Chromosomal Location and Linkage Analysis of Loose Smut Resistance in Wheat

BY

RONALD E. KNOX

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CHROMOSOMAL LOCATION AND LINKAGE ANALYSIS OF LOOSE SMUT RESISTANCE IN WHEAT

BY

RONALD E. KNOX

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

ABSTRACT

The present knowledge of the genetics of loose smut resistance in wheat is neither substantial nor coherent enough to allow researchers to efficiently develop molecular markers linked to loose smut resistance genes. Knowledge of the chromosome location of loose smut resistance genes can improve the efficiency of analysis of genetic linkage to mapped molecular markers. The primary objective of this study was to determine the chromosome location of a loose smut resistance gene in common wheat. A second objective was to evaluate seed proteins and monoclonal antibodies to certain of those proteins as chromosome markers for their role in cytogenetic analysis of loose smut resistance. Linkage analysis between a loose smut resistance gene and genetic markers located within the same chromosome, and analysis of information about the inheritance of loose smut resistance were ancillary objectives. Segregating populations were generated by crossing loose smut susceptible cytogenetic stocks with sources of loose smut resistance, and the progeny were evaluated for seed protein and loose smut resistance traits. Seed protein and monoclonal antibody chromosome markers assisted in chromosome detection, and were found to be a reliable tool in cytogenetic analysis. The chromosome-marker-assisted cytogenetic analysis was used to determine the chromosomal location of a loose smut resistance gene in chromosome 6A. Evidence of an association between the chromosome 6A loose smut resistance gene and a restriction fragment length polymorphism was found. Evaluation of cytogenetic stocks for loose smut resistance placed the 6A loose smut resistance gene in the short arm of chromosome 6A. Limited results on the inheritance of loose smut resistance pointed to the occurrence of recessive and dominant genes, and cultivars possessing multiple genes for resistance. Loose smut resistance genes can be located to chromosomes by using molecular markers to assist in the cytogenetic

analysis, and the knowledge of the chromosome location of a loose smut resistance gene allows the efficient establishment of linkage with markers known to be in the same chromosome.

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FORWARD

This thesis starts with a general abstract, introduction and literature review, followed by the presentation of four chapters of experimental research, each representing a particular research theme. The thesis concludes in a general discussion and conclusion followed by the list of references cited throughout the thesis. The format of each chapter is as follows: the abstract, introduction, materials and methods, results, and discussion on the theme of the research within the chapter. The thesis is written in the style of the *Canadian Journal of Plant Science*.

Chapter 3 entitled 'The application of chromosome-specific monoclonal antibodies to wheat genetics' is published in *Genome*. Chapter 4 entitled 'A monoclonal antibody chromosome marker analysis used to locate a loose smut resistance gene in wheat chromosome 6A' is submitted to *Theoretical and Applied Genetics*. Chapter 5 illustrates linkage analysis between a chromosome 6A loose smut resistance gene and other traits in the same chromosome. Chapter 6 brings together a collection of data generated toward the objective of locating a loose smut resistance gene to a chromosome. Although providing no conclusive results the data in Chapter 6 is of sufficient impact to affect the direction of future research and therefore this information is reported within the main body of the thesis. The list of references contains all references used throughout the thesis. An appendix presents the lists of lines and results of their inoculation with a series of loose smut races and which were used as background information for the development of the thesis.

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CHAPTER 1

INTRODUCTION

Loose smut is a disease of historical economic importance (Fischer and Holton 1957), and the disease continues to inflict losses and add to production costs by forcing farmers to chemically treat seed of susceptible cultivars. Input costs to farmers can be reduced by providing loose smut resistant varieties. A thorough understanding of the genetics of loose smut resistance provides more efficient means of maintaining resistance during selection, and thus of reducing the cost of breeding for resistance to loose smut. The development of genetic markers linked to loose smut resistance genes provides the potential of further reducing this cost.

Knowledge of the significance of the host crop is important to put into perspective the impact of a disease such as loose smut. Wheat is a widely adapted crop, and is grown in most parts of the world at various altitudes and at various times throughout the year. Because of its wide adaptation, wheat covers more hectares of land, and total production is greater, than any other crop in the world (Briggle and Curtis 1987). A susceptible variety of wheat grown on the Canadian prairies, without any means of control of loose smut, will suffer an annual average yield loss of eight percent (Nielsen unpublished). Such a potential loss on a world-wide basis is considerable.

In Canada, genetic resistance to loose smut in wheat is the preferred method of control, although through the 1980s, several susceptible wheat varieties were registered (ADF 1993). Genetic resistance provides a favourable alternative or supplement to chemical control, and effective resistance to a comprehensive collection of races from around the world does exist (Nielsen 1983). The greatest barrier to the use of loose smut resistance is the difficulty in evaluating breeding material for

this trait. Little useful information is available on the genetics of loose smut resistance in wheat, partly because few studies have been done, and partly because the information from the different studies cannot be interrelated due to inconsistent characterization of races. An understanding of the genetics of loose smut resistance will lead to more efficient breeding, by allowing the characterization of genes in terms of the number required for effective resistance, the breadth of resistance of each individual gene, and the expressivity and penetrance of individual genes. Breeding efficiency will also be gained by improved ability to determine population size, and better characterization of races. Determining the chromosome location assists in identifying genes, and is useful in the development of markers to loose smut resistance genes. The development of markers closely linked to loose smut resistance genes provides the potential of more efficient selection for resistance if the markers are more easily evaluated than loose smut resistance.

Many terms have been created to explain cytogenetic conditions, a few which can be confusing are defined here. The phrase chromosome location will be used to refer to the chromosome in which a gene resides, and will be used in a different sense from gene mapping. Gene mapping will be used to refer to the position of a gene relative to other genes or to the centromere. The term linkage will be used to indicate the degree of association of genes with each other. The traditional definition of testcross is a cross of a homozygous recessive parent onto an individual which is either homozygous dominant or heterozygous, the segregation of which is used to measure the condition of alleles at the loci in question. For the purpose of cytogenetic analysis the traditional definition is expanded to include the null allele, which results from the use of particular cytogenetic stocks, and which will be treated as a recessive allele (e.g. a nullisomic 1B stock will be considered to have the null allele for any gene in that chromosome).

Like breeding for loose smut resistance, genetic analysis of loose smut resistance is difficult because of a requirement for a large family size, imposed by the failure of the fungus to infect all plants with a susceptible genotype. This requirement for large families is compounded for routine cytogenetic analyses used to locate genes to chromosomes, because the most common procedures also require a large population size.

The two primary objectives at the beginning of this thesis project were to lay the groundwork for more efficient cytogenetic analysis using molecular chromosome markers in conjunction with segregating populations derived from cytogenetic stocks, and to identify the chromosome location of at least one loose smut resistance gene using the chromosome marker technique. If time permitted, a further objective was to establish a linkage between a loose smut resistance gene located to a chromosome and markers located to the same chromosome. The strategy used was to focus analysis on a few chromosomes for which good chromosome markers were available, and to cross several sources of loose smut resistance onto cytogenetic stocks for the chosen chromosomes. A series of races was used to inoculate segregating progeny to improve the chances of characterizing individual genes in lines which are multigenic for loose smut resistance. Because chromosome markers were used, small populations provided sufficient information to determine the association of loose smut resistance with a chromosome. Even though small populations were used, information on the number of genes segregating could still be established because a series of races was used to inoculate segregating progeny. A fourth objective of this project was to analyze the data from all populations to provide preliminary evidence of the nature and number of genes involved in loose smut resistance in the lines studied. Finally, many genetic stocks were inoculated to determine how they should be used in crossing, and loose smut resistant cytogenetic stocks were inoculated to try to obtain evidence of the chromosome location of the resistance.

CHAPTER 2

LITERATURE REVIEW

This literature review covers, in detail, aspects of the biology and genetics of wheat and of the pathogen causing loose smut, *Ustilago tritici* (Pers.) Rostrup f.sp. *tritici* (Boerema et al. 1992) (referred to henceforth as *Ustilago tritici* based on Nielsen (1985)). The information presented attempts to provide insight into the factors affecting the study of genetics of wheat resistance to *Ustilago tritici*, and provides background for the development of genetic markers in wheat, markers for both chromosomes and resistance genes. Such basic information is essential if mapping genes for resistance to this pathogen is to be successful. The disciplines from which information is drawn include the anatomy and morphology, genetics, and pathology of the *U.tritici*/wheat aegricorpus, and the cytogenetics, seed protein chemistry, and molecular biology of wheat. Review articles will be reported where possible for the reader who desires more background. Primary reports are normally referred to when the information has a potential impact on the course of research into the development of molecular markers to loose smut resistance genes.

Wheat

Genetics

Common or bread wheat (*Triticum aestivum*) is an allopolyploid (hexaploid) cereal grain of relatively recent evolutionary origin, and is a member of the tribe Triticeae of the family Gramineae (Feldman 1976; Kimber and Sears 1987). Such a complex genome confers both advantages and disadvantages on its study (Sorrells 1992). The significance of three genomes is that many genes occur in more than one

dose (Schmidt 1973; Hart 1987), providing the plant with a high degree of genetic plasticity, but making genetic analysis more difficult (e.g. the three seed colour genes (Schmidt 1973)). The three genomes, however, allow aneuploid manipulations that cannot be accomplished in a diploid (Allard 1960). Wheat lines with such cytogenetic aberrations are known and used in locating genes to chromosome arms, and in mapping genes relative to the centromere. Because wheat is of relatively recent evolutionary origin, diversity at the DNA level is expected to be relatively low, in fact little polymorphism is detected at this level (He et al. 1992; Joshi and Nguyen 1993).

To accomplish the objective of locating a loose smut resistance gene to a chromosome, techniques previously used for locating other traits to chromosomes can be adapted. These principles also apply to locating traits with potential as genetic markers to chromosomes. A discussion of the principles behind different methods used to locate genes to chromosomes, along with the advantages and disadvantages of each method, is left to the introduction to Chapter 4. Evaluation of substitution lines or alien addition lines carrying pairs of homoeologous chromosomes, monosomic analysis, telosomic analysis, in situ hybridization, and linkage to a trait previously located to a chromosome are all methods by which genes can be located to chromosomes.

Analysis of two series of substitution lines was used by Dhitaphichit and coworkers (1989) to locate genes for loose smut resistance. Nicholson and coworkers (1993) used the method to determine the chromosomes involved in *Septoria nodorum* resistance. This method, along with analysis of ditelosomic, nullisomic-tetrasomic, and alien addition lines, is the method of choice for locating potential genetic markers to chromosomes. Examples of potential markers located using these methods include: Random Amplified Polymorphic DNAs (RAPDs) (Devos and Gale 1992); Restriction Fragment Length Polymorphisms (RFLPs) (Chao et al. 1988, 1989; Sharp et al. 1988a, 1989; Gill et al. 1991; Harcourt and

Gale 1991; Heun et al. 1991; Liu and Tsunewaki 1991; Anderson et al. 1992; Devos et al. 1992b); isozymes (Hart et al. 1980; Diaz et al. 1986; Chenicek and Hart 1987; Koebner et al. 1988; Liu et al. 1990; Petchey et al. 1990; Thiele and Seidel 1990; Liu and Gale 1991; Friebe et al. 1992; Sun and Dvorak 1992; Thiele and Melz 1992); and seed proteins (Orth and Bushuk 1974; Aragoncillo et al. 1975; Bietz et al. 1975; Payne et al. 1980, 1981, 1982, 1984; Brown and Flavell 1981; Galili and Feldman 1983; Jackson et al. 1983; Fra-Mon et al. 1984; Lafiandra et al. 1984; Burnouf and Bietz 1985; Sanchez-Monge et al. 1986; Liu et al. 1989; Garcia et al. 1990; Graybosch and Morris 1990; Gupta and Shepherd 1990; Masojc et al. 1993a).

Monosomic analysis is commonly applied to locating genes for resistance to pests such as the root knot nematode (Kaloshian et al. 1991), leaf rust (Dyck and Kerber 1971, 1977 and 1981; Dyck et al. 1987), stem rust (McIntosh 1972 and 1978; Kerber and Dyck 1973; Sanghi and Baker 1974; Sawhney et al. 1981), and yellow rust (Kema and Lange 1992; Bariana and McIntosh 1993; Jiazhi et al. 1993). In addition, monosomic analysis is used to identify the chromosome location of genes of traits which have potential as genetic markers. For example, seed proteins (Hueros et al. 1988; Sanz et al. 1988) as well as visual traits such as kernel colour and glume shape (Bares and Kosner 1975) are evaluated for chromosome location by monosomic analysis.

The identification of the chromosomal location of eyespot resistance provides an example whereby the association of a previously located trait with that of a trait to be located provides evidence of the chromosomal location of the trait to be located (Mena et al. 1992). The evaluation of linkage of traits previously located to chromosomes with traits such as RFLPs which are being newly mapped allows the identification of the chromosome location of the newly mapped traits (Liu and Tsunewaki 1991). This strategy also allows mapping of the traits previously located to chromosomes for which no linkage data was

available by taking advantage of newly discovered associations of previously located traits with common, for example, RFLP loci.

Ditelosomic or alien substituted chromosome lines are used to locate genes by segregation analysis whereby a correlation is established between, for example, loose smut resistance and a particular chromosome observed microscopically in the segregating progeny. This method, however, depends on much microscopy and is often restricted to verifying gene location done by other means or for locating a gene to a chromosome arm. This method is used to map genes for resistance to leaf rust (Rowland and Kerber 1974; Kerber 1988), stem rust (McIntosh 1972, 1978; Jones et al. 1991) and yellow rust (Kema and Lange 1992; Bariana and McIntosh 1993).

Anatomy

An understanding of the morphology and anatomy of the wheat plant is important to understanding the disease cycle of loose smut and the response of particular forms of resistance at the cellular level. Such information is the basis of inoculation and assessment strategies used for selecting for resistance to loose smut. As a member of the Gramineae, wheat displays the common grassy traits of this family. Cultivated wheat is a self pollinated annual (Allard 1960). After pollination and fertilization of the ovary, the embryo develops sometimes as far as the beginning of the fourth leaf (Briggle 1967). Upon germination, the embryo expands to expose the coleorhiza and the coleoptile, and the second internode, the internode just above the point of attachment of the coleoptile, elongates within the coleoptile. The apical meristem continues to divide, laying down further leaf initials and tiller buds. After the plant enters the reproductive phase, the spike develops and is pushed upward with internode elongation. Anthesis normally takes place after the spike has emerged from the boot (the last leaf sheath), with the lemma being forced away from the palea by the

lodicules. Cross pollination, or the entry of loose smut spores into the florets, can occur at this time (Agarwal and Gupta 1989).

The fertilized ovary develops into the seed while the pericarp and integuments form the seed coat of the fruit, called a caryopsis (Esau 1977). The embryo develops fully, prior to the full development of the endosperm (Briggle 1967). From the standpoint of genetics of resistance to loose smut, it is important to consider that the integuments and pericarp develop from maternal tissue, and reflect the genetics of the female parent. Another consideration regarding genetic analysis is that the endosperm is 3n with 2 doses of each chromosome being maternal and one paternal. The dosage effect of chromosomes, imparted on seed proteins, can be taken advantage of in certain types of genetic studies (Uhlen and Ringlund 1987; Hueros et al. 1988), and will be discussed later as it relates to the use of monoclonal antibodies to seed proteins as chromosome markers.

Loose Smut

Disease Cycle

The infection and invasion processes of *Ustilago tritici* in wheat and *Ustilago nuda* in barley are similar, and have been reviewed thoroughly by Shinohara (1976). In fact, there has been much debate over the years as to whether or not *Ustilago tritici* and *Ustilago nuda* should be classed as a single species (Nielsen 1972). Major studies on the anatomy of infection are presented by Batts (1955a and b), Gaskin (1958) and, in barley only, Gabor (1985).

The loose smut fungus infects wheat at the time of flowering, when a spore (or spores) of *U. tritici* enters a floret and germinates to form haploid hyphae which fuse to form dikaryotic hyphae. The dikaryotic hyphae form an appressorium and infection peg on the ovary wall, whereby the developing ovary is penetrated (Batts 1955b; Gabor 1985). The fungus grows through the parenchyma of the pericarp to the integuments

(testa) and nucellus; the fungus permeates these tissues but, for the most part, does not penetrate the endosperm. The fungus grows toward the base of the ovary, where it enters the embryo (taking about five days), before passing through the scutellum and establishing in the apical meristem approximately 20 days after inoculation (Batts 1955a and b; Gabor 1985). During maturation of the seed, the fungus goes dormant and is revitalized upon germination of the seed.

During seedling development the fungus grows with the meristem (Mantle 1961c). At the time when the head is developing, the fungal mycelium forms sporogenous hyphae, from which spores form by segmentation. Nuclei fuse within the spores to form diploid nuclei. When the infected spike emerges, most of the tissue has been converted to a mass of dark brown spores. The spores are disseminated by wind and rain, and are particularly suited to impaction on narrow surfaces such as glumes (Gregory 1952). Those spores which enter florets at anthesis start the disease cycle over again.

Environmental Effects on Loose Smut Expression

Obtaining consistent and optimum expression of disease is important for accuracy in genetic analysis. The level of loose smut expression of a particular wheat line is influenced by genotype of the host, environment (Agarwal and Gupta 1989, Dean 1969, Kavanagh 1961, Tapke 1948), and by the genotype of the pathogen. The penetrance of a trait is also often affected by the genetic background in which a gene resides (Allard 1960). Minor genes imparting a low level of resistance (Mackenzie 1991) may contribute to the penetrance of major genes. Sample size can be adjusted to account for the level of random variation for a particular expected difference between resistant and susceptible classes, such that a real difference can be detected. Replication is required to determine the extent of variation beyond random variation. A further discussion of the statistical approach to analyzing loose smut data is presented in

Chapter 4.

There is evidence that the environmental conditions at the time of inoculation can affect the amount of infection. Whereas spike wetness plays only a minor role, humidity has an influence on the level of infection (Tapke 1931; Dean 1965; Loria et al. 1982). The importance of humidity in artificial inoculation (presumably by water suspension methods) was debated by Nielsen (1987a). Tapke (1948) indicated, however, the significance of humidity on natural infection, and that spores on plants in drier air germinate more slowly. Tapke (1948) also pointed out the importance of duration of flowering on the level of infection and that plants under water stress proceed through flowering more quickly than those with ample water. In studies of barley, Malik and Batts (1960) came up with similar results, showing that pollination is delayed under moist cloudy conditions, and that florets remain open longer than in dry warm weather. Perhaps such a delay in ovary development is important in providing adequate time for infection. Certainly, prolonging floret opening provides more time for spores to enter.

Water stress, it should be noted, is not only a function of humidity, temperature, and air speed, but also of the water-supplying capability of the soil. Care should be taken during the watering of plants grown under artificial growth conditions and to be inoculated with loose smut, so that water stress does not become a variable associated with the level of infection. Gregory (1952) showed that smut spores can be carried in raindrops. Malik and Batts (1960) did three tests of spore transmission. In one treatment, water droplets carried spores and were allowed to impact on florets. In a second treatment, dry spores were blown onto spikes, followed by the application of water droplets; thirdly, dry spores were applied by brush or wind, without water. Loose smut infection was greater in the former two treatments in which water droplets were involved. Inoculation techniques carried out under high

humidity and simulated light showers may be the best for promoting infection and thereby ensuring detection of susceptibility and resistance.

The optimum temperature for infection by the loose smut pathogen is between 22 and 25°C (Tropova 1938; Agarwal et al. 1984). Temperature also affects the expression of loose smut symptoms, particularly in certain combinations of host and pathogen genotypes (Kavanagh 1959, 1961a and c; Anon. 1963; Dean 1965 and 1969). The optimum temperature for loose smut expression is around 23°C, with lower temperatures having less of an effect on expression than higher temperatures. Tapke (1948) discussed conflicting reports of the effect of the temperature to which infected seedlings are exposed on the level of loose smut at maturity. Kavanagh (1961c) studied the effect of temperature on the level of smut expression, and found that high temperature in some genotypes reduces sporulation. The effect of temperature is most critical during internode elongation, with sporulation being reduced after longer exposure to high temperature. Temperature is also associated with mycelial production of *U. nuda* in culture (Kavanagh 1961c). As the temperature increased, the growth in culture declined. Dean (1969) provided an example of low temperature reducing sporulation. Although mycelium is not eliminated from heads of some genotypes when sporulation is absent, the heads are abnormal.

Another factor which affects the level of loose smut incidence is depth of seeding, with shallow seeding being more favourable to loose smut infection (Agarwal and Gupta 1989). The effect of seeding depth may, in turn, be related to seed size, because small seeds are more highly infected than other seed sizes under natural infection conditions (Taylor 1927). Low soil fertility favours loose smut expression (Fromme 1920). No evidence of the effect of light quality, or intensity on loose smut infection could be found in the literature. In contrast to the previously mentioned studies, where environment played a role in the

level of loose smut expression, Batts and Jeater (1958a) found no association between environmental conditions during the growing period of susceptible plants derived from inoculated seed, and the incidence of loose smut. The discrepancies between different reports on the effect of environment on the expression of loose smut may be caused by the differential response of the genotypes (Kavanagh 1961c) used by the different workers.

Inoculum and Inoculations

Method and timing of inoculation are further variables in the evaluation of the level of resistance of a particular genotype. Therefore, it is important to understand the nature of inoculum, and timing and methods of inoculation. These were thoroughly reviewed by Nielsen (1987a).

Sexual recombination in *Ustilago tritici* occurs just prior to infection; therefore, variability in the level of infection can occur because of the inoculum source used. Races of *U. tritici* that are homozygous for virulence genes are necessary for genetic studies on resistance. A race homozygous for virulence loci may be obtained by a series of passages of the isolate through a differential host. However, the approach to homozygosity will be delayed by linkage of chromatin to the mating type locus, and because the probability of gamete fusion is represented by sampling without replacement (once two of the four gametes fuse there is no choice but for the remaining two gametes to fuse) (Kirby 1984).

The inoculation procedure is dependent on knowledge of the disease cycle. Knowing that spores pass from an infected plant to healthy florets, where they germinate to infect the ovary and developing embryo around the time of anthesis, is critical to the success of artificial inoculation. Inoculation methods must attempt to simulate this natural process. Many different methods of inoculation have been tried over the years (Nielsen 1987a); examples include the use of: inoculum suspended

in talc and placed in a bag over the wheat head (Loria et al. 1982), spores brushed onto the stigmas (Kilduff 1933), dusting spores onto florets with the upper portion of the lemma and palea removed (Tingey and Tolman 1934), high pressure jet inoculation (Anderson 1961), partial vacuum with spores suspended in water (Heyne and Hansing 1955), and the Poehlmann method (Poehlmann 1945), involving a hypodermic needle and rubber bulb or syringe to inject a spore-water suspension into florets. The Poehlmann method has a number of advantages for genetic studies, such as avoiding contamination, which is more difficult with the partial vacuum and high pressure jet inoculation methods. Furthermore, the injection of spores with a syringe is simpler and easier than the bag or brush methods. Finally, infection levels tend to be higher with the Poehlmann method compared to the partial vacuum method (Malik and Batts 1960).

Timing and Method

Timing of inoculation is critical because a rather narrow infection window is created by the requirement that spores must enter florets around the time of flowering so that infection can occur. Several studies were done to determine the optimum stage of inoculation. Studies by Piekenbrock (1927) and Malik and Batts (1960) favoured mid-anthesis or later for inoculation, whereas Tingey and Tolman (1934) and Gothwal (1972) suggested the optimum inoculation period to be just prior to anthesis. However, the majority of studies point to mid-anthesis as the optimum time for inoculation (Tapke 1929; Oort 1939; Ohms and Bever 1956; Bever and Ohms 1966; Jones and Dhitaphichit 1991). The range in time around mid anthesis for which optimum infection is maintained varies across the different studies, as well varietal differences were noted in some studies (Tapke 1929; Gothwal 1972). Nielsen (1987a) considered mid-anthesis (growth stages 60-65 on the Zadocks scale) to be a reasonable compromise as the optimum time for inoculation.

Malik and Batts (1960) discovered that infection was greatest in seed from mid-head, probably indicating that there is an optimum stage of ovary development for infection, and that florets in a head of wheat are at various stages of development at any one time, with the lower and upper florets on the head usually being less mature than the middle. The concentration of inoculum also influences the level of infection (Oort 1939; Chatrath and Bahl 1966; Jones and Dhitaphichit 1991). One gram of spores per litre of water is recommended (Nielsen 1987a). When the Poehlmann method is used, the inoculum is injected into individual florets on an entire spike, and at maturity the seed is harvested for planting in rows in the greenhouse for evaluation.

Host Range

Ustilago tritici, in addition to being a pathogen of wheat, attacks a number of relatives of wheat, including *Aegilops* spp., *Agropyron* spp., *Elymus* spp., *Hordeum* spp., *Secale cereale* and *Triticale hexaploide*, and other genera of the tribe Triticeae (Nielsen 1973, 1978a, b, 1985, 1987b). The host range of *U. tritici* has implications on the use of alien cytogenetic stocks in locating, within chromosomes, genes for resistance to loose smut, and on the potential of obtaining new sources of resistance for wheat from alien sources. It is important that cytogenetic stocks be tested for loose smut reaction; relying on the infection information of the progenitor stock can lead to wrong conclusions if an alien chromosome is carrying a loose smut resistance gene.

Genetics of *Ustilago tritici*

Many races of *Ustilago tritici* exist and the interaction with wheat tends to follow a gene-for-gene relationship (Holton 1953; Oort 1963; Nielsen 1987a). Because of a gene-for-gene interaction, insight into the genetics of the pathogen also provides insight into the genetics of

resistance in the host. Furthermore, as Nielsen (1987a) pointed out, successful breeding for resistance to loose smut depends on a knowledge of the biology of the pathogen, particularly knowledge of the genetics of virulence.

As mentioned in the discussion on inoculum, sexual recombination is considered to take place during spore germination, with karyogamy or diploidization having taken place previously during the process of spore maturation (Shinohara 1976). Meiosis results in haploid nuclei which are distributed within a promycelium during spore germination. Haploid hypha formation, hyphal fusion, and dikaryotization were described in detail by Popp (1955).

The fact that recombination occurs just prior to infection must be considered in the race concept of this pathogen (Christensen and Rodenhiser 1940; Fischer and Holton 1957). The major implication is that spores are not clonally propagated as are urediospores in the rusts. In fact, when considering virulence loci, a sorus collected from the field represents a heterogeneous, heterozygous population if the infection originated from the fusion of genetically different haploid hyphae. This heterozygosity will be expressed as variation in subsequent infections of cultivars with particular resistance genes (differentials), and this variation may be misconstrued as instability in the pathogen. Cherewick (1958) found variability remained in isolates even after 5-10 cycles of inbreeding.

Nielsen (1987a) considered the event of spores of differing races landing in the same floret to be rare. An extrapolation of this consideration would mean races will be highly inbred. One would expect this situation only to apply when incidence of loose smut is low. In one study where the incidence of two races of *Ustilago nuda* was high, over seven percent of infections were by hybrids (Thomas 1984). This relatively high incidence of hybridization was found by Thomas (1978) to be as much as 43% of the expected frequency, based on random mating of

gametes produced by *U. nuda* in barley.

Because of the great deal of technical expertise and effort required in crossing races and characterizing the progeny for virulence, reports on genetics of virulence of *Ustilago* are few. Nielsen (1988) reviewed the subject of the genetics of *Ustilago nuda* and *U. tritici*, and has been the sole investigator in the genetic analysis of virulence in *U. tritici* (Nielsen 1977, 1982). In Nielsen's work, four virulence genes were identified. A summary of virulence for each race and the infection of the differentials is given in Table 2.1A (Nielsen 1977, 1982, 1987a).

Nielsen's work demonstrated that races possess multiple genes for virulence and differentials possess multiple genes for resistance. Nielsen (1977) first crossed races T1 and T2. He found that no segregation for virulence in the progeny occurred on TD2 (where TD represents *U. tritici* differential (Nielsen 1987)) and TD3, and concluded the virulence was the same in both, or that T2 has two closely linked virulence genes. Similarly, no segregation occurs on TD4 and TD5, and here, too, the conclusion is that virulence is the same in both, or that T1 has two closely linked virulence genes. Segregation does occur between the TD2/TD3 group and the TD4/TD5 group. Such segregation is expected from two races which give the pattern of resistance and susceptibility against two sources of resistance that these lines do (Table 2.1B group IV). Any other combination of fewer than two resistance genes produces a pattern of three susceptible types (Table 2.1B group I-II) instead of two susceptible and two resistant lines. Nielsen (1977) pointed out that there are races which have additional but differing virulence than T1 and T2 because there are races which give the pattern of group IV of Table 2.1B on differentials TD2 and TD3 or TD4 and TD5. The complement of the virulence interaction is that TD2 and TD3, in addition to having a gene for resistance in common, must have a gene difference to produce the susceptible/ resistant combination seen in Table 2.1C. Likewise, TD4 and TD5 must

Table 2.1A. Summary of races, virulence genes, and differentials used in genetic studies by Nielsen (1977, 1982) and resistance genes assigned by McIntosh (1988) based on the gene-for-gene principle, along with race reaction as susceptible (S) or resistant (R)

Virulence Genes			Race			
			T5 utv4	T1 utv2	T2 utv1 utv3	
Differential		Resistance Gene				Number of Races to Which Susceptible
TD2	(Renfrew)	Ut1	R	R	S	5
TD3	(FlorenceXAurore)	Ut1	R	R	S	14
TD10	(Red Bobs)	Ut1	R	R	S	15
TD4	(Kota)	Ut2	R	S	R	17
TD5	(Little club)	Ut2	R	S	R	23
TD8	(Carma)	Ut3	R	R	S	16
TD12	(ThatcherXRegent)	Ut4	S	R	R	5

Table 2.1B. Phenotypes when combinations of resistance alleles at one locus and genes at two loci are compared with virulence alleles at one locus and genes at two loci where (-) = susceptible or avirulent condition at other loci

	I			II	
	Virulence			Virulence	
Resistance	-	Utv1	Resistance	Utv1	Utv2
Ut1	R	S	Ut1	S	R
-	S	S	-	S	S

	III			IV	
Ut1	R	S	Ut1	S	R
Ut2	R	R	Ut2	R	S

Table 2.1C. Evidence for a gene difference for resistance between TD2 and TD3, and between TD4 and TD5

Differential	Race		Differential	Race	
	T9	T31		T6	T19
TD2	R	S	TD4	S	R
TD3	S	R	TD5	R	S

Table 2.1D. Evidence that TD2 has an additional gene to TD10 and likewise TD3 has an additional gene to TD10

Differential	Race		Differential	Race	
	T9	-		T31	-
TD2	R	-	TD3	R	-
TD10	S	-	TD10	S	-

have a gene difference. TD4 has an additional gene over TD5, and TD2 has an additional gene over TD3, because TD4 and TD2 are resistant to more races than TD5 and TD3 respectively (Table 2.1A), based on Person's (1959) theoretical analysis of resistance and virulence.

Nielsen (1982) reported virulence on TD10 is the same as on TD2 and TD3, indicating a gene in common among the three. The pair TD2 and TD10, as well as the pair TD10 and TD3, also differ in a resistance gene (Table 2.1D), and TD2 is likely to have one gene more than TD10 and TD3 because TD2 is resistant to more races than TD10 or TD3 (Table 2.1A). Virulence in races T5 and T2 on TD8 and TD12 segregate from each other as expected from the race pattern shown in Table 2.1A. Virulence on TD8 also segregates from TD2, 3 and 10, indicating a virulence gene difference in race T2 compared to race T5, and thus a different resistance gene in TD8. The genes responsible for virulence on TD12 and TD8 are different from the gene in T1, because neither T5 nor T2 is virulent on TD4 or TD5. Therefore, based on the gene-for-gene concept, TD12 and TD4 differ in genes for resistance. TD12 lost a gene during improvement of this differential for agronomic traits by Nielsen and Dyck (1988), demonstrating that TD12 has more than one gene. Based on Person's (1959) method of analysis, TD12 differs by two genes over TD4 (Table 2.1A).

Loose Smut Races and the Wheat Differential Set

Forty one races of loose smut have been identified by Nielsen (1987a), thereby implying several genes for resistance. Given that 35 races on hexaploid wheat exist, and assuming a gene-for-gene relationship is in effect, at least six resistance genes are required for differentiation (2 to the power 6 = 64). Nielsen's differentials contain 15 hexaploid wheat lines, although this number could be reduced to 10 with some deletions and additions (Table 2.2). Some races have been pooled in Table 2.2 because the incompatibility reaction is not

Table 2.2. A condensed differential set of *Triticum aestivum* (data collected by J. Nielsen)

	Race (T-)																												
Differential	27	16	11	34	35	37	28	5	1	7	6	23	41	18	21	17	38	8	31	20	9	2	12	15	10	22	13	19	39
Prelude/Diamant	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Chinook			S	S	S	S		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
TD5A			S	S	S		S		S	S		S		S	S		S	S		S	S		S	S	S	S	S	S	S
TD4	S								S	S	S			S				S		S	S		S	S	S	S	S		S
TD8A		S			S							S	S	S	S			S				S			S	S	S	S	S
TD10						S							S			S	S		S	S	S	S		S		S	S	S	S
Moscovka											S				S	S	S		S	S		S		S		S	S		
Sinton								S											S			S	S		S	S		S	S
TD9												S		S	S			S							S	S	S	S	S
TD15							S			S					S			S			S						S		
TD2																S			S			S		S					

considered. Given even 10 differentials, the number of races is either severely under-represented (2 to the power 10 = 1024), or some of the differentials possess more than one gene for resistance, and existing races which could be further subdivided are not, or a combination of the two problems exists.

In the same manner that differential wheat lines can identify races, races can be selected which can differentiate the wheat lines. In the same way that differentials with more than one gene for resistance will underestimate the number of virulence combinations and thus races (Person 1959), races not possessing full virulence minus one virulence gene will underestimate the number of resistance genes. The objective in choosing races for genetic work on resistance is to use the races to identify the segregation of different genes for resistance. Ideally one would like to have a set of races, with each race containing all virulence genes except one, and each race deficient in a different gene. However, the next best choice is those races with as great a virulence as possible, which will differentiate the differential cultivars. Eight differential races selected from Nielsen's (1987a) collection are listed in Table 2.3. Genetic analysis to locate loose smut resistance genes to chromosomes will be made more difficult by the segregation of multiple genes, requiring the use of larger populations. Resistance to loose smut in a cultivar may be conferred by a single gene to which no race with virulence is yet known, or resistance may be conferred by a combination of resistance genes to which no corresponding combination of virulence genes is yet known in the pathogen. Because of the effort involved in locating genes to chromosomes, single gene lines would be ideal. Barring this ideal, the next best material to use for initial studies would consist of lines about which something of the inheritance of resistance is known, or lines with a pedigree and to which the known races have been tested. Given that the latter is represented by the differentials, these are the first choice for genetic studies.

Table 2.3. Races of Ustilago tritici which differentiate the *Triticum aestivum* differentials (from Nielsen 1987a)

Race	Differential (TD-)														
(T-)	2	3	4	5	6	7	8	9	10	12	14	15	16	17	18
2	S	S			S	S	S		S						
6			S								S				
8			S	S		S	S	S				S			
10			S	S		S	S	S		S					S
15	S	S	S	S		S			S				S		
19		S		S	S	S	S	S	S	S			S	S	
31	S					S			S	S					
39		S	S	S	S	S	S	S	S	S			S	S	S

Many sources of resistance to all known races have been described by Nielsen (1983), indicating that other resistance genes may still be available for identification; however, combinations of existing resistance genes, to which the corresponding virulence combinations have not yet been found, cannot be ruled out.

Anatomy of Resistance

From the developmental morphology and anatomy of the wheat plant and the disease cycle of loose smut, five stages at which resistance can be expressed have been identified (Gaskin and Schafer 1962; Loria et al. 1982). Loria and coworkers (1982) talked about field resistance in which floret accessibility is considered the important feature. Any restriction to the entry of loose smut spores constitutes a form of resistance by the escape of infection, and has been termed morphological resistance by Nielsen (personal communication), and will not be considered further in this thesis. Mantle (1961b), on the other hand, talked about resistance at the tissue level, through the use of light microscopy. He described four tissues where mycelial proliferation is stopped. These tissues are: ovary, scutellum, crown, and apical growing point. Three of these locations were examined by Gabor and Thomas (1987) in barley, namely: prevention of ovary penetration, embryo resistance, and resistance in the seedling. Mantle (1961c) further observed that the fungus in susceptible cultivars maintains itself very near the apical growing point, dispelling the hypothesis that the progress of the mycelium can fall behind the development of the growing point and that the mycelium catches up to the growing point at some later stage.

Gaskin and Schafer (1962) summarized the types of histological reactions which can occur. Resistance to penetration is the second site of defence by the host, after exclusion of spores from the floret, but is the first site for physiological resistance. An important

consideration in the study of genetics of loose smut resistance is that the outer layers of tissue of the ovary are maternal, and represent the genotype of the female. In such an instance, a recessive gene for loose smut resistance would appear dominant upon inoculation of the F_1 seed. Schaller (1949) believed a maternal tissue effect occurs for the barley cultivar Trebi, which is resistant to *U. nuda*. Necrosis of invaded cells occurs during the resistant reaction in Trebi (Shinohara 1976). Batts (1958b), Gaskin and Schafer (1962) and Nielsen and Dyck (1988) observed a maternal tissue effect in wheat. It is important to consider that even susceptible genotypes become resistant to infection as the ovary matures. The effect of ovary maturity was discussed by Batts (1955b), and is indicated in the section on Timing and Method of Inoculation in this review.

Resistance to the fungus that succeeds in penetrating the ovary can occur at two levels in relation to the embryo: exclusion from the embryo (Gaskin and Schafer 1962; Popp 1959), and invasion of the embryo but exclusion from the plumular bud (Ohms and Bever 1955; Batts and Jeater 1958b; Popp 1959). These two forms of resistance were not discriminated by Mantle (1961a) or Popp (1951). A fifth location of resistance occurs within the seedling, in which case the mycelium does not keep pace with the apical meristem (Gaskin and Schafer 1962). Even if the fungus maintains itself within the meristem, resistance may be manifested by the inhibition of growth and further development of the seedling, referred to as the incompatibility reaction (Oort 1944; Mantle 1961b). If the fungus becomes established in the growing point, it may be excluded from the inflorescence during the rapid meristematic growth of the seedling (Gaskin and Schafer 1962). Should the fungus succeed in invading the tissue of the immature inflorescence, it will be transported upwards with the inflorescence as the stem elongates.

An attempt to speed up the process of evaluating loose smut resistance by evaluating embryos was demonstrated by Popp (1951 and 1959), Batts

and Jeater (1958b), and Gaskin and Schafer (1962). Inoculating seedlings was demonstrated by Kavanagh (1959 and 1961b) and Jones and Dhitaphichit (1991) as a means of reducing the time from inoculation to infection. Such methods, however, are unsatisfactory because only a few forms of resistance might meet the selection criteria. In the case of embryo analysis, only resistance manifested in the maternal tissue up to the scutellum, along the infection path, would be selected for; other forms of resistance would be discarded. This method does not avoid the difficult and time-consuming inoculation procedure, and, in fact, more inoculations would have to be done because certain forms of resistance would be discarded. More information is needed to conclude whether or not seedling inoculation would circumvent certain types of resistance.

