INHERITANCE OF RESISTANCE TO COMMON BUNT (*TILLETIA CARIES* AND *T. FOETIDA*) AND IDENTIFICATION OF RAPD MARKERS LINKED TO BUNT RESISTANCE IN WHEAT

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Inheritance of Resistance to Common Bunt (*Tilletia caries* and *T. foetida*) and identification of RAPD Markers Linked to Bunt Resistance in Wheat

Common bunt causes yield loss and reduces grain quality in common and durum wheats in western Canada. In order to aid bunt resistance breeding, this study was conducted to investigate the race-specificity and the inheritance of resistance to common bunt and to identify RAPD markers linked to bunt resistance for use in marker-assisted selection. In the study on race specificity of resistance, significant differences in resistance to bunt among cultivars and in virulence among races were found. Race T1 was found to be the least virulent and T19 was the most virulent. Race-specific resistance was demonstrated in all experiments.

Both common (Kite and Triple Dirk) and spelt (RL5407 and SK0263) wheats were used for studies on the inheritance of resistance to bunt. Generation mean analysis showed that additive effects were the main genetic effects and dominance effects were not significant in any cross. Epistatic effects may exist in the cross Laura/Kite. Gene estimates from the qualitative analysis indicated that Triple Dirk and RL5407 may carry a single gene for resistance to race T1, T13 and L7. Kite may carry two genes and SK0263 carries either one or two genes for resistance to race T1. Heritability was estimated to be moderate to high (54%-90%) for bunt resistance to races T1, T13 and L7.

An allelic study demonstrated that RL5407 may not carry *Bt2, Bt3, Bt6, Bt8* or *Bt10* genes for bunt resistance. RL5407 and SK0263 likely carry the same gene for resistance to race T1 and this gene is different from the gene carried by SK0505. RL5407 possibly carries a gene conditioning resistance to both races T13 and L7 in addition to the one for resistance to race T1. The genes carried by Triple Dirk for resistance to races T1, T13 and L7 are different from each other. No cytoplasmic effect was found for resistance to race T1 in the crosses Genesis/RL5407 and RL5407/Genesis.

Two flanking RAPD markers linked to resistance to bunt race T1, $UBC548_{590}$ (in repulsion) and $UBC274_{988}$ (in coupling), were identified in Laura/RL5407. They had linkages of 9.1±4.0 cM and 18.2±5.6 cM, respectively.

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ABSTRACT

Common bunt is one of the major wheat diseases, reducing yield and grain quality in both common and durum wheats in western Canada. The most economic and effective way to control this disease is by the development of cultivars with bunt resistance. In order to aid bunt resistance breeding, this study was conducted to investigate the race-specificity and the inheritance of resistance to common bunt and to identify RAPD markers linked to bunt resistance for use in marker-assisted selection.

In the study on race specificity, significant differences in resistance to common bunt among cultivars and in virulence among different races were found. Race T1 was found to be the least virulent and T19 was the most virulent. Race-specific resistance was demonstrated in all experiments.

Both common (Kite and Triple Dirk) and spelt (RL5407 and SK0263) wheats were used for studies on the inheritance of resistance to bunt. Generation mean analysis showed that additive effects were the main genetic effects and dominance effects were not significant in any cross. Epistatic effects may exist in the cross Laura/Kite. Gene estimates from the qualitative analysis indicated that Triple Dirk carries a single gene controlling bunt resistance to each of the races T1 and L7, and one or two genes for resistance to race T13. RL5407 carries a gene conferring resistance to both races T13 and L7, plus a single gene for resistance to race T1. Kite possibly carries two genes and SK0263 carries at least two genes for resistance to race T1. Heritability estimates ranged from 0.38 to 0.77 for bunt resistance to race T1, from 0.48 to 0.67 for resistance to race T13 and from 0.75 to 0.81 for resistance to race L7.

An allelic study demonstrated that RL5407 may not carry *Bt2*, *Bt3*, *Bt6*, *Bt8* or *Bt10* genes for bunt resistance. RL5407 and SK0263 likely carry the same gene for resistance to race T1 and this gene is different to the gene carried by SK0505. The study of allelic genes for resistance to different races indicated that RL5407 possibly carries another gene conditioning resistance to both races T13 and L7 in addition to the one for resistance to race T1. The genes carried by Triple Dirk for resistance to races T1, T13 and L7 are likely different from each other.

No cytoplasmic effect was found by testing bunt resistance to race T1 in the crosses Genesis/RL5407 and RL5407/Genesis.

Two flanking RAPD markers, $UBC548_{500}$ and $UBC274_{988}$, were identified to be linked to bunt resistance to race T1. The linkages were 9.1 cM and 18.2 cM, respectively.

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1. INTRODUCTION

Common bunt, also called stinking smut or covered smut, is caused by *Tilletia caries* (DC.) Tul. (syn. *T. tritici* (Bjerk) Wint.) and *T. foetida* (Wallr.) Liro. (syn. *T. laevis* Kuhn) and was first recognised as an infectious disease of wheat in the eighteenth century (Wiese, 1977). This disease was once considered as the second most destructive disease of wheat in the United States (Bressman, 1931) and has occurred in all wheat growing countries of the world (Munjal, 1966; Bahadur and Singh, 1987). It causes yield loss in common and durum wheats (*Triticum aestivum* L. em. Thell and *T. durum* Desf.) and reduces grain quality through production of toxic black fungal spores releasing a fishy odour (Flor et al., 1932; Cherewick, 1953; Martens et al., 1984).

