Chromosome location of the host response to Ptr ToxB produced by race 5 *Pyrenophora tritici –repentis.*

BY

LARISA MORIER

A Thesis
Submitted to the Faculty of Graduate Studies
In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE

Department of Plant Science
University of Manitoba
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FORWARD

This thesis is written in traditional thesis style. The thesis begins with a general abstract, introduction, literature review and materials and methods, followed by results and discussion of each experiment and general discussion and conclusions. The referencing style used in this thesis conforms to the requirements of the Canadian Journal of Plant Science.

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ABSTRACT

Pyrenphora tritici-repentis (Died.), the causal agent of tan spot of wheat, is an important leaf-spotting disease affecting wheat across western Canada. Six races of tan spot have been identified, which cause two symptom types: necrosis and extensive chlorosis. Host-specific toxins, Ptr ToxA and Ptr ToxB, have been identified as the main pathogencity factors causing necrosis and chlorosis, respectively. Understanding the inheritance of resistance to tan spot is critical to effectively breed for resistance. The objective of this study was to determine the number of resistance genes in Chinese Spring to race 5 and Ptr ToxB and to identify the chromosome location of the resistance gene(s). Chinese Spring a resistant wheat parent was crossed with Katepwa, a susceptible wheat parent. Segregation of resistant and susceptible genotypes in F_2 and F_2-derived F_3 families indicated that both resistance to race 5 Algerian isolate 3-24 and insensitivity to Ptr ToxB are controlled by the same single, recessive, nuclear gene. Twenty-one monosomic lines of Chinese Spring were crossed with Katepwa and the F_1 and F_2 generations were evaluated for reaction to Ptr ToxB. The gene for reaction to Ptr ToxB was located on chromosome 2B but the chromosome arm that carried resistance to Ptr ToxB could not be identified. The monosomic series of Chinese Spring crossed with Katepwa was also used to confirm that resistance to Ptr ToxA is located on chromosome 5B.

1. INTRODUCTION

Leaf-spotting diseases cause significant yield losses to wheat (*Triticum aestivum* L.) worldwide (Shabeer and Bockus 1988; Rees and Platz 1989; Sissons 1996; Gilbert et al. 1998). In 1997 the incidence of leaf-spotting diseases in Manitoba fields was 70% (Gilbert et al. 1998) compared to 31% in 1974 (Tekauz 1976). Tan spot (or Yellow Spot) is an important leaf-spotting disease caused by the homothallic ascomycete *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph *Drechslera tritici-repentis* (Died.)shoemaker). Over the last several decades the incidence of *P. tritici-repentis* has increased in Western Canada (Gilbert et al.1998) due mainly to a shift from conventional tillage practices that bury or remove crop residue, to conservation tillage which retains crop residues on the soil surface (Schuh 1990).

P. tritici-repentis reduces yield by decreasing the grain weight of kernels and the number of kernels per spike (Shabeer and Bockus 1988; Rees and Platz 1989; Sissons 1996). The average annual yield loss in north-eastern Australia was 12.7 percent, but under conditions that favoured the disease yield losses as high as 60 percent occurred (Rees et al. 1981; Rees and Platz 1989).

Wheat is an important crop in western Canada. In 1999, 3,212,000 acres of wheat were seeded in Manitoba, 14,470,000 acres were seeded in Saskatchewan, and 7,160,000 acres were seeded in Alberta (Manitoba Agriculture and Food 2001; Saskatchewan Agriculture and Food 2001; Alberta Agriculture, Food and Rural Development 2001). Because of the large wheat acreage, even small losses in yield represent large economic losses to the prairie region. Since wheat is an important crop in Western Canada and *P. tritici-repentis* contributes to significant yield losses, control of

this disease is economically important in western Canada. Incorporation of genetic resistance is the most economically and environmentally sound method of control. Understanding the inheritance of resistance is essential to effectively breed for resistance. The objectives of this study were to:

1) Identify the number of resistance genes in Chinese Spring to isolate Algerian 3-24 of race 5 and the toxin Ptr ToxB.

2) Identify the chromosome location of the gene(s) for host reaction to the race 5 toxin, Ptr ToxB.

3) Confirm the chromosome location of the gene for host reaction to the race 2 toxin, Ptr ToxA.

2. LITERATURE REVIEW

2.1 *Pyrenophora tritici-repentis*

2.1.1 Infection process

The life cycle of the causal agent of Tan spot (*Pyrenophora tritici-repentis*) includes two forms, sexually produced ascospores and asexually produced conidia (Appendix 7.1). Pseudothescia live saprophytically on crop residues. Once mature they produce ascospores (primary inoculum), that are released and can initiate the primary infection cycle (Hosford 1971). A moist environment favours the production and saprophytic growth of pseudothescia on crop residues and the production of next generation ascocarps (Pfender et al. 1988). Conservation tillage practices allow pseudothescia to stay viable for several years on crop residues (Summerell and Burgess 1989). In the spring ascospores are dispersed from pseudothescia during wet periods (Hosford 1972). Dispersed ascospores infect young wheat seedlings. Conidia are produced on infected plants and form the secondary inoculum (Hosford 1972). Conidia can cause multiple infection cycles, approximately eight days in length (Riaz et al. 1991). Secondary inoculum, conidia, can also move in from fields large distances away (Francl 1997). However, Sone et al. (1994) suggest that disease spread by either the primary or the secondary inoculum is very limited and neighbouring fields are unlikely to cause epidemics where no primary inoculum already exists.

Primary and secondary inoculum are both wind borne and require 12 to 48 hours of leaf wetness for optimum infection, but as little as 6 hours of leaf wetness will allow

infection to proceed (Hosford et al. 1987). Conidiation on leaves can occur with only a nightly dew or evening precipitation and could be enhanced by the short wetness period at sunrise (Francl 1998). Temperatures between 20 °C and 25 °C are optimum for infection to proceed (da Luz and Bergstrom 1986; Lamari and Bernier 1994).

Ascospores contribute to increased disease severity incidence, and increased area under the disease progress curve by providing primary inoculum early in spring (Schuh 1990, Adee and Pfender 1989, Rees and Platz 1980). Shabeer and Bockus (1988) found that these early season ascospore infections contribute 17 percent of the total yield loss due to *P. tritici-repentis* infection. The remainder of the yield losses caused by *P. tritici-repentis* results from the secondary infection cycle caused by conidia (secondary inoculum). Therefore, ascospore production is important in the initiation of disease development and conidia production is the primary contributor to late season infection and yield losses.

2.1.2 Symptom and Race Differentiation

P. tritici-repentis of wheat produces two distinct symptom types on susceptible wheat genotypes, tan necrosis and extensive chlorosis. Necrosis is characterized by tan-coloured tissue which develops around a small dark brown to black spot (Lamari and Bernier 1989b). Extensive chlorosis is characterized by the development of diffuse, progressive yellowing of leaf tissue, without tissue disintegration (Lamari and Bernier 1991; Lamari et al. 1991; Lamari et al. 1995b).

Lamari and Bernier (1989b) identified more than one pathotype of *P. tritici-repentis*. Their initial results grouped isolates into four pathotypes: pathotype 1

(nec+chl+) caused both necrosis and chlorosis on susceptible host genotypes, pathotype 2 (nec + chl -) caused only necrosis, pathotype 3 (nec – chl +) caused only chlorosis on hexaploids and only necrosis on tetraploids and pathotype 4 (nec – chl -) was neither necrosis or chlorosis-inducing and is thus termed avirulent (Lamari and Bernier 1989a, b, 1991). The pathotypes were later classified into six different races based on the symptoms produced on a differential host set (Table 2.1) (Lamari et al. 1995b, Pers. Com. Dr. L. Lamari 2001). Races 1 to 4 correspond to pathotypes 1 to 4, respectively. Race 5 (nec – chl +) induces only chlorosis on hexaploid wheats and necrosis on tetraploid wheats, however, the genotypes which are susceptible differ from race 3 (Lamari et al. 1995b; Gamba and Lamari 1998). Race 6 (nec - chl +) induces chlorosis on susceptible host genotypes that is also susceptible to either race 3 chlorosis or race 5 chlorosis, suggesting that this race is a combination of race 3 and race 5 virulence factors. Races 1 and 2 are the most common races found in western Canada (Lamari et al. 1995b; Lamari et al. 1998). Both races 3 and 4 are found at very low levels in Western Canada with race 3 found predominately on durum wheat cultivars (Lamari et al. 1995b; Lamari et al. 1998). A single isolate of race 5, which originates from Algeria, has been found in Canada and was characterized by low virulence (Strelkov et. al., *in progress*).

A rating scale from 1 to 5 is used to characterize reactions to *P. tritici repentis* (Lamari and Bernier 1989a). Lesion type 1 = small dark brown to black spots without any surrounding chlorosis or tan necrosis (resistant); 2 = small dark brown to black spots with very little chlorosis or tan necrosis (moderate resistance); 3 = small dark brown to black spots completely surrounded by a distinct chlorotic or tan necrotic ring and the lesions are

Table 2.1: Differential host set used to differentiate the six races of *Pyrenophora triciti-repentis* (Lamari et al. 1998; Gamba et al. 1998; Lamari 2001, unpublished data).

Genotype	Ploidy	Race 1	Race 2	Race 3	Race 4	Race 5	Race 6	Ptr ToxA	Ptr ToxB
Salamouni	6X	R [z]	R	R	R	R	R	R	R
Katepwa	6X	S (N) [y]	S (N)	R	R	S (C)	S (C)	S (N)	S (C)
6B365	6X	S (C) [x]	R	S (C)	R	R	S (C)	R	R
6B662	6X	R	R	R	R	S (C)	S (C)	R	S (C)
Glenlea	6X	S (N)	S (N)	R	R	R	R	S (N)	R
Sceptre	4X	S (N)	S (N)	S (N)	R	S (N)	S (N)	S (N)	R
4B1149	4X	R	R	R	R	R	R	R	R

[z] R = The host displays a resistant reaction when the race is inoculated on the plant.

[y] S (N) = The host displays a susceptible necrotic reaction when the race is inoculated on the plant or the toxin is infitrated.

[x] S (C) = The host displays a susceptible chlorotic reaction when the race is inoculated on the plant, or the toxin is infiltrated.

generally not coalescing (moderately susceptible); 4 = small dark brown to black spots completely surrounded with chlorotic or tan necrotic zones and some of the lesions are coalescing (susceptible); 5 = dark brown to black centres that may or may not be distinguishable and most infected zones consist of coalescing chlorotic or tan necrotic lesions (highly susceptible).

2.1.3 Toxins

Several *P. tritici-repentis* toxins have been isolated from different races. Several toxins were isolated from race 1, 2, 3 and 5 (Orolaza et al. 1995; Tuori et al. 1995; Strelkov et al. 1998; Balance et al. 1989; Brown and Hunger 1993). Host toxicity to each toxin is genotype specific (Tomas and Bockus 1987). Resistance is expressed as insensitivity to (a) toxin(s).

Several studies have correlated the host-pathogen reaction and the host-toxin reaction (Tomas and Bockus 1987; Lamari and Bernier 1989c; Duguid 1995; Tuori et al. 1995; Stock 1996; Gamba et al. 1998). Thus an insensitive host reaction to a toxin can be correlated with a resistant host reaction to the pathogen that produces that same toxin and a sensitive host reaction to a toxin can be correlated with a susceptible host reaction to the pathogen that produces that same toxin. Ciuffetti et al. (1997) concluded that toxin production is the main pathogencity factor since a single gene for toxin production can be moved from a virulent isolate to an avirulent isolate resulting in virulence. Other host-selective toxins responsible for plant diseases also have a correlation between a host's susceptibility to a pathogen and the host's sensitivity to the toxin produced by that pathogen, suggesting that both are controlled by the same gene, or set of genes (Scheffer

and Livingston 1984). Scheffer and Livingston (1984) suggested that for host-selective toxins a single-gene for susceptibility and sensitivity supports the theory that sensitive plants possess a receptor site for the toxin and that resistant plants lack this receptor.

To eliminate confusion, toxin designations were standardized so that all toxins purified thus far are designated Ptr ToxX and genes are designated ToxX, where X = A, B, C.... (Ciuffetti et al. 1998). Ptr ToxA is a necrosis-inducing toxin produced by isolates of race 1 and 2 and the gene coding for toxin production has been designated as ToxA. Chlorosis toxin designations have also been standardized so that all chlorosis-inducing race 5 toxins are designated Ptr ToxB and all chlorosis-inducing race 1 or 3 toxins are designated Ptr ToxC. The corresponding genes for these chlorosis toxins are designated ToxB and ToxC, respectively (Ciuffetti et al. 1998).

2.1.3.1 Necrosis Toxins

Ballance et al. (1989) isolated and purified a necrosis toxin that was a large monomeric protein with a molecular weight of about 13900 Da. This heat-labile purified toxin, produces necrosis at infiltration levels as low as 0.2nM on sensitive host tissue. Tomas et al. (1990) also purified a necrosis toxin, however, the toxin they purified is a heat-stable protein with a molecular weight of about 14700 Da. This protein, would only produce necrosis at a minimum infiltration level of 90nM, much higher than the toxin purified by Ballance et al. (1989). Tuori et al. (1995) wished to clarify the characteristics of the toxic components in necrosis-inducing isolates. They identified several chromatograpically and immunologically distinct toxins, however, they too purified one major necrosis toxin. They concluded that this component which they designated Ptr

ToxA was most likely the same toxin as that purified by Tomas et al. (1990). Other minor toxic components were obtained, the cationic minor toxins were concluded to be most likely the same as the toxin purified by Ballance et al. (1989) and the anionic minor toxins may be yet another novel group of toxins. Tuori et al. (1995) hypothesized that all of these toxins may be produced in lower or higher amounts depending on the isolate being used in the study. Ciuffetti et al. (1997) confirmed the results of Tuori et al. (1995) by using the gene that encodes for Ptr ToxA, ToxA. Ciuffetti and Tuori (1999) hypothesized that all of the above toxins could be produced by the same gene but undergo different processing to yield toxins with slightly different properties but that elicit the same response. Moreover, two independent sequencing projects revealed that the toxins isolated by Ballance et al. (1989) and by Tomas et al. (1990) had the same sequence except for one base pair and that this change did not change the coding sequence (Tomas et al. 1990; Ciuffetti and Tuori 1999; Zhang et al. 1997; Ballance et al. 1989).

Lamari et al. (1995a) found that Ptr ToxA is produced *in planta* only by necrosis-inducing isolates and both sensitive and insensitive plants show *in planta* production. Toupin (2000) found that the tonoplast was the primary site of action of Ptr ToxA. In addition Toupin (2000) suggested that Ptr ToxA passes freely across the cell wall but passes across the plasma membrane via endocytosis. Zhang et al. (1997) established the secondary structure of the Ptr ToxA protein and hypothesized that a membrane adhesion site along with several phosphorylation sites exist and may be important in the phytotoxic action of the pathogen. Kwon et al. (1996, 1998) suggested that Ptr ToxA caused the leakage of K^+ and H^+ ions out of the plasmalemma and that low

temperature and metabolic inhibitors suppressed this movement. As well, Kwon et al. (1998) hypothesized that Ptr ToxA requires active host processes including transcription, translation and functional H^+-ATPase enzymes in order for a susceptible response to occur.

2.1.3.2 Chlorosis Toxins

Studies on the chlorosis-inducing toxin(s) were lead by Orolaza et al. (1995). Culture filtrates from chlorosis-inducing race 5 isolates produced a sensitive reaction on susceptible hosts and an insensitive toxin reaction on resistant hosts, indicating that chlorosis toxin was most likely the main pathogenicity factor (Orolaza et al. 1995). The toxin isolated in this study, designated Ptr ToxB, is a hydrophilic protein larger than 3.5 kDa that is stable when exposed to organics (Orolaza et al. 1995). Strelkov (1998) isolated a chlorosis toxin that was 6.61 kDa in size, hydrophilic, heat stable, a monomeric protein and was considered to be the same toxin as that isolated by Orolaza et al. (1995).

Strelkov et al. (1998) determined that Ptr ToxB is involved in the light-dependant degradation of chlorophyll, or photo-chemical bleaching. Chlorophyll synthesis was not affected. Photo-oxidation of the chlorophyll occurred and was the result of the direct or indirect inhibition of photosynthesis.

2.1.4 Inheritance of Host Reaction to *P. tritici-repentis*

Reaction to *P. tritici-repentis* is controlled by the genotypic interaction between the wheat plant and the pathogen. A susceptible reaction occurs when the host has a susceptible genotype and the pathogen has a virulent genotype (Lamari and Bernier

1989b; Schilder and Bergstrom 1990). If either one of these two criteria are not met, resistance will result. Host susceptibility may be dominant or recessive depending on the cultivar and the race of *P. tritici-repentis* tested (Lamari and Bernier 1991; Duguid 1995). Reactions to the necrosis symptom type and the chlorosis symptom type are controlled independently of each other (Lamari and Bernier 1991; Lamari et al. 1991; Duguid 1995; Gamba et al. 1998).

False resistance can appear when the temperature either dips below or rises above the optimum temperatures for *P. tritici-repentis* disease development (da Luz and Bergstrom 1986; Lamari and Bernier 1994). The optimum temperatures for disease development were reported as 18-20 °C under controlled environments and 24-28 °C in the field (da Luz and Bergstrom 1986 ; Lamari and Bernier 1994; Sone et al. 1994)

2.1.4.1 Inheritance of Resistance to Necrosis-Inducing Races

Prior to the recognition of two symptoms, necrosis and chlorosis, several studies indicated that resistance to race 1 was quantitatively inherited (Elias et al. 1989; da Luz and Hosford 1980; Schilder and Bergstrom 1990; Krupinsky 1992). Lamari et al. (1991) and Sykes and Bernier (1991) concluded that contradictory genetic results in previous studies were due to the failure to separate necrosis and chlorosis symptoms within the susceptible response and that resistance to race 1 was qualitatively inherited in all ploidy levels.

Resistance to race 1 was identified as being controlled by two independent, recessive loci in hexaploid wheats, one recessive locus in diploid wheats, and either one or two recessive loci in tetraploid wheats (Sykes and Bernier 1991). Duguid and Brûlé-

Babel (2001b) further characterized race 1 resistance as being controlled by two independent, nuclear genes, in which resistance to the necrosis-inducing component and its corresponding toxin was recessively controlled and resistance to the chlorosis-inducing component was dominantly controlled.

Resistance to race 2 necrosis and insensitivity to the necrosis toxin, Ptr ToxA, is controlled by a single, recessive, nuclear gene (Lee and Gough 1984; Lamari and Bernier 1991; Duguid and Brûlé-Babel 2001a ; Stock 1996; Stock et al. 1996; Faris et al. 1996). A gene controlling reaction to race 2 was located on the long arm of chromosome 5B, designated *tsn1* (Faris et al. 1996; Stock et al. 1996). Two RAPD (Random Amplified Polymorphic DNA) markers were loosely linked (approximately 23 to 27cM) to the *tsn1* resistance gene (Stock 1996). Faris et al. (1996) identified two RFLP (Restriction Fragment Length Polymorphisms) loosely linked to *tsn1* (approximately 6cM and 17cM).

2.1.4.2 Inheritance of Resistance to Chlorosis-Inducing Races

Resistance to yellow spot (common name of *P. tritici-repentis* in Australia) was reported as incomplete and possibly possessing a complex and additive form of resistance (Rees and Platz 1989; Rees and Platz 1990). However, Rees and Platz (1990) later found high levels of resistance in many Brazilian wheat varieties suggesting that their initial reports, limited only to Australian wheat varieties which do not have high levels of resistant genotypes, were incomplete.

Resistance to race 3 chlorosis is controlled by one recessive, nuclearly inherited gene which functions independently from the one recessive, nuclearly inherited gene that controls resistance to race 5 chlorosis (Gamba et al. 1998). Resistance to race 3 isolate

D308 was reported to be dominant in the cross between 6B365 and Glenlea and incompletely dominant in the cross between 6B365 and Salamouni (Lamari and Bernier 1991). Duguid and Brûlé-Babel (2001a) found that resistance to race 3 was controlled by a single, dominant, nuclear gene in crosses between ST15 and Erik, ST6, 6B367 or 6B1043. However, in the crosses between BH1146 and ST15 there were two dominant, nuclear genes and between Katepwa and ST15 there were two recessive, nuclear genes conferring resistance (Duguid and Brûlé-Babel 2001a). As well, resistance to race 3 was discovered to be associated with RFLP markers on the long arm of both 1B and 3B (Faris et al. 1997).

Susceptibility to race 5 and sensitivity to the race 5 chlorosis toxin, Ptr ToxB, are controlled by the same dominant, nuclear gene in hexaploid wheats (Orolaza et al. 1995; Gamba et al. 1998).

Resistance to race 1 chlorosis can be associated with several RFLP markers with the major effect established on the short arm of chromosome 1A (Faris et al. 1997; Effertz et al. 1998).

Gamba and Lamari (1998) found that on susceptible (sensitive) tetraploid wheats race 5 and race 3 produced necrosis and Ptr ToxB does not produce cholorsis. The necrosis reaction induced by race 3 and race 5 in tetraploid wheats is controlled independently from each other and independently from the chlorosis induced by race 1 (Gamba and Lamari 1998).

2.2 Cytogenetics

2.2.1 The Wheat genome

The wheat genus *Triticum* includes diploid, tetraploid and hexaploid species. Hexaploid wheat is comprised of three different sets of seven chromosomes for a total of 21 chromosomes in haploid tissue or 42 chromosomes in diploid tissue (Sears 1953). Cytologically, wheat behaves as a diploid due to the *Ph1* gene on chromosome 5BL that inhibits homoeologous pairing (Riley 1974). The three 'genomes' found in hexaploid wheat designated genome A, B and D originated from other species in the *Gramineae* family that contain a basic chromosome number of seven (Riley et al. 1969). Each of the seven chromosomes in one genome have corresponding homoeologous chromosomes in the other two genomes.

The D genome in wheat originates from *T. tauschii* (*Aegilops squarrosa)*, since both *T. tauschii* and the wheat D genome are nearly identical (Sarkar and Stebbins 1956; Sears 1953; Bailey 1999). The A genome on the other hand originates from a diploid Einkorn species (Sears 1953; Sarkar and Stebbins 1956), most likely *Triticum urartu,* not *Triticum monococcum* (Dvorak 1998). The origin of the B genome is more precarious since it is thought that many changes have occurred since the B genome was first introduced into the wheat genus. The most likely candidate for the B genome is *Aegilops speltoides* (*T. speltoides*) (Sarkar and Stebbins 1956; Riley et al. 1969; Alam and Gustafson 1988; Dvorak 1998; Liu et al. 1998a; Bailey 1999). Kimber and Athwal (1972) hypothesized that *Aegilops speltoides* was not the B genome donor, despite the

fact that they were unable to identify a more likely candidate. Blake et al. (1998) and Liu et al. (1998b) suggested that the lack of complete chromosome pairing between the wheat B genome and the genome of *Aegilops speltoides* was due to differentiation of genetic material in both species after polyploidization and not because the B genome did not originate from *Aegilops speltoides*. Liu et al. (1998b) proposed that shortly after polyploidization, homeologous chromosomes underwent non-random loss of low-copy, non-coding DNA sequences causing a divergence in homeologous chromosomes leading to the survival of the polyploid species.

2.2.2 Aneuploids

One of the most powerful tools used in determining genetic composition since the 1940's is the use of aneuploid series (Singh 1993). An aneuploid is the genomic condition in which not all of the chromosomes in a plant genome are present in equal numbers so the total chromosome number is not an exact multiple of the haploid set (Bailey 1999). In aneuploidy there can be either additional copies of one or more chromosomes (i.e. 20+III, 20+IV or 2n+1, 2n+2) or reduced copies of one or more chromosomes (i.e. 20+I , 20 or 2n-1, 2n-2). An aneuploid series is a series of lines in which a single chromosome in part, or in whole, is missing or multiplied and the chromosome affected by this change is different for each line in the series.

Aneuploid series allow cytogeneticists to identify differences in phenotype between plants which have the normal chromosome complement and plants that are lacking the normal chromosome complement. Differences in phenotype indicate the change in a specific gene or allele which controls the trait in question and provides

cytogenetists with information about how the genome is organized. Spanning many years, Sears (1953) developed monosomic, nulli-tetrasomic and ditelosomic aneuploid series from the wheat cultivar Chinese Spring.

A few genes in the wheat genome are vital for normal diploid reproduction. The homology between the three 'genomes' of wheat is such that homeologous chromosome pairing can occur forming what is known as multivalents (association of three or more chromosomes). The *Ph1* gene on the long arm of chromosome 5B prevents mulitvalents from forming, making it a vital component for normal reproduction (Riley and Chapman 1958; Sears 1969; Kimber and Athwal 1972; Riley 1974). If this gene is missing mulitvalents will form and normal reproduction will be inhibited. Similarly, chromosome 3D possesses a gene controlling homoeologous pairing (Riley 1974). Chromosomes 2A, 3A and 3B are known to have genes that control chiasma formation (Sears 1969), however these genes are not as potent as the *Ph1* gene, so their loss can often be tolerated. Since these genes are important for normal diploid reproduction the aneuploid series developed by Sears is not complete.

Due to abnormalities in pairing unexpected chromosome aberrations can occur in the crossing and selfing of aneuploid lines. These aberrations include telocentric chromosomes (only one arm), isochromosomes (two arms which are genetically identical), univalent shifts and other irregularities in chromosome structure (Unrau 1950; Sears 1953; Person 1956). Univalent shift is the change in the monosomic condition from one homologous pair of chromosomes to another homologous pair of chromosomes. Univalent shift often results from the formation of several (usually 3) univalents at meiosis (Person 1956). These univalents can divide normally or misdivide (lost) (Person

1956).

2.2.2.1 Monosomics

A monosomic series consists of a set of wheat lines in which each line is missing one chromosome in a homologous pair of chromosomes (Figure 2.1). When monosomic plants are selfed, three types of progeny are produced; monosomic, missing one chromosome of the homologous pair, nullisomic, missing both chromosomes of the homologous pair, or disomic, containing both chromosomes of the homologous pair. Female gametes are more likely to tolerate the loss of a chromosome compared to male gametes (Sears 1953). Therefore, a higher proportion of monosomic progeny than nullisomic or disomic progeny are produced when monosomics are selfed (Appendix 7.2) (Sears 1953). The expected percentages of monosomic, nullisomic and disomic progeny from a selfed monosomic are approximately 73 percent, 3 percent and 24 percent, respectively (Appendix 7.2)(Sears 1953).

When trying to identify the chromosomal location of a trait, the line in which the desired phenotypic difference occurs is termed the critical monosomic (Sears 1953). This critical monosomic possesses only one allele so both recessive and dominant phenotypes will be expressed. When a critical monosomic with either the recessive or dominant allele is crossed with a normal homozygous individual after one selfing generation, the allele carried by the homozygous individual will be the only allele expressed in the monosomic state instead of the normal 3 dominant to 1 recessive ratio (Figure 2.2). The expected segregation ratios of the progeny from the critical monosomic will differ if the trait of interest is expressed as a recessive or dominant trait, however, both recessive and dominant traits will differ significantly from the normal 3:1 ratio (Figure 2.2).

A – Monosomic for 1A, one homologous chromosome only (purple). Normal homoelogous chromosome pair 1B(blue).

B - Nulli-tetrasomic for 1A, no homologous pair 1A (purple) and two copies of homoelogous pair 1B (blue).

C – Ditelosomic for 1A, one arm of the homologous pair 1A. Normal homoelogous pair 1B (blue).