Genetic Studies of Resistance

Several studies of inheritance of resistance to loose smut have been done in wheat. Studies determining numbers of genes and allele interaction are summarized in Table 2.4. Many workers agree that it is not easy to determine the number of genes involved in resistance to loose smut, although some workers do indicate the number of resistance genes. In large part, the difficulties arise from not being able to classify segregating progeny clearly as susceptible or as resistant. Compared to determining the number of segregating genes, allelic relationships are perhaps not so difficult to establish, based on the inoculation of F_1 (crossed seed) or segregation in the F_2. Of course, the particular race or races used in a study will influence the outcome, because only those genes in wheat which are interacting with the avirulence genes of the pathogen will be observed. Metzger et al. (1979) demonstrated the use of multiple races for evaluating the genetic nature of common bunt resistance. Such a procedure can be easily adapted to the evaluation of loose smut resistance by inoculating different heads of the same plant with different races. More

Table 2.4. A summary of genetic studies of resistance to loose smut of wheat

Cross	Dominance of Resistance	Number of Genes Segregating	Reference
Grune Dame* X Rumkers Dickkopf and Rimpaus red schlanstedt	Recessive		Piekenbrock 1927
Grune Dame* X Roter Schlandstedter	Recessive		Grevel 1930
Kota X Red Bobs* Kota X Garnet*			Kilduff 1933
San Martin X 38 MA*	Recessive	3	Rudorf and Rosenstiel 1934
Hope* X Federation Hope* X Dicklow No. 3 Preston* X 01-24	Incomplete Dominance	3	Tingey and Tolman 1934
Pansar III* X -	Dominant		Larose and Vandervalle 1937
Federation/Khapli* X Mentana	Dominant		Milan 1938
-	Dominant		Milan 1939
Vilmorin 27 X Jubile*	Dominant		Larose and Vandervalle 1937
Thatcher* X Redman		1	Campbell 1948
Dundee 48* X Ranee White Federation* X Ranee	Dominant Dominant	1 1	Pugsley 1953
Kawvale* X Clarkan	Dominant	2	Heyne and Hansing 1955

(continued on next page)

Table 2.4. continued

Todd* X Knox or	Partly	2	Richards 1961
Kawvale* X Clarkan	recessive	2	
Richelle* X ↓	↓	2	
Hope-Hussar* X Knox	Recessive	1	Gaskin and Schafer 1962
PI191533* X Knox	|	1	
Kawvale* X Knox	|	2	
Tremexino* X Knox	|	2	
Rieti* X Knox	↓	2	
NP824* X Rio Negro	Dominant	1	Mathur and Kohli 1963
NP775 X NP798*	Dominant	2	Agrawal et al. 1963
Cappelle* X Hybrid 46	Dominant	1	Ribeiro 1963
Picardie* X and	|	1	
Kota* X Intercrosses	↓	1	
NP790* X NP775	Dominant	1	Agrawal and Jain 1965
Bexencukskaya 98*	Additive	3	Shestakova and Vjushkov 1974
Saratovskaya 36*			
Saratovskaya 29*			
Kenya 340Y4A1 X IAS52	Dominant	2	Medeiros 1976

* Indicates resistant parent.
- Indicates information was not available.

information about the segregation of resistance genes can be obtained by multiple race inoculations.

Because there is no standard race classification for loose smut, it is difficult to compare the commonality of resistance genes in published studies. However, data for dominant and recessive modes of allelic interaction show that recessive alleles occur frequently. Workers report on both dominant (Tingey and Tolman 1934; Milan 1938, 1939; Pugsley 1953; Richards 1961; Mathur and Kohli 1963; Ribeiro 1963; Agrawal and Jain 1965; Nielsen and Dyck 1988) and recessive alleles (Piekenbrock 1927; Gaskin and Schafer 1962; Agrawal and Jain 1965; Nielsen and Dyck 1988), with some expressing complete resistance (immunity) (Grevel 1930; Rudorf and Rosenstiel 1934). Others have demonstrated incomplete dominance (Tingey and Tolman 1934; Richards 1961), while Campbell (1948) attempted to study inheritance of resistance of parents in which the susceptible parent had some level of resistance. The study of moderately expressive genes allows for the study of gene interaction with respect to a given race. Shestakova and Vjushkov (1974) reported on additive gene action (although the source does not indicate whether or not such action was inter- or intragenic or both), while Kilduff (1933) reviewed reports describing genes having cumulative effects, a condition also mentioned by Tingey and Tolman (1934). Heyne and Hansing (1955) talked about minor genes. The existence of minor genes and genes with cumulative effects indicates that combinations of genes can result in a greater level of resistance to a given race than a single moderately expressive gene alone. This information, if true, would validate the development of genetic markers to minor genes for the purpose of pyramiding the genes.

There are two reports of the inheritance of the incompatibility reaction. Ribeiro (1963) reported that incompatibility in Cappelle, Picardie, and Kota is inherited as a single dominant gene, and Medeiros (1976) indicated incompatibility in Kenya 340Y4A1 is conferred by a

single dominant gene.

Dhitaphichit and coworkers (1989) studied substitution lines of wheat. They demonstrated that resistance to race T6 may be influenced by genes in a number of chromosomes in the wheat cultivars Hope and Thatcher, but that the most effective resistance in Hope resides in chromosome 7A, and in Thatcher in chromosome 7B. The Hope and Thatcher genes are of interest because of their potential use as sources of resistance in breeding, and their potential durability. Hope resistance is considered to derive from Vernal emmer, whereas Thatcher resistance derives from Iumillo durum (Nielsen 1969).

Ribeiro (1963) recommended that genes for loose smut resistance in wheat be designated 'Ut' and McIntosh (1988) used these symbols to characterize putative genes, based on the resistance-virulence interaction worked out by Nielsen (1977 and 1982).

Genetic Markers

The advantages of knowledge of the genetics of resistance to loose smut to breeding for resistance have been pointed out in the introduction to this dissertation. The chromosomal location of a gene is one aspect of genetic knowledge which can assist in the determination of genetic markers located in the same chromosome. Improving the efficiency of cytological analysis by using biochemical chromosome markers can speed up the rate at which genes such as those controlling loose smut resistance can be located in chromosomes (Anderson et al. 1992). This section provides a discussion of genetic markers because of their role as chromosome markers in cytogenetic analysis, and because of their potential as markers to genes for loose smut resistance. A more detailed discussion of the use of chromosome markers for locating genes to chromosomes is left to Chapter 3.

The phenotype of any gene previously located to a chromosome can be used as a genetic marker for the chromosome in which it resides.

However, the most useful genetic markers are those which are easy to observe. Other than a few simple morphological traits, the easiest markers to observe are seed proteins visualized by electrophoresis. This is because seed proteins can be sampled by taking a portion of the endosperm, leaving the embryo end of the seed intact and viable (Bietz et al. 1975; du Cros and Hare 1985). In addition, the seeds do not have to be grown until the test results are known, saving the effort and space involved in planting the seed and growing the plants. The analysis of seed proteins with antibodies is especially useful. Antibodies exist which discriminate between proteins (Skerritt and Underwood 1986; Dawood et al. 1989; Donovan et al. 1989; Skerritt and Robson 1990; Zawistowski et al. 1992; Brett et al. 1993; Graybosch et al. 1993; Masojc et al. 1993b). The presence or absence of a protein indicates the presence or absence of a gene, and perhaps even allelic forms of the protein coded by a gene at a particular locus. Monoclonal antibodies have the added advantage over electrophoresis of discriminating among lines having different gene dosage. Isozymes, disease reaction, and DNA polymorphism can also be used as markers.

Seed Proteins

Because of the impact of seed proteins on quality, a great deal of research has been done on them. In fact, several reviews are available on the subject of seed proteins (Garcia-Olmedo et al. 1978 and 1982; Payne and Rhodes 1982; Kreis et al. 1985; Shewry and Miflin 1985; Payne 1987; de Man 1990; MacRitchie et al. 1990; Tatham et al. 1990).

Seed proteins are classified based on differential solubilities of the different protein components, and specific polypeptides are identified by electrophoretic procedures which separate the polypeptides based on size and charge. It is useful to study the chemistry of these proteins in a little more depth if they are to be used effectively as chromosome markers. De Man (1990) described the Osborne classification of wheat

seed proteins, whereby proteins are classified and named based on solubility. The Osborne system is as follows: Albumins are water soluble proteins; Globulins are neutral salt soluble proteins; Prolamins are aqueous-ethanol extractable; Glutelins are soluble in dilute acid or alkali. Shewry and Miflin (1985) considered an additional subclass of proteins the CM or chloroform/methanol soluble proteins, which, as the name implies, are soluble in chloroform-methanol. These proteins are a subclass within the prolamins. As well, Shewry and Miflin (1985) grouped the glutenins as prolamins because the glutenins are soluble in aqueous alcohol, with the distinction between the glutenins and other prolamins being that a reducing agent is required for solubility of the glutenins. Solubility of gliadins (a class of prolamins) is not dependent on the presence of a reducing agent. The glutenins contain cysteine which can participate in intermolecular disulphide bonds (Shewry et al. 1992). Gliadins, which possess sulphur containing amino acids (sulphur rich gliadins), form intramolecular disulphide bonds. The glutenins and gliadins combine to form gluten.

The important consideration for using seed proteins as molecular markers is how they can be separated and visualized. Normally, the glutenins are separated by SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis), which also allows an estimation of molecular weight of proteins (Bietz et al. 1975; Ng and Bushuk 1987). The remaining groups of proteins are normally separated using acid-PAGE in a one step system (Garcia-Olmedo et al. 1982). However, because of the large number of proteins present in seeds, various modifications have been applied both to extraction procedures and separating procedures, including two-dimensional methods. These will not be discussed further because this thesis emphasizes the rapid screening of lines based upon markers that can be identified by one dimensional electrophoresis. Resolution during the separation of gliadins is optimized when the gels are run at between 7 and 10°C (Lookhart et al.

1985). Firm gels are obtained by lowering the levels of catalyst (Khan et al. 1985), and when acrylamide concentration is in the range of 11 to 16% (Graybosch and Morris 1990).

The albumins and globulins usually coincide with many of the seed proteins which have metabolic and structural activity (Shewry and Miflin 1985; Gupta et al. 1991); whereas the gliadins and glutenins are associated with the starch matrix, and, as such, provide a role in nitrogen, carbon, and sulphur storage. Because of the diversity of albumins and globulins, two-dimensional separation often has been the only way to characterize the individual components (Aragoncillo et al. 1975; Fra-Mon et al. 1984; Sanchez-Monge et al. 1986; Forsyth and Koebner 1992). Therefore, more work needs to be done to improve resolution in a one-dimensional system.

Locating genes coding seed proteins to chromosomes is an important area of research. Reviews on this subject were presented by Garcia-Olmedo and coauthors (1978 and 1982). Gliadin loci are located in the group 1 and the group 6 chromosomes (Brown and Flavell 1981; Galili and Feldman 1983; Lafiandra et al. 1984; Payne et al. 1984; Graybosch and Morris 1990). Trans-regulation may occur, considering Howes et al. (1990) found suppression of band 45 in Prelude and desuppression of band 40 in Tetra Prelude; however, this could also be explained by post-translational processing. Brown and Flavell (1981) found that chromosome 2A affects the synthesis of gliadins genes in chromosome 6D.

The high molecular weight glutenins are located in the group 1 chromosomes (Orth and Bushuk 1974; Bietz et al. 1975; Payne et al. 1980, 1981, 1982), although Bietz et al. (1975) indicated such a locus also occurs in 4D. Low molecular weight glutenins are coded by group 1 chromosomes (Jackson et al. 1983; Burnouf and Bietz 1985; Gupta and Shepherd 1990); whereas globulins and albumins are located in a variety of chromosomes (Aragoncillo et al. 1975; Brown and Flavell 1981; Fra-Mon et al. 1984; Sanchez-Monge et al. 1986; Liu et al. 1989; Garcia et al.

Table 2.5. Chromosome and seed proteins associated with that chromosome. (Adapted from Garcia-Olmedo et al. (1978, 1982) with additions)

Chromosome	Protein (chromosome arm location)
1A	Gliadin (S), Glutenins (L), Purothionins (L)
1B	Gliadin (S), Glutenins (L), Purothionins (L), Globulin
1D	Gliadin (S), Glutenins (L), Purothionins (L)
2A	Gliadin, Alpha-amylase inhibitor
2B	Alpha-amylase inhibitor
2D	Gliadin, Alpha-amylase inhibitor
3A	Albumin (S)
3B	Albumin (S), Alpha-amylase inhibitor (S), Globulin
3D	Albumin (S), Alpha-amylase inhibitor (S), Globulin
4A	Albumin, Globulin
4B	CM, Albumin -HMW (L)
4D	CM, Albumin -HMW (L), Globulin
5A	Albumin -HMW (L), Purothionin
5B	Albumin (L), Purothionin, Globulin
5D	Albumin (L), Purothionin
6A	Gliadin (S), Albumin
6B	Gliadin (S), Globulin
6D	Gliadin (S), Alpha-amylase inhibitor (S), Globulin
7A	LMW gliadins (S), Albumin (L)
7B	CM, Albumin (L), Globulin
7D	CM, LMW gliadins (L), Albumin (L), Globulin

HMW = high molecular weight, LMW = low molecular weight, CM = chloroform-methanol, L = long arm, S = short arm.

1990; Forsyth and Koebner 1992; Masojc et al. 1993a). Each chromosome is known to be associated with at least one type of seed protein (Table 2.5). Therefore, it is possible to have a seed protein chromosome marker for each chromosome in wheat.

An important requirement of a genetic marker is that it demonstrate allelic variation. Although alleles of the HMW glutenins have been sorted out (Lawrence and Shepherd 1981; Payne et al. 1981; Payne and Lawrence 1983; Gupta and Shepherd 1988), much work remains to sort out the alleles of other types of seed proteins.

Monoclonal Antibodies

Monoclonal antibodies (MAbs) are antibodies derived from a clonal line of b-lymphocytes that have immortality as a result of being fused with an immortal myeloma to produce a hybridoma (Milstein 1980; Galfre and Milstein 1981). Antibodies produced by such cells are often highly specific. MAbs are created by purifying an antigen, such as a seed storage protein, which is injected into a mouse to stimulate an immune response. The b-cells are extracted from the spleen of the mouse, and cultured and fused with myeloma cells. The resulting clones continue to divide, and most continue to produce antibodies. These cells are then diluted out, and colonies derived from single cells are screened for specificity using, for example, an enzyme linked immunosorbant assay (ELISA). There are many general references such as Klein (1990) which provide the fundamentals of immunology. Monoclonal antibodies in particular are discussed by Milstein (1980) and Galfre and Milstein (1981). Immunology as it applies to certain aspects of cereal research is reviewed by Vaag and Munck (1987), and Skerritt and coauthors (1990). However, the experimental work reported in this dissertation is mainly directed at the exploitation of existing MAbs through their use as genetic markers, and further discussion will be focussed on monoclonal antibodies to seed proteins as chromosome and genetic markers.

Because advantages exist from using seed proteins as genetic markers, and because of the convenience and rapid testing through the use of monoclonal antibodies (Skerritt et al. 1990), applying monoclonal antibodies to seed proteins as markers takes advantage of the benefits of both (Konarev 1981). To date, MAbs as chromosome markers are limited to the detection of alien rye and *Agropyron* chromosome segments in wheat (Howes et al. 1989, 1993; Brett et al. 1990).

Antibodies have been produced to a number of seed proteins ranging from gliadins to alpha-amylase inhibitor proteins (Skerritt and Underwood 1986; Dawood et al. 1989; Donovan et al. 1989; Skerritt and Robson 1990; Zawistowski et al. 1992; Brett et al. 1993; Graybosch et al. 1993; Masojc et al. 1993b). The binding specificity of antibodies varies, but for antibodies to be used as chromosome markers the following qualities are useful: The antibody binds to most if not all strains of wheat possessing the chromosome; The antibody has a high affinity and specificity for the protein, with little cross reaction to proteins coded by other chromosomes; The level of binding is high, allowing quantification. One of the advantages of such an antibody over electrophoresis is that the protein content can be quantified (Iannetta et al. 1993), which allows the discrimination of gene dosage (Skerritt et al. 1987; Masojc et al. 1993b). Such discrimination allows the detection of heterozygosity, or the detection of a single dose of a chromosome. In the case of a complex electrophoretic gel pattern, or where a null allele exists at a locus, or where two cultivars in a cross share the same allelic form of a protein used as a marker, electrophoresis may not allow discrimination between a heterozygote and a homozygote.

Skerritt and Robson (1990) pointed out that antibodies made to glutenins often cross-react with glutenins coded by loci in other chromosomes. MAbs raised against total gliadin extracts range in specificities to gliadins, with some even binding to glutenins (Mills et

al. 1990; Skerritt et al. 1991). The type of immunoassay (e.g. ELISA) affects the level of cross-reactivity (Skerritt and Hill 1990). Furthermore, the type of extractant and solid phase affects cross reactivity.

The potential exists for antibodies developed to other cereals to be used in wheat. Singh and coworkers (1988) found that an antibody to an oat globulin also binds to triticin of wheat. Skerritt and Lew (1990) found antibodies to wheat gliadins bind to proteins from rye, oats, and barley. In fact, 230/9, an antibody discussed in Chapter 4, is among this group. Although cross-reaction of MAbs to proteins coded by different genes is undesirable for use as a chromosome marker, being able to draw on antibodies developed to other cereal grain seed proteins expands the potential battery of antibodies available for a particular task, if conditions can be manipulated to obtain the desired specificity.

DNA and Isozyme Markers

Isozymes as genetic markers offer great diversity, which is an advantage for their use as genetic markers (Koebner et al. 1988; McIntosh 1988; Liu et al. 1990; Petchey et al. 1990; Thiele and Seidel 1990; Liu and Gale 1991; Sacco et al. 1992; Sun and Dvorak 1992; Thiele and Melz 1992). However, as pointed out by Sharp and coworkers (1989), isozyme analysis requires a diversity of methods be used to evaluate each marker. Because of this difficulty with isozymes, DNA markers, with their uniformity of analysis and potentially great diversity, have greater potential as genetic markers.

A number of types of DNA marker strategies on cereal crops, including sequence tagged sites (Tragoonrung et al. 1992), random amplified polymorphic DNA (RAPD) markers (Devos and Gale 1992; He et al. 1992; Dweikat et al. 1993), variable number tandem repeats (VNTRs) (Talbert and Clack 1991; Szurmak and Dobrzanska 1993), and restriction fragment

length polymorphism (RFLP) markers (Chao et al. 1988; Sharp et al. 1988a), have been evaluated. The level of polymorphism for DNA markers is low in wheat. However, some researchers have found that denaturing gradient gel electrophoresis improves the detection of polymorphism in RAPD markers in wheat (He et al. 1992; Dweikat et al. 1993). Because RAPD markers are a more recent development, the pool of useful markers is low, and map development has not be as great as with RFLP markers. In fact, a number of maps in wheat of various types has been produced using RFLP markers (Chao et al. 1988, 1989; Sharp et al. 1988a, 1989; Gill et al. 1991; Heun et al. 1991; Liu and Tsunewaki 1991; Anderson et al. 1992; Devos and Gale 1993; Devos et al. 1993). RFLPs were chosen as markers as part of the research for this thesis because of the knowledge of their chromosome location, and, in some cases, their map location has already been determined. Mapped RFLPs allow their selective use in linkage analysis to loose smut resistance genes which have been located to particular chromosomes.

Generally, RFLPs make good chromosome markers because there is often polymorphism among genomes when no polymorphism occurs within a genome (Sharp et al. 1988a, 1989; Talbert et al. 1991; Anderson et al. 1992). In fact, Gale and coworkers (1989) listed a number of applications for RFLPs in cytogenetic analysis. Many of the polymorphisms detected are considered to be caused by deletions or insertions of DNA in the noncoding region associated with the gene which the RFLP probe detects, as opposed to point mutations which affect restriction sites (Sharp et al. 1988b; Sabelli et al. 1992). Particular restriction enzymes are capable of producing polymorphism, whereas others will not (Chao et al. 1989; Graner et al. 1990). Among the most informative enzymes in wheat are Eco RV, Ssp I, Xba I, Hind III, and Dra I. Some probes detect only single-copy sequences, whereas others detect multiple-copy sequences (Chao et al. 1989). Such multiple-copy sequences may be due to homology of sequences of homoeologous chromosomes, or due to multiple copies of a

gene within a chromosome (Chao et al. 1988; Liu and Tsunewaki 1991; Anderson et al. 1992).

CHAPTER 3

The Application of Chromosome-Specific Monoclonal Antibodies to Wheat Genetics

ABSTRACT: A monoclonal antibody (P24B) to a wheat gliadin protein coded by a gene in the short arm of chromosome 1B was used as a chromosome marker. Somatic chromosome number, as predicted by the level of binding of the antibody to extracts from seeds of single cross F_2 and testcross F_1 populations, was confirmed with SDS-PAGE gel electrophoresis and root-tip analysis. The implications of the use of monoclonal antibodies as tools for cytogenetic analysis are discussed.

INTRODUCTION

Cytogenetics has played an important role in wheat breeding, both in transferring new sources of stress and disease resistance from related wild species, and in identifying the chromosome location of specific genes once transferred (Allard 1960; Knott 1987b; McIntosh 1987). Locating a gene to a chromosome requires the analysis of progeny for nullisomic, monosomic, and disomic chromosome composition, as well as evaluating the expression of the stress or disease resistance phenotype. However, cytological analysis is time-consuming (Gale et al. 1989), thus the method is limited to the study of relatively small populations. Biochemical markers are available to aid in identifying the presence of specific chromosomes. These include DNA probes (Chao et al. 1989; Sharp et al. 1989), isozymes (Masojc and Gale 1990), polyacrylamide gel

electrophoretic kernel protein banding patterns (Bietz et al. 1975), and monoclonal antibodies (MAbs) (Howes et al. 1989 and 1993). The use of biochemical markers to track chromosomes promises to increase the efficiency of cytogenetic analysis (Gale et al. 1989). MAbs to seed storage proteins are very appealing as markers because they are easy to use and allow selection to be done on seeds rather than at some later stage in plant development. In wheat, MAbs specific to endosperm gliadin proteins coded by the 1BS chromosome arm have been used to rapidly screen wheat lines for the presence of the 1BL/1RS wheat-rye translocation (Howes et al. 1989).

Monosomic analysis has been used extensively in wheat to locate dominant genes, such as disease resistance, to specific chromosomes (McIntosh 1987). This method requires the analysis of sufficient numbers of progeny to distinguish aberrant genetic ratios, such as those deviating from a 3:1 in the case of a single (dominant) gene segregation (Dyck et al. 1987; Kaloshian et al. 1991). Genetic studies of resistance to some diseases, such as loose smut, requires progeny testing using F_3 or testcross F_2 families to provide sufficient data to accommodate problems such as disease escapes or low penetrance of the trait (Thomas and Metcalfe 1984). Although the amount of cytological analysis is not extensive, population sizes required to characterize the segregation of the disease resistance trait are unwieldy. Crossing a resistant parent to a nullisomic or monosomic series, and classifying selfed or testcross progeny as nullisomic, monosomic, and euploid types, require relatively few of these plants in a progeny test to establish a correlation between the presence or absence of the trait of interest and presence or absence of a particular chromosome. Nevertheless, the locating of genes to chromosomes has been restricted to those that are simply inherited with high penetrance and high expressivity, because of large population sizes otherwise required for either cytogenetic analysis or segregation analysis. Rapid identification of chromosomes

with markers will improve the efficiency of cytogenetic analysis of traits which are less easily studied by conventional means.

In this study, the usefulness of a MAb to identify nullisomic, monosomic, and disomic lines for the 1B chromosome was evaluated. The MAb procedure was verified with polyacrylamide gel electrophoresis and conventional cytogenetic techniques.

MATERIALS AND METHODS

Plant Material

Five lines listed in Table 3.1 were used in crosses to produce segregating populations for examination with the MAb method of chromosome analysis. The crosses were as follows: Roblin N1B/Prelude, Roblin N1B/Canthatch//Prelude/Diamant, Roblin N1B/TD6//Prelude. The two testcross populations allowed independent verification of the MAb's ability to quantify protein as a reflection of 1B chromosome number by providing differences in seed protein electrophoretic banding pattern. As demonstrated in Table 3.1, Prelude and TD6 differ in their chromosome 1B alleles for high molecular weight (HMW) glutenins. As well, Prelude is missing a lower molecular weight band present in TD6.

Table 3.1. Somatic chromosome number, electrophoretic banding pattern, and reaction to MAb P24B of cultivars used in crosses for cytogenetic analysis

Cultivar	Somatic Chromosome Number (2n=)	HMW Glutenin 1BL bands	Binding by MAb P24B to 1BS gliadin
Roblin Nulli 1B (801-1)	40	null	-
Prelude	42	7+8	+
Prelude/Diamant F_6	42	7+9	+
Canthatch	42	7+9	+
TD6 (PI69282)	42	7+9	+

- = no binding, + = binding occurs, HMW = high molecular weight, 1BS = chromosome 1B short arm, 1BL = chromosome 1B long arm, nulli = nullisomic.

Similarly, the line Prelude/Diamant is missing the lower molecular weight band present in Canthatch. The Roblin N1B (nullisomic for chromosome 1B) used in these crosses was identified from a population of five thousand half kernels of breeder seed of Roblin (Campbell and Czarnecki 1987) examined with MAb P24B. In all crosses Roblin N1B was used as the female, and in the three-way crosses the F_1 plant was used as the female. Another line identified from the Roblin breeder seed population designated 796-1 was used as a low MAb binding control instead of the nullisomic 1B because of scarcity of seed of this latter line. Parents, F_1 plants, and testcross F_1 plants were grown in a growth cabinet at 18 and 15°C on a 16 and 8 h day/night cycle.

Enzyme Linked Immunosorbant Assay (ELISA)

The procedure for ELISA determinations was the same as that described previously (Howes et al. 1989), with the following modifications. The propan-2-ol extracts were diluted 1:15 in 70% ethanol, and triplicate 100-uL aliquots were bound 1 h at 25°C and rinsed in 20 mM TRIS - 0.15 M NaCl - Tween 80 (TBS-Tween). Blocking and MAb P24B binding was as described previously. Bound MAb was detected with a 1 in 2000 dilution of affinity purified goat anti-mouse alkaline phosphatase (Bio-Rad Laboratories), and developed with substrate solution (0.5 mg/mL p-nitrophenyl phosphate, 1 M diethanolamine pH 9.6, 0.5 mM $MgCl_2$) 1 to 16 h until the control samples gave readings of one to two absorbance units read at 405 nm. Neepawa or Prelude was used as the control within and across ELISA plates.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using the method of Payne and Corfield (1979), but using an 11% acrylamide - 0.05% bisacrylamide separating gel for the Roblin N1B/Prelude population, and 17% acrylamide - 0.08% bisacrylamide for the

Roblin N1B/Canthatch//Prelude/Diamant and Roblin N1B/TD6//Prelude populations. Endosperm pieces, following extraction with propan-2-ol, were extracted with 150 uL 16% dimethylformamide - 5% 2-mercaptoethanol - 0.062 M TRIS pH 6.8 - 2% SDS (Smith et al. 1986) at 60°C for 1 h. Extracted samples (30-40 uL) were applied to gel slots. Neepawa was used as a reference for the identification of HMW glutenin bands (Ng and Bushuk 1987).

After electrophoresis, the gels were fixed in 12% TCA for 1-3 h, stained in 0.04% Coomassie Brilliant Blue R250 in 50% methanol - 10% acetic acid - H_2O overnight, rinsed 4 h in 50% methanol - 10% acetic acid - H_2O, 15 min in H_2O, stained in PAGE 90 stain (Blakesley and Boezi 1977) 16 h, and cleared in 20% ammonium sulfate solution. Photography was performed with cool white fluorescent back lighting, using an orange filter.

Cytogenetic Analysis

Root-tip analysis was done on a sample of plants from the Roblin N1B/Prelude F_2, Roblin N1B/TD6//Prelude testcross F_1, and Roblin N1B/Canthatch//Prelude/Diamant testcross F_2 populations. Embryo ends of seed were placed on moist sand for five days. Root tips were collected in ice water, held at 0-1°C for 22 h, then fixed in a 3:1 solution of 95% ethanol to glacial acetic acid, and stained with Feulgen solution following hydrolysis in 1 M HCl for 8 min at 60°C. The root tips were squashed in 1.5% acetocarmine solution, and the morphology and number of mitotic chromosomes were recorded.

RESULTS

Isolation of Spontaneous 1B Nullisomic

Out of the 5,000 Roblin breeder seed kernels analyzed by ELISA, 12 kernels were identified as having low or zero binding to MAb P24B. Eight out of the 12 kernels germinated, but two failed to develop a

shoot, and of the six seeds that grew, four were normal (2n=42), with progeny kernels producing a normal binding reaction to MAb P24B. One plant, designated 796-1, had 42 chromosomes, but none of the progeny kernels reacted to MAb P24B. SDS-PAGE showed that 796-1 lacked 1BS gliadins, but had 1BL HMW subunits 7+8. Root tip analysis of this line produced no visual evidence of a deletion of chromatin, however a mutation or small intrastitual deletion in the gliadin locus could explain the results. The other plant was monosomic (2n=41) with three satellite chromosomes, and selfed progeny kernels segregated for MAb binding. Of the low-reacting progeny kernels, most were monosomic (2n=41) but one was nullisomic (2n=40), and SDS-PAGE showed that the 1BS gliadins and 1BL HMW glutenins were absent in the nullisomic. This plant, designated 801-1, had a slower growth rate and a lower seed set (five kernels/spike) than Roblin (27 kernels/spike). The 801-1 line was used as the female parent in the Roblin N1B/Canthatch cross, while selfed seeds confirmed to be nullisomic 1B (chromosome 1B is characterized by its large satellite, very similar to chromosome 6B, yet the 1B satellite is smaller and its short arm is shorter than that of 6B) were used for the Roblin N1B/TD6 and Roblin N1B/Prelude crosses.

Roblin N1B/Prelude F_2 Analysis

F_2 seed of the cross Roblin N1B/Prelude was examined to determine the ability of the antibody to distinguish aneuploid lines within a segregating population. One hundred and sixty F_2 half kernels were examined by ELISA using MAb P24B, resulting in a range of absorbance values which are summarized in Fig. 3.1 (in Fig. 3.1 and subsequent histograms, dividing marks on the horizontal axis represent boundaries of the class intervals, and the values between the interval boundaries represent the class midpoints). A large peak was apparent over the range of intermediate relative absorbance readings, while smaller peaks were observed in each tail. Ten kernels which had low antibody binding

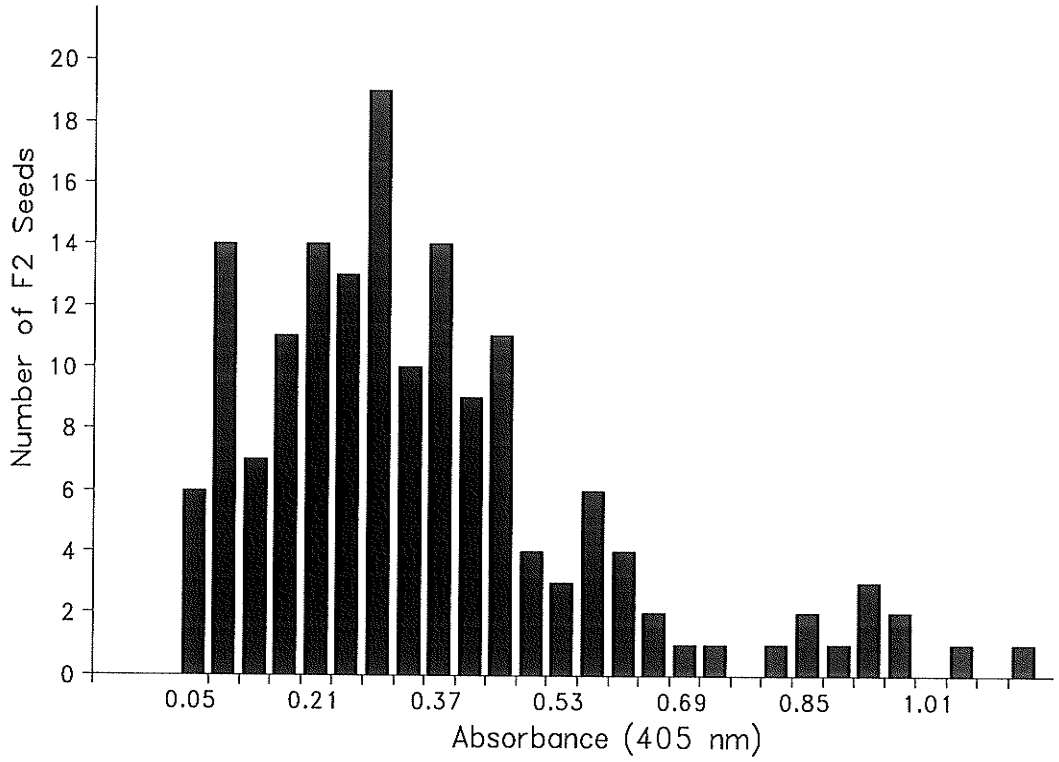
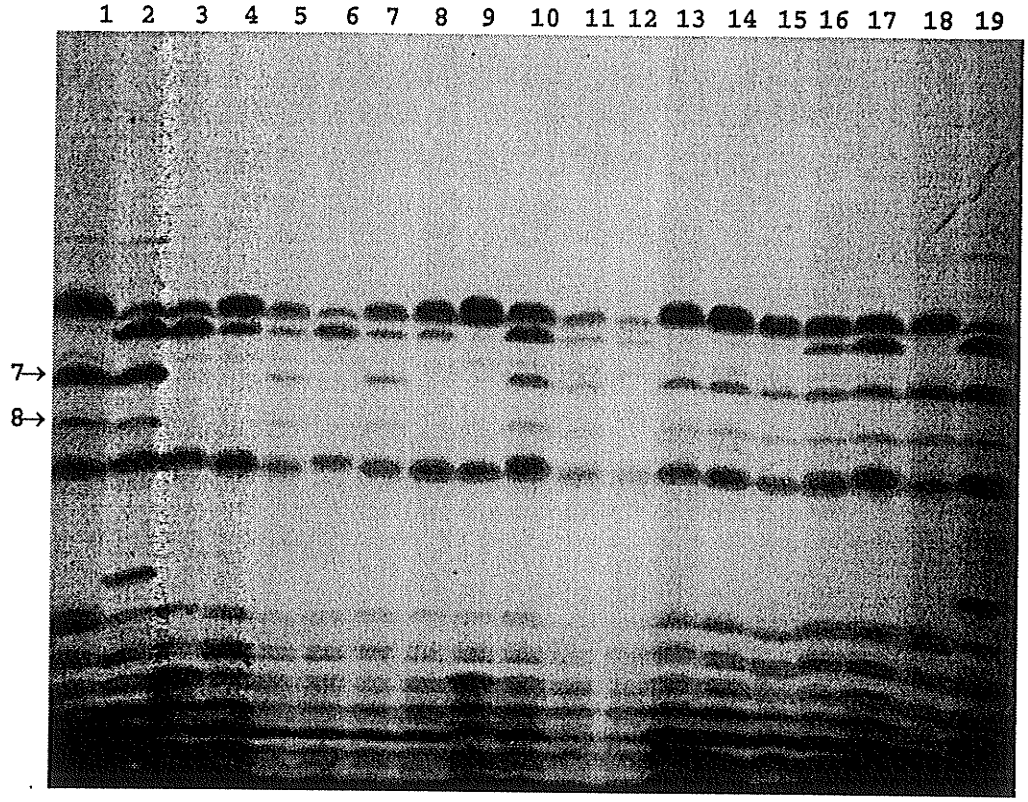


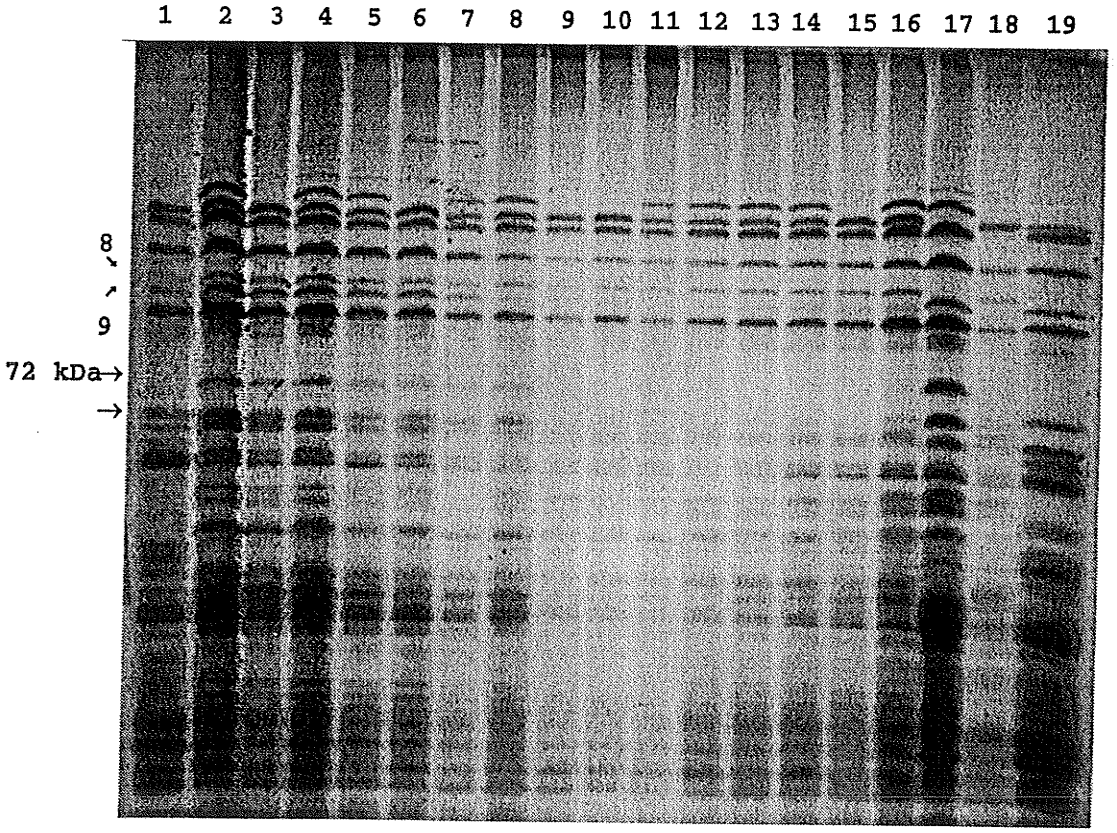
Fig. 3.1. Relative binding (to Prelude) of MAb P24B to 50% propan-2-ol extracts of F_2 half kernels from the cross Roblin N1B/Prelude.

reactions determined by low relative absorbance values, and five kernels which had intermediate antibody binding reactions determined by intermediate relative absorbance values, were selected for SDS-PAGE. All five of the intermediate-reacting kernels had HMW subunits 7+8 characteristic of the Prelude chromosome 1B (Fig. 3.2). Six of the 10 low-reacting kernels did not have HMW subunits 7+8, indicating the absence of chromosome 1B, while the others (except one) had fainter bands for HMW subunits 7+8, relative to the euploid control, suggesting they were monosomic 1B. The intensity of bands is in part related to the seed size used for extraction. The one extract expected to have a week band 7+8 (Fig. 3.2 lane 10), but did not, may have been caused by the extraction of a larger piece of seed.

Fig. 3.2. SDS-PAGE separation of F_2 kernels of the Roblin/Prelude cross selected on the basis of MAb binding: slot 1 and 18, Prelude; slot 2 and 19, Roblin; slot 3-12, selection of seeds with a low MAb reading (monosomic and nullisomic); slot 13-17, selection of seeds with a intermediate MAb reading (all monosomic).

Fig. 3.6. SDS-PAGE separation of extracts from testcross F_1 kernels from Roblin N1B/TD-6//Prelude cross: slot 1, Neepawa and Prelude; slots 2-8, selections with high MAb reaction (disomic); slots 9-16, selections with intermediate MAb reaction (monosomic); slot 17, TD-6; slot 18, Prelude; slot 19, Neepawa.


