GENETIC ANALYSES OF HESSIAN FLY RESISTANCE IN KS 94U275

by

MICHELLE SUSAN SAMUEL-FOO

(Under the Direction of Jerry W. Johnson)

ABSTRACT

The need for enhanced genetic diversity in wheat (*Triticum aestivum* L.) for resistance against the Hessian fly (HF) [*Mayetiola destructor* Say] is a continuous one, as the HF remains a seriously damaging pest throughout the wheat producing regions of the United States and the world over. Breeding for resistance has been the most practical and economical method for effecting HF control as new genes for resistance are needed because of the continuous evolution of virulence in HF populations. KS 94U275 is an experimental line which indicated HF resistance in greenhouse and field trails. On the basis of segregation ratios (1:1 resistant/susceptible in BC_1F_1 and 3:1 resistant/susceptible in F_2 populations), it was determined that resistance in KS 94U275 is conditioned by a single dominant gene. Microsatellite marker analysis was also performed to identify a DNA marker linked to this gene.

INDEX WORDS: Wheat, *Triticum aestivum*, KS 94U275, AGS 2000, GA 901146, Hessian fly resistance, *Mayetiola destructor*, Genetic analyses, Segregation ratios, SSR

GENETIC ANALYSES OF HESSIAN FLY RESISTANCE IN KS 94U275

by

MICHELLE SUSAN SAMUEL-FOO

B.A., Brewton-Parker College, Georgia, 2000

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2003

GENETIC ANALYSES OF HESSIAN FLY REISTANCE IN KS 94U275

by

MICHELLE SUSAN SAMUEL-FOO

Major Professor:	Jerry W. Johnson
Committee:	Joe Bouton
	G. David Buntin

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
December 2003

DEDICATION

For Cornell and Yohan.

ACKNOWLEDGEMENTS

I feel truly blessed to have had the kind of overwhelming support that I did during the past couple of years, from so many people. I would first of all like to thank Jerry for having me as a graduate student and for helping me cultivate and nurture my love of agriculture. Jerry, you have been great as a major professor, mentor and friend to not only me, but to my family as well, and I sincerely thank you for everything that you have done. You and your family have been tremendously kind and generous to me…thank you, this work and me receiving this degree could not have been possible without your help.

I would like to also thank David McMillin for recognizing potential in me and for introducing me to Jerry in the first place. It was purely coincidental that we got to talking one afternoon and he told me about Jerry and his breeding program in Griffin. From that moment on, my faith was sealed and I have not looked back since. Dr McMillin, you opened the gate for me…I am greatly indebted to you for convincing me to go on to graduate school.

The following people have helped me in various ways:

The faculty at Brewton-Parker College's Math and Science department, especially William Said, Mariam George and Haywood Boyd, for their support and encouragement from the commencement of my undergraduate studies right up through today, thank you all.

The faculty and staff at the Crop and Soil Sciences department here at the University of Georgia, most notably my advisory committee members: Joe Bouton and G. David Buntin. Thank you both for your support for being patient with me. Also special mention has to be made of the faculty members whom I have been privileged to learn from: M. Adang, J. All, K. Braman, M. Cabrera, H. Chen, K. Dawe, M. Pierce, J. Ruberson, M. Scanlon, P. Schliekelman, A. Smith, W. Parrot, W. Vencill and S. Wessler.

I cannot forget Vivienne Sturgill for lending a sympathetic and reassuring ear and for putting to rest all my queries and fears, and most importantly for all her little reminders, ensuring that I got all my documents turned in to the right places at the right time, thanks Viv.

Special thanks to all that I have worked with over the past two years especially the staff at the Georgia Experiment Station and PGRCU in Griffin: Zhenbang Chen, Melanie Newman, Wook Kim, SJ Chang, Dan Bland, Steve Sutton, Mark Hopkins, Stephanie Dunn and Tracey Jenkins. Chen, thanks for teaching me to run markers, gels, sequencing…the complete package of marker analysis. You were patient with me and never faltered in your reassurances right up through the day of my final defense. You are one of the good ones. I am extremely grateful for you helping me out all those times that you did, running those gels for me when I had to be out, busy with the things that new first-time moms have to do.

SJ and So Young, I will never forget your kindness. Thanks SJ for chauffeuring me to and from classes whilst I was pregnant with Yohan.

Finally, Cornell my husband, and Yohan my son of nine months, you two are always on my side, encouraging me and helping me to remain focused. Your love and support serves as a constant reminder to me of what is truly important.

There are many other people that have helped me in various ways but time and space does not permit me to go into length, but for anyone that I may have failed to name personally in these writings, thank you…

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS v

LIST OF TABLES viii

LIST OF FIGURES ix

CHAPTER

1 INTRODUCTION 1

2 LITERATURE REVIEW 5

Mayetiola destructor as a Major pest of Wheat 5

Using Molecular Markers in Breeding Programs 10

References 14

3 INHERITANCE OF HESSIAN FLY RESISTANCE IN KS 94U275 26

Abstract 27

Introduction 28

Materials and Methods 29

Results and Discussion 32

Acknowledgements 34

References 35

4 MOLECULAR MARKER STUDY OF HF RESISTANCE IN KS 94U275 41

Abstract 42

Introduction 42

Materials and Methods 44

Results and Discussion 47

Acknowledgements 49

References 50

LIST OF TABLES

Page

Table 3.1: Inheritance of resistance to Hessian fly biotype L in KS 94U275 39

Table 3.2: Segregation analysis for reaction to *Mayetiola destructor* Say in F_2 populations.....40

Table 4.1: Polymorphic primer pairs detected from initial screenings.....54

Table 4.2: List of potential microsatellite markers for Hessian fly resistance in KS 94U275 57

LIST OF FIGURES

Page

Figure 4.1: SSR polymorphism between parentals KS 94U275(R) and GA 901146(S).....58

Figure 4.2: DNA fragments from amplification of wheat genotypes with microsatellite ……59

CHAPTER 1

INTRODUCTION

The Hessian fly (HF), [*Mayetiola destructor* Say], is a major pest of winter wheat [*Triticum aestivum* L.] in the southern United States, with outbreaks occurring periodically in Georgia since the mid-1800s (Webster 1899). The HF chooses wheat as its primary host although the fly has been found to infest triticale, barley and rye occasionally (Ratcliffe and Hatchett, 1997). Several wild grasses including *Agropyron repens* (L.) *A. smithii* Rydb., *Elmus virginicus* L., *E. canadensis* and *Aegilops* sp. may also serve as hosts. In the southeastern United States, volunteer wheat also may serve as a reservoir for flies that are able to infest fall-planted small grains (Buntin and Raymer, 1989; Johnson et al., 1987). HF infestations have been a major limiting factor in wheat production in this region, causing reductions of grain production by killing plants, stunting vegetative tillers and preventing spike development. Forage losses occur when infestations exceed approximately 10% of infested tillers (McColloch 1923, Hill et al. 1943, Buntin and Raymer 1989). As wheat is one of the most important food crops in the world, understanding its genetics and particularly how it interacts with its major yield reducing pests is extremely important for producing resistant cultivars and general plant breeding purposes.

The HF was first found infesting wheat on Long Island New York in 1779 and is believed to have been accidentally introduced here by Hessian soldiers in straw bedding during the American Revolutionary War (Ratcliffe and Hatchett, 1997). Damage caused as a result of HF infestations has resulted in extensive crop losses in all major wheat growing regions of the United States. Damages of $100 million has been estimated to have occurred in a single year

(Cartwright and Jones, 1953). More recently, in Georgia, the HF losses have been estimated at $4 million in 1986 and $28 million in 1989 (Hudson et al., 1988; Hudson at al., 1991). It was also estimated that another 99,000 to 124,000 ha (40,000-50,000 acres) of wheat was left unharvested in Georgia in 1989 because of severe damage caused by HF infestations. Although a decline in losses have occurred due to HF infestation since its peak in 1989 (as a result of a switch to mostly resistant varieties), substantial losses are still observed with estimates being $200,000, $669,000, $528,000, $1,000,000 respectively for the period 1999 through 2002 (G. David Buntin, personal communication).

In the mid-western United States, Hessian fly aestivate as puparia in wheat stubble during the summer and produces a single generation for the fall and spring seasons although additional generations have been periodically observed in some years (McCollich 1923, Walton and Packard 1930). In the coastal plain region of the southern United States, 4-5 generations are observed annually, with 2 generations occurring in the fall, 1 generation occurring in the winter, and 1-2 generations occurring in the spring (Buntin and Chapin 1990). The most practical and economically feasible method of controlling this pest has been the planting of resistant cultivars. Numerous sources of resistance to HF in wheat have been identified and incorporated into breeding programs.

Studies conducted on the inheritance of resistance to Hessian fly in tetraploid and hexaploid wheat (Patterson and Gallun 1973; Hatchett and Gill 1983; Oellermann et al., 1983; Stebbins et al., 1983; Maas et al., 1987; Obanni et al., 1989) have shown that resistance is either dominant, partially dominant or recessive and conditioned by single, duplicate, or multiple genetic factors derived from common wheat and durum wheats [*Triticum turgidum* L. var. *durum*], the wild wheat [*Triticum tauschii* (Coss) Schmal.] and rye [*Secale cereale* L.] (Gallum

1977; Friebe et al., 1990, 1991; Ratcliffe and Hatchett, 1997). Their increasing usage however, applies selection pressure on populations of the insect, increasing the frequency of alleles for virulence on deployed resistance genes (Gallum 1977).

To date, 29 major genes, designated *H1* to *H29*, conferring resistance to *M. destructor* have been identified and are being used in cultivar improvement (McIntosh et al., 1998). Most of these genes confer resistance only against specific biotypes of the HF (Friebe et al., 1994). Additionally, specific HF biotypes have evolved that that are unaffected by resistance alleles that have been deployed in These biotypes are said to be virulent to wheat with such resistant alleles. wheat cultivars. Virulence appears to be conditioned by recessive alleles at specific virulence loci in the HF genome in a manner that is characteristic of a classic gene for gene interaction (Gallum 1977; Stuart et al., 1997). To date 16 HF biotypes have been identified, designated as GP (Great Plains) and A to O (Gallum 1977) and are distinguished only by their ability (virulence) or inability (avirulence) to survive on and stunt wheat cultivars carrying specific resistance genes (Ratcliffe et al., 1994). Biotypes L and O are the most common in the Southeast, with HF populations in winter wheat for 2001 being predominantly biotype L in northern Georgia with O as the dominant biotype in the southern part of the state (Buntin et al., 2001).