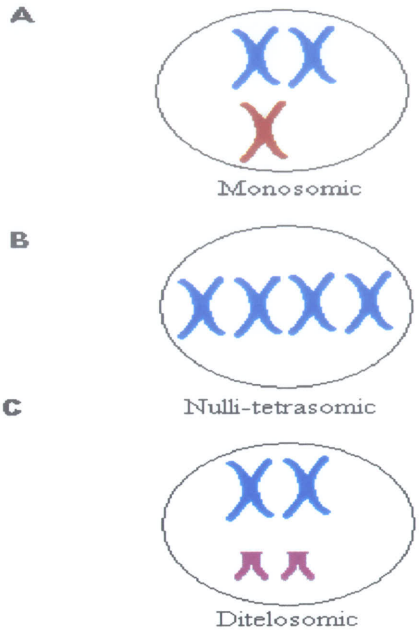


Figure 2.1 : Aneuploid series available in wheat.

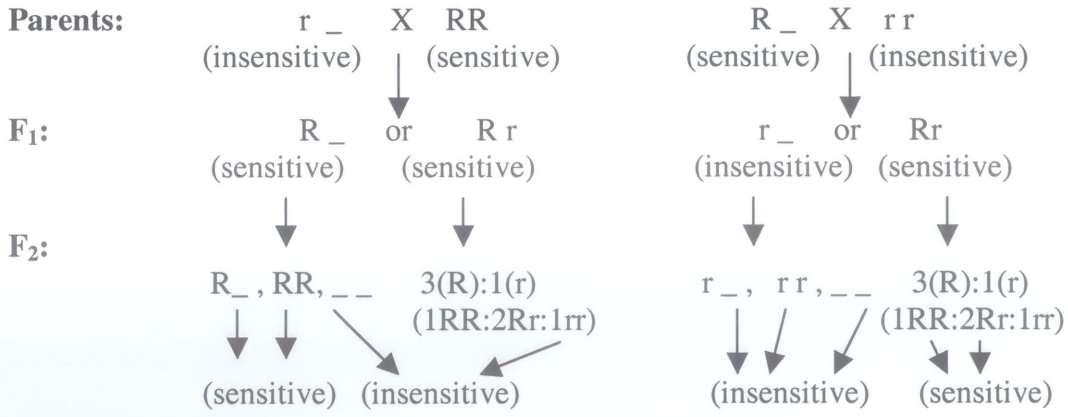


Figure 2.2: The resulting progeny when a monosomic plant is crossed with either a homozygous recessive or dominant plant.

2.2.2.2 Nulli-tetrasomics

A nulli-tetrasomic series can similarly be used to identify the chromosome location of a trait. A nulli-tetrasomic series consists of a set of wheat lines in which each line is missing both chromosomes of a homologous pair (nulli) and are replaced by a compensating pair of chromosomes (tetra – because they have four copies of the homologous chromosome) (Figure 2.1). In wheat the compensating pair of chromosomes is one of the two homeologous pairs of chromosomes. For example, if the homologous chromosome 4A is missing it will be replaced with either the homologous chromosome 4B or 4D, which are homeologous to 4A.

When nulli-tetrasomic individuals are selfed they produce only nulli-tetrasomic progeny. The critical nulli-tetrasomic, like the critical monosomic refers to the chromosome which the trait of interest lies on. The critical nulli-tetrasomic will exhibit a different phenotype than the non-critical nulli-tetrasomics in the first generation since no alleles for the trait are present in the critical nulli-tetrasomic. Crossing or selfing generations for both dominant and recessive traits are not required.

2.2.2.3 Ditelosomics

Ditelosomic plants lack one arm on both chromosomes in a homologous pair (Figure 2.1). A ditelosomic series contains a set of lines in which a different arm is missing in each line. The genetics of a ditelosomic series works in the same manner as a nulli-tetrasomic series, however, only involving one arm of a homologous pair instead of

the whole chromosome. Ditelosomics are used to identify the chromosome arm that carries the trait of interest. Deletion stocks also exist and operate on the same principles as ditelosomics, however, in this case only a small portion of one arm is missing (Endo and Gill 1996).

3. MATERIALS AND METHODS

3.1 Plant Material

3.1.1 Sources

Monosomic, nulli-tetrasomic and ditelosomic lines of the cultivar Chinese Spring were obtained from two sources. The first source, Agriculture and Agri-food Canada Cereal Research Centre, Winnipeg, Manitoba, Canada, provided 10 seeds of each available monosomic line. The second source, United States Department of Agriculture - ARS, Aberdeen, Idaho provided 10 seeds of each available monosomic and nulli-tetrasomic lines, 5 seeds of each available ditelosomic line and 20 seeds of disomic Chinese Spring.

Within the aneuploid series a line consists of all plants missing or suspected to be missing the same chromosome. For example, in the monosomic population all plants missing chromosome 1A are considered to be a part of the line 1A and all plants missing chromosome 2A are considered to be a part of the line 2A, and so forth. In the case of the nulli-tetrasomic lines both the missing chromosome and the replacing chromosome were used in designating one line. For example, for the line 4A(4B), chromosome 4A is missing and chromosome 4B is present in duplicate. In the case of the ditelosomic lines the chromosome arm which is present is used in the line designation (i.e. 2AL – the long arm of chromosome 2A is present and the short arm of chromosome 2A is missing).

3.1.2 Monosomic Screening

Two repetitions of 5 plants for each line and from each source of monosomic seed were tested for reaction to Ptr ToxB and Ptr ToxA. The seed planted orginated from a selfed monosomic plant. However, these seeds were not confirmed as monosomic therefore, some plants were monsomic and some were not (appendix 7.2). Repetitions 1 and 2 originated from Agriculture and Agri-food Canada and repetitions 3 and 4 originated from United States Department of Agriculture (USDA). Seeds from each monosomic line were planted and crossed with Katepwa as the male parent and the Chinese Spring monosomic line as the female parent. However, the Chinese Spring individuals were not confirmed as monosomic in nature, therefore, some plants will be monosomic and segregate in the expected outcome of a monosomic and some plants will be nullisomic or disomic and segregate in the expected outcome of the nullisomic and disomic. The identity of each parental Chinese Spring plant was maintained.

In repetitions 3 and 4, 20 F_1 individuals from each Chinese Spring monosomic line/Katepwa (CS mono/Katepwa) were self-fertilized and in repetitions 1 and 2 , four F_1 individuals from each CS mono/Katepwa line were self-fertilized. Glycine bags were placed on the spikes of each plant to ensure the purity of the self fertilization. The identity of both parental and F_1 individuals were maintained.

Initially, 15 F_2 individuals from each selfed CS mono/Katepwa F_1 individual in repetition 4 were grown out and tested for their reaction to Ptr ToxB and some to Ptr ToxA. If a line looked like it might be the critical chromosome then 15 F_2 plants from each F_1 individual from repetition 3 were grown out. Finally, an additional 15 F_2 plants

from specific F_1 individuals from repetitions 1, 2, 3 and 4 were grown out and tested for reaction to Ptr ToxB and Ptr ToxA.

3.1.3 Nulli-tetrasomic and Ditelosomic Screening

A maximum of 36 and a minimum of 9 individuals from each nulli-tetrasomic line were planted and evaluated for reaction to Ptr ToxB and Ptr ToxA. Each of these plants were self-fertilized using glycine bags to maintain seed stocks.

A maximum of 28 and a minimum of 8 individuals from each ditelosomic line were planted and evaluated for reaction to Ptr ToxB and Ptr ToxA. Each of these plants were self-fertilized using glycine bags to maintain seed stocks.

3.1.4 Inheritance Population

Normal Chinese Spring from USDA was reciprocally crossed with Katepwa and evaluated for reaction to Ptr ToxB. F_1 progeny were self-fertilized using glycine bags and evaluated for their reaction to Ptr ToxB. One hundred and forty-six Chinese Spring/Katepwa and 54 Katepwa/Chinese Spring F_2 individuals were evaluated for reaction to Ptr ToxB and the race 5 isolate Algerian 3-24. F_2 progeny were self-fertilized and the identity of the F_2 individuals were maintained to produce F_2-derived F_3 families. Twenty individuals from each of 103 Chinese Spring/Katepwa and 40 Katepwa/Chinese Spring F_2-derived F_3 families were evaluted for their reaction to Ptr ToxB.

3.2 Growing Conditions

Individuals from all parental and F_1 generations were planted in 10.16 cm (4") square plastic pots with a mix of 2 parts soil, 1 part sand and 1 part peat moss. Individuals from the CS mono/Katepwa F_1-derived F_2 generation and Chinese Spring/Katepwa F_2-derived F_3 generation were planted in 53.34 cm by 27.94 cm flats with 48 cells per flat (each cell was 6.35 cm by 5.08 cm) containing Terra-Lite 2000 Metro Mix growing media.

All plant material was initially grown in growth rooms with 16 h light (950 $\mu Em^{-2} s^{-1}$ of light intensity from florescent and GrowLux lighting) at 20 °C and 8 h darkness at 16 °C. Once evaluation for reaction to toxins or pathogen was complete, individuals to be grown to maturity were moved to the greenhouse. Parental individuals involved in crossing were kept in the growth rooms until crossing was complete.

3.3 Cytological Examination

Root tips of germinating seeds were excised from CS mono/Katepwa parental, F_1 and F_2 generations, as well as, Chinese Spring nulli-tetrasomic and ditelosomic populations, to view mitotic cell division and determine chromosome number.

3.3.1 Preparation of Tissue for Root Tip Extraction

Seeds were first sterilized in 1 part Javex : 10 parts water for five minutes and then rinsed with sterile water three times. Procedures outlined by Singh (1993) and T. Aung (Per. Com., Agriculture and Agri-food Canada, Cereal Research Center, Winnipeg, Manitoba) were followed. Seeds were then allowed to imbibe in sterile water at room

temperature overnight. Imbibed seeds were placed on sterile sand in petri plates and kept at 5 °C for 24 h. The plates were then moved to 20 °C for approximately 70 h or until root lengths reached 2.5 to 3.8 cm. Root tips from roots that were 2.5 to 3.8 cm long were excised and placed in pre-chilled sterile distilled water and kept at 1 °C for 19 h. The water was removed and root tips were fixed in 3 parts 95% ethanol and 1 part glacial acetic acid (Carnoy's solution I) (Singh 1993; Smith 1947). Root tip samples were stored in the fixative until processed (24 h to 2 years). Once the root tips were excised, the seedlings were planted for further study (see 3.2 Growing Conditions for specific planting conditions).

3.3.2 Root Tip Preparation for Cytological Examination

Procedures outlined by Singh (1993), Smith (1947) and T. Aung (Per. Com., Agriculture and Agri-food Canada, Cereal Research Center, Winnipeg, Manitoba) were followed. To prepare root tip samples for viewing under a microscope, the samples were removed from the fixative and placed in 60 °C *1N* HCl (Smith 1947; Singh 1993). Samples were then incubated for 14 minutes in a water bath at 60 °C and placed in Feulgen staining solution (Singh 1993) (Appendix 7.3). Samples remained in the staining solution for at least 30 minutes or at most 3 days. Root tips were removed from the staining solution and placed on a glass slide. A drop of aceto-carmine solution was placed on the glass slide. The darkly coloured root tip was excised from the rest of the root material and macerated using only perpendicular motions. A cover slip was gently placed on the glass slide and the slide was warmed slightly on a hot plate. Firm pressure was applied to the cover slip and glass slide, once again using only perpendicular

motions. The slide was then viewed using bright field light microscopy at 65x to 1600x magnification.

3.3.3 Chromosome Counting

Dividing cells at metaphase were observed to determine chromosome number. Chromosome number was reported for a sample only after at least three cells with the same chromosome number were observed.

Chromosome counts were only observed on selected individuals from the F_1 and F_1-derived F_2 families of CS mono 2B/Katepwa, CS mono 5B/Katepwa and CS mono 7D/Katepwa.

3.4 Algerian 3-24 Inoculum and Toxin Production

3.4.1 Single Spore Stock Plate Production

Dried leaf material, infected with *P. tritici-repentis* isolate, Algerian 3-24 obtained from L. Lamari (Department of Plant Science,University of Manitoba, Winnipeg, Manitoba) was used as the source for stock plate production as described by Lamari and Bernier (1989a). The leaf material was cut into 2-4 cm pieces and placed on wet filter paper in a sterile glass petri plate and incubated under fluorescent lighting at room temperature for 18 h followed by incubation in the dark at 15 °C for 24 h. Single conidia were then picked from the leaf surfaces and individually placed on V8-PDA media (Appendix 7.4). These single spore plates were incubated for 6-8 days in darkness at 20 °C or until mycelial growth was approximately 6 cm in diameter. These plates were then stored as single spore stock plates at 5 °C for a maximum of 1 month.

3.4.2 Inoculum Production

The procedures outlined by Lamari and Bernier (1989a) were followed for inoculum production. Plugs, 0.5 cm in diameter, were taken from single spore stock plates and individually placed on V8-PDA media. These plates were incubated at 22 °C for 5 days or until mycelial growth was approximately 4 cm in diameter. The mycelial growth was then smashed using the end of a sterile test tube and sterile distilled water. Once mycelial growth was completely flattened, the water was decanted off the plates. The plates were incubated once again under fluorescent lighting at room temperature for 18 h followed by incubation in darkness at 15 °C for 24 h. Conidia produced were harvested with a wire loop and distilled water. Each plate was washed twice and all water and conidia from all plates were placed in a large beaker. The conidial suspension was blended in a blender for a few seconds and the conidia concentration estimated using a hemocytometer (Hausser Scientific 3720, Blue Bell, Pa). The final inoculum concentration was adjusted to 3500 spores ml^{-1} with sterile distilled water. One drop of Tween 20 (polyoxyethylene sorbitan monolaurate) per 100 ml of inoculum was added prior to inoculation to reduce surface tension on the leaf.

3.4.3 Inoculation

Inoculation procedures outlined by Larmari and Bernier (1989a) were followed. A DeVilbis sprayer connected to an air outlet generating 67 KPa of pressure was used to apply the inoculum. Inoculum was evenly sprayed onto individual plants at the 2 to 3 leaf stage until run-off. Once inoculation was completed plants were placed in a misting

chamber to ensure continuous leaf wetness for 24 h. Leaf wetness was provided by an ultrasonic humidifier.

The F_2 progeny from the reciprocal cross between Chinese Spring and Katepwa were screened with the inoculum produced from the Algerian 3-24 isolate along with six plants from three check varieties, resistant Chinese Spring and Erik, and susceptible Katepwa. Plants were rated according to the 1 to 5 scale described by Lamari and Bernier (1989a) (see 2.1.2 Symptom and Race Differentiation).

3.4.4 Toxin Production

To produce partially purified toxin the procedures similar to those outlined by Strelkov (1998) were followed. Four to 6 plugs were taken from single spore stock plates of the isolate Algerian 3-24 and placed into Roux bottles containing 150 ml of Fries liquid media (Appendix 7.5). Roux bottles were placed into an incubator set at 20 °C for 21 days. Culture filtrates were then filtered from the mycelial mats using Whatman No. 1 filter paper. Once the culture filtrates were isolated they were aliquoted into approximately 20 ml volumes and freeze dried.

Ten grams of freeze dried culture filtrate were dissolved into 20 ml of 20 mM sodium acetate and 20 µl of 100 µM PMSF (Phenylmethylsulfonyl fluoride). This solution was then split into equal volumes and centrifuged (Beckman model J2-21 centrifuge) at 1700 xg and 4 °C for 10 minutes. The supernatant was removed and placed into a clean beaker stored in the fridge. The pellets were dissolved with 10 ml of 20 mM sodium acetate and 10 µl of 100 µM PMSF and centrifuged as above. This supernatant was added to the existing supernatant.

Two hundred grams of ammonium sulfate crystals were ground into a fine powder and the desired amount of ammonium sulfate was added to the supernatant according to the calculation (0-25% saturation) (Strelkov 1998):

$$\frac{144.5 * (\text{ml of supernatant})}{1000} = \text{number of grams of ammonium sulfate needed}$$

The ammonium sulfate was completely dissolved in the supernatant and spun at 1700 xg and 4 °C for 10 minutes. In the 0-25% saturation, large, hydrophobic proteins precipitated out. The pellet was discarded and the desired amount of ammonium sulfate was added to the supernatant according to the calculation (25-80% saturation) (Strelkov 1998):

$$\frac{385.72 * (\text{ml of supernatant})}{1000} = \text{number of grams of ammonium sulfate needed}$$

Once again the ammonium sulfate was completely dissolved in the supernatant and the solution spun at 1700x g and 4 °C for 10 minutes. In the 25-80% saturation the smaller, hydrophillic proteins precipitated out. The supernatant was discarded and the pellet was dissolved in 10 ml of 20 mM sodium acetate at pH 4.6.

The final solution was poured into approximately 20 cm of dialysis tubing with a molecular mass cut-off of 3500 Da and dialysized against distilled water for 24 h at 4 °C.

A dilution series of the partially purified toxin was then run so that the concentration that gives the best reaction could be determined. A dilution series of 1/10, 1/20, 1/30, 1/50, 1/100, 1/150 and 1/200 was infiltrated into both the insensitive (Erik and Chinese Spring) and sensitive (Katepwa) checks. The dilution with the best differentiation among checks was then used for further infiltrations with that stock of toxin. Once the best dilution was determined, the concentrated toxin was aliquoted into

0.5 ml volumes and frozen for future use. The dilution that gave the best results for that set of concentrated toxin was recorded for future use.

3.4.5 Toxin Infiltration

A Hagborg device (Hagborg 1970) (Figure 3.1) was used to infiltrate Ptr ToxB toxin into leaves. The plants were then placed under a light intensity of 950 $\mu Em^{-2} s^{-1}$ for 7 days after which they were rated for sensitivity (development of chlorosis) or insensitivity (lack of any discolouration of leaf tissue) (Lamari and Bernier 1989c). Six plants from each check variety were used for each screening, Chinese Spring and Erik were both insensitive to Ptr ToxB and Katepwa was sensitive to Ptr ToxB.

All nulli-tetrasomic and ditelsomic plants were grown and infiltrated with Ptr ToxB. A maximum of 36 plants or a minimum of 8 plants from each line were evaluated for reaction to Ptr ToxB.

In the CS mono/Katepwa population four repetitions of 5 plants from each monosomic line were evaluated in the parental generation for reaction to Ptr ToxB. Twenty F_1 individuals from each CS mono/Katepwa parent in repetitions 3 and 4 and four F_1 individuals from each CS mono/Katepwa parent in repetitions 1 and 2 were evaluated for reaction to Ptr ToxB. Fifteen individuals from CS mono/Katepwa F_1-derived F_2 families from repetitions 3 and 4 were evaluated for reaction to Ptr ToxB. An addition 15 plants from specific CS mono/Katepwa F_1-derived F_2 families were evaluated for reaction to Ptr ToxB from all four repetitions.

All parental and F_1 progeny from the reciprocal crosses between Chinese Spring/Katepwa and Erik/Katepwa were screened with Ptr ToxB. Two hundred F_2

progeny from the reciprocal cross Chinese Spring/Katepwa were evaluated for reaction to Ptr ToxB. Twenty individuals from each of the 143 F_2-derived F_3 families of Chinese Spring/Katepwa and 35 F_2-derived F_3 families of Erik/Kaptewa were also evaluated for their reaction to Ptr ToxB.

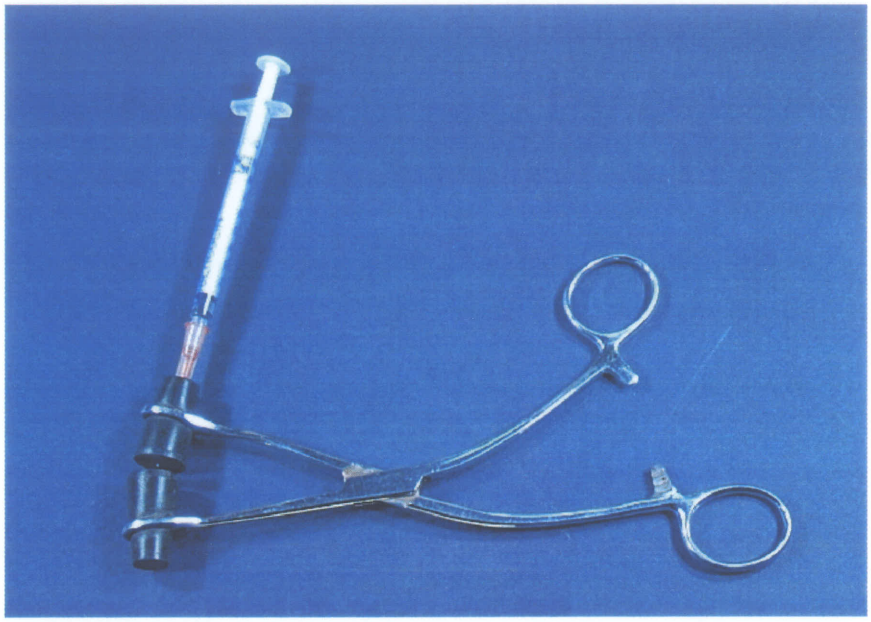


Figure 3.1 : Hagborg device used for infiltration of Ptr ToxB and Ptr ToxA (Hagborg 1970). Leaf tissue is placed between the two stoppers and firm pressure is applied. The syringe is depressed to release toxin into the leaf tissue.

3.5 Infiltration of 86-124 Culture Filtrates (Ptr ToxA)

Culture filtrate from the race 2 isolate 86-124 was obtained from L. Lamari (Department of Plant Science, University of Manitoba, Winnipeg, Manitoba) and was used to test for sensitivity to the race 2 necrosis toxin (Ptr ToxA).

Nulli-tetrasomic and ditelosomic populations, all CS mono/Katepwa lines in the parental and F_1 generations and CS mono 5B/Katepwa and CS mono 7D/Katepwa lines in the F_1-derived F_2 generation were infiltrated with Ptr ToxA using a hagborg device. Once infiltrated, the plants were placed in a growth room under a light intensity of 950 $\mu Em^{-2} s^{-1}$ (the same as growing conditions outlined in 3.2). Plants were rated after 3 days for sensitivity (development of necrosis) or insensitivity (lack of necrosis development).

Six plants from each of Ptr ToxA-insensitive Chinese Spring and Erik and Ptr ToxA-sensitive Katepwa were used as check varieties for evaluating host reaction. A maximum of 36 plants to a minimum of 8 plants from each nulli-tetrasomic and ditelosomic line were evaluated for reaction to Ptr ToxA. CS mono/Katepwa lines were evaluated for reaction to Ptr ToxA, five parental plants from each line in repetitions 3 and 4 and four F_1 plants from each parent in repetitions 3 and 4 were tested. Fifteen to thirty individuals from each F_1-derived F_2 families of CS mono 5B/Katepwa and CS mono 7D/Katepwa from both repetitions 3 and 4 were evaluated for reaction to Ptr ToxA.

3.6 Statistical Analysis

Chi-square goodness of fit tests (Strickberger 1985) were preformed on all F_1-derived F_2 families in CS mono/Katepwa and F_2 and F_2-derived F_3 families in the reciprocal cross Chinese Spring/Katepwa. Test for homogenity was preformed on F_2 and F_2-derived F_3 families in the reciprocal cross Chinese Spring/Katepwa to ensure that data could be pooled (Strickberger 1985).

4. RESULTS AND DISCUSSION

4.1 Inheritance of reaction to Ptr ToxB and the *P. tritici-repentis* isolate Algerian 3-24 in Chinese Spring/Katepwa.

A reciprocal cross between Chinese Spring and Katepwa was used to test the inheritance of reaction to the chlorosis toxin, Ptr ToxB and the *P. tritici-repentis* race 5 isolate, Algerian 3-24. All Chinese Spring parents were insensitive/resistant and all Katepwa parents were sensitive/susceptible to Ptr ToxB and the race 5 pathogen, respectively (Figure 4.1 and 4.2). The F_1 progeny of Chinese Spring/Katepwa and its reciprocal cross were all sensitive to Ptr ToxB indicating that insensitivity to Ptr ToxB is recessive in nature.

Two hundred F_2 progeny from Chinese Spring/Katepwa and its reciprocal cross were screened with both Ptr ToxB and the isolate Algerian 3-24. Individuals that were sensitive to Ptr ToxB were also susceptible to Algerian 3-24. Similarly, individuals that were insensitive to Ptr ToxB were also resistant to Algerian 3-24. No reciprocal differences were observed, therefore, resistance to Ptr ToxB is not controlled by cytoplasmic inheritance but by nuclear inheritance. A chi-square test for homogenity of the F_2 progeny data from both Chinese Spring/Katepwa and Katepwa/Chinese Spring crosses showed that the data could be pooled (chi-square = 0.27, P = 0.60). Given that cytoplasmic inheritance is not involved all data from reciprocal crosses were pooled. The F_2 progeny fit a 3 sensitive/susceptible : 1 insensitive/resistant segregation ratio with 146 sensitive/susceptible individuals and 54 insensitive/resistant individuals (chi-square =

0.43, P = 0.51). Therefore, in the cross between Chinese Spring and Katepwa a single, nuclear gene controls reaction to both Ptr ToxB and the isolate Algerian 3-24 where insensitivity/resistance is recessive and sensitivity/susceptibility is dominant.

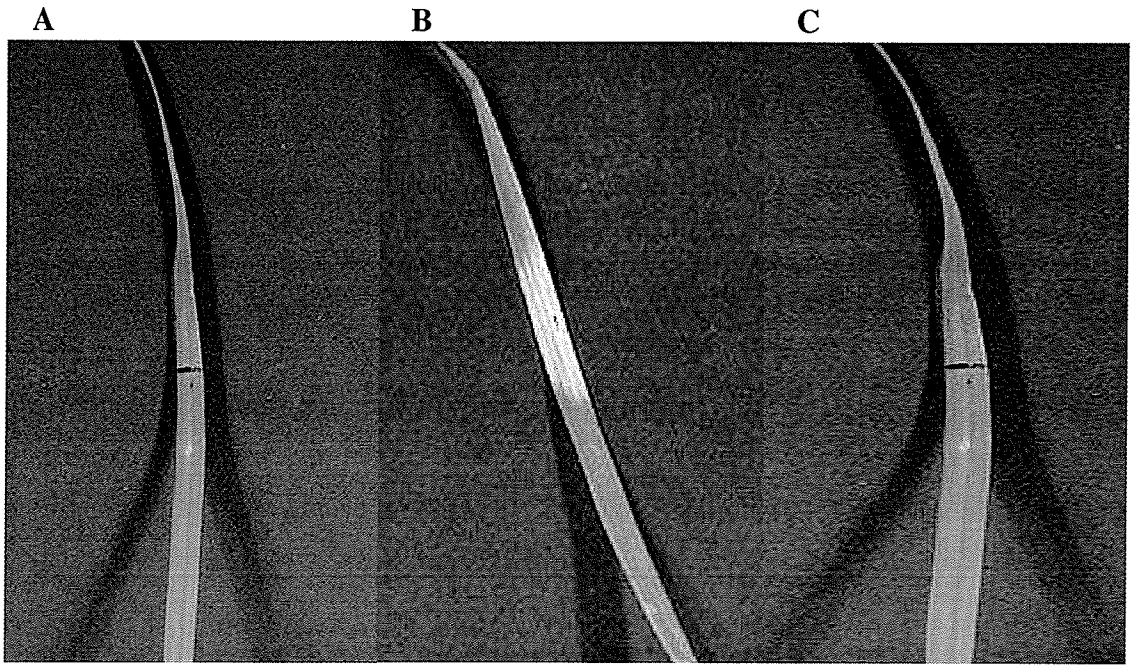


Figure 4.1 : Reaction of parental and check lines infiltrated with Ptr ToxB. A- Erik insensitive check, B- Katepwa sensitive parent/check, C- Chinese Spring insensitive parent/check.