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
7→
8→


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
8
9
72 kDa→
→

The MAb and PAGE analyses were followed up with cytological

Table 3.2. Comparison of cytological analysis of root tips to P24B MAb reaction of populations Roblin N1B/Prelude and Roblin N1B/TD6//Prelude

Population	Somatic cell number (2n=)	MAb P24B reaction Low	Intermediate	High
Roblin N1B/Prelude	40	2[Y]	0	-[Z]
	41	4	5	-
	42	0	0	-
Roblin N1B/TD6//Prelude	40	-	0	0
	41	-	9	0
	42	-	0	6

[Y]Number of plants in each class.
[Z]Indicates no samples were taken.

examination of root tips. The results are shown in Table 3.2. The five seeds which had an intermediate MAb binding reaction and PAGE bands 7+8 had 41 chromosomes. Four of the 10 low samples could not be obtained because of lack of germination. Of the remaining six, two were 2n=40. These two seeds corresponded to both low MAb binding reaction and the absence of PAGE bands 7+8. The other four seeds were 2n=41. Three of the seeds corresponded to low MAb binding and PAGE bands 7+8, while one seed corresponded to low MAb binding and the absence of bands 7+8. The absence of 7+8 can be explained by the possible loss of the gene coding for these bands. However, other explanations are possible, such as the occurrence of a univalent shift, or technical error such as poor extraction, sample transfer, too small a seed piece, or other error.

Roblin N1B/Canthatch//Prelude/Diamant

The testcross Roblin N1B/Canthatch//Prelude/Diamant was studied because the endosperm of the progeny of such a cross is genetically more uniform than for an F_2 population. To demonstrate the chromosome dosage discrimination of MAb P24B the extracts of F_1, half-kernels of the cross Roblin N1B/Canthatch were compared with extracts of Roblin, 796-1 (a low binding control) and Canthatch in an ELISA. Binding of the MAb P24B to

F_1 seeds was less than 33% of the value of Canthatch or Roblin (Fig. 3.3). The Roblin N1B/Canthatch F_1 plants were testcrossed with Prelude/Diamant as a pollen parent, and relative binding of MAb P24B to each testcross F_1 kernel is shown in Fig. 3.4. Seeds could be separated into two groups based on antibody binding, those with an intermediate binding (11-60% of Neepawa) and those with high binding (80-115% of Neepawa). The embryo ends of seeds used in the above analysis were planted, and one spike on each plant was bagged. To confirm the results of the ELISA done on F_1 seed, 10 testcross F_2 kernels from the bagged heads were analyzed by ELISA, and SDS-PAGE and root-tip counts were done on the seeds with the lowest binding extracts (Table 3.3).

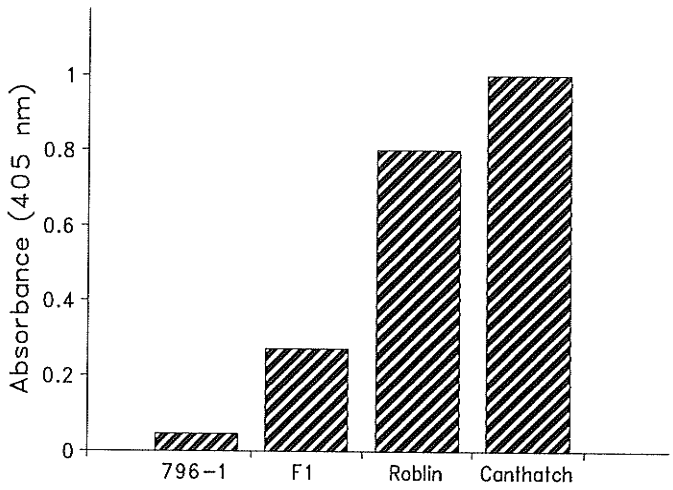


Fig. 3.3. Relative binding (to Canthatch) of MAb P24B to 50% propan-2-ol extracts of half kernels of Roblin, 796-1, Canthatch and F_1 Roblin N1B/Canthatch.

Without exception, all families with uniformly high MAb binding reactions derived from parents having a high MAb binding reaction. Likewise, all families segregating for MAb binding reaction derived from parents with an intermediate MAb binding reaction. The Prelude/Diamant and Roblin N1B lines lacked a protein band when compared with Canthatch. SDS-PAGE indicated, again without exception, that families which did not contain the band derived from parents with intermediate MAb binding reactions. The intermediate MAb reaction is expected to correspond to

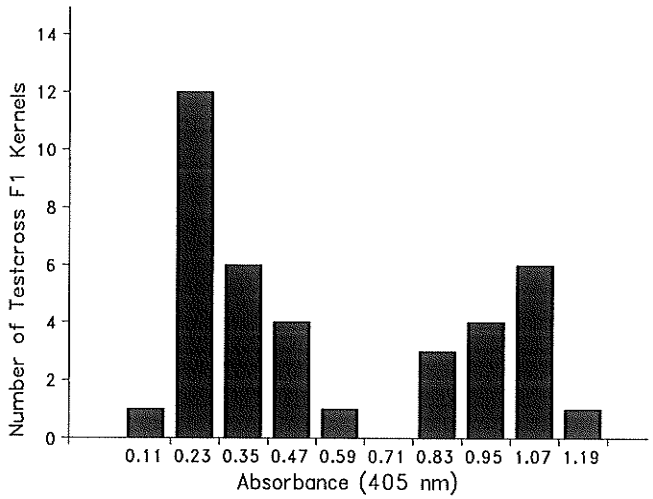


Fig. 3.4. Relative binding (to Neepawa) of MAb P24B to 50% propan-2-ol extracts of testcross F_1 kernels from the cross Roblin N1B/Canthatch//Prelude/Diamant.

the hemizygous condition, with the single 1B chromosome being derived from Prelude/Diamant. Families which contained the band derived from parents with high MAb binding reaction, which were expected to be disomic, with the Canthatch chromosome producing the band. A sample of testcross F_2 seeds was analyzed for somatic chromosome number. All low-binding reaction seeds contained three satellite chromosomes, and 88% of these seeds contained 41 chromosomes. All high-reacting seeds contained four satellite chromosomes, and all contained 42 chromosomes.

Roblin N1B/TD6//Prelude

Allelic differences in seed storage proteins occurred between parents TD6 and Prelude (Table 3.1). The gene locus for the 7+8 bands is in a different arm than that of the gene coding a product recognized by MAb P24B (Dawood et al. 1989; Payne 1987). SDS-PAGE of this population was considered to be a very good confirmation of the MAb's performance in predicting the segregation and dosage of the 1B chromosome.

The endosperm portion of F_1 seeds of the Roblin N1B/TD6//Prelude cross were assayed by an ELISA (Fig. 3.5). As with Roblin N1B /Canthatch//Prelude /Diamant, a bimodal distribution was produced. The samples with the

Table 3.3. Genetic assortment of MAb P24B reaction, occurrence of PAGE band and root tip count and chromosome morphology of Roblin N1B/Canthatch//Prelude/Diamant testcross F_2 progeny derived from F_1 seed classed as intermediate or high MAb binding

Testcross F_2 Characteristic	Testcross F_1 MAb reaction	
	Intermediate	High
Genetic assortment to MAb	Segregating (16)[z]	Nonsegregating (11)
PAGE band occurrence	Absent (13)	Present (11)
Root-tip count	40 or 41 (9)	42 (7)
Number of satellite chromosomes	3 (9)	4 (7)

[z]Number of seeds or plants tested are in parentheses.

highest and lowest absorbance readings were run using SDS-PAGE (Fig. 3.6, page 45). A close relationship occurred between the magnitude of ELISA values and presence or absence of HMW subunit 9 characteristic of TD6. Gel slots two to eight contained samples with high MAb binding reaction and all contained band 8 (from Prelude) and band 9 (from TD6). In slot eight, band 9 was present but weak. Two other bands (arrows Fig. 3.6, page 45) absent in Prelude appeared only in the high MAb samples. The one band measured at 72 kDa (Ng and Bushuk 1987)

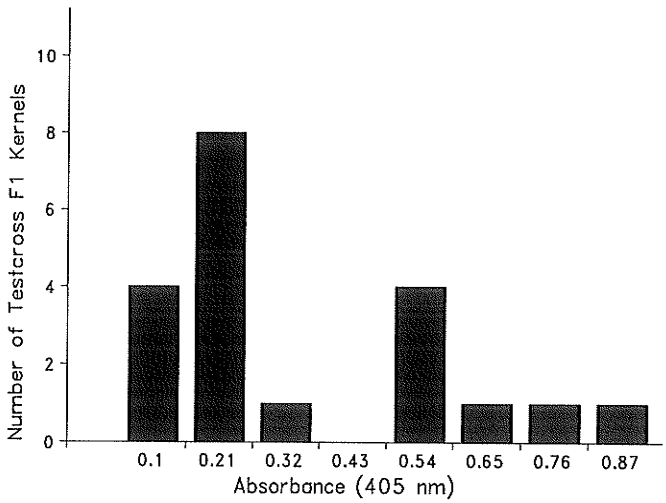


Fig. 3.5. Relative binding (to Prelude) of MAb P24B to 50% propan-2-ol extracts of testcross F_1 kernels from the cross Roblin N1B/TD6//Prelude.

has previously been shown to be associated with the 1B short chromosome arm (Howes et al. 1990). Samples in slots 9 to 16 had an intermediate MAb binding reaction and contained only band 8.

Chromosome counts were carried out on root-tip cells from testcross F_1 seed of Roblin N1B/TD6//Prelude. A strong relationship between chromosome counts and MAb binding reaction was observed (Table 3.2). Those plants with high MAb binding reactions possessed 42 chromosomes with four satellite chromosomes, while those with low binding reactions possessed only 41 chromosomes with three satellite chromosomes.

DISCUSSION

The MAb test for wheat chromosome 1B was applied to two types of genetic analysis: (1) a single-cross monosomic-1B F_1 derived F_2 population from which disomic, monosomic and nullisomic kernels could be identified, and (2) a testcrosses from which monosomic or disomic kernels for chromosome 1B could be identified. Throughout this study, high molecular weight glutenins coded by chromosome 1BL (Glu B1) and an unidentified protein separated by SDS-PAGE were used as an independent check on the MAb test. Monosomic and disomic plants derived from the selfed monosomic could not be distinguished from each other with SDS-PAGE because of the same banding pattern. The results demonstrate that gel electrophoresis can be used to further enrich the population after MAb analysis, so that cytogenetic analysis need only be applied to a very few seeds. Cytological analysis is still required to confirm the identity of prescreened lines, because regulatory mutants or small deletions can also result in a low binding reaction to the MAb, without the loss of the chromosome.

The MAb screen enables very large numbers of seeds to be non-destructively examined (Howes et al. 1989) so that even spontaneous 1B monosomic plants could be detected. In wheat, on average, a selfed monosomic plant segregates to produce about 73% monosomic, 24% euploid,

and 3% nullisomic offspring (McIntosh 1987). A trimodal distribution was observed using the MAb P24B as a chromosome marker (Fig. 3.1). The peaks were not well separated, but because the seed was from a heterogeneous and heterozygous population of F_2 seed, a range of expression of genes could be expected. Furthermore, the endosperm tissue is triploid, and MAb binding values of monosomic lines will be of two types (3n=62 with 2 doses of 1B and 3n=61 with 1 dose of 1B, although 3n=61 types will predominate), depending on whether chromosome 1B is passed through the male or the female gamete. Although the MAb may not reliably reflect the dosage of a particular chromosome, it can be used to enrich the number of aneuploids within a population of seed.

Monosomic kernels were generated from testcrosses using monosomic 1B plants as the female parent, and euploid lines as the pollen parent. The Roblin N1B/TD6//Prelude testcross was chosen with cultivars differing in Glu B1 alleles, subunit 7+8 or 7+9, so that monosomic and disomic kernels could also be identified by the presence of one (monosomic) or two (disomic) Glu B1 alleles. The Roblin N1B/Canthatch//Prelude/Diamant testcross population fortuitously segregated for the presence or absence of a different band. The strong relationship between MAb reaction and banding pattern for both populations confirmed the cytogenetic interpretation made based on the MAb binding reaction.

The triploid nature of the endosperm tissue was exploited through the testcross method. The monosomic F_1 female either contributes 2 doses of the 1B chromosome or does not contribute any 1B chromosome to the triploid endosperm tissue. The pollen parent contributes only one-third of the chromosome number in endosperm tissue, which is reflected in the amount of protein, that is, endosperm tissue of all testcross kernels is either 3n=61 with chromosome 1B present in a single dose or 3n=63 with 1B present in 3 doses. As a result of the differential chromosome dosage effect, the quantitative nature of the MAb test could be applied.

Every kernel could be identified as being monosomic 1B (and thus derived from the fertilization with the euploid pollen parent) or disomic 1B, which was reflected in the bimodal distributions (Figs. 3.4 and 3.5) of the testcross F_1 seed of the two different populations tested. As expected, the testcross produced a preponderance of intermediate-MAb-reacting kernels compared to the number of high-MAb-reacting kernels. Testing a number of seeds of a testcross F_2 family by MAb analysis or by gel electrophoresis can be used as a check on the MAb result of the one progenitor testcross F_1 seed used to classify that seed as monosomic or disomic. The testcross method can be expected to produce more consistent and clear results because of consistency in chromosome number in the endosperm tissue, but also because of homogeneity among all testcross individuals.

In these studies, disease-resistant genotypes were crossed to a plant nullisomic for chromosome 1B. The method can be extended by crossing the euploid, for example a disease-resistant genotype (male parent), to a susceptible monosomic plant (female parent), and selecting by MAb analysis only monosomic kernels which must contain the 1B chromosome from the resistant genotype. The monosomic plant can then be testcrossed, and MAb analysis used to classify monosomics and disomics. Because many monosomic series are available, this would often be the method of choice. As few as 10 monosomic plants are needed to be tested to determine if a disease resistance gene is located in chromosome 1B ($P>0.001$). A disadvantage is that recessive genes cannot be as readily studied. The method applied to hybrid progeny of a selfed double monosomic wheat alien substitution line has the advantages of both of the above methods (Howes et al. 1993).

The results demonstrate that MAbs can be used as chromosome markers in aneuploid analysis, particularly in a testcross, thereby improving the efficiency of cytogenetic analysis. At present, this method cannot be applied to all chromosomes since only MAbs specific to 1B, 6A and 6D

chromosomes have been identified (Howes et al. 1989 and 1993; Skerritt 1991; Skerritt et al. 1991). Endosperm proteins are coded by group 1 and 6 chromosomes (Wrigley and Shepherd 1973; Payne et al. 1981), and an alpha amylase inhibitor has been associated with a group 2 chromosome (Masojc and Gale 1990). Albumins and globulins have been associated with groups 3, 4, 5, 6 and 7 chromosomes (Fra-Mon et al. 1984; Gupta et al. 1991), and alpha amylase inhibitor genes in the group 3 and group 6 chromosomes (Sanchez-Monge et al. 1986). Thus, it should be possible to isolate specific MAbs for all wheat chromosomes.

CHAPTER 4

A Monoclonal Antibody Chromosome Marker Analysis Used to Locate a Loose Smut Resistance Gene in Wheat Chromosome 6A

ABSTRACT: Many genes have been located in specific wheat chromosomes, yet little is known about the location of genes for resistance to *Ustilago tritici*, which causes loose smut. Crosses were made between the loose smut susceptible alien substitution lines Cadet 6Ag(6A) and Rescue 6Ag(6A) (lines in which wheat chromosome 6A is substituted by *Agropyron* chromosome 6), and four cultivars resistant to race T19: Cadet, Kota, Thatcher and TD18. The segregating progeny were tested for reaction to loose smut race T19, and for the level of binding with a monoclonal antibody specific to a chromosome-6A-coded seed protein. The antibody, which binds only in the presence of the 6A chromosome, was used as a chromosome marker. An association was established between resistance to *U. tritici* race T19 and the presence of chromosome 6A for each of the cultivars tested. The results indicated that resistance to race T19 resides in chromosome 6A for all four cultivars. Race T19 resistance in Cadet appears to be located in the short arm of chromosome 6A, based on the evaluation of the Cadet 6A long ditelosomic stock, which was susceptible, and the Cadet 6A-short: 6 *Agropyron*-short alien translocation stock, which was resistant. Chromosome markers can be used in conjunction with cytogenetic stocks to locate genes to chromosomes.

INTRODUCTION

Control measures for loose smut are difficult to develop and maintain because they must take into account the intimate relationship between this obligate fungal pathogen and its host, but control is justified because of low, but consistent yearly yield losses (Schafer 1987, Wiese 1987). Genetic resistance, which obviates the environmental hazards posed by seed treatment, has been used effectively to control loose smut (Schafer 1987). Knowledge of the chromosome location of loose smut resistance genes can be used to improve the efficiency of breeding for resistance to this disease.

Breeding and genetic analysis of loose smut resistance are difficult because florets of the wheat spike must be individually inoculated, and progeny evaluation requires extensive growth cabinet and greenhouse space (Jones and Dhitaphichit 1991). Not only is the evaluation of loose smut reaction done on a family basis (Gaskin and Schafer 1962), nearly two generations of the host must be grown: one for inoculation and the second for symptom expression. With advances in the development of molecular markers, the breeding for resistance to loose smut can potentially be more efficient through the use of markers closely linked to loose smut resistance genes.

As maps of molecular markers in the Triticeae become commonplace (Chao et al. 1989; Sharp et al. 1989; Gill et al. 1991; Heun et al. 1991; Liu and Tsunewaki 1991; Anderson et al. 1992; Devos et al. 1992 and 1993; Devos and Gale 1993), the use of such markers will be limited by the number of genes mapped to chromosomes within the particular populations used to develop the maps. Genes for traits not polymorphic within existing mapping populations will not be so easily mapped, thus requiring the study of parents and populations for which molecular markers and maps have not been developed, but which possess the desired traits of interest such as loose smut resistance.

Analysis of two chromosome substitution series was done by Dhitaphichit et al. (1989) to locate two loose smut resistance genes to wheat chromosomes 7A and 7B. Results of analysis of varietal substitution lines, however, should not be considered conclusive evidence of the location of a gene (McIntosh 1987; Koebner et al. 1988; Yen and Baenziger 1992) because such backcross lines are prone to incomplete genotype restoration. Furthermore, heterogeneity or residual heterozygosity in either or both the recurrent and donor parents used in the development of substitution lines can result in misleading conclusions. In addition, chromosomal localization is restricted to those genes within the available cytogenetic stocks. Strategies involving segregation analysis, using confirmed cytogenetic stocks, not only provide stronger evidence of the chromosomal location of a gene, but also allow analysis of traits not carried within existing cytogenetic stocks.

Monosomic analysis involving segregating populations is commonly used for locating genes in chromosomes (Sears 1969). The problem with this method is that large F_2 populations are required to distinguish segregation ratios. As an example, a line possessing a dominant loose smut resistance gene can be crossed onto a monosomic series. Five percent nullisomic progeny will be assumed in the case of the critical cross. A minimum population size of 57 is required to distinguish the ratio involving the critical chromosome of 19:1 disomic and monosomic (resistant) to nullisomic (susceptible), from the ratio of 3:1 resistant to susceptible for the noncritical chromosome populations (99 percent probability) (Hanson 1959). Because of the wide variation for loose smut reaction within a genotype, analysis is complicated by overlap in the reaction of resistant and susceptible families such that delimitation of the classes is difficult (Ribeiro 1963), and arbitrary delimitation should be avoided (Knott 1987a).

Chapter 3 provides a discussion on a method of chromosome tracking

using seed proteins as chromosome specific molecular markers, so that traits can be located to chromosomes using relatively small populations (i.e. fewer than the 57 families required for monosomic analysis). The crosses involved the use of nullisomic cytogenetic stocks to avoid crossing over for a particular chromosome. The advantage of rapid analysis using monoclonal antibodies (MAb) to seed proteins is utilized (Howes et al. 1989) when MAbs are used as chromosome markers . MAbs are also used as chromosome markers in populations involving alien substitution lines (Howes et al. 1993). The present study made use of alien substitution lines to avoid crossing over while using a MAb as a chromosome marker.

In principle, to use alien substitution lines in conjunction with chromosome markers, crosses would be made as in monosomic analysis, but instead of crossing onto a monosomic series, crosses would be made between a source of loose smut resistance and a series of susceptible alien chromosome substitution lines, each representing a different chromosome. For each cross, progeny would be classified using (from a series of chromosome markers such as antibodies specific to particular chromosomes) the marker which is polymorphic between the resistant parent and the corresponding alien chromosome substitution line in that cross. Complete association of the resistance trait with a wheat chromosome, and susceptibility with the alien chromosome in one of the crosses, would indicate the chromosome location of the gene. All other crosses would show random segregation for resistance and susceptibility within each chromosome class.

Rather than use a full series of 21 alien chromosome substitution lines crossed onto a single source of resistance, the approach used here was to evaluate those chromosomes for which good seed protein markers are available, and to cross cytogenetic stocks that represent the chromosomes with markers onto different sources of loose smut resistance. The group 1 and group 6 wheat chromosomes coding for the

seed storage proteins offered the greatest potential of providing polymorphic chromosome markers (Payne 1987). The sources of resistance chosen for evaluation included: lines from the *U. tritici* race differential set (Nielsen 1987a), other sources of resistance of historical significance in spring wheat breeding, and the progenitors of cytogenetic stocks found to be resistant during the course of this study.

This study was undertaken primarily to provide evidence of the chromosome location of a loose smut resistance gene or genes, but also to validate the use of MAbs as molecular chromosome markers in conjunction with alien substitution lines. After a first round of crossing of cytogenetic stocks with sources of resistance had been initiated, the concurrent evaluation of cytogenetic stocks for loose smut reaction indicated that the cultivar Cadet was resistant to loose smut race T19, while the *Agropyron* alien substitution line Cadet 6Ag(6A) (Whelan 1988) was susceptible to this race. This information indicated the location of a resistance gene in chromosome 6A. Attention was focused on the study of the segregation analysis of this 6A/T19 resistance, and chromosome location of this resistance on chromosome 6A in four cultivars is the main subject of this report.

Initial testing of populations from the first round of crosses indicated a T19 resistance gene to be in the 6A chromosome for Kota, a progenitor of the differential TD4 (Hanna 1937; Nielsen 1987a). A second round of crossing, including Cadet and Thatcher crossed onto Cadet 6Ag(6A), was initiated after the inoculation of Cadet and Cadet 6Ag(6A) indicated resistance to be in chromosome 6A. Thatcher is a parent of Cadet (Zeven and Zeven-Hissink 1976) and progenitor to the differential TD12A (Nielsen 1987a). The Cadet 6Ag(6A)/Cadet cross was made because it could allow assessment of the chromosome marker technique for locating loose smut resistance genes to chromosomes, based on the evidence for the location of the T19/6A resistance gene already

available from the inoculation of Cadet and Cadet 6Ag(6A). Evaluation of the Cadet 6Ag(6A)/Cadet and Cadet 6Ag(6A)/Thatcher populations indicated a T19 resistance gene in chromosome 6A for both populations. Evaluation of a fourth population having TD18, a loose smut differential, and Rescue 6Ag(6A) (Whelan 1988) as parents indicated yet another line with a T19 resistance gene in chromosome 6A.

Using the chromosome 6A seed-protein specific MAb 230/9 (Skerritt et al. 1991; Howes et al. 1993) as a chromosome marker and the alien substitution lines Cadet 6Ag(6A) and Rescue 6Ag(6A) to avoid crossing over of the 6A chromosome, T19 resistance in Cadet, Kota, Thatcher and TD18 was found to be associated with wheat chromosome 6A. The gene location was accomplished using a minimum of resources, and confirms the value of molecular markers as chromosome markers (in particular, MAbs to seed proteins), in combination with alien substitution lines, as a method of locating genes to chromosomes.

Knowledge of the chromosomal location of loose smut resistance genes within the differential set will make easier the eventual development of single gene differential lines used to assess races of *U. tritici*.

MATERIALS AND METHODS

Cadet 6Ag(6A) and Rescue 6Ag(6A) (alien chromosome substitution lines in which the 6A chromosome is substituted with the homoeologous *Agropyron elongatum* chromosome 6Ag) were two of a group of substitution lines used as females in crosses with several sources of loose smut resistance. Dr. E.D.P. Whelan of the Agriculture Canada Research Station, Lethbridge is gratefully acknowledged for the provision of the group 6 cytogenetic stocks. The loose smut reaction of the cytogenetic stocks was unknown during a first round of crossing. Because at least half a year is required to evaluate a genotype for resistance, resistance evaluation of stocks was done concurrently with crossing. Kota and TD18 were among the sources of resistance in this round of crossing. In a second round

of crossing, the resistant cultivars Cadet and Thatcher were included. TD18 was crossed onto Rescue 6Ag(6A), whereas the other three resistant sources were crossed onto Cadet 6Ag(6A). F_1 plants were grown from each cross and the heads were bagged.

Eight races of loose smut were initially chosen as inoculum, with an attempt to select races which maximize virulence and at the same time allow for differentiation of resistance within the loose smut differentials described by Nielsen (1987a). Only races T8, T15, T19, and T31 were pertinent and therefore used on the parental combinations in this study.

The MAb 230/9 was provided by Dr. J.H. Skerritt at CSIRO in Sydney Australia, to whom we are grateful. F_2 seed was analyzed for the presence or absence of the wheat 6A chromosome, based on the binding of MAb 230/9. Antibody binding was based on an ELISA (Enzyme Linked Immunosorbant Assay) described by Howes and coworkers (1989). A portion of the brush end of each seed was removed and placed in a multiwell plate, and extracted in 150 uL of 50% propan-2-ol overnight at 37°C. Seed pieces were squashed with a blunt-ended rod and allowed to incubate another 30 min. Three replicates, of a 1 in 10 dilution of each extract in 70% ethanol, were bound to a plate at room temperature for 30 min and blocked with 1% milk for 15 min. The MAb 230/9 was diluted 1 in 10,000 and applied to plates for 90 min, followed by the addition of goat-antimouse antibody conjugated with alkaline phosphatase diluted 1 in 2000 applied for 2 h. Ethanolamine buffer (1.0 M, pH 9.5) containing 1 mg/mL p-nitrophenyl phosphate as substrate for the phosphatase was added, and the colour reaction was allowed to develop. Absorbance was measured using a Titre Tek multichannel spectrophotometer at wavelength 405 nm. Incubations of milk and antibody steps were done at 37°C. Washes between each step were done using 20 mM Tris pH 7.4 - 0.15 M NaCl - 0.05% Tween 80 (TBS-Tween). Antibodies were prepared in 0.05% milk diluted in TBS-Tween.

Up to 80 F_2 seeds for each population were analyzed with the MAb 230/9, along with Thatcher and Cadet as high antibody binding controls. Ten of the highest binding seeds, expected to be disomic for the wheat 6A chromosome, and 10 of the lowest binding seeds, expected to be absent for the 6A but disomic for the *Agropyron* 6 chromosome were selected (Howes et al. 1993). Absorbance readings were converted to relative readings, using the high controls as a basis. The population size of 10 high-binding and 10 low-binding seed was determined based on distinguishing between the two most likely causes of the expected outcome of all low-binding seed (disomic *Agropyron* 6Ag and no antigen for antibody) selections being susceptible and all high-binding seed (disomic wheat 6A and possessing antigen to antibody) selections being resistant. These two causes are chance segregation or a real association between loose smut resistance and the wheat chromosome. Considering the segregation possibility, chance segregation would have to work in favour of the one phenotype (e.g. resistant) for the one chromosome class (e.g. wheat), and at the same time working in favour of the other phenotype (susceptible) for the other chromosome class (*Agropyron*) to provide the same outcome as a real association between the chromosome and resistance. Because races with complex virulence were chosen, few multiple-gene segregations for resistance to the same race were assumed. In an F_2 population, 3:1 (or 1:3) is the expected segregation ratio for a single gene. If resistance was dominant and there was no association between resistance and the chromosome in a particular cross, then by chance the susceptible genotype was expected to occur 25 percent of the time within lines classified as disomic for the alien chromosome. To minimize the chance to one percent of deciding in favour of a real association of resistance with a chromosome, when in fact chance segregation occurred, a population of 4 individuals must be studied ($p=.25^4=0.004$) within the alien chromosome class. If the resistance was recessive, the wheat chromosome class is the most

important to consider. When the dominance relationship is unknown, as was the case in this study, both classes must be considered for a total population of eight. The population of 20 chosen in this study was considered ample.

The embryo end of the seeds having the desired antibody reactions were pregerminated and planted in sterile soil to minimize microbial infestation of the seed and prevent foreign seed contamination. The Cadet 6Ag(6A)/Kota population was inoculated with race T19 as a priority, and, if fertile heads remained inoculation was performed with race T31 to determine the association between T31 and T19 resistance. Likewise, the Rescue 6Ag(6A)/TD18 population was inoculated with T19 first, and T15, T31 and T8 were inoculated onto later spikes. The Cadet 6Ag(6A)/Cadet and Cadet 6Ag(6A)/Thatcher populations were inoculated with only race T19.

Plants to be inoculated were grown in a growth cabinet at 18°C, with 16 h light and 8 h dark. Plants were inoculated using a 10 mL hypodermic syringe with a 22 gauge needle to inject a water suspension of spores (approximately 1 mg mL^{-1}) into florets at mid-anthesis. Following inoculation, the head was bagged to minimize cross pollination and to improve infection conditions (Nielsen 1987a).

A minimum sample size was calculated using loose smut values of the parental lines (Table 4.1). The family size needed to distinguish between p1=.74 (the probability of occurrence of factor one, here equal to the proportion of smutted plants in Cadet 6Ag(6A)) and p2=.26 (the probability of occurrence of factor two, here equal to the proportion of smutted plants in Kota) at a 99 percent probability is 16 (Hanson 1959) for the population Cadet 6Ag(6A) and Kota, which required the greatest family size of any of the parental combinations studied. Where possible, at least two heads (often 3 to 4 heads) on each F_2 plant were inoculated. Because of the statistical unreliability of data on families of fewer than 16, due to the influence of small sample effects,

such data was not used for the analysis and interpretation of the data presented here. However, it should be noted that the data of families of fewer than 16 would not have distorted the interpretation of the data.

Inoculated heads were harvested and threshed separately at maturity. Seed from one head from each family for each race was planted in the greenhouse, followed by planting of a second head when the space became available, and so on until all heads had been planted and evaluated. The numbers of loose smut infected plants and healthy plants were counted. Proportions of smutted plants were calculated, with the range in sample (family) sizes occurring for each population (mean sample size in parentheses) as follows: Cadet 6Ag(6A)/Kota - T19: 18 to 144 (75), - T31: 16 to 49 (36); Cadet 6Ag(6A)/Thatcher - T19: 34 to 106 (66); Cadet 6Ag(6A)/Cadet - T19: 48 to 142 (102); Rescue 6Ag(6A)/TD18 - T8: 18 to 71 (45), - T15: 19 to 107 (61), - T19: 19 to 112 (60), - T31: 21 to 112 (41).

To confirm the MAb 230/9 binding of the single F_2 seeds, 10 F_3 seeds of populations Cadet 6Ag(6A)/Kota, Cadet 6Ag(6A)/Thatcher, and Cadet 6Ag(6A)/Cadet were tested to the antibody in an ELISA.

The alien translocation line Cadet 6AgS:6AS (Whelan and Lukow 1990), in which the long arm of chromosome 6 of Cadet is replaced by the short arm of chromosome 6 from *Agropyron*, and the ditelosomic Cadet 6AL t", missing the short arms of the homologous 6A chromosomes, were inoculated. These inoculations were done with race T19 after reaction to race T19 of the Cadet 6Ag(6A) and Cadet had been determined. The ditelosomic and translocation lines were inoculated with race T19 to provide information on the arm location of the resistance gene.

Statistical analysis of much of the data throughout this study was superfluous because of the magnitude of differences between treatments; nonetheless, statistical analyses were done and will be reported. A t-test was performed on the absorbance readings of the parents (Table

4.1). Thatcher, Cadet, Kota and Cadet 6Ag(6A), and TD18 and Rescue 6Ag(6A) loose smut reactions were compared using Fisher's exact 2-tail test. Pairwise, 2 X 2 tests of independence using Fisher's exact 2-tail test were done using the loose smut reaction data from Cadet, Cadet 6Ag(6A), Cadet 6AgS:6AS, and Cadet 6ALt" (a t-test could not be used because values of the resistant cultivars were not normally distributed). Loose smut data from the segregating populations were arcsine transformed to obtain normality before applying a t-test. All tests were run using the computer procedures of the Statistical Analysis System Institute (1989).

RESULTS

This section presents data from a selection of lines and populations taken from a larger group of lines and populations used initially in the study. The results represent those lines and populations analyzed after the focus was turned to the 6A chromosome, based on initial results from the inoculation of the Cadet 6Ag(6A) parent and its progenitor Cadet. Only results of populations that indicated T19 resistance to be in chromosome 6A are presented; however, results of the reaction of these populations to other races are also presented to provide a contrast between results that demonstrated resistance to be associated with the wheat 6A chromosome, and results that demonstrated resistance not to be associated with the wheat 6A chromosome.

The MAb 230/9 binding to seed extracts of the two alien substitution lines Rescue 6Ag(6A) and Cadet 6Ag(6A) was significantly ($p>0.005$ when the null hypothesis assumes no difference in mean absorbance readings) lower than that of the euploid cultivars, providing the polymorphism necessary to use this antibody as a chromosome marker (Table 4.1). Absorbance readings from the alien substitution lines and the Cadet 6AL t" were significantly lower than the euploid lines or Cadet 6AgS:6AS ($p> 0.005$).

Table 4.1. Proportion of smutted to total plants (P) and total plants (N) of wheat cultivars and lines inoculated with loose smut races T19, T31, T15 and T8 and monoclonal antibody 230/9 binding (OD) relative to Cadet and the standard deviation (sd) using a sample size of six

Race	T19		T31		T15		T8			
	P	N	P	N	P	N	P	N	OD	sd
Cadet	0.11	309	0.96	28	0.00	118	0.00	104	1.00	0.295
Cadet 6*Ag*(6A)	0.74	204	0.93	28	0.13	103	0.00	62	0.05	0.028
Cadet 6AL t"	0.55	299	-[z]	-	-	-	-	-	0.09	0.034
Cadet 6*Ag*S:6AS	0.22	93	-	-	-	-	-	-	1.26	0.517
Kota	0.26	23	0.00	38	0.16	37	0.65	49	0.86	0.323
Thatcher	0.16	107	0.73	56	0.00	36	0.00	59	1.05	0.361
TD18	0.00	38	0.00	35	0.00	37	0.00	36	1.08	0.054
Rescue 6*Ag*(6A)	0.39	56	0.17	18	0.64	55	0.33	48	0.10	0.014

[z]Inoculation not done.

The loose smut reactions of the wheat lines used as parents in this study are also presented in Table 4.1. Of the four loose smut races shown, the most noteworthy results were for the cultivars Cadet and Cadet 6Ag(6A) because of their significant ($p>0.001$) difference in response to race T19. These results indicated that resistance to race T19 resides in the wheat 6A chromosome. To get an indication of the chromosome arm location and a further affirmation of the chromosomal location, two additional cytogenetic stocks were evaluated: the Cadet 6AL t" and the Cadet 6AgS:6AS translocation. Results of the inoculation with T19 of the Cadet 6AgS:6AS and the Cadet 6AL t" are also shown in Table 4.1. There was no reason to inoculate these two lines with the other three races. The proportion of smutted plants in Cadet 6AgS:6AS was significantly lower than in Cadet 6Ag(6A) or Cadet 6AL t", and likewise for Cadet compared with Cadet 6Ag(6A) or Cadet 6AL t", demonstrating that loose smut reaction was not independent of chromosome condition ($p>0.001$ for each of the tests).

Whereas the loose smut reaction to each of the races was reasonably clear-cut for most lines in Table 4.1, the reactions for Rescue 6Ag(6A) were less clear. For example, the proportion of infected plants to race T31 was 0.17 for Rescue 6Ag(6A) whereas the proportion in Rescue was 0.82 ($n=22$). Thatcher and Kota differed significantly for reaction to loose smut race T19 from Cadet 6Ag(6A), and TD18 from Rescue 6Ag(6A) ($p>0.001$). The results of the inoculations of the parents dictated which races could be used on the segregating populations.