The presence of these virulent HF biotypes in Georgia increases the urgency for the development of cultivars that carry resistance to the HF as well as the need for an effective molecular marker system that would be enable earlier identification of cultivars containing HF resistance genes. To this end, one objective of this research was to evaluate the number of genes conferring suspected resistance in KS 94U275 from evaluations of BC_1F_1 and F_2-derived lines. Additionally, this work also endeavors to identify molecular markers linked to this HF resistance gene using the molecular marker technique of Simple Sequence Repeats (SSRs).

CHAPTER 2

LITERATURE REVIEW

Mayetiola destructor as a Major Pest of Wheat

The Hessian fly (HF), [*Mayetiola destructor* (Say)], belongs to the class of insects known as gall midges (Diptera: Cecidomyiidae). The fly is a seriously damaging pest of wheat (*Triticum aestivum* L.) both worldwide and in the United States. It is believed to have been introduced into the United States from Europe during the late 1700s by Hessian soldiers during the American Revolutionary war (Briggle et al., 1982). HF can be found infesting all major wheat growing areas of the US, from the Atlantic coast to the Great Plains and parts of California, Idaho, Oregon and Washington (Ratcliffe 1997). In particular, the pest has been noted in the Southeastern US periodically for many years (Hatchett 1969, Morrill and Nelson 1976). Wheat is the preferred host of the HF, but the pest may also infest barley, rye and triticale. Additionally several wild grasses including *Agropyron repens* (L.), *A. smithii pusillum* Nutt., and *Aegilops* spp. may serve as hosts in the absence of these preferred crops. Volunteer wheat may also act as a reservoir for HF (Buntin and Chapin, 1989; Ratcliff and Hatchett, 1997).

Adult HF bear a resemblance to small mosquitoes. They are smoky gray, fragile, have pointed abdomens and are about 3 mm in length (Ratcliffe 1997). The abdomen of the female fly appears reddish in color and this coloration is caused by the color of the eggs developing inside. The eggs are glossy red, cylindrical and approximately 0.5 mm in length. Initially, the first-instar

is also red in color but eventually transforms to a white color, whilst the second-instar larva is white and reaches a length on about 4 mm once mature. The puparium or “flaxseed” is darkish-brown in color and ranges from 3-5 mm in length. Gagne and Hatchett (1989) provide a detailed description of larval instars.

Adult flies are weak fliers and only live about two to three days. In winter wheat areas, the HF life cycle begins with the fall emergence of the adults from volunteer or infested wheat. Emergence occurs during the early morning hours with the males emerging first followed shortly by the females. Newly emerged females cling to a plant leaf and extend their abdomens, releasing a sex pheromone, which attracts the males for mating (McKay and Hatchett, 1984). Shortly after mating, females deposit reddish, elongated eggs in rows in grooves on the upper surfaces of wheat leaves. Seedling wheat plants or tillers are preferred for egg-laying. A single female fly lays between 200-300 eggs which hatch in 3-10 days, depending on temperature.

The HF has 4-5 generations annually in the coastal plain region of the southern United States, with 2 generations occurring in fall, 1 generation occurring in the winter and 1-2 generations occurring in spring (Buntin and Chapin 1990). In the northern spring wheat regions, only one spring generation is produced annually. Supplementary broods are sometimes observed in between the main fall or spring generations. When a summer or supplementary brood does develop, early-sown non-resistant wheat is often severely damaged (Buntin and Chapin, 1990; Lidell and Schuster 1990). With the onset of fall (early-late September and sometimes late August in Georgia), the HF appears and lay eggs on the leaves of seedling wheat or volunteer wheat plants. Larvae emerge from these eggs and begin feeding. They complete their growth before winter sets in and they pass the cold season as shiny brown, seed-like puparium or

“flaxseeds”. The flaxseeds may be found at the base of old plant crowns or near the nodes behind the leaf sheaths. Within the flaxseed is the fourth-stage larva, which, in dry areas of the Great Plains, does not resume development until a general soaking rain breaks dormancy. In Georgia, the breaking of dormancy is thought to be temperature related (Buntin, personal communication). Shortly thereafter, development is completed and the larva pupates and emerges as an adult fly in about two weeks.

The larval stage of the HF is the most injurious to wheat. In the spring, adult flies emerge from the flaxseeds to lay eggs on the wheat leaves. Once hatched, the first-stage larvae crawl downward, gradually reaching the base of the leaf. Here, beneath the leaf sheath, the young larva initiates feeding. Larvae press their mouthparts against the plant, injecting their saliva into the plant, which causes the tissue to release the cell contents. Feeding here causes the stem to weaken which may result in the stalks breaking and the plant lodging before harvest. A single larva, feeding for just three days, is capable of permanently stunting a young wheat plant or tiller. Thus, obvious reductions in both quality and quantity of grain are observed.

The larvae will develop into puparia about the time that the wheat begins to head and they will remain in the wheat stubble as “flaxseeds” until fall. The amount of damage caused is related to degree of infestation by the HF larvae. Even a single larva can cause significant damage to a wheat plant because salivary toxins released while feeding interfere with normal wheat growth. Plants attacked at the one-leaf stage may be killed outright. Wheat attacked later will be severely stunted, with perhaps the first tillers killed and plant growth delayed. Plants infested in the fall can easily be recognized by their darker than normal green coloration, and leaves with unusually broad blades. Young plants or tillers infested in the fall often die during

the winter. Plants attacked in the spring have shortened and weakened stems that may eventually break just above the first or second node, causing plants to lodge near harvest. Heavily infested fields will have reduced yields and lower quality grain caused by adverse physiological effects (reduced plant growth and kernel size and number) and mechanical damage (breakage due to weakening of stems).

The control methods for *Mayetiola destructor* are preventative rather than curative in nature as many biotic and abiotic factors play a role in the abundance of HF populations and hence the potential of the populations to cause damage. If these factors favor the insects continued existence and development, increases in HF populations may occur rapidly and consequently the ability to predict the occurrence and location of economic infestations becomes near impossible (Ratcliff and Hatchett, 1997). Among the more effective methods for preventing infestations are: planting resistant varieties, delayed seeding of winter wheat to escape fall infestations and completely destroying volunteer wheat. During the last 50 years, resistant varieties have offered the most reliable and economical control (Ratcliffe 1997). Resistant cultivars and delayed planting have been quite effective for preventing losses to this pest in the upper Midwest, but in the South, late planting is a poor strategy for HF management (Buntin and Chapin 1990, Buntin et al., 1990) because oviposition and larval survival can occur through the winter. However, studies conducted in Georgia (Buntin and Raymer 1989, Buntin et al., 1991) have demonstrated the value of resistant cultivars in the south. HF infestations have been significantly reduced to <1% in areas where resistant cultivars have been grown for several years (Maxwell et al., 1972). The combined practices of delayed seeding and proper management of volunteer wheat has also proven effective in reducing HF infestations in winter wheat throughout most of the wheat growing regions of the US (Ratcliffe 1997). Chemical control of HF

infestations remains among the least feasible of control methods, in as much as Buntin and Chapin (1990) demonstrated that with employing resistant cultivars, comparable levels of HF control can be achieved as with systemic insecticides, minus any ill-effects that may be associated with the usage of the chemical insecticides. Temperature plays an important role as it governs development of the HF (Foster and Taylor 1975) and can affect the plant's level of resistance. Therefore during warm periods no fly-free date may occur in Georgia and other southeastern states, and resistant cultivars may become susceptible (Johnson et al., 1984).

Resistance to HF in wheat have been identified from a number of various sources and successfully integrated into wheat breeding programs. Among the more popular sources are the wild *Triticum* species and rye [*Secale cereale* L.]. Of the *Triticum* species, *T. tauschii* (Coss) Schmal has been a major source of resistance to HF (Friebe et al., 1990,1991; Hatchett et al., 1993; Ratcliff and Hatchett, 1997). Resistance may be dominant, partially dominant or recessive, and governed by single, duplicate or multiple genetic factors derived from these common and durum wheats.

To date, 29 major genes, designated *H1* to *H29*, conferring resistance to *M. destructor* have been identified and are being used in cultivar improvement (McIntosh et al., 1998). Most of these genes confer resistance only against specific biotypes of the Hessian fly (Friebe et al., 1994). Of the 16 HF biotypes that have been identified, biotype L is thought to be the most virulent (Sosa 1981; Obanni et al., 1989; Amri et al., 1990). Genes that confer resistance to biotype L include *H9, H10, H12, H13, H14, H16, H17, H18* and *H19* (Patterson et al., 1988; Maas et al., 1989; Ratcliffe et al., 1994; Ratcliff et al., 1996). Additionally, in recent years,

resistance to biotype L has also been identified in rye and wheat-rye translocations (Hatchett et al., 1993).

Genes for resistance to the HF exhibit differential sensitivities to high temperature, and cultivars carrying the genes react differently when infested by various biotypes (Cartwright et al. 1946, Sosa and Foster 1976, Sosa 1979, Tyler and Hatchett 1983, Ratanatham and Gallun 1986). For example, the resistance of plants heterozygous for the *H13* gene to HF biotypes was significantly reduced at 28^{o} C, whereas resistance of plants homozygous for this gene was relatively stable at this temperature (Tyler and Hatchett 1983). Alternatively, Ratanatham and Gallun (1986) showed that the interactions of larval density, fly biotype and temperature did not significantly affect resistance expression of plants possessing the *H3, H5 or H6* genes. Temperature governs development of the HF (Foster and Taylor 1975) and can affect the plant's level of resistance. Therefore during warm periods no fly-free date may occur in Georgia and other southeastern states, and resistant cultivars may become susceptible.