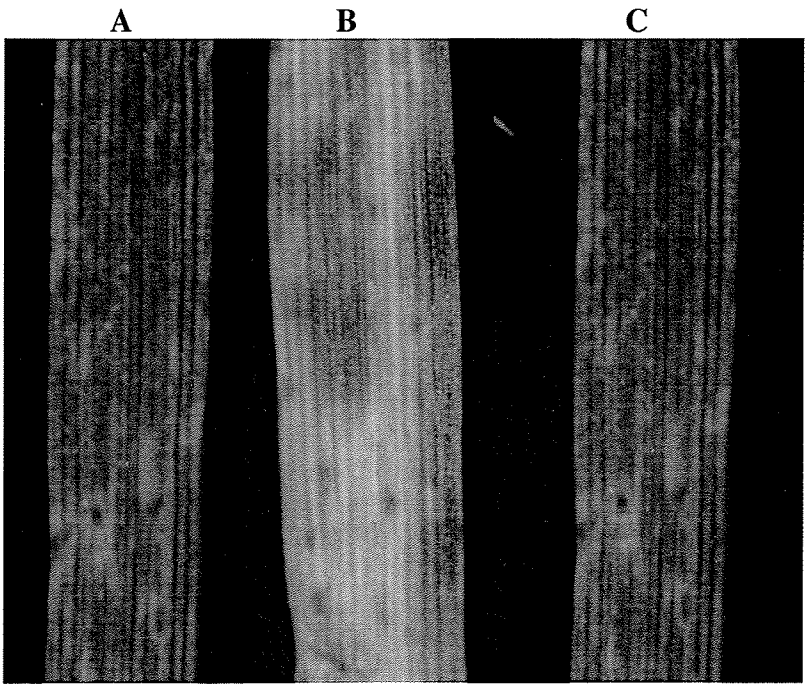


Figure 4.2 : Reaction of parental and check lines inoculated with the race 5 isolate Algerian 3-24. A- Erik resistant check, B- Katepwa susceptible parent/check, C- Chinese Spring resistant parent/check.

A chi-square test for homogenity showed that the F_2-derived F_3 data from the reciprocal Chinese Spring/Katepwa cross could be pooled (chi-square = 0.54, P = 0.46). The F_2-derived F_3 families comprised of 20 individuals per family, fit a 1 homozygous sensitive (33 families): 2 segregating (76 families): 1 homozygous insensitive (34 families) ratio (chi-square = 0.58 and P = 0.45) confirming that Ptr ToxB is controlled by a single recessive, nuclear gene.

Results from Orolaza et al. (1995) suggested that a sensitive/susceptible reaction to both Ptr ToxB and the race 5 pathogen were controlled by a single, dominant, nuclearly

inheritated gene in the reciprocal cross Katepwa/ST15. Gamba et al. (1998) further characterized the genetic control of reaction to Ptr ToxB and the race 5 pathogen by examining crosses between Katepwa, 6B662, 6B365, Erik, Glenlea and ST15. Gamba et al. (1998) also found that sensitivity/susceptibility to both Ptr ToxB and the race 5 pathogen were controlled by a single, nuclear, dominant gene.

The results obtained in this study with the cross between Chinese Spring and Katepwa corroborate the results from both Gamba et al. (1998) and Orolaza et al. (1995) showing that a single, nuclear, recessive gene controls insensitivity/resistance to Ptr ToxB and the race 5 isolate Algerian 3-24.

4.2 Screening of Nulli-tetrasomic and Diteolsomic Populations with Ptr ToxB

Since insensitivity to Ptr ToxB is controlled by a single, recessive, nuclear gene and the Chinese Spring aneuploid series carries the insensitive gene, the easiest way to identify which chromosome possesses the gene for host reaction to Ptr ToxB would be to use nulli-tetrasomic and ditelosomic lines. All available nulli-tetrasomic and ditelosomic lines were screened for reaction to Ptr ToxB (Table 4.1). All lines tested were insensitive to Ptr Tox B. However, ditelosomic and nulli-tetrasomic lines were not available for chromosome arms 2AS, 4BS and 5BL.

Table 4.1: Number of Individuals of ditelosomic and nulli-tetrasomic lines with sensitive or insensitive reactions to Ptr ToxB.

Ditelosomic Chromosome	Number of Individuals Exhibiting Reactions		Nulli-tetrasomic Chromosome	Number of Individuals Exhibiting Reactions	
	- [z]	+ [y]		-	+
1AS	25	0	1A(1B)	17	0
1AL	24	0	1A(1D)	13	0
2AS	10	0	2A(2B)	n.a.	n.a.
2AL	n.a. [x]	n.a.	2A(2D)	n.a.	n.a.
3AS	10	0	3A(3B)	31	0
3AL	11	0	3A(3D)	36	0
4AS	20	0	4A(4B)	22	0
4AL	28	0	4A(4D)	9	0
5AS	n.a.	n.a.	5A(5B)	24	0
5AL	9	0	5A(5D)	29	0
6AS	16	0	6A(6B)	30	0
6AL	21	0	6A(6D)	32	0
7AS	18	0	7A(7B)	29	0
7AL	24	0	7A(7D)	29	0
1BS	8	0	1B(1A)	29	0
1BL	7	0	1B(1D)	32	0
2BS	4	0	2B(2A)	35	0
2BL	3	0	2B(2D)	30	0
3BS	24	0	3B(3A)	n.a.	n.a.
3BL	27	0	3B(3D)	34	0
4BS	8	0	4B(4A)	n.a.	n.a.
4BL	n.a.	n.a.	4B(4D)	n.a.	n.a.
5BS	n.a.	n.a.	5B(5A)	n.a.	n.a.
5BL	24	0	5B(5D)	n.a.	n.a.
6BS	28	0	6B(6A)	n.a.	n.a.
6BL	27	0	6B(6D)	34	0
7BS	21	0	7B(7A)	27	0
7BL	18	0	7B(7D)	32	0
1DS	7	0	1D(1A)	n.a.	n.a.
1DL	8	0	1D(1B)	20	0
2DS	6	0	2D(2A)	18	0
2DL	9	0	2D(2B)	13	0
3DS	8	0	3D(3A)	10	0
3DL	12	0	3D(3B)	13	0
4DS	13	0	4D(4A)	16	0
4DL	11	0	4D(4B)	n.a.	n.a.
5DS	n.a.	n.a.	5D(5A)	13	0
5DL	9	0	5D(5B)	14	0
6DS	10	0	6D(6A)	12	0
6DL	11	0	6D(6B)	15	0
7DS	14	0	7D(7A)	14	0
7DL	16	0	7D(7B)	36	0

[z] insensitive reaction to Ptr ToxB , [y] sensitive reaction to Ptr ToxB

[x] n.a. = not available

Three hypotheses can be used to explain the results in the nulli-tetrasomic and ditelosomic populations. The first hypothesis is that since there are three chromosome arms not represented in the series (2AS, 4BS and 5BL) that the gene controlling reaction to Ptr ToxB maybe present on one of these three arms. If this were the case then the result would be that all available lines are insensitive to Ptr ToxB. We would expect when screening the monosomic population that one of these three chromosomes displays the segregation ratio of the critical chromosome. The study of the segregation ratio of the monosomic series (section 4.3) will indicate whether any one of these arms is the critical chromosome.

The second hypothesis is that the polyploid nature of wheat could lead to a compensation effect (i.e. when the critical chromosome is not present a gene which would not normally be expressed may be expressed masking the lack of the critical chromosome). This could be due to duplicate genes as wheat has been identified as having many duplications and triplications of DNA sequences (Sears 1953; Gill and Gill 1998). Or the (these) masking gene(s) could be (a) suppressor(s) or other epistatic interaction(s). In this situation the screening of the monosomic population would tell us no more than the screening of the ditelosomic and nulli-tetrasomic populations because all monosomic lines would also show 100% insensitivity to Ptr ToxB.

The third hypothesis that would explain the results from the ditelosomic and nulli-tetrasomic populations is that the mode of action of the gene for reaction to Ptr ToxB is passive in nature. If the mode of action is active then one line should be sensitive to the toxin and all other lines should be insensitive (Figure 4.3 - B). In nulli-tetrasomic lines one entire chromosome is not present in the genome, if the resistance gene lies on the

missing chromosome, the toxin reaction will be sensitive, since there is no insensitivity/resistance gene expression to inhibit the effect of the toxin on the plant cell. If the gene lies on any other chromosome in the genome, the presence of the insensitivity/resistance gene will inhibit the effect of the toxin on the plant cell and insensitivity will result. In this case a receptor inhibits the toxin from entering or affecting the cell. The theory is similar for ditelosomic lines, although, instead of an entire chromosome missing only one arm of the chromosome is missing.

However, if the mode of action for resistance is passive in nature then the critical nulli-tetrasomic and all other chromosomes will have the same reaction since the presence of the insensitivity/resistance gene or the absence of the insensitivity/resistance gene still results in an insensitive reaction (Figure 4.3 -A). In this case the toxin must be recognized by a receptor for sensitivity to occur.

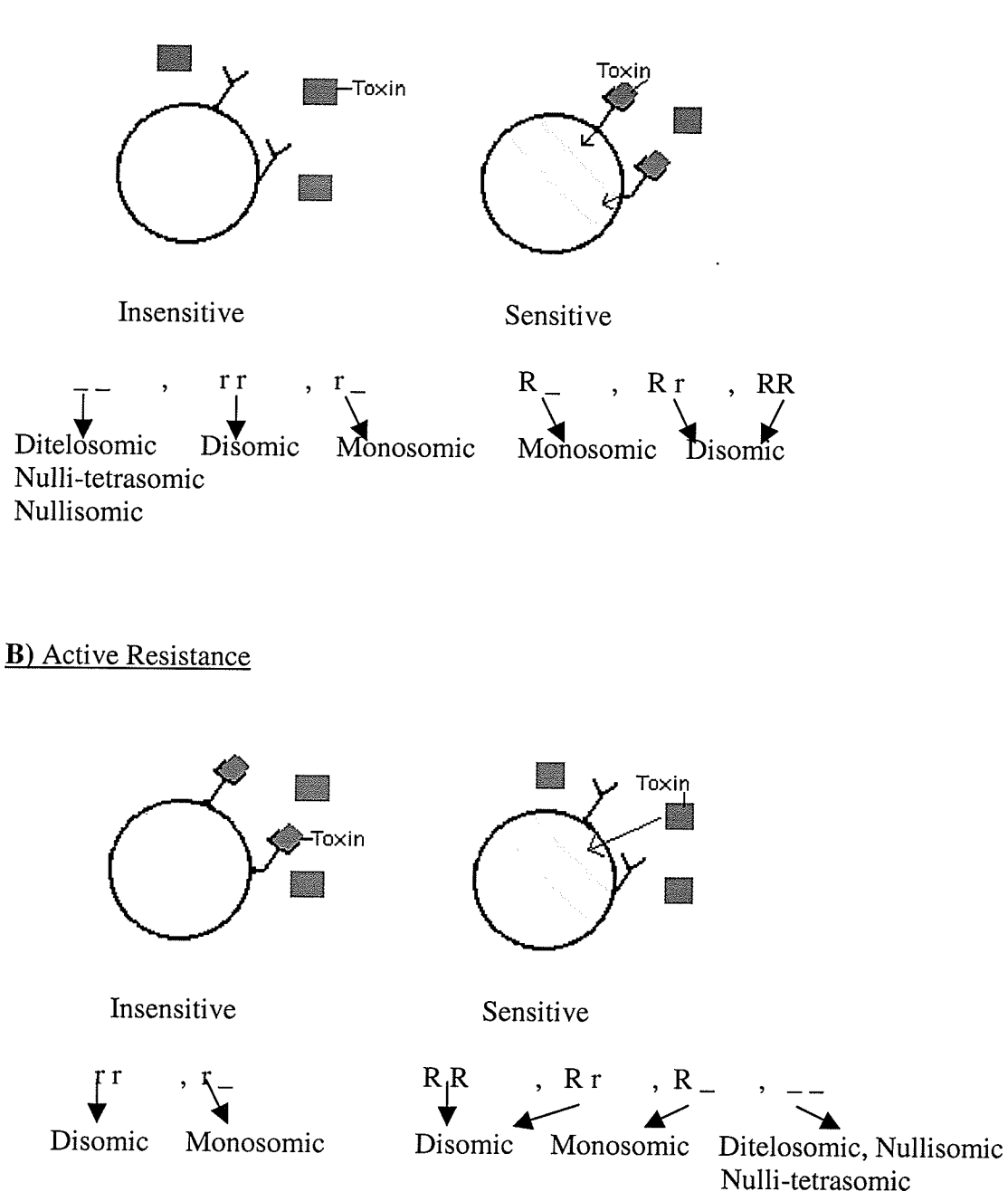


Figure 4.3 : Two modes of action that control a plant's response to host-selective-toxins: passive resistance and active resistance. R = susceptible allele, r = resistant allele.

4.3 Screening of Monosomic Populations with Ptr ToxB

All 434 selfed monosomic Chinese Spring (CS mono) parents (expect: 73% will be monosomic, 24% will be disomic and 3% will be nullisomic, Appendix 7.1) were insensitive and 60 selfed Katepwa parents were sensitive to Ptr ToxB (Table 4.2). Root tips from the Chinese Spring parents were taken prior to crossing with the Katepwa parents in hope that only monosomic individuals would be used in crossing with Katepwa. Unfortunately, due to time constraints, chromosome counts were not finished prior to crossing. Since 73% of the Chinese Spring selfed monosomics were expected to be true monosomics extra crosses were made with the intention of eliminating the non-monosomic crosses later. However, problems with the pretreatment made it impossible to conduct accurate chromosome counts. Therefore, results consist of all CS mono/Katepwa crosses which may include non-monosomic Chinese Spring parents.

All 2129 F_1 progeny from the 21 different lines of CS mono/Katepwa were sensitive to Ptr ToxB (Table 4.2). This was expected, regardless of the type of mode of action (passive vs active), since resistance is recessively inherited.

The 1039 F_1-derived F_2 families from the cross CS mono/Katepwa screened with Ptr ToxB segregated into four different groups: 1 sensitive:1 insensitive, 3 sensitive:1 insensitive, 1 insensitive, and all sensitive (Table 4.2 and Appendix 7.2).

In a cross that is monosomic for the non-critical chromosome, the expected outcome is a 3 sensitive:1 insensitive ratio (Figure 4.4). The majority of the F_1-derived F_2 families segregated into this non-critical chromosome ratio (Table 4.2).

Table 4.2 : Summary of results from screening of all monosomic individuals with Ptr ToxB.

Monosomic Chromosome	Chinese Spring Parental generation		Chinese Spring mono / Katepwa F_1		F_1-derived F_2 families						
	- [z]	+ [y]	-	+	1:1 [x]	3:1 [w]	One Insensitive Individual	All Sensitive	Total families	% Critical Families out of Total # of	% 1:1 Families out of Total # of Families
1A	9	0	0	130	5	58	0	0	63	0.0	7.9
2A	30	0	0	137	4	57	0	0	61	0.0	6.6
3A	14	0	0	142	8	51	3	1	63	6.3	12.7
4A	13	0	0	154	1	130	1	1	133	1.5	0.8
5A	24	0	0	79	6	62	1	0	69	1.4	8.7
6A	13	0	0	130	2	34	0	0	36	0.0	5.6
7A	15	0	0	97	2	32	1	1	36	5.6	5.6
1B	24	0	0	117	1	35	0	0	36	0.0	2.8
2B	30	0	0	158	2	107	22	6	137	20.4	1.5
3B	15	0	0	108	2	41	1	1	45	4.4	4.4
4B	17	0	0	69	0	68	0	1	69	1.4	0.0
5B	14	0	0	86	3	31	1	1	36	5.6	8.3
6B	31	0	0	104	0	48	1	0	49	2.0	0.0
7B	31	0	0	86	1	18	0	0	19	0.0	5.3
1D	10	0	0	93	1	57	2	1	61	4.9	1.6
2D	30	0	0	78	4	59	1	0	64	1.6	6.3
3D	18	0	0	101	0	35	0	0	35	0.0	0.0
4D	30	0	0	71	1	47	1	0	49	2.0	2.0
5D	29	0	0	34	0	33	1	0	34	2.9	0.0
6D	27	0	0	100	9	54	0	0	63	0.0	14.3
7D	10	0	0	55	3	28	2	0	33	6.1	9.1

[z] insensitive reaction to Ptr ToxB , [y] = sensitive reaction to Ptr ToxB

[x] one sensitive to one insensitive ratio of one family to Ptr ToxB

[w] three sensitive to one insensitive ratio of one family to Ptr ToxB

In the nulli-tetrasomic and ditelosomic populations, three chromosome arms were unavailable for screening with Ptr ToxB. These three chromosome arms could carry the gene for reaction to Ptr ToxB and could not be eliminated as possible critical chromosomes. However, monosomic screening of the chromosomes in question (2A, 4B and 5B) revealed that the percentage of critical families were 0% for 2A, 1.5% for 4B and 5.5% for 5B (table 4.2), thus none of these chromosomes carry the gene for reaction to Ptr ToxB.

Two hypotheses can explain the 1 sensitive:1 insensitive ratio. One hypothesis is that the sample size was too small and by chance alone more insensitive individuals were screened than sensitive. The second hypothesis is that Katepwa used in these crosses may contain some unknown compensating effect with Ptr ToxB. Compensating effects between Katepwa and other wheat genotypes have been seen previously with other races of *P. tritici-repentis* (Duguid 1995). The percentage of 1:1 families out of the total number of families screened for each line was well under 10% for all but two lines (3A and 6D). A compensating effect from Katepwa may have a significant effect on the results from monosomic cross involving 6D but has little effect on the results from all other monosomic crosses. Sample size explains the 1:1 ratio in all of the monosomic crosses except 6D. Further work should be done on all monosomic lines to determine whether the 1:1 ratio is a significant factor in the host reaction or is just an artifact of sample size.

Theoretically, if a cross has a monosomic parent then this individual should produce approximately 73% monosomic progeny, 3% nullisomic progeny, and 24% disomic progeny (Sears 1953, Figure 4.4). Therefore, the F_1-derived F_2 families of the

CS mono/Katepwa that have all sensitive or only 1 insensitive individual should have a monosomic parent (F_1) where 3% of the F_2 individuals should be nullisomic (Figure 4.4). If this is the critical monosomic then the disomic and monosomic individuals of the F_2 population should be sensitive to Ptr ToxB because they will still have at least one copy of the allele for sensitivity to Ptr ToxB from Katepwa. The nullisomic individuals in the F_2 population of a critical monosomic will be insensitive to Ptr ToxB because they do not have any copies of the gene for reaction to Ptr ToxB. One insensitive individual in a population of 30 individuals is 3% of that population. A population size that is less than 30 individuals most likely would have no nullisomic individuals, hence no insensitive individuals.

In the parental generation of Chinese Spring 73% are monosomic, 24% are disomic and 3% are nullisomic. When crossed with homozygous sensitive Katepwa the F_1 progeny will be approximately 39.5% monosomic sensitive and 60.5% disomic sensitive. In the F_1-derived F_2 families it would be expected that 39.5% of the families from one line would have the segregation ratio for the critical chromosome if that chromosome carries the gene and all other families would segregate into the normal 3:1 ratio.

In all lines the families that possessed only 1 insensitive individual or no insensitive individuals gave the expected outcome of the critical monosomic (Figure 4.4). Critical F_1-derived F_2 families in all lines, except 2B, made up less than 6.5% of the total families in their respective lines. This means that all lines but 2B are unlikely candidates for the critical chromosome.

In total 22 families out of 137 F_1-derived F_2 families from the cross CS mono 2B/Katepwa possessed only 1 insensitive individual (Table 4.2). Some of these families had a population size of 30 individuals that made 1 insensitive individual 3% of the population. Six families out of 137 from the cross CS mono 2B/Katepwa possessed all sensitive individuals (Table 4.2). Most of these families had a smaller population size of about 15 individuals. The population size was so small in the families with all sensitive individuals that the probability of having an insensitive nullisomic individual was low ($P = 0.37$) but the probability of having an insensitive disomic individual (non-critical chromosome) was still high ($P = 0.99$). Consequently, these families were regarded as having monosomic parents (F_1) of the critical chromosome. These two categories made up 20% of the CS mono 2B/Katepwa families which is slightly less than expected (39.5%) however can be explained by the small sample size.

Chromosome counts on mitotic cell division were conducted to determine the number of chromosomes F_1 and F_1-derived F_2 individuals possessed (Table 4.3). In the cross CS mono 2B/Katepwa all individuals that were thought to have monosomic F_1 parents were in fact monosomic. As well, all F_1-derived F_2 individuals that were hypothesized to be nullisomic (insensitive to Ptr ToxB) were in fact nullisomic.

Table 4.3 **The number of chromosomes present at mitotic metaphase in the F_1 & F_2 progeny of Chinese Spring mono 2B / Katepwa individuals screened with Ptr ToxB.**

Monosomic Chromosome	F_1 Individual	Number of Chromosomes	# Cells Counted [z]	Reaction to Ptr ToxB [w]	Monosomic Chromosome	F_2 Individual	Number of Chromosomes	# Cells Counted	Reaction to Ptr ToxB
2B	2-11 [y]	41	4	+	2B	2-11-28 [x]	40	3	-
2B	2-12	41	4	+	2B	2-12-3	40	3	-
2B	2-20	41	3	+	2B	2-12-10	40	4	+
2B	4-6	41	3	+	2B	2-20-1	41	3	+
2B	4-7	41	3	+	2B	2-20-15	40	4	-
2B	5-3	41	3	+	2B	4-6-5	40	4	-
2B	5-9	41	4	+	2B	4-6-3	41	3	+
2B	6-2	41	3	+	2B	4-7-2	40	3	-
2B	6-7	41	4	+	2B	4-7-15	41	4	+
2B	6-10	41	3	+	2B	5-3-4	40	3	-
2B	6-16	41	4	+	2B	5-3-12	41	4	+
2B	7-2	41	4	+	2B	5-9-18	40	3	-
2B	7-8	41	3	+	2B	5-9-6	41	4	+
2B	7-11	41	4	+	2B	6-7-8	40	3	-
2B	7-14	41	3	+	2B	6-7-5	41	3	+
2B	7-15	41	3	+	2B	7-11-8	40	4	-
2B	8-11	41	3	+	2B	7-11-12	41	3	+
2B	8-12	41	3	+	2B	7-14-7	40	3	-
2B	8-18	41	3	+	2B	7-14-3	41	4	+
2B	8-19	41	3	+	2B	7-15-15	40	4	-
2B	9-1	41	3	+	2B	7-15-8	41	3	+
2B	9-5	41	3	+	2B	7-2-11	40	3	-
2B	9-9	41	3	+	2B	7-2-1	41	3	+
2B	10-4	41	4	+	2B	7-8-9	40	3	-
2B	10-6	41	3	+	2B	7-8-7	41	3	+
2B	10-8	41	3	+	2B	8-11-13	40	4	-
					2B	8-11-5	41	3	+
					2B	8-12-19	40	3	-
					2B	8-12-6	41	3	+
					2B	8-18-3	40	3	-
					2B	8-18-9	41	3	+
					2B	8-19-1	41	4	+
					2B	8-19-17	40	4	-
					2B	9-1-2	41	4	+
					2B	9-1-21	40	3	-
					2B	9-9-2	41	4	+
					2B	9-9-4	40	3	-
					2B	10-4-16	40	3	-
					2B	10-4-2	41	3	+
					2B	10-6-15	40	3	-
					2B	10-6-12	41	3	+

[z] The number of cells counted with the same chromosome number.

[y] First number is the number assigned to the individual in the parental generation and the second number is the number assigned to the individual in the F1 generation.

[x] First and second number same as 'b', the third number is the number assigned to the individual in the F2 generation.

[w] A'+' means sensitive to Ptr ToxA and a '-' means insensitive to Ptr ToxA.

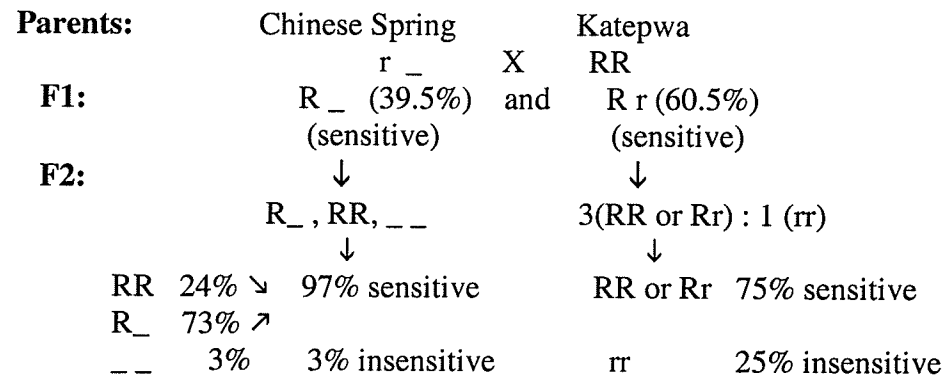


Figure 4.4 : Progeny outcome of the critical monosomic Chinese Spring crossed with Katepwa. Adapted from Sears 1953.

The mode of action of Ptr ToxB is important in determining the number of insensitive individuals to expect. In passive resistance, if an individual possesses the insensitive allele in homozygous state or no allele for insensitivity/sensitivity (chromosome missing) then the individual's reaction to the toxin is insensitivity (Figure 4.3-A). In all other instances with passive resistance the individual's response will be sensitivity. In active resistance, if the sensitive (dominant) allele is present or no allele for insensitivity/sensitivity (chromosome missing) is present then the individual will be sensitive to the toxin (Figure 4.3-B). Therefore, passive resistance must control the response to Ptr ToxB because nullisomic individuals in the F_1-derived F_2 families and all Chinese Spring parents were insensitive to Ptr ToxB. If the response to Ptr ToxB was controlled by active resistance, a small number of sensitive individuals may be present in the selfed Chinese Spring monsomic parents of CS mono 2B/Katepwa and no insensitive individuals would be present in the F_1-derived F_2 families of CS mono 2B/Katepwa.

Therefore, using the monosomic populations screened with Ptr ToxB the insensitivity/resistance gene lies on chromosome 2B since 20% of the CS mono 2B/Katepwa families displayed the characteristic segregation ratios of a critical

monosomic and all other crosses segregated in a 3:1 ratio. The chromosome counts performed on the F_1 and F_1-derived F_2 families substantiated the monosomic or nullisomic nature of individuals with the respective phenotypes. It was concluded that insensitivity (resistance) to Ptr ToxB is passive in nature, that is, that the host plant contains receptors that recognize the toxin and cause sensitivity to Ptr ToxB. Analysis of monosomic, ditelosomic and nulli-tetrasomic populations support the theory that the gene for reaction to Ptr ToxB displays a mode of action of passive resistance.