MAb 230/9 binding to F_2 seed extracts was expected to represent the wheat chromosome dosage. The results of ELISAs for the four populations Cadet 6Ag(6A)/Cadet, Cadet 6Ag(6A)/Kota, Cadet 6Ag(6A)/Thatcher and Rescue 6Ag(6A)/TD18 are shown in Fig. 4.1A to 4.1D respectively (in this figure the values of the horizontal axes represent relative absorbance readings; in this and subsequent histograms, tick marks of horizontal

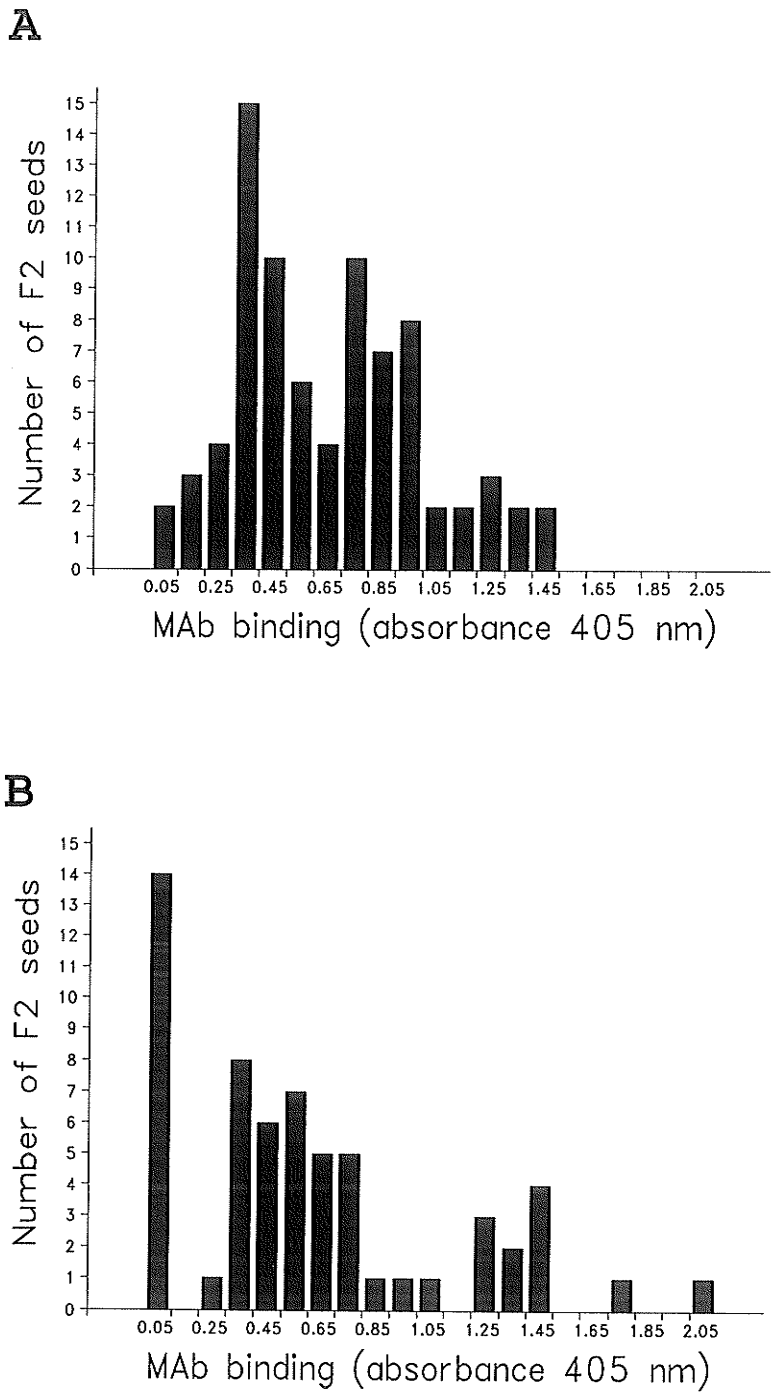


Fig. 4.1. MAb 230/9 binding measured by absorbance of ELISAs on seed extracts from F_2 populations: (A) Cadet 6Ag(6A)/Cadet; (B) Cadet 6Ag(6A)/Kota;

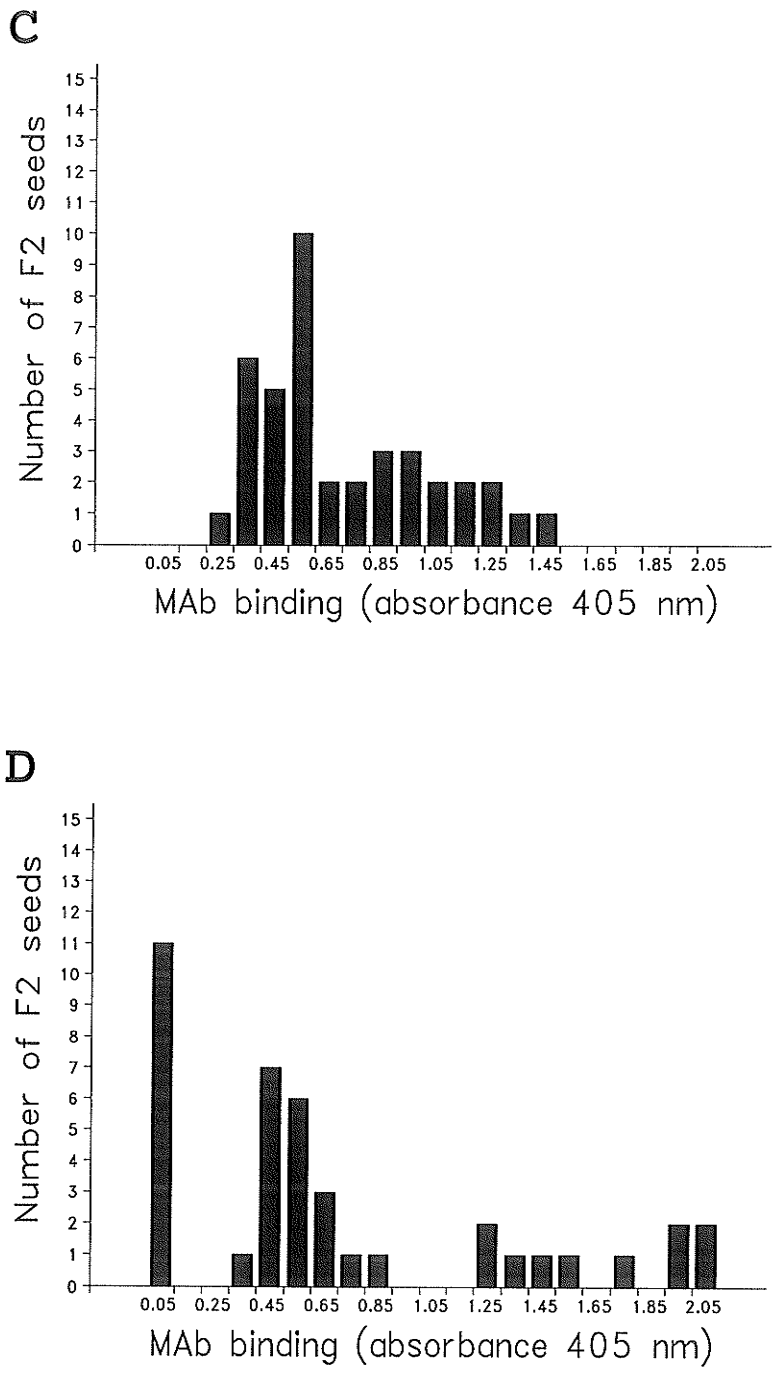


Fig. 4.1. cont'd (C) Cadet 6Ag(6A)/Thatcher; (D) Rescue 6Ag(6A)/TD18 (note: population and treatment sizes are not all equal).

axes demarcate class intervals, and the values represent the midpoint of the interval). The seeds represented by the far left-hand tail of the distribution were expected to lack wheat chromosomes, reflected in a low level of binding of the antibody. Instead of possessing the wheat 6A chromosome, these embryos were expected to be mostly disomic for the 6-*Agropyron* chromosome (endosperm trisomic 6Ag), whereas embryos of seed represented by the far right-hand tail were expected to be disomic for the 6A-wheat chromosome (endosperm trisomic 6A and high binding of the antibody). As seen in Fig. 4.1B, analysis of the Cadet 6Ag(6A)/Kota population produced a trimodal distribution, as did the Rescue 6Ag(6A)/TD18 (Fig. 4.1D), whereas the Cadet 6Ag(6A)/Cadet (Fig. 4.1A) produced few low-reacting seed, and Cadet 6Ag(6A)/Thatcher produced none (Fig. 4.1C). The seeds with extremely high or extremely low MAb binding selected from the tails of each distribution were carried on for loose smut analysis and further MAb analysis. The intermediate-binding seeds were expected to be double monosomics, and, with the exception of the Thatcher population, were dropped from further study. A 10-seed sample from each F_2 plant (F_3 seed) was used to confirm the reaction of the single F_2 seeds. The number of double monosomics accidentally selected was 1 in 20 for the Cadet 6Ag(6A)/Kota population, and 5 in 20 for the Cadet 6Ag(6A)/Cadet population, where the 20 seeds consisted of both low and high types. However, none of the Cadet 6Ag(6A)/Thatcher population were disomic for 6Ag. These results were consistent with the distributions shown in Fig. 4.1, which showed that the Cadet 6Ag(6A)/Cadet and the Cadet 6Ag(6A)/Thatcher produced few very low-binding seeds. F_3 seed of TD18 was not evaluated.

The results of segregation of F_2 progeny for loose smut in lines previously classified for chromosome 6A or 6Ag are shown for Cadet 6Ag(6A)/Cadet in Fig. 4.2A, Cadet 6Ag(6A)/Thatcher in Fig. 4.2B, Cadet 6Ag(6A)/Kota in Fig. 4.2C and D, and Rescue 6Ag(6A)/TD18 in Fig. 4.2E to 4.2H. Segregation response to different races are shown for cultivars

Kota and TD18 in Fig. 4.2C to 4.2D and 4.2E to 4.2H respectively. The reactions of the Cadet 6Ag(6A)/Kota F_2 segregating lines to *U. tritici* race T19 (based on the evaluation of F_3 families) are shown in Fig. 4.2C for the disomic 6A and the disomic 6Ag chromosome classes (based on MAb 230/9 binding). Those progeny with the wheat 6A chromosome had a significantly lower proportion of smut than those with the 6Ag chromosome, with no overlap between the two chromosome classes (the probability of 't' being near infinitely small when the null hypothesis assumes no effect of chromosome). The Cadet 6Ag(6A)/Kota population sample size used was 18 divided into two groups of 9 based on type ofchromosome. The probability of not obtaining the alternative phenotype in one chromosome class, based on chance segregation, was less than 0.0001. Similar results for reaction to race T19 were obtained with the Rescue 6Ag(6A)/TD18 (Fig. 4.2E) and the Cadet 6Ag(6A)/Cadet (Fig. 4.2A) populations; however, the size of the alien chromosome class was only five for the Cadet 6Ag(6A)/Cadet population. The low MAb binding progeny (representing chromosome 6Ag) shown in Fig. 4.2B for the Cadet 6Ag(6A)/Thatcher population represented double monosomic plants inoculated with race T19. Plotted values for race T19 infection of all four populations produced similar distributions (Fig. 4.2). The mean infected proportion for F_3 families derived from F_2 plants, selected as having the substituted 6Ag chromosome for each population, was of similar infection level to the susceptible parent in the cross, while the mean of lines selected as having the wheat 6A chromosome for each population was similar to the mean of the resistant parent in the cross.

In contrast to the results from inoculation with T19, when the same plants from the population Cadet 6Ag(6A)/Kota were inoculated with race T31, to which Kota was resistant but Cadet 6Ag(6A) was susceptible, no association (t-test probability is 0.65) between chromosome 6A and resistance could be ascertained (Fig. 4.2D). A further illustration of

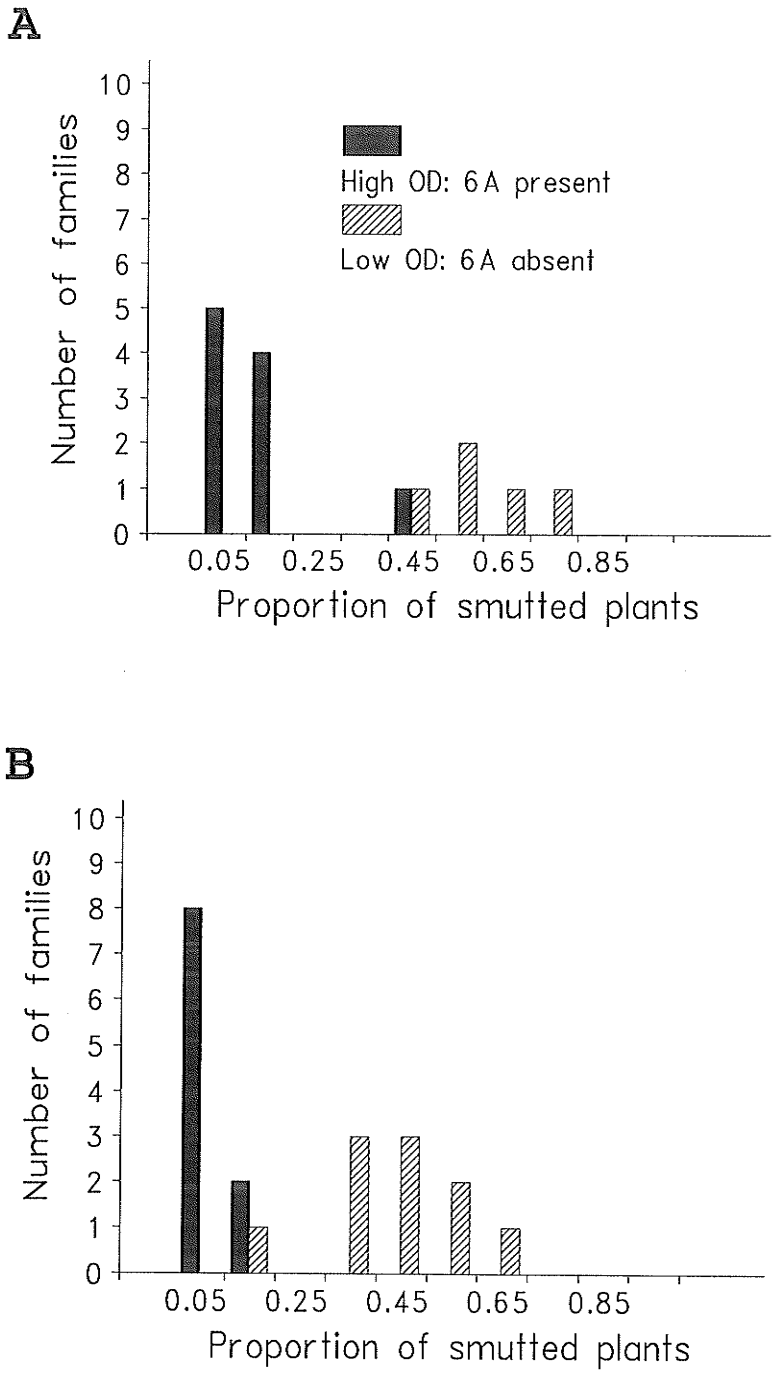


Fig. 4.2. Distribution of loose smut proportions of F_3 families classified by chromosome based on antibody readings: (A) Cadet 6Ag(6A)/Cadet to race T19; (B) Cadet 6Ag(6A)/Thatcher to race T19;

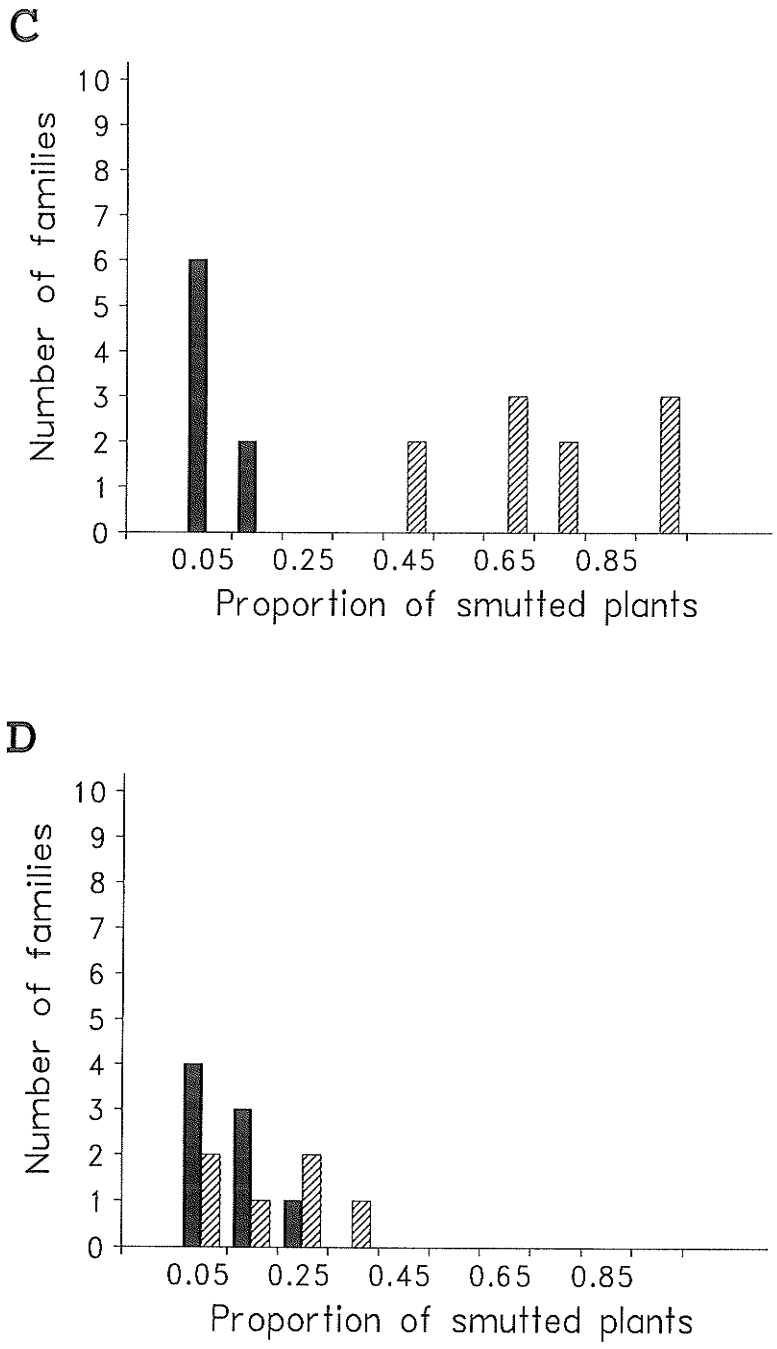


Fig. 4.2 cont'd (C) Cadet 6Ag(6A)/Kota to race T19; (D) Cadet 6Ag(6A)/Kota to race T31;

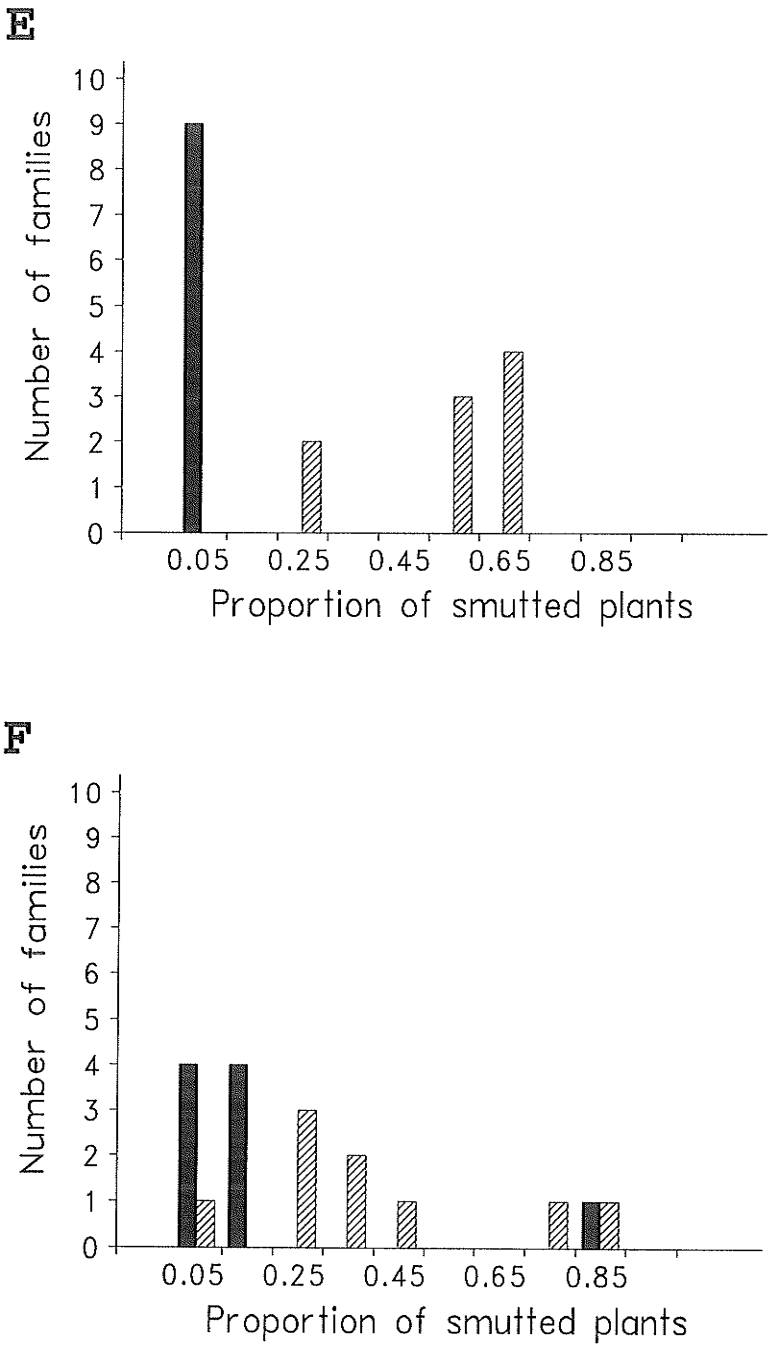


Fig 4.2 cont'd (E) Rescue 6Ag(6A)/TD18 to race T19; (F) Rescue 6Ag(6A)/TD18 to race T31;

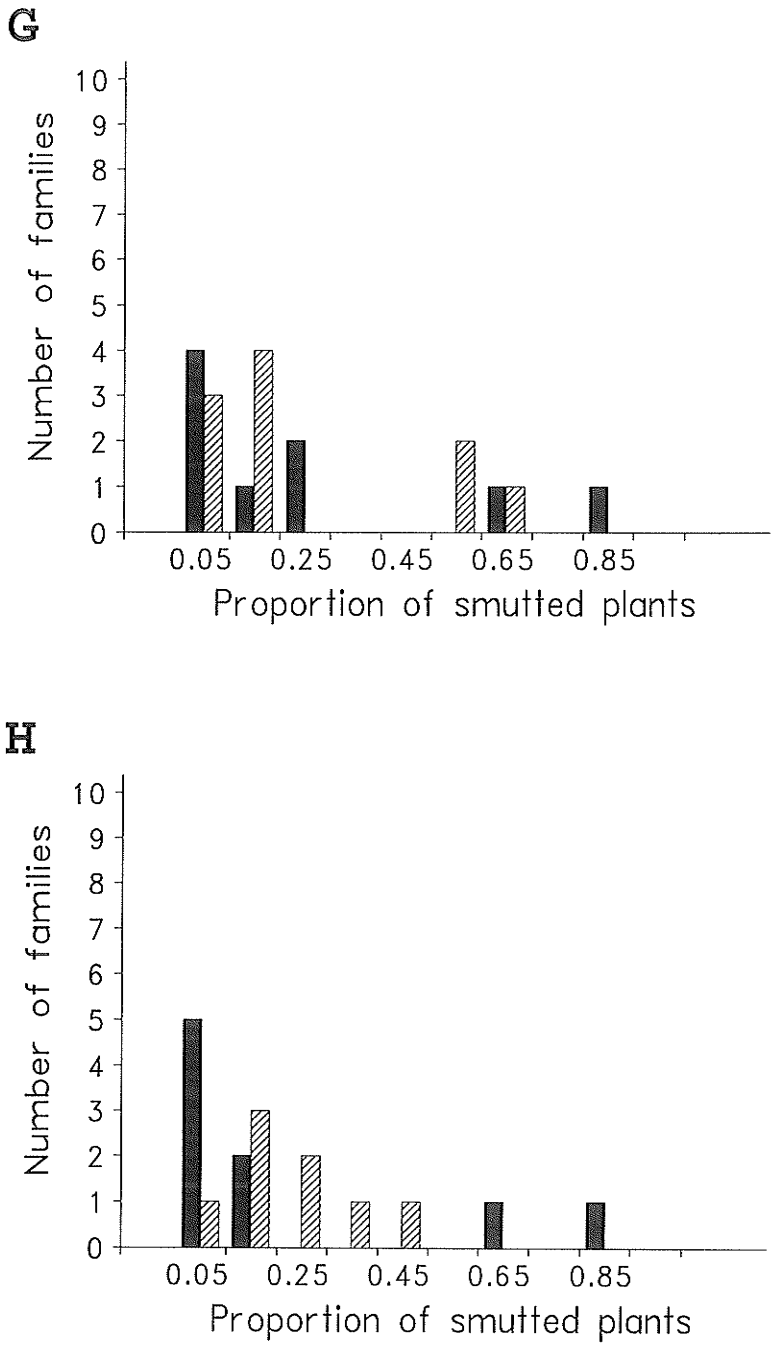


Fig 4.2 cont'd (G) Rescue 6Ag(6A)/TD18 to race T15; (H) Rescue 6Ag(6A)/TD18 to race T8 (note: population and treatment sizes are not all equal).

the distribution of progeny when no association occurred is demonstrated in Fig. 4.2G by the progeny of the Rescue 6Ag(6A)/TD18 population when tested to race T15 (t-test probability of 0.80). The lack of association of the 6A chromosome with resistance to races T31 and T8 was not so clear cut in the Rescue 6Ag(6A)/TD18 population (Fig. 4.2F and H).

One further result noted from this study was the lower fertility and viability of lines containing alien chromosomes, compared to euploid plants. This was demonstrated by the number of F_3 seed and plants in each chromosome group, and the fact that most families that were excluded from analysis because of being too small were classed as having the 6Ag chromosome. As an example, essentially all seed of the Rescue 6Ag(6A)/TD18 population was inoculated. From the 2982 inoculated seeds planted from plants that were classed as having the wheat 6A chromosome, 2304 plants, representing the first 83 heads inoculated, survived to be rated. Of the 2617 inoculated seeds from plants classed as having the 6Ag chromosome, only 1457 plants, representing seed from the first 88 heads, survived to be rated.

DISCUSSION

The location of a T19 resistance gene appeared to be in chromosome 6A of Cadet, based on evidence from segregation analysis and cytogenetic stock evaluation. A MAb chromosome marker enabled the use of small populations for testing the chromosomal location of the loose smut resistance genes. T19 resistance in three other cultivars, which have relationships to loose smut differentials, or to Cadet, was found to be associated with the 6A chromosome with the aid of the MAb marker. The chromosomal location of genes within the loose smut differential set can be used to help identify the genes for resistance in the differential set. The T19 resistance gene in Cadet appeared to be in the 6A short chromosome arm, based on cytogenetic stock evaluation.

The prime objective of this study was to locate a gene or genes for loose smut resistance to a chromosome in wheat through analysis of a segregating population, with the aid of a chromosome marker and an alien chromosome substitution stock. The low level of loose smut race T19 infection of Cadet, compared to the high level of infection of Cadet 6Ag(6A), indicated that the location of resistance to race T19 was in chromosome 6A. This information allowed greater effort to be focused on the study of segregating populations possessing resistance to race T19, in which one of the parents possessed an alien chromosome substitution in which the wheat 6A chromosome was replaced. The Cadet 6Ag(6A)/Cadet and Cadet 6Ag(6A)/Thatcher populations were generated, and the Cadet 6AgS:6AS and Cadet 6AL t" were inoculated based on the preliminary information that a loose smut resistance gene resided in chromosome 6A. Analysis of the Cadet 6Ag(6A)/Cadet population not only allowed confirmation of the chromosomal location of a loose smut race T19 resistance gene in the Cadet 6A chromosome, it also affirmed the value of the MAb chromosome marker/alien substitution line method for locating genes to chromosomes. One segregate from the Cadet 6Ag(6A)/Cadet population, classed as having the 6A chromosome, was more susceptible than its other 6A siblings (Fig. 4.2A). This may be simply random variation or a second gene for partial resistance to race T19 may be segregating. The important point to note is that if the resistance gene were not in 6A, the probability that all the lines classed as not having the 6A chromosome also segregated as susceptible is remote.

The results also demonstrated that T19 resistance in Thatcher, TD18, and Kota was associated with the 6A chromosome. Nielsen (1987a) reported that TD4, a selection from Kota, produced infection levels of less than 10% when inoculated with race T19. The Kota used in this study was inoculated to confirm Nielsen's report (Table 4.1); however, the Kota used in this study did not appear to be as resistant to T19 as the TD4 described by Nielsen. The segregation for the T19 resistance in

the Kota used in this study confirmed that the Kota possesses resistance. The difference in reaction between the Kota here and TD4 used by Nielsen (1987a) may be due to random variation, or Nielsen's TD4 may have a different gene or modifier gene if the original Kota were heterogeneous. In addition to the results indicating that Kota had a gene for resistance in chromosome 6A, the results also indicated that Kota had a second gene for resistance to T31, which was not in chromosome 6A. The same progeny that were inoculated with T19 were inoculated with T31, yet progeny within each chromosome class were mainly resistant.

Although no disomic 6Ag progeny were identified in the Cadet 6Ag(6A)/Thatcher population, double monosomics were evaluated for resistance to race T19 along with the lines disomic for the wheat 6A chromosome. The susceptibility of the Cadet 6Ag(6A)/Thatcher double monosomics to race T19 indicates the gene is hemizygous ineffective or recessive. Because all of the lines classed as disomic for the wheat 6A were resistant, and all of the lines double monosomic for the wheat 6A and *Agropyron* 6Ag chromosomes were susceptible, the evidence is strong that the T19 resistance in Thatcher resides in the wheat 6A chromosome.

Unlike Cadet 6Ag(6A), which was highly susceptible, Rescue 6Ag(6A) was only moderately susceptible to race T19. The moderate susceptibility of the Rescue 6Ag(6A) may have been due to mis-inoculation. The results showing that the susceptible segregates of the Rescue 6Ag(6A)/TD18 population were more susceptible than the Rescue 6Ag(6A) tends to support the mis-inoculation hypothesis. As for the reaction to race T31, Rescue 6Ag(6A) appeared moderately resistant. Yet, Rescue inoculated to T31 was highly susceptible. Highly susceptible progeny did segregate from the cross of Rescue 6Ag(6A) with TD18, as shown by inoculations with race T31 (Fig. 4.2F), indicating that the data for T31 on Rescue 6Ag(6A) may be an underestimate of the true susceptibility of this cultivar to race T31. In general, workers evaluating the genetics

of loose smut resistance over the years have been frustrated by the variable reaction of this disease (Ribeiro 1963).

The T31 and T8 results were not clear-cut for the Rescue 6Ag(6A)/TD18 population. A two-gene system could explain the results of loose smut incidence values in response to inoculation with T31 and T8. A major gene segregating for resistance, but not in chromosome 6A, would explain the approximate 3:1 segregation (actual numbers 7:2) of resistant to susceptible in the 6Ag chromosome class (Fig 4.2F). But, in general, the 6A lines were more resistant than the 6Ag lines. A second gene in 6A could explain the additional resistance. The difference in loose smut reaction between the 6A classified lines and the 6Ag classified lines was not statistically significant for the Rescue 6Ag(6A)/TD18 population inoculated to T31 or T8 (the probabilities being 0.10 and 0.65 respectively), but these values were confounded by the susceptible segregation class. Given the hypothesis that two genes are functioning in a cumulative fashion, four classes result: R1R2, R1S2, S1R2, S1S2 where R is resistant and S susceptible and 1 is locus one and 2 is locus 2. If a second gene residing on 6A is removed (i.e. 6Ag lines) two groups of resistance can be formed, those segregating for two genes (6A lines R1R2, R1S2, S1R2) and those segregating for one gene (6Ag lines R1). The null hypothesis is that chromosome 6A does not possess a loose smut resistance gene influencing the reaction to race T31 or T8. Susceptible segregates are excluded from this analysis to determine the effect of R2. When the t-test was recalculated with the susceptible segregates excluded, a significant difference between the 6A and 6Ag groups was detected at the 5% probability level, indicating that chromosome 6A indeed had an effect on loose smut resistance to races T8 and T31 in the TD18 population. Modifier genes, and genes with cumulative and additive effects, have previously been implicated in loose smut resistance (Kilduff 1933; Heyne and Hansing 1955; Shestakova and Vjushkov 1974). Further study is needed to confirm this potential

cumulative effect of resistance genes on loose smut.

The segregates susceptible to race T8 from the population Rescue 6Ag(6A)/TD18 were also the most susceptible to race T15, indicating that the same gene conferred resistance to these two races. The ratio of 14 resistant to 5 susceptible suggests that genetic control for resistance to race T15 could be from one dominant gene. A progeny susceptible to T15 and T8 was resistant to T31, and a different progeny susceptible to T31 was resistant to both T15 and T8, indicating yet another gene in TD18. That TD18 could have 3 genes for resistance to loose smut is supported by the few races virulent on it as a differential (Nielsen 1987a, Person 1959).

The low loose smut reaction of Cadet 6AgS:6AS and the high reaction of Cadet 6AL t" indicated the presence of the T19 resistance gene to be in the short arm of chromosome 6A.

With regard to the method used to locate the T19 resistance genes to chromosome 6A, the MAb 230/9 is particularly useful as a 6A chromosome marker. This antibody binds to gliadins produced at the 6A locus (Skerritt et al. 1991; Howes et al. 1993), but did not bind to seed extracts which had the 6A chromosome substituted by the 6 *Agropyron* chromosome (Table 4.1). The absorbance readings of the alien substitution lines were consistent with those reported by Howes et al. (1993). The low antibody-binding of the Cadet 6AL t" is consistent with the fact that the gliadin locus is located in the 6AS chromosome arm (Garcia-Olmedo et al. 1982). Because this antibody bound to seed extracts possessing the wheat 6A chromosome, but did not bind to extracts of seed in which the wheat 6A chromosomes were replaced by 6Ag chromosomes of *Agropyron*, the polymorphism needed to classify segregating progeny was satisfied.

In principle, the MAb chromosome marker/alien chromosome procedure is more efficient in terms of the population size required to make a decision on chromosome location of a gene than is monosomic analysis (a

population of 57 for monosomic analysis compared to 8 for chromosome marker analysis). The procedure appears to be robust, accommodating the sometimes wide variation seen in loose smut observations. The chromosome marker/alien substitution line method seems to be able to accommodate problems of variation within a trait being analyzed, because of precise classification of the chromosome condition. Because small populations are used, re-evaluation of outliers is feasible for classification of the seed by the antibody, and, if necessary, highly deviant smut reactions can be verified by inoculating a sample of F_3 plants. The method provides clear results when the genotype being studied possesses a single highly expressive gene. The method could also be used in more complex genetic situations, where, for the cross involving the critical chromosome, the segregation ratio for n genes in the wheat chromosome class is compared to a ratio of n-1 genes in the alien chromosome substitution class, where n is the number of genes. Obviously, in systems involving more than one gene, population sizes must be larger when races cannot be selected to selectively remove the effects of all but one of the resistance genes.

The minimum population of lines with and without a particular wheat chromosome required to determine the chromosomal residence of a single gene may be small, but a population size somewhat larger is useful. This is because misclassification of F_2 seeds may occur. Although three chromosome classes are expected, four endosperm classes are expected in the F_2 progeny of a double monosomic plant. As was pointed out in Chapter 3, the level of endosperm protein encoded by a particular allele will vary depending on whether or not the allele is contributed by the female or the male (i.e. whether or not the endosperm is triploid). Therefore, the class of double monosomics actually represented two classes (one class of endosperm possessing two 6-*Agropyron* chromosomes and one 6A-wheat, and the second class possessing two 6A-wheat chromosomes and one 6-*Agropyron*). The other two classes are represented

by progeny possessing disomic alien chromosomes and by progeny possessing disomic wheat chromosomes. Figure 4.1 demonstrates that peaks tend to merge. Among the most obvious factors which can affect the level of antibody binding and cause chromosome dosage classes to merge are (in addition to gene dosage (Chapter 3)) the environmental conditions for which the developing seed is exposed (Shewry and Miflin 1985), the size of seed piece used for extraction, and the precision with which samples are diluted and handled. Other reasons for having a greater than theoretical minimum population size include, that not all seeds may survive, and plants may possess problems such as poor vigour or fertility.

With loose smut, there is a second population factor to be considered, namely the family size of inoculated plants. When the level of disease expression between the resistant and susceptible parent declines, then the family size (i.e. the number of inoculated seed from a single plant) must be increased. In this study, there is evidence for a reduction in fertility and/or viability when a line possesses an alien chromosome in place of a wheat chromosome. This factor must be accounted for when determining the number of heads to be inoculated.

The success of the chromosome marker method depends on being able to evaluate a large initial F_2 population of seed for their chromosome constitution. Theoretically the alien chromosome should segregate with its homoeologous wheat chromosome in a 1:2:1 ratio. In this study the proportion of disomics was considered when large populations of F_2 seed were initially evaluated to classify the seed based on chromosome make up (Fig. 4.1). A major advantage of monoclonal antibodies to seed proteins is their ease of use in an ELISA, in which many seeds can be evaluated with minimal effort (Howes et al. 1989). In the experiments here, even though initial populations were large (between 60 and 80 seeds) they may not have been large enough, as demonstrated by the Cadet 6Ag(6A)/Thatcher and Cadet 6Ag(6A)/Cadet populations (Fig. 4.1A and

4.1C). No explanation can be offered for the Cadet 6Ag(6A)/Thatcher population not generating 6Ag disomic progeny, except that perhaps poor maintenance of the alien chromosome in the disomic form in certain crosses may be a potential drawback of the use of alien substitution lines. The fact that segregation for the disomic alien chromosome 6Ag was under represented was indicated by the antibody, and, in hindsight, larger populations of F_2 seed could have been evaluated to improve the opportunity of selecting disomic 6Ag lines. Not just seeds with the lowest readings should be selected, but those with the lowest readings which are comparable to the low check should be selected. Obviously, simplicity and precision of classifying the chromosome marker trait contribute to the efficiency of the overall analysis.

The common chromosome location of resistance to race T19 in the cultivars Kota, Cadet, Thatcher and TD18 is evidence that these cultivars may possess the same or a related gene. Knowledge of the chromosomal location of genes within the differential set would provide some indication of the relationship of genes possessed by those differentials, and would assist in the development of single gene differential lines. The crosses in this study provide a preliminary study of the relationship of resistance to race T19 among the four cultivars, all of which are related to or are differentials. Kota and Thatcher are progenitors to differentials TD4 and TD12 respectively, and TD18 is a differential (Nielsen 1987a). The Thatcher cross was made based on the observation that TD12 was resistant to race T19, and when the differential was crossed to make it day-length insensitive as described by Nielsen and Dyck (1988), T19 resistance was lost in the improved differential TD12A. Nielsen and Dyck (1988) showed that Thatcher's value as a differential was not diminished by the loss of T19 resistance during agronomic improvement of this differential. This can be explained by the fact that the gene lost was duplicated in another differential, for which the present study provides evidence (both TD4 or

TD18 may have the same T19 resistance gene as Thatcher). Thatcher and Kota are of interest not only because of their use in race differentials, but also because their differential derivatives TD4 and TD12 were used in the analysis of virulence of races of loose smut (Nielsen 1977 and 1982). Genes for resistance to loose smut in TD4 and TD12 have been named by McIntosh (1988), based on virulence interactions of loose smut races (Nielsen 1977 and 1982).

Nielsen's (1977, 1982) genetic analyses of virulence showed Kota has a gene (Ut2) different from TD12 (Ut4). Because TD12A became susceptible to only race T19 during agronomic improvement as a differential, the T19 resistance must be conditioned by a second resistance gene in TD12. Race T5 is virulent on TD12 but avirulent on TD4, and was one of the races used by Nielsen (1982) as a parent in the cross for virulence segregation analysis. For this difference in reaction between TD4 and TD12 to occur, T5 must have virulence on the T19 resistance gene in TD12. The avirulence of T5 on TD4 indicates Ut2 is the effective gene, and the T19 resistance gene (assuming the T19 resistance in Kota and TD12 is the same) is a third gene between the two differentials. Ribeiro (1963) first suggested Ut as a symbol for genes resistant to loose smut of wheat. He labelled Kota resistance to race C2 as $Ut_{c2.4}$, meaning the fourth gene discovered to be resistant to race C2. McIntosh (1988) designated 4 genes for resistance based on the gene-for-gene concept of host pathogen interactions and Nielsen's (1977 and 1982) identification of virulence genes. McIntosh designated FlorenceXAurore, Renfrew, and Red Bobs as having Ut1, Kota and Little Club as having Ut2, Carma Ut3, and ThatcherXRegent Ut4. Therefore, I propose the second gene in TD12 be designated Ut5. Further study is required to determine whether or not this is the same gene as that found in Kota, Cadet, and TD18.

The results of this study demonstrate the value of using a seed-protein-specific monoclonal antibody as a chromosome marker in

conjunction with an alien substitution line in the cytogenetic analysis of wheat. Gains in efficiency are realized in two ways: The correlative marker method is robust and requires a smaller population size than monosomic analysis; By using MAbs, more rapid analysis of a population is achieved through an ELISA and sampling seed endosperm, in contrast to conventional cytogenetic analysis of root tips of seedlings or of pollen mother cells with microscopic observation. The use of alien substitution lines, and the production and analysis of F_2 populations for disomic individuals, allows for evaluation of recessive genes with the same ease as dominant genes.

The MAb 230/9 is a good indicator of chromosome 6A constitution based on the relationship achieved with loose smut reaction in the segregating Cadet 6Ag(6A)/Cadet population compared with the evidence from inoculation of the cytogenetic stocks indicated a 6A short location for T19 loose smut resistance in Cadet. This study is indicative of the potential value of other chromosome marker/alien chromosome substitution line combinations for locating genes to chromosomes. These results provide the first demonstration, through genetic segregation analysis, of the chromosomal location of a loose smut resistance gene, and the first tentative location of such a gene in a chromosome arm. The monoclonal-antibody-based cytogenetic technique applied here can be used to assist the chromosomal location of a wide variety of difficult-to-evaluate wheat traits. The identification of the chromosomal location of genes should be seen as a way of facilitating future efforts of gene tagging with biochemical markers such as RFLPs (restriction fragment length polymorphisms) or RAPD (random amplified polymorphic DNA).