Using Molecular Markers in Breeding Programs

The potential for marker assisted selection (MAS) to improve the efficacy of trait selection in crop plants is being widely studied (Staub et al., 1996; Mohan et al., 1997). As such, the science of plant breeding and plant genetics is currently undergoing a great revolution with the incorporation of molecular (DNA) markers to complement traditional breeding practices. Through this integration of molecular techniques with the customary breeding practices, it is now becoming possible to accelerate the time that would normally be required to develop improved cultivars. No longer are breeders limited to trait selection at the phenotypic level, as selection of

desirable traits at the molecular level is now becoming the reality that allows for introgression/transfer of genes to be completed in minimal time.

Molecular markers are able to reveal neutral sites of variation at the DNA sequence level, variation that would normally not be otherwise discernible at the phenotypic level. As a result of this, they occur more frequently that morphological markers and have the additional advantage of not disturbing the plant/organism being investigated (Jones et al., 1997). Initially, when using DNA markers in a breeding program, regardless of the species, a genetic map has to be constructed with markers regularly distributed across the species' genome. Next, selecting among the many different DNA marker systems requires a careful analysis of the ultimate project objectives, time available for investigation and completion, the genomic diversity of the species of interest, and the amount of financial and other pertinent resources available (Staub et al., 1996).

Molecular marker technologies have facilitated the construction of genetic maps of many organisms including many important crop species (Boerma, 2000). Roder et al., (1998) recently reported the construction of a microsatellite map of wheat. Eighty percent of the primer sets that were developed were genome specific and able to detect only a single locus in one of the three genomes of wheat (A, B, or D).

Microsatellites or Simple Sequence Repeats (SSRs) are tandem repeats of 2-4 nucleotides that are frequent and almost randomly distributed throughout most eukaryotic genomes which makes them desirable markers for genetic mapping (Pestova et al., 2000). Markers based on SSR sequences allow for the detection of high levels of polymorphism within a species, which can then be easily assayed using polymerase chain reactions (PCR).

Among the other available molecular marker systems, Restriction fragment length polymorphisms (RFLPs) have been extensively used in bread wheat and detailed linkage maps are available for all 7 homeologous groups (Gupta et al., 1997). Despite this, RFLP marker development has been unusually difficult in wheat because of the polyploidy nature of the crop, the high proportion of repetitive DNA and unusually low levels of polymorphism within the genome (Chao et al., 1989). Not only does simple sequence repeats reveal a higher incidence of detectable polymorphism, but additionally, they are more informative than any other DNA marker (Powell et al., 1996) and they have a greater uniformity in distribution throughout the genome, are codominant, and their screening relies on simple Polymerase chain reactions (PCR), requiring only small amounts of DNA (Roder et al., 1998). The high density microsatellite map of bread wheat constructed by Roder et al., (1998), consists of more than 279 markers, which have been put to a variety of uses including studies of genetic diversity (Plaschke et al., 1995; Fahima et al., 1998), and gene mapping (Korzun et al., 1998; Peng et al., 1999; Salina et al., 2000.

The Random Amplified Polymorphic DNA (RAPD) assay, detects nucleotide sequence polymorphisms by means of the polymerase chain reaction (PCR) and a single primer of arbitrary nucleotide sequence (Wang et al., 1995). This technique is a useful method for generating molecular markers (Williams et al., 1990; Welsh and McClelland, 1990) but the results tend to be difficult to reproduce and repeat, hence the understandable caution on its acceptance. In wheat, two independent studies showed that SSR provide a greater level of intraspecific polymorphism than RFLP (Roder et al., 195) and prompted the development of more than 400 SSR loci in wheat (Roder at al., 1995, Devos et al., 1995, Plaschke et al., 1996, Bryan et al., 1997; Roder et al., 1998; Stephenson et al., 1998).

AFLPs are fragments of DNA that have been amplified using directed primers from restriction digested genomic DNA (Matthes at al., 1998; Karp et al., 1997). The AFLP technique combines the RFLP reliability with the power of PCR to amplify simultaneously many restriction fragments (Vos et al., 1995). AFLP is highly efficient at generating a large number of polymorphisms, no prior sequence information is required with this method and a high multiplex ratio is possible (Rafalski et at., 1996), however, the method can be quite costly as several expensive components are in AFLP analysis, also the method of scoring fragments, like RAPDS is open to a certain amount of "interpretation" (Robinson and Harris, 1999).

Molecular markers are part of the intrusive 'new genetics' that is trusting its way into all areas of modern biology, from genomics to traditional plant breeding (Jones et al., 1997). The speed, sensitivity and ease of implementation of PCR based markers will allow greater direct application in breeding programs, increasing throughput and efficiency (Bryan et al., 1999).

References

Amri, A., J.H. Hatchett, T.S. Cox, M. El-Bouhssini and R.G. Sears. 1990.

Resistance to Hessian fly from North African durum wheat germplasm. Crop Science 30:378-381.

Bryan, G.J., A.J. Collins, P. Stephenson, A. Orry, J.B. Smith and M.D. Gale. 1997. Low levels of DNA sequence variation among adapted genotypes of hexaploid wheat. Theor. Appl. Genet. 94:557-563.

Bryan, G.J., P. Stephenson, A. Collins, J. Kirby, J.B. Smith and M.D. Gale. 1999. Low levels of DNA sequence variation among adapted genotypes of hexaploid wheat. Theor. Appl. Genet. 99:192-198.

Buntin , G.D., and J.W. Chapin. 1990. Hessian fly (Diptera: Cecidomyiidae) Biology in the southeastern United States: Geographic variation and temperature-dependent phenology. J. Econ. Entomology. 85:1015-1024.

Cartwright, W.B., R.M. Caldwell and L.E. Compton. 1946. Relation of temperature to the expression of resistance of wheats to Hessian fly. J. Am. Soc. Agron. 38:259-263.

Cambron, S.E., F.L. Patterson, H.W. Ohm, R.H. Ratcliffe, and G.G. Safranski. 1995. Genetic analyses of Hessian fly resistance in eight durum wheat introductions. Crop Sci. 35:708-714.

Chao, S., P. Sharp, A. Worland, E. Warham, R. Koebner, M.D. Gale. 1989. RFLP-based genetic maps of wheat homoeologous group-7 chromosomes. Theor Appl Genet 78:495-504.

De Loose M., and G. Gheysen. 1995. Identification methods based on molecular techniques. UPOV (International Union for the protection of New varieties of Plants). Working Group on Biochemcial and molecular techniques and DNA-Profiling in Particular, Wageningen, the Netherlands.

Devos, K.M., G.J. Bryan, A.J. Collins, P. Stephenson and M.D. Gale. 1995. Application of two microsatellite sequences in wheat storage proteins as molecular markers. Theor. Appl. Genet. 90:247-253.

Dweikat, I., H. Ohm, S. Mackenzie, F. Patterson, S. Cambron, and R. Ratcliffe. 1994. Association of a DNA marker with Hessian fly resistance gene *H9* in wheat. Theor. Appl. Genet. 89:964-968.

Fahima, T., M.S. Roder, A. Grama, and E. Nevo. 1998. Microsatellite DNA polymorphism divergence in *Triticum dicoccoides* accessions highly resistant to yellow rust. Theor. Appl. Genet. 96:187-195.

Formusoh, E.S., J.H. Hatchett, W.C. Black IV, and J.J. Stuart. 1996. Sex-linked inheritance of virulence against wheat resistance gene *H9* in the Hessian fly. Ann. Entomol. Soc. Amer. 89:428-434.

Friebe, B., J.H. Hatchett, B.S. Gill, Y. Mukai and E.E. Sebesta. 1991. Transfer of Hessian fly resistance from rye to wheat via radiation-induced terminal and intercalary chromosomal translocations. Theor. Appl. Genet. 83:33-40.

Friebe, B., J.H. Hatchett, R.G. Sears, and B.S. Gill. 1990. Transfer of Hessian fly resistance from 'Chaupon' rye to hexaploid wheat via a 2BS/2RL wheat-rye chromosome translocation. Theor. Appl. Genet. 79:385-389.

Gagne, R.J. and J.H. Hatchett. 1989. Instars of the Hessian fly (Diptera: Cecidomyiidae). Ann. Entomol. Soc. Am. 82:73-79.

Gallun, R.L. and L.P. Reitz. 1971. Wheat cultivars resistant to Hessian fly. USDA, ARS, Production Res. Rept. 134:16.

Grover, P.B., Jr. 1995. Hypersensitive response of wheat to the Hessian fly. Entomol. Exp. Appl. 74:283-294.

Hatchett, J.H., R.G. Sears, and T.S. Cox. 1993. Inheritance of resistance to Hessian fly in rye and wheat-rye translocation lines. Crop Sci. 33:730-734.

Hatchett, J.H., G.L. Kreitner, and R.J. Elzinga. 1990. Larval mouthparts and feeding mechanism of the Hessian fly (Diptera: Cecidomyiidae). Ann. Entomol. Soc. Am. 83:1137-1147.

Hatchett, J.H. and B.S. Gill. 1983. Expression and genetics of resistance to Hessian fly in *Triticum tauschii* (Coss) Schmal. pp 807-811 in S. Sakamoto (ed.), 6th Int. Wheat Genet. Symp., Kyoto University, Kyoto, Japan.

Hatchett, J.H., T.J. Martin, and R.W. Livers. 1981. Expression and inheritance of resistance to Hessian fly in synthetic hexaploid wheats derived from Triticum tauschii (Coss) Schmal. Crop Sci. 21:731-734.

Johnson, J.W., J.J. Roberts, W.A. Gardner and J.E. Foster. 1984. Occurrence and Importance of Hessian fly in Georgia. J. GA. Entomol. Soc. 19, no. 4. p: 538-542.

Johnson, J.W., G.D. Buntin, J.E. Foster, J.J. Roberts and P.L. Raymer. 1987. Response of triticale to the Hessian fly (Diptera: Cecidomyiidae). J. Entomol. Sci. 22:512-554.