4.4 Screening of Nulli-tetrasomic and Ditelosomic Populations with Ptr ToxA

Chinese Spring nulli-tetrasomic and ditelosomic populations were screened with Ptr ToxA to determine if the gene for host reaction to Ptr ToxA is truly on chromosome 5BL as published by both Stock et al. (1996) and Faris et al. (1996). All available nulli-tetrasomic and ditelosomic populations were evaluated for reaction to Ptr ToxA and all were found to be insensitive (Table 4.4). However, three chromosome arms were not available for screening with Ptr ToxA (5BL, 2AS and 4BS) so the gene for host reaction to Ptr ToxA could exist on one of these three chromosomes. This result was identical to the result obtained by Stock et al. (1996).

Like Ptr ToxB three hypotheses can be used to explain the results in the nulli-tetrasomic and ditelosomic populations evaluated for reaction to Ptr ToxA. The first hypothesis is that there are three chromosome arms not represented in the series (2AS, 4BS and 5BL) and that the gene controlling reaction to Ptr ToxA is present on one of these three arms. So one of these three chromosomes would show the segregation ratio

for the critical chromosome in the monosomic population if that chromosome possessed the gene for reaction to Ptr ToxA. The second hypothesis, a compensation effect (i.e. when the critical chromosome is not present a gene which would not normally be expressed may be expressed masking the lack of the critical chromosome), would result in all monosomic lines showing 100% insensitivity to Ptr ToxA. This theory is not confirmed in the screening of the monosomic population (see section 4.5). The third hypothesis is that the mode of action for resistance to Ptr ToxA is passive (Figure 4.3 – A). If the mode of action for resistance is passive then both the critical and non-critical nulli-tetrasomic and ditelosomic lines will all have the same reaction, insensitivity. In this case the toxin must be recognized by a receptor for sensitivity to occur. Secondly, the F_1-derived F_2 families of the critical chromosome would show a segregation ratio of 97% sensitive to 3% insensitive instead of all sensitive individuals (active resistance).

Since all of the lines screened were insensitive to Ptr ToxA it can be concluded that either the mode of action that is controlling the plant's response to Ptr ToxA is passive resistance or one of the three chromosomes not available for screening carries the gene for reaction to Ptr ToxA.

Table 4.4 : Number of individuals of ditelosomic and nulli-tetrasomic lines with sensitive and insensitive reactions to Ptr ToxA.

Ditelosomic Chromsome	Number of Individuals Exhibiting Reactions		Nulli-tetrasomic Chromsome	Number of Individuals Exhibiting Reactions	
	- [z]	+ [y]		- [z]	+ [y]
1AS	5	0	1A(1B)	5	0
1AL	5	0	1A(1D)	5	0
2AS	5	0	2A(2B)	n.a.	n.a.
2AL	n.a. [x]	n.a.	2A(2D)	n.a.	n.a.
3AS	5	0	3A(3B)	4	0
3AL	5	0	3A(3D)	5	0
4AS	5	0	4A(4B)	5	0
4AL	5	0	4A(4D)	4	0
5AS	n.a.	n.a.	5A(5B)	5	0
5AL	5	0	5A(5D)	5	0
6AS	5	0	6A(6B)	5	0
6AL	5	0	6A(6D)	5	0
7AS	5	0	7A(7B)	5	0
7AL	5	0	7A(7D)	5	0
1BS	5	0	1B(1A)	5	0
1BL	5	0	1B(1D)	5	0
2BS	4	0	2B(2A)	5	0
2BL	3	0	2B(2D)	5	0
3BS	5	0	3B(3A)	n.a.	n.a.
3BL	5	0	3B(3D)	5	0
4BS	5	0	4B(4A)	n.a.	n.a.
4BL	n.a.	n.a.	4B(4D)	n.a.	n.a.
5BS	n.a.	n.a.	5B(5A)	n.a.	n.a.
5BL	5	0	5B(5D)	n.a.	n.a.
6BS	5	0	6B(6A)	n.a.	n.a.
6BL	5	0	6B(6D)	3	0
7BS	5	0	7B(7A)	5	0
7BL	5	0	7B(7D)	5	0
1DS	5	0	1D(1A)	n.a.	n.a.
1DL	5	0	1D(1B)	5	0
2DS	5	0	2D(2A)	5	0
2DL	5	0	2D(2B)	5	0
3DS	5	0	3D(3A)	5	0
3DL	5	0	3D(3B)	4	0
4DS	5	0	4D(4A)	n.a.	n.a.
4DL	5	0	4D(4B)	n.a.	n.a.
5DS	n.a.	n.a.	5D(5A)	5	0
5DL	5	0	5D(5B)	5	0
6DS	5	0	6D(6A)	n.a.	n.a.
6DL	5	0	6D(6B)	5	0
7DS	5	0	7D(7A)	n.a.	n.a.
7DL	5	0	7D(7B)	6	0

[z] = Insensitive reaction to Ptr ToxA , [y] = Sensitive reaction to Ptr ToxA, [x] n.a. = not available

4.5 Screening of Monosomic Populations with Ptr ToxA

Selfed Chinese Spring monosomic parents from USDA parents 1-5 (repetition #3), also used in the monosomic screening of Ptr ToxB, were all screened with Ptr ToxA. The Chinese Spring parents that were crossed with Katepwa would have 73% monsomic, 24% disomic and 3% nullisomic. Unfortunately, chromosome number of these Chinese Spring parents could not be confirmed (see section 4.3) so all parents were crossed with Katepwa and carried through to the F_1-derived F_2 generation.

All 188 Chinese Spring monosomic parents screened with Ptr ToxA were insensitive to Ptr ToxA (Table 4.5). All 317 F_1 CS mono/Katepwa individuals were sensitive to Ptr ToxA (table 4.5). Since all nulli-tetrasomic and ditelosomic lines were insensitive correlating with results from Stock et al. (1996) F_1-derived F_2 CS mono/Katepwa screening was only carried out on CS mono 5B/Katepwa and CS mono 7D/Katepwa families. Screening of the substitution lines of Chinese Spring mono/Kenya Farmer by Stock et al. (1996) identified CS mono 5B/Kenya Farmer(5B) and CS mono 7D/Kenya Farmer(7D) as possible critical chromosomes.

In the 51 F_1-derived F_2 families of CS mono 5B/Katepwa 14 families possessed one insensitive individual and 11 families possessed no insensitive individuals to Ptr ToxA (Table 4.5). These 25 critical families made up 49% of the CS mono 5B/Katepwa families. In the parental generation of Chinese Spring 73% are monosomic, 24% are disomic and 3% are nullisomic. When crossed with homozygous sensitive Katepwa the F_1 progeny will be approximately 39.5% monosomic sensitive and 60.5% disomic sensitive. In the F_1-derived F_2 families it would be expected that 39.5% of the families

Table 4.5 : Summary of results for all monosomic individuals screened with Ptr ToxA

Monosomic Chromosome	Chinese Spring Parental generation		Chinese Spring mono / Katepwa F_1		F_1-derived F_2 families					
	- [z]	+ [y]	-	+	1:1 [x]	3:1 [w]	One Insensitive Individual	All Sensitive	Total Families	% Critical Families out of Total # of Families
1A	8	1	0	18						
2A	10	0	0	14						
3A	9	0	0	18						
4A	9	0	0	14						
5A	8	0	0	20						
6A	9	0	0	14						
7A	10	0	0	13						
1B	10	0	0	16						
2B	10	0	0	19						
3B	10	0	0	18						
4B	8	0	0	14						
5B	9	0	0	35	1	25	14	11	51	49.0
6B	10	0	0	16						
7B	9	0	0	16						
1D	9	0	0	3						
2D	9	0	0	16						
3D	7	0	0	10						
4D	9	0	0	14						
5D	7	0	0	6						
6D	9	0	0	14						
7D	9	0	0	9	2	24	1	0	27	3.7

[z] = insensitive reaction to Ptr ToxA , [y] = sensitive reaction to Ptr ToxA

[x] = one sensitive to 1 insensitive ratio of one family to Ptr ToxA, [w]= 3 sensitive to 1 insensitive ratio of one family to Ptr ToxA

would have the segregation ratio for the critical chromosome if that chromosome carries the gene. In the crosses of CS mono 5B/Katepwa 49% of the families fit the monosomic F_1 category which is more than the expected 39.5%.

Chromosome counts on F_1 progeny and F_1-derived F_2 individuals of CS mono 5B/Katepwa supported the results that families in the 1 insensitive or all sensitive categories had monsomic F_1 parents and that F_2 individuals that were insensitive were in fact nullisomic (Table 4.6).

In the Ptr ToxB screenings lines that were not the critical monosomic contained less than 10% of the families in the critical two categories. In crosses of CS mono 7D/Katepwa screened with Ptr ToxA only 4% of the 27 F_1-derived F_2 families (Table 4.5) fit into the critical chromosome categories making it unlikely that it possesses the gene for reaction to Ptr ToxA. As well the single F_1-derived F_2 individual that showed insensitivity to Ptr ToxA was not nullisomic when chromosome counts were preformed (Table 4.6).

The theories explaining mode of action, critical monosomic identification and monosomic segregation in section 4.2 and 4.3 for Ptr ToxB are the same for Ptr ToxA. Therefore, the gene that controls reaction to Ptr ToxA is located on chromosome 5B and its mode of action is passive resistance. The chromosome location for Ptr ToxA correlates with Faris et al. (1996) and Stock et al. (1996). However, the mode of action resulting from this study contradicts the results obtained by Stock et al. (1996). Stock et al.'s (1996) findings were that of active resistance not passive resistance. Stock et al. (1996) used several different populations in their study, a set of selfed monsomics for chromosome 5B and 7D, a cross consisting of CS mono 5B/CS(KF 5B) and CS mono

Table 4.6 : The number of chromosomes present at mitotic metaphase in the F_1 & F_2 progeny of Chinese Spring mono/Katepwa individuals screened with Ptr ToxA.

Monosomic Chromosome	F_1 Individual	Number of Chromosomes	# Cells Counted [z]	Reaction to Ptr ToxA [y]	Monosomic Chromosome	F_2 Individual	Number of Chromosomes	# Cells Counted	Reaction to Ptr ToxA
5B	2-2 [x]	41	3	+	5B	2-2-4 [w]	41	3	+
5B	2-5	41	3	+	5B	2-5-15	40	3	-
5B	2-6	41	3	+	5B	3-2-4	40	3	-
5B	2-10	41	3	+	5B	3-4-9	40	3	-
5B	3-2	41	4	+	5B	6-5-7	40	4	-
5B	3-3	41	3	+	5B	6-8-12	40	3	-
5B	3-4	41	4	+	5B	6-14-3	40	4	-
5B	3-5	41	3	+	5B	7-1-7	40	3	-
5B	4-3	41	4	+	5B	7-2-5	40	3	-
5B	5-4	41	3	+	5B	7-5-6	40	3	-
5B	5-6	41	3	+	5B	8-9-12	40	3	-
5B	6-5	41	3	+	5B	9-1-7	40	3	-
5B	6-8	41	4	+	5B	9-3-4	40	3	-
5B	6-9	41	4	+	5B	10-12-15	40	3	-
5B	6-13	41	4	+	7D	1-6-5	42	4	-
5B	6-14	41	3	+					
5B	7-1	41	3	+					
5B	7-2	41	3	+					
5B	7-5	41	4	+					
5B	8-9	41	3	+					
5B	9-1	41	2	+					
5B	9-3	41	3	+					
5B	10-5	41	4	+					
5B	10-12	41	3	+					

[z] The number of cells counted with the same chromosome number. [y] A'+' means sensitive to Ptr ToxA and a '-' means insensitive to Ptr ToxA.

[x] First number is the number assigned to the individual in the parental generation and the second number is the number assigned to the individual in the F1 generation.

[w] First and second number same as 'b', the third number is the number assigned to the individual in the F2 generation

7D/CS(KF 7D) and CS/CS(KF 5B) and CS/CS(KF 7D). The last two sets of crosses (CS mono 5B/CS(KF 5B) and the CS/CS(KF 5B)) identified 5B as the critical chromosome but the selfed monosomics confirmed the critical chromosome and identified the mode of action as active resistance. It is highly likely that the cross between CS mono/Katepwa which was carried out in this study is influenced by the Katepwa parent possessing a compensating gene on another chromosome. Background effects or masked genes were seen in a study on race 3 by Duguid (1995) in Katepwa. It may be possible that Katepwa has additional genes throughout the genome that are only expressed when more dominant genes are deleted. This would lead to an insensitive reaction in nullisomic individuals when you expected a sensitive reaction. Analysis of selfed monosomics would confirm whether the result of passive resistance in this study is an artifact of the cross with Katepwa or is truly passive resistance. The Chinese Spring monosomic parents used to cross with Katepwa for the analysis of reaction to Ptr ToxA were selfed monosomics. All of the Chinese Spring parents were insensitive; if this is active resistance we would expect that a small portion of the CS mono 5B parents would be sensitive to Ptr ToxA since 3% of this population would be expected to be nullisomic. Since chromosome counts on these parents could not be done it is unknown whether there were no nullisomic parents used to cross with Katepwa or if this is truly passive resistance. However, a nullisomic parent would produce 100% monosomic progeny in the F_1 so it would be expected that all the families from that parent would have the critical chromosome segregation in the F_1-derived F_2 generation. As shown, in Appendix 7.7 there were a few parents that had all families segregating as a critical chromosome, however, the population size for these parents were very small and a conclusive answer

can not be drawn from this without either further testing of this parent or the chromosome number of this parent.

Chromosome arm location for Ptr ToxA could not be determined using the nulli-tetrasomic or ditelosomic populations, however, both Faris et al. (1996) and Stock (1996) located molecular markers (RFLPs and RAPDs respectively) on the long arm of chromosome 5B loosely linked to the gene for reaction to Ptr ToxA. It is assumed that since chromosome location was identical to the previous studies that it is highly likely that the chromosome arm identified by both Faris et al. (1996) and Stock et al. (1996) is accurate.

5. GENERAL DISCUSSION

The incorporation of genetic resistance into commercial culitvars of wheat is the most economical and environmentally sustainable method of *P. tritici-repentis* disease control. The identification of two symptom types, necrosis and chlorosis, and 6 races of *P. tritici-repentis* has allowed for a more accurate identification of monogenic resistance. Single gene resistance is much easier to incorporate into existing wheat breeding programs than polygenic resistance. Monogenic resistance also allows for screening of germplasm with molecular markers closely linked to the resistance gene in question.

5.1 Inheritance of Ptr ToxB and the race 5 isolate Algerian 3-24

The inheritance of resistance to Ptr ToxB and the race 5 pathogen in the cross Chinese Spring/Katepwa was confirmed as a single, recessive, nuclearly inherited gene which correlated with the results reported in other hexaploid wheat crosses made by both Orolaza et al. (1995) and Gamba et al. (1998). A single gene for resistance to both Ptr ToxB and the race 5 pathogen also confirmed that Ptr ToxB is the main pathogencity factor in race 5 isolates on Chinese Spring/Katepwa. This means that the toxin can be used with confidence in genetic and molecular studies for both the development of resistant lines and the study of host-pathogen interactions in this cross. Single gene resistance traits are much easier to move into existing culitivars than quanitative traits. Single gene resistance should also mean quicker identification of the pathogen's mode of action.

5.2 Analysis of Nulli-tetrasomic and Ditelosomic Populations Screened with Ptr ToxB and Ptr ToxA

The nulli-tetrasomic and ditelosomic screening for both Ptr ToxB and Ptr ToxA were not as informative as was hoped, since no critical chromosome could be identified for either toxin. However, this does not discredit their usefulness in future studies on chromosome location for other traits of interest. The nulli-tetrasomic and ditelosomic analysis did in the case of the Ptr ToxB analysis confirm the finding of passive resistance. If the ditelosomic or nulli-tetrasomic lines had elucidated the chromosome location of the gene for reaction to Ptr ToxB then deletion stocks could have been used in a similar fashion to get closer to the exact location of the resistance gene.

5.3 Inheritance of Ptr ToxB in Monosomic Chinese Spring crossed with Katepwa

In Ptr ToxB analysis, CS mono/Katepwa F_1-derived F_2 populations showed that chromosome 2B carries the gene for reaction to Ptr ToxB. However, the exact chromosome arm location could not be identified using any of the aneuploid series. Attempts were made to locate the chromosome arm using microsatellite molecular markers, however, due to lack of polymorphisms and limited map saturation attempts were unsuccessful.

The mode of action for resistance to Ptr ToxB was also determined to be passive resistance. Therefore, in order to have sensitivity a receptor site must exist for the toxin (Figure 4.3). Unlike Ptr ToxA, very little work on the mode of action has been completed to date. However, Strelkov (1998) determined that sensitivity to Ptr ToxB is

light-dependent and involves the degradation of chlorophyll but not the inhibition of chorophyll synthesis. Hypothesizing that Ptr ToxB directly or indirectly inhibits electron flow in the light-dependant reactions resulting in the inability of thylakoid membrane to dissipate excited energy causing the degradation of chlorophyll (Strelkov 1998). It is not likely that the receptor site for sensitivity to Ptr ToxB is in the light-dependant reactions themselves since we would probably not see difference in sensitivity between hosts. However, like Ptr ToxA there could be a receptor site for Ptr ToxB on the plasma membrane which leads to the entry of the toxin into the cell. This type of active recognition of the toxin for entry into the cell is conducive to passive resistance which was determined in this study. Once in the cell it could undergo some process so that it is now able to affect the light-dependant reactions of photosynthesis in some manner. Strelkov (Per. Com. 2001) has characterized a partial amino acid sequence of Ptr ToxB and found that most likely the original protein is cleaved into a smaller one once inside the host tissue.

5.4 Inheritance of Ptr ToxA in Monosomic Chinese Spring crossed with Katepwa

Analysis of resistance to Ptr ToxA confirmed its location on chromosome 5B as reported by both Stock et al. (1996) and Faris et al. (1996). However, the mode of action of resistance was different from that reported by Stock et al. (1996). In this study, resistance to Ptr ToxA was determined as passive, therefore, a receptor site for susceptibility is required. Stock (1996) reported that resistance to Ptr ToxA was an active form of resistance where the gene for reaction to Ptr ToxA is required for a resistant reaction to occur. This type of resistance is similar to the defense response system or the

gene-for-gene model where hosts actively recognize pathogen invasion. To defend themselves from attack resistant hosts will produce phytoalexins, excess lignin and cellulose, let neighbouring cells die to deter the spread of hyphae, and control nutrients into and out of infected areas (Deacon 1997). Although, Dushnicky et al. (1998) found that production of lignin was increased in surrounding mesophyll cells and intercellular spaces this increase in production was more of a physical barrier than a nutritive barrier. This suggests that perhaps active resistance is not the mode of action. Similarly, Scheffer and Livingston (1984) stated that most host-selective toxins such as Ptr ToxA have a single gene for reaction to the toxin and that this single gene supports the theory that sensitive plants possess a receptor site for the toxin and resistant plant lack this receptor site. Deshpande (1993) suggested that Ptr ToxA binds to a site on the plasma membrane that alters the permeability of the membrane causing cell death and necrotic lesions, indicating that cell recognition is necessary for a susceptible reaction. Results obtained by Toupin (2000) also support the theory of passive resistance. Toupin (2000) reported that the primary site of action was that of the tonoplast resulting in the disruption of normal cell function. Toupin (2000) suggested that perhaps Ptr ToxA slowly but freely passes across the cell wall and then through endocytosis is brought across the plasma membrane to the tonoplast where its primary site of action may occur. If the mode of action of resistance is passive, then in order for a host to be susceptible it must recognize the toxin. There are two locations in which this recognition could occur, at the tonoplast or at the plasma membrane. The most likely site is at the plasma membrane, since endocytosis requires the recognition of some molecule by a glycoprotein receptor site. Although some animal viruses are transferred passively across the plasma membrane

through endocytosis by "riding" along with molecules taken up for normal cell function (Prescott et al. 1993) Ptr ToxA is a large protein molecule of 13.9 kDa so it is unlikely that it would be able to "ride" along with other molecules normally taken up by the cell. It is more likely that Ptr ToxA directly binds to a receptor site normally used for endocytosis. Zhang et al. (1997) established the secondary structure of Ptr ToxA and hypothesized that a membrane adhension site along with several phosphorylation sites exist. This membrane adhension site may be important in the recognition of Ptr ToxA at the plasma membrane, and phosphorylation sites are also important for signal reconigtion. Kwon et al. (1998) reported that sensitivity to Ptr ToxA required transcription, translation and active H^+-ATPases which suggests that Ptr ToxA is further restructured once inside the host cell.

Although many of the mode of action studies done on the pathogen support the passive form of resistance it is still unclear if the study done by Stock et al. (1996) is an accurate depiction of mode of action (active resistance). Analysis in this study with Chinese Spring/Katepwa to race 5 and the study by Duguid and Brûlé-Babel (2001a) to race 2, suggest that Katepwa possesses only one gene for reaction to each race. However, if one gene is removed it is still uncertain if another gene compensates for the lost of the initial gene in Katepwa. Comparing results from this study and the study by Stock et al. (1996) suggests that perhaps there is a compensation by another gene in Katepwa when the initial gene is lost.

5.5 Conclusions and Future Research

Hexaploid wheat's response to Ptr ToxB was located on chromosome 2B, however, the chromosome arm could not be elucidated from the aneuploid series used. The linkage of molecular markers to this trait would allow for the confirmation of the chromosome identified in this study, as well as, which arm it lies on. Inheritance of hexaploid wheat's response to Ptr ToxB was confirmed as being a single, nuclearly inherited trait with resistance being recessive. The gene that controls host response to Ptr ToxA was confirmed in this study as being on chromosome 5B; once again the chromosome arm could not be verified. Similarly, molecular markers, especially microsatellite markers closely linked to this trait would confirm chromosome location and could be useful in a resistance breeding program.

Further screening of accession lines may identify other sources of resistance. It is still unclear as to whether some cultivars possess more than one gene for response to Ptr ToxB and Ptr ToxA since crosses with Katepwa seem to be showing background effects. Further work looking at Chinese Spring selfed monosomics with confirmed chromosome counts would eliminate background effects from Katepwa or other cultivars. Further screening with Ptr ToxA of already existing F_1-derived F_2 families from the cross CS mono/Katepwa may also identify some parents from the original cross that were nullisomic which would confirm or dispute the mode of action. Screening with Ptr ToxB with the already existing F_1-derived F_2 families from the cross CS mono/Katepwa needs to be carried out to confirm whether the 1:1 ratio seen in some families was just an artifact of sampling size or was background effect from Katepwa.

Often identifying other genes located on the same chromosome as a gene of interest can help determine the location and elucidate the function and organization of the genome. Other disease resistance genes located on chromosome 2B are: several of the QTLs for *Puccinia graminis tritici* (stem rust) on both the long and short arms (Loegering 1978), cereal cyst nematode on the long arm (Williams et al 1996), one of the QTLs for *Puccinia recondita* (leaf rust) *Lr23* on the short arm (Nelson et al. 1997), one of the genes for *Ustilago tritici* (loose smut) (Procunier et al. 1997) race T10 on the long arm. F_2-derived F_3 families of Chinese Spring/Katepwa were screened for segregation for reaction to Ptr ToxB, this population could be used to screen against one of the above disease resistance genes if the parents are also polymorphic for that resistance gene. This would allow the identification of chromosome location in relation to other known genes.

As well, two genes that are part of the defense response system – peroxidase and superoxide dismutase (Li et al. 1999) are located on the long arm of chromosome 2B. The peroxidase and superoxide dismutase genes are active oxygen scavengers, Strelkov (1998) found that active oxygen scavengers reduced the development of chlorosis in susceptible Katepwa plants. Perhaps this tells us about how the genome is organized.

6. LIST OF REFERENCES

Alam, K.B. and Gustafson, J.P. 1988. Tan-spot resistance screening of *Aegilops* species. Plant Breeding. **100**: 112-118.

Alberta Agriculture, Food and Rural Development. [online] Available: Http://www.agric.gov.ab.ca/economic/stats/stats_fldcrop.html. [8 March , 2001].

Adee, S.R. and Pfender, W.F. 1989. The effect of primary inoculum level of *Pyrenophora tritici-repentis* on tan spot epidemic development in wheat. Phytopathology **79**: 833-877.

Bailey, J. 1999. Dictionary of plant sciences. Penguin Books, Toronto. 504 pp.

Ballance, G.M., Lamari, L. and Bernier, C.C. 1989. Purification and characterization of a host-selective necrosis toxin from *Pyrenophora tritici-repentis*. Phy. Mol. Plant Path. **35**: 203-213.

Blake, N.K., Lehfeldt, B.R., Hemphill, A., Shan, X. and Talbert, L.E. 1998. DNA sequence analysis suggests a monophyletic origin of the wheat B genome. Proceedings of the 9th International Wheat Genetics Symposium. **2**: 14-16.

Brown, D.A., and Hunger, R.M. 1993. Production of a chlorosis-inducing, host-specific, low-molecular weight toxin by isolates of *Pyrenophora tritici-repentis*, cause of tan spot of wheat. J. Phytopathology. **137**:221-232.

Ciuffetti, L.M. and Tuori, R.P. 1999. Advances in the characterization of the *Pyrenophora tritici-repentis*-wheat interaction. Phytopathology **89:** 444-449.

Ciufffetti, L.M., Tuori, R.P. and Gaventa, J.M. 1997. A single gene encodes a selective toxin causal to the development of tan spot of wheat. Plant Cell. **9**: 135-144.

Ciuffetti, L.M., Francl, L.J., Ballance, G.M., Bockus, W.W., Lamari, L., Meinhardt, S.W. and Rasmussen, J.B. 1998. Standardization of toxin nomenclature in the *Pyrenophora tritici-repentis* / wheat interaction. Can.J.Plant Path. **20**: 421-424.

Da Luz, W.C. and Bergstrom, G.C. 1986. Effect of temperature on tan spot development in spring wheat cultivars differing in resistance. Can. J. Plant.Path. **8**: 451-454.

Da Luz, W.C. and Hosford jr., R.M. 1980. Twelve *Pyrenophora trichostoma* races for virulence to wheat in the central plains of North America. Phytopathology **70**: 1193-1196.

Deacon, J.W. 1997. Fungi as plant parasites. p. 224-253. In: Modern Mycology. Blackwell Science Ltd., London.

Deshpande, U.S. 1993. Effects and mechanism of action of *Pyrenophora tritici-repenits* (Ptr) necrosis toxin of wheat. M.Sc. Thesis.University of Manitoba. Winnipeg, MB. 118 pp.

Dhingra, O.D., and Sinclair, J.B. 1985. Basic plant pathology methods: culture media and their formulations. CRC Press Inc., Boca Raton, Fl. 355 pp.

Duguid, S.D. 1995. Genetics of resistance to *Pyrenophora tritici-repentis* (Died.) Drechs. in hexaploid wheat (*Triticum aestivum L.*) PhD. Thesis. University of Manitoba, Winnipeg, MB. 130 pp.

Duguid, S.D., and Brûlé-Babel, A.L. 2001a. Inheritance and interaction of spring Wheat (Triticum aestivum L.) resistance to race 2 and race 3 *Pyrenophora tritici-repentis* (Died.) Can. J. Plant Sci. **81**: 527-533.

Duguid, S.D., and Brûlé-Babel, A.L. 2001b. Inheritance of resistance to a necrosis- and chlorosis-inducing isolate from race 1 of *Pyrenophora tritici-repentis* in spring wheat. Can. J. Plant Sci. **81**: 519-525.

Dushnicky, L.G., Ballance, G.M., Sumner, M.J. and MacGregor, A.W. 1998. The role of lignification as a resistance mechanism in wheat to a toxin-producing isolate of *Pyrenophora tritici-repentis*. Can. J. Plant. Path. **20**: 35-47.