CHAPTER 5

The Association Between a Wheat Chromosome 6A Gene Controlling Loose Smut Resistance in Wheat, the Sr8 and Gliadin Gene loci, and an RFLP in the Same Chromosome

ABSTRACT: Linkage was tested among a group of chromosome 6A traits. Homozygous recombinant progeny for the 6A short chromosome arm of the population Cadet 6AgS:6AS/Chinese Spring Sr8//Cadet 6Ag(6A) (where Ag represents *Agropyron* chromosome and S, short arm) were tested to stem rust race C10 and loose smut race T19, and evaluated for gliadin banding pattern using polyacrylamide gel electrophoresis. DNA samples taken from the progeny were cut using restriction enzymes, and the cut samples were probed to detect RFLPs. The loose smut resistance gene was neither linked to the gliadin, nor to the stem rust resistance genes. However, the 6A loose smut resistance gene was associated with an RFLP.

INTRODUCTION

Genetic markers tightly linked to genes conferring loose smut resistance are potentially easier to evaluate than reaction to the disease. A gene for resistance to loose smut race T19 has been located to chromosome 6A

(Chapter 4 of this thesis), and is likely in the short arm of this chromosome. Also located in the short arm of the 6A chromosome are a gliadin gene (Shepherd 1968; Garcia-Olmedo et al. 1978, 1982; Payne 1987; McIntosh 1988) and the stem rust resistance gene Sr8 (Sears et al. 1957; McIntosh 1972; Sawhney et al. 1981). As well, RFLPs have been located to this chromosome (Sharp et al. 1988a, 1989; Heun et al. 1991; Liu and Tsunewaki 1991; Anderson et al. 1992).

In this study, a population was established which demonstrated polymorphism for alleles at the gliadin locus and the loose smut resistance locus. Progeny were also evaluated for the Sr8 stem rust resistance gene, and a sample of RFLP probes was evaluated for polymorphism between the parents and among the progeny. The purpose of this study was to determine the linkage relationship between a loose smut resistance gene located in chromosome 6A with the gliadin locus also in the same chromosome, thus allowing assessment of the value of the gliadin locus as a marker for the loose smut resistance gene. Linkage analysis was also done between the loose smut resistance, gliadin and the Sr8 stem rust resistance gene loci, and a group of restriction fragment length polymorphism (RFLP) probes located in this chromosome.

MATERIALS AND METHODS

Plant Material

The translocation line Cadet 6AgS:6AS (Dr. E.D.P. Whelan, Agriculture Canada Research Station, Lethbridge, Alberta provided this and other Cadet cytogenetic stocks), resistant to *U. tritici* race T19, was used as the female parent in a cross with Chinese Spring Sr8 (from the late Dr. E.R. Sears, Dep. of Agronomy, University of Missouri, Columbia, MO) (Fig. 5.1). The latter line was developed by backcrossing the Sr8 stem rust resistance gene into the cultivar Chinese Spring (Sears et al. 1957). The Cadet 6AgS:6AS is an alien translocation line in which the

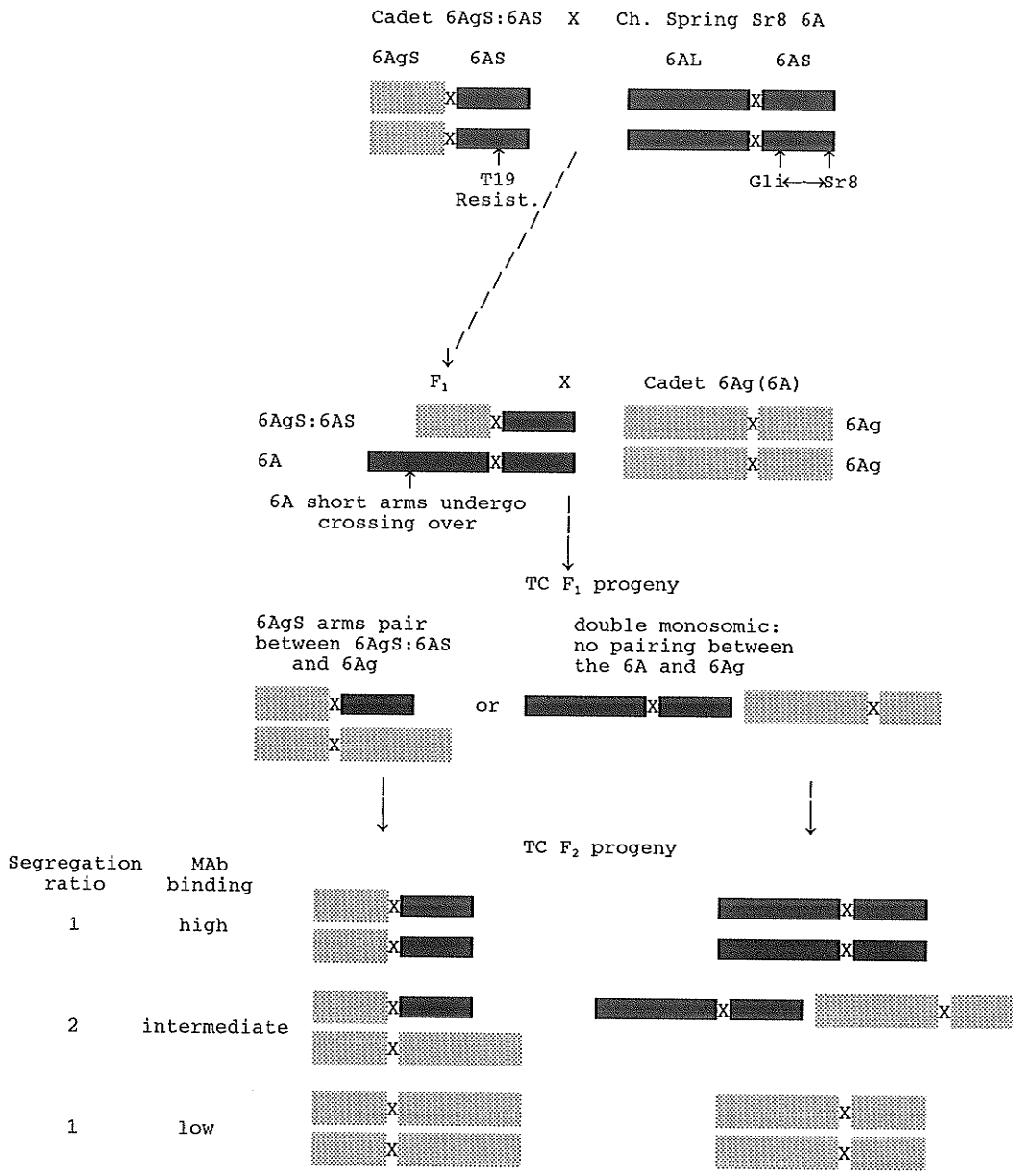


Fig. 5.1. Crossing scheme used for the Cadet 6AgS:6AS alien translocation line in a cross with Chinese (Ch.) Spring Sr8 testcrossed with the alien chromosome substitution line Cadet 6Ag(6A).

long arm of chromosome 6 of Cadet is replaced by the short arm of chromosome 6 from *Agropyron* (Whelan and Lukow 1990). Two plants of Chinese Spring Sr8 (designated CS Sr 8A and CS Sr 8B) were used as pollen sources. When selfed progeny of each were tested with stem rust race C10, only the CS Sr8A plant was found to be resistant. A testcross was made using Cadet 6Ag(6A) as the testcross female parent. This line lacks the wheat 6A chromosome and the T19 resistance gene (Chapter 4 this thesis). The wheat 6A chromosome from the Chinese Spring Sr8 and the 6AS chromosome arm from Cadet 6AgS:6AS will have no pairing partner when the F_1 of Chinese Spring Sr8 and Cadet 6AgS:6AS is crossed with Cadet 6Ag(6A). In such a cross, the only pairing event allowing crossing over, occurs in the 6A short arm of chromosome 6A during meiosis of the initial cross.

Eighty testcross (TC) F_1 seeds were planted and grown to maturity. In an attempt to minimize growth cabinet usage, five TC seeds per pot were planted, with tillers trimmed in an attempt to produce one good head per plant. All eighty seeds developed plants, only two of which produced less than 10 seeds. Up to 10 seeds (TC F_2) from each plant were evaluated with the monoclonal antibody (MAb) 230/9 (provided by Dr. J.H. Skerritt CSIRO, Sydney Australia), which binds to the protein coded by the gliadin locus in chromosome 6A (Howes et al. 1993). The level of antibody binding indicates gene and therefore chromosome dosage (Chapter 4 of this thesis). The one seed with the highest binding from each group of 10 seeds was planted, in an attempt to recover plants disomic for either the 6A chromosome or the 6AgS:6AS chromosome. The resulting plants were grown in the growth cabinet in individual pots, and two to three heads were inoculated with a water suspension of teliospores of *U. tritici* race T19 spores (1 mg mL^{-1}). The loose smut inoculated heads (TC F_3 seed) were harvested individually and planted in the greenhouse. Seed was collected from 63 plants, being those remaining after some families produced no high an antibody binding seed in the F_2, and lack

of emergence or sterility in others. Five uninoculated TC F_3 seeds from each TC F_2 plant were used for electrophoretic and antibody analysis. The embryo ends of these seeds were grown out and plant material was harvested for DNA extraction. Two sets of five TC F_3 seeds of plants derived from the CS Sr 8A rust resistant parent were also grown and inoculated with stem rust race C10, and, as well CS Sr 8A and Cadet were planted as controls.

Rust inoculations were done with race C10, which is avirulent on the Sr8 gene. Rust spores were rubbed on damp leaves with the fingers, or by misting of a spore suspension from a spray bottle. Inoculated plants were placed in a dew chamber overnight. Plants were removed from the dew chamber in the morning, and covered with clear plastic caps to maintain moisture. The plants were placed under bright light for the remainder of the day at which time the plastic caps were removed.

Growth cabinets and greenhouses were operated at 15 to 18°C, with supplemental light provided in the greenhouse. Day length was 16 h light and 8 h dark.

Enzyme Linked Immunosorbant Assay

MAb analysis was done using an enzyme linked immunosorbant assay (ELISA). For detailed procedures, see Chapter 4 of this thesis. Seeds were cut in half so that the embryo end could be grown for other analyses, while the endosperm end was extracted in 50% propan-2-ol. The extract was bound to microtitre plates, and ELISAs were performed using MAb 230/9, which is specific for protein coded by wheat chromosome 6A. As mentioned, 10 TC F_2 seed from each plant were analyzed in an attempt to obtain disomic seed for either the 6AgS:6AS chromosome or for the 6A chromosome. Five TC F_3 seeds were evaluated to confirm the disomic condition.

Polyacrylamide Gel Electrophoresis

Acid PAGE gels were run on seed extracts of Cadet, Cadet 6AgS:6AS, Cadet 6Ag(6A), Chinese Spring Sr8, and Chinese Spring 6AL ditelosomic (t"), Chinese Spring 6AS t", Prelude Sr13 (the Chinese Spring cytogenetic stocks were originally from the late Dr. E.R. Sears, and maintained and provided by Dr. E.R. Kerber, and Prelude Sr13 by Dr. D. Harder both at Agriculture Canada Research Station, Winnipeg, Manitoba) to determine which gliadin band(s) corresponded to the 6A short arm locus. The extracts from TC F_3 seed used for the ELISA were also used in polyacrylamide gel electrophoresis (PAGE) at low pH. Six percent acrylamide with 1.25% bis-acrylamide was prepared at pH 3.1 using aluminum lactate buffer. Gels were run for 1 3/4 h at 540 V at 7°C with sodium lactate bottom buffer pH 3.1 and aluminum lactate top buffer pH 3.1. The antibody binding was correlated to PAGE band variation to determine whether or not the antibody 230/9 could detect allelic variation.

Loose Smut Inoculations

Inoculum was prepared and inoculations were done using a syringe and needle as described in Chapter 4 of this thesis. At least two heads of each TC F_2 plant were inoculated, and selected F_3 plants were inoculated to confirm results of the F_2 inoculation.

DNA Analysis

Plants derived from the embryo end of the seeds used for acid PAGE analysis were germinated and allowed to grow to the four to five leaf stage. The leaf material from the up to five seedlings of the same family was harvested into perforated polyethylene bags as bulks. Bulking of leaf material from the up to 5 plants was based their homozygosity for the 6AS chromosome arm. Immediately after each sample was harvested the leaf material was cut into 2 to 3 cm pieces and

submerged in liquid nitrogen. The plant material was transferred from liquid nitrogen to a freeze dryer, and lyophilized at 10 um Hg vacuum pressure at room temperature for two days. Following drying, samples were stored on silica gel at -20°C until the DNA was extracted.

The following steps were used for DNA extraction. Up to 250 mg of plant material was ground in a 115 mm mortar with a pestle, with 3 g of acid-washed sand and 25 mL of liquid N_2. The sample was transferred to a 50 mL Teflon centrifuge tube. To each tube was added 20 mL of lysis buffer consisting of 1.1% CTAB (hexadecyltrimethylammonium bromide), 55 mM Tris pH 8, 55 mM EDTA (ethylenediaminetetraacetic acid) pH 8 and 1.54 M NaCl at pH 8, followed immediately by the addition of 50 uL Proteinase K (1 mg/mL). The sample was vortexed and 2.2 mL 20% SDS was added, followed by gentle mixing. The samples were allowed to digest for 2 h at 65°C with intermittent gentle mixing. Tubes were filled with 24:1 chloroform:isoamyl alcohol (about 15 mL). Samples were rocked and allowed to mix for 1/2 h. Samples were centrifuged for 20 min in a table-top centrifuge at about 2,500 rpm at room temperature. The upper liquid phase was transferred to a clean Teflon tube. A volume of propan-2-ol equal to 0.6 times the volume of the aqueous extract (about 9 mL) was added to precipitate the DNA. The DNA was spooled onto a glass rod and washed with 70% ethanol. The ethanol was allowed to drain from the DNA, which was then dissolved in 2 mL of sterile water in a 30 mL Teflon tube overnight at 4°C. Each sample was treated with 7.2 uL of 10 mg/mL heat-treated ribonuclease (RNAase) in TE (10 mM Tris, 1 mM EDTA) for 2 h at 37°C. To reduce the amount of carbohydrate extracted with the DNA (the carbohydrate interfered with dissolving the samples in water), a second extraction with 1% CTAB, 1 M NaCl and 1X TE was done for each sample. Six millilitres of phenol:chloroform:isoamyl alcohol (25:24:1) was added to each sample with mixing. Samples were centrifuged for 15 min, and the upper aqueous solution was transferred to a clean tube. Chloroform:isoamyl alcohol (4.5 mL, 24:1) was added

and mixed, and again centrifuged for 15 min. The upper aqueous solution was saved in a clean tube, precipitated with 95% ethanol and 1/10 volume 3 M sodium acetate (pH 5.2), and stored at -20°C. The DNA was rinsed twice in 70% ethanol and dissolved in sterile water. The concentration of the DNA in each sample was determined and adjusted to 1.5 ug/uL after quantification spectrophotometrically at 260 nm. Restriction of the DNA of each of the parents was done with restriction enzymes Eco RV, Hind III, Dra I, Xba I, and Bst IIE (Gibco BRL), and probed with a series of chromosome group 6 probes (provided by Dr. A. Graner, Institut fur Resistenzgenetik, Grunbach, Germany; Dr. M.D. Gale, IPSR, Cambridge, UK; and Dr. Won Kim, Agriculture Canada Research Station, Winnipeg, Canada as part of the North American Barley Genome Mapping group) to determine those which produced polymorphism between CS Sr8 and Cadet 6AgS:6AS and to see which bands were associated with the 6A chromosome. Eco RV and Hind III restrictions resulted in polymorphism, and these enzymes were used on DNA of the progeny. Fifteen micrograms of DNA was cut with 40 units of the restriction enzyme in the appropriate reaction buffer. The samples were incubated overnight, loaded on a 0.9% agarose gel (20 X 25 cm and 5 mm thick), and run for 1100 V-h. The gel was blotted onto a Zeta Probe GT (Bio-Rad) nylon membrane for 14 h overnight, using an alkaline blotting system. The membrane was rinsed in 2X SSC (sodium chloride-sodium citrate made as a 20X stock with 173 g NaCl 88.2 g sodium citrate adjusted to pH 7.0 with NaOH and made to 1 L with deionized (DI) water)(Sambrook et al. 1989) and baked at 80°C for 40 min.

Probing involved first blocking the membrane with 3 mL of 5 mg/mL of autoclaved salmon sperm DNA boiled 10 min (to denature the DNA) in a prehybridization solution consisting of 18 mL of water, 6 mL of 5X HSB (30.3 g PIPES into 300 mL DI water pH 6.8 and 600 mL 5 M NaCl and 40 mL 0.5 M EDTA $Na_2 2H_2O$ made to 1 L with DI water), and 3 mL of modified Denhart's III (2 g 1% BSA (bovine serum albumin), 2 g Ficoll-400, 2 g

PVP-360 (polyvinylpyrolidone), 10 g SDS, 5 g $Na_4P_2O_7$-H_2O and 100 mL DI water, pH 8.0) at 65°C for 6 h in a HYBaid oven. The probes were labelled by the random primer labelling method using ^{32}P adenosine (BRL Random Primers DNA Labelling System), and precipitated with the addition of 10 uL of autoclaved salmon sperm DNA, 1/10 volume of 3 M sodium acetate, pH 5.2, and 2.5 volume of 95% ethanol. The solution was placed at -20°C for 2 h, centrifuged for 25 min in a microcentrifuge, washed once with cold 70% ethanol, dissolved in TE pH 7.6, and placed in a boiling water bath for 10 min. The probe was suspended in 30 mL fresh prehybridization solution to produce the hybridization solution. The prehybridization solution was poured off the blots and the hybridization solution was added. Probes were allowed to bind for 16 h at 65°C. Blots were removed from the hybridization tubes, transferred to trays, and washed two times for 15 min in 2X SSC with 1% SDS, and two times for 10 min in 0.35X SSC with 1% SDS at 65°C. Excess wash solution was blotted from the membranes, and blots were wrapped in Saran plastic wrap. The blots were exposed to Kodak XAR5 XO mat X-ray film in cassettes containing intensifying screens for up to five days at -70° C.

RESULTS

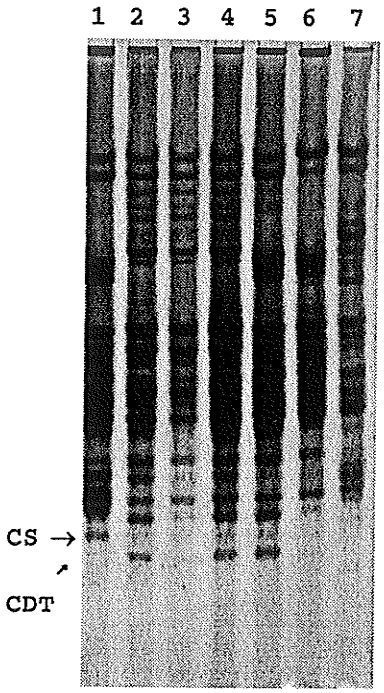
Segregates of the cross Cadet 6AgS:6AS/Chinese Spring Sr8//Cadet 6Ag(6A) were classified for loose smut resistance, 6A gliadin proteins, stem rust resistance, and an RFLP. The TC F_2 seed within a family were expected to consist of disomics for the 6AgS:6AS translocation, heterozygotes for the 6AgS:6AS translocation, and 6Ag, and disomics for 6Ag in a 1:2:1 ratio (Fig. 5.1). Alternatively, a family could possess disomics for the 6A, double monosomics for the 6A and 6Ag, and disomics for the 6Ag in a 1:2:1 ratio. The seeds with the highest antibody binding were expected to be either those disomic for the 6AgS:6AS, or

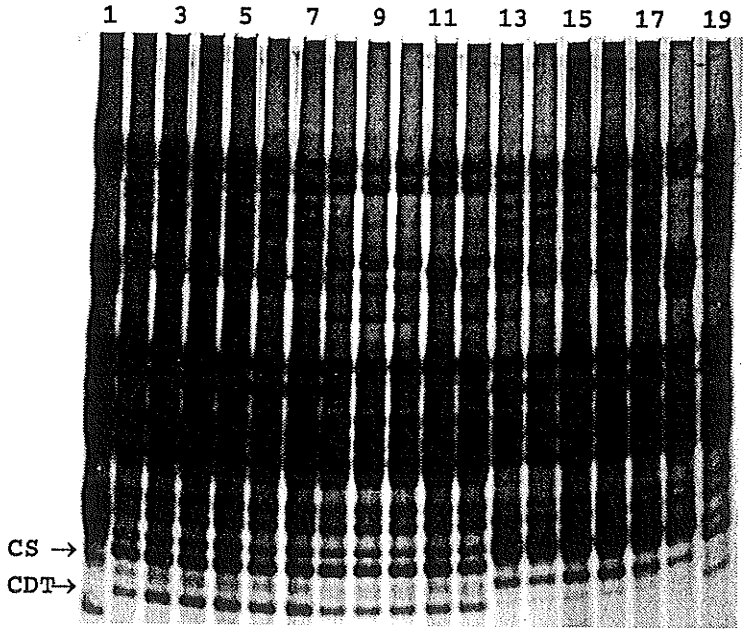
those disomic for the 6A chromosomes. Eight of the families did not contain seeds producing high binding, therefore seed from those families was not planted. The remaining 72 families possessed seed ranging from low binding to high binding. Because double monosomics were not available as checks to provide a mean and standard deviation to which to compare the segregating seeds, and because the binding values of the double monosomic distribution probably overlapped with disomic 6A values, it was impossible to predict which intermediate values were from double monosomics, allowing some double monosomics to be selected. The lowest binding seed kept had a corrected absorbance (relative to the high control) of 0.2. Nine TC F_2 plants either did not survive or did not produce seed, leaving 63 families. One plant was inoculated with loose smut but no additional seed was available for PAGE and the ELISA, dropping the total number of useful families to 62.

The PAGE protein band designated Cdt in Fig. 5.2A was absent in Cadet 6Ag(6A) and Chinese Spring 6AL t" indicating this band is coded by the 6A gliadin locus on 6AS. Chinese Spring Sr8 had a different band in the same region of the gel as 6A band in Cadet. Five TC F_3 seeds representing each family were evaluated for their gliadin banding pattern, using Acid PAGE, and the same extracts were also used for the ELISA. Fig. 5.2B provides an example of the segregation for the 6A band in three families. Note that Chinese Spring Sr8A has an additional fast running band at the bottom of lane 1 (Fig. 5.2B) compared to Chinese Spring Sr8B (lane 18), adding to the evidence of heterogeneity in this stock. There were no discrepancies between Acid PAGE bands and the level of MAb binding for those families which uniformly showed either the Cadet 6A gliadin allele or the Chinese Spring Sr8 gliadin allele. That is to say, there were no families in which a low absorbance corresponded to a 6A band. However, this was not the case for families identified as homogeneous not expressing a 6A band; four families showed

Fig. 5.2A. PAGE analysis is as follows for the lane and cultivar: 1, Chinese Spring Sr8; 2, Cadet; 3, Cadet 6Ag(6A); 4, Cadet 6AgS:6AS; 5, Chinese Spring 6AS t"; 6, Chinese Spring 6AL t"; 7, Prelude Sr13.

Fig. 5.2B. PAGE analysis of five TC F_3 seeds representing three families (lanes 3-7, 8-12, 13-17) of the Cadet 6AgS:6AS/Chinese Spring Sr8//Cadet 6Ag(6A) population and controls Chinese Spring Sr8A (lane 1) and Chinese Spring Sr8B (lane 18) and Cadet (lanes 2 and 19).


1 2 3 4 5 6 7
CS →
CDT


1 3 5 7 9 11 13 15 17 19
CS →
CDT→

discrepancies between Acid PAGE results and antibody binding (i.e. some with low antibody binding possessed the PAGE band).

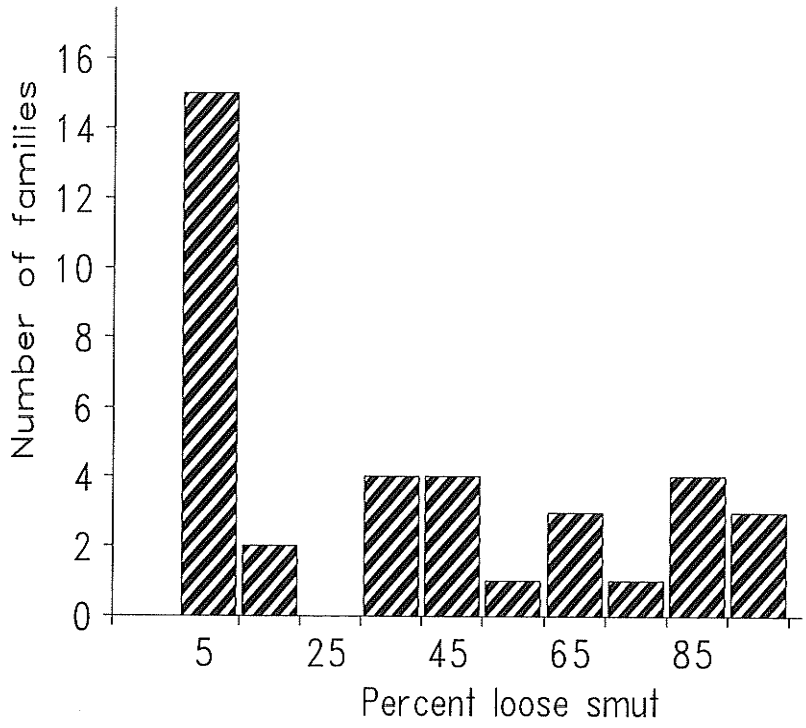


Fig. 5.3. Segregation of Cadet 6AgS:6AS/Chinese Spring Sr8//Cadet 6Ag(6A) TC F_3 families for reaction to loose smut race T19.

Thirty-nine families were uniform for Acid PAGE banding (and ELISA) across the five seeds tested. Thirty-seven of the thirty-nine homogeneous families had sister seed inoculated to race T19 of loose smut. The segregation of families for loose smut reaction is shown in Fig. 5.3 (tick marks on the horizontal axis demarcate class intervals and the values represent the midpoint for each interval). There appeared to be two populations: those having greater than 25% loose

smut, and those having less than 25% loose smut. To determine whether or not loose smut resistance to race T19 was linked to the gliadin locus, the population was divided into families having the Cadet 6A gliadin allele and those having the Chinese Spring Sr8 gliadin allele. Each of the gliadin classes were further divided as being resistant or susceptible to T19 (Table 5.1). A Chi square test showed only random assortment of alleles, indicating that these genes were not linked.

Table 5.1. Segregation of Cadet 6AgS:6AS and Chinese Spring Sr8 for a 6A gliadin band and loose smut race T19 resistance and a chi square to test deviation from a 1:1:1:1 segregation ratio

Resistance to Race T19	Gliadin Allele		Total
	Cadet 6AgS:6AS	Chinese Spring	
Resistant	10	7	17
Susceptible	8	12	20
Total	18	19	37

Ratio	Trait	Chi Square	df	Prob. = 0.05	
1:1	Gliadin	0	1	3.84	NS
1:1	Loose smut	0.108	1	3.84	NS
1:1:1:1	Combined	1.054	3	7.82	NS

Several setbacks occurred in trying to obtain stem rust reactions of the population and checks. Seedlings from the two parental lines of Chinese Spring Sr8 showed variation for reaction to race C36. One line gave a type-two reaction, whereas the other gave a type-four reaction. See Knott (1987a) for a description of reaction types. Based on this information, only those families derived from the resistant Chinese Spring Sr8 parent were tested for stem rust reaction. Although race C36 is avirulent on Sr8, it was found that Cadet and Cadet 6AgS:6AS were also resistant (giving a type two reaction) to this race. Race C10 was selected because it has wide virulence, yet is avirulent on Sr8 (Green

1981). C10 was found to be virulent on Cadet (reaction type 3 to 4), while avirulent on Chinese Spring Sr8 (type-two reaction). A shortage of Cadet 6AgS:6AS seed hindered the evaluation of rust reaction on this line. In a first round of inoculations of the progeny, up to five plants per family were inoculated with race C10. Although few clearly resistant lines could be detected (i.e. characterized by the type-two reaction), some lines showed a greater level of resistance than others. The families were classified as susceptible, questionable, and resistant. A second inoculation of the families, this time at the jointing stage, produced similar results. Again, there were those which were clearly susceptible and those which were difficult to classify. An attempt to evaluate additional races on the parents for further study of the progeny failed because of a powdery mildew infestation.

One option was to group the stem rust susceptible families together and group those with questionable stem rust reactions together with the resistant class. When the results were compared with the values for loose smut reaction and with gliadin banding pattern of the same families, linkage was indicated by the low number of recombinant families (Tables 5.2A and 5.2C). The parental types are circled in these tables, and are the predominant classes. The low number of recombinant progeny was true for both the stem rust resistance and the loose smut resistance combination (Table 5.2D), and for the rust resistance and gliadin combination (Table 5.2B) when the families with questionable rust ratings were classified as rust susceptible. The population size of 18 is somewhat small for the Chi square test, where a minimum of five observations for the smallest class for a total of 20 is recommended. With the caution of the population size in mind, the Chi square test was run on each of the groupings, and is shown in Table 5.2. When tested to a 1:1:1:1 expected segregation ratio, the Chi square probabilities were not significant at a 5% level for each grouping of

Table 5.2. Chi square values for goodness of fit of segregation ratios for gliadin against Sr8 rust resistance, and loose smut resistance against Sr8 rust resistance

A

Questionable rust ratings as resistant

	Rust Susc.	Rust Resist.	Total
Ch. Spring-gli	2	5	7
Cadet-gli	7	4	11
Total	9	9	18

B

Questionable rust ratings as susceptible

	Rust Susc.	Rust Resist.	Total
Ch. Spring-gli	3	4	7
Cadet-gli	9	2	11
Total	12	6	18

C

Questionable rust ratings as resistant

	Rust Susc.	Rust Resist.	Total
Loose smut Resistant	8	3	11
Loose smut Susceptible	1	6	7
Total	9	9	18

D

Questionable rust ratings as susceptible

	Rust Susc.	Rust Resist.	Total
Loose smut Resistant	9	2	11
Loose smut Susceptible	3	4	7
Total	12	6	18

	Ratio	Trait	Chi Square	df	Prob. = 0.05
A					
	1:1	Gliadin	0.500	1	3.84 NS
	1:1	Sr8 Rust	0.056	1	3.84 NS
	1:1:1:1	Combined	2.889	3	7.82 NS
B					
	1:1	Gliadin	0.500	1	3.84 NS
	1:1	Sr8 Rust	1.389	1	3.84 NS
	1:1:1:1	Combined	6.444	3	7.82 NS
C					
	1:1	Sr8 Rust	0.0555	1	3.84 NS
	1:1	Loose smut	0.5	1	3.84 NS
	1:1:1:1	Combined	6.4444	3	7.82 NS
D					
	1:1	Sr8 Rust	1.3889	1	3.84 NS
	1:1	Loose smut	0.5	1	3.84 NS
	1:1:1:1	Combined	6.4444	3	7.82 NS

values. Because the probability of obtaining the Chi square value obtained for three of the combinations was reasonably low (between 5 and 10%), and considering the small sample size used, linkage of these traits should not be ruled out without further testing. Ten DNA probes were evaluated against Chinese Spring Sr8 and Cadet 6AgS:6AS and Cadet 6Ag(6A) DNA (Table 5.3) in search for polymorphism that could be used as

Table 5.3. A list of DNA probes located to the Triticeae 6 chromosomes and the source of the probe

Probe identity	Arm probed	Source
MWG514	L	Dr. A. Graner
MWG620	S	Federal Biological Research Centre
CMWG652	S	for Agriculture and Forestry
CMWG653	S	Institute for Resistance Genetics
CMWG669	L	Grunbach, Germany
MWG820	S	
223B		Dr. W. Kim (NABGM)
458		Agriculture Canada
		Research Station
		Winnipeg, Canada
167	S	Dr. M.D. Gale
154	L	Cambridge Laboratory
		Norwich, U.K.

additional markers for loose smut resistance, and as a chromosome marker for locating the loose smut resistance gene relative to the centromere. Up to five restriction enzymes were evaluated in an attempt to improve the chance of obtaining a useful polymorphism. All probes bound to southern blots producing two or more bands. One probe was found to be associated with the 6A chromosome because a band produced by Cadet 6AgS:6AS was not produced by Cadet 6Ag(6A), but no polymorphism between Cadet 6AgS:6AS and Chinese Spring Sr8 was found. In most cases no polymorphism and no association with the 6A chromosome occurred. When polymorphism did occur it was inconsistent across enzymes, i.e., some enzymes produced polymorphism and some did not. One probe (167)

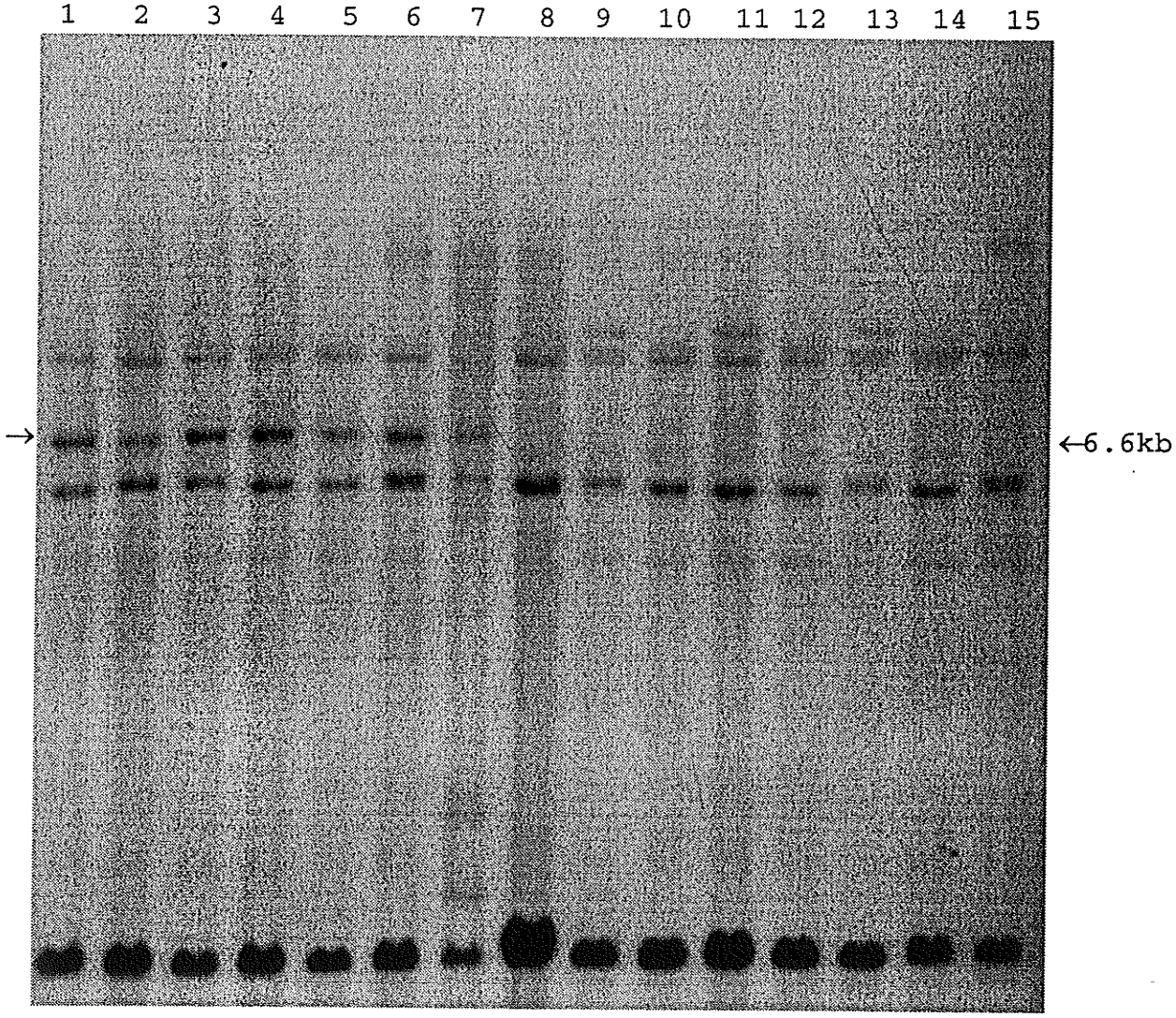
Table 5.4. Segregation of progeny from the Cadet 6AgS:6AS/Chinese Spring Sr8//Cadet 6Ag(6A) population for a 6A Hind III RFLP band and (A) loose smut race T19 resistance, (B) stem rust resistance, (C) gliadin band

		Probe 167, Hind III		
A	Resistance to Race T19	Band Present	Band Absent	Total
	Resistant	0	10	10
	Susceptible	12	2	14
	Total	12	12	24
B	Resistance to Race C10			
	Resistant	1	4	5
	Susceptible	1	2	3
	Total	2	6	8
C	Gliadin locus			
	Cadet 6AgS:6AS	5	6	11
	Chin. Sp. Sr8	7	6	13
	Total	12	12	24

	Ratio	Trait	Chi Square	df	Prob. = 0.05	
A						
	1:1	Probe 167	0.042	1	3.84	NS
	1:1	Loose smut	0.375	1	3.84	NS
	1:1:1:1	Combined	17.333	3	7.82	*
B						
	1:1	Probe 167	1.125	1	3.84	NS
	1:1	Sr8 Rust	0.125	1	3.84	NS
	1:1:1:1	Combined	3.000	3	7.82	NS
C						
	1:1	Probe 167	0.042	1	3.84	NS
	1:1	Gliadin	0.042	1	3.84	NS
	1:1:1:1	Combined	0.333	3	7.82	NS

detected polymorphism in the progeny that was not apparent in the parents when DNA samples were cut with both Hind III and Eco RV enzymes. The Chi square results and data are presented for only the Hind III enzyme in Table 5.4 because the Eco RV pattern of association was, for the most part, the same. The polymorphism is shown in Fig. 5.4. The 1:1 ratios were not significant when single loci were considered, indicating no deviant segregation at a locus. This was true for

Fig. 5.4. DNA of homozygous recombinant lines for the 6AS chromosome arm cut with Hind III and probed with probe 167 where lane and description of line are as follows: 1-7, loose smut susceptible lines with 6.6 kb band; 8-12, loose smut resistant lines without 6.6 kb band; 13, Chinese Spring Sr8; 14, Cadet 6Ag(6A); 15, Cadet 6AgS:6AS.


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
→
←6.6kb

segregation involving the RFLP (Hind III and Eco RV generated) with the stem rust, gliadin, and loose smut loci. A significant Chi square was produced for the combined segregation of the loose smut locus and the RFLP generated by the Hind III enzyme, (as was the case with the loose smut locus and two polymorphisms generated by Eco RV).

DISCUSSION

The cross between a normal wheat and an alien translocation line in which the long arm is replaced will allow pairing only of the wheat short arms. Traits in the short arm only are free to recombine, allowing linkage analysis. Because a testcross was done with the alien substitution line, homozygous recombinant lines were produced for the 6AS chromosome arm. The process of generating the homozygous recombinant lines is similar to the process of developing recombinant substitution lines described by Jones et al. (1991). Homozygous recombinant lines allow the lines to be propagated indefinitely without disturbing, due to crossing over, the linkage relationship of genes in the short arm of 6A.

The most reliable results from this study were those for loose smut reaction to race T19, and gliadin evaluation for which the population size was the largest. These data indicated that the T19 resistance of chromosome 6A was not linked to the gliadin locus in the same chromosome. The rust data were the least reliable because of the small sample size, and because of the poor difference in reaction between resistant and susceptible types. There was a preponderance of parental types for both the rust resistance and gliadin combination and the rust resistance and loose smut resistance combination. Detection of a weak linkage between the rust resistance locus and both the gliadin and the loose smut resistance loci may be possible. With a larger sample size, precision of the linkage estimate could be improved. Better conditions for expression of the Sr8 gene, or the use of a different race which may

better detect the Sr8 gene, could also improve the reliability of this linkage analysis.