Control of common bunt by chemical treatment of the seed is possible but not always effective. Besides, chemical control is usually not the control method of choice because of the cost of seed treatment and its potentially adverse effects on the environment. These aspects have resulted in the prevalence of this disease (Singh and Chopra, 1986). The most economic and effective means of controlling common bunt of wheat is by development of wheat cultivars with resistance to the pathogen (Smeltzer, 1952).

Knowledge of genetics and mode of inheritance for common bunt resistance is essential in determining breeding strategies. Identification of new sources of bunt resistance in spelt and common wheats can provide useful information which could

facilitate breeding for resistance to this disease.

Conventional plant breeding is based on selection of individuals in the segregating generations following a sexual cross. Since this selection is based on a visible phenotype, its efficiency may be reduced by environmental variation and by polygenic inheritance. However, phenotypic selection can now be done by direct selection for genotypes using molecular markers. Restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers have been demonstrated to be useful tools for genetic mapping and plant breeding (Williams et al., 1990; Rafalski et al., 1991; O'Brien, 1993). Since it is more labour-intensive, the RFLP assay is less suitable for plant genetics and breeding. However, the RAPD assay does avoid some of the technical limitations of the RFLP method.

The objectives of this study were:

(1) to determine if race specific resistance to common bunt exists.

(2) to determine the genetic control of resistance to common bunt in selected wheat cultivars.

(3) to identify RAPD markers linked to common bunt resistance.

2. LITERATURE REVIEW

2.1 The biology of common bunt of wheat

2.1.1 Morphology and taxonomy of the pathogen

Common bunt of wheat, one of the oldest known plant diseases, is caused by two closely related species, *Tilletia caries* (DC.) Tul. (syn. *T. tritici* (Bjerk) Wint.) and *T. foetida* (Wallr.) Liro. (syn. *T. laevis* Kühn). These two species are mainly distinguished by their spore morphology (Munjal, 1966; Kollmorgen and Ballinger, 1987). *T. caries* has globose, brownish black teliospores (15-25 µm in diameter) with distinct reticulations 0.5 to 1.2 µm deep. *T. foetida* has globose to elongated, smooth-walled teliospores (17-22 µm in diameter) (Stockwell and Trione, 1986; Mathur and Cunfer, 1993).

The genus *Tilletia*, family Tilletiaceae is classified in the order Ustilaginales, class Teliomycetinae, subdivision Basidiomycotina (Jones, 1987; Agrios, 1988). In addition to the two species mentioned, there are two closely related species called *T. controversa* and *T. indica* (syn. *Neovossia indica*) which cause dwarf bunt and karnal bunt (syn. partial bunt) of wheat, respectively (Zillinsky, 1983).

2.1.2 Host range

The major economically important host of the pathogen is wheat (Bahadur and Singh, 1987). However, rye, barley and some grasses including *Aegilops*, *Lolium* and

Agropyron may also be hosts (Kühn, 1876; Reichert, 1931; Duran and Fischer, 1961; Wiese, 1977), but the disease is not common on these species (Martens et al., 1984).

Early in 1876, Kühn found that rye was naturally infected by a *Tilletia* pathogen and named it *T. secalis* (Kühn, 1876). Later, this disease occurred frequently in Russia and western Europe (Reichert, 1931). Koernicke (1877) discovered a *Tilletia* species on barley and named it *T. hordei*. However, Schellenberg (1911) found that the species infecting rye and barley, i.e. *T. secalis* and *T. hordei*, were morphologically identical to *T. tritici*, the species infecting wheat, and proposed that *T. secalis* and *T. hordei* should not be used. Gaines and Stevenson (1923) also confirmed that *T. tritici* and *T. secalis* were the same fungus.

2.1.3 Epidemiology of common bunt

2.1.3.1 Disease cycle and disease development

The bunt fungi persist as teliospores in soil and on seed. Spores on planted seed or in the soil germinate in response to low temperature (5-15°C) and soil moisture (15-60%) (Martens et al., 1984, Bahadur and Singh, 1987) to produce a basidium (promycelium) on which 8-16 uninucleate, hyaline basidiospores (primary sporidia) develop. The basidiospores in pairs fuse near their middle, forming H-shaped structures with a heterokaryon. These may germinate to form infection tubes or produce secondary sporidia which are hyaline and dikaryotic (Munjal, 1966; Wiese, 1977). The secondary sporidia germinate to produce infection tubes (hyphae) that penetrate the coleoptile of the germinating seed or the tiller initials (Hoffmann, 1982), often before young seedlings emerge from the soil (Zillinsky, 1983). Later, the mycelium within the seedling develops

as the plant grows and progresses to the terminal meristematic tissues, eventually infecting the developing head and replacing all kernel tissues within the pericarp with teliospores to form a "bunt ball" (Fig. 2.1). Each bunt ball may contain 6 to 9 million spores (Munjal, 1966). The fragile pericarps of bunt balls remain intact but rupture during harvest to release black powdery spores which contaminate the soil and healthy seeds (Wiese, 1977; Mathur and Cunfer, 1993). A fishy odour is released by the fungal spores, resulting from the production of the chemical *trimethylamine*. Hence, common bunt is often called 'stinking smut' (Martens et al., 1984). Teliospores from the ruptured bunt balls may also be spread mechanically or by wind (Wiese, 1977). The dispersed teliospores become the source of the next disease cycle.