Dvorak, J. 1998. Genome analysis in the Triticum-Aegilops alliance. Proceedings of the 9th International Wheat Genetics Symposium. **1**: 8-11.

Endo, T.R., and Gill, B.S. 1996. The deletion stocks of common wheat. J. Hered. **87**: 295-307.

Effertz, R.J., Anderson, J.A. and Francl, L.J. 1998. QTLs associated with resistance to chlorosis induction by *Pyrenophora tritici-repentis* in adult wheat. Can. J. Plant Path. **20**: 438-439.

Elias, E., Cantrell, R.G. and Hosford Jr., R.M. 1989. Heritability of resistance to tan spot in durum wheat and its association with other agronomic traits. Crop Sci. **29**: 299-304.

Faris, J.D, Anderson, J.A., Francl, L.J. and Jordahl, J.G. 1996. Chromosomal location of a gene conditioning insensitivity in wheat to a necrosis-inducing culture filtrate from *Pyrenphora tritici-repentis*. Phytopathology **86**: 459-463.

Faris, J.D., Anderson, J.A., Francl, L.J. and Jordahl, J.G. 1997. RFLP mapping of resistance to chlorosis induction by *Pyrenophora tritici-repentis* in wheat. Theor. Appl. Gen. **94**: 98-103.

Francl, L.J. 1997. Local and mesodistance dispersal of *Pyrenophora tritici-repentis* conidia. Can. J. Plant Path. **19**: 247-255.

Francl, L.J. 1998. Genesis and liberation of conidia of *Pyrenophora tritici-repentis*. Can. J. Plant Path. **20**: 387-393.

Gamba, F.M. and Lamari, L. 1998. Mendelian inheritance of resistance to tan spot [*Pyrenphora tritici-repentis*] in selected genotypes of durum wheat (*Triticum turgidum*). Can. J. Plant Path. **20**: 408-414.

Gamba, F.M., Lamari, L. and Brûlé-Babel, A.L. 1998. Inheritance of race-specific necrotic and chlorotic reactions induced by *Pyrenphora tritici-repentis* in hexaploid wheats. Can. J. Plant Path. **20**: 401-407.

Gilbert, J., Tekauz, A., Kaethler, R., Muller, E. Krome, U., Heath, J., and Stultzer, M. 1998. 1997 survey of leaf spot diseases of wheat in Manitoba. Can. Plant Dis. Sur. **78**: 84.

Gill, B.S. and Gill, K.S. 1998. Molecular cytogenetic explorations into the genome of wheat. Proceedings of the 9th International Wheat Genetics Symposium. **1**: 29-32.

Hagborg, W.A.F. 1970. A device for injecting solutions and suspensions into thin leaves of plants. Can. J. Bot. **48**: 1135-1136.

Hosford jr., R.M. 1971. A form of *Pyrenophora trichostoma* pathogenic to wheat and other grasses. Phytopathology **61**: 28-32.

Hosford jr., R.M. 1972. Propagules of *Pyrenophora trichostoma*. Phytopathology **62**: 627-629.

Hosford, jr., R.M., Larez, C.R., Hammond, J.J. 1987. Interaction of wet period and temperature on Pyrenophora tritici-repentis infection and development in wheats of differing resistance. Phytopathology. **77**: 1021-1027.

Kimber, G. and Athwal, R.S. 1972. A reassessment of the course of evolution of wheat. Proc. Nat. Acad. Sci. **69**: 912-915.

Krupinsky, J.M. 1992. Aggressiveness of isolates of *Pyrenophora tritici-repentis* obtained from wheat in the northern great plains. Plant Dis. **76**: 87-91.

Kwon, C.Y., Rasmussen, J.B., Francl, L.J. and Meinhardt, S.W. 1996. A quantitative bioassay for necrosis toxin from *Pyrenophora tritici-repentis* based on electrolyte leakage. Phytopathology **86**: 1360-1363.

Kwon, C.Y., Rasmussen, J.B. and Meinhardt, S.W. 1998. Activity of Ptr ToxA from *Pyrenophora tritici-repentis* requires host metabolism. Phy. Mol. Plant Path. **52**: 201-212.

Lamari, L. and Bernier, C.C. 1989a. Evaluation of wheat lines and cultivars to tan spot [*Pyrenophora tritici-repentis*] based on lesion type. Can. J. Plant Path. **11**: 49-56.

Lamari, L. and Bernier, C.C. 1989b. Virulence of isolates of *Pyrenophora tritici-repentis* on 11 wheat cultivars and cytology of the differential host reactions. Can. J. Plant Path. **11**: 284-290.

Lamari, L. and Bernier, C.C. 1989c. Toxin of *Pyrenphora tritici-repentis*: Host-specificity, significance in disease, and inheritance of host reaction. Phytopathology **79**: 740-744.

Lamari, L. and Bernier, C.C. 1991. Genetics of tan necrosis and extensive chlorosis in tan spot of wheat caused by *Pyrenophora tritici-repentis*. Phytopathology **81**: 1092-1095.

Lamari, L. and Bernier, C.C. 1994. Temperature-induced resistance to tan spot [*Pyrenophora tritici-repentis*] of wheat. Can. J. Plant Path. **16**: 279-286.

Lamari, L., Ballance, G.M., Orolaza, N.P. and Kowatsch, R. 1995a. In planta production and antibody neutralization of the Ptr necrosis toxin from *Pyrenophora tritici-repentis*. Phytopathology **85**: 333-338.

Lamari, L., Bernier, C.C. and Smith, R.B. 1991. Wheat genotypes that develop both tan necrosis and extensive chlorosis in response to isolates of *Pyrenophora tritici-repentis*. Plant Dis. **75**: 121-122.

Lamari, L., Gilbert, J. and Tekauz, A. 1998. Race differentiation in *Pyrenophora tritici-repentis* and survey of physiological variation in western Canada. Can. J. Plant Path. **20**: 396-400.

Lamari, L., Sayoud, R., Boulif, M. and Bernier, C.C. 1995b. Identification of a new race in *Pyrenophora tritici-repentis*: implications for the current pathotype classification system. Can. J. Plant Path. **17**: 312-318.

Lee, T.S. and Gough, F.J. 1984. Inheritance of Septoria leaf blotch (*S. tritici*) and Pyrenophora tan spot (*P. tritici-repentis*) resistance in *Triticum aestivum* cv. Carifen 12. Plant Dis. **68**: 848-851.

Li, W.L, Faris, J.D., Chitoor, J.M., Leach, J.E., Hulbert, S.H., Liu, D.J., Chen, P.D. and Gill, B.S. 1999. Genomic mapping of defense response genes in wheat. Theor. App. Gen. **98**: 226-233.

Liu, B., Vega J.M. and Feldman, M. 1998b. Differentiation of homoeologous chromosomes in polyploid wheat. Proceedings of the 9th International Wheat Genetics Symposium. **2**: 70-71.

Liu, X., Wang, R.R.C., Jia, J. and Dong, Y. 1998a. Genome relationships among *Sitopsis* species of *Aegilops* and the B/G genome of *Triticum* assessed by RAPD markers. Proceedings of the 9th International Wheat Genetics Symposium. **2**: 79-81.

Loegering, W.Q. 1978. Allelism and linkage of three genes in chromosome 2B of wheat for reaction to *Puccinia graminis tritici*. Genetics. **68**: 1202-1203.

Manitoba Agriculture and Food. [online]. Available: Http://www.gov.mb.ca/agriculture/statistics/aac10s02.html [8 March, 2001].

Martens, J.W., Seaman, W.L. and Atkinson, T.G. 1988. Diseases of field crops in Canada : An illustrated compendium. Canadian Phytopathological Society, Harrow. 160 pp.

Nelson, J.C., Singh, R.P., Autrique, J.E., Sorrells, M.E. 1997. Mapping genes conferring and suppressing leaf rust resistance in wheat. Crop Sci. **37**: 1928-1935.

Orolaza, N.P., Lamari, L. and Ballance, G.M. 1995. Evidence of a host-specific chlorosis toxin from *Pyrenophora tritici-repentis*, the causal agent of tan spot of wheat. Phytopathology **85**: 1282-1287.

Person, C. 1956. Some aspects of monosomic wheat breeding. Can. J. Bot. **34**: 60-70.

Pfender, W.F., Pacey, C.A. and Zhang W. 1988. Saprophytic growth and pseudothecia production by *Pyrenophora tritici-repentis* in plant tissue held at controlled water potentials. Phytopathology **78**: 1205-1210.

Prescott, I.M., Harley, J.P., Klein, D.A. 1993. Microbiology: Second Edition: Volume One. Wm. C. Publishers. Dubuque. IA. 126 pp.

Procunier, J.D., Knox, R.E., Bernier, A.M., Gray, M.A. and Howes, N.K. 1997. DNA markers linked to a *T10* loose smut resistance gene in wheat (*Triticum aestivum L.*). Genome. **40**: 176-179.

Rees, R.G., Mayer, R.J. and Platz, G.J. 1981. Yield losses in wheat from yellow spot: a disease-loss relationship derived from single tillers. Aust. J. Agric. Res. **32**: 851-859.

Rees, R.G. and Platz, G.J. 1980. The epidemiology of yellow spot of wheat in southern Queensland. Aust. J. Agric .Res. **31**: 259-267.

Rees, R.G. and Platz, G.J. 1989. Effectiveness of incomplete resistance to *Pyrenophora tritici-repentis* in wheat. Aust. J. Agric. Res. **40**: 43-48.

Rees, R.G. and Platz, G.J. 1990. Sources of Resistance to *Pyrenophora tritici-repentis* in bread wheats. Euphytica **45**: 59-69.

Riaz, M., Bockus, W.W. and Davis, M.A. 1991. Effects of wheat genotype, time after inoculation, and leaf age on conidia production by *Drechslera tritici-repentis*. Phytopathology **81**: 1298-1302.

Riley, R. 1974. Cytogenetics of chromosome pairing in wheat. Genetics. **78**: 193-203.

Riley, R. and Chapman, V. 1958. Genetic control of the cytologically diploid behavior of hexaploid wheat. Nature. 182: 713-715.

Riley, R., Unrau, J. and Chapman, V. 1969. Evidence on the origin of the B genome of wheat. J. Hered. **49**: 90-98.

Sarkar, P. and Stebbins, G.L. 1956. Morphological evidence concerning the origin of the B genome in wheat. Amer. J. Bot. **43**: 297-304.

Saskatchewan Agriculture and Food. [online]. Available: Http://www.agr.g…/Prodsc.asp?firstPick=Statistics&secondpick=Crops&thirdpick=Production [8 March, 2001].

Scheffer, R.P. and Livingston, R.S. 1984. Host-selective toxins and their role in plant diseases. Science **223**: 17-21.

Schilder, A.M.C. and Bergstrom, G.C. 1990. Variation in virulence within the population of *Pyrenohpora tritici-repentis* in New York. Phytopathology **80**: 84-90.

Schuh, W. 1990. The influence of tillage systems on incidence and spatial pattern of tan spot of wheat. Phytopathology **80**: 804-807.

Sears, E.R. 1953. Nullisomic analysis in common wheat. Am. Naturalist **87**: 245-252.

Sears, E.R. 1969. Wheat cytogenetics. Ann. Rev. Genet. **3**: 451-468.

Shabeer, A. and Bockus, W.W. 1988. Tan spot effects on yield and yield components relative to growth stage in winter wheat. Plant Dis. **72**: 599-602.

Singh, R.J. 1993. Chapter 2: The handling of plant chromosomes. Plant Cytogenetics. CRC Press Inc. Ann Arbor. 7-24 pp.

Sissons, N.D. 1996. Effect of leaf wetness period on infection by *Pyrenophora tritici-repentis* and the influence of residue management on the development of leaf spot diseases of wheat. M. Sc. Thesis. University of Manitoba, Winnipeg, MB. 122 pp.

Smith, L. 1947. The Acetocarmine Smear Technique. Stain Technology. **22**: 17-31.

Sone, J., Bockus,W.W. and Claassen, M.M. 1994. Gradients of tan spot of wither wheat from a small-area source of *Pyrenophora tritici-repentis*. Plant Dis. **78**: 622-627.

Strelkov, S.E. 1998. Purification and mode of action of Ptr (*Pyrenophora tritici-repentis*) chlorosis toxin. M.Sc. Thesis. University of Manitoba, Winnipeg, MB. 77 pp.

Strelkov, S.E., Lamari, L. and Ballance, G.M. 1998. Induced chlorophyll degradation by a chlorosis toxin from *Pyrenophora tritici-repentis*. Can. J. Plant Path. **20**: 428-435.

Strickberger, M.W. 1985. Probability and statistical testing. In: Genetics, 3rd. ed. Macmillan Publishing Company, New York.126-146 pp.

Stock, W.S. 1996. Chromosomal location and RAPD marker development for tan spot resistance in hexaploid wheat. MSc. Thesis. University of Manitoba, Winnipeg, MB. 92 pp.

Stock, W.S., Brûlé-Babel, A.L. and Penner, G.A. 1996. A gene for resistance to a necrosis-inducing isolate of *Pyrenophora tritici-repentis* located on 5BL of *Triticum aestivum* cv. Chinese Spring. Genome **39**: 598-604.

Summerell, B.A. and Burgess, L.W. 1989. Factors influencing survival of *Pyrenophora tritici-repentis*: stubble management. Mycol. Res. **93**: 38-40.

Sykes, E.E. and Bernier, C.C. 1991. Qualitative inheritance of tan spot resistance in hexaploid, tetraploid and diploid wheat. Can. J. Plant Path. **13**: 38-44.

Tekauz, A. 1976. Distribution, severity, and relative importance of leaf spot diseases of wheat in western Canada in 1974. Can. Plant Dis. Sur. **56**: 36-40.

Tomas, A. and Bockus, W.W. 1987. Cultivar-specific toxicity of culture filtrates of *Pyrenophora tritici-repentis.* Phytopathology **77**: 337-1340.

Tomas, A., Feng, G.H., Reeck, G.R., Bockus, W.W. and Leach, J.E. 1990. Purification of a cultivar-specific toxin from *Pyrenophora tritici-repentis*, causal agent of tan spot of wheat. Mol. Plant-Micro. Inter. **3**: 221-224.

Toupin, E.D. 2000. Microscopic Examination of the Effects of Ptr (*Pyrenophora tritici-repentis*) ToxA on wheat. M.Sc. Thesis. University of Manitoba. Winnipeg, MB. 105 pp.

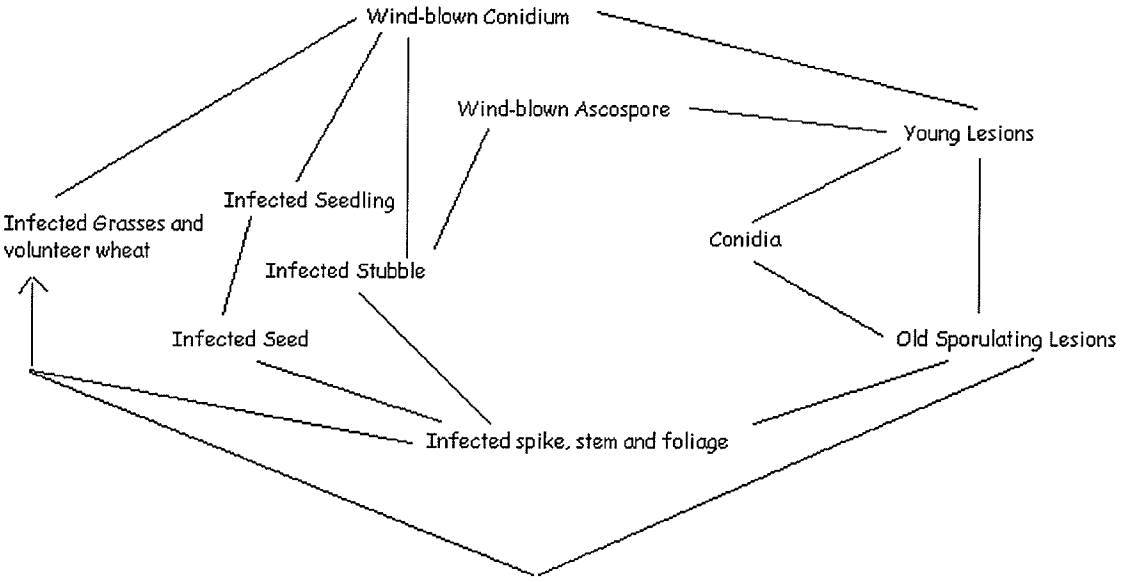
Tuori, R.P., Wolpert, T.J. and Ciuffetti, L.M. 1995. Purification and immunological characterization of toxic components from cultures of *Pyrenophora tritici-repentis.* Mol. Plant-Micro. Inter. **8**: 41-48.

Unrau, J. 1950. The use of monosomes and nullisomes in cytogenetic studies of common wheat. Sci. Agric. **30**: 66-89.

Willams, K.J., Fisher, J.M., and Langridge, P. 1996. Development of a PCR-based allele-specific assay from an RFLP probe linked to resistance to cereal cyst nematode in wheat. Genome. **39**: 798-801.

Zhang, H.F., Francl, L.J., Hordahl, J.G. and Meinhardt, S.W. 1997. Structural and physical properties of a necrosis-inducing toxin from *Pyrenophora tritici-repentis.* Phytopathology **87**: 154-160.

Appendix 7.1: The life cycle of *Pyrenophora tritici-repentis* (tan spot) (Martens et al 1988).



Appendix 7.2: The resulting progeny when a monosomic plant is selfed (Sears 1953).

	21 Chromosome Pollen grain (96%)	**20 Chromosome pollen grain (4%)**
21 Chromosome egg cell (25%)	42 Chromosomes (24%)	41 Chromosomes (1%)
20 Chromosome egg cell (75%)	41 Chromosomes (72%)	40 Chromosomes (3%)

42 Chromosomes : 24 %
41 Chromosomes : 73%
40 Chromosomes: 3%

Appendix 7.3 : Feulgen Solution for staining root tip preparations.
(Pers. Com. Dr. T. Aung, Cereal Research Center, Agriculture and Agri-food Canada, Winnipeg, Manitoba; Singh 1993)

I) Boil 400ml of distilled deionized water, add 2 g basic fuchsin and slowly boil for 2 more minutes stirring constantly until all dissolved.
II) Cool to 50°C and add 60 ml *1N*-HCL (hydrochloric acid).
II) Cool to 25°C and add 6 g $K_2S_2O_5$ (potassium metabisulfite)
IV) Store for 24 h in dark (should be straw in colour).
V) Add 2 g decolorizing carbon, filter using Whatman #2 filter paper and no water. Store in fridge in dark bottle (Should be colorless).

Appendix 7.4 : V8-PDA media for growing *Pyrenophora tritici-repentis* spores.
(Dhingra and Sinclair 1985)

V8 juice	150 ml
PDA (potato dextrose agar)(difco)	10 g
$CaCO_3$	3 g
Agar	10 g
Distilled Water	850 ml

Mix all ingredients together and autoclave.
Pour autoclaved mixture into sterile petri plates while still hot. Let cool and harden.

Appendix 7.5 : Fries Media for isolation of culture filtrates from growing *Pyrenophora tritici-repentis* plugs amended with 0.1% yeast extract. (Dhingra and Sinclair 1985)

NH_4 (Tartrate)	2.5 g
NH_4NO_3(Ammonium Nitrate)	0.5 g
KH_2PO_4(Potassium Phosphate Monobasic)	0.65 g
Sucrose	15 g
Yeast Extract	0.5 g
Trace Elements*	2 ml
Distilled Water	500 ml

Mix all ingredients together and autoclave.

*Trace Elements:

LiCl (Lithium chloride)	83.5 mg
$CuCl_2 \cdot H_20$(Copper chloride)	53.5 mg
H_2MOO_4 (Molybalyic Acid)	17 mg
$MnCl_2 \cdot 4H_2O$ (Manganese chloride)	36 mg
$CoCl_2 \cdot 4H_2O$ (Colbalt chloride)	40 mg

Appendix 7.6: Screening of Monosomic F_1-derived F_2 individuals with Ptr ToxB.

Line [z]	Parent [y]	F_1 Family [x]	F_1-derived F_2 individuals			3:1 Ratio Tested [w]		1:1 Ratio Tested [v]	
			# Insensitive	# Sensitive	Total	X^2	Probability	X^2	Probability
1A	6	1	3	10	13	**0.18**	*0.67*		
1A	6	2	2	11	13	**1.00**	*0.32*		
1A	6	3	3	10	13	**0.18**	*0.67*		
1A	6	4	5	10	15	**0.42**	*0.52*		
1A	6	7	2	13	15	**1.49**	*0.22*		
1A	6	8	4	12	16	**0.08**	*0.77*		
1A	6	9	5	22	27	**0.83**	*0.36*		
1A	6	10	3	13	16	**0.58**	*0.45*		
1A	6	11	7	9	16	**2.58**	*0.11*	**0.31**	0.58
1A	6	12	5	11	16	**0.25**	*0.62*		
1A	6	13	6	10	16	**1.08**	*0.30*		
1A	6	15	6	8	14	**2.00**	*0.16*	**0.36**	0.55
1A	6	16	2	15	17	**2.02**	*0.16*		
1A	6	17	3	20	23	**2.13**	*0.14*		
1A	6	18	2	14	16	**1.75**	*0.19*		
1A	6	19	5	9	14	**0.67**	*0.41*		
1A	6	20	3	13	16	**0.58**	*0.45*		
1A	7	1	4	12	16	**0.08**	*0.77*		
1A	7	3	2	13	15	**1.49**	*0.22*		
1A	7	4	2	11	13	**1.00**	*0.32*		
1A	7	5	6	25	31	**0.72**	*0.40*		
1A	7	7	3	13	16	**0.58**	*0.45*		
1A	7	8	4	11	15	**0.07**	*0.80*		
1A	7	9	2	14	16	**1.75**	*0.19*		
1A	7	10	6	8	14	**2.00**	*0.16*	**0.36**	0.55
1A	7	11	4	11	15	**0.07**	*0.80*		
1A	7	12	4	12	16	**0.08**	*0.77*		
1A	7	13	6	9	15	**1.49**	*0.22*	**0.67**	0.41
1A	7	14	2	15	17	**2.02**	*0.16*		
1A	7	15	8	7	15	**5.76**	*0.02*		
1A	7	16	4	11	15	**0.07**	*0.80*		
1A	7	17	4	11	15	**0.07**	*0.80*		
1A	7	18	6	10	16	**1.08**	*0.30*		
1A	7	19	5	11	16	**0.25**	*0.62*		
1A	7	20	5	10	15	**0.42**	*0.52*		
1A	8	1	3	13	16	**0.58**	*0.45*		
1A	8	2	3	13	16	**0.58**	*0.45*		
1A	8	4	2	9	11	**0.58**	*0.45*		
1A	8	5	4	11	15	**0.07**	*0.80*		
1A	8	8	2	13	15	**1.49**	*0.22*		
1A	8	9	2	14	16	**1.75**	*0.19*		
1A	8	10	4	11	15	**0.07**	*0.80*		
1A	8	11	3	12	15	**0.42**	*0.52*		
1A	8	12	6	8	14	**2.00**	*0.16*	**0.36**	0.55
1A	8	13	4	12	16	**0.08**	*0.77*		
1A	8	14	4	12	16	**0.08**	*0.77*		
1A	8	15	4	12	16	**0.08**	*0.77*		
1A	8	16	3	10	13	**0.18**	*0.67*		
1A	10	1	4	12	16	**0.08**	*0.77*		
1A	10	3	5	11	16	**0.25**	*0.62*		
1A	10	4	3	10	13	**0.18**	*0.67*		
1A	10	5	3	13	16	**0.58**	*0.45*		
1A	10	6	3	12	15	**0.42**	*0.52*		
1A	10	7	3	13	16	**0.58**	*0.45*		
1A	10	8	3	13	16	**0.58**	*0.45*		
1A	10	10	2	14	16	**1.75**	*0.19*		
1A	10	11	5	9	14	**0.67**	*0.41*		
1A	10	12	4	12	16	**0.08**	*0.77*		

Appendix 7.6: Screening of Monosomic F_1-derived F_2 individuals with Ptr ToxB.

Line [z]	Parent [y]	F_1 Family [x]	F_1-derived F_2 individuals			3:1 Ratio Tested [w]		1:1 Ratio Tested [v]	
			# Insensitive	# Sensitive	Total	X^2	Probability	X^2	Probability
1A	10	13	3	13	16	**0.58**	*0.45*		
1A	10	15	4	10	14	**0.10**	*0.76*		
1A	10	17	5	11	16	**0.25**	*0.62*		
1A	10	18	2	13	15	**1.49**	*0.22*		
1A	10	20	2	14	16	**1.75**	*0.19*		
1B	6	2	2	10	12	**0.78**	*0.38*		
1B	6	3	2	8	10	**0.40**	*0.53*		
1B	6	8	4	9	13	**0.18**	*0.67*		
1B	6	11	4	11	15	**0.07**	*0.80*		
1B	6	12	3	8	11	**0.09**	*0.76*		
1B	6	14	5	11	16	**0.25**	*0.62*		
1B	6	17	3	12	15	**0.42**	*0.52*		
1B	7	2	2	8	10	**0.40**	*0.53*		
1B	7	3	2	11	13	**1.00**	*0.32*		
1B	7	4	3	8	11	**0.09**	*0.76*		
1B	7	6	4	6	10	**0.93**	*0.33*		
1B	7	7	5	8	13	**1.00**	*0.32*		
1B	7	8	3	13	16	**0.58**	*0.45*		
1B	7	9	2	14	16	**1.75**	*0.19*		
1B	7	10	2	12	14	**1.24**	*0.27*		
1B	7	11	2	11	13	**1.00**	*0.32*		
1B	7	12	2	11	13	**1.00**	*0.32*		
1B	7	14	3	9	12	**0.11**	*0.74*		
1B	7	15	4	12	16	**0.08**	*0.77*		
1B	7	16	3	9	12	**0.11**	*0.74*		
1B	8	2	2	9	11	**0.58**	*0.45*		
1B	8	3	4	10	14	**0.10**	*0.76*		
1B	8	4	7	8	15	**3.27**	*0.07*	**0.13**	0.72
1B	8	6	4	10	14	**0.10**	*0.76*		
1B	8	7	2	11	13	**1.00**	*0.32*		
1B	8	8	5	8	13	**1.00**	*0.32*		
1B	8	9	3	13	16	**0.58**	*0.45*		
1B	8	11	4	12	16	**0.08**	*0.77*		
1B	8	13	6	10	16	**1.08**	*0.30*		
1B	8	14	2	10	12	**0.78**	*0.38*		
1B	9	1	2	9	11	**0.58**	*0.45*		
1B	9	2	2	11	13	**1.00**	*0.32*		
1B	9	3	2	14	16	**1.75**	*0.19*		
1B	9	4	4	12	16	**0.08**	*0.77*		
1B	9	11	3	12	15	**0.42**	*0.52*		
1B	10	8	2	13	15	**1.49**	*0.22*		
1D	6	1	3	8	11	**0.09**	*0.76*		
1D	6	2	3	10	13	**0.18**	*0.67*		
1D	6	3	3	13	16	**0.58**	*0.45*		
1D	6	4	2	13	15	**1.49**	*0.22*		
1D	6	5	3	9	12	**0.11**	*0.74*		
1D	6	7	4	12	16	**0.08**	*0.77*		
1D	6	9	3	29	32	**4.63**	*0.03*		
1D	6	10	6	10	16	**1.08**	*0.30*		
1D	6	11	3	12	15	**0.42**	*0.52*		
1D	6	13	2	14	16	**1.75**	*0.19*		
1D	6	14	3	13	16	**0.58**	*0.45*		
1D	6	15	3	12	15	**0.42**	*0.52*		
1D	6	18	3	12	15	**0.42**	*0.52*		
1D	6	19	4	14	18	**0.22**	*0.64*		
1D	7	2	4	11	15	**0.07**	*0.80*		
1D	7	3	3	12	15	**0.42**	*0.52*		
1D	7	4	5	10	15	**0.42**	*0.52*		

Appendix 7.6: Screening of Monosomic F_1-derived F_2 individuals with Ptr ToxB.