Although a number of studies have described linkage to seed protein loci (McIntosh (1988); Jones et al. (1990); Czarnecki and Lukow (1992)), only a few studies have shown linkage between RFLPs and disease or pest resistance loci in wheat (Chao et al. 1989; Mena et al. 1992; Conner et al. 1993). Only a limited number of markers are available at this time for such studies in wheat. However, in the present study, an association was found between an RFLP and the loose smut resistance gene, although further testing is required to determine if this is a chance occurrence or if it is truly linked, and to answer the question why the polymorphism did not show up in the parents. A linkage value cannot be assigned because the parents do not show the polymorphism. However, if the most prevalent types are assumed to be the parental types, the recombination frequency is eight percent. A recombination frequency of eight percent would make this RFLP a useful marker. One possibility for the lack of polymorphism in the parents is that only one plant of each parent was used for DNA extraction and a genetically aberrant plant may have been chosen. In which case in future studies more parental plants should be used as a DNA source.

Because the long arm of Cadet is replaced by the 6Ag short arm, the Cadet 6AgS:6AS/Chinese Spring Sr8//Cadet 6Ag(6A) population will provide the opportunity in the future to determine the recombination frequency of wheat short arm traits with the centromere. Therefore, any marker identified for the long arm of Chinese Spring Sr8 can be used as a centromere marker, because the long arm will remain unchanged throughout meiosis. Because appropriate polymorphism for the group 6 long chromosome arms could not be found from the probes used in this study, possible linkage of the loose smut resistance to the centromere could not be tested. In addition to RFLP markers, a number of isozymes may act as centromere markers in this population, or as markers for the

loose smut resistance gene. Isozymes which have been associated with the 6A chromosome (Hart et al. 1980; McIntosh 1988) include esterase (Jaaska 1980), aminopeptidase (Hart and Langston 1977), aconitase (Chenicek and Hart 1987), glutamic oxaloacetic transaminase (Hart 1975), alpha amylase (Nishikawa and Nobuhara 1971; Gale et al. 1983), and dipeptidase, (Goldenberg 1986).

In conclusion, the gliadin locus is not a suitable marker for race T19 loose smut resistance located in the 6A chromosome, but an RFLP is promising.

CHAPTER 6

The Genetics of Loose Smut Resistance in Wheat

ABSTRACT: Information on the number and location of loose smut resistance genes residing in various wheat cytogenetic stocks, lines used as loose smut differentials, and cultivars commonly used in breeding programs can assist in their further genetic analysis and use in resistance breeding. A number of sources of resistance, and cytogenetic stocks were inoculated with up to eight races of *U. tritici* (T2, T6, T8, T10, T15, T19, T31, and T39). A survey of a number of small segregating populations from crosses designed to locate loose smut resistance to chromosomes allowed preliminary information on the number of genes segregating, because of inoculation of each progeny with a series of races. Some lines demonstrated multi-gene segregation for loose smut resistance. Both dominant and recessive genes were found. A gene for resistance to race T15 in the cultivar H44 was located in chromosome 6A by segregation analysis, and evidence was found that a gene resistant to race T10 was located in chromosome 2B in the cultivar Chinese Spring. The location of the T10 resistance was found by inoculation of Chinese Spring-Thatcher substitution lines. Preliminary information about the number of genes can be obtained by the inoculation of each member of small populations with a number of races. Information obtained about the location of loose smut resistance genes from the inoculation of cytogenetic stocks can help to focus future genetic research.

INTRODUCTION

Many sources of loose smut resistance in wheat have been identified, several of which display complete resistance to existing races (Anderson 1961; Nielsen 1983). Multiple genes seem to contribute to resistance in at least some of the *U. tritici* race differential lines (Chapter 4 of this thesis; Tingey and Tolman 1934). Few cytogenetic stocks have been evaluated for resistance to loose smut (McIntosh 1988), with the exception of the Chinese Spring-Hope and Chinese Spring-Thatcher inter-varietal substitution lines (Dhitaphichit et al. 1989). Inoculation of such cytogenetic stocks can immediately yield useful information such as the influence of Hope and Thatcher chromosomes 7A and 7B respectively on dramatically reducing the incidence of infection by race T6 (Dhitaphichit et al. 1989). Not only will breeding for resistance to loose smut benefit from the direct knowledge of number and combinations of genes involved in resistance, but benefits will be seen through the better characterization of races and the easier development of genetic markers (Allard 1960, Fleischmann and Baker 1971, Morris 1987). The assessment of cytogenetic stocks for loose smut resistance will allow for their systematic use, as well as occasionally providing information on chromosome location should the stocks bring together the right combination of genetic factors, such as a susceptible chromosome substituted for a resistant chromosome (Chapter 4 this thesis; Dhitaphichit et al. 1989). The determination of the genetic makeup of the lines used to differentiate races will contribute to the development of single-gene differential lines, and to a set of lines which can be used as a reference set to which all other genes can be compared systematically (Allard 1960, Fleischmann and Baker 1971). Characterized genes in resistant lines used in breeding can be compared to previously identified genes, so that new genes or alleles of existing genes can be identified (Dyck and Kerber 1985). Knowledge of the chromosomal

location of a gene helps in the gene identification process (Bariana and McIntosh 1993). Knowledge of the number of genes and dominance relationship of alleles helps in locating genes to chromosomes, and knowledge of the chromosomal location of genes helps in the development of markers to those genes (Morris 1987).

The purpose of this chapter is to report preliminary information on the number and nature of genes found in a number of differential and resistant stocks. Information on the reaction of cytogenetic stocks to races of loose smut is also provided, so that genes can be more efficiently located to chromosomes. Preliminary testing of cytogenetic stocks and lines of potential value in breeding or genetic studies was done to allow an understanding of segregation when these lines were intercrossed, and to confirm findings reported by others such as Anderson (1961) and Nielsen (1983 and 1987a).

The differential lines of Nielsen (1987a) and three highly resistant Canadian hexaploid wheats (Glenlea, H44 and HY377) were used as parents in crosses in the present study. Tentative evidence for a resistance gene in chromosome 1B from HY377 was obtained from the backcross development of this cultivar (Howes, personal communication). HY377 is a backcross line possessing a protein band that occurred in the loose smut resistant donor parent, but not the recurrent loose smut susceptible parent. The protein was known to be coded by chromosome 1B. The recurrent parent allele was expected to be transferred to HY377 after five backcrosses, but was not. In the present study a cross was made with HY377 onto the Prelude monosomic 1B (M1B) cytogenetic stock to try to confirm the location of a 1B loose smut resistance gene. The cultivars Glenlea and H44 were evaluated because they possess complete resistance to all races in the Nielsen collection (Nielsen 1983) and are used as a source of resistance in breeding programs.

MATERIALS AND METHODS

F_2 Populations were established using Rescue 6Ag(6A), Rescue 6Ag(6B), Rescue 6Ag(6D), and Cadet 6Ag(6A) as the female parents in selected crossing combinations with the cultivars Glenlea, H44, Kota, Red Bobs, TD2, TD3, TD6, TD9, and TD16. Testcross populations were established using Roblin nullisomic 1B (N1B) as the female in crosses with Kota, Canthatch, TD2, and TD18, with the resulting single cross F_1 plants crossed with the susceptible Prelude. Prelude M1B was used as the female in a cross with HY377 and the subsequent monosomic F_1 was crossed with Prelude. Segregation of the 1B chromosome was evaluated by the same methods as reported in Chapter 3 using monoclonal antibody P24B as a chromosome marker, and segregation of the 6A chromosome was evaluated using the same methods reported in Chapter 4 using monoclonal antibody 230/9 as a chromosome marker. Polyacrylamide gel electrophoresis was used to track protein bands as chromosome markers for the populations in which the 6B and 6D chromosomes were being studied. Electrophoretic methods and staining of gels are described in Chapter 4. Progeny of these populations were inoculated with one or more of races T2, T6, T8, T10, T15, T19, T31, and T39. The procedures used for inoculation were the same as those described in Chapter 4.

Inter-varietal substitution lines were grown as described for lines in Chapter 4. The Chinese Spring-Thatcher lines were those developed by J. Kuspira (Genetics Department, University of Alberta), and maintained and provided by Dr. P.L. Dyck (Agriculture Canada Research Station, Winnipeg, Manitoba). The Cadet-Rescue lines were those developed by R.I. Larson, and maintained and provided by Dr. E.D.P. Whelan (Agriculture Canada Research Station, Lethbridge, Alberta). The Chinese Spring-Thatcher inter-varietal substitution lines were evaluated with races T6, T10, T15, T19, T31, and T39 to identify chromosomes affecting resistance to these races. The inter-varietal substitution lines of Cadet-Rescue were inoculated with races T8, T15, and T19 to identify

chromosomes affecting resistance to these races.

RESULTS

The results of inoculations of progeny of segregating populations will be provided first, followed by the results of inoculations of the substitution lines. The proportion of loose smut in the parental lines, from the inoculation with up to eight races, are listed in Appendix 1 (results of inoculations of other lines not discussed in this chapter are also found in Appendix 1). The interpretation of the segregation of the progeny of populations derived from these cytogenetic stocks and lines is based on the loose smut reactions of these lines.

Figure 6.1 (the values for proportion of smutted plants on the horizontal axis represent the mid-point of the interval for which the endpoints are marked by ticks, for this and subsequent figures) demonstrates that those progeny of the Rescue 6Ag(6A)/H44 population missing the 6A chromosome were, in general, more susceptible to loose smut race T15 than those progeny possessing the 6A chromosome. The most susceptible plants of this population were characterized as weak and much less fertile than plants in the resistant group. One plant which derived from a seed with a low antibody-binding had a low loose smut infection, and was a vigorous, fertile plant like the plants derived from high antibody-binding seed.

The results obtained from the remaining populations, in which chromosomes 1B, 6A, 6B and 6D segregated, did not show the chromosomal location of any further genes. These populations did provide some indication of the presence of alleles dominant for loose smut resistance, and, for those populations inoculated with more than one race, an indication of the number of genes was also obtained. The presence of dominant genes was the most likely explanation for the results of segregation of populations involving: Glenlea to all races tested (Fig. 6.2, 6.9D); Kota and Cadet 6Ag(6A) to T15 (Fig. 6.3A); TD6

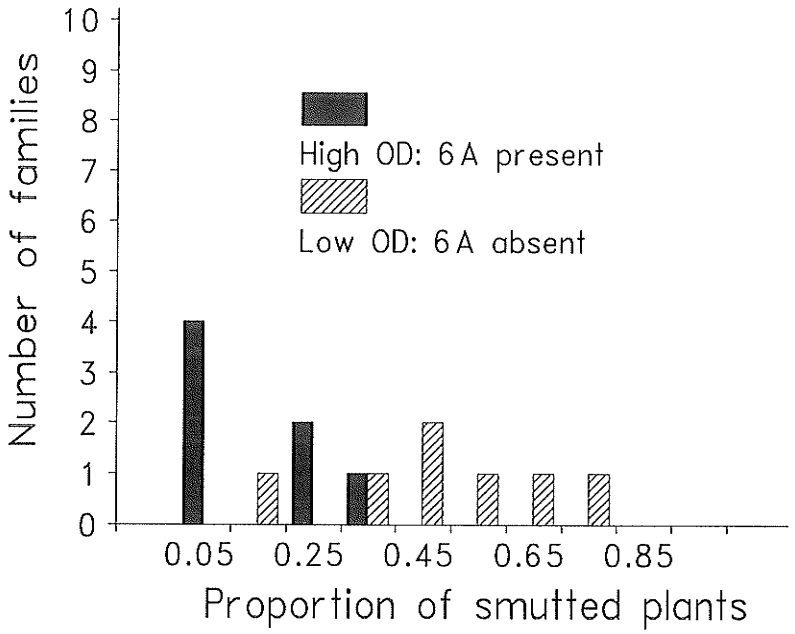


Fig 6.1. Distribution of progeny of the Rescue 6Ag(6A)/H44 population inoculated to race T15.

to T15 and T31 (Fig. 6.3D and E); TD16 to T10 and T31 (Fig. 6.4D and E); Canthatch to T19 and T39 (Fig. 6.6C and D); HY377 to T2, T10, T31, and T39 (Fig. 6.7A, B, D, and E); TD9 to T15 (Fig. 6.9B); and TD2 to T19 (Fig. 6.9C). Recessive genes or genes with partial dominance are indicated in the results of segregation of: TD6 to races T8 and T10 (Fig. 6.3B and C); TD3 to T10 (Fig. 6.4B and Fig. 6.8B) and T8 (Fig. 6.8A); TD16 to T8 (Fig. 6.4C); Red Bobs to T8 and T10 (Fig. 6.8C, Fig. 6.4A and 6.8D); Roblin N1B to T10 and T15 (Fig. 6.5A and B); Kota to T19 (Fig. 6.5C); TD18 to T10 and T19 (Fig. 6.6A and B); HY377 to T19 (Fig. 6.7C); and TD2 to T39 (Fig. 6.9C). Not enough data was available to assess the allelic relationship for resistance to races T8 and T10 in TD2, although, considering Fig. 6.9A and 6.9C, complete dominance did not seem to occur for any race except, perhaps, to race T19.

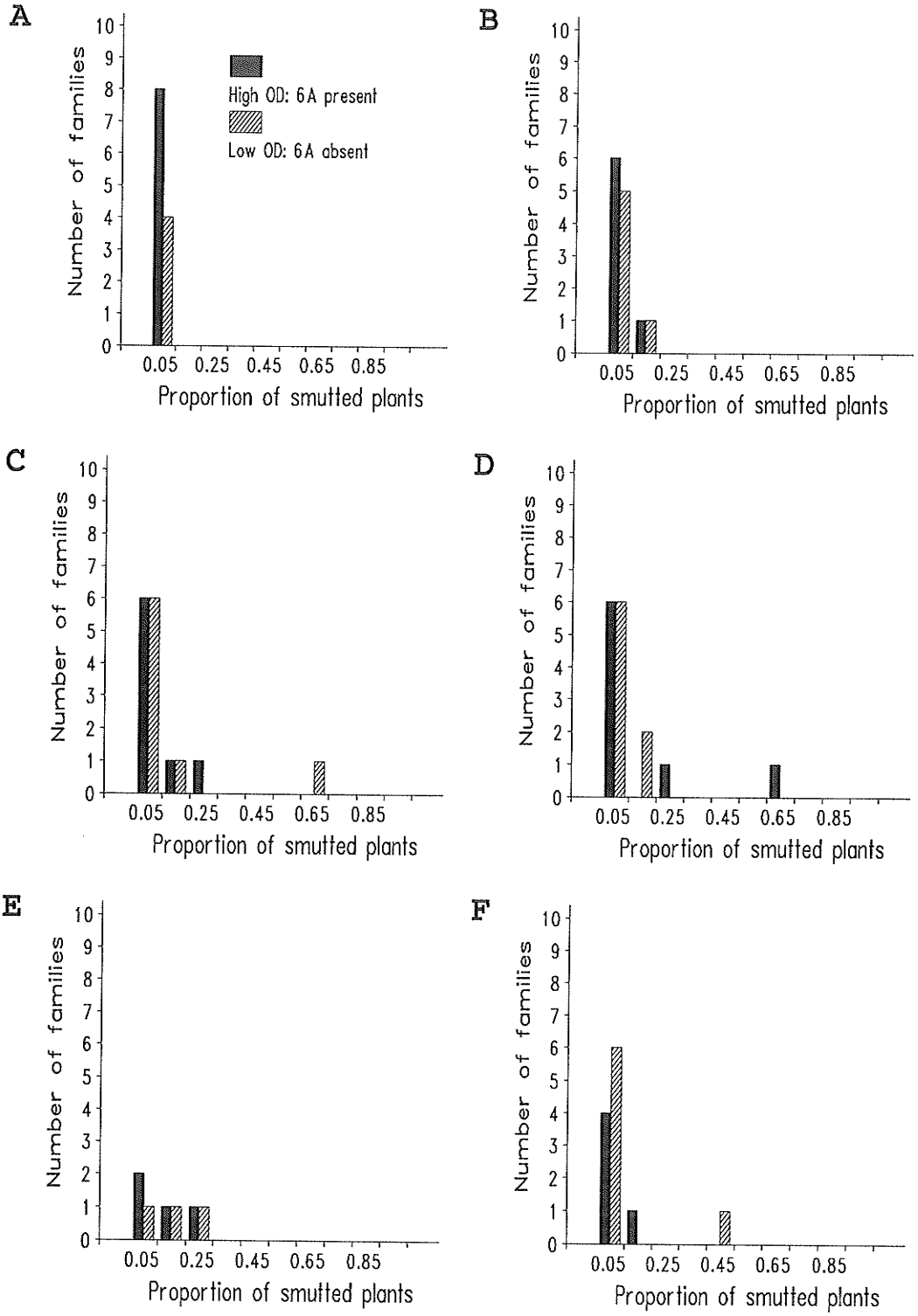


Fig. 6.2. Loose smut reaction of families of the population Rescue 6Ag(6A)/Glenlea inoculated with (A) T8, (B) T10, (C) T15, (D) T19, (E) T31, (F) T39.

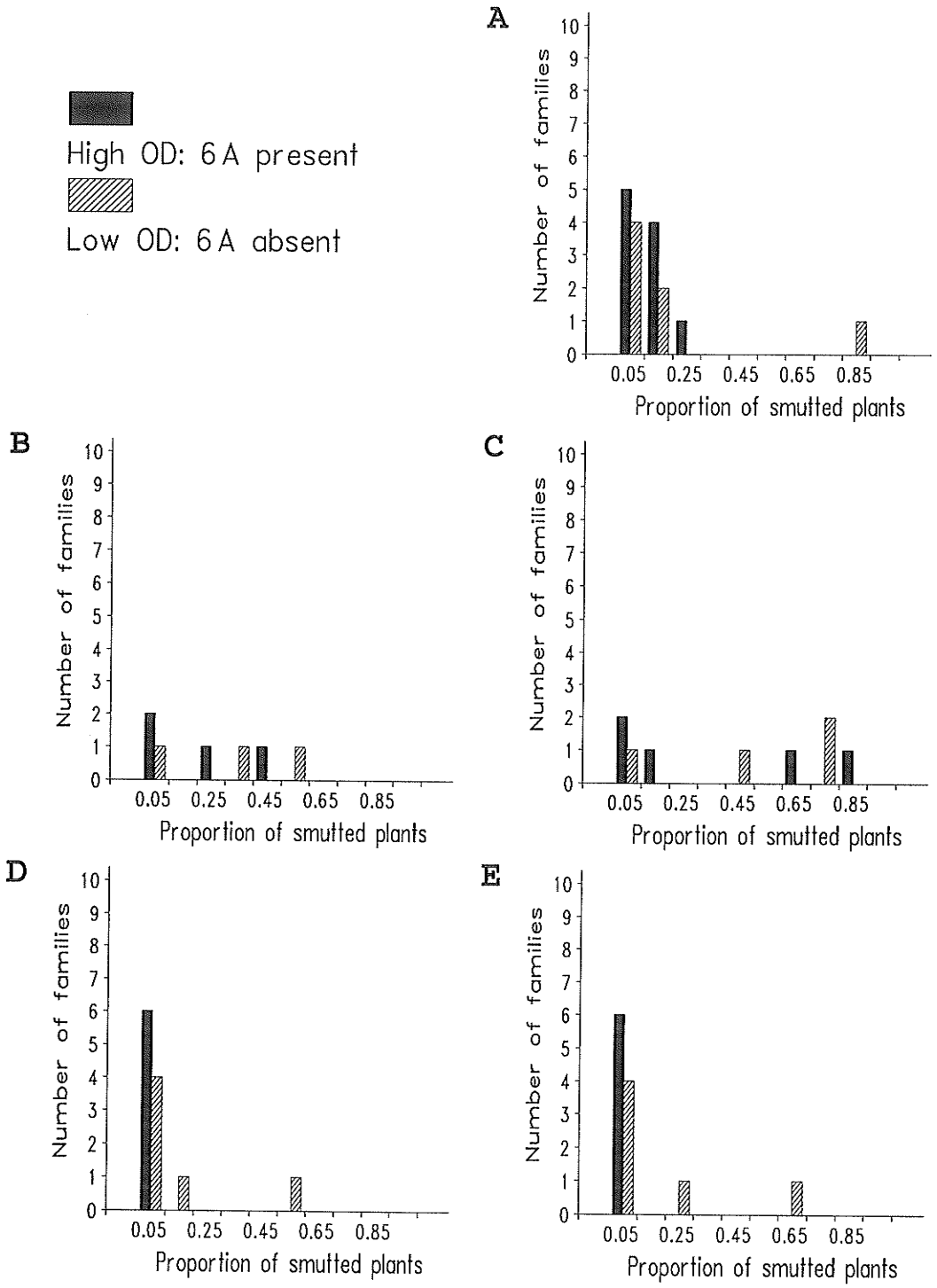


Fig. 6.3. Loose smut reaction of families of the population Cadet 6Ag(6A)/Kota inoculated with (A) T15, and Rescue 6Ag(6A)/TD6 with (B) T8, (C) T10, (D) T15, (E) T31.

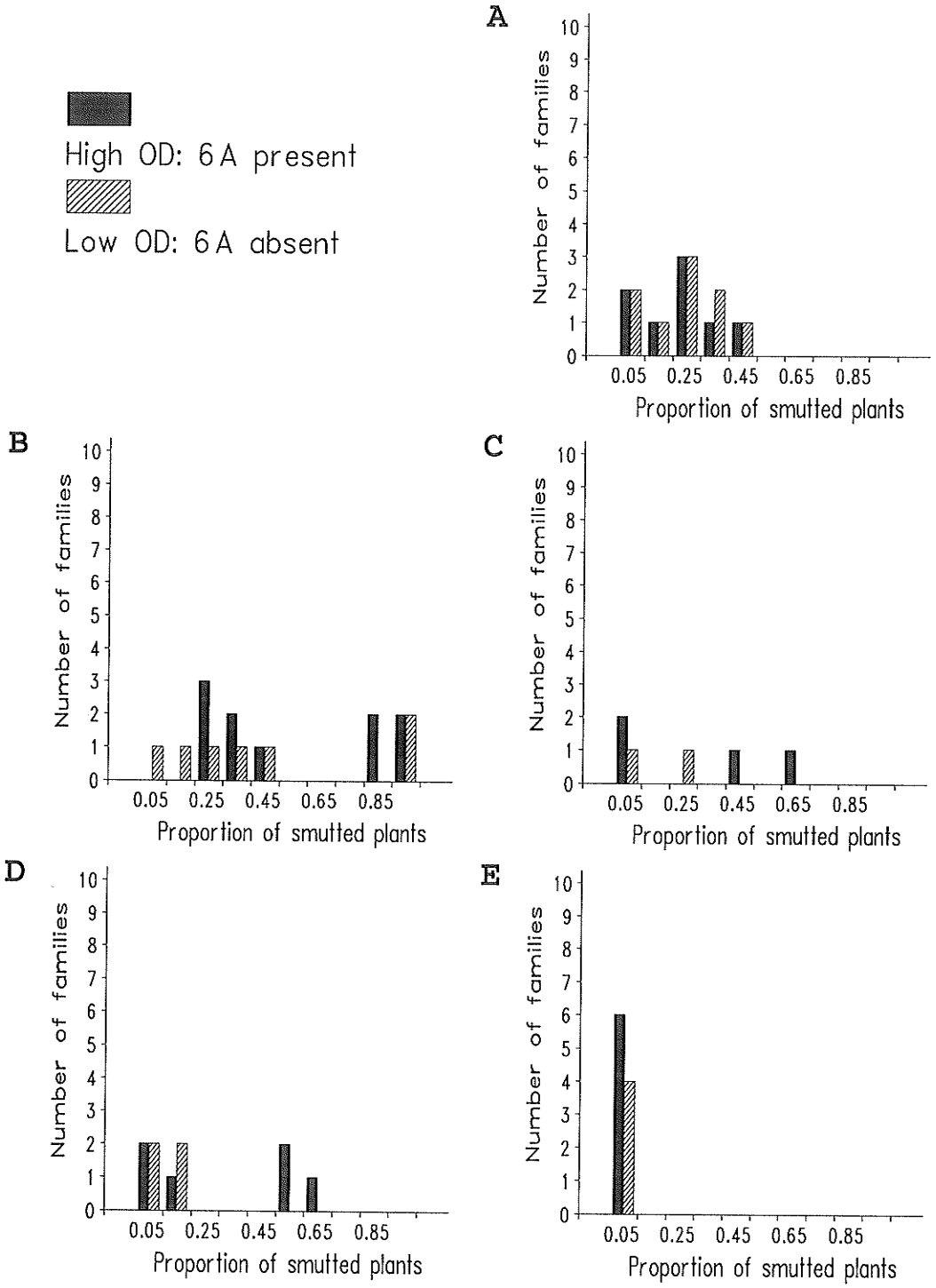


Fig. 6.4. Loose smut reaction of families of the population Cadet 6Ag(6A) crossed with (A) Red Bobs and inoculated with T10, (B) TD3 and T10 and Rescue 6Ag(6A) crossed and inoculated with (C) TD16 and T8, (D) TD16 and T10, (E) TD16 and T31.

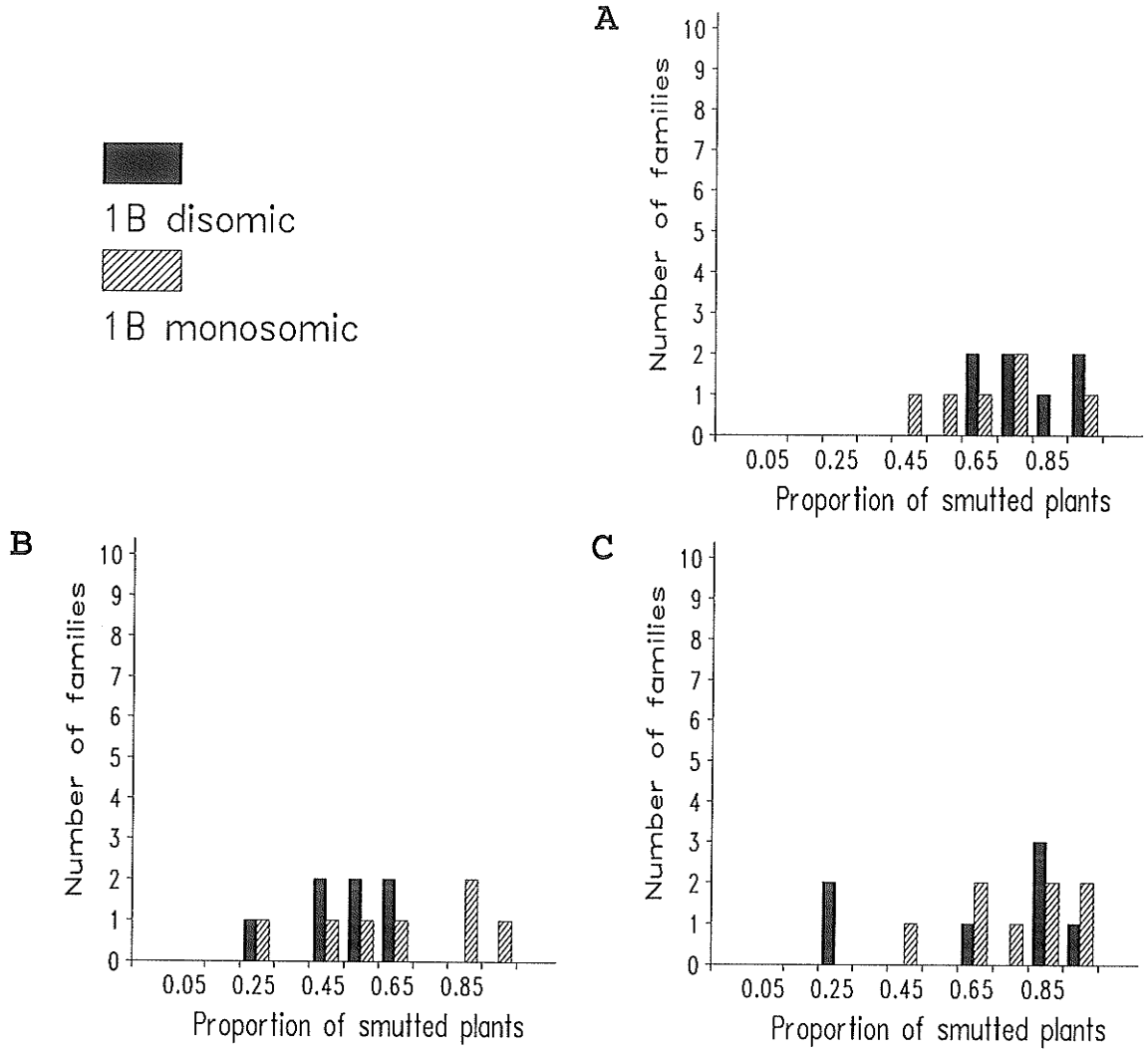


Fig. 6.5. Loose smut reaction of families from populations of Roblin N1B crossed onto a Kota and testcrossed with Prelude and inoculated with races (A) T10, (B) T15, (C) T19.

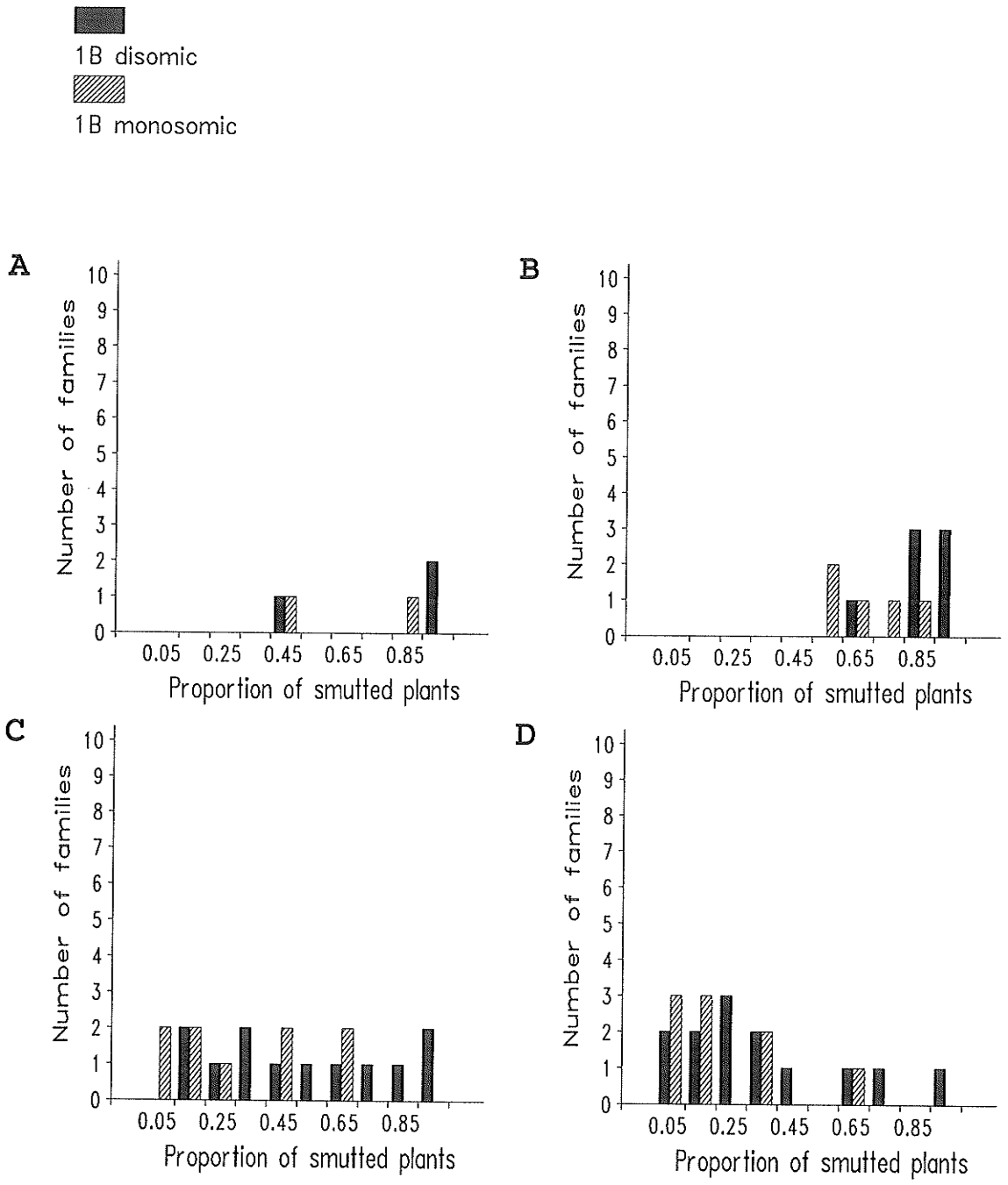


Fig. 6.6. Loose smut reaction of families of the populations on which a loose smut resistant line was crossed onto Roblin N1B and test crossed with Prelude, cultivar and race are as follows (A) TD18 and T10, (B) TD18 and T19, (C) Canthatch and T19, (D) Canthatch and T39.

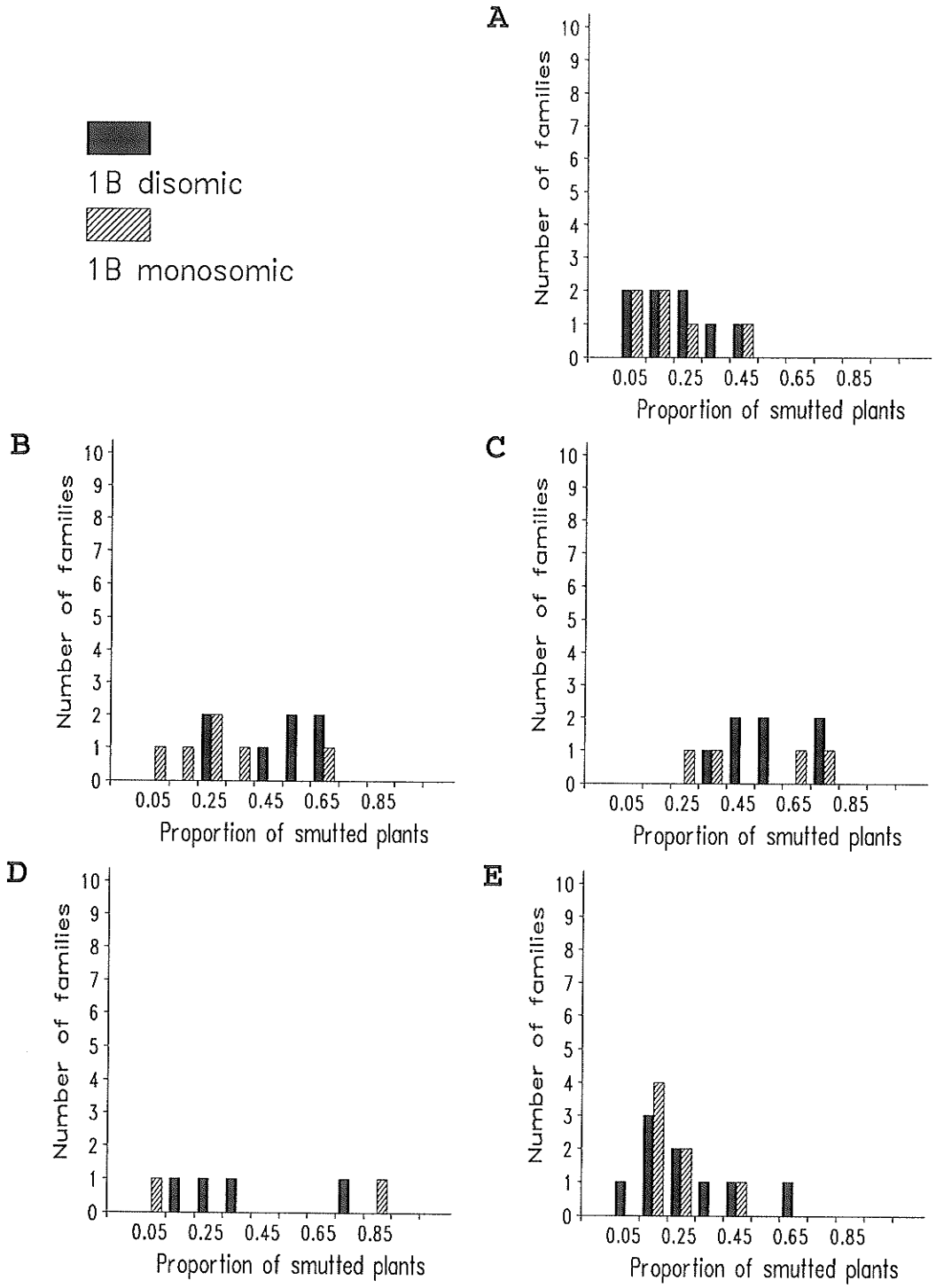


Fig. 6.7. Loose smut reaction of families from the population with which HY377 was crossed onto Prelude M1B and test crossed with Prelude, inoculated with (A) T2, (B) T10, (C) T19, (D) T31 (E) T39.

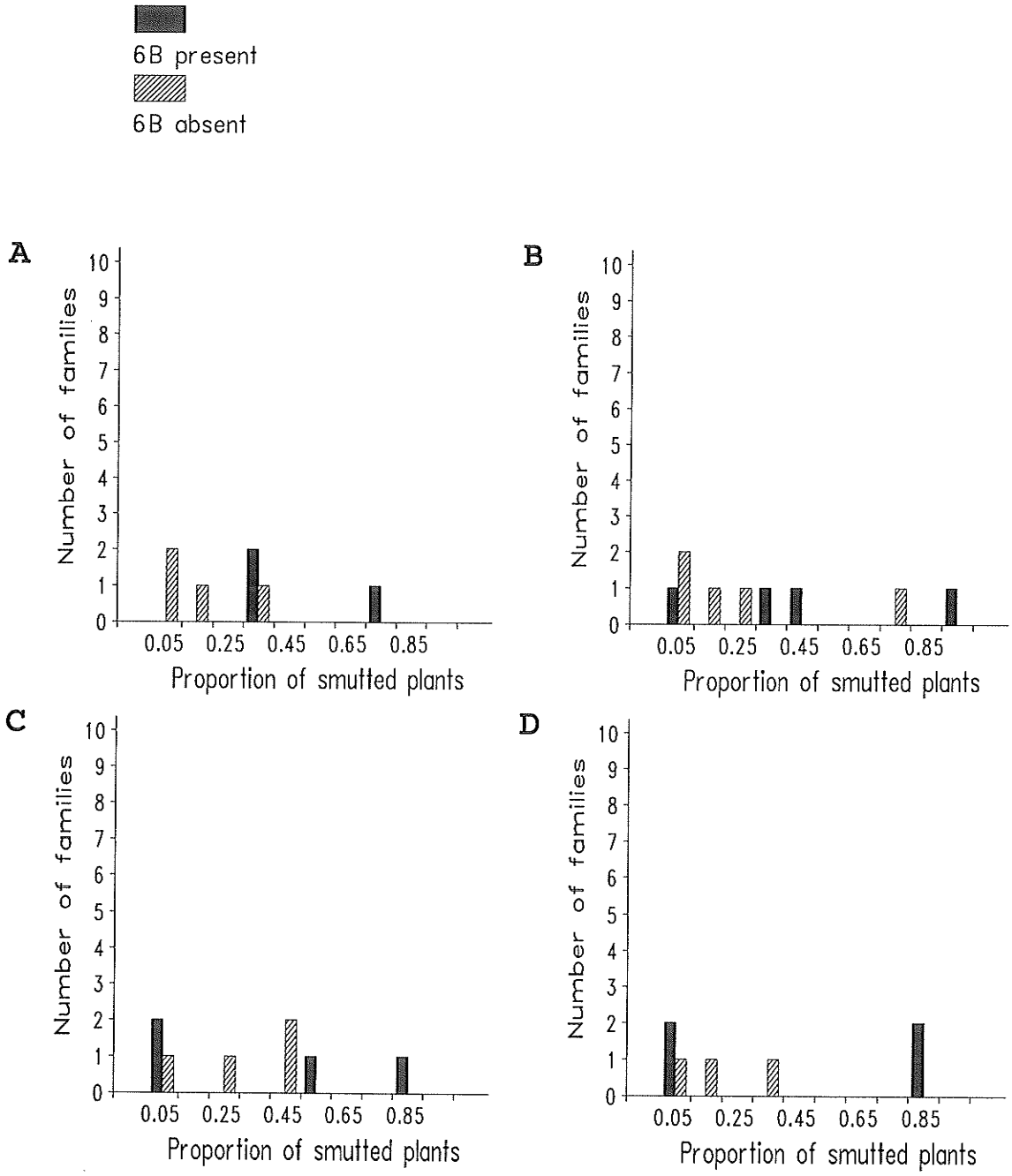


Fig. 6.8. Loose smut reaction of families from the populations in which Rescue 6Ag(6B) was crossed and inoculated with (A) TD3 and T8, (B) TD3 and T10, (C) Red Bobs and T8, (D) Red Bobs and T10.