Korzun, V., M.S. Roder, M. W. Ganal, A.J. Worland, C.N. Law. 1998. Genetic analyses of the dwarfing gene(Rht80 in wheat. Part I. Molecular mapping of *Rht8* on the short arm of chromosome 2D of bread wheat (*Triticum aestivum* L.) Theor. Appl. Genet. 96: 1104-1109.

Maas, F.B., F.L. Patterson, J.E. Foster and J.H. Hatchett. 1987. Expression and inheritance of resistance of 'Marquillo' wheat to Hessian fly in rye and in wheat-rye translocation lines. Crop Science 33: 730-734.

McColloch, J.W. 1923. The Hessian fly in Kansas. Manhattan, Kansas: Agricultural Experiment Station, Kansas State Agricultural College, 1923.

McKay, P.A. and J.H. Hatchett. 1984. Mating behavior and evidence of a female sex pheromone in the Hessian fly, Mayetiola destructor (Say) (Diptera: Cecidomyiidae). Ann. Entomol. Soc. Amer. 77:616-620.

Mohan, M., S. Nair, A. Bhagwat, T.G. Krishana and M. Yano. 1997. Genome Mapping, molecular markers and marker assisted selection in crop plants. Molecular Breeding 3:87-103.

Obanni, M.H., W. Ohm, J.E. Foster and F.L. Patterson. 1989. Genetics of resistance of PI 422297 durum wheat to the Hessian fly. Crop Science 29:249-252.

Ohm, H.W., H.C. Sharma, F.L. Patterson, R.H. Ratcliffe, and M. Obanni. 1995. Linkage relationships among genes on wheat chromosome 5A that condition resistance to Hessian fly. Crop Sci. 35:1603-1607.

Ollermann, C.M., F.L. Patterson and R.L. Gallun. 1983. Inheritance of resistance in ‘Luso” wheat to Hessian fly. Crop Science 23: 221-224.

Patterson, F.L. and R.L. Gallun. 1973. Inheritance and resistance of Seneca wheat race E of Hessian fly. Pages 445-449 in E.R. Sears and L.M.S. Sears (eds.) 4th Int. Wheat Genet. Symp., University of Missouri, Columbia, Missouri.

Peng, J.H., T. Fatima, M.S. Roder, Y.C. Li., A. Dahan, A. Grama, Y.I. Ronin, A.B. Korol and E. Nevo. 1999. Microsatellite tagging of the stripe-rust resistance Gene *YrH52* derived from wild emmer wheat, *Triticum dicoccoides*, and suggested negative crossover interference on chromosome 1B. Theor. Appl. Genet. 98: 862-872.

Pestsova, E., M.W. Ganal and M.S. Roder. 2000. Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome Vol. 43:689-697.

Plaschke, J., A. Borner, K. Wendehake, M.W. Ganal, and M.S. Roder. 1996. The use of wheat aneuploids for the chromosomal assignment of microsatellite loci. Euphitica 89:33-40.

Plaschke, J., M.W. Ganal and M.S. Roder. 1995. Detection of genetic diversity in

closely related bread wheat using microsatellite markers. Theor. Appl. Genet. 91:1001-1007.

Ratanatham, S., and R.L. Gallun. 1986. Resistance to Hessian fly (Diptera: Cecidomyiidae) in wheat as affected by temperature and larval density. Environ. Entomol. 15:305-310.

Ratcliffe, R.H., G.G. Safranski, F.L. patterson, H.W. Ohm and P.L. Taylor. 1994. Biotype status of Hessian fly (Diptera:Cecidomyiidaae) populations from the eastern United States and their response to 14 Hessian fly resistance genes. Jour. Econ. Entomol. 87:1113-1121.

Ratcliffe, R .H., H.W. Ohm, F.L. Patterson, S.E. Cambron, and G.G. Safranski. 1996. Response of resistance genes H9 to H19 in wheat to Hessian fly (Diptera: Cecidomyiidae) laboratory biotypes and field populations from the Eastern United States. J. Econ. Entomol. 89:1309-1317.

Ratcliffe, R.H. and J.H. Hatchett. 1997. Biology and genetics of the Hessian fly and resistance in wheat. p. 47-56. In K. Bondari [ed], New Developments in Entomology. Publisher, Research Signpost, Scientific Information Guild, Trivandrum, India.

Ratcliffe, R.H., S.E. Cambron, K.L. Flanders, N.A. Bosque-Perez, S.L. Clement, and H.W. Ohm. 2000. Biotype composition of Hessian fly (Diptera: Cecidomyiidae) populations from the southeastern, Midwestern, and northwestern United States and virulence to resistance genes in wheat. Journal of Economic Entomology 93:1319-1328.

Roder, M.S., J. Plaschke, S.U. Koning, A. Borner, M.E. Sorrells, S.D. Tanksley and M.W. Ganal. 1995. Abundance, variability and chromosomal location of microsatellite in wheat. Mol, Gen. Genet. 246:327-333.

Roder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M. Tixier, P. Leroy and M.W. Ganal. A Microsatellite Map of Wheat. Genetics 49:2007-2023.

Roder, M.S. 2000. Comparative microsatellite mapping of the induced Sphaerococcoid mutation genes in *Triticum aestivum*. Theor. Appl. Genet. 100:686-689.

Salina. E., A. Borner, I. Leonova, V. Korzun, L. Laikova, O. Maystrenko and M.S. Roder. 2000. Comparative microsatellite mapping of the induced sphaerococcoid mutation genes in *Triticum aestivum*. Theor. Appl. Genet. 100: 686-689.

Sosa, O. 1981. Biotype J and L of the Hessian fly discovered in Indiana wheat field. Journal of Economic Entomology 74: 182-182.

Sosa, O. Jr. 1979. Hessian fly: Resistance of wheat as affected by temperature and duration of exposure. Environ. Entomol. 8:280-281.

Sosa, O. Jr., and J.E. Foster. 1976. Temperature and the expression of resistance in wheat to the Hessian fly. Environ. Entomol. 5:333-336.

Staub, J.E., F.C. Serquen, and M. Gupta. 1996. Genetic Markers, map construction, and their application in plant breeding. HortScience 31:729-740.

Stebbins, N.B., F.L. Patterson and R.L. Gallun. 1983. Inheritance of resistance of PI94587 wheat to biotype B and D of Hessian fly. Crop Science 23:251-253.

Stephenson, P., B. Glenn, J. Kirby, A. Collins, K.M. Devos C. Busso and M.D. Gale. 1998. Fifty new microsatellite loci for the wheat genetic map. Theor. Appl. Genet. 97:946-949.

Stuart, J.J., S.G. Wellso and R.H. Ratcliffe. 1997. Induction and Inheritance of Chromosome Rearrangements in the Hessian Fly (Diptera:Cecidomyiidae). Ann. Entomol. Soc. Amer. 90(4): 480-486.

Tyler, J.M. and J.H. Hatchett. 1983. Temperature influence on expression of resistance to Hessian fly (Diptera:cecidomyiidae) in wheat derived from *Triticum tauschii*. J. Econ. Etomol. 76: 323-326.

Vos, P., R. Hogers, M. Bleekeer, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acid Res. 23:4407-4414.

Wang, R-C., J. Chen and L.R. Joppa. 1995. Production of Chromosome Specific RAPD Markers for Langdon Durum Wheat Disomic Substitution Lines. Crop Sci. 35:886-888.

Welsh, J., and M. McClelland. 1992. Fingerprinting Genomes Using PCR with Arbitrary Primers. Nucleic Acids Res. 18:7213-7218.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA Polymorphisms Amplified by Arbitrary Primers are Useful as Genetic Markers. Nucleic Acid Res. 18:6531-6535.

CHAPTER 3

INHERITANCE OF HESSIAN FLY RESISTANCE IN KS 94U275[1]

[1] Samuel-Foo, M.S. and J.W. Johnson 2003. To be submitted to The Journal of Plant Breeding

Abstract

The need for enhanced genetic diversity in wheat (*Triticum aestivum* L.) for resistance against the Hessian fly (HF) [*Mayetiola destructor* Say] is a continuous one, as the HF remains a seriously damaging pest throughout the wheat producing regions of the United States and the world over. Breeding for resistance has been the most practical and economical method for effecting HF control as new genes for resistance are needed because of the evolution of virulence in HF populations. Thus far, twenty nine major genes conferring resistance to HF have been identified and described (*H1* to *H29*), and these continue to be used in cultivar improvement programs. KS 94U275 is an experimental line developed by the Wheat Genetics Research Center (WGRC) at Kansas State University, which is suspected to have derived HF resistance from its *Aegilops tauschii* lineage. The objectives of this study were to (i) determine the number of resistant genes in KS 94U275 to Hessian fly biotype L, and (ii) determine the mode of inheritance of this resistance. On the basis of segregation ratios (1:1 resistant/susceptible in BC_1F_1 populations; 3:1 resistant/susceptible in F_2 populations), it was determined that resistance in KS 94U275 is conditioned by a single dominant gene. This finding holds great promise in the campaign for novel HF resistant genes that can be incorporated into superior cultivars to aid in withstanding assault from the HF.

Introduction

The Hessian fly (HF) is a serious pest of wheat (*Triticum aestivum* L.) that is capable of causing considerable damage to crops in all major wheat producing regions of the United States. Genetic resistance has been used extensively to protect wheat cultivars from damage by this pest (Hatchett et al., 1987). To date 29 major genes that condition resistance to HF larvae have been identified and described, for use in cultivar improvements. These genes have been denoted *H1* through *H29* (McIntosh et al., 1998; Raupp at al., 1993; Patterson et al., 1992). The mechanism of resistance conditioned by most of these genes is antibiosis, whereby the first instar larvae die after feeding on resistant plants (El Bouhssini et al., 1996).