Line [z]	Parent [y]	F_1 Family [x]	F_1-derived F_2 individuals			3:1 Ratio Tested [w]		1:1 Ratio Tested [v]	
			# Insensitive	# Sensitive	Total	X^2	Probability	X^2	Probability
1D	7	5	5	11	16	**0.25**	*0.62*		
1D	7	6	0	27	27	**9.72**	*0.00*		
1D	7	7	3	13	16	**0.58**	*0.45*		
1D	7	8	3	13	16	**0.58**	*0.45*		
1D	7	9	1	16	17	**3.90**	*0.05*		
1D	7	10	6	10	16	**1.08**	*0.30*		
1D	7	11	3	12	15	**0.42**	*0.52*		
1D	7	13	3	13	16	**0.58**	*0.45*		
1D	7	14	4	12	16	**0.08**	*0.77*		
1D	7	15	5	11	16	**0.25**	*0.62*		
1D	7	19	2	13	15	**1.49**	*0.22*		
1D	7	20	3	13	16	**0.58**	*0.45*		
1D	8	1	2	14	16	**1.75**	*0.19*		
1D	8	2	3	14	17	**0.76**	*0.38*		
1D	8	3	4	12	16	**0.08**	*0.77*		
1D	8	4	5	11	16	**0.25**	*0.62*		
1D	8	6	3	12	15	**0.42**	*0.52*		
1D	8	7	3	10	13	**0.18**	*0.67*		
1D	8	8	3	12	15	**0.42**	*0.52*		
1D	8	9	2	15	17	**2.02**	*0.16*		
1D	8	11	4	11	15	**0.07**	*0.80*		
1D	8	13	4	12	16	**0.08**	*0.77*		
1D	8	14	1	15	16	**3.58**	*0.06*		
1D	9	1	4	12	16	**0.08**	*0.77*		
1D	9	2	5	10	15	**0.42**	*0.52*		
1D	9	3	5	11	16	**0.25**	*0.62*		
1D	9	4	2	14	16	**1.75**	*0.19*		
1D	9	6	3	11	14	**0.29**	*0.59*		
1D	9	7	3	12	15	**0.42**	*0.52*		
1D	9	8	2	14	16	**1.75**	*0.19*		
1D	9	9	3	13	16	**0.58**	*0.45*		
1D	9	10	2	14	16	**1.75**	*0.19*		
1D	9	11	6	10	16	**1.08**	*0.30*		
1D	9	13	4	12	16	**0.08**	*0.77*		
1D	9	14	4	27	31	**2.78**	*0.10*		
1D	9	15	2	14	16	**1.75**	*0.19*		
1D	9	16	4	14	18	**0.22**	*0.64*		
1D	9	19	3	26	29	**3.76**	*0.05*		
1D	9	20	5	10	15	**0.42**	*0.52*		
1D	10	2	3	13	16	**0.58**	*0.45*		
1D	10	3	2	13	15	**1.49**	*0.22*		
1D	10	5	3	13	16	**0.58**	*0.45*		
1D	10	9	7	9	16	**2.58**	*0.11*	**0.31**	0.58
1D	10	10	4	12	16	**0.08**	*0.77*		
2A	2	1	11	21	32	**1.29**	*0.26*		
2A	2	2	4	11	15	**0.07**	*0.80*		
2A	2	3	4	12	16	**0.08**	*0.77*		
2A	2	4	4	12	16	**0.08**	*0.77*		
2A	2	5	3	13	16	**0.58**	*0.45*		
2A	2	6	3	13	16	**0.58**	*0.45*		
2A	2	7	5	26	31	**1.58**	*0.21*		
2A	2	8	5	11	16	**0.25**	*0.62*		
2A	2	9	5	11	16	**0.25**	*0.62*		
2A	2	10	3	12	15	**0.42**	*0.52*		
2A	2	11	6	10	16	**1.08**	*0.30*		
2A	2	12	5	27	32	**1.79**	*0.18*		
2A	2	13	4	10	14	**0.10**	*0.76*		
2A	3	1	3	11	14	**0.29**	*0.59*		

Appendix 7.6: Screening of Monosomic F_1-derived F_2 individuals with Ptr ToxB.

Line [z]	Parent [y]	F_1 Family [x]	F_1-derived F_2 individuals			3:1 Ratio Tested [w]		1:1 Ratio Tested [v]	
			# Insensitive	# Sensitive	Total	X^2	Probability	X^2	Probability
2A	3	2	4	11	15	**0.07**	*0.80*		
2A	3	3	4	22	26	**1.59**	*0.21*		
2A	3	4	7	9	16	**2.58**	*0.11*	**0.31**	0.58
2A	3	5	2	14	16	**1.75**	*0.19*		
2A	3	6	6	10	16	**1.08**	*0.30*		
2A	3	7	5	11	16	**0.25**	*0.62*		
2A	3	8	4	12	16	**0.08**	*0.77*		
2A	3	9	2	13	15	**1.49**	*0.22*		
2A	3	10	5	26	31	**1.58**	*0.21*		
2A	3	11	3	12	15	**0.42**	*0.52*		
2A	3	12	7	17	24	**0.17**	*0.68*		
2A	3	17	4	12	16	**0.08**	*0.77*		
2A	3	18	4	11	15	**0.07**	*0.80*		
2A	3	19	3	13	16	**0.58**	*0.45*		
2A	3	20	2	14	16	**1.75**	*0.19*		
2A	4	1	10	23	33	**0.39**	*0.53*		
2A	4	4	5	10	15	**0.42**	*0.52*		
2A	4	5	3	11	14	**0.29**	*0.59*		
2A	4	6	5	10	15	**0.42**	*0.52*		
2A	4	7	4	11	15	**0.07**	*0.80*		
2A	4	8	6	8	14	**2.00**	*0.16*	**0.36**	0.55
2A	4	9	5	6	11	**2.03**	*0.15*		
2A	4	10	3	9	12	**0.11**	*0.74*		
2A	4	11	4	10	14	**0.10**	*0.76*		
2A	4	12	4	22	26	**1.59**	*0.21*		
2A	4	13	4	8	12	**0.33**	*0.56*		
2A	4	14	5	10	15	**0.42**	*0.52*		
2A	4	15	3	7	10	**0.13**	*0.72*		
2A	4	16	3	14	17	**0.76**	*0.38*		
2A	5	1	4	12	16	**0.08**	*0.77*		
2A	5	2	5	10	15	**0.42**	*0.52*		
2A	5	3	4	11	15	**0.07**	*0.80*		
2A	5	5	6	10	16	**1.08**	*0.30*		
2A	5	7	4	11	15	**0.07**	*0.80*		
2A	5	8	5	20	25	**0.52**	*0.47*		
2A	5	9	4	10	14	**0.10**	*0.76*		
2A	5	10	3	8	11	**0.09**	*0.76*		
2A	5	11	2	11	13	**1.00**	*0.32*		
2A	5	12	3	10	13	**0.18**	*0.67*		
2A	5	13	4	11	15	**0.07**	*0.80*		
2A	5	14	4	9	13	**0.18**	*0.67*		
2A	5	15	2	13	15	**1.49**	*0.22*		
2A	5	16	3	21	24	**2.39**	*0.12*		
2A	5	17	3	9	12	**0.11**	*0.74*		
2A	5	18	7	7	14	**4.10**	*0.04*	**0.07**	0.79
2A	5	19	4	26	30	**2.53**	*0.11*		
2A	5	20	7	9	16	**2.58**	*0.11*	**0.31**	0.58
2B	1	4	2	24	26	**4.67**	*0.03*		
2B	1	5	3	19	22	**1.88**	*0.17*		
2B	1	6	3	27	30	**4.04**	*0.04*		
2B	1	8	5	20	25	**0.52**	*0.47*		
2B	1	9	2	26	28	**5.29**	*0.02*		
2B	1	10	3	22	25	**2.65**	*0.10*		
2B	1	11	4	9	13	**0.18**	*0.67*		
2B	1	12	2	23	25	**4.36**	*0.04*		
2B	1	13	3	12	15	**0.42**	*0.52*		
2B	1	14	2	9	11	**0.58**	*0.45*		
2B	1	15	2	14	16	**1.75**	*0.19*		

Appendix 7.6: Screening of Monosomic F_1-derived F_2 individuals with Ptr ToxB.

Line [z]	Parent [y]	F_1 Family [x]	F_1-derived F_2 individuals			3:1 Ratio Tested [w]		1:1 Ratio Tested [v]	
			# Insensitive	# Sensitive	Total	X^2	Probability	X^2	Probability
2B	2	1	5	19	24	**0.39**	*0.53*		
2B	2	2	5	11	16	**0.25**	*0.62*		
2B	2	3	5	9	14	**0.67**	*0.41*		
2B	2	4	3	13	16	**0.58**	*0.45*		
2B	2	5 (US)	4	16	20	**0.47**	*0.49*		
2B	2	5 (AgCan)	0	20	20	**7.40**	*0.01*		
2B	2	6	9	22	31	**0.20**	*0.65*		
2B	2	7	3	12	15	**0.42**	*0.52*		
2B	2	9	7	19	26	**0.05**	*0.82*		
2B	2	11	0	28	28	**10.05**	*0.00*		
2B	2	12	1	28	29	**7.80**	*0.01*		
2B	2	13	6	19	25	**0.09**	*0.76*		
2B	2	14	3	25	28	**3.48**	*0.06*		
2B	2	16	3	24	27	**3.20**	*0.07*		
2B	2	19	2	10	12	**0.78**	*0.38*		
2B	2	20	1	26	27	**7.15**	*0.01*		
2B	3	1	7	17	24	**0.17**	*0.68*		
2B	3	2	2	12	14	**1.24**	*0.27*		
2B	3	4	2	14	16	**1.75**	*0.19*		
2B	3	5	2	13	15	**1.49**	*0.22*		
2B	3	6	3	27	30	**4.04**	*0.04*		
2B	3	7	2	13	15	**1.49**	*0.22*		
2B	3	8	2	14	16	**1.75**	*0.19*		
2B	3	9	4	11	15	**0.07**	*0.80*		
2B	3	10	2	13	15	**1.49**	*0.22*		
2B	4	1	2	11	13	**1.00**	*0.32*		
2B	4	2	7	24	31	**0.20**	*0.65*		
2B	4	3	2	28	30	**5.91**	*0.02*		
2B	4	4	4	13	17	**0.14**	*0.71*		
2B	4	5	2	14	16	**1.75**	*0.19*		
2B	4	6	1	29	30	**8.13**	*0.00*		
2B	4	7	1	25	26	**6.82**	*0.01*		
2B	4	8	4	29	33	**3.30**	*0.07*		
2B	4	9	5	11	16	**0.25**	*0.62*		
2B	4	10	3	22	25	**2.65**	*0.10*		
2B	4	11	2	14	16	**1.75**	*0.19*		
2B	4	12	3	13	16	**0.58**	*0.45*		
2B	4	13	3	25	28	**3.48**	*0.06*		
2B	4	14	2	24	26	**4.67**	*0.03*		
2B	4	15	2	14	16	**1.75**	*0.19*		
2B	5	1	2	20	22	**3.45**	*0.06*		
2B	5	2	2	12	14	**1.24**	*0.27*		
2B	5	3	1	27	28	**7.48**	*0.01*		
2B	5	4	2	25	27	**4.98**	*0.03*		
2B	5	5	3	17	20	**1.40**	*0.24*		
2B	5	6	2	12	14	**1.24**	*0.27*		
2B	5	7	2	14	16	**1.75**	*0.19*		
2B	5	8	2	10	12	**0.78**	*0.38*		
2B	5	9	1	26	27	**7.15**	*0.01*		
2B	5	10	3	27	30	**4.04**	*0.04*		
2B	5	11	2	10	12	**0.78**	*0.38*		
2B	5	12	2	24	26	**4.67**	*0.03*		
2B	5	13	3	29	32	**4.63**	*0.03*		
2B	5	14	5	24	29	**1.18**	*0.28*		
2B	5	15	3	23	26	**2.92**	*0.09*		
2B	5	16	3	11	14	**0.29**	*0.59*		
2B	5	17	2	23	25	**4.36**	*0.04*		
2B	5	18	2	11	13	**1.00**	*0.32*		

Appendix 7.6: Screening of Monosomic F_1-derived F_2 individuals with Ptr ToxB.

Line [z]	Parent [y]	F_1 Family [x]	F_1-derived F_2 individuals			3:1 Ratio Tested [w]		1:1 Ratio Tested [v]	
			# Insensitive	# Sensitive	Total	X^2	Probability	X^2	Probability
2B	5	19	2	12	14	**1.24**	*0.27*		
2B	6	2	0	16	16	**6.08**	*0.01*		
2B	6	3	6	10	16	**1.08**	*0.30*		
2B	6	5	2	14	16	**1.75**	*0.19*		
2B	6	6	4	11	15	**0.07**	*0.80*		
2B	6	7	1	15	16	**3.58**	*0.06*		
2B	6	9	2	15	17	**2.02**	*0.16*		
2B	6	10	0	16	16	**6.08**	*0.01*		
2B	6	14	3	12	15	**0.42**	*0.52*		
2B	6	15	4	9	13	**0.18**	*0.67*		
2B	6	16	0	16	16	**6.08**	*0.01*		
2B	6	17	2	14	16	**1.75**	*0.19*		
2B	6	20	1	15	16	**3.58**	*0.06*		
2B	7	2	1	14	15	**3.27**	*0.07*		
2B	7	6	3	13	16	**0.58**	*0.45*		
2B	7	7	4	12	16	**0.08**	*0.77*		
2B	7	8	1	14	15	**3.27**	*0.07*		
2B	7	9	2	12	14	**1.24**	*0.27*		
2B	7	10	2	14	16	**1.75**	*0.19*		
2B	7	11	1	15	16	**3.58**	*0.06*		
2B	7	14	1	15	16	**3.58**	*0.06*		
2B	7	15	1	15	16	**3.58**	*0.06*		
2B	7	17	4	11	15	**0.07**	*0.80*		
2B	7	18	2	12	14	**1.24**	*0.27*		
2B	8	1	2	13	15	**1.49**	*0.22*		
2B	8	2	2	22	24	**4.06**	*0.04*		
2B	8	3	3	13	16	**0.58**	*0.45*		
2B	8	5	5	11	16	**0.25**	*0.62*		
2B	8	6	3	13	16	**0.58**	*0.45*		
2B	8	7	3	28	31	**4.33**	*0.04*		
2B	8	8	2	14	16	**1.75**	*0.19*		
2B	8	9	4	11	15	**0.07**	*0.80*		
2B	8	10	3	12	15	**0.42**	*0.52*		
2B	8	11	1	14	15	**3.27**	*0.07*		
2B	8	12	1	21	22	**5.52**	*0.02*		
2B	8	14	6	10	16	**1.08**	*0.30*		
2B	8	15	5	10	15	**0.42**	*0.52*		
2B	8	16	7	9	16	**2.58**	*0.11*	**0.31**	0.58
2B	8	17	3	12	15	**0.42**	*0.52*		
2B	8	18	1	28	29	**7.80**	*0.01*		
2B	8	19	1	29	30	**8.13**	*0.00*		
2B	8	20	6	10	16	**1.08**	*0.30*		
2B	9	1	1	23	24	**6.17**	*0.01*		
2B	9	3	3	8	11	**0.09**	*0.76*		
2B	9	5	0	16	16	**6.08**	*0.01*		
2B	9	6	2	25	27	**4.98**	*0.03*		
2B	9	7	3	24	27	**3.20**	*0.07*		
2B	9	9	1	19	20	**4.87**	*0.03*		
2B	9	10	4	10	14	**0.10**	*0.76*		
2B	9	12	4	12	16	**0.08**	*0.77*		
2B	9	13	3	31	34	**5.22**	*0.02*		
2B	9	14	2	17	19	**2.58**	*0.11*		
2B	9	15	3	12	15	**0.42**	*0.52*		
2B	9	17	5	26	31	**1.58**	*0.21*		
2B	9	18	4	23	27	**1.81**	*0.18*		
2B	9	19	5	10	15	**0.42**	*0.52*		
2B	10	1	3	12	15	**0.42**	*0.52*		
2B	10	2	3	13	16	**0.58**	*0.45*		

Appendix 7.6: Screening of Monosomic F_1-derived F_2 individuals with Ptr ToxB.

Line [z]	Parent [y]	F_1 Family [x]	F_1-derived F_2 individuals			3:1 Ratio Tested [w]		1:1 Ratio Tested [v]	
			# Insensitive	# Sensitive	Total	X^2	Probability	X^2	Probability
2B	10	3	7	9	16	**2.58**	*0.11*	**0.31**	0.58
2B	10	4	1	21	22	**5.52**	*0.02*		
2B	10	5	4	12	16	**0.08**	*0.77*		
2B	10	6	1	15	16	**3.58**	*0.06*		
2B	10	7	3	12	15	**0.42**	*0.52*		
2B	10	8	0	18	18	**6.74**	*0.01*		
2B	10	9	6	10	16	**1.08**	*0.30*		
2B	10	10	4	11	15	**0.07**	*0.80*		
2B	10	12	5	9	14	**0.67**	*0.41*		
2D	6	1	4	10	14	**0.10**	*0.76*		
2D	6	2	4	24	28	**2.05**	*0.15*		
2D	6	3	2	14	16	**1.75**	*0.19*		
2D	6	5	2	13	15	**1.49**	*0.22*		
2D	6	6	4	10	14	**0.10**	*0.76*		
2D	6	7	4	11	15	**0.07**	*0.80*		
2D	6	8	2	13	15	**1.49**	*0.22*		
2D	6	9	2	13	15	**1.49**	*0.22*		
2D	6	10	4	10	14	**0.10**	*0.76*		
2D	6	11	6	8	14	**2.00**	*0.16*	**0.36**	0.55
2D	6	12	3	29	32	**4.63**	*0.03*		
2D	6	13	4	11	15	**0.07**	*0.80*		
2D	6	16	4	11	15	**0.07**	*0.80*		
2D	6	17	3	13	16	**0.58**	*0.45*		
2D	6	18	4	10	14	**0.10**	*0.76*		
2D	6	19	3	13	16	**0.58**	*0.45*		
2D	6	20	6	9	15	**1.49**	*0.22*	**0.67**	0.41
2D	7	1	9	18	27	**0.83**	*0.36*		
2D	7	2	5	11	16	**0.25**	*0.62*		
2D	7	3	6	8	14	**2.00**	*0.16*	**0.36**	0.55
2D	7	4	3	13	16	**0.58**	*0.45*		
2D	7	5	2	12	14	**1.24**	*0.27*		
2D	7	7	5	10	15	**0.42**	*0.52*		
2D	7	8	5	10	15	**0.42**	*0.52*		
2D	7	9	5	10	15	**0.42**	*0.52*		
2D	7	10	3	12	15	**0.42**	*0.52*		
2D	7	12	4	12	16	**0.08**	*0.77*		
2D	7	13	5	10	15	**0.42**	*0.52*		
2D	7	14	4	12	16	**0.08**	*0.77*		
2D	7	15	2	13	15	**1.49**	*0.22*		
2D	7	16	4	12	16	**0.08**	*0.77*		
2D	7	17	1	15	16	**3.58**	*0.06*		
2D	7	18	4	11	15	**0.07**	*0.80*		
2D	7	19	4	12	16	**0.08**	*0.77*		
2D	7	20	2	13	15	**1.49**	*0.22*		
2D	8	1	3	15	18	**0.96**	*0.33*		
2D	8	3	3	9	12	**0.11**	*0.74*		
2D	8	4	4	14	18	**0.22**	*0.64*		
2D	8	5	2	13	15	**1.49**	*0.22*		
2D	8	6	5	9	14	**0.67**	*0.41*		
2D	8	7	4	13	17	**0.14**	*0.71*		
2D	8	13	2	13	15	**1.49**	*0.22*		
2D	8	14	5	11	16	**0.25**	*0.62*		
2D	8	16	4	12	16	**0.08**	*0.77*		
2D	8	17	5	10	15	**0.42**	*0.52*		
2D	8	18	4	10	14	**0.10**	*0.76*		
2D	8	19	3	11	14	**0.29**	*0.59*		
2D	8	20	5	26	31	**1.58**	*0.21*		
2D	9	1	2	13	15	**1.49**	*0.22*		

Appendix 7.6: Screening of Monosomic F_1-derived F_2 individuals with Ptr ToxB.

Line [z]	Parent [y]	F_1 Family [x]	F_1-derived F_2 individuals			3:1 Ratio Tested [w]		1:1 Ratio Tested [v]	
			# Insensitive	# Sensitive	Total	X^2	Probability	X^2	Probability
2D	9	2	3	13	16	**0.58**	*0.45*		
2D	9	4	5	11	16	**0.25**	*0.62*		
2D	9	5	2	14	16	**1.75**	*0.19*		
2D	9	6	3	12	15	**0.42**	*0.52*		
2D	9	7	3	28	31	**4.33**	*0.04*		
2D	9	9	6	9	15	**1.49**	*0.22*	**0.67**	0.41
2D	9	10	3	10	13	**0.18**	*0.67*		
2D	9	11	4	9	13	**0.18**	*0.67*		
2D	10	1	5	10	15	**0.42**	*0.52*		
2D	10	4	3	12	15	**0.42**	*0.52*		
2D	10	7	4	12	16	**0.08**	*0.77*		
2D	10	11	5	11	16	**0.25**	*0.62*		
2D	10	12	5	10	15	**0.42**	*0.52*		
2D	10	13	4	12	16	**0.08**	*0.77*		
2D	10	14	5	11	16	**0.25**	*0.62*		
3A	7	1	6	9	15	**1.49**	*0.22*	**0.67**	0.41
3A	7	2	5	10	15	**0.42**	*0.52*		
3A	7	4	3	13	16	**0.58**	*0.45*		
3A	7	5	4	10	14	**0.10**	*0.76*		
3A	7	7	3	11	14	**0.29**	*0.59*		
3A	7	11	5	15	20	**0.07**	*0.80*		
3A	7	14	2	12	14	**1.24**	*0.27*		
3A	7	16	5	10	15	**0.42**	*0.52*		
3A	7	17	2	13	15	**1.49**	*0.22*		
3A	7	18	1	14	15	**3.27**	*0.07*		
3A	7	19	4	11	15	**0.07**	*0.80*		
3A	8	1	2	14	16	**1.75**	*0.19*		
3A	8	2	5	11	16	**0.25**	*0.62*		
3A	8	5	2	13	15	**1.49**	*0.22*		
3A	8	6	4	11	15	**0.07**	*0.80*		
3A	8	7	2	12	14	**1.24**	*0.27*		
3A	8	8	3	13	16	**0.58**	*0.45*		
3A	8	9	3	13	16	**0.58**	*0.45*		
3A	8	10	4	10	14	**0.10**	*0.76*		
3A	8	11	3	12	15	**0.42**	*0.52*		
3A	8	12	4	12	16	**0.08**	*0.77*		
3A	8	13	5	10	15	**0.42**	*0.52*		
3A	8	14	5	11	16	**0.25**	*0.62*		
3A	8	15	6	9	15	**1.49**	*0.22*	**0.67**	0.41
3A	8	16	3	13	16	**0.58**	*0.45*		
3A	8	17	3	10	13	**0.18**	*0.67*		
3A	8	19	5	9	14	**0.67**	*0.41*		
3A	9	1	6	9	15	**1.49**	*0.22*	**0.67**	0.41
3A	9	2	2	14	16	**1.75**	*0.19*		
3A	9	3	4	12	16	**0.08**	*0.77*		
3A	9	4	1	14	15	**3.27**	*0.07*		
3A	9	5	5	10	15	**0.42**	*0.52*		
3A	9	6	6	11	17	**0.76**	*0.38*		
3A	9	7	9	7	16	**7.58**	*0.01*	**0.31**	0.58
3A	9	8	4	11	15	**0.07**	*0.80*		
3A	9	9	2	13	15	**1.49**	*0.22*		
3A	9	10	6	8	14	**2.00**	*0.16*	**0.36**	0.55
3A	9	11	5	11	16	**0.25**	*0.62*		
3A	9	12	2	14	16	**1.75**	*0.19*		
3A	9	13	5	10	15	**0.42**	*0.52*		
3A	9	14	4	12	16	**0.08**	*0.77*		
3A	9	15	3	13	16	**0.58**	*0.45*		
3A	9	16	3	12	15	**0.42**	*0.52*		

Appendix 7.6: Screening of Monosomic F_1-derived F_2 individuals with Ptr ToxB.

Line [z]	Parent [y]	F_1 Family [x]	F_1-derived F_2 individuals			3:1 Ratio Tested [w]		1:1 Ratio Tested [v]	
			# Insensitive	# Sensitive	Total	X^2	Probability	X^2	Probability
3A	9	17	4	6	10	**0.93**	*0.33*		
3A	10	1	6	9	15	**1.49**	*0.22*	**0.67**	0.41
3A	10	2	3	13	16	**0.58**	*0.45*		
3A	10	3	7	9	16	**2.58**	*0.11*	**0.31**	0.58
3A	10	4	4	12	16	**0.08**	*0.77*		
3A	10	5	4	10	14	**0.10**	*0.76*		
3A	10	6	2	13	15	**1.49**	*0.22*		
3A	10	7	2	14	16	**1.75**	*0.19*		
3A	10	8	1	13	14	**2.95**	*0.09*		
3A	10	9	0	15	15	**5.76**	*0.02*		
3A	10	10	4	11	15	**0.07**	*0.80*		
3A	10	11	2	14	16	**1.75**	*0.19*		
3A	10	13	3	11	14	**0.29**	*0.59*		
3A	10	15	4	12	16	**0.08**	*0.77*		
3A	10	16	3	12	15	**0.42**	*0.52*		
3A	10	17	4	12	16	**0.08**	*0.77*		
3A	10	18	3	14	17	**0.76**	*0.38*		
3A	10	19	3	13	16	**0.58**	*0.45*		
3A	10	20	6	9	15	**1.49**	*0.22*	**0.67**	0.41
3B	6	1	5	10	15	**0.42**	*0.52*		
3B	6	2	2	8	10	**0.40**	*0.53*		
3B	6	5	3	12	15	**0.42**	*0.52*		
3B	6	6	2	13	15	**1.49**	*0.22*		
3B	6	9	3	8	11	**0.09**	*0.76*		
3B	6	10	5	10	15	**0.42**	*0.52*		
3B	7	1	4	12	16	**0.08**	*0.77*		
3B	7	3	5	10	15	**0.42**	*0.52*		
3B	7	4	8	8	16	**4.75**	*0.03*	**0.06**	0.80
3B	7	5	4	12	16	**0.08**	*0.77*		
3B	7	8	3	13	16	**0.58**	*0.45*		
3B	7	9	5	11	16	**0.25**	*0.62*		
3B	7	10	5	9	14	**0.67**	*0.41*		
3B	7	11	4	18	22	**0.79**	*0.37*		
3B	7	12	4	11	15	**0.07**	*0.80*		
3B	7	13	6	10	16	**1.08**	*0.30*		
3B	7	15	4	18	22	**0.79**	*0.37*		
3B	7	18	4	12	16	**0.08**	*0.77*		
3B	7	20	2	13	15	**1.49**	*0.22*		
3B	8	4	4	10	14	**0.10**	*0.76*		
3B	8	5	3	12	15	**0.42**	*0.52*		
3B	8	10	3	12	15	**0.42**	*0.52*		
3B	8	11	3	12	15	**0.42**	*0.52*		
3B	8	15	1	14	15	**3.27**	*0.07*		
3B	8	16	4	9	13	**0.18**	*0.67*		
3B	8	17	5	10	15	**0.42**	*0.52*		
3B	8	18	3	11	14	**0.29**	*0.59*		
3B	8	19	5	9	14	**0.67**	*0.41*		
3B	10	1	3	12	15	**0.42**	*0.52*		
3B	10	2	4	11	15	**0.07**	*0.80*		
3B	10	3	0	16	16	**6.08**	*0.01*		
3B	10	4	4	10	14	**0.10**	*0.76*		
3B	10	5	3	12	15	**0.42**	*0.52*		
3B	10	6	6	8	14	**2.00**	*0.16*	**0.36**	0.55
3B	10	7	3	12	15	**0.42**	*0.52*		
3B	10	9	6	10	16	**1.08**	*0.30*		
3B	10	10	4	11	15	**0.07**	*0.80*		
3B	10	11	4	11	15	**0.07**	*0.80*		
3B	10	12	5	10	15	**0.42**	*0.52*		

Appendix 7.6: Screening of Monosomic F_1-derived F_2 individuals with Ptr ToxB.