2.1.3.2 Factors influencing disease infection

There is a very close relationship between environmental factors and bunt infection and development (Yarham and McKeown, 1989). These environmental factors include soil temperature (Mathur and Cunfer, 1993), soil moisture (Bahadur and Singh, 1987), inoculum load (Holton and Heald, 1941) and planting date and depth (Gaudet and Puchalski, 1990).

Soil temperature and moisture seem to be the most important of the factors affecting infection (Faris, 1923). Temperatures of 5°C to 15°C are optimal for infection (Faris, 1923; Wiese, 1977), while lower temperatures may inhibit the germination of both seeds and bunt spores (Bahadur and Singh, 1987; Mathur and Cunfer, 1993). Higher temperatures may cause the plants to escape infection (Munjal, 1966). Holton and Heald (1941)

found no smut in inoculated wheat plants growing at soil temperatures of either 20°C to 25°C or at 2°C to 4°C.

Soil moisture levels of 15-60% favour infection (Bahadur and Singh, 1987). Holton and Heald (1941) found that very little or no smut developed when soil moisture content was only 7%, but there was a gradual increase in infection as the moisture content increased to 38%.

The spore load on wheat seed or in the soil is also an important factor affecting disease infection and development. Infection was light for seeds carrying under 500 spores per grain and moderate for seeds carrying 500 to 2500 spores per grain (Holton and Heald, 1941). Heald and Boyle (1923) reported that maximum infection occurred when one gram of teliospores was used to dust 100 grams of seed. Mackie and Briggs (1923) found a direct relationship between spore load and percentage bunt. Infection levels declined with reduction in bunt spore load.

Planting date and planting depth can also affect infection intensity (Munjal, 1966). Gaudet and Puchalski (1990) reported that bunt infection for most of western Canadian spring wheats was lower when the crop was planted in late May than in April. However, the degree of correlation between bunt infection and planting dates may vary among bunt races since there was an interaction between the aggressiveness of races and planting dates (Gaudet and Puchalski, 1990). Holton and Heald (1941) found that bunt infection was directly correlated with planting depth and bunt infection was highest when the seeding depth was 5 to 8 cm.

2.1.4 Relationship between bunt infection and resistance to other diseases

Infection by common bunt fungi can modify the physiology of the wheat plant and cause increased susceptibility to other diseases such as rust, seedling blight and root rot (Fischer and Holton, 1957). In 1917, Lang reported that all bunted wheat plants had short culms and were "laden" with stripe rust. He found that rows of a rust-resistant cultivar, which suddenly became infected with rust, were those inoculated with bunt. Fischer and Holton (1957) suggested that the metabolic effects of metabolic by-products from the fungal hyphae could cause susceptibility to stripe rust. Increased susceptibility of wheat to winter freezing injury was also positively correlated to the degree of bunt infection (Holton and Heald, 1936). However, the incidence of common bunt in near-isogenic lines with stem rust resistance gene *Sr26* was significantly lower than in those lines without it, suggesting that *Sr26* can induce increased resistance (Fisher and Kuiper, 1977). Bunt infection can also induce increased resistance to powdery mildew, possibly the result of release of substances inhibitory to the powdery mildew fungi by the bunt fungi (Fischer and Holton, 1957).

2.1.5 Disease infection and symptom identification

The symptoms of common bunt are not usually obvious until after the heading stage, although the pathogens attack the plant very early in its development (Goel and Singh, 1975; Hoffmann, 1982). Usually, plants infected by *T. caries* are shorter than healthy ones especially the infected tillers, but *T. foetida* causes no height reduction. However, bunted plants are recognized most easily when the heads emerge. The infected wheat spikes remain erect, dark or olive green (Bahadur and Singh, 1987) and the glumes may be

spread apart by bunt balls which are usually slightly larger and more spherical than healthy kernels (Goel and Singh, 1975; Wiese, 1977; Mathur and Cunfer, 1993). The infected grain (bunt ball) is often discoloured because the interior is filled with black teliospores. Common bunt of wheat can be detected in the field by visual inspection of individual spikelets for bunt balls. Identification is often easier immediately following rain (Popp, 1947).

2.1.6 Disease assessment and economic importance

Common bunt occurs worldwide (Wiese, 1977; Hoffmann, 1982) and reduces wheat yield and grain quality (Flor et al., 1932; Munjal, 1966; Jones, 1987). Common bunt was once considered the most destructive disease of wheat in the pacific Northwest of USA (Flor et al., 1932; Mathur and Cunfer, 1993). In 1927, the yield loss due to common bunt in the United States was estimated at more than 760,000 metric tons (Mathur and Cunfer, 1993). Severe yield losses have also been reported in Germany, Canada, Australia, China and Argentina (Goel and Singh, 1975). In Canada, extensive yield losses due to common bunt in spring and winter wheats were reported in the early 1950's (Cherewick, 1953). However, the application of seed treatments and use of resistant varieties and cultural practices such as correct time and depth of seeding and crop rotation have almost eliminated yield losses due to this disease in spring wheat in western Canada (Goel and Singh, 1975; Gaudet and Puchalski, 1989a, 1989b, 1990).

Yield reduction estimates range from 25% to 50%, even up to 100% in particular fields (Holton, 1947; Goel and Singh, 1975). The disease also affects wheat market value.

Grain contaminated with bunt spores is toxic for human consumption and contaminated straw is dangerous to cattle (Goel and Singh, 1975, Mathur and Cunfer, 1993).