Despite the large number of genes that are available, breeding for resistance has evolved into a very complex process owing to the identification of virulent biotypes of the insect that are unaffected by resistant genes (Chen et al., 1990, Sosa, 1981; Hatchett, 1969; Hatchett and Gallun, 1968). The genetic interaction observed between resistance in *Triticum* and avirulence in HF is consistent with a gene for gene relationship, with virulence in the insect being conferred by homozygous recessive pairs of genes (Hatchett and Gallun, 1970). Evidence for this gene for gene relationship has been supported by investigations involving six different resistance genes in wheat: *H3* (Gallun and Hatchett 1969), *H7H8* (Hatchett and Gallun 1970, Patterson and Gallun 1973); *H6* (Gallun 1978), *H5* (Perry 1990), *H9* (Formusoh et al., 1996) and *H13* (Zantoko and Shukle 1997). In the studies conducted, it was demonstrated that each of these resistance genes had a corresponding avirulence gene in the HF. In wheat, resistance alleles are usually dominant to susceptible alleles and in the HF, all known avirulence alleles are dominant to virulence alleles (Stuart et al., 1998).

The HF has a reputation for causing extensive crop losses in all major wheat growing regions of the United States. Damage as much as $100 million has been estimated to have occurred in a single year (Cartwright and Jones, 1953). More recently, in Georgia, the HF caused losses estimated at $200,000, $669,000, $528,000 and $1,000,000 for the period 1999 through 2002 (G. David Buntin, personal communication).

Control of the HF through the use of resistant cultivars has offered the most practical, economical and environmentally effective means of reducing the damage caused by this pest. The widespread use of resistant cultivars, however, exerts selection pressure on the insect for the development of new biotypes that are virulent to those resistance genes already identified (Cox and Hatchett, 1986). Thus the identification and effective utilization of new sources of resistance remain continual challenges for plant breeders and entomologists alike.

The experimental line KS 94U275, developed by the Wheat Genetics Resource Center of Kansas State University, possesses *Triticum tauschii* lineage. Previous studies have been reported in which genes for resistance have been successfully transferred from *Aegolips* into *Triticum* spp. including genes *H13, H22, H23, H24* and *H26* (Hatchett and Gill, 1983; Ratcliff and Hatchet, 1997). Thus the *Ae. tauschii* ancestry of KS 94U275, is suspected to be the source of the resistance that has been observed with this line.

Materials and Methods

KS 94U275 is an experimental line developed by the Wheat Genetics Research Center (WGRC) at Kansas State University. The pedigree of the KS 94U275 is KS 90WGRC10 x KS 92WGRC16 (Brown-Guedira, personal communication). KS 90WGRC10 is a hard red winter cultivar which has the pedigree: TAM 107*3/ TA 2460 (*Aegilops tauschii*).

KS 92WGRC16 (PI 592728) is also a hard red winter cultivar with the pedigree: Triumph 64/3/KS 8010-71/TA 2470 (*Aegilops tauschii*)//TAM200. This line (KS 94U275) was selected for this genetics study as it performed impressively in field trials. HF resistance in this line was first noticed in field trials grown in experimental plots at the University of Georgia's Experiment Station, located in Plains Georgia in 1999. In 2000, it was included in both greenhouse and field testing for HF resistance conducted at the Georgia Experiment Station in Griffin, Georgia. That year only a few of our elite lines produced good results and amongst them, KS 94U275 was exceptional. Additionally, the line possesses the *Aegilops tauschii* lineage, which has been used as a source of HF resistance genes in previous inheritance studies and molecular marker development programs (Pestsova et al., 2000; Raupp et al., 1993; Gill et al., 1987). KS 94U275 was used in crosses with "GA 901146" and "AGS 2000". KS 94U275 served as the resistant check in tests of segregating populations.

Hessian fly biotype L, maintained by the Insect and Weed Control Unit, USDA-ARS, at Purdue University, was used in the seedling tests. Biotype L was chosen because of it being the most virulent of the HF biotypes (Buntin et al., 2001) presently found in the fields. Larvae can infest wheats carrying *H1H2, H3, H4, H5 H6, H7H8, H11* and *H15* HF resistant genes (Friebe et al., 1989). HF biotypes are designated as GP (Great Plains) and as A to O as determined by the 16 combinations of reactions possible with four differential cultivars each containing one gene: *H3, H5, H6, H7H8* (Patterson et al., 1992).

The number of genes of KS 94U275 and their effectiveness in conferring resistance to HF biotype L was determined by the segregation of BC_1F_1 families from testing conducted in greenhouses/growth chambers. F_1 plants from the cross KS 94U275 x GA 901146 were crossed

to susceptible GA 901146 to obtain BC_1F_1 families, and F_2 populations were obtained from the cross AGS 2000 x KS 94 U275 and its reciprocal cross.

Screening for resistance to the HF was similar to that as described by Cartwright and La Hue (1944), Maas et al., (1987) and Cambron et al., (1996). Testing was conducted in growth chambers both at the University of Georgia and at Purdue University. In general, screening requires the maintenance of HF biotypes, a three week post-infestation period during which symptoms are screened for, proper incubation, and a controlled environment and containment facilities (Dweikat et al., 1994).

For this experiment, seedlings were grown in greenhouse soil mixture with peat moss in wooden flats that each contained 10 evenly spaced rows. BC_1F_1 plants (about 25-30) were planted in eight or nine of the 10 rows. The two end rows in each flat were seeded with appropriate resistant and susceptible checks. Hessian flies were allowed to emerge from a stock of infested wheat plants to infest seedlings in the first-leaf stage under cheesecloth tents placed over the flats. After oviposition for that one day, adults were removed and flats were placed in growth chambers. The temperature inside the growth chambers was maintained at 20^oC ($\pm$ 1^oC), with a 12-h light period and a photosynthetic photon density of approximately 300 mmol m^{-2} s^{-1}. Eighteen to 21 days post-infestation, plants were scored as either being resistant or susceptible. All backcross families were classified as homozygous susceptible or segregating.

Susceptible plants displayed typical HF infestation symptoms: plants appeared stunted with dark green leaves, and live larvae were detectable at the base of the first leaf (Cambron et al., 1995). The resistant plants appeared normal. To verify resistance, the ‘normal’ plants were examined for the presence of dead larvae which confirmed that the seedling was infested but

resistant. All F_2 populations were tested for goodness of fit to known genetic ratios using the Chi-square test, adjusted for small sample size and with one degree of freedom (Steel and Torrie, 1980).

Results and Discussion

Because the pedigree of KS 94U275 contains the *Aegilops tauschii* lineage, and Hessian fly resistance has previously been successfully transferred from this wild relative of wheat into common bread wheat, it was suspected that KS 94U275 would have obtained HF resistance from *Ae. tauschii*. The parents of KS 94U275 have also been tested and found to be susceptible with the exception of TA 2470 (Johnson, unpublished data). This hypothesis was also supported by the fact that KS 94U275 performed well in both field and greenhouse conditions with HF resistance potential being noted as early as 1999. Resistance, as noted by Friebe et al., (1990) was expressed as normal looking plants that contained dead first instars upon closer examination.

The F_1 population from the cross of KS 94U275 x GA 901146 were all resistant (data not shown). This observation suggested to us that a dominant gene was involved in this resistance. The number of genes of KS 94U275 involved in conferring resistance to HF biotype L was evaluated by the segregation of F_2 populations obtained from crosses between AGS 2000 and KS 94U275. In the F_2 analysis of the resistance of AGS 2000 x KS 94U275 and it's reciprocal cross, the populations segregated in a 3:1 (resistant/susceptible) ratio, a manner consistent with that of a trait that is governed by a single dominant gene (Table 3.1). When a F_2 family is segregating for one gene for resistance, one-fourth of the plants are expected to be homozygous resistant, one half heterozygous resistant and one fourth susceptible. Even if resistance of the parental resistant type is not expressed in all parents, resistance would be expected to be expressed in one or more

plants in the F_2 family (Cambron et al., 1995). There were 14 susceptible F_2 plants out of a total of 73 plants in the test of KS 94U275 x AGS 2000, whereas 18 susceptible progeny were expected ($\chi^2 = 0.633, P > 0.50$). Similarly, of the 89 plants that comprised the test of AGS 2000 x KS 94U275, 19 susceptible plants were observed out of an expected 22 susceptible plants ($\chi^2 = 1.319, 0.25 < P < 0.50$). In both instances the deviations were less than or equal to 4 from ideal numbers thus strongly supporting a one governing gene hypothesis. The combined data from these crosses showed 80 % resistance amongst F_2 plants ($\chi^2 = 1.852, P > 0.50$).

The number of genes of KS 94U275 involved in conferring resistance to HF biotype L was additionally evaluated by the segregation of the BC_1F_1 generation obtained from the cross of KS 94U275 x GA 901146. In this BC_1F_1 population, resistance to HF biotype L appeared to be conditioned by one dominant gene (Table 3.2). The Backcross F_1 population segregated in a 1:1 ratio of resistant to susceptible plants, indicating that KS 94U275 possesses a simply inherited dominant resistant gene. Out of the 55 plants tested, 27 resistant and 28 susceptible plants were observed. This gave a χ^2 value of 0.18 ($P > 0.75$).

Hessian fly resistant loci are widely distributed in the D genome of wheat, having been found on chromosomes 1D, 3D, 4D and 6D in hexaploid genotypes derived from *T. tauschii* (Gill et al., 1987; Raupp et al., 1993). It remains unknown just how many additional, nonalleleic genes are carried by the dozens of resistant *T. tauschii* accessions that have not been used in wheat breeding. Continuous efforts in the search for HF resistance genes need to be maintained. To increase the durability of resistance, a good deployment strategy needs to be adopted (El Bouhssini, et al., 1999), otherwise large areas being cultivated with varieties carrying the same

genes would increase the selection pressure on the insect population and result in a more dynamic development of HF biotypes.

To stay ahead of biotype development of HF, entomologists, geneticists and plant breeders alike, have to continue to identify new HF resistant genes and integrate them into superior cultivars. This research has identified another possible gene for resistance to HF that is potentially useful in allowing for greater diversity among genes deployed in breeding resistant wheat cultivars against attack by the Hessian fly.