Line [z]	Parent [y]	F_1 Family [x]	F_1-derived F_2 individuals			3:1 Ratio Tested [w]		1:1 Ratio Tested [v]	
			# Insensitive	# Sensitive	Total	X^2	Probability	X^2	Probability
3B	10	14	2	13	15	**1.49**	*0.22*		
3B	10	15	4	12	16	**0.08**	*0.77*		
3B	10	17	5	11	16	**0.25**	*0.62*		
3B	10	18	2	13	15	**1.49**	*0.22*		
3B	10	19	2	12	14	**1.24**	*0.27*		
3B	10	20	4	11	15	**0.07**	*0.80*		
3D	1	2	5	8	13	**1.00**	*0.32*		
3D	6	1	4	12	16	**0.08**	*0.77*		
3D	6	2	2	12	14	**1.24**	*0.27*		
3D	6	4	5	25	30	**1.38**	*0.24*		
3D	6	5	5	28	33	**2.01**	*0.16*		
3D	6	6	4	10	14	**0.10**	*0.76*		
3D	6	10	2	9	11	**0.58**	*0.45*		
3D	6	11	3	11	14	**0.29**	*0.59*		
3D	6	12	4	12	16	**0.08**	*0.77*		
3D	6	14	5	10	15	**0.42**	*0.52*		
3D	6	15	5	10	15	**0.42**	*0.52*		
3D	6	18	2	10	12	**0.78**	*0.38*		
3D	7	1	2	9	11	**0.58**	*0.45*		
3D	7	2	10	22	32	**0.54**	*0.46*		
3D	7	4	2	12	14	**1.24**	*0.27*		
3D	7	5	10	17	27	**1.81**	*0.18*		
3D	7	8	2	9	11	**0.58**	*0.45*		
3D	9	2	6	9	15	**1.49**	*0.22*		
3D	9	4	2	12	14	**1.24**	*0.27*		
3D	9	5	4	28	32	**3.04**	*0.08*		
3D	9	6	4	8	12	**0.33**	*0.56*		
3D	9	7	4	7	11	**0.58**	*0.45*		
3D	10	1	5	10	15	**0.42**	*0.52*		
3D	10	3	3	11	14	**0.29**	*0.59*		
3D	10	4	4	10	14	**0.10**	*0.76*		
3D	10	5	2	13	15	**1.49**	*0.22*		
3D	10	6	4	12	16	**0.08**	*0.77*		
3D	10	7	9	20	29	**0.45**	*0.50*		
3D	10	8	5	10	15	**0.42**	*0.52*		
3D	10	9	3	9	12	**0.11**	*0.74*		
3D	10	11	4	8	12	**0.33**	*0.56*		
3D	10	12	3	23	26	**2.92**	*0.09*		
3D	10	13	3	9	12	**0.11**	*0.74*		
3D	10	17	4	12	16	**0.08**	*0.77*		
3D	10	18	4	10	14	**0.10**	*0.76*		
4A	1	1	5	10	15	**0.42**	*0.52*		
4A	1	2	4	11	15	**0.07**	*0.80*		
4A	1	3	2	13	15	**1.49**	*0.22*		
4A	1	4	3	13	16	**0.58**	*0.45*		
4A	1	5	3	14	17	**0.76**	*0.38*		
4A	1	7	3	14	17	**0.76**	*0.38*		
4A	1	8	5	11	16	**0.25**	*0.62*		
4A	1	9	4	12	16	**0.08**	*0.77*		
4A	1	10	2	14	16	**1.75**	*0.19*		
4A	1	11	3	13	16	**0.58**	*0.45*		
4A	1	12	7	25	32	**0.29**	*0.59*		
4A	1	13	0	32	32	**11.38**	*0.00*		
4A	1	14	4	8	12	**0.33**	*0.56*		
4A	1	15	2	14	16	**1.75**	*0.19*		
4A	1	17	5	10	15	**0.42**	*0.52*		
4A	1	19	3	12	15	**0.42**	*0.52*		
4A	1	20	3	13	16	**0.58**	*0.45*		

Appendix 7.6: Screening of Monosomic F_1-derived F_2 individuals with Ptr ToxB.

Line [z]	Parent [y]	F_1 Family [x]	F_1-derived F_2 individuals			3:1 Ratio Tested [w]		1:1 Ratio Tested [v]	
			# Insensitive	# Sensitive	Total	X^2	Probability	X^2	Probability
4A	2	1	4	11	15	**0.07**	*0.80*		
4A	2	2	5	9	14	**0.67**	*0.41*		
4A	2	3	3	13	16	**0.58**	*0.45*		
4A	2	5	4	12	16	**0.08**	*0.77*		
4A	2	6	5	10	15	**0.42**	*0.52*		
4A	2	7	3	12	15	**0.42**	*0.52*		
4A	2	8	3	12	15	**0.42**	*0.52*		
4A	2	9	4	12	16	**0.08**	*0.77*		
4A	2	10	5	24	29	**1.18**	*0.28*		
4A	2	11	3	11	14	**0.29**	*0.59*		
4A	2	12	3	13	16	**0.58**	*0.45*		
4A	2	13	2	8	10	**0.40**	*0.53*		
4A	2	14	3	10	13	**0.18**	*0.67*		
4A	2	15	4	10	14	**0.10**	*0.76*		
4A	2	16	5	8	13	**1.00**	*0.32*		
4A	2	17	3	11	14	**0.29**	*0.59*		
4A	2	18	4	27	31	**2.78**	*0.10*		
4A	2	19	3	10	13	**0.18**	*0.67*		
4A	2	20	2	13	15	**1.49**	*0.22*		
4A	3	1	4	12	16	**0.08**	*0.77*		
4A	3	2	4	12	16	**0.08**	*0.77*		
4A	3	3	3	12	15	**0.42**	*0.52*		
4A	3	4	3	12	15	**0.42**	*0.52*		
4A	3	5	7	26	33	**0.39**	*0.53*		
4A	3	6	6	22	28	**0.33**	*0.56*		
4A	3	7	4	12	16	**0.08**	*0.77*		
4A	3	8	3	8	11	**0.09**	*0.76*		
4A	3	9	3	12	15	**0.42**	*0.52*		
4A	3	10	5	11	16	**0.25**	*0.62*		
4A	3	11	3	12	15	**0.42**	*0.52*		
4A	3	12	3	11	14	**0.29**	*0.59*		
4A	3	13	2	13	15	**1.49**	*0.22*		
4A	3	14	3	13	16	**0.58**	*0.45*		
4A	3	15	5	10	15	**0.42**	*0.52*		
4A	3	16	3	12	15	**0.42**	*0.52*		
4A	3	17	5	10	15	**0.42**	*0.52*		
4A	3	18	4	25	29	**2.29**	*0.13*		
4A	4	1	6	11	17	**0.76**	*0.38*		
4A	4	2	3	24	27	**3.20**	*0.07*		
4A	4	3	5	9	14	**0.67**	*0.41*		
4A	4	4	5	11	16	**0.25**	*0.62*		
4A	4	6	3	12	15	**0.42**	*0.52*		
4A	4	8	3	12	15	**0.42**	*0.52*		
4A	4	9	5	12	17	**0.14**	*0.71*		
4A	4	10	6	21	27	**0.23**	*0.63*		
4A	4	11	4	10	14	**0.10**	*0.76*		
4A	4	12	4	10	14	**0.10**	*0.76*		
4A	4	13	2	12	14	**1.24**	*0.27*		
4A	4	14	2	13	15	**1.49**	*0.22*		
4A	4	15	2	12	14	**1.24**	*0.27*		
4A	4	16	2	25	27	**4.98**	*0.03*		
4A	4	17	4	11	15	**0.07**	*0.80*		
4A	4	18	4	11	15	**0.07**	*0.80*		
4A	5	2	7	22	29	**0.08**	*0.78*		
4A	5	3	5	8	13	**1.00**	*0.32*		
4A	5	4	5	8	13	**1.00**	*0.32*		
4A	5	5	2	13	15	**1.49**	*0.22*		
4A	5	6	2	12	14	**1.24**	*0.27*		

Appendix 7.6: Screening of Monosomic F_1-derived F_2 individuals with Ptr ToxB.

Line [z]	Parent [y]	F_1 Family [x]	F_1-derived F_2 individuals			3:1 Ratio Tested [w]		1:1 Ratio Tested [v]	
			# Insensitive	# Sensitive	Total	X^2	Probability	X^2	Probability
4A	5	8	7	8	15	**3.27**	*0.07*	**0.13**	0.72
4A	5	11	1	14	15	**3.27**	*0.07*		
4A	5	12	5	11	16	**0.25**	*0.62*		
4A	5	13	4	7	11	**0.58**	*0.45*		
4A	5	16	4	11	15	**0.07**	*0.80*		
4A	6	5	3	14	17	**0.76**	*0.38*		
4A	6	6	2	13	15	**1.49**	*0.22*		
4A	6	7	2	14	16	**1.75**	*0.19*		
4A	6	8	2	13	15	**1.49**	*0.22*		
4A	6	12	3	13	16	**0.58**	*0.45*		
4A	6	14	4	12	16	**0.08**	*0.77*		
4A	6	16	2	13	15	**1.49**	*0.22*		
4A	6	17	2	13	15	**1.49**	*0.22*		
4A	6	18	4	9	13	**0.18**	*0.67*		
4A	6	19	3	13	16	**0.58**	*0.45*		
4A	6	20	2	13	15	**1.49**	*0.22*		
4A	7	1	4	12	16	**0.08**	*0.77*		
4A	7	2	2	14	16	**1.75**	*0.19*		
4A	7	3	3	24	27	**3.20**	*0.07*		
4A	7	5	2	11	13	**1.00**	*0.32*		
4A	7	6	2	24	26	**4.67**	*0.03*		
4A	7	9	3	10	13	**0.18**	*0.67*		
4A	7	10	2	12	14	**1.24**	*0.27*		
4A	7	12	4	10	14	**0.10**	*0.76*		
4A	7	14	4	11	15	**0.07**	*0.80*		
4A	7	15	5	10	15	**0.42**	*0.52*		
4A	7	16	2	11	13	**1.00**	*0.32*		
4A	7	17	3	23	26	**2.92**	*0.09*		
4A	7	18	4	25	29	**2.29**	*0.13*		
4A	7	19	4	13	17	**0.14**	*0.71*		
4A	7	20	3	20	23	**2.13**	*0.14*		
4A	8	1	2	12	14	**1.24**	*0.27*		
4A	9	1	3	10	13	**0.18**	*0.67*		
4A	9	3	2	14	16	**1.75**	*0.19*		
4A	9	5	5	11	16	**0.25**	*0.62*		
4A	9	6	5	24	29	**1.18**	*0.28*		
4A	9	7	3	13	16	**0.58**	*0.45*		
4A	9	8	2	21	23	**3.75**	*0.05*		
4A	9	9	4	9	13	**0.18**	*0.67*		
4A	9	10	4	8	12	**0.33**	*0.56*		
4A	9	11	3	11	14	**0.29**	*0.59*		
4A	9	12	2	12	14	**1.24**	*0.27*		
4A	9	14	2	9	11	**0.58**	*0.45*		
4A	9	16	3	10	13	**0.18**	*0.67*		
4A	9	17	3	8	11	**0.09**	*0.76*		
4A	9	18	3	11	14	**0.29**	*0.59*		
4A	9	19	2	13	15	**1.49**	*0.22*		
4A	10	4	4	25	29	**2.29**	*0.13*		
4A	10	5	2	19	21	**3.16**	*0.08*		
4A	10	8	3	29	32	**4.63**	*0.03*		
4A	10	9	4	6	10	**0.93**	*0.33*		
4A	10	10	2	11	13	**1.00**	*0.32*		
4A	10	14	3	10	13	**0.18**	*0.67*		
4A	10	15	3	13	16	**0.58**	*0.45*		
4A	10	16	2	12	14	**1.24**	*0.27*		
4A	10	17	2	22	24	**4.06**	*0.04*		
4A	10	18	2	13	15	**1.49**	*0.22*		
4A	10	19	5	10	15	**0.42**	*0.52*		

Appendix 7.6: Screening of Monosomic F_1-derived F_2 individuals with Ptr ToxB.

Line [z]	Parent [y]	F_1 Family [x]	F_1-derived F_2 individuals			3:1 Ratio Tested [w]		1:1 Ratio Tested [v]	
			# Insensitive	# Sensitive	Total	X^2	Probability	X^2	Probability
4B	2	1	9	21	30	**0.31**	*0.58*		
4B	2	2	9	22	31	**0.20**	*0.65*		
4B	2	3	7	23	30	**0.13**	*0.72*		
4B	2	4	6	24	30	**0.58**	*0.45*		
4B	3	1	8	24	32	**0.04**	*0.84*		
4B	3	3	8	22	30	**0.04**	*0.83*		
4B	3	4	5	23	28	**1.00**	*0.32*		
4B	3	5	4	26	30	**2.53**	*0.11*		
4B	3	6	9	21	30	**0.31**	*0.58*		
4B	3	7	5	24	29	**1.18**	*0.28*		
4B	3	8	5	16	21	**0.11**	*0.74*		
4B	3	10	5	27	32	**1.79**	*0.18*		
4B	4	1	7	23	30	**0.13**	*0.72*		
4B	4	2	7	22	29	**0.08**	*0.78*		
4B	5	1	7	25	32	**0.29**	*0.59*		
4B	5	2	7	24	31	**0.20**	*0.65*		
4B	5	3	8	20	28	**0.14**	*0.71*		
4B	5	4	7	25	32	**0.29**	*0.59*		
4B	5	5	8	23	31	**0.03**	*0.86*		
4B	5	6	6	24	30	**0.58**	*0.45*		
4B	5	7	10	22	32	**0.54**	*0.46*		
4B	5	8	7	21	28	**0.05**	*0.83*		
4B	5	9	8	22	30	**0.04**	*0.83*		
4B	5	10	7	24	31	**0.20**	*0.65*		
4B	6	1	6	21	27	**0.23**	*0.63*		
4B	6	2	5	18	23	**0.28**	*0.60*		
4B	6	3	5	14	19	**0.05**	*0.82*		
4B	6	4	7	23	30	**0.13**	*0.72*		
4B	7	2	5	10	15	**0.42**	*0.52*		
4B	7	3	7	16	23	**0.28**	*0.60*		
4B	7	5	4	9	13	**0.18**	*0.67*		
4B	7	7	2	12	14	**1.24**	*0.27*		
4B	7	9	0	15	15	**5.76**	*0.02*		
4B	7	16	5	11	16	**0.25**	*0.62*		
4B	7	17	4	9	13	**0.18**	*0.67*		
4B	7	18	4	12	16	**0.08**	*0.77*		
4B	8	1	6	19	25	**0.09**	*0.76*		
4B	8	3	8	25	33	**0.07**	*0.79*		
4B	8	4	6	18	24	**0.06**	*0.81*		
4B	8	6	4	11	15	**0.07**	*0.80*		
4B	8	7	5	11	16	**0.25**	*0.62*		
4B	8	8	3	8	11	**0.09**	*0.76*		
4B	8	9	3	9	12	**0.11**	*0.74*		
4B	8	10	4	12	16	**0.08**	*0.77*		
4B	8	14	3	8	11	**0.09**	*0.76*		
4B	8	15	2	14	16	**1.75**	*0.19*		
4B	9	1	9	18	27	**0.83**	*0.36*		
4B	9	2	4	11	15	**0.07**	*0.80*		
4B	9	4	3	12	15	**0.42**	*0.52*		
4B	9	5	7	19	26	**0.05**	*0.82*		
4B	9	6	6	20	26	**0.15**	*0.69*		
4B	9	9	7	23	30	**0.13**	*0.72*		
4B	9	10	2	13	15	**1.49**	*0.22*		
4B	9	13	4	11	15	**0.07**	*0.80*		
4B	9	14	4	12	16	**0.08**	*0.77*		
4B	9	17	4	11	15	**0.07**	*0.80*		
4B	10	3	3	16	19	**1.18**	*0.28*		
4B	10	5	3	9	12	**0.11**	*0.74*		

Appendix 7.6: Screening of Monosomic F_1-derived F_2 individuals with Ptr ToxB.

Line [z]	Parent [y]	F_1 Family [x]	F_1-derived F_2 individuals			3:1 Ratio Tested [w]		1:1 Ratio Tested [v]	
			# Insensitive	# Sensitive	Total	X^2	Probability	X^2	Probability
4B	10	7	4	11	15	**0.07**	*0.80*		
4B	10	9	4	11	15	**0.07**	*0.80*		
4B	10	10	4	8	12	**0.33**	*0.56*		
4B	10	11	3	9	12	**0.11**	*0.74*		
4B	10	12	3	12	15	**0.42**	*0.52*		
4B	10	13	3	9	12	**0.11**	*0.74*		
4B	10	15	5	14	19	**0.05**	*0.82*		
4B	10	16	4	10	14	**0.10**	*0.76*		
4B	10	17	3	11	14	**0.29**	*0.59*		
4B	10	18	4	13	17	**0.14**	*0.71*		
4B	10	19	4	12	16	**0.08**	*0.77*		
4D	1	2	2	15	17	**2.02**	*0.16*		
4D	1	3	4	12	16	**0.08**	*0.77*		
4D	6	1	3	13	16	**0.58**	*0.45*		
4D	6	2	3	11	14	**0.29**	*0.59*		
4D	7	1	5	11	16	**0.25**	*0.62*		
4D	7	4	1	14	15	**3.27**	*0.07*		
4D	7	5	3	12	15	**0.42**	*0.52*		
4D	7	6	5	10	15	**0.42**	*0.52*		
4D	7	7	7	8	15	**3.27**	*0.07*	**0.13**	0.72
4D	7	8	5	9	14	**0.67**	*0.41*		
4D	7	10	5	11	16	**0.25**	*0.62*		
4D	7	11	7	27	34	**0.51**	*0.48*		
4D	7	13	4	10	14	**0.10**	*0.76*		
4D	7	14	5	11	16	**0.25**	*0.62*		
4D	7	15	3	13	16	**0.58**	*0.45*		
4D	7	17	5	11	16	**0.25**	*0.62*		
4D	7	18	5	9	14	**0.67**	*0.41*		
4D	7	19	9	22	31	**0.20**	*0.65*		
4D	7	20	4	12	16	**0.08**	*0.77*		
4D	8	1	5	11	16	**0.25**	*0.62*		
4D	8	2	3	13	16	**0.58**	*0.45*		
4D	8	5	10	21	31	**0.72**	*0.40*		
4D	8	6	3	13	16	**0.58**	*0.45*		
4D	8	10	4	10	14	**0.10**	*0.76*		
4D	8	11	10	20	30	**0.93**	*0.33*		
4D	9	1	12	20	32	**2.38**	*0.12*		
4D	9	2	3	29	32	**4.63**	*0.03*		
4D	9	3	7	27	34	**0.51**	*0.48*		
4D	9	4	3	12	15	**0.42**	*0.52*		
4D	9	5	5	11	16	**0.25**	*0.62*		
4D	9	6	4	12	16	**0.08**	*0.77*		
4D	9	8	3	13	16	**0.58**	*0.45*		
4D	9	9	3	13	16	**0.58**	*0.45*		
4D	9	11	2	11	13	**1.00**	*0.32*		
4D	9	12	6	10	16	**1.08**	*0.30*		
4D	9	13	3	13	16	**0.58**	*0.45*		
4D	10	1	3	10	13	**0.18**	*0.67*		
4D	10	3	4	11	15	**0.07**	*0.80*		
4D	10	4	4	9	13	**0.18**	*0.67*		
4D	10	5	2	11	13	**1.00**	*0.32*		
4D	10	6	2	13	15	**1.49**	*0.22*		
5A	1	1	4	10	14	**0.10**	*0.76*		
5A	1	2	3	10	13	**0.18**	*0.67*		
5A	1	3	4	12	16	**0.08**	*0.77*		
5A	1	4	5	10	15	**0.42**	*0.52*		
5A	1	5	4	10	14	**0.10**	*0.76*		
5A	2	1	4	11	15	**0.07**	*0.80*		

Appendix 7.6: Screening of Monosomic F_1-derived F_2 individuals with Ptr ToxB.

Line [z]	Parent [y]	F_1 Family [x]	F_1-derived F_2 individuals			3:1 Ratio Tested [w]		1:1 Ratio Tested [v]	
			# Insensitive	# Sensitive	Total	X^2	Probability	X^2	Probability
5A	2	2	3	13	16	**0.58**	*0.45*		
5A	2	3	4	10	14	**0.10**	*0.76*		
5A	2	4	4	10	14	**0.10**	*0.76*		
5A	2	5	4	12	16	**0.08**	*0.77*		
5A	2	6	6	9	15	**1.49**	*0.22*	**0.67**	0.41
5A	2	7	4	11	15	**0.07**	*0.80*		
5A	2	8	4	11	15	**0.07**	*0.80*		
5A	2	9	5	10	15	**0.42**	*0.52*		
5A	2	10	3	13	16	**0.58**	*0.45*		
5A	2	11	4	11	15	**0.07**	*0.80*		
5A	2	12	4	12	16	**0.08**	*0.77*		
5A	2	13	7	9	16	**2.58**	*0.11*	**0.31**	0.58
5A	2	14	4	11	15	**0.07**	*0.80*		
5A	2	15	5	9	14	**0.67**	*0.41*		
5A	2	16	4	11	15	**0.07**	*0.80*		
5A	3	1	7	10	17	**2.02**	*0.16*	**0.59**	0.44
5A	3	2	3	12	15	**0.42**	*0.52*		
5A	3	3	4	12	16	**0.08**	*0.77*		
5A	3	4	4	12	16	**0.08**	*0.77*		
5A	3	5	5	11	16	**0.25**	*0.62*		
5A	3	6	4	8	12	**0.33**	*0.56*		
5A	3	7	4	13	17	**0.14**	*0.71*		
5A	3	8	3	11	14	**0.29**	*0.59*		
5A	3	9	4	8	12	**0.33**	*0.56*		
5A	3	10	5	11	16	**0.25**	*0.62*		
5A	3	11	6	9	15	**1.49**	*0.22*	**0.67**	0.41
5A	3	12	4	9	13	**0.18**	*0.67*		
5A	3	13	6	9	15	**1.49**	*0.22*	**0.67**	0.41
5A	3	14	5	10	15	**0.42**	*0.52*		
5A	4	1	6	8	14	**2.00**	*0.16*	**0.36**	0.55
5A	4	2	4	11	15	**0.07**	*0.80*		
5A	4	3	3	13	16	**0.58**	*0.45*		
5A	5	1	4	12	16	**0.08**	*0.77*		
5A	5	2	3	11	14	**0.29**	*0.59*		
5A	5	3	4	11	15	**0.07**	*0.80*		
5A	7	1	8	22	30	**0.04**	*0.83*		
5A	7	2	8	24	32	**0.04**	*0.84*		
5A	7	5	6	25	31	**0.72**	*0.40*		
5A	7	6	7	24	31	**0.20**	*0.65*		
5A	7	9	6	24	30	**0.58**	*0.45*		
5A	7	10	6	25	31	**0.72**	*0.40*		
5A	7	13	7	24	31	**0.20**	*0.65*		
5A	7	15	10	26	36	**0.11**	*0.74*		
5A	8	1	4	6	10	**0.93**	*0.33*		
5A	8	2	4	21	25	**1.37**	*0.24*		
5A	8	6	9	22	31	**0.20**	*0.65*		
5A	8	8	2	14	16	**1.75**	*0.19*		
5A	8	9	4	18	22	**0.79**	*0.37*		
5A	8	14	9	18	27	**0.83**	*0.36*		
5A	9	1	7	24	31	**0.20**	*0.65*		
5A	9	5	5	27	32	**1.79**	*0.18*		
5A	9	6	6	25	31	**0.72**	*0.40*		
5A	9	9	5	23	28	**1.00**	*0.32*		
5A	9	10	5	25	30	**1.38**	*0.24*		
5A	9	11	7	23	30	**0.13**	*0.72*		
5A	9	12	6	22	28	**0.33**	*0.56*		
5A	9	13	9	12	21	**3.16**	*0.08*		
5A	9	15	4	27	31	**2.78**	*0.10*		

Appendix 7.6: Screening of Monosomic F_1-derived F_2 individuals with Ptr ToxB.