2.1.7 Physiologic specialization

Physiologic specialization in common bunt was first reported by Faris in 1923 (Faris, 1923). From 1925 to 1927, Reed (1928) discovered significant differences in the reaction of cultivars to certain bunt collections, providing evidence for the presence of physiologic races in *T. caries* and *T. foetida*, and enabling identification of pathogenic races (Reed, 1928). Bressman (1931) identified races of *T. caries* and *T. foetida* based on reaction of cultivars Albit, Hussar, Ridit and Oro. So far, more than 40 races of *Tilletia caries* and *T. foetida* have been identified.

In western Canada, surveys conducted from 1949 to 1955 identified five races of *T. caries* and seven races of *T. foetida* present in the western Canadian prairies (Gaudet and Puchalski, 1989a). In later surveys (from 1975 to 1982), 44 bunt races were identified from winter and spring wheat in western Canada. Of these, 70% were races of *T. foetida* (Gaudet and Puchalski, 1989a).

2.1.8 Control of common bunt

2.1.8.1 Chemical control

As a seed- and soil- borne disease, common bunt can be controlled by the use of seed and fields free of bunt spores (Munjal, 1966; Mathur and Cunfer, 1993). Early in 1755, Tillet discovered that common bunt of wheat was more prevalent in plants grown from contaminated seeds than healthy seeds and the occurrence of this disease could be

diminished by seed treatments (Agrios, 1988). Chemicals are usually effective in blocking the germination of the pathogen either on the seed surface or around the vicinity of the seed (Trione, 1973; Singh et al., 1977). Many seed treatment fungicides, including copper carbonate, copper sulfate, sodium chloride, formaldehyde and hexachlorobenzene, can provide good protection and control (Thompson, 1921; Mackie and Briggs, 1921; Purdy, 1955). Chemicals such as carboxin (Gaudet et al., 1992), phenylpyrroles and guanidines (Noon and Jackson, 1992) were also found to provide effective control of the bunt disease. In addition, the fungicides chosen for controlling common bunt can be non-systemic as the source of initial inoculum is the bunt spores present on the surface of seeds. The active ingredients of non-systemic seed treatments include maneb and formaldehyde (Saskatchewan Agriculture and Food, 1996a).

2.1.8.2 The use of resistant cultivars

The use of resistant cultivars is considered the most desirable control strategy, since use of fungicides for seed treatment may have economic, environmental and health-related disadvantages. In the 1950's, many wheat varieties with combined resistance from cultivars Martin and Turkey were released (Briggs and Holton, 1950; Schmidt et al., 1969). In western Canada, spring wheat cultivars having Hope in their ancestry, e.g. Katepwa (Campbell and Czarnecki, 1987) and Columbus (Campbell and Czarnecki, 1981), are bunt resistant (Puchalski and Gaudet, 1991). The *Bt10* resistance gene has been incorporated into spring wheats such as AC Taber, M78-9505, AC Karma and AC Foremost, and winter wheats such as Weston, Ranger, Moro, Franklin and M82-2102

(Demeke et al., 1996). Durum wheats are usually very resistant (Puchalski and Gaudet, 1991).

2.1.8.3 Agronomic practices

Wheat seedlings can be infected when conditions, such as soil temperature and moisture, are suitable for infection. Therefore, infection can be effectively limited by changing agronomic practices such as crop rotation, planting date, and rate and depth of seeding (Goel and Singh, 1975). Since the teliospores can remain in the soil for a few years, crop rotation with non-host crops such as canola and pulse crops for a few years can greatly reduce bunt inoculum. These measures are most applicable to areas where soil contamination is the main source of common bunt inoculum. Shallow seeding may reduce bunt infection because of reduced inoculum and soil moisture but higher temperature near to the soil surface (Goel and Singh, 1975; Duczek and Piening, 1982; Goates, 1996).

2.1.8.4 Biological control

Because of the possible health and environmental risks associated with fungicide use as seed treatment, environment-friendly agents are increasingly preferred. As long as an agent capable of disrupting the initial infection of germinating seeds by the fungal spores is found, biological control of common bunt is possible. Fluorescent *Pseudomonas* spp. have been reported to produce anti-fungal compounds, siderophores, for the control of soilborne fungal pathogens (Thomashow and Weller, 1990; McManus et al., 1993). McManus et al. (1993) found that a rifampicin-resistant derivative of *Pseudomonas fluorescens* strain 2-79 (Pf2-79r) can inhibit teliospore germination of *T. laevis*. After

wheat seeds or 2-week-old seedlings were treated with this bacterial strain, the disease incidence of common bunt was reduced by 50-65%. Hokeberg et al. (1997) found that a pseudomonas isolate, MA342, can suppress *T. caries* in the field by over 70%.

2.2 Genetic control of resistance to common bunt in wheat

2.2.1 Types of disease resistance in plants

Nelson (1973) classified disease resistance into two major types according to host response to plant pathogens. The plant either (a) resists the establishment of a successful parasitic relationship by restricting the infection site and infection process, or (b) resists the colonization and growth of the parasite after a successful infection. The former is generally referred to as hypersensitive resistance, non-uniform resistance, specific resistance, vertical resistance or major gene resistance. The latter has been termed field resistance, generalized resistance, non-specific resistance, uniform resistance, partial resistance, horizontal resistance, multigenic or polygenic resistance, or minor gene resistance.

Van der Plank (1963) proposed two terms for disease resistance: (a) vertical and (b) horizontal resistance. He used *vertical resistance* to describe resistance which is effective against one or several races of a pathogen, and *horizontal resistance* to describe resistance which is effective against all races of a pathogen. However, they are not considered the best terms to use since neither indicates the type of host response incited by the parasite nor the degree of effectiveness of the resistance to different races (Nelson, 1973).

