categories				
		1	2	3	4	5
Tomahawk	25			19	6	
RonL	21		1	20		
Brawl CL Plus	31			31		
KS020638-M-5	23			19	4	
KS06HW79	31	30		1		

Table 3. Segregation of resistance to wheat streak mosaic virus in $F_{2:3}$ families derived from resistant F_2 plants in crosses of KS06HW79/Brawl CL Plus and KS06HW79/KS020638-M-5.

Cross	Segregation type of $F_{2:3}$ families	No. of lines	$\chi 2$ (1 R : 2 M)	*P* value
KS06HW79/Brawl CL Plus	All resistant individuals (R)[1]	10		
	Mix of resistant and susceptible individuals (M)	24		
	Total	34	0.13	0.71
KS06HW79/KS020638-M-5	All resistant individuals (R)	14		
	Mix of resistant and susceptible individuals (M)	48		
	Total	62	3.53	0.06

[1]Plants with a score of 1 were classified as resistant and plants with a score of 2 or greater were classified as susceptible. In an R line, all the plants are resistant. In an M line, there are both resistant and susceptible plants.

Table 4. Wheat streak mosaic virus resistance scores of check and parental lines and varieties grown with doubled haploid populations derived from crosses of KS06HW79/KS12HM57 and KS06HW79/KS13-6126.

Cross	Parents and checks	Total plants inoculated	Number of plants in rating scale				
			1	2	3	4	5
KS06HW79/KS12HM57	Tomahawk	23		1	4	2	16
	RonL	25		2	8	4	11
	KS12HM57	23				23	
	KS06HW79	20	19	1			
KS06HW79/KS13-6126	Tomahawk	8				2	6
	RonL	7	2		5		
	2013-6126	8				6	2
	KS06HW79	8	8				

Table 5. Segregation of resistance to wheat streak mosaic virus in doubled haploid derived from crosses of KS06HW79/KS12HM57 and KS06HW79/KS13-6126.

Cross	Segregation of doubled haploid lines	No. of lines	$\chi 2$ (1 R : 3 S)	*P*
KS06HW79/KS12HM57	Susceptible lines (S)[1]	71		
	Resistant lines (R)	12		
	Total	83	6	0.03
KS06HW79/KS13-6126	Susceptible lines (S)	52		
	Resistant lines (R)	19		
	Total	71	0.43	0.73

[1] A double haploid line comprised of asymptomatic plants was classified as resistant while a double haploid line comprised of plants with a score of 2 or above was classified as susceptible.

Table 6. ELISA values and GHV scores for plants with unclear symptoms in the KS06HW79/Brawl CL Plus F_2 population.

Sample No.	ELISA	GHV[1]
3	0.170	10.0
4	0.015	0.9
5	0.014	0.8
6	0.181	10.6
7	0.17	10
8	0.011	0.6
9	0.02	1.2
10	0.013	0.8
11	0.019	1.1
12	0.057	3.4
13	0.014	0.8
14	0.036	2.1
15	0.071	4.2
16	0.01	0.6
17	0.03	1.8
18	0.152	8.9
19	0.014	0.8
20	0.014	0.8
21	0.017	1
22	0.164	9.6
23	0.015	0.9
24	0.013	0.8
25	0.011	0.6
127	0.036	2.1
Healthy wheat	0.013	0.8
Healthy wheat	0.021	1.2
Average Healthy	0.017	1.0
WSMV	0.239	14.1
WSMV	0.183	10.8

[1]GHV= (sample ELISA value/healthy ELISA), or number of times greater than equivalent healthy value wheat samples. Plants were considered as uninfected if their values were less than 200% of the value of healthy control plants.

Table 7. ELISA values and GHV scores for plants with unclear symptoms in the KS06HW79/KS020638-M-5 F_2 population.

Sample No.	ELISA	GHV[1]
27	0.019	1.1
28	0.088	5.2
29	0.011	0.6
30	0.012	0.7
31	0.182	10.7
32	0.108	6.4
33	0.013	0.8
34	0.116	6.8
35	0.013	0.8
36	0.006	0.4
37	0.009	0.5
38	0.041	2.4
41	0.078	4.59
42	0.008	0.47
43	0.107	6.29
44	0.187	11.00
45	0.119	7.00
46	0.013	0.76
47	0.131	7.71
48	0.01	0.59
49	0.061	3.59
50	0.007	0.41
51	0.014	0.82
52	0.009	0.53
53	0.111	6.53
54	0.013	0.76
55	0.013	0.76
124	0.007	0.41
125	0.008	0.5
126	0.008	0.5
Healthy wheat	0.013	0.8
Healthy wheat	0.021	1.2
Average Healthy	0.017	1.0
WSMV	0.239	14.1
WSMV	0.183	10.8

[1]GHV= (sample ELISA value/healthy ELISA), or number of times greater than equivalent healthy value wheat samples. Plants were considered as uninfected if their values were less than 200% of the value of healthy control plants.