Acknowledgements

The work of Sue Cambron (USDA-ARS), Insect and Weed Control Research Unit and Dept of Entomology, Purdue University in the rearing of Hessian fly cultures and testing of cultivars is gratefully acknowledged and appreciated. Additionally, this work could not have been completed without the assistance and expertise of Dan Bland and Steve Sutton, research assistants at the Georgia Experiment Station, Griffin, GA. Zinbang Chen, Wook Kim and Melanie Newman for their critical review of this manuscript and overall assistance are also gratefully acknowledged.

References

Cambron, S.E., F.L. Patterson, H.W. Ohm, R.H. Ratcliffe, and G.G. Safranski. 1995. Genetic analyses of Hessian fly resistance in eight durum wheat introductions. Crop Sci. 35:708-714.

Dweikat, I., H. Ohm, S. Mackenzie, F. Patterson, S. Cambron, and R. Ratcliffe. 1994. Association of a DNA marker with Hessian fly resistance gene *H9* in wheat. Theor. Appl. Genet. 89:964-968.

El Bouhssini, M., J.H. Hatchett and S. Lhaloui. 1996. Larval survival on wheat plants carrying resistance genes to Hessian fly (Diptera:Cecidomyiidae) in Morocco. Arab J. Plant Prot. 13(2):103-105.

Formusoh, E.S., J.H. Hatchett, W.C. Black IV and J.J. Stuart. 1996. Sex-linked inheritance of virulence against wheat resistance gene *H9* in the Hessian fly (Diptera: cecidomyiidae). Ann. Entomol. Soc. Am. 89:428-434.

Friebe, B., J.H. Hatchett, R.G. Sears and B.S. Gill. 1990. Transfer of Hessian fly resistance from 'Chaupon' rye to hexaploid wheat via a 2BS/2RL wheat–rye chromosome translocation. Theor Appl Genet. 79:385-389.

Gill, B.S., and W.J. Raupp. 1987. Direct genetic transfers from *Aegilops squarrosa* to hexaploid wheat. Crop Sci. 27: 445-450.

Hatchett, J.H. and R.L. Gallun. 1970. Genetics of the ability of the Hessian fly, *Mayetiola destructor* (Say) to survive on wheats having different genes for resistance. Ann Entomol. Soc. Am. 63:1400-1407.

Hatchet, J.H., K.J. Starks, and J. A.Webster. 1987. Insect and mite pests of wheat. p. 625-675. In E.G. Heyne (ed.) Wheat and wheat Improvement. Agron. Monogr. 13. ASA, CSSA, and SSSA, Madison, WI.

Patterson, F.L., J.E. Foster, H.W. Ohm, J.H. Hatchett, and P.L. Taylor. 1992. Proposed system of nomenclature for biotypes of Hessian fly (Diptera: Cecidomyiidae) in North America. J. Econ. Entomol. 85:307-311.

Patterson, F.L., and R.L. Gallun. 1973. Inheritance of resistance of Seneca wheat to race E of the Hessian fly. Proc. Int. Wheat Genet. Symp. 4: 445-449.

Perry, J. 1990. The genetic relationship between resistance in wheat, *Triticum aestivum* L., and Biotypes C and L of the Hessian fly, *Mayetiola destructor* (Say). M.Sc. Thesis, Department of Entomology, Purdue University, West Lafayette, Indiana, 1990.

Pestsova, E., M.W. Ganal and M.S. Roder. 2000. Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome Vol. 43:689-697.

Raupp, W.J., A. Amri, J.H. HAtchett, B.S. Gill, D.L. Wilson and T.S. Cox. 1993. Chromosomal location of Hessian fly-resistance genes *H22, H23* and *H24* derived from *Triticum tauschii* in the D genome of wheat. J. Hered. 84:142-145.

Sosa, O., Jr. 1981. Biotypes J and L of Hessian Fly discovered in an Indiana wheat field. J. Econ Entomol. 74:180-182.

Steel, R.G.D. and J.H. Torrie. 1980. Principles and procedures of statistics: A biometrical approach. 2nd ed. McGraw-Hill Book Co., New York.

Stuart, J.J., S.J. Schulte, P.S. hall and K.M. Mayer. 1998. Genetic mapping of Hessian Fly avirulence gene *vH6* using bulk segregant analysis. Genome 41: 702-708.

Zantoko, L., and R.H. Shuckle. 1997. Genetics of virulence in the Hessian fly to resistance gene *H13* in wheat. J. Hered. 88:120-123.

Table 3.1. Inheritance of resistance to Hessian fly biotype L in KS 94U275 as shown by reaction of BC_1F_1 populations from the cross between KS 94U275(R) and GA 901146(S).

	BC_1F_1 Plants				
Cross	Resistant	Susceptible	Total	χ^2	P-value
Expected Ratio 1:1	———	number	———		
KS 94U275*2/GA 901146	27	28	55	0.18	> 0.75

Table 3.2. Segregation analysis for reaction to *Mayetiola destructor* Say in F_2 populations developed from crosses between KS 94U275(R) and AGS 2000(S).

	F_2 Plants				
Cross	Resistant	Susceptible	Total	χ^2	P-value
Expected Ratio 3:1	number				
AGS2000 x KS 94U275	70	19	89	0.633	> 0.50
KS 94U275x AGS2000	59	14	73	1.319	0.25-0.50
Total	129	33	162	1.852	0.10-0.25

CHAPTER 4

MOLECULAR MARKERS LINKED TO HF RESISTANCE IN KS 94U275[2]

2 Samuel-Foo, M.S. and J.W. Johnson. 2003. To be submitted to The Journal of Plant Breeding.

Abstract

The Hessian fly (HF) [*Mayetiola destructor* (Say)] is a seriously damaging pest of winter wheat (*Triticum aestivum* L.) in the United States and throughout the wheat growing regions of the world. Genetic resistance is the most effective and economical means that has been employed to effect HF control in wheat. To date, 29 Hessian fly resistance genes, designated *H1* to *H29,* have been identified in wheat. The genetic interactions between wheat resistance genes and HF biotypes are highly specific and exhibit a gene for gene relationship. Molecular markers are potentially useful in aiding breeders in their pursuit of superior cultivars that are resistant to HF infestation as they can substantially accelerate the time needed to develop these cultivars. In this study, F_2-derived wheat populations from KS 94U275(R) x AGS 2000(S) and KS 94U275(R) x GA 901146(S) were used to attempt to identify DNA markers through effective screening of these lines against 274 publicly available primer pairs. The F_2 lines and parents were screened for HF resistance at the USDA-ARS Insect and Weed Control Research Unit and Department of Entomology at Purdue University. The wheat plants were infested with Biotype L of HF in all tests. Simple Sequence Repeats (SSRs) analysis was conducted using a combination of bis-acrylamide (29:1) vertical gel, and agarose-metaphor (1.2 - 1.5%) horizontal gel electrophoresis to identify DNA markers associated with HF resistance in KS 94U275.

Introduction

The Hessian fly (HF) [*Mayetiola destructor* Say] is a destructive pest of wheat (*Triticum aestivum* L.) in many parts of the world including the southern United States (Hatchett et al., 1987). Wheat is the primary host of the HF, although the insect pest may infest triticale, barley and rye occasionally (Ratcliffe and Hatchett, 1997). In the southeastern United States, HF infestations has been a major limiting factor in wheat production as larval injury reduces grain quality and size, weakens the stem at the site of feeding and causes stem breakage as the grain matures (Buntin, 1999).

Wheat is grown world wide and is the most widely adapted cereal. Usually, breeders attempt to improve several different characters in their breeding programs, including: yield, quality, adaptation and pest resistance among others. With the arthropod pest, *Mayetiola destructor*, permanence of insect resistance is a main consideration as HF biotypes continue to make it difficult to achieve lasting resistance (Allan, 1987).

The science of plant breeding and plant genetics is currently undergoing a great revolution with the incorporation of molecular (DNA) markers to complement traditional breeding practices. Through the integration of molecular techniques with customary breeding practices, it is now becoming possible to accelerate the time that would normally be required to develop improved cultivars. No longer are breeders limited to trait selection at the phenotypic level, as selection of desirable traits at the molecular level is now becoming the reality that allows for the introgression/transfer of genes to be completed in minimal time.

Microsatellites or Simple Sequence Repeats (SSRs) are tandem repeats of 2-4 nucleotides that are frequent and almost randomly distributed throughout most eukaryotic genomes (Pestova

et. al., 2000). Molecular markers based on SSR sequences allow for the detection of high levels of polymorphism within a species, which can then be easily assayed using polymerase chain reactions (PCR). The SSR technique has gained rapid acceptability because of its codominant nature, reproducibility, and high information content (De Loose and Gheysen, 1995). The variation in the number of repeats present in these loci is what determines the length differences of the amplified fragments (Manifesto et al., 2001).

In this investigation, SSRs analysis was performed to identify DNA markers associated with HF resistance in KS 94U275, an experimental line developed by the WGRC at Kansas State University, using a set of publicly availably SSR primers (Roder et al., 1998; Pestsova et al., 2000; Cregan et al., 2002).

Materials and Methods

F_2 populations were derived lines from the crosses of KS 94U275 (R) x AGS 2000 (S) and KS 94U275 (R) x GA 901146 (S). The parents were initially crossed in the fall of 1999. F_1 generations were grown in greenhouses at the Georgia Experiment Station, Griffin Georgia followed by the F_2's the next year. Prior to maturity, young leaves of F_2 plants were individually harvested and frozen for later DNA analysis.

In the laboratory, DNA was extracted from the frozen leaf tissue according to the procedure as described by Sharp (1998), with slight modifications. Approximately 300 mg of tissue was ground in liquid nitrogen, using a mortar and pestle, to a very fine powder to which 1 ml of DNA extraction buffer [4% Sarkosyl (4g), 0.1M-Tris-HCL (10 ml), 10 mM EDTA pH 8.0 (2 ml)] was added. The light green powdery tissue was then transferred to labeled 2 ml

eppendorf tubes to which 750 µl phenol:chloroform:isoamyl alcohol (25:24:1) was added followed by centrifugation at 10 000 RPM for 10 minutes. After careful extraction of the top aqueous layer, the phenol:chloroform:isoamyl alcohol treatment was repeated. Fifty microliters of Sodium acetate ($NaCH_3COOH$) was then added to each tube followed by 2 volumes (1000ul) absolute ethanol. The tubes were placed in a -70^{o}C freezer for 5 minutes to allow maximum precipitation of DNA. After centrifugation, the DNA pellet was washed with 1ml 70% ethanol and allowed to dry in a speed vacuum for 1 hour and then subsequently resuspended in 20µl sterile water. RNAse treatment was performed as necessary.