2.2.2 Race specific resistance to common bunt

2.2.2.1 Genetic basis of race-specific resistance

Race-specific resistance has long been a major tool for controlling plant disease by genetic means. This type of resistance is highly effective against one or more races of a pathogen and ineffective against other races, in other words, it is an "all-or-nothing" resistance (Nelson, 1973).

Race-specific resistance functions only when the host plant carries a resistance gene (*R*) and the pathogen carries an avirulence gene. Flor (1955), working with flax rust (*Melampsora lini*), suggested a gene-for-gene relationship; for each gene determining rust resistance in flax (*Linum usitatissimum*) there was a specific and related gene determining pathogenicity in the rust fungus. Beynon (1997) suggested that the products from the pathogen interact with the corresponding host *R*-gene products to elicit the disease resistance response. Either the absence of the avirulence gene in the pathogen or the absence of *R*-gene in the host could result in host invasion. Thus, the resistance response occurs only when the pathogen releases a gene product which can be detected by the presence of a specific resistance gene *(R)* in the host plant (Herbers et al., 1992).

2.2.2.2 Characterization of race-specific resistance

Race-specific resistance genetically behaves as a single-gene trait (Nelson, 1973). This single gene can confer resistance to one or many races of a pathogen and is considered the most dramatic resistance reaction exhibited by plants against their corresponding pathogens. Clarke (1997) indicated that while race-specific resistance provides host plants with a selective advantage over plants with no specific resistance, its

use can also lead to the rapid appearance of new virulence genes and rapid changes in the structure of the pathogen population. Race-specific and race non-specific resistance to the same fungal parasite may operate through genetically distinct pathways (Schulze-Lefert et al., 1997).

Race-specific resistance functions primarily by suppressing the pathogens from penetrating the host plant through hypersensitive reaction (Nelson, 1973). Gaines (1918; 1920) reported two distinct factors controlling the resistance of wheat to common bunt: (a) one prevents infection, and (b) the other prevents the development of the fungus within the plant tissue and the formation of bunt-balls.

2.2.2.3 Common bunt fungi *(Tilletia caries* and *T. foetida)* and race-specific resistance genes

The development of bunt resistant wheat cultivars requires a systematic breeding program based on genetic studies of this host-pathogen system. The virulence formulae of races of the common bunt fungi are listed in Table 2.1 (Hoffmann and Metzger, 1976).

Among the ten resistant genes, only *Bt8* confers resistance to all reported races (Sharp, 1973). *Bt10* provides resistance to most races except *T-25, T-26,* and *T-27; Bt9* confers resistance to 36 of the 41 known races of common bunt (Hoffmann and Metzger, 1976; Metzger et al., 1979).

Currently in western Canada, the most effective *Bt* genes are *Bt5, Bt8* and *Bt10* (Gaudet and Puchalski, 1989b). A composite of six bunt races, L-7, L-16, T-1, T-6, T-13, and T-19, is used in western Canada for testing wheat cultivars proposed for registration

(Gaudet and Puchalski, 1989b). This composite is virulent against the *Bt1, Bt2, Bt3, Bt4, Bt6* and *Bt7* resistance genes (Kendrick and Holton, 1961: Hoffmann and Metzger, 1976).

Table 2.1. The pathogenic relationship between the common bunt fungi, *Tilletia caries* (T-races) and *T. foetida* (L-races), and the 10 bunt resistance *(Bt)* genes in wheat (Hoffmann and Metzger, 1976).

Race designation	Virulence formula against *Bt* genes	
	Virulence	Avirulence
T-14	1	2, 3, 4, 5, 6, 7, 8, 9, 10
T-10	5	1, 2, 3, 4, 6, 7, 8, 9, 10
T-1, L-1, L-2	7	1, 2, 3, 4, 5, 6, 8, 9, 10
T-20	1, 2	3, 4, 5, 6, 7, 8, 9, 10
T-2, T-4, T-6, L-4	1, 7	2, 3, 4, 5, 6, 8, 9, 10
T-11	2, 3	1, 4, 5, 6, 7, 8, 9, 10
T-3, L-3	2, 7	1, 3, 4, 5, 6, 8, 9, 10
T-9	5, 7	1, 2, 3, 4, 6, 8, 9, 10
T-13, L-9	1, 2, 3	4, 5, 6, 7, 8, 9, 10
T-5, T-7, T-8, L-5, L-6, L-7	1, 2, 7	3, 4, 5, 6, 8, 9, 10
T-12	1, 5, 7	2, 3, 4, 6, 8, 9, 10
T-25	1, 7, 10	2, 3, 4, 5, 6, 8, 9
L-10	2, 3, 7	1, 4, 5, 6, 8, 9, 10
T-17	2, 4, 6	1, 3, 5, 7, 8, 9, 10
T-19	1, 2, 3, 7	4, 5, 6, 8, 9, 10
T-15	1, 2, 5, 7	3, 4, 6, 8, 9, 10
T-26	1, 2, 7, 10	3, 4, 5, 6, 8, 9
T-18	1, 4, 6, 7	2, 3, 5, 8, 9, 10
T-22	2, 4, 6, 7	1, 3, 5, 8, 9, 10
T-21, L-16	1, 2, 4, 6, 7	3, 5, 8, 9, 10
T-24	2, 3, 4, 6, 7	1, 3, 5, 8, 9, 10
T-16	2, 4, 5, 6, 7	1, 3, 8, 9, 10
T-28, L-8	2, 4, 6, 7, 9	1, 3, 5, 8, 10
T-23	1, 2, 4, 6, 7, 9	3, 5, 8, 10
T-27	1, 2, 4, 6, 7, 10	3, 5, 8, 9

2.2.3 Genetic sources of resistance to common bunt in western Canada

Prior to the 1950s, most durum and hard red spring wheats were susceptible to common bunt (Popp, 1947). Since then, bunt infection of cultivated wheats has decreased

due to (1) fungicide use, (2) development of shorter season cultivars, and (3) use of resistant cultivars (Gaudet and Puchalski, 1989b).