Line [z]	Parent [y]	F_1 Family [x]	F_1-derived F_2 individuals			3:1 Ratio Tested [w]		1:1 Ratio Tested [v]	
			# Insensitive	# Sensitive	Total	X^2	Probability	X^2	Probability
5A	10	2	8	24	32	**0.04**	*0.84*		
5A	10	3	10	20	30	**0.93**	*0.33*		
5A	10	5	1	14	15	**3.27**	*0.07*		
5A	10	5	6	26	32	**0.88**	*0.35*		
5A	10	9	9	21	30	**0.31**	*0.58*		
5B	2	1	3	13	16	**0.58**	*0.45*		
5B	2	2	3	11	14	**0.29**	*0.59*		
5B	2	3	5	12	17	**0.14**	*0.71*		
5B	2	4	5	11	16	**0.25**	*0.62*		
5B	2	6	3	13	16	**0.58**	*0.45*		
5B	2	7	7	9	16	**2.58**	*0.11*	**0.31**	0.58
5B	2	8	6	9	15	**1.49**	*0.22*	**0.67**	0.41
5B	2	10	4	12	16	**0.08**	*0.77*		
5B	2	11	4	12	16	**0.08**	*0.77*		
5B	3	1	5	11	16	**0.25**	*0.62*		
5B	3	2	6	10	16	**1.08**	*0.30*		
5B	3	3	3	13	16	**0.58**	*0.45*		
5B	3	4	4	12	16	**0.08**	*0.77*		
5B	3	5	6	9	15	**1.49**	*0.22*	**0.67**	0.41
5B	3	6	4	12	16	**0.08**	*0.77*		
5B	3	7	3	13	16	**0.58**	*0.45*		
5B	3	8	6	10	16	**1.08**	*0.30*		
5B	3	9	2	14	16	**1.75**	*0.19*		
5B	3	10	2	14	16	**1.75**	*0.19*		
5B	3	11	5	11	16	**0.25**	*0.62*		
5B	3	12	2	14	16	**1.75**	*0.19*		
5B	3	13	1	15	16	**3.58**	*0.06*		
5B	3	14	3	13	16	**0.58**	*0.45*		
5B	3	15	0	15	15	**5.76**	*0.02*		
5B	3	16	4	12	16	**0.08**	*0.77*		
5B	3	17	6	10	16	**1.08**	*0.30*		
5B	3	18	3	12	15	**0.42**	*0.52*		
5B	3	19	1	15	16	**3.58**	*0.06*		
5B	4	1	5	11	16	**0.25**	*0.62*		
5B	4	2	5	10	15	**0.42**	*0.52*		
5B	4	3	3	13	16	**0.58**	*0.45*		
5B	5	1	4	12	16	**0.08**	*0.77*		
5B	5	3	2	11	13	**1.00**	*0.32*		
5B	5	4	6	10	16	**1.08**	*0.30*		
5B	5	5	4	11	15	**0.07**	*0.80*		
5B	5	6	2	11	13	**1.00**	*0.32*		
5D	5	1	9	21	30	**0.31**	*0.58*		
5D	5	2	3	12	15	**0.42**	*0.52*		
5D	5	3	7	25	32	**0.29**	*0.59*		
5D	5	4	6	22	28	**0.33**	*0.56*		
5D	5	5	7	25	32	**0.29**	*0.59*		
5D	5	6	3	12	15	**0.42**	*0.52*		
5D	5	7	3	13	16	**0.58**	*0.45*		
5D	5	8	4	12	16	**0.08**	*0.77*		
5D	5	9	4	11	15	**0.07**	*0.80*		
5D	5	14	3	12	15	**0.42**	*0.52*		
5D	5	15	3	7	10	**0.13**	*0.72*		
5D	5	16	3	12	15	**0.42**	*0.52*		
5D	7	1	6	16	22	**0.06**	*0.81*		
5D	7	2	6	22	28	**0.33**	*0.56*		
5D	7	3	7	21	28	**0.05**	*0.83*		
5D	8	1	6	14	20	**0.20**	*0.65*		
5D	8	2	5	17	22	**0.18**	*0.67*		

Appendix 7.6: Screening of Monosomic F_1-derived F_2 individuals with Ptr ToxB.

Line [z]	Parent [y]	F_1 Family [x]	F_1-derived F_2 individuals			3:1 Ratio Tested [w]		1:1 Ratio Tested [v]	
			# Insensitive	# Sensitive	Total	X^2	Probability	X^2	Probability
5D	8	3	6	19	25	**0.09**	*0.76*		
5D	8	4	6	20	26	**0.15**	*0.69*		
5D	8	5	8	18	26	**0.36**	*0.55*		
5D	8	6	7	16	23	**0.28**	*0.60*		
5D	8	7	6	18	24	**0.06**	*0.81*		
5D	8	8	6	19	25	**0.09**	*0.76*		
5D	8	9	6	15	21	**0.11**	*0.74*		
5D	8	10	6	20	26	**0.15**	*0.69*		
5D	8	11	8	21	29	**0.08**	*0.78*		
5D	8	12	7	20	27	**0.04**	*0.85*		
5D	8	13	6	19	25	**0.09**	*0.76*		
5D	8	16	6	22	28	**0.33**	*0.56*		
5D	8	17	6	14	20	**0.20**	*0.65*		
5D	8	18	4	23	27	**1.81**	*0.18*		
5D	8	19	8	25	33	**0.07**	*0.79*		
5D	8	20	8	19	27	**0.23**	*0.63*		
6A	6	1	3	12	15	**0.42**	*0.52*		
6A	6	2	3	9	12	**0.11**	*0.74*		
6A	6	3	5	11	16	**0.25**	*0.62*		
6A	6	4	3	7	10	**0.13**	*0.72*		
6A	6	5	4	11	15	**0.07**	*0.80*		
6A	6	7	3	9	12	**0.11**	*0.74*		
6A	6	8	3	12	15	**0.42**	*0.52*		
6A	6	9	5	11	16	**0.25**	*0.62*		
6A	6	10	3	11	14	**0.29**	*0.59*		
6A	6	11	4	9	13	**0.18**	*0.67*		
6A	6	16	4	12	16	**0.08**	*0.77*		
6A	7	2	2	8	10	**0.40**	*0.53*		
6A	7	9	2	12	14	**1.24**	*0.27*		
6A	7	13	5	9	14	**0.67**	*0.41*		
6A	7	14	3	9	12	**0.11**	*0.74*		
6A	7	19	3	10	13	**0.18**	*0.67*		
6A	7	20	4	7	11	**0.58**	*0.45*		
6A	9	2	4	10	14	**0.10**	*0.76*		
6A	9	11	3	9	12	**0.11**	*0.74*		
6A	9	12	3	11	14	**0.29**	*0.59*		
6A	9	13	4	10	14	**0.10**	*0.76*		
6A	9	14	7	9	16	**2.58**	*0.11*	**0.31**	0.58
6A	9	17	8	9	17	**3.90**	*0.05*	**0.12**	0.73
6A	9	18	5	11	16	**0.25**	*0.62*		
6A	9	19	6	11	17	**0.76**	*0.38*		
6A	9	20	5	10	15	**0.42**	*0.52*		
6A	10	1	3	13	16	**0.58**	*0.45*		
6A	10	2	3	11	14	**0.29**	*0.59*		
6A	10	4	3	12	15	**0.42**	*0.52*		
6A	10	5	4	10	14	**0.10**	*0.76*		
6A	10	6	3	11	14	**0.29**	*0.59*		
6A	10	7	4	11	15	**0.07**	*0.80*		
6A	10	8	4	12	16	**0.08**	*0.77*		
6A	10	9	4	12	16	**0.08**	*0.77*		
6A	10	10	4	9	13	**0.18**	*0.67*		
6A	10	11	4	11	15	**0.07**	*0.80*		
6B	1	2	3	14	17	**0.76**	*0.38*		
6B	1	3	4	10	14	**0.10**	*0.76*		
6B	1	4	2	14	16	**1.75**	*0.19*		
6B	6	3	2	11	13	**1.00**	*0.32*		
6B	6	5	3	11	14	**0.29**	*0.59*		
6B	6	17	1	13	14	**2.95**	*0.09*		

Appendix 7.6: Screening of Monosomic F_1-derived F_2 individuals with Ptr ToxB.

Line [z]	Parent [y]	F_1 Family [x]	F_1-derived F_2 individuals			3:1 Ratio Tested [w]		1:1 Ratio Tested [v]	
			# Insensitive	# Sensitive	Total	X^2	Probability	X^2	Probability
6B	6	20	4	11	15	**0.07**	*0.80*		
6B	7	1	5	8	13	**1.00**	*0.32*		
6B	7	2	2	12	14	**1.24**	*0.27*		
6B	7	3	4	12	16	**0.08**	*0.77*		
6B	7	4	5	10	15	**0.42**	*0.52*		
6B	7	5	2	15	17	**2.02**	*0.16*		
6B	7	8	3	12	15	**0.42**	*0.52*		
6B	7	9	3	13	16	**0.58**	*0.45*		
6B	7	10	4	11	15	**0.07**	*0.80*		
6B	7	13	2	14	16	**1.75**	*0.19*		
6B	7	15	5	11	16	**0.25**	*0.62*		
6B	7	17	4	7	11	**0.58**	*0.45*		
6B	7	20	2	8	10	**0.40**	*0.53*		
6B	8	2	4	9	13	**0.18**	*0.67*		
6B	8	4	2	12	14	**1.24**	*0.27*		
6B	8	5	6	22	28	**0.33**	*0.56*		
6B	8	6	5	21	26	**0.67**	*0.41*		
6B	8	7	3	10	13	**0.18**	*0.67*		
6B	8	8	2	11	13	**1.00**	*0.32*		
6B	9	1	4	11	15	**0.07**	*0.80*		
6B	9	2	4	23	27	**1.81**	*0.18*		
6B	9	3	2	12	14	**1.24**	*0.27*		
6B	9	4	9	20	29	**0.45**	*0.50*		
6B	9	5	5	11	16	**0.25**	*0.62*		
6B	9	7	2	12	14	**1.24**	*0.27*		
6B	9	8	3	10	13	**0.18**	*0.67*		
6B	9	9	4	10	14	**0.10**	*0.76*		
6B	9	10	5	10	15	**0.42**	*0.52*		
6B	9	14	3	8	11	**0.09**	*0.76*		
6B	9	15	5	24	29	**1.18**	*0.28*		
6B	9	16	3	13	16	**0.58**	*0.45*		
6B	10	1	3	11	14	**0.29**	*0.59*		
6B	10	4	2	12	14	**1.24**	*0.27*		
6B	10	5	11	18	29	**2.29**	*0.13*	**1.72**	0.19
6B	10	6	3	11	14	**0.29**	*0.59*		
6B	10	7	4	8	12	**0.33**	*0.56*		
6B	10	8	3	13	16	**0.58**	*0.45*		
6B	10	9	11	17	28	**2.71**	*0.10*	**1.32**	0.25
6B	10	10	6	11	17	**0.76**	*0.38*		
6B	10	11	3	12	15	**0.42**	*0.52*		
6B	10	12	2	12	14	**1.24**	*0.27*		
6B	10	13	3	9	12	**0.11**	*0.74*		
6B	10	14	2	13	15	**1.49**	*0.22*		
6D	6	3	3	14	17	**0.76**	*0.38*		
6D	6	4	4	12	16	**0.08**	*0.77*		
6D	6	5	3	13	16	**0.58**	*0.45*		
6D	6	6	4	12	16	**0.08**	*0.77*		
6D	6	7	3	13	16	**0.58**	*0.45*		
6D	6	9	5	10	15	**0.42**	*0.52*		
6D	6	12	4	11	15	**0.07**	*0.80*		
6D	6	13	2	13	15	**1.49**	*0.22*		
6D	6	14	2	14	16	**1.75**	*0.19*		
6D	6	15	3	12	15	**0.42**	*0.52*		
6D	6	16	7	13	20	**0.87**	*0.35*		
6D	6	17	4	13	17	**0.14**	*0.71*		
6D	6	18	4	12	16	**0.08**	*0.77*		
6D	6	19	6	9	15	**1.49**	*0.22*	**0.67**	0.41
6D	8	1	8	8	16	**4.75**	*0.03*	**0.06**	0.80

Appendix 7.6: Screening of Monosomic F_1-derived F_2 individuals with Ptr ToxB.

Line [z]	Parent [y]	F_1 Family [x]	F_1-derived F_2 individuals			3:1 Ratio Tested [w]		1:1 Ratio Tested [v]	
			# Insensitive	# Sensitive	Total	X^2	Probability	X^2	Probability
6D	8	2	3	12	15	**0.42**	*0.52*		
6D	8	3	4	11	15	**0.07**	*0.80*		
6D	8	4	6	10	16	**1.08**	*0.30*		
6D	8	5	5	10	15	**0.42**	*0.52*		
6D	8	6	3	13	16	**0.58**	*0.45*		
6D	8	7	6	10	16	**1.08**	*0.30*		
6D	8	8	4	10	14	**0.10**	*0.76*		
6D	8	8	7	9	16	**2.58**	*0.11*	**0.31**	0.58
6D	8	9	4	12	16	**0.08**	*0.77*		
6D	8	10	4	10	14	**0.10**	*0.76*		
6D	8	11	3	12	15	**0.42**	*0.52*		
6D	8	12	5	11	16	**0.25**	*0.62*		
6D	8	13	5	10	15	**0.42**	*0.52*		
6D	8	14	3	13	16	**0.58**	*0.45*		
6D	8	15	2	12	14	**1.24**	*0.27*		
6D	8	16	5	11	16	**0.25**	*0.62*		
6D	9	1	7	9	16	**2.58**	*0.11*	**0.31**	0.58
6D	9	3	5	11	16	**0.25**	*0.62*		
6D	9	4	4	11	15	**0.07**	*0.80*		
6D	9	5	4	12	16	**0.08**	*0.77*		
6D	9	6	6	10	16	**1.08**	*0.30*		
6D	9	7	5	11	16	**0.25**	*0.62*		
6D	9	8	4	12	16	**0.08**	*0.77*		
6D	9	9	5	11	16	**0.25**	*0.62*		
6D	9	10	3	14	17	**0.76**	*0.38*		
6D	9	11	4	12	16	**0.08**	*0.77*		
6D	9	14	2	14	16	**1.75**	*0.19*		
6D	9	15	3	13	16	**0.58**	*0.45*		
6D	9	16	4	11	15	**0.07**	*0.80*		
6D	9	17	3	13	16	**0.58**	*0.45*		
6D	9	19	8	8	16	**4.75**	*0.03*	**0.06**	0.80
6D	9	20	3	12	15	**0.42**	*0.52*		
6D	10	1	2	13	15	**1.49**	*0.22*		
6D	10	2	4	11	15	**0.07**	*0.80*		
6D	10	3	2	13	15	**1.49**	*0.22*		
6D	10	4	4	12	16	**0.08**	*0.77*		
6D	10	5	6	9	15	**1.49**	*0.22*	**0.67**	0.41
6D	10	8	4	11	15	**0.07**	*0.80*		
6D	10	9	5	9	14	**0.67**	*0.41*		
6D	10	10	8	8	16	**4.75**	*0.03*	**0.06**	0.80
6D	10	11	7	9	16	**2.58**	*0.11*	**0.31**	0.58
6D	10	12	7	9	16	**2.58**	*0.11*	**0.31**	0.58
6D	10	14	4	12	16	**0.08**	*0.77*		
6D	10	15	5	11	16	**0.25**	*0.62*		
6D	10	17	3	13	16	**0.58**	*0.45*		
6D	10	18	3	10	13	**0.18**	*0.67*		
6D	10	19	3	11	14	**0.29**	*0.59*		
6D	10	20	2	13	15	**1.49**	*0.22*		
7A	6	1	5	10	15	**0.42**	*0.52*		
7A	6	2	3	13	16	**0.58**	*0.45*		
7A	6	3	8	8	16	**4.75**	*0.03*	**0.06**	0.80
7A	7	1	2	14	16	**1.75**	*0.19*		
7A	7	2	3	12	15	**0.42**	*0.52*		
7A	7	3	5	11	16	**0.25**	*0.62*		
7A	7	5	5	10	15	**0.42**	*0.52*		
7A	7	6	2	14	16	**1.75**	*0.19*		
7A	7	9	5	11	16	**0.25**	*0.62*		
7A	7	11	3	13	16	**0.58**	*0.45*		

Appendix 7.6: Screening of Monosomic F_1-derived F_2 individuals with Ptr ToxB.

Line [z]	Parent [y]	F_1 Family [x]	F_1-derived F_2 individuals			3:1 Ratio Tested [w]		1:1 Ratio Tested [v]	
			# Insensitive	# Sensitive	Total	X^2	Probability	X^2	Probability
7A	7	12	3	13	16	**0.58**	*0.45*		
7A	7	13	3	11	14	**0.29**	*0.59*		
7A	7	14	4	11	15	**0.07**	*0.80*		
7A	7	15	4	12	16	**0.08**	*0.77*		
7A	7	16	3	13	16	**0.58**	*0.45*		
7A	8	1	3	12	15	**0.42**	*0.52*		
7A	8	2	2	13	15	**1.49**	*0.22*		
7A	8	3	5	10	15	**0.42**	*0.52*		
7A	8	4	6	10	16	**1.08**	*0.30*		
7A	8	5	5	11	16	**0.25**	*0.62*		
7A	9	1	0	15	15	**5.76**	*0.02*		
7A	9	3	4	11	15	**0.07**	*0.80*		
7A	9	4	4	11	15	**0.07**	*0.80*		
7A	9	5	3	9	12	**0.11**	*0.74*		
7A	9	6	2	11	13	**1.00**	*0.32*		
7A	9	7	6	10	16	**1.08**	*0.30*		
7A	9	9	4	12	16	**0.08**	*0.77*		
7A	9	10	5	11	16	**0.25**	*0.62*		
7A	9	12	6	10	16	**1.08**	*0.30*		
7A	9	13	7	8	15	**3.27**	*0.07*	**0.13**	0.72
7A	9	14	1	13	14	**2.95**	*0.09*		
7A	9	15	6	10	16	**1.08**	*0.30*		
7A	9	17	4	10	14	**0.10**	*0.76*		
7A	9	18	4	12	16	**0.08**	*0.77*		
7A	9	19	4	12	16	**0.08**	*0.77*		
7A	9	20	4	9	13	**0.18**	*0.67*		
7B	6	1	5	11	16	**0.25**	*0.62*		
7B	6	2	3	20	23	**2.13**	*0.14*		
7B	6	3	3	14	17	**0.76**	*0.38*		
7B	8	2	5	21	26	**0.67**	*0.41*		
7B	8	3	2	15	17	**2.02**	*0.16*		
7B	8	5	2	11	13	**1.00**	*0.32*		
7B	8	6	2	14	16	**1.75**	*0.19*		
7B	8	10	6	10	16	**1.08**	*0.30*		
7B	8	11	2	13	15	**1.49**	*0.22*		
7B	8	13	6	10	16	**1.08**	*0.30*		
7B	8	14	2	13	15	**1.49**	*0.22*		
7B	8	15	3	12	15	**0.42**	*0.52*		
7B	8	17	4	25	29	**2.29**	*0.13*		
7B	8	18	4	11	15	**0.07**	*0.80*		
7B	8	19	3	12	15	**0.42**	*0.52*		
7B	8	20	2	4	6	**0.22**	*0.64*		
7B	9	1	3	13	16	**0.58**	*0.45*		
7B	10	1	5	11	16	**0.25**	*0.62*		
7B	10	3	7	8	15	**3.27**	*0.07*	**0.13**	0.72
7D	1	1	4	8	12	**0.33**	*0.56*		
7D	1	5	3	10	13	**0.18**	*0.67*		
7D	1	9	3	13	16	**0.58**	*0.45*		
7D	1	13	2	12	14	**1.24**	*0.27*		
7D	2	2	3	11	14	**0.29**	*0.59*		
7D	2	5	3	13	16	**0.58**	*0.45*		
7D	3	1	4	12	16	**0.08**	*0.77*		
7D	5	1	3	12	15	**0.42**	*0.52*		
7D	5	4	5	10	15	**0.42**	*0.52*		
7D	6	1	4	11	15	**0.07**	*0.80*		
7D	6	2	5	11	16	**0.25**	*0.62*		
7D	7	1	3	12	15	**0.42**	*0.52*		

Appendix 7.6: Screening of Monosomic F_1-derived F_2 individuals with Ptr ToxB.

Line [z]	Parent [y]	F_1 Family [x]	F_1-derived F_2 individuals			3:1 Ratio Tested [w]		1:1 Ratio Tested [v]	
			# Insensitive	# Sensitive	Total	X^2	Probability	X^2	Probability
7D	7	4	5	11	16	**0.25**	*0.62*		
7D	7	5	3	14	17	**0.76**	*0.38*		
7D	7	6	4	11	15	**0.07**	*0.80*		
7D	7	7	5	11	16	**0.25**	*0.62*		
7D	7	8	7	9	16	**2.58**	*0.11*	**0.31**	0.58
7D	7	9	4	12	16	**0.08**	*0.77*		
7D	7	10	1	15	16	**3.58**	*0.06*		
7D	7	11	3	13	16	**0.58**	*0.45*		
7D	7	12	4	11	15	**0.07**	*0.80*		
7D	7	13	5	10	15	**0.42**	*0.52*		
7D	7	14	6	10	16	**1.08**	*0.30*		
7D	7	15	1	15	16	**3.58**	*0.06*		
7D	7	16	6	10	16	**1.08**	*0.30*		
7D	7	17	5	10	15	**0.42**	*0.52*		
7D	7	18	7	11	18	**1.56**	*0.21*	**0.94**	0.33
7D	7	19	4	11	15	**0.07**	*0.80*		
7D	7	20	4	12	16	**0.08**	*0.77*		
7D	8	1	6	10	16	**1.08**	*0.30*		
7D	8	2	3	13	16	**0.58**	*0.45*		
7D	10	1	7	8	15	**3.27**	*0.07*	**0.13**	0.72
7D	10	2	5	10	15	**0.42**	*0.52*		

[z] = the chromosome that is monosomic for that line.

[y] = the number assigned to each parent Chinese Spring plant at the time of crossing with Katepwa.

[x] = the number assigned to each F_1 plant at the time of F_1 selfing, and the number used to designate tha F_1-derived F_2 family.

[w] = Chi-square and Probability values calculated for each family fitting the expected 3 sensitive to 1 insensitive ratio.

[v] = Chi-square and Probability values calculated for each family fitting the expected 1 sensitive to 1 insensitive ratio.

= Families that are displaying the segregation ratio of a critical chromosome.
= Families that are displaying the 1:1 segregation ratio.

Appendix 7.7: Screening of Monosomic F_1-derived F_2 individuals with Ptr ToxA.

Line [z]	Parent [y]	F_1 Family [x]	F_1-derived F_2 individuals			3:1 Ratio Tested [w]		1:1 Ratio Tested [v]	
			# Insensitive	# Sensitive	Total	X^2	Probability	X^2	Probability
5B	1	1	0	15	15	**5.76**	*0.02*		
5B	2	1	3	13	16	**0.58**	*0.45*		
5B	2	2	0	14	14	**5.43**	*0.02*		
5B	2	3	3	14	17	**0.76**	*0.38*		
5B	2	4	4	12	16	**0.08**	*0.77*		
5B	2	5	1	18	19	**4.54**	*0.03*		
5B	2	6	0	32	32	**11.38**	*0.00*		
5B	2	7	4	12	16	**0.08**	*0.77*		
5B	2	8	2	27	29	**5.60**	*0.02*		
5B	2	10	0	25	25	**9.05**	*0.00*		
5B	2	11	6	10	16	**1.08**	*0.30*		
5B	3	1	3	13	16	**0.58**	*0.45*		
5B	3	2	1	28	29	**7.80**	*0.01*		
5B	3	3	1	30	31	**8.46**	*0.00*		
5B	3	4	1	28	29	**7.80**	*0.01*		
5B	3	5	0	30	30	**10.71**	*0.00*		
5B	3	6	5	11	16	**0.25**	*0.62*		
5B	3	7	7	9	16	**2.58**	*0.11*	**0.31**	0.58
5B	3	8	6	10	16	**1.08**	*0.30*		
5B	3	9	6	10	16	**1.08**	*0.30*		
5B	3	10	4	12	16	**0.08**	*0.77*		
5B	3	11	4	12	16	**0.08**	*0.77*		
5B	3	12	3	13	16	**0.58**	*0.45*		
5B	3	13	5	11	16	**0.25**	*0.62*		
5B	3	14	3	12	15	**0.42**	*0.52*		
5B	3	15	3	12	15	**0.42**	*0.52*		
5B	3	16	2	14	16	**1.75**	*0.19*		
5B	3	17	3	28	31	**4.33**	*0.04*		
5B	3	18	2	26	28	**5.29**	*0.02*		
5B	3	19	2	14	16	**1.75**	*0.19*		
5B	4	1	3	29	32	**4.63**	*0.03*		
5B	4	2	3	27	30	**4.04**	*0.04*		
5B	4	3	0	31	31	**11.04**	*0.00*		
5B	5	1	4	12	16	**0.08**	*0.77*		
5B	5	3	2	26	28	**5.29**	*0.02*		
5B	5	4	0	16	16	**6.08**	*0.01*		
5B	5	5	5	11	16	**0.25**	*0.62*		
5B	5	6	0	21	21	**7.73**	*0.01*		
5B	6	5	1	13	14	**2.95**	*0.09*		
5B	6	8	1	13	14	**2.95**	*0.09*		
5B	6	9	0	14	14	**5.43**	*0.02*		
5B	6	13	0	15	15	**5.76**	*0.02*		
5B	6	14	1	15	16	**3.58**	*0.06*		
5B	7	1	1	13	14	**2.95**	*0.09*		
5B	7	2	1	13	14	**2.95**	*0.09*		
5B	7	5	1	14	15	**3.27**	*0.07*		
5B	8	9	1	14	15	**3.27**	*0.07*		
5B	9	1	1	16	17	**3.90**	*0.05*		
5B	9	3	1	9	10	**1.73**	*0.19*		
5B	10	5	0	16	16	**6.08**	*0.01*		
5B	10	12	1	11	12	**2.33**	*0.13*		
7D	1	1	6	11	17	**0.76**	*0.38*		
7D	1	2	4	13	17	**0.14**	*0.71*		
7D	1	3	3	14	17	**0.76**	*0.38*		
7D	1	4	7	8	15	**3.27**	*0.07*	**0.13**	0.72
7D	1	5	5	13	18	**0.07**	*0.79*		
7D	1	6	1	14	15	**3.27**	*0.07*		

Appendix 7.7: Screening of Monosomic F_1-derived F_2 individuals with Ptr ToxA.

Line [z]	Parent [y]	F_1 Family [x]	F_1-derived F_2 individuals						
						3:1 Ratio Tested [w]		1:1 Ratio Tested [v]	
			# Insensitive	# Sensitive	Total	X^2	Probability	X^2	Probability
7D	1	7	4	13	17	**0.14**	*0.71*		
7D	1	8	2	14	16	**1.75**	*0.19*		
7D	1	9	3	13	16	**0.58**	*0.45*		
7D	1	10	2	16	18	**2.30**	*0.13*		
7D	1	11	6	10	16	**1.08**	*0.30*		
7D	1	12	5	13	18	**0.07**	*0.79*		
7D	1	13	5	9	14	**0.67**	*0.41*		
7D	1	14	7	7	14	**4.10**	*0.04*	**0.07**	0.79
7D	2	1	4	11	15	**0.07**	*0.80*		
7D	2	2	4	11	15	**0.07**	*0.80*		
7D	2	3	4	12	16	**0.08**	*0.77*		
7D	2	4	5	11	16	**0.25**	*0.62*		
7D	2	5	3	14	17	**0.76**	*0.38*		
7D	2	6	3	10	13	**0.18**	*0.67*		
7D	3	1	6	10	16	**1.08**	*0.30*		
7D	3	2	3	16	19	**1.18**	*0.28*		
7D	5	1	3	14	17	**0.76**	*0.38*		
7D	5	2	5	13	18	**0.07**	*0.79*		
7D	5	3	3	11	14	**0.29**	*0.59*		
7D	5	4	3	13	16	**0.58**	*0.45*		
7D	5	5	3	14	17	**0.76**	*0.38*		

[z] = the chromosome that is monosomic for that line.

[y] = the number assigned to each parent plant at the time of crossing with Katepwa.

[x] = the number assigned to each F_1 plant at the time of F_1 selfing, and the number used to designate that F_1-derived F_2 family.

[w] = Chi-square and Probability values calculated for each family fitting the expected 3 sensitive to 1 insensitive ratio.

[v] = Chi-square and Probability values calculated for each family fitting the expected 1 sensitive to 1 insensitive ratio.

: Families that are displaying the segregation ratio of a critical chromosome.
: Families that are displaying the 1:1 segregation ratio.