The DNA was quantified using the Hoefer® DyNA Quant 200 Fluorometer and diluted to 50ng/µl. Five microliters of the extracted DNA was electrophoresed on a 1.5% agarose gel to ensure high-quality DNA. Microsatellite markers that were developed by Roder et al., (1998), Pestsova et al., (2000) and Cregan et al., (2002) were then tested. The primer pairs were obtained from QIAGEN Operon (Operon Technologies, Alameda CA) to survey for polymorphism across the lines. PCR reactions were prepared with each reaction mixture consisting of 250nM of each primer, 0.2mM of each deoxynucleotide, 1.5mM MgCl2, 1 unit Taq Polymerase, and 50-100 ng of DNA in a total reaction volume of 25ul. PCRs were conducted in Perkin-Elmer (Norfolk, CA) thermocyclers. The cycling consisted of 3min at 94^{o} C, followed by 45 cycles of 94^{o} C at 1 min, with 1 min at either 50, 55 or 60^{o} C (as per the specific annealing temperature of the individual microsatellite), 2 min at 72^{o} C with a final extension step of 10 min at 72^{o} C. Upon completion of the cycling procedure, the reaction mixtures were held at 4^{o}C.

Initially, primer pairs were selected for screenings from the D genome as we suspected that the HF resistance was coming from the *T. tauschii* lineage of the parent KS 94U275. PCR

reactions were set up as described above surveying for polymorphisms between the parental lines KS 94U275 (R), GA 901146 (S) and AGS 2000 using each of the selected primer pairs. The reaction products were then electrophoresed on 1.2-1.5% agarose-metaphor and/or 29:1 bisacrylamide gels in 1X TBE buffer (Tris-Borate EDTA) at 90-130 V for 3 to 4 h, stained with ethidum bromide and photographed under ultra violet light. The size of each band was estimated simultaneously by means of a 50-bp DNA Ladder (life Technologies-Gibco BRL) that was loaded in adjacent lanes of the gels. From these initial screenings, a total of 30 potential polymorphisms were detected between the parental lines and set aside for further analysis, including 6 polymorphic primer pairs [XGDM006, XGDM077, Xgwm102, Xgwm296, Xgwm301 and Xgwm484] which were verified in screenings conducted at Purdue University (Table 4.1).

Once the initial screening for polymorphism between the parents was completed, additional PCR reactions, comprising KS 94U275, GA 901146 and/or AGS 2000 and at least nine resistant and susceptible F_2 progeny in most cases, were set up following similar conditions as described above, to screen the primer pairs that had previously been shown to be polymorphic for either KS 94U275, GA 901146 or AGS 2000. Leaves from resistant and susceptible F_2 had been harvested and frozen to provide a source of DNA for this analysis. The PCR reaction components were manipulated (varying $MgCl_2$ concentrations, annealing temperatures etc) in an attempt to arrive at optimal conditions for the reactions. The PCR products were then separated on 1.2-1.5% agarose-metaphor gels. In those instances where bands appeared ambiguous and it was difficult to distinguish between bands, the PCR products were run out on 29:1 bis/acrylamide gels for greater resolution of bands.

Results and Discussion

Out of the 274 publicly available primer sets (Roder et al., 1998; Pestsova et al., 2000 and Cregan et al., 2002) that were surveyed for polymorphism between the parentals KS 94U275(R), GA 901146(S) and/or AGS 2000(S), a total of 30 polymorphic primer pairs were detected (Table 4.1). Additional and more thorough screenings of these lines along with resistant and susceptible F_2 progeny, revealed what appeared to be distinct polymorphisms in five out of the 30 lines (Table 4.2). Polymorphisms were detected at the following loci: Xgwm030, Xgwm102, Xgwm174, Xgwm484 and Xgwm539 (Figure 4.1).

Subsequent attempts to duplicate these findings however, produced ambiguous results. The banding patterns that were initially observed proved difficult to reproduce at times. This was the case in both the agarose and acrylamide gels that were run in efforts to confirm the separation of fragments. For instance, for primer pair Xgwm174, the marker that was revealed from initial screenings was estimated to be in the range 325-350bp. The band was present in eight out of eleven (8/11) resistant F_2 progenies and absent in four out of nine (4/9) F_2 susceptible progenies. Three lanes failed to amplify any bands (Figure 4.2). The band was very visible across the resistant progenies, despite variations in the intensity across the gel which could be attributed to a number of factors including, inconsistencies in loading of the gel and "slippage" of the enzyme during amplification reactions. The fact that this band appeared in two of the susceptible lines could be attributed to a number of different factors including misclassification of the susceptible plant and recombination between the marker and gene. If crossing over occurred between the resistant gene and the SSR in a heterozygous plant, then the SSR could generate a positive result in a susceptible plant. Furthermore, if the loci are dispersed sufficiently far apart on a

chromosome they may not be able to detect the SSR and if frequent crossover events occur, independent segregation will appear to take place even though the SSR and the gene are on the same chromosome. The situation was similar for all five of the other primer pairs that had revealed distinct polymorphisms from initial screenings between parental lines (data not shown).

Generally screening for SSRs is a time-consuming, laborious and expensive procedure and despite best intentions and careful screening, quite often, as was the case with this study, only a small number of potential microsatellites loci might be revealed and even then these may not produce the targeted results. Also, during the amplification process, the Taq polymerase enzyme can "slip", leading to the production of products that differ by about 1-5 repeat units from the expected products (Ciof et al., 1998) and frequently this results in products with decreased intensity which may inadvertently be missed during visualization of the bands. Bueteler et al., (1999) suggested that caution be used when relying on band size in the interpretation of SSR polymorphism. Also, as reported by Devos et al., (1995), missing amplification products can be attributed to sequence alterations (such as point mutations, deletions and inversions) within the priming sites.

In conclusion, the identification of markers linked to resistance genes in wheat remains a difficult task due to low levels of polymorphism among cultivars. Hexaploid bread wheat exhibits approximately 1 polymorphic nucleotide per 1000 basepairs. Also, Plaschke et al., (1995) stated that the D genome of wheat shows less variation, possibly due to its more recent incorporation into the wheat genome and maybe concentrating screening efforts in this genome was a dire decision. Pestsova, et al., (2000), reported that even though, *Ae tauschii* has a high potential as a source of microsatellite markers for the D genome of wheat, microsatellites present

in *Ae. tauschii* may be deleted in the D genome of wheat and alternatively, the flanking regions of the microsatellites in these two genomes may be different. What's more, considering the high polymorphism of microsatellites themselves, Pestsova et al. (2000) thinks that it should be expected that the sizes of the amplifying fragments from wheat and *Ae. tauschii* are different.

In summary, wheat remains an elusive crop to work with due to its very nature. Molecular markers, however, have been successfully developed and used for wheat genome analysis by a number of investigators (Devos et al., 1995; Dweikat et al., 1997; Roder at al., 1998; Khan et al., 2000; Anderson et al., 2001, etc), and furthermore, holds great promise for the integration of marker assisted breeding into traditional breeding programs. Microsatellite markers have been shown to be a highly informative and reliable marker system once adequate time and resources can be allocated to the marker development program. The primer pair Xgwm174 may prove to be a weak marker linked to the HF resistance gene in KS 94U275. Further efforts need to be concentrated in the development of markers that show greater linkage to the resistance loci in KS 94U275 as this will allow for easier discrimination between breeding lines that may/may not contain the resistance gene and in overall future marker assisted selections.

Acknowledgements

Zinbang Chen is gratefully acknowledged for giving of his time and efforts to aid in this project and in the review of this manuscript. Melanie Newman and Wook Kim are also acknowledged for reviewing and assisting with this work.

References

Allan, R.E. 1987.Wheat. p. 699-713. In W.E. Fehr [ed], Principles of Cultivar Development. Vol. 2. Crop Species. MacMillian Publishing Company, New York.

Anderson, J.A., R.W. Stack, S. Liu, B.L. Waldron, A.D Fjeld, C. Coyne, B. Moreno-Sevilla, J. Mitchell Fetch, Q.J. Song, P.B. Cregan, R.C. Frohberg. 2001. DNA markers for Fusarium head blight resistance QTLs in two wheat populations. Theor. Appl. Genet. 102: 1164-1168.

Bueteler, M.I., R.L. Jarret, D.R. LaBonte. 1999. Sequence characterization of microsatellites in diploid and polyploidy Ipomoea. Theor Appl Genet 99:123-132.

Buntin, G.D., and P.L. Raymer. 1989. Hessian fly (Diptera: Cecidomyiidae) damage and forage production of winter wheat. J. Econ. Entomol. 82: 301-306.

Ciofi, C. S.M. Funk, T. Coote, D.J. Cheesman, R.L. Hammond, I.J. Saccheri, .W.

Bruford. 1998. Genotyping with microsatellites markers. In: Karp A, Isaac PG, Ingram DS (eds). Molecular Tools for Screening Biodiversity. Chapman and Hall, London, pp. 195-201.

De Loose M., and G. Gheysen. 1995. Identification methods based on molecular techniques. UPOV (International Union for the protection of new varieties of Plants). Working Group on Biochemcial and molecular techniques and DNA-Profiling in Particular, Wageningen, the Netherlands.

Devos, K.M., G.J. Bryan, A.J. Collins, P. Stephenson and M.D. Gale. 1995. Application of two microsatellite sequences in wheat storage proteins as molecular markers. Theor. Appl. Genet. 83: 497-505.