Of the wheat cultivars grown in western Canada, all durum wheats are resistant to common bunt under field conditions, but are susceptible to the majority of races under controlled environmental conditions (Gaudet and Puchalski, 1989a). Cultivars with Mexican semi-dwarf cultivars in their ancestry, such as Laura (Depauw et al., 1988), have poor resistance (Saskatchewan Agriculture and Food, 1996b). Most of the currently registered hard red spring wheats possess good resistance to bunt.

2.2.4 Bunt resistance genes and their relationships

The first report on the inheritance of resistance to common bunt was in 1920 when Gaines studied crosses involving the resistance cultivar Turkey (Gaines, 1920). He concluded that (a) different wheat varieties possessed different kinds of resistance and (b) bunt resistance was not a simple Mendelian trait since a continuous range of infection from complete immunity to complete susceptibility was observed (Gaines, 1920, 1923). However, Briggs (1926) reported that resistance to common bunt was due to one factor in Martin and two factors in Hussar. By using different physiologic races in bunt inheritance studies, Bressman (1931) concluded that Martin carried two factors for bunt resistance (M1 and M2), Hussar and White Odessa carried the same factor for bunt resistance and the genes in Martin, Albit and White Odessa were also allelic.

Briggs (1930, 1932, 1933 and 1936) found three major genes for bunt resistance to race T-1 and designated them as the Martin (M), Hussar (H), and Turkey (T) genes,

respectively. The Martin gene was also found in White Odessa, Banner Berkeley, Odessa, and Sherman wheats (Briggs, 1933). Likewise, the Turkey gene carried by Turkey 3055 was also found in Turkey 1558, Turkey 1558B, Turkey 2578 and Oro (Briggs, 1936). The Martin gene and the Turkey gene are loosely linked (34cM) (Briggs, 1940). Stanford (1941) reported a new gene for bunt resistance in Rio wheat, the R gene, which is closely linked to the Turkey (T) gene and more loosely linked to the Martin (M) gene. Later, these four genes and other bunt resistance genes were designated as different *Bt* genes (Table 2.2). Of these four known genes M, H, T, and R, the Martin and the Turkey genes together can give resistance to 25 known races (Briggs and Holton, 1950).

Using aneuploid analysis, Sears et al. (1960) discovered that the principal Martin gene was located on chromosome 2B. Later, a bunt resistance gene carried by the cultivars Bison, Omaha, Nebred and Kaw, each of which had the same Turkey factor, was shown to be located on chromosome 1B (Sears, 1960; Schmidt et al., 1969). The status of chromosome locations and original source for the known bunt resistance genes is listed in Table 2.2.

Among the *Bt* genes, *Bt4* is linked with *Bt5* (30cM) and *Bt6* (15cM) (McIntosh, 1983). *Bt9* was inherited independently of *Bt1*, *2*, *4*, *6*, and *7* (Metzger et al., 1979).

2.2.5 Association of common bunt resistance with agronomic traits

To select indirectly for bunt resistance, possible relationships between bunt resistance and other agronomic traits have been investigated (Churchward, 1932; Smith, 1933). Platt (1950) reported linkage between wheat stem sawfly (*Cephus cinctus* Nort.) resistance and

Table 2.2. Chromosome locations and origins for the known bunt resistance genes.

Gene Symbol	Original Source	Chromosome Location	Reference
Bt1	Martin (M1)	2B	Sears, 1960; Hart et al., 1993
Bt2	Hussar (H)	Unknown	Waud and Metzger, 1970
Bt3	Ridit (rd)	Unknown	Waud and Metzger, 1970
Bt4	Turkey (T)	1B	Schmidt et al., 1969; McIntosh, 1983; Hart et al., 1993
Bt5	Hohenheimer	1B	McIntosh, 1983; Hart et al., 1993
Bt6	Rio (R)	1B	Schmidt et al., 1969; Nelson, 1973; McIntosh, 1983
Bt7	Martin (M2)	2D	Schaller et al., 1960; Hart et al., 1993
Bt8	Yayla 305	Not on 5A, 1B, or 2D	Waud and Metzger, 1970
Bt9	C.I. 7090	Unknown	Metzger et al., 1979
Bt10	PI 113061, PI 113063	1B	Metzger and Silbaugh, 1971
Bt11	PI 554119	Unknown	Goates, 1996
Bt12	PI 119333	Unknown	Goates, 1996
Bt13	Thule III	Unknown	Goates, 1996
Bt14	Doubbi	Unknown	Goates, 1996
Bt15	Carleton	Unknown	Goates, 1996

bunt susceptibility, but McKenzie (1964) did not find any association between sawfly reaction and bunt reaction to race T-2. Similarly, Kilduff (1933) indicated that awnedness was associated with bunt resistance, but Smith (1933) found no linkage between bunt resistance and awnedness or glume colour. No linkage was also found between bunt reaction and chaff colour (Churchward, 1932), plant height, glume colour or heading date (McKenzie, 1964). However, Metzger and Silbaugh (1971) found that the *Bt10* gene

was loosely linked with glume colour on chromosome 1B. Red glume colour (*Rg1*) was linked with the Turkey gene *Bt4* (23cM) but not with the other Turkey gene *Bt5* (McIntosh, 1983).

2.3 Identification of RAPD markers linked to common bunt resistance genes

2.3.1 Introduction

Selection based on phenotype sometimes may not be effective due to environmental influence, or is difficult because of the complex inheritance of polygenic characters. This relates particularly to the current disease testing procedure for common bunt resistance which is not only time consuming and labour intensive, but also frequently inconclusive due to environmental effects on disease infection (Gaudet et al., 1993). Thus, testing must be repeated for a few years to secure accurate disease data.

The use of DNA markers can avoid the short-comings of phenotype-based selection (Rafalski et al., 1991; Waugh and Powell, 1992) and allows plant breeders to select at any time since molecular markers are reflecting information on DNA sequences which are not influenced by the environment. Thus, the advantage of using molecular markers over traditional selection is their ability to predict the presence or absence of the resistance genes in question based on the linkage between the resistance genes and the markers (Williams et al., 1990). Therefore, the identification of molecular markers linked to bunt resistance could substantially facilitate the screening of wheat breeding lines and accelerate the development of new resistant cultivars.

2.3.2 RAPD assay

The polymerase chain reaction (PCR) was first used in medical diagnosis for the detection of sickle cell anaemia (Saiki et al., 1985). It involved a series of cycles each comprising three steps: a) heat denaturation of DNA, b) primer annealing to DNA template, and c) extension of the target DNA. Use of random amplified polymorphic DNA (RAPD) was developed by Williams et al. (1990). The RAPD assay is based on PCR amplification of DNA fragments without knowledge of the target DNA sequence. Technically, it relies on differential amplification of DNA fragments by the polymerase chain reaction, using a single oligonucleotide primer of arbitrary sequence. The polymorphic DNA sequences caused by base changes in the primer binding sites or by chromosome rearrangements within the amplified sequence are separated by gel electrophoresis. This procedure requires only a small amount of DNA.

2.3.3 Comparison of RAPDs with other molecular markers

Since morphological markers are likely associated with deleterious effects and not easy to analyse in breeding populations, and the number of polymorphisms for isozymes is very limited (Tanksley et al., 1989), DNA-based markers can avoid these limitations. DNA-based markers include restriction fragment length polymorphism (RFLPs) (Grodzicker et al., 1974; Beckmann and Soller, 1986), randomly amplified polymorphic DNA (Williams et al., 1990; Welsh and McClelland, 1990), sequence-characterized amplified regions (SCAR) (Paran and Michelmore, 1993; McDermott et al., 1994), amplified fragment length polymorphism (AFLP) (Vos et al., 1995), sequence-tagged-

sites (STS) (Olson et al., 1989), and simple sequence repeats or microsatellites (SSR) (Wu and Tanksley, 1993; Becker and Heun, 1995).

Restriction fragment length polymorphism (RFLP) is based on the cleavage of genomic DNA by restriction enzymes into different lengths and the length polymorphism at a specific site is then detected after hybridization with a labelled DNA probe (Ragot and Hoisington, 1993). RFLP has been used to develop detailed genetic maps and for selection of genotypes without the need of phenotypic expression (Paran et al., 1991, Rafalski et al., 1991). The disadvantages of RFLP are that this assay requires large amounts of DNA, requires DNA transfer and Southern blot hybridization and is therefore laborious and time-consuming (Rafalski et al., 1991). Random amplified polymorphic DNA (RAPD) is widely used in many different organisms, including plants, animals and micro-organisms (Williams et al., 1990; Welsh and McClelland, 1990; Rafalski et al., 1991; Michelmore et al., 1991; Waugh and Powell, 1992; Devos and Gale, 1992; Vierling and Nguyen, 1992). RAPD analysis does not have the technical limitations of RFLP analysis, is rapid, requires only small amounts of DNA which does not have to be of high quality, and does not involve use of radioactive probes (Williams et al., 1990, Rafalski et al., 1991, Devos and Gale 1992).

Both RAPD and RFLP have been used to detect polymorphisms in DNA sequence (Rafalski et al., 1991; Tinker et al., 1993). The RFLP assay usually needs species-specific probes (cDNAs or randomly chosen genomic clones) to detect genetic loci that share sequence homology. One or more alleles can be detected at a genetic locus. In segregating progenies, homozygotes can be distinguished from the heterozygotes because of the property of codominance. For RAPD, however, the complete sequence of the amplified

products is unknown. RAPD markers are usually dominant, thus can not immediately deduce heterozygosity or homozygosity (Paran et al., 1991, Carlson et al., 1991). The RAPD technique is also more sensitive to changes in the reaction conditions (Devos and Gale, 1992).

A more recent type of molecular marker is the amplified fragment length polymorphism (AFLP) (Vos et al., 1995). This procedure includes the restriction of total DNA by endonucleases, ligation of oligonucleotide adapters to the ends of the fragments, followed by the selective amplification of the restricted DNA fragments. AFLP can produce reproducible patterns and high levels of polymorphism (Becker et al., 1995; Prabhu and Gresshoff, 1994). However, a major disadvantage is that AFLP markers are dominant.

Paran and Michelmore (1993) developed sequence-characterized amplified regions (SCARs) as additional PCR-based molecular markers. A SCAR is a DNA sequence at a single genetically defined locus which can be identified by PCR amplification using a pair of specific primers which are derived by cloning and sequencing the two ends of the amplified products of RAPD markers. The advantages of SCARs over RAPD markers are that they can detect a single locus, their amplification is less sensitive to PCR reaction conditions and they can be converted into codominant markers (Paran and Michelmore, 1993; McDermott et al., 1994). SCARs are similar to the sequence-tagged site (STSs) proposed by Olson et al. (1989) in the physical map of human genome since SCARs can be derived from RAPD or cloned sequences, such as RFLP probes, with a known location on a chromosome (Talbert et al., 1994). SCARs derived from RFLP probes are actually identical to STSs but are not useful as markers. STSs are safer to use, more efficient than

RFLP and more reproducible than RAPDs (Talbert et al., 1994). Simple sequence repeats (SSR) or microsatellites are tandemly arranged repeats of oligonucleotides on a chromosome (Becker and Heun, 1995). The major advantages of SSR are their high level of polymorphism and that they are genetically codominant (Wu and Tanksley, 1993).

2.3.4 Bulked segregant analysis and identification of RAPD markers linked to disease resistance genes

Bulked segregant analysis was developed by Michelmore et al. (1991) as a rapid procedure for identifying markers and has been employed to develop RAPD markers for disease resistance genes in many crops, such as wheat (Hartl et al., 1995; Demeke et al., 1996), barley (Barua et al., 1993; Krasichynska, 1997), oat (Penner et al., 1993a, 1993b), common bean (Haley et al., 1993), lettuce (Michelmore et al., 1991; Paran et al., 1991), and muskmelon (Wechter et al., 1995). Bulked segregant analysis uses two bulked DNA samples collected from individuals segregating in a population from one cross and is carried out by screening, using random primers, for differences between the two DNA samples (e.g. resistant and susceptible bulks). Each pool contains individuals having identical genotypes for a specific genomic region of interest but arbitrary genotypes at other loci. Thus, the two DNA bulks are considered to differ genetically only in the region of interest and the DNA fragment amplified in one sample but not in the other should be genetically linked to the loci controlling the trait of interest.

2.3.5 Application of RAPD markers to the mapping of disease-resistance genes

RAPDs are considered as informative as RFLPs in genomic mapping for homozygous lines such as double haploid lines or advanced recombinant inbred lines (Reiter et al., 1992; He et al., 1995), and considerable efforts to convert RFLP markers to specific PCR-based amplicons are underway (Penner, 1996). Undoubtedly, RAPD analysis will play an increasingly important role in genomic mapping (Weining and Langridge, 1991; Uphoff and Wricke, 1992; Mouzeyar et al., 1995; Jacobs et al., 1996; Zhang et al., 1996).

3. RACE-SPECIFIC RESISTANCE TO COMMON BUNT (*TILLETIA CARIES* AND *T. FOETIDA*) IN WHEAT

3.1 Introduction

Race-specific resistance normally refers to resistance effective against some races of a pathogen and ineffective against other races (Nelson, 1973). This type of resistance prevents the establishment of a successful infection site and thus is usually expressed as a hypersensitive reaction (Nelson, 1973; Broers, 1989). Based on the gene-for-gene hypothesis, race-specific resistance is considered to be due to the recognition between the product of a resistance gene and an elicitor produced by the corresponding avirulence gene (Herbers et al., 1992; Knogge and Marie, 1997). Race-specific resistance is usually controlled by a single gene (Nelson, 1973; Broers, 1989; Hovmøller, 1989; Bonman, 1992; Robinson, 1996; Brown et al., 1997), but examples of control by more than one gene exist (Hovmøller, 1989).

Race-specific resistance has been considered a major tool for control of plant disease. Therefore, it was important to determine if race-specific resistance to the bunt races currently prevalent in western Canada exists so that bunt resistance genes can be used effectively. Accordingly, the objectives of these experiments were (1) to investigate the existence of race-specific bunt resistance in selected common and spelt wheat cultivars and (2) to provide a basis for choosing appropriate genotypes and common bunt races for

genetic studies of bunt resistance in wheat.

3.2 Materials and methods

3.2.1 Materials

Three separate experiments were conducted using 17 different wheat cultivars/lines including landraces, improved cultivars and nine single *Bt*-gene lines (Table 3.1).

Table 3.1. Genotypes tested for race-specific resistance to common bunt in 1994.

Experiment number	Total number of cultivars/lines	Cultivar/line
1	9	Columbus[a], Katepwa, Laura[a], Triple Dirk, Glaive, PI428536, PI428552, PI414707, RL5407
2	12	SK0263, SK0505, Kite, Bowie, PI469271, SUN118A, USDA25, Lake, M2145-2-5, Columbus, Katepwa, Laura
3	11	Columbus, Laura and nine *Bt*-gene lines

[a] Columbus and Laura were used in all three experiments.

Prior to seeding, seed of the host genotypes was inoculated with six common bunt races, two races (L7, L16) of *T. foetida* and four races (T1, T6, T13, and T19) of *T. caries*. Spores of each race were obtained from infected spikes which were ground to produce a powder of teliospores. Although many bunt races have been identified in western Canada (Gaudet and Puchalski, 1989a), these six races are considered the most important in western Canada because of their prevalence.

3.2.2 Experimental design

Split-plot designs with four replications were used in each of the three experiments. Cultivars and *Bt*-gene lines were treated as the main plots while the six races and a non-inoculated