Table 8. ELISA values for plants with unclear symptoms in $F_{2:3}$ family lines derived from the cross of KS06HW79/Brawl CL Plus.

Sample No.	ELISA	GHV[1]
1	0.189	5.9
2	0.200	6.3
3	0.230	7.2
4	0.224	7
5	0.090	2.8
6	0.079	2.5
7	0.146	4.6
8	0.100	3.1
9	0.088	2.8
10	0.148	4.6
11	0.185	5.8
12	0.148	4.6
13	0.128	4
14	0.097	3.0
15	0.032	1.0
16	0.059	1.8
17	0.209	6.5
18	0.033	5.4
19	0.174	1.8
20	0.059	5.6
21	0.179	5.3
22	0.168	3.7
23	0.118	6.6
24	0.211	0.1
25	0.004	5.9
26	0.190	3.7
27	0.119	3.1
28	0.100	6
29	0.193	2.4
30	0.076	5.1
31	0.164	6.3
32	0.202	5
Healthy wheat	0.032	1.0
Healthy wheat	0.032	1.0
Healthy wheat	0.031	1.0

[1]GHV= (sample ELISA value/healthy ELISA), or number of times greater than equivalent healthy value wheat samples. Plants were considered as uninfected if their values were less than 200% of the value of healthy control plants.

Table 9. ELISA values for plants with unclear symptoms in $F_{2:3}$ family lines derived from the cross of KS06HW79/KS020638-M-5.

Sample No.	ELISA score	GHV[1]
33	0.161	5.0
34	0.214	6.7
35	0.087	2.7
36	0.230	7.2
37	0.068	2.1
38	0.135	4.2
39	0.091	2.8
40	0.084	2.6
41	0.166	5.2
42	0.070	2.2
43	0.214	6.7
44	0.002	0.1
45	0.088	2.8
46	0.049	1.5
47	0.115	3.6
48	0.056	1.8
49	0.211	6.6
50	0.084	2.6
51	0.014	0.4
52	0.236	7.4
53	0.079	2.5
54	0.242	7.6
55	0.230	7.2
56	0.147	4.6
57	0.143	4.5
58	0.128	4.0
59	0.147	4.6
60	0.117	3.7
61	0.107	3.3
62	0.235	7.3
63	0.127	4.0
64	0.232	7.3
65	0.061	1.9
66	0.063	2.0
67	0.200	6.3
68	0.038	1.2
69	0.146	4.6
70	0.181	5.7
71	0.031	1.0
72	0.204	6.4

Healthy wheat	0.032	1.0
Healthy wheat	0.032	1.0
Healthy wheat	0.031	1.0

[1]GHV= (sample ELISA value/healthy ELISA), or number of times greater than equivalent healthy value wheat samples. Plants were considered as uninfected if their values were less than 200% of the value of healthy control plants.

Table 10. ELISA scores and GHV scores for plants with unclear symptoms in the KS06HW79/KS12HM57 DH population.

Sample	ELISA	GHV[1]
1	0.341	34.1
2	0.403	40.3
3	0.725	72.5
4	0.317	31.7
5	0.307	30.7
6	0.391	39.1
7	0.338	33.8
8	0.449	44.9
9	0.577	57.7
10	0.621	62.1
11	0.965	96.5
12	0.609	60.9
13	0.437	43.7
14	0.646	64.6
15	0.579	57.9
16	0.656	65.6
17	0.524	52.4
18	0.522	52.2
19	0.797	79.7
20	0.540	54.0
21	0.513	51.3
22	0.466	46.6
23	0.457	45.7
Average Healthy Wheat	0.01	1.0

[1]GHV= (sample ELISA value/healthy ELISA), or number of times greater than equivalent healthy value wheat samples. Plants were considered as uninfected if their values were less than 200% of the value of healthy control plants.

Table 11. ELISA scores and GHV scores for plants with unclear symptoms in the KS06HW79/KS13-6126 DH population.

Sample	ELISA	GHV[1]
24	0.425	42.5
25	0.259	25.9
26	0.283	28.3
27	0.325	32.5
28	0.288	28.8
29	0.292	29.2
30	0.278	27.8
31	0.251	25.1
32	0.276	27.6
33	0.155	15.5
34	0.132	13.2
35	0.177	17.7
36	0.250	25.0
37	0.102	10.2
38	0.052	5.2
39	0.009	0.9
40	0.007	0.7
41	0.079	7.9
42	0.040	4.0
43	-0.010	-1.0
44	0.225	22.5
45	-0.045	-4.5
46	-0.081	-8.1
Average Healthy Wheat	0.01	1.0

[1]GHV= (sample ELISA value/healthy ELISA), or number of times greater than equivalent healthy value wheat samples. Plants were considered as uninfected if their values were less than 200% of the value of healthy control plants.