Hatchet, J. H., K.J. Starks, and J. A.Webster. 1987. Insect and mite pests of wheat. P. 625-675. In E.G . Heyne (ed.) Wheat and wheat Improvement. Agron. Monogr. 13. ASA, CSSA, and SSSA, Madison, WI

Hill, C.C, E.J. Udine, and J.S. Pinckney. 1943. A method of estimating reduction in yield of wheat caused by Hessian fly infestation. Washington, D.C: US Dept of Agriculture.

Khan, I.A., J.D. Procunier, D.G. Humphreys, G. Tranquilli, A.R. Schlatter, S. Marcucci-Poltri, R. Frohberg and J. Dubcovsky. 2000. Development of PCR-Based Markers for a High Grain Protein Content gene from *Triticum turgidum* spp. dicoccoided transferred to bread Wheat. Crop Sci. 40:518-524.

Manifesto, M.M., A.R. Schlatter, H.E. Hopp, E.Y. Suarez and J. Dubcovsky. 2001. Quantative evaluation of genetic Diversity in Wheat Germplasm Using Molecular Markers. Crop Sci. 41:682-690.

McColloch, J.W. 1923. The Hessian fly in Kansas. Manhattan, Kansas: Agricultural Experiment Station, Kansas State Agricultural College, 1923

Plaschke, J. M.W. Ganal and M.S. Roder. 1995. Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor. Appl. Genet. 91:1001-1007.

Pestsova, E., M.W. GAnal and M.S. Roder. 2000. Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689-697.

Roder, M.S., J. Plaschke, S.U. Konig, A. Borner, M.E. Sorrells, S.D. Tanksley and M.W. Ganal. 1995. Abundance, variability and chromosomal location of microsatellites in wheat. Mol Gen Genet 246: 327-333.

Roder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M. Tixier, P. Leroy, and M.W. Ganal. 1998. A Microsatellite Map of Wheat. Genetics 49: 2007-2023.

Ratcliffe, R.H. and J.H. Hatchett. 1997. Biology and genetics of the Hessian fly and resistance in wheat. p. 47-56. In K. Bondari [ed], New Developments in Entomology. Publisher, Research Signpost, Scientific Information Guild, Trivandrum, India.

Table 4.1. Polymorphic primer pairs detected from initial screenings of publicly available microsatellites with KS 94U275(R) and AGS2000(S). (Susceptible).

Loci	Chrom. Location	Primer Sequence	Approx. size range (bp)
BARC006	3DL	TTCGGTCGTTGAGGTGACCAATTATG	175-250
		GACAAAGGATTAGCCCAAAGTAAGAG	
BARC026	7D	GCGCTGGGTAAAAAGTGAAATTC	200-300
		TGCAAGTGGAGGGGGAGGCGAGAG	
BARC071	3DL	GCGCTTGTTCCTCACCTGCTCATA	250-350
		GCGTATATTCTCTCGTCTTCTTGTTGGTT	
Xgdm006*	2D	GATCAATCAAGCATGTGTGTGT	150-200
		GGATGCCATGCCAAAGTATT	
Xgdm033	1A/1D	GGCTCAATTCAACCGTTCTT	150-200
		TACGTTCTGGTGGCTGCTC	
Xgdm046	7D	TGTGTTGGCCTTGTGGTG	120-150
		CTACCCAATGCATCCCCTTA	
Xgdm77*	1D	GACACACAATAGCCAAAGCA	180-250
		TGATGTCGGCACTATTTTGG	
Xgdm107	2D	AGCAACAAACGCGAGAGC	150-200
		TGACACCCGGTTGTTGG	
Xgdm111	1D	CACTCACCCCAAACCAAAGT	180-220
		GATGCAATCGGGTCGTTAGT	
Xgdm138	5D	CATGAGCCGATTCAGCG	180-250

Loci	Chrom. Location	Primer Sequence	Approx. size range (bp)
		CGCTTAAATTGAAGTACCGC	
Xgdm141	6D	ATGGAGACCATGGACCAGAG	150-180
		GGCGGTGTTCCTATGCC	
Xgwm003	3D	GCAGCGGCACTGGTACATTT	80-100
		AATATCGCATCACTATCCCA	
Xgwm030	3A/2D	ATCTTAGCATAGAAGGGAGTGGG	130-160
		TTCTGCACCCTGGGTGAT	
Xgwm102*	2D	TCTCCCATCCAACGCCTC	140-160
		5’TGTTGGTGGCTTGACTATTG	
Xgwm165	4A/4B/4D	TGCAGTGGTCAG[ATG][a]TTTCC	160-200
		CTTTTCTTTCAGATTGCGCC	
Xgwm174	5D	GGGTTCCTATCTGGTAAATCCC	210-230
		GACACACATGTTCCTGCCAC	
Xgwm186	5A	GCAGAGCCTGGTTCAAAAAG	110-130
		CGCCTCTAGCGAGAGCTATG5’	
Xgwm276	7A	ATTTGCCTGAAGAAAATATT	100-120
		AATTTCACTGCATACACAAG	
Xgwm282	7A	TTGGCCGTGTAAGGCAG	180-200
		TCTCATTCACACACAACACTAGC	
Xgwm292	5D	TCACCGTGGTCACCGAC	180-220
		CCACCGAGCCGATAATGTAC	
Xgwm296*	2D	AATTCAACCTACCAATCTCTG	180-185
		GCCTAATAAACTGAAAACGAG	

Loci	Chrom. Location	Primer Sequence	Approx. size range (bp)
Xgwm301*	2D	GAGGAGTAAGACACATGCCC	150-170
		GTGGCTGGAGATTCAGGTTC	
Xgwm341	3D	TTCAGTGGTAGCGGTCGAG	155-165
		CCGACATCTCATGGATCCAC	
Xgwm368	4B	CCATTTCACCTAATGCCTGC	255-270
		AATAAAACCATGAGCTCACTTGC	
Xgwm484*	2D	ACATCGCTCTTCACAAACCC	150-155
		AGTTCCGGTCATGGCTAGG	
Xgwm493	3B	TTCCCATAACTAAAACCGCG	175-180
		GGAACATCATTTCTGGACTTTG	
Xgwm539*	2D	CTGCTCTAAGATTCATGCAACC	140-155
		GAGGCTTGTGCCCTCTGTAG	
Xgwm566	3B	TCTGTCTACCCATGGGATTTG	120-130
		CTGGCTTCGAGGTAAGCAAC	
Xgwm617	5A/6A	GATCTTGGCGCTGAGAGAGA	150-165
		CTCCGATGGATTACTCGCAC	
Xgwm644	6B/7B	GTGGGTCAAGGCCAA[GG][b]	140-160
		AGGAGTAGCGTGAGGGGC	

*denotes polymorphisms between parentals that were verified at Purdue University

[a] [AGT] replaces [ATG] in Xgwm165-2D.

[b][AGA] replaces [GG] in Xgwm644-7B.

Table 4.2. List of Potential Microsatellite Markers for HF resistance in KS 94U275

Loci	Chromosome Location	Annealing temperature	Approximate band size (bp)
Xgwm030	3A/2D	60/60	150
Xgwm102	2D	60	180
Xgwm174	5D	55	200
Xgwm301	2D	55	260
Xgwm484	5A	60	150
Xgwm539	2D	60	20

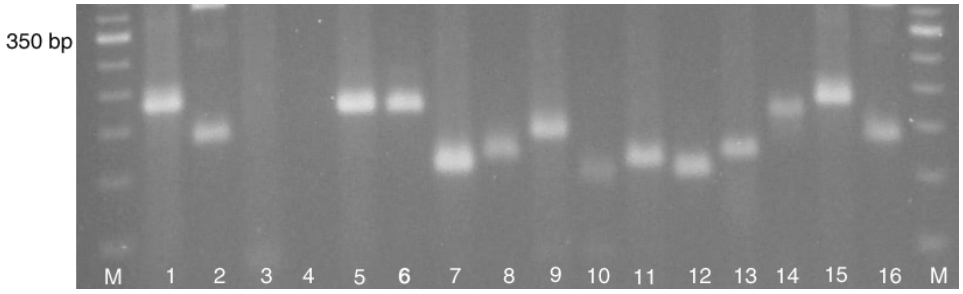

Figure 4.1. SSR polymorphism between parentals KS 94U275 (R) and GA 901146 (S). Odd numbers represent KS 94U275 and even numbers represent GA 901146. Each pair of reactions were screened against SSR primers: Xgwm174, Xgwm276, Xgwm301, Xgwm484, Xgwm539, Xgwm102, Xgwm030 and Xgwm174 (duplicate) respectively. M represents 50bp marker.

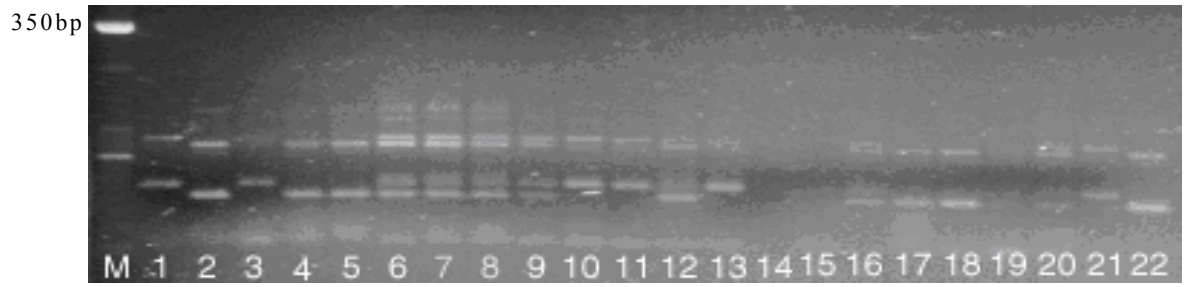

Figure 4.2. DNA fragments from amplification of wheat genotypes with microsatellite marker Xgwm174. Lanes 1 and 2 are the parents of the population KS 94U275 (R) and GA 901146 (S); 3-13 and 14-22 represent the resistant and susceptible F_2 progeny respectively. M indicates 50 bp marker.

-