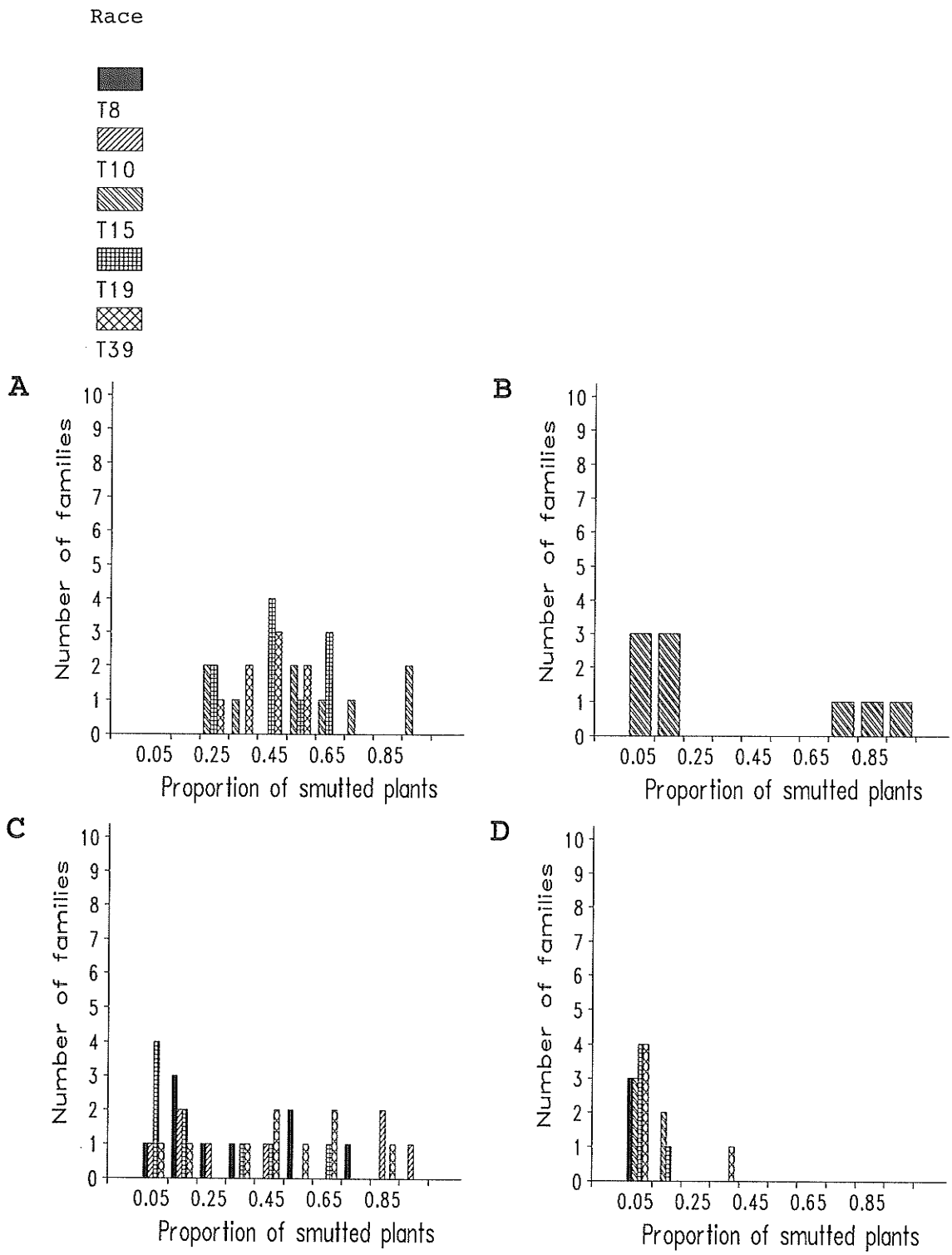


Fig. 6.9. Loose smut reaction to various families from the populations of (A) Roblin N1B/TD2/Prelude, (B) Rescue 6Ag(6A)/TD9, (C) Rescue 6Ag(6B)/TD2, (D) Rescue 6Ag(6D)/Glenlea.

More than one gene segregating was determined by the differential segregation of the same progeny to different races. Some of the populations tested to more than one race indicated more than one segregating gene. The inoculation of the Rescue 6Ag(6A)/Glenlea population with three different races (T15, T19, and T39) resulted in a different susceptible segregate to each race (Fig. 6.2C, D and F). There was a preponderance of resistant types to all races, and no susceptible segregates occurred upon inoculation with races T8, T10, and T31 (Fig. 6.2A, B and E).

The two lines of the Rescue 6Ag(6A)/TD6 population which were most susceptible to race T31 were also the most susceptible to race T15, indicating that the gene or genes in TD6 resistant to race T31 were the same genes conferring resistance to T15 (Fig. 6.3D and E). Comparing Fig. 6.3B and C to Fig. 6.3D and E shows that more susceptible segregates occurred when the progeny were inoculated with races T8 or T10 than when inoculated with T15 or T31. Most families susceptible to race T8 were also susceptible to race T10. Only one family differed in this respect.

In the Cadet 6Ag(6A)/Kota population (Fig. 6.3A), both parents were resistant to race T15. One susceptible segregate was produced, indicating that Cadet 6Ag(6A) and Kota each possess different single dominant genes for resistance to race T15. When the same progeny were inoculated with races T15 and T19, the progeny most susceptible to T15 were not the same as the progeny most susceptible to T19 (Chapter 4).

No highly susceptible segregates to race T10 were observed among the 17 families from the population Cadet 6Ag(6A)/Red Bobs (Fig. 6.4A). Like the progeny of the Cadet 6Ag(6A)/Red Bobs population, the resistant segregates from the population Cadet 6Ag(6A)/TD3 were only moderately so (Fig.6.4B). The progeny of the Rescue 6Ag(6A)/TD16 population most susceptible to T8 (Fig. 6.4C) were the same progeny which were most susceptible to T10 (Fig. 6.4D) from the cross of . The progeny

susceptible to races T8 and T10 were resistant to T31 because no segregates susceptible to race T31 were identified (Fig 6.4 E).

In the testcross population of Roblin N1B/Canthatch//Prelude resistant progeny predominated when inoculated with T39 (Fig. 6.6D), but not when inoculated with T19 (Fig. 6.6C). Like Canthatch, HY377 was evaluated in a testcross, and most progeny clustered toward the resistant end of the spectrum when inoculated with T2 (Fig. 6.7A) and T39 (Fig. 6.7E), and to the susceptible end when inoculated with T19 (Fig. 6.7C). The two progeny most susceptible to race T31 (Fig. 6.7D) were not among the progeny most susceptible to other races. In a testcross involving Kota, a susceptible phenotype to race T19 predominated in most of the progeny (Fig. 6.5C). Kota and Roblin are resistant to T15, whereas only Roblin is resistant to T10. The progeny of the cross Roblin N1B/Kota//Prelude were susceptible to race T10 (Fig. 6.5A) and T15 (Fig. 6.5B), as mentioned earlier, indicating segregation of a recessive gene or genes.

Susceptible and moderately susceptible segregates were common among the progeny of TD3 and Red Bobs when inoculated with either T8 or T10 (Fig. 6.8). The segregates most susceptible to race T8 are the same progeny susceptible to T10 when TD3 was the parent (Fig. 6.8A and B), and similarly for Red Bobs (6.8C and D).

Results of inoculation of Cadet-Rescue and Chinese Spring-Thatcher inter-varietal substitution lines are highlighted in the remainder of this section. Thatcher was found to be susceptible to *U. tritici* races T10, T31, T39, with incomplete resistance to race T19, and certain Chinese Spring sources were susceptible to T6 and T15 (Table A.4). Cadet is resistant to races T8, T15, and T19 (Table A.1), and Rescue is susceptible to races T15 and T19, with incomplete resistance to race T8 (Table A.2). Table 6.1 lists the loose smut reaction of the series of Cadet varietal substitution lines, in which each Rescue chromosome was substituted separately into Cadet, replacing its Cadet homologous chromosome counterpart. The data were incomplete for race T8, but the

Table 6.1. Loose smut race T8, T15 and T19 reaction of Cadet substitution lines into which individual Rescue chromosomes have been substituted

Race	T8				T15				T19			
Line	mean	sd	n	rep	mean	sd	n	rep	mean	sd	n	rep
Cdt Res 1A	.	.	.	.	0.91	0.37	69	3	0.12	.	17	1
Cdt Res 1B	0.00	.	13	1	0.00	0.00	58	3	0.07	.	14	1
Cdt Res 1D	0.00	.	26	1	0.00	0.00	68	3	0.04	.	23	1
Cdt Res 2A	0.00	.	12	1	0.00	0.00	66	3	0.18	0.96	62	3
Cdt Res 2B	0.00	.	19	1	0.00	0.00	111	4	0.02	0.12	60	2
Cdt Res 2D	0.00	.	13	1	0.00	0.00	56	3	0.03	0.15	38	2
Cdt Res 3A	0.62	0.30	39	2	0.84	0.15	62	3	0.00	0.00	35	2
Cdt Res 3B	0.00	0.00	26	2	0.00	0.00	86	3	0.05	0.02	39	2
Cdt Res 3D	.	.	0	0	0.79	0.44	52	3	0.15	0.25	33	2
Cdt Res 4A	0.00	0.00	32	2	0.00	0.00	75	3	0.00	0.00	40	2
Cdt Res 4B	0.00	0.00	31	2	0.00	0.00	55	2	0.00	.	6	1
Cdt Res 4D	0.00	0.00	67	3	0.00	0.00	80	3	0.03	.	31	1
Cdt Res 5A	0.00	.	32	1	0.00	0.00	43	2	0.00	0.00	42	2
Cdt Res 5B	.	.	.	.	0.82	0.04	87	3	0.15	0.24	40	2
Cdt Res 5D	0.00	0.00	46	2	0.00	0.00	84	3	0.03	0.15	34	2
Cdt Res 6A	0.81	0.99	47	2	0.78	0.92	83	3	0.84	0.44	56	2
Cdt Res 6B	0.00	0.00	52	2	0.00	0.00	76	3	0.00	.	13	1
Cdt Res 6D	0.00	0.00	41	2	0.00	0.00	57	3	0.11	.	19	1
Cdt Res 7A	0.00	0.00	35	2	0.00	0.00	61	3	0.07	0.53	42	2
Cdt Res 7B	.	.	.	.	0.71	0.58	63	3	0.00	.	21	1
Cdt Res 7D	0.00	0.00	46	2	0.00	0.00	100	3	0.32	0.16	62	2

.= No data, sd = standard deviation, n = number of plants rated, rep = number of inoculated heads, Cdt = Cadet, Res = Rescue where the chromosome designation is the chromosome of Rescue substituted into Cadet.

values that were obtained matched line for line those of race T15 (Cadet and Rescue reactions were 0.0 and 0.72 smutted to race T15, respectively). Unexpectedly, six lines were highly susceptible to race T15. The Cadet-Rescue 6A line highly susceptible to race T19 should be noted (Cadet and Rescue reactions were 0.11 and 0.55 smutted to race T19 respectively).

The data from the Chinese Spring-Thatcher substitution series were most clear-cut for three races (Table 6.2). The single high rating to race T10 when the Thatcher chromosome 2B is substituted for the Chinese

Table 6.2. Loose smut race T6, T10, T15, T19, T31 and T39 reaction of Chinese Spring substitution lines into which individual Thatcher chromosomes have been substituted

Race	T6		T10		T15	
Line	mean	n	mean	n	mean	n
CS Tc 1A	0.00	25	0.00	5	0.67	30
CS Tc 1B	0.68	31	0.00	11	0.66	32
CS Tc 1D	0.07	14	0.00	6	0.62	13
CS Tc 2A	0.08	12	0.00	20	0.76	33
CS Tc 2B	0.59	34	0.75	20	0.88	25
CS Tc 2D	0.30	30	0.00	19	0.62	50
CS Tc 3A	0.67	24	0.00	33	0.60	25
CS Tc 3B	0.00	15	0.00	18	0.72	25
CS Tc 3D	0.59	27	0.00	33	0.71	41
CS Tc 4A	0.23	30	0.00	21	0.87	23
CS Tc 4B	0.54	26	0.00	19	0.76	29
CS Tc 4D	0.69	29	0.00	33	0.71	34
CS Tc 5A	0.20	60	0.00	29	0.49	41
CS Tc 5B	0.88	25	0.00	9	0.70	30
CS Tc 5D	.	.	0.00	2	0.67	21
CS Tc 6A	0.33	33	0.00	6	0.86	42
CS Tc 6B	0.42	45	0.00	6	0.74	19
CS Tc 6D	0.00	32	0.00	14	0.42	19
CS Tc 7A	0.30	20	0.00	11	0.37	19
CS Tc 7B	0.00	16	0.00	7	0.00	18
CS Tc 7D	0.17	29	0.00	26	0.74	27

Race	T19		T31		T39	
Line	mean	n	mean	n	mean	n
CS Tc 1A	.	.	0.00	38	0.00	9
CS Tc 1B	0.00	4	0.00	31	0.00	16
CS Tc 1D	0.00	14	0.00	20	0.00	15
CS Tc 2A	0.00	9	0.00	18	0.00	22
CS Tc 2B	1.00	2	0.00	33	0.37	27
CS Tc 2D	.	.	0.00	15	0.00	28
CS Tc 3A	0.06	17	0.00	32	0.00	14
CS Tc 3B	0.00	5	0.00	24	0.00	8
CS Tc 3D	0.00	20	0.00	33	0.00	14
CS Tc 4A	0.00	26	0.00	17	0.00	10
CS Tc 4B	0.00	23	0.00	15	0.00	20
CS Tc 4D	0.00	18	0.00	25	0.00	10
CS Tc 5A	0.00	34	0.00	36	0.00	30
CS Tc 5B	.	.	0.00	22	0.43	21
CS Tc 5D	.	.	0.00	9	0.00	1
CS Tc 6A	0.00	18	0.00	39	0.00	3
CS Tc 6B	0.00	17	0.00	40	0.23	13
CS Tc 6D	0.00	13	0.00	37	0.00	8
CS Tc 7A	0.00	16	0.00	19	0.00	27
CS Tc 7B	0.00	9	0.39	23	0.49	72
CS Tc 7D	0.00	22	0.00	33	0.00	20

.= No data, n = number of plants CS = Chinese Spring, Tc = Thatcher, where the chromosome designation is the chromosome of Thatcher substituted into Chinese Spring.

Spring 2B, indicated that the Chinese Spring resistance to race T10 resides in chromosome 2B (Chinese Spring and Thatcher reactions to race T10 are 0.0 and 0.96 respectively). Likewise, the only susceptible reaction to race T31 in the Chinese Spring-Thatcher series was for the chromosome 7B line, which indicated that resistance to this race resides in chromosome 7B (Chinese Spring and Thatcher reactions to race T31 were 0.0 and 0.73 respectively). The Chinese Spring-Thatcher 7B line was the only line fully resistant to race T15 (Chinese Spring and Thatcher reactions to race T15 were 0.61 and 0.0 respectively). Limitations on the number of heads available for inoculation meant not all inoculations could be accomplished with race T19. Four lines showed no incidence when inoculated with T6, with one of these lines being Chinese Spring-Thatcher 7B. Inoculation of the Chinese Spring-Thatcher substitution lines with T39 produced the unusual result of four susceptible. The level of loose smut infection to T39 was moderate in each of the four lines (Chinese Spring and Thatcher reactions to race T39 are 0.0 and 0.64 respectively).

DISCUSSION

The data demonstrate the occurrence of genes with different levels of dominance. Multiple genes for resistance are indicated by inoculation results from both the segregating populations and the inter-varietal substitution lines. Evidence for the number of genes acting in a particular genotype comes from the different reaction of the same progeny to different races. Ribeiro (1963) used two races to help sort out the number of resistance genes to loose smut, while Ward and Metzger (1970), and Metzger et al. (1979) used multiple races to indicate the number of resistance genes segregating against common bunt. The proportion of resistant to susceptible progeny also gives an idea of the number of genes involved (Heyne and Hansing 1955). A population inoculated to different races can demonstrate different levels of

dominance to each of the races. This may indicate different resistance genes or modifying factors (Samborski 1963). Combining these forms of evidence from multiple populations, which agree in terms of the number of genes estimated, strengthens the conclusions about the number of genes in the resistant parent.

When most of the F_2 progeny of a segregating population are resistant, dominance is indicated (Heyne and Hansing 1955), which was the case with most of the populations studied. Conversely, when most of the progeny are susceptible and a parent is known to be resistant, then the gene or genes are recessive (Gaskin and Schafer 1962). If the progeny cluster in the midrange region, then the resistance is partially dominant (Lamari and Bernier 1991). Examples of both of these latter situations were also seen. Considering Table 2.4 of Chapter 2, the appearance of dominant, partially dominant, and recessive alleles is not surprising.

An argument can be made to support the hypothesis that Glenlea possesses at least three genes for resistance. The progeny from the population Rescue 6Ag(6A)/Glenlea susceptible to races T15, T19 and T39 differed, indicating that each progeny had a resistance gene; however, the proportion of resistant to susceptible progeny either approached or was a 15:1 ratio, depending on the race, indicating two genes for resistance to any one race, for a total of three genes. Metzger and coworkers (1979) used the same strategy to help determine the number of common bunt resistance genes involved in the line CI7090. The lack of progeny susceptible to races T8, T10, and T31 further supports the hypothesis of more than one gene for resistance in Glenlea (Fig. 6.2). That Glenlea would have three genes for resistance is not surprising, considering its background. One of the parents of Glenlea is derived from lines known to possess high levels of loose smut resistance, including Pembina, Thatcher, Redman, Regent, Marquis and H44 (Anderson 1961, Nielsen 1983, Zeven and Zeven-Hissink 1976). Tingey and Tolman (1934) reported three genes for loose smut resistance in Hope, a sib of

H44 (Zeven-Hissink 1976). The other parent of Glenlea (CB100) is also derived from lines possessing loose smut resistance (Nielsen personal communication). An important implication of the finding that virulence exists to the genes making up the resistance in Glenlea, should it be borne out with more detailed study, is that virulence exists to an important source of resistance used in breeding of Canadian prairie wheat. All that is required to overcome the Glenlea resistance is a recombination of the existing virulence genes. Another consideration is that when Glenlea is used as a source of resistance, a large breeding population will be required to recover all three genes. One segregate moderately susceptible to race T39 was produced out of the five members of the Rescue 6Ag(6D)/Glenlea population, indicating as little as one gene, or providing evidence that a gene resides in chromosome 6D. The population size of Rescue 6Ag(6A) was not big enough to determine whether or not any of the genes are in the 6A chromosome of Glenlea. The population size would have to be big enough (about 40 progeny in each chromosome class (Hanson 1959)) to distinguish between a 3:1 ratio in the class missing the 6A chromosome, and a 15:1 ratio in the class in which the 6A chromosome is present.

The simplest explanation of the segregation data from the TD6-derived population is that two genes are segregating (Fig. 6.3B to E). Resistance to races T8 and T10 is common because the susceptible segregates were, for the most part, the same for the two races, and the gene appears to be partly dominant. Resistance to races T15 and T31 is common because the susceptible segregate was the same for the two races, and the gene appears to be dominant. Progeny segregating for a partially dominant gene to one group of races, and for a dominant gene to a second group of races, is an indication of two genes. That a gene resides in 6A in TD6 cannot be ruled out. TD6 is the line PI69282 and was used by Nielsen (1987a) in place of the late-maturing cultivar van Hoek from Oort's differential set (Nielsen 1987a).

Both Kota and Cadet 6Ag(6A) are resistant to T15. The segregation of resistance from Kota when crossed onto Cadet 6Ag(6A) would indicate that these two cultivars differ in two dominant genes for resistance to race T15 (Fig. 6.3A). The testcross of Roblin N1B/Kota with Prelude does not contradict dominance to race T15 in Kota (Fig. 6.5B). Segregation to T31 was also dominant (Fig. 4.2D). The testcross of Roblin N1B/Kota//Prelude indicates that resistance in Kota to race T19 is recessive. The difference in reaction of the progeny of the Cadet 6Ag(6A)/Kota to race T19 and T31 indicated two genes in Kota (Chapter 4). The one dominant and one recessive response to two different races is a further indication of two genes for resistance in Kota. Ribeiro (1963) reported that Kota possesses a single dominant gene to the race designated as C2, although in a study by Kilduff (1933) Kota was the susceptible parent. Kota was added to Oort's differential set by Cherewick (Nielsen 1987a).

Only one partly dominant gene seemed to be segregating from Red Bobs (Fig. 6.8C and D). Kilduff (1933) used Red Bobs as a loose smut resistant source in a study of the genetics of loose smut resistance. Although he did not feel confident enough to make a conclusion about the inheritance of resistance in Red Bobs, his data indicate a single dominant gene. Red Bobs, like Kota, was added to Oort's differential set by Cherewick (Nielsen 1987a). TD3 displayed incomplete dominance to races T8 and T10, and only one gene can be said to be segregating (Fig. 6.8A and B). TD3 is a cultivar derived from Flourence X Aurore, and is one of Oort's original differential lines (Nielsen 1987a). The segregation pattern of TD16 is best explained by two genes, because of the lack of a susceptible type from the 10 progeny inoculated with race T31, yet susceptible progeny segregated when inoculated with T8 and T10 (Fig. 6.4C to E). One gene would seem to confer resistance to both race T8 and T10. TD16 is the line Marroqui 588, and was added to the differential set by Nielsen (1987a).

All chromosome 1B analyses were set up as testcrosses. No chromosome association with resistance was seen; however, there are some interesting points to note. The testcross can give an indication of the dominance of alleles. Testcross F_1 plants are all either heterozygous resistant or homozygous susceptible, if resistance is dominant (Ribeiro 1963). Roblin N1B was obtained from a monosomic 1B plant from Roblin breeder seed (Chapter 3). Roblin N1B has the same plant type and seed protein profile as Roblin except for the missing 1B protein bands in the nullisomic when seed proteins were run using PAGE. Roblin is resistant to race T10 (Table A.4). The resistance in Roblin N1B to race T10 is either recessive, or Roblin, the progenitor of Roblin N1B, has lost a gene for resistance (Kota is susceptible to T10) (Fig. 6.5A).

Indications are that T19 resistance in TD18 recessive, like that in Kota. TD18 is the line C.T. 439, and was added to the differential set by Nielsen (1987a). Recessive resistance to T19 in TD18 and Kota would support a hypothesis that TD18 and Kota possess the same gene. As indicated in Chapter 4, TD18 may have two other dominant genes for resistance. This is not surprising, considering that TD18 has both Thatcher and Regent in its background (Zeven and Zeven-Hissink 1976).

Two dominant genes were indicated to race T39 in Canthatch (Fig. 6.6D) because of a greater number of resistant to susceptible progeny in the testcross, where a 3:1 segregation indicates two genes (Ribeiro 1963). Neither the number of genes involved, nor the gene action for resistance to race T19 is indicated (Fig. 6.6C). A study by Campbell (1948) indicated at least one gene for loose smut resistance in Thatcher, and from his data the gene appeared to be dominant. Canthatch is a backcross derivative of Thatcher (Zeven and Zeven-Hissink 1976).

Two genes can explain the segregation in crosses involving HY377 (Fig. 6.7). Resistance to races T2, T31, and T39 shows up as dominant, whereas resistance to T10 and T19 seems to be only partly dominant, although dominance reversal cannot be ruled out. It is reasonable to

assume that two genes confer resistance to races T2 and T39, because of few susceptible segregates to these two races. Furthermore, T31-susceptible progeny are resistant when inoculated with other races.

There appears to be a single dominant gene segregating to race T15 in TD9 (Fig. 6.9B). TD9 is the cultivar Kearney, and was added to the differential set by Nielsen (1987a). Resistance in TD2 may be partly dominant (Fig. 6.9A and C). TD2 is Renfrew, and is one of Oort's original differential lines (Nielsen 1987a).

The Cadet-Rescue inter-varietal substitution lines were originally developed to evaluate milling and baking quality (Kosmolak et al. 1980). Rescue and Cadet also differ in their loose smut reaction to various races (Tables A.1 and A.2). Inoculation of the Cadet-Rescue substitution lines with different *U. tritici* races produced some unexpected results. Two of the substitution lines tested to race T8 were susceptible (four lines were not inoculated), and six lines were susceptible to race T15. Possible explanations for multiple line susceptibility include that either Rescue or Cadet were heterogeneous for resistance to races T8 and T15, or that a univalent shift may have occurred during the development of certain lines (Gale et al. 1989). As well, mixing may have occurred during the handling of the seed stocks. Translocations or mutations would also be difficult to detect, although it is difficult to explain six susceptible lines when only one was expected. There is no evidence to indicate that the resistance to race T15 is different from the resistance to race T8. Even though the data is questionable, the results still suggest a focus for future research. It is likely that one of the six susceptible lines truly represents the Cadet chromosome possessing the resistance gene to T15, which has been knocked out by substitution with one of the Rescue chromosomes. A more detailed study of six chromosomes in cytogenetic stocks is a substantial reduction in effort from analysis with 21 cytogenetic stocks. The inoculations of the Cadet-Rescue series with race T19 provided clear

evidence that the T19 resistance is in chromosome 6A, which supports the findings discussed in Chapter 4. The levels of infection for the other lines are within the expected range of infection for Cadet.

Some of the same problems of high infection on more than one substitution line occurred, as well, with inoculations of Chinese Spring-Thatcher substitution lines (Table 6.3). The Chinese Spring-Thatcher lines were developed to assess many traits, but not loose smut resistance (Kuspira and Unrau 1957). Chinese Spring is resistant to T10, T19, T31, and T39, whereas Thatcher is susceptible to these races (Table A.4). Conversely, Thatcher is resistant to races T6 and T15, yet Chinese Spring is susceptible. Therefore, inoculation of these substitution lines offered an opportunity to locate resistance genes in Chinese Spring when knocked out by a susceptible Thatcher chromosome, and to locate resistance genes in Thatcher when a particular Thatcher chromosome makes Chinese Spring resistant to race T6 or T15.

It is not unreasonable to believe that the gene conditioning resistance to race T10 in chromosome 2B of Chinese Spring also conditions resistance to race T19, because of the similar reaction of the lines to these two races. Results for T6 and T39 are less straightforward, and may be due to factors previously described as a possible explanation for six susceptible lines in the Cadet-Rescue substitution line series. Considering that race T6 virulence is not broad across the differentials, indicating this race has few virulence genes (Nielsen 1987a), it would not be surprising that Thatcher has more than one gene for resistance to race T6, although four genes in four chromosomes seems a very high number. Dhitaphichit et al. (1989) also inoculated the Chinese Spring-Thatcher substitution lines to race T6, and they found infection in all lines but the Chinese Spring-Thatcher 7B. They concluded that the major factor controlling loose smut resistance in Thatcher is in chromosome 7B. The fact that four lines are susceptible to race T39 is most difficult to explain. It is

interesting that one susceptible line to race T39 is Chinese Spring-Thatcher 2B (chromosome 2B of Chinese Spring seems to confer resistance to race T10), and a second is Chinese Spring-Thatcher 7B (chromosome 7B of Chinese Spring seems to confer resistance to race T31). It is also interesting that only partial susceptibility occurs. Perhaps there is a series of chromosomes, each contributing some resistance to race T39, such that only partial resistance occurs when genes are removed, but when together, complete resistance occurs. Tingey and Tolman (1934) proposed that such a mechanism may occur in the cultivar Hope.

T15 resistance in Thatcher was demonstrated to be in chromosome 7B, whereas resistance to T31 in Chinese Spring resides in chromosome 7B. If they are at the same locus, obviously they must be different alleles. However, it is possible that more than one loose smut resistance locus occurs in chromosome 7B, which would favour breeding for resistance, because multiple loci can be recombined so resistance genes are together, whereas multiple alleles cannot. Resistance to T6 may be conferred by the gene that confers resistance to T15 in Thatcher.

In summary, this report provides evidence either through segregation ratio or differences in race infection of different progeny, that more than one loose smut resistance gene occurs in lines in the loose smut race differential set or their progenitors (Kota, Thatcher, TD18, TD6, TD10, and TD16) and in Glenlea and HY377. Examples of dominance, partial dominance, and recessive allele interaction exists. Because of the small sample size used in the present study, further work needs to been done to be conclusive as to the nature of the genetic makeup of the differentials and of key genetic sources of resistance used in breeding programs. If Glenlea does carry three genes for resistance, and only the combination of genes prevents disease, it follows that if this source of resistance became widespread it may not be long-lasting. The dependency of breeding for resistance on a narrow gene base emphasizes the need for molecular markers to genes for resistance to allow

pyramiding of genes, even into a background such as Glenlea for which no known race possesses all the required corresponding virulence.

The evidence that a second resistance gene to race T15 may reside in chromosome 6A in H44 should be followed up using a larger population. It can be shown that the putative H44 gene cannot be the same gene as that controlling resistance to race T19 in Kota, because Kota is susceptible to race T15. However, the H44 resistance may be allelic to T19 resistance, which could be determined by an intercross of H44 and a T19 resistant source.

Evidence that the T19 resistance in Kota and TD18 is recessive comes from the testcross progeny of populations Roblin N1B/Kota//Prelude and Roblin N1B/TD18/Prelude. That the T19 resistance is recessive, and in chromosome 6A strengthens the hypothesis that the T19 resistance in Kota and TD18 is due to the same gene. Stronger evidence that one gene is shared among these three cultivars would come from crossing each combination and determining whether or not segregation had occurred. Inoculation of the Cadet-Rescue chromosome substitution series provided further evidence that a gene conferring resistance to race T19 resides in chromosome 6A.

The information on race T15 from the Cadet-Rescue substitution lines indicates that further study should be focused on chromosomes 1A, 3A, 3D, 5B, 6A, and 7B. Cadet is a derivative of Thatcher (Zeven and Zeven-Hissink 1976), and Chinese Spring was made resistant to race T6 when the Chinese Spring chromosome was replaced by the Thatcher 7B chromosome, as determined in a study by Dhitaphichit et al. (1989). These results were confirmed in the present study, and inoculation of these substitution lines with T15, to which Chinese Spring is susceptible, showed that when the Thatcher 7B chromosome replaces the Chinese Spring 7B, resistance occurs. All of these pieces of information strongly support the contention that a loose smut resistance gene in Thatcher is located in chromosome 7B. The inoculation of the Chinese Spring-Thatcher

substitution lines further indicates a resistance gene to race T10 in Chinese Spring residing in chromosome 2B. This information combined with the results of Chapter 4, that a T19 resistance gene resides in chromosome 6A of Thatcher, would indicate Thatcher has three genes for loose smut resistance.

Future work should follow up with segregation analysis of the tentative gene locations determined by inoculations of inter-varietal substitution lines. Identifying the chromosomal location and intercrossing of the differential lines is required to help identify the genes and to produce single-gene lines for loose smut resistance. Such single gene lines will aid in better discrimination of races and the identification of new loose smut resistance genes.

GENERAL DISCUSSION

A monoclonal antibody to a seed storage protein coded by the 1B chromosome was used as a chromosome marker. The MAb chromosome marker was used to track the 1B chromosome in a hybrid population in which one of the parents was nullisomic for the 1B chromosome. Through the use of such a cytogenetic stock, the 1B chromosome could segregate without recombination of the genes within the chromosome. Although a MAb to a seed storage protein has several advantages as a chromosome marker, there are drawbacks to the use of nullisomic lines, the biggest being low fertility. In an effort to avoid the fertility problems of nullisomic lines, the usefulness of other cytogenetic stocks was investigated. Alien chromosome substitution lines tend to compensate for the loss of a homologous pair of chromosomes (Sears 1969). A MAb to a chromosome 6A seed protein was used as a chromosome marker in a population in which one parent was an alien substitution line. Not only did the study confirm the value of a MAb as a marker, but also demonstrated that alien substitution lines can be easier to work with. By using MAbs in conjunction with the appropriate cytogenetic stocks, chromosome analysis with a microscope is reduced to investigating the occasional unusual result based on MAb analysis, and the population size can be reduced compared to monosomic analysis. Use of the MAb chromosome marker/alien chromosome substitution line allowed the chromosomal location of a loose smut resistance gene to be in chromosome 6A. With the knowledge of the chromosomal location of the loose smut resistance gene, the focus of attention was turned to linkage analysis and mapping of the loose smut resistance gene with other chromosome 6A traits. An association of the loose smut resistance gene with an RFLP was found, although no association could be found between the loose smut resistance gene and the gliadin locus. The use of several races to

inoculate each population allowed discrimination of more than one segregating gene even when the population size was small.

Sharp and coworkers (1989) point out the convenience of using RFLPs as chromosome markers because they provide a uniform protocol of analysis used to mark all traits, compared to isozymes, for example, which require many different assays. An ELISA is a uniform protocol for the analysis of monoclonal antibodies, and such a test is easier to perform than either RFLP or isozyme analysis. When MAbs are used to assay seed proteins, they provide a genetic marker method with a level of efficiency unlike any other marker protocol. However, because the potential number of seed protein gene loci is low relative to the number of RFLP loci, seed proteins and MAbs to those proteins should be considered mainly as chromosome markers and not as general genetic markers. Another disadvantage to MAbs is the large amount of effort required to obtain a suitable antibody (Klein 1990). Seed proteins observed through electrophoresis are more simply evaluated than RFLPs, and sufficient seed protein loci have been identified to mark each chromosome in wheat (Table 2.5). RFLP analysis requires a relatively large sample of DNA, too much to be obtained from the endosperm end of a seed. Therefore, RFLP analysis requires the germination of seeds and their development into a tillering plant to provide sufficient DNA, whereas seed protein analysis can be done on a portion of the endosperm of a resting seed. By using seed proteins as markers, the seed does not have to be germinated and grown until after the results of the assay are known.

Random amplified polymorphic DNA (RAPD) marker analysis is a promising alternative to seed proteins as chromosome markers. RAPD analysis requires less DNA than RFLP analysis (Williams and St. Clair 1993), however, a question to be answered is whether or not enough DNA can be extracted from an ungerminated seed to make this method as efficient as seed protein analysis. Another drawback to the use of both RFLPs and

RAPDs in wheat is the lack of polymorphism (He et al. 1992; Dweikat et al. 1993; Joshi and Nguyen 1993), which may be overcome through the use of denaturing gradient gel electrophoresis (He et al. 1992; Dweikat et al. 1993). Being able to detect chromosome dosage based on the amount of binding of the MAb is another advantage of the MAb. Classification of progeny by the number of chromosomes, in addition to identity of the chromosome, improves the efficiency of the analysis. RAPD markers tend not to be codominant (Devos and Gale 1992). The inability to distinguish heterozygotes, hemizygotes or double monosomics diminishes the value of RAPD markers as chromosome markers.

An important condition for the success of any chromosome marker technique is that the chromosome be inherited without recombination taking place so that a correlation between a particular trait and its chromosome can be established. Although crosses were performed between a loose-smut-susceptible nullisomic and a loose-smut-resistant source, a testcross was required to recover the aneuploid gametes. Monosomic lines were suggested as a substitute for nullisomic lines (Chapter 3), however, one more evaluation step is required to confirm that the monosomic line is indeed monosomic and a testcross is still required to recover the aneuploid gametes. Alien substitution lines were used with good success. Advantages of the alien substitution lines were the good fertility of the lines, and, as a result, the lack of a requirement of a testcross. An alien substitution line lacks a homologous pair of chromosomes, but this deficiency is compensated for by the addition of a pair of homoeologous chromosomes (Sears 1969). When the loose-smut-susceptible 6 *Agropyron* alien substitution line was crossed to a loose-smut-resistant line, a double monosomic was generated in which a single 6A chromosome from the resistant parent was present with a single 6Ag chromosome from the susceptible alien substitution line. Because of the lack of pairing between the alien chromosome and the wheat chromosome, no recombination can occur within the wheat chromosome.

Loose smut resistance is located in the 7A and 7B chromosomes, based on the inoculation of inter-varietal substitution lines (Dhitaphichit et al. 1989), the drawbacks to the use of which were previously described (Chapter 4). This study successfully used the MAb chromosome marker/ alien substitution line method to locate a loose smut resistance gene to chromosome 6A in four cultivars. Inoculation of inter-varietal substitution lines and alien chromosome substitution lines confirmed the gene location in the cultivar Cadet. Inoculation of a ditelosomic and an alien translocation line provided evidence that the loose smut resistance gene in the cultivar Cadet is in the short arm of chromosome 6A. Inoculation of the Chinese Spring-Thatcher substitution lines confirmed the results of Dhitaphichit and coworkers (1989) that a gene resides in chromosome 7B. Evidence of a gene for resistance in Chinese Spring in chromosome 2B was also found.

A question that needs to be addressed is why locate genes to chromosomes at all? The location of genes to chromosomes is part of the process used to identify and characterize a gene (Bariana and McIntosh 1993). Locating a gene to a chromosome makes mapping the gene easier, and allows the more efficient determination of linkage with markers already mapped to the same chromosome. A putative RFLP marker was found by knowing the chromosomal location of the gene and focusing linkage analysis on RFLPs associated with that chromosome. Knowledge of the 6A chromosomal location of the loose smut resistance gene provided the opportunity to do linkage analysis with the gliadin locus although no linkage was detected. RAPD markers can be evaluated quickly compared to RFLP markers (Devos and Gale 1992), for example, making it feasible to concentrate on the development of a marker to a single gene. A map of RAPD markers would, however, allow the more efficient development of markers to specific genes if the chromosomal location of the gene is known. Linkage analysis could be performed between the gene and all RAPD markers known to be in the same chromosome, rather than to all RAPD

markers.

When considering the use of genetic markers, it is important to consider the drawbacks as well as the advantages so that the most useful markers can be found: 1. The marker must be more efficient to use than the evaluation of the trait it replaces (i.e., cheaper, faster, less labour intensive); 2. The marker must be sufficiently closely linked (up to 15 centimorgans would be useful) to the trait of interest to provide a high probability that under selection for the marker the trait will be pulled along, ideally with the marker being pleiotropic with the trait of interest; 3. The marker cannot confer any detrimental effect on individuals which carry the marker, and, ideally, the marker itself should be a trait with positive effects; 4. The marker must be easily identified; 5. The marker must be in a *cis* configuration with the trait so that the known allele can be transferred without concern that detrimental alleles exist for the same marker locus; and 6. Allelic forms of the marker must exist, with the rare allele of the marker being most useful.

The location of a loose smut resistance gene to a chromosome, using a monoclonal antibody as a chromosome marker, in conjunction with an alien chromosome substitution line, indicates that not only can loose smut resistance genes be located to chromosomes with this method, but that other genes could be located to chromosomes in a similar manner. If monoclonal antibodies were developed to seed proteins coded by each chromosome, and a complete set of alien substitution lines were brought together, any gene could be located to any chromosome using this method. These seed protein loci, in conjunction with cytogenetic stocks, could be used to determine the chromosomal location of genes controlling traits of interest, and thus make the search for genetic markers easier.

The work here is just the beginning of what can be done not only in loose smut genetics, but in the development of molecular chromosome markers. Future research can take a number of directions, including:

the study of the inheritance of sources of loose smut resistance; the breakdown of sources of loose smut resistance into single-gene components to simplify marker development and to provide an improved race differential set; the development of MAbs to the seed proteins coded by additional chromosomes in wheat; the determination of the chromosomal location of additional loose smut resistance genes; and genetic analysis to identify closely linked markers which could be applied in breeding programs. More specific questions arising from this research include: is the T19 resistance in chromosome 6A in Kota, Thatcher, Cadet and TD18 allelic; and does the race T15 resistance gene in H44 reside in chromosome 6A, and is it allelic to the T19 resistance gene? Segregation analysis should be done to confirm the location of a Chinese Spring loose-smut-resistance gene in chromosome 2B, and, similarly, to follow up on the work done by Dhitaphichit and coworkers (1989) for the location of resistance genes in chromosomes 7A and 7B. As new markers located to chromosome 6A become available, linkage analysis should be done with the 6A loose smut resistance gene in an attempt to develop a more closely linked marker in the event that the Cadet gene, or a more effective allele of the Cadet gene should become important in breeding. The T19 loose smut resistance gene should be isolated into a susceptible genetic background to determine how effective the gene is against the spectrum of races, and to determine its penetrance in the absence of other genes for resistance.

CONCLUSION

A gene for resistance to loose smut race T19 was shown to be in chromosome 6A, after the classification of segregating populations for chromosome make-up and for loose smut reaction. Chromosomal location analysis is very efficient when monoclonal antibodies are used as chromosome markers in conjunction with alien chromosome substitution lines. The population size used to locate a loose smut resistance gene to a chromosome was 20 or fewer. The location of a gene to a chromosome facilitates the analysis of linkage with other traits and markers located to the same chromosome. Analysis of the race T19 loose smut resistance indicated that the gliadin locus in chromosome 6A was not linked; however, an RFLP located to the 6A chromosome might be associated with the resistance. It should be possible to develop molecular markers linked to loose smut resistance genes, for use in breeding for resistance to loose smut.

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APPENDIX A

INTRODUCTION

This appendix lists a series of lines inoculated with up to eight races of *U. tritici*. The data is presented mainly for reference purposes. Although, some unusual findings are pointed out in the discussion section to follow. These results were not included in a separate chapter in the body of the thesis because the data as it stands was not considered strong enough for any conclusions to be made.

MATERIALS AND METHODS

Lines listed in the tables of this appendix were grown and inoculated as described for lines in Chapter 4. The Chinese Spring-*Aegilops umbellulata* (CS-U),and -*Hordeum chilense* (CS-Hch) lines were provided by Dr. T.E. Miller (Cambridge Laboratory, Colney Lane, Norwich, UK.), the Chinese Spring-*Agropyron intermedium* (CS-Ai) lines were provided by Dr. R.A. McIntosh (University of Sydney, Plant Breeding Institute, Cobbitty, NSW, Australia), the Chinese Spring-Hope (CS-Hope) substitution lines were those of Dr. J. Kuspira, maintained and provided by Dr. P.L. Dyck (Agriculture Canada Research Station, Winnipeg, Manitoba), the Cadet and Rescue lines were provided by Dr. E.D.P. Whelan (Agriculture Canada Research Station, Lethbridge, Alberta) and the loose smut differential lines (TD-) were provided by Dr. J. Nielsen (Agriculture Canada Research Station, Winnipeg, Manitoba).

RESULTS AND DISCUSSION

Table A.1 provides a list of reactions of all the Cadet cytogenetic stocks evaluated during this study. The most significant results were pointed out in Chapter 4. However, a few things are worth noting. The progeny of the double monosomic 6A/6Ag population inoculated to race T19 were highly susceptible providing evidence that the T19 resistance is recessive. Substitution of the wheat chromosomes 4A, 4D and 5D with the alien chromosomes 4Ag, 4Ag and 5Ag respectively did not affect the level of resistance of Cadet to any of the races tested. Substitution of the 6A chromosome with the 6Ag in addition to affecting resistance to T19 also seemed to affect the level of expression to race T15. Whereas, all other Cadet lines were completely resistant to T15 Cadet 6Ag(6A) showed 13% loose smut. This is an indication that loose smut resistance may work jointly to produce cumulative effects in some cases.

Table A.2 summarizes data of races inoculated onto various Rescue cytogenetic stocks. There were no interesting reactions that made any sense. Three reactions that did not make sense were the lack of infection in Rescue 6Ag(6A) to race T2, Rescue 6Ag(6D) to race T2 and Rescue 6Ag(6D) to race T31. In general Rescue and the Rescue lines produced quite variable infection responses both among and within race-line combinations (see standard deviations in Table A.2 compared to, for example, the standard deviations for the *U. tritici* race differentials in Table A.3). Inconsistent inoculation may be responsible for these extreme deviations. Another explanation is that heterogeneity occurs among the *Agropyron* chromosomes, however, these usually derive from one stock. Alternatively heterogeneity in Rescue may be another explanation.

Inoculations of the differentials were done to verify race reactions and the results for the most part reflected the expected reaction (Table

A.3) (Nielsen 1987). Discrepancies which should be noted were resistance to T15 by Red Bobs (TD10), moderate susceptibility of Kota (TD4) to race T19, susceptibility of TD3 to T31. The reactions of Red Bobs (TD10) and Kota (TD4) can be explained based on heterogeneity of the sample. The genetic research was initiated on Red Bobs and Kota before consideration had been given to the use of the differentials as the genetic studies. For these two lines the inbred differential was not used for race evaluation, but instead the original variety had been used. The problem of heterogeneity was noticed too late in the study for re-evaluations of lines to be performed. However, the consistency of reactions of the other differentials to the races used in this study compared to the reactions expected based on Nielsen (1987) would indicate the races are correctly classified. There is no explanation for the resistance in TD3 to race T31.

A number of other lines were inoculated to evaluate levels of resistance for a variety of reasons. Some were inoculated for evaluation as suscepts, some as sources of resistance. Some were inoculated as cytogenetic stocks to see if chromosome variation affected the level of resistance. A series of lines were evaluated to determine whether or not Canthatch resistance was due to loose smut resistance being carried with stem rust resistance during the development of Canthatch or to see if the recurrent parent Thatcher was heterogeneous for resistance.

Canthatch received from Dr. Eric Kerber (Agriculture Canada, Winnipeg), Canthatch RL2936, Egypt Na101/Mq12/12, FKN Prelude16 Sr7, Hope 4B 5aR, Kenya Farmer, Lee6/KF, Prel 4 Sr7b, Red Thatcher, Tc7/KF, Thatcher and Thatcher RL1945 were inoculated with race T39. The common element of several of these lines is the possession of the Sr7 stem rust resistance gene at various levels of backcrossing. The Sr7 gene was introduced into Canthatch or derivatives of Thatcher. The backcross lines were evaluated to determine if resistance to race T39 was associated with Sr7 and the different sources of Thatcher were inoculated to determine if heterogeneity existed within Thatcher. The low reaction of Canthatch relative to Thatcher to race T39 (Table A.4) was what spurred the study. The Eric Kerber Canthatch is a single plant selection which subsequently was further inbred several generations. A look at Canthatch RL2936 showed a higher reaction to T39 with a high standard deviation. The results were based on up to five inoculated heads from up to five different plants. These results suggest heterogeneity. Comparison of three Thatcher lines Red Thatcher, Thatcher and Thatcher RL1945 also indicated heterogeneity when the variation in reaction to T39 was compared (Table A.4). Evidence that loose smut resistance was not carried with Sr7 resistance was seen when Kenya Farmer was evaluated showing 49% incidence of susceptible plants to race T39. Kenya Farmer was the source of Sr7 resistance in Canthatch.

Comparing Chinese Spring 1U(1A), 1U(1B), 1U(1D) to Chinese Spring showed no difference in resistance to seven of the eight races. However, reaction to T6 may be somewhat different which again may be due to heterogeneity of the lines or the 1U chromosome may carry some resistance to loose smut. Analysis of the group 7 cytogenetic stocks of Chinese Spring is complicated by the fact that the Chinese Spring used to produce the alien cytogenetic stocks was not provided. Therefore three Chinese Spring sources were used as controls. Chinese Spring (EK) received from Dr. Eric Kerber, Chinese Spring (Pl Sc) received from the Department of Plant Science, University of Manitoba and Chinese Spring (NH) provided by Dr. Neil Howes were used as controls. These stocks differed somewhat in there resistance to T6 but were most different in reaction to T15. Whereas, Chinese Spring PlSc and NH were susceptible to T15 Chinese Spring EK was resistant. Comparison of the group 7 cytogenetic stocks indicates that there may be resistance carried in these chromosomes in Chinese Spring. Chinese Spring 7Ai(7D) was

susceptible to T39 and T19. However, none of the other chromosome seven substitution lines were susceptible indicating that the alien chromosomes 7U and 7Hch carry resistance or an error occurred in the development of the Chinese Spring 7Ai(7D) line. A similar response of Chinese Spring 7Ai(7B) to T6 and 7Ai(7B) to T2 and T6 occurred as happened with Chinese Spring 7Ai(7D) with the same explanation. However, it is odd that the 7A and 7B substitution lines were susceptible while the 7D substitution line was resistant when substituted with 7Ai, indicating that the Chinese Spring used here may have been susceptible to T6 and that the 7D substitution line was mis-inoculated. Considering resistance to race T15 most of the group 7 stocks were susceptible, indicating that the original Chinese Spring was also susceptible. The 7Ai group of chromosomes may be an exception, where only Chinese Spring 7Ai(7A) was susceptible. This and the susceptible reaction of Chinese Spring 7Ai(7A) to T2 was the strongest evidence that a gene for loose smut resistance in Chinese Spring resides in chromosome 7A. The fact that Dhitaphichit and coworkers (1989) reported resistance in Hope in chromosome 7A and which was confirmed here supports the fact that Chinese Spring could and Hope does have resistance in 7A. A cross between Chinese Spring and Hope would have to be made to determine if they have the same gene, alleles of the same gene or a different gene. It is interesting that Chinese Spring Sr8 was susceptible to several races to which even Chinese Spring PlSc was resistant. Because the Sr8 resistance is in chromosome 6A it is possible that incorporation of the Sr8 resistance also resulted in the incorporation of a loose smut susceptible allele in 6A or that yet another genotype of Chinese Spring was used to develop the Sr8 line.

Table A.1 Infection results of a group of Cadet cytogenetic stocks evaluated with races T2, T6, T8, T10, T15, T19, T31 and T39 of loose smut

Race	T2				T6			
Line	mean	sd	n	rep	mean	sd	n	rep
Cadet	0.00	0.00	40	2	0.00	0.00	105	4
Cadet 4Ag(4A)	0.00	0.00	28	6	0.00	0.00	37	4
Cadet 4Ag(4D)	.	.	.	.	0.00	.	13	1
Cadet 5Ag(5D)	0.00	0.00	16	2	0.00	0.00	51	3
Cadet 6AL ditelo	.	.	.	.	.	.	.	.
Cadet 6Ag(6A)	0.00	0.00	48	4	0.00	0.00	54	2
Cadet 6Ag/6A	.	.	.	.	.	.	.	.
Cadet 6AgS:6AS	.	.	.	.	.	.	.	.

Race	T8				T10			
Line	mean	sd	n	rep	mean	sd	n	rep
Cadet	0.00	0.00	127	5	0.84	0.27	56	3
Cadet 4Ag(4A)	0.00	0.00	33	5	0.60	0.10	15	2
Cadet 4Ag(4D)	.	.	.	.	0.67	.	6	1
Cadet 5Ag(5D)	0.00	0.00	54	3	0.61	0.81	56	4
Cadet 6AL ditelo	.	.	.	.	.	.	.	.
Cadet 6Ag(6A)	0.00	0.00	62	3	0.88	0.45	105	5
Cadet 6Ag/6A	.	.	.	.	.	.	.	.
Cadet 6AgS:6AS	.	.	.	.	.	.	.	.

Race	T15				T19			
Line	mean	sd	n	rep	mean	sd	n	rep
Cadet	0.00	0.00	142	7	0.11	0.54	463	23
Cadet 4Ag(4A)	0.00	0.00	82	13	.	.	.	.
Cadet 4Ag(4D)	.	.	.	.	.	.	.	.
Cadet 5Ag(5D)	0.00	0.00	33	2	0.26	0.16	98	4
Cadet 6AL ditelo	.	.	.	.	0.55	0.83	463	22
Cadet 6Ag(6A)	0.13	1.55	103	5	0.74	0.60	204	9
Cadet 6Ag/6A	.	.	.	.	0.82	1.19	416	16
Cadet 6AgS:6AS	.	.	.	.	0.22	0.62	141	14

Race	T31				T39			
Line	mean	sd	n	rep	mean	sd	n	rep
Cadet	0.96	0.11	28	2	0.31	1.02	124	6
Cadet 4Ag(4A)	0.33	.	3	1	0.44	0.43	59	8
Cadet 4Ag(4D)	.	.	.	.	.	.	.	.
Cadet 5Ag(5D)	0.55	1.92	31	2	0.67	0.76	33	3
Cadet 6AL ditelo	.	.	.	.	.	.	.	.
Cadet 6Ag(6A)	0.93	.	28	1	0.51	1.76	43	2
Cadet 6Ag/6A	.	.	.	.	.	.	.	.
Cadet 6AgS:6AS	.	.	.	.	.	.	.	.

.= No data, sd = standard deviation, n = number of plants rated, rep = number of inoculated heads.

Table A.2. Loose smut race T2, T6, T8, T10, T15, T19, T31 and T39 reaction of various Rescue cytogenetic stocks

Line	mean	sd	n	rep	mean	sd	n	rep
Race		T2				T6		
Rescue	0.19	0.31	27	2	0.00	0.00	112	10
Rescue (C-6D)	.	.	.	.	0.00	0.00	61	9
Rescue 5Ag(5D)	0.44	0.47	9	2	0.00	.	2	1
Rescue 6Ag(6A)	0.00	.	20	1	0.00	0.00	15	2
Rescue 6Ag(6B)	0.43	0.43	21	3	0.00	0.00	7	3
Rescue 6Ag(6D)	0.00	.	33	1	0.00	0.00	198	10
Rescue 6AgS:6DL	.	.	.	.	0.00	0.00	52	4
Rescue 6DS t"	.	.	.	.	0.00	0.00	44	8
Rescue N6A/T6B	.	.	.	.	0.07	0.37	15	3
Rescue N6D	.	.	.	.	0.03	0.30	176	10
Race		T8				T10		
Rescue	0.17	0.90	77	3	0.30	0.38	33	2
Rescue (C-6D)	.	.	.	.	.	.	.	.
Rescue 5Ag(5D)	0.25	.	24	1	0.65	.	17	1
Rescue 6Ag(6A)	0.33	0.60	48	2	0.63	.	16	1
Rescue 6Ag(6B)	0.66	1.15	65	3	0.86	0.20	28	3
Rescue 6Ag(6D)	0.66	1.22	77	3	0.80	.	25	1
Rescue 6AgS:6DL	.	.	.	.	.	.	.	.
Rescue 6DS t"	.	.	.	.	.	.	.	.
Rescue N6A/T6B	0.64	1.60	11	2	.	.	.	.
Rescue N6D	0.06	.	17	1	.	.	.	.
Race		T15				T19		
Rescue	0.72	0.77	39	3	0.55	1.67	107	5
Rescue (C-6D)	.	.	.	.	.	.	.	.
Rescue 5Ag(5D)	0.90	.	20	1	.	.	.	.
Rescue 6Ag(6A)	0.64	0.78	55	3	0.39	0.91	56	3
Rescue 6Ag(6B)	0.83	0.18	42	3	1.00	0.00	14	2
Rescue 6Ag(6D)	0.37	1.39	87	5	0.22	1.51	77	4
Rescue 6AgS:6DL	.	.	.	.	.	.	.	.
Rescue 6DS t"	.	.	.	.	.	.	.	.
Rescue N6A/T6B	0.67	0.66	63	4	0.33	0.86	54	4
Rescue N6D	0.63	0.92	24	2	0.17	.	12	1
Race		T31				T39		
Rescue	0.82	0.28	22	2	0.60	0.76	87	4
Rescue (C-6D)	.	.	.	.	.	.	.	.
Rescue 5Ag(5D)	0.69	0.85	42	3	.	.	.	.
Rescue 6Ag(6A)	0.17	.	18	1	0.58	1.21	57	3
Rescue 6Ag(6B)	0.97	0.11	30	3	0.88	0.24	65	4
Rescue 6Ag(6D)	0.00	.	26	1	0.46	0.79	46	4
Rescue 6AgS:6DL	.	.	.	.	.	.	.	.
Rescue 6DS t"	.	.	.	.	.	.	.	.
Rescue N6A/T6B	.	.	.	.	0.24	0.74	54	4
Rescue N6D	.	.	.	.	0.33	.	9	1

See Table 6.1 description of abbreviations

Table A.3. Loose smut incidence in the hexaploid wheat differentials inoculated to T2, T6, T8, T10, T15, T19, T31, T39

Race	T2				T6			
Line	mean	sd	n	rep	mean	sd	n	rep
TD 2	0.63	0.62	32	2	0.00	0.00	28	2
TD 3	1.00	0.00	10	2	0.00	.	8	1
Kota (TD 4)	0.00	0.00	30	2	0.31	0.04	51	2
TD 5A	0.03	0.15	37	2	0.00	.	5	1
TD 6	0.72	0.82	39	2	0.00	0.00	40	2
TD 7	0.86	0.62	21	2	0.00	0.00	20	2
TD 8A	0.97	0.23	33	2	0.00	0.00	29	2
TD 9	0.00	0.00	43	2	0.00	0.00	34	2
Red Bobs (TD10)	0.51	2.96	35	2	0.00	0.00	22	2
TD12A	0.00	0.00	25	3	0.00	0.00	34	2
TD14	0.00	0.00	6	2	0.96	0.15	28	2
TD15	0.00	0.00	43	2	0.00	0.00	23	4
TD16	0.00	0.00	52	2	0.00	0.00	13	2
TD17	0.00	0.00	38	3	0.00	0.00	34	2
TD18	0.00	0.00	35	2	0.00	0.00	37	2
Race	T8				T10			
Line	mean	sd	n	rep	mean	sd	n	rep
TD 2	0.00	0.00	37	2	0.00	0.00	56	2
TD 3	0.00	0.00	38	2	0.00	0.00	30	2
Kota (TD 4)	0.65	2.30	49	2	0.96	0.05	46	2
TD 5A	1.00	0.00	11	2	0.94	0.13	53	2
TD 6	0.00	.	8	1	.	.	0	0
TD 7	0.65	1.75	31	2	0.90	0.09	29	2
TD 8A	0.55	0.31	67	4	0.91	0.65	34	2
TD 9	0.69	0.17	36	2	0.95	0.08	58	2
Red Bobs (TD10)	0.03	0.20	35	2	0.19	0.64	32	2
TD12A	0.00	0.00	36	2	0.93	0.25	59	4
TD14	0.00	.	12	1	0.00	0.00	22	2
TD15	0.13	0.21	64	4	0.00	0.00	2	2
TD16	0.00	0.00	37	2	0.00	0.00	39	2
TD17	0.00	0.00	9	2	0.00	0.00	7	2
TD18	0.00	0.00	36	2	0.98	0.13	58	3

continued on next page.

Table A.3 continued

Race	T15				T19			
Line	mean	sd	n	rep	mean	sd	n	rep
TD 2	0.12	0.21	81	4	0.00	0.00	39	2
TD 3	0.88	0.38	34	2	0.88	0.31	24	2
Kota (TD 4)	0.16	0.04	37	2	0.43	1.20	42	2
TD 5A	0.88	.	34	1	0.94	0.43	32	2
TD 6	0.00	0.00	38	2	0.78	0.10	32	2
TD 7	0.95	0.01	39	2	0.26	1.42	35	2
TD 8A	0.00	0.00	37	2	0.25	0.47	28	2
TD 9	0.00	0.00	35	2	0.19	0.79	91	4
Red Bobs (TD10)	0.03	0.15	38	2	0.12	0.75	17	2
TD12A	0.00	0.00	34	2	0.71	1.37	150	7
TD14	0.16	0.37	25	2	0.06	0.12	36	3
TD15	0.11	0.32	28	3	0.00	0.00	21	4
TD16	0.95	0.31	39	2	0.93	0.47	40	2
TD17	0.03	0.16	32	2	1.00	.	20	1
TD18	0.00	0.00	37	2	0.00	0.00	38	2

Race	T31				T39			
Line	mean	sd	n	rep	mean	sd	n	rep
TD 2	0.90	0.72	63	2	0.00	0.00	39	2
TD 3	1.00	0.00	32	2	0.94	0.03	35	2
Kota (TD 4)	0.00	0.00	38	2	0.91	0.03	44	2
TD 5A	0.00	0.00	34	2	0.70	0.77	37	2
TD 6	0.00	.	24	1	0.56	1.89	34	2
TD 7	0.92	0.00	26	2	0.61	1.55	28	2
TD 8A	0.00	0.00	33	2	0.81	0.04	36	2
TD 9	0.00	0.00	49	2	0.33	0.47	40	2
Red Bobs (TD10)	0.97	0.26	31	2	0.27	0.87	48	3
TD12A	1.00	0.00	55	3	0.95	1.92	22	2
TD14	0.00	0.00	12	2	0.09	0.56	32	2
TD15	0.00	0.00	82	3	0.00	0.00	31	3
TD16	0.00	0.00	57	3	0.81	0.77	36	2
TD17	0.00	0.00	51	3	1.00	0.00	33	2
TD18	0.00	0.00	35	2	0.52	1.27	29	2

See Table 1 for definition of abbreviations

Table A.4. Inoculation of miscellaneous cultivars and cytogenetic stocks with races T2, T6, T8, T10, T15, T19, T31, T39

Race	T2				T6			
Line	mean	sd	n	rep	mean	sd	n	rep
783	0.00	0.00	12	2	0.00	0.00	26	4
8021	0.00	0.00	32	2	0.00	.	19	1
A8	.	.	.	.	0.00	.	17	1
Berillo	.	.	.	.	0.00	.	12	1
Biggar BSR	0.00	.	28	1	0.00	.	31	1
Bihar	1.00	.	5	1	0.78	.	23	1
CS	0.00	0.00	42	2	0.12	0.46	157	9
CS 1U(1A)	0.00	.	29	1	0.00	.	39	1
CS 1U(1B)	0.00	0.00	23	2	0.00	0.00	25	2
CS 1U(1D)	0.00	.	14	1	0.00	.	5	1
CS 7Ai(7A)	0.42	0.64	66	5	0.88	.	17	1
CS 7Ai(7B)	0.00	.	31	1	0.71	.	31	1
CS 7Ai(7D)	0.00	.	23	1	0.00	.	22	1
CS 7Hch(7A)	0.00	.	31	1	0.03	.	39	1
CS 7Hch(7B)	.	.	.	.	.	.	.	.
CS 7Hch(7D)	0.00	.	11	1	0.00	.	52	1
CS 7U(7A)	0.00	.	26	1	0.00	.	24	1
CS 7U(7B)	0.00	0.00	53	2	0.02	.	43	1
CS 7U(7D)	0.00	0.00	56	2	0.02	.	44	1
CS Hope 5A	0.00	.	18	1	0.38	1.23	69	6
CS Hope 6B	0.00	0.00	78	4	0.68	0.58	62	4
CS Hope 7A	0.00	0.00	18	2	0.05	0.21	44	2
CS N1D/T1B	.	.	.	.	.	.	.	.
CS NH	0.00	.	12	1	0.25	.	8	1
CS P1Sc	0.00	.	33	1	0.35	.	17	1
CS Sr8	.	.	.	.	0.91	0.54	46	2
Canthatch	0.00	0.00	39	2	0.00	0.00	27	2
Canthatch N7D	.	.	.	.	0.00	.	7	1
Canthatch RL2936	.	.	.	.	.	.	.	.
Diamant	0.82	0.61	38	2	1.00	0.00	52	2
EgyptNa101/Mq12/12	.	.	.	.	.	.	.	.
FKN Prel6 Sr7	.	.	.	.	.	.	.	.
Glenlea	0.00	0.00	54	2	0.00	0.00	35	2
H44	0.00	.	25	1	0.00	0.00	48	3
HY368	0.85	0.45	40	2	0.00	0.00	15	3

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Table A.4 continued

Race	T2				T6			
Line	mean	sd	n	rep	mean	sd	n	rep
HY377	0.00	0.00	24	2	0.00	0.00	21	2
Hope 4B 5aR	.	.	.	.	.	.	.	.
Katepwa	0.00	0.00	40	2	0.00	0.00	33	2
Kenya Farmer	.	.	.	.	.	.	.	.
L8474	0.73	.	11	1	0.00	.	6	1
Laura	0.86	0.12	64	2	0.93	0.19	80	2
Lee6/KF	.	.	.	.	.	.	.	.
NH301 70 3	.	.	.	.	.	.	.	.
Pembina	0.00	0.00	56	2	0.00	0.00	31	2
Prel 4 Sr7b	.	.	.	.	.	.	.	.
Prel./Dia 192	0.89	0.41	27	2	.	.	.	.
Prelude	0.90	.	10	1	0.89	0.41	56	3
Prelude Sr13	.	.	.	.	0.78	.	40	1
RL4137	.	.	0	0	.	.	.	.
RL4555	0.00	.	14	1	0.00	.	1	1
RL5404	0.00	.	13	1	0.00	0.00	41	2
Red Thatcher	.	.	.	.	.	.	.	.
Roblin	0.00	0.00	44	2	0.03	0.17	66	4
Selkirk	0.04	0.07	48	2	0.09	0.23	47	2
Tc7/KF	.	.	.	.	.	.	.	.
Tetracanthatch	.	.	.	0	.	.	.	0
Tetrarescue	.	.	.	.	.	.	.	.
Thatcher	0.00	0.00	49	2	0.00	0.00	41	2
Thatcher RL1945	.	.	.	.	.	.	.	.
Timgalen	0.00	.	29	1	0.00	.	16	1
Vic A	.	.	.	.	0.00	0.00	43	2

Table A.4 continued

Race	T8				T10			
Line	mean	sd	n	rep	mean	sd	n	rep
783	0.00	0.00	31	3	0.00	0.00	40	3
8021	0.00	.	8	1	0.00	0.00	32	2
A8	1.00	.	17	1	.	.	.	.
Berillo	.	.	.	.	.	.	.	.
Biggar BSR	0.08	.	50	1	0.00	.	38	1
Bihar	1.00	.	16	1	1.00	.	17	1
CS	0.00	0.00	40	6	0.00	0.00	29	3
CS 1U(1A)	0.00	.	8	1	0.00	.	17	1
CS 1U(1B)	0.00	0.00	7	2	0.00	0.00	28	2
CS 1U(1D)	0.00	.	5	1	0.00	.	3	1
CS 7Ai(7A)	0.00	.	2	1	0.00	.	19	1
CS 7Ai(7B)	0.07	.	30	1	0.00	.	23	1
CS 7Ai(7D)	0.00	.	9	1	0.00	.	19	1
CS 7Hch(7A)	0.00	0.00	15	2	0.00	.	5	1
CS 7Hch(7B)	.	.	.	.	0.00	.	9	1
CS 7Hch(7D)	0.00	.	11	1	0.00	.	20	1
CS 7U(7A)	0.00	.	3	1	0.00	.	13	1
CS 7U(7B)	0.00	.	16	1	0.00	.	21	1
CS 7U(7D)	0.00	.	7	1	0.00	.	6	1
CS Hope 5A	0.00	0.00	15	2	0.00	0.00	51	4
CS Hope 6B	0.00	0.00	25	4	0.00	0.00	71	4
CS Hope 7A	0.00	0.00	24	2	0.00	.	12	1
CS N1D/T1B	.	.	.	.	.	.	.	.
CS NH	0.00	.	1	1	0.00	.	5	1
CS PlSc	.	.	.	.	0.00	.	8	1
CS Sr8	.	.	.	.	0.00	.	7	1
Canthatch	0.00	0.00	43	2	0.10	0.08	48	2
Canthatch N7D	0.00	.	17	1	.	.	.	.
Canthatch RL2936	.	.	.	.	.	.	.	.
Diamant	0.99	0.12	68	3	0.92	0.54	38	2
EgyptNa101/Mq12/12	.	.	.	.	.	.	.	.
FKN Prel6 Sr7	.	.	.	.	.	.	.	.
Glenlea	0.00	0.00	48	2	0.00	0.00	44	2
H44	0.00	0.00	44	2	0.00	.	23	1
HY368	0.67	1.81	95	3	0.96	0.22	49	2
HY377	0.35	1.87	74	3	0.00	0.00	17	2
Hope 4B 5aR	.	.	.	.	.	.	.	.
Katepwa	0.00	0.00	47	2	0.59	0.61	39	2
Kenya Farmer	.	.	.	.	.	.	.	.
L8474	0.92	.	13	1	0.68	.	19	1

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Table A.4 continued

Race	T8				T10			
Line	mean	sd	n	rep	mean	sd	n	rep
Laura	0.92	0.19	62	2	0.63	1.24	63	2
Lee6/KF	.	.	.	.	.	.	.	.
NH301 70 3	.	.	.	.	.	.	.	.
Pembina	0.00	0.00	45	2	0.00	0.00	28	2
Prel 4 Sr7b	.	.	.	.	.	.	.	.
Prel./Dia 192	1.00	0.00	42	3	0.91	0.22	44	2
Prelude	0.98	0.19	81	4	0.88	0.51	67	4
Prelude Sr13	.	.	.	.	0.74	.	27	1
RL4137	0.94	.	18	1	.	.	.	.
RL4555	0.17	.	24	1	0.87	.	23	1
RL5404	0.00	0.00	23	2	0.47	.	15	1
Red Thatcher	.	.	.	.	.	.	.	.
Roblin	0.00	0.00	43	4	0.06	0.00	36	2
Selkirk	0.12	0.48	43	2	0.15	1.10	40	2
Tc7/KF	.	.	.	.	.	.	.	.
Tetracanthatch	.	.	.	.	.	.	.	.
Tetrarescue	.	.	.	.	.	.	.	.
Thatcher	0.00	0.00	59	2	0.96	0.05	53	2
Thatcher RL1945	.	.	.	.	.	.	.	.
Timgalen	0.79	.	38	1	0.46	.	13	1
Vic A	.	.	.	.	.	.	.	.

continued on next page

Table A.4 continued

Race	T15				T19			
Line	mean	sd	n	rep	mean	sd	n	rep
783	0.00	0.00	46	4	0.87	0.42	54	5
8021	0.00	.	20	1	0.55	.	20	1
A8	0.68	.	25	1	1.00	.	21	1
Berillo	.	.	.	.	0.00	.	14	1
Biggar BSR	0.00	.	42	1	0.00	.	23	1
Bihar	0.61	.	28	1	0.90	.	30	1
CS	0.01	0.11	98	5	0.00	0.00	103	5
CS 1U(1A)	0.00	.	44	1	0.00	.	25	1
CS 1U(1B)	0.00	.	12	1	0.00	0.00	11	2
CS 1U(1D)	0.00	.	17	1	0.00	.	7	1
CS 7Ai(7A)	0.38	0.65	116	4	0.14	.	14	1
CS 7Ai(7B)	0.06	.	33	1	0.00	.	32	1
CS 7Ai(7D)	0.00	.	21	1	0.33	.	24	1
CS 7Hch(7A)	0.62	0.40	65	2	0.00	0.00	40	2
CS 7Hch(7B)	0.00	.	1	1	.	.	.	.
CS 7Hch(7D)	0.52	0.71	86	2	0.00	.	62	1
CS 7U(7A)	0.16	.	25	1	0.00	.	23	1
CS 7U(7B)	0.56	.	34	1	0.00	.	24	1
CS 7U(7D)	0.29	0.58	65	2	0.00	.	30	1
CS Hope 5A	0.66	0.61	53	3	0.00	0.00	56	3
CS Hope 6B	0.72	0.62	96	4	0.00	0.00	31	2
CS Hope 7A	0.00	.	4	1	0.00	.	19	1
CS N1D/T1B	0.00	.	9	1	0.00	.	20	1
CS NH	0.72	.	25	1	0.00	.	18	1
CS PlSc	0.66	.	29	1	0.00	.	23	1
CS Sr8	0.93	.	29	1	0.92	0.48	71	2
Canthatch	0.00	0.00	48	2	0.00	0.00	50	2
Canthatch N7D	0.00	0.00	23	2	0.00	.	11	1
Canthatch RL2936	.	.	.	.	.	.	.	.
Diamant	0.92	0.51	37	2	0.95	0.30	39	2
EgyptNa101/Mq12/12	.	.	.	.	.	.	.	.
FKN Prel6 Sr7	.	.	.	.	.	.	.	.
Glenlea	0.00	0.00	47	2	0.00	0.00	45	2
H44	0.03	0.21	31	2	0.00	.	18	1
HY368	0.79	0.89	89	3	0.91	0.22	53	3

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Table A.4 continued

Race	T15				T19			
Line	mean	sd	n	rep	mean	sd	n	rep
HY377	0.03	0.12	29	2	0.00	0.00	18	2
Hope 4B 5aR	.	.	.	.	.	.	.	.
Katepwa	0.00	.	22	1	0.00	0.00	29	2
Kenya Farmer	.	.	.	.	.	.	.	.
L8474	0.82	.	17	1	1.00	.	20	1
Laura	0.81	0.53	88	2	0.65	0.86	79	2
Lee6/KF	.	.	.	.	.	.	.	.
NH301 70 3	.	.	.	.	.	.	.	.
Pembina	0.00	0.00	41	2	0.00	0.00	58	2
Prel 4 Sr7b	.	.	.	.	.	.	.	.
Prel./Dia 192	.	.	.	.	1.00	.	18	1
Prelude	0.87	0.38	99	5	0.90	0.43	82	4
Prelude Sr13	0.78	1.94	40	2	0.81	.	36	1
RL4137	.	.	.	.	.	.	.	.
RL4555	0.82	.	22	1	0.00	.	22	1
RL5404	0.00	0.00	52	2	0.00	0.00	35	2
Red Thatcher	.	.	.	.	.	.	.	.
Roblin	0.01	0.10	87	4	0.77	1.02	77	4
Selkirk	0.33	2.04	48	2	0.00	0.00	40	2
Tc7/KF	.	.	.	.	.	.	.	.
Tetracanthatch	.	.	.	0	.	.	.	.
Tetrarescue	0.00	.	2	1	0.00	.	1	1
Thatcher	0.00	0.00	36	2	0.16	0.56	107	6
Thatcher RL1945	.	.	.	.	.	.	.	.
Timgalen	0.54	.	28	1	0.33	.	9	1
Vic A	.	.	.	.	0.00	0.00	55	3

continued on next page

Table A.4 continued

Race	T31				T39			
Line	mean	sd	n	rep	mean	sd	n	rep
783	0.47	0.45	38	3	0.62	0.62	21	2
8021	0.00	.	14	1	0.20	.	20	1
A8	.	.	.	.	0.77	.	13	1
Berillo	.	.	.	.	.	.	.	.
Biggar BSR	0.00	.	56	1	0.00	.	17	1
Bihar	0.87	.	31	1	1.00	.	17	1
CS	0.00	0.00	33	2	0.00	0.00	34	3
CS 1U(1A)	0.00	.	32	1	0.00	.	8	1
CS 1U(1B)	0.00	.	12	1	0.00	0.00	16	2
CS 1U(1D)	0.00	.	8	1	0.00	.	3	1
CS 7Ai(7A)	.	.	.	.	0.04	.	24	1
CS 7Ai(7B)	0.00	.	22	1	.	.	.	.
CS 7Ai(7D)	0.00	.	16	1	0.24	.	17	1
CS 7Hch(7A)	0.00	.	23	1	0.00	.	17	1
CS 7Hch(7B)	0.00	0.00	9	2	.	.	.	.
CS 7Hch(7D)	0.00	.	23	1	0.00	.	15	1
CS 7U(7A)	0.00	.	19	1	0.00	.	16	1
CS 7U(7B)	0.00	.	37	1	0.00	.	25	1
CS 7U(7D)	0.00	.	29	1	0.00	.	16	1
CS Hope 5A	0.00	0.00	35	2	0.00	0.00	35	2
CS Hope 6B	0.00	0.00	49	2	0.00	0.00	37	2
CS Hope 7A	.	.	0	0	.	.	.	.
CS N1D/T1B	.	.	.	.	.	.	.	.
CS NH	0.00	.	10	1	0.00	.	10	1
CS PlSc	0.00	.	14	1	0.00	.	12	1
CS Sr8	.	.	.	.	0.75	0.25	51	2
Canthatch	0.49	0.39	43	2	0.08	0.90	448	16
Canthatch N7D	.	.	.	.	0.00	.	8	1
Canthatch RL2936	.	.	.	.	0.24	1.18	127	5
Diamant	0.91	0.25	55	2	0.98	0.15	45	2
EgyptNa101/Mq12/12	.	.	.	.	0.58	0.74	212	10
FKN Prel6 Sr7	.	.	.	.	0.48	0.83	153	8
Glenlea	0.00	0.00	52	2	0.00	.	50	1
H44	0.02	0.13	45	2	0.00	0.00	33	2
HY368	0.88	0.29	32	2	0.85	0.33	68	3

continued on next page

Table A.4 continued

Race	T31				T39			
Line	mean	sd	n	rep	mean	sd	n	rep
HY377	0.00	.	15	1	0.00	0.00	21	2
Hope 4B 5aR	.	.	.	.	0.11	0.80	108	6
Katepwa	0.04	.	24	1	0.08	0.27	49	2
Kenya Farmer	.	.	.	.	0.49	1.49	204	9
L8474	0.79	.	19	1	0.89	.	18	1
Laura	0.90	0.30	71	2	0.45	1.66	53	2
Lee6/KF	.	.	.	.	0.61	0.68	157	10
NH301 70 3	.	.	.	.	0.00	0.00	64	3
Pembina	0.02	0.18	45	2	0.00	0.00	43	2
Prel 4 Sr7b	.	.	.	.	0.69	0.85	162	8
Prel./Dia 192	0.93	0.04	29	2	.	.	.	.
Prelude	0.93	0.23	44	3	0.74	0.59	73	4
Prelude Sr13	.	.	.	.	.	.	.	.
RL4137	.	.	.	.	.	.	.	.
RL4555	0.00	.	31	1	0.00	0.00	32	2
RL5404	0.89	.	19	1	0.06	0.31	32	2
Red Thatcher	.	.	.	.	0.76	0.56	182	9
Roblin	0.00	0.00	51	2	0.45	1.71	65	4
Selkirk	0.26	1.25	38	2	0.00	0.00	41	2
Tc7/KF	.	.	.	.	0.44	0.72	181	9
Tetracanthatch	.	.	.	.	.	.	.	0
Tetrarescue	.	.	.	.	.	.	.	.
Thatcher	0.73	1.01	56	2	0.64	0.42	45	2
Thatcher RL1945	.	.	.	.	0.28	0.45	211	8
Timgalen	0.00	.	40	1	0.28	0.16	47	2
Vic A	.	.	.	.	.	.	.	.

See Table 6.1 for description of abbreviations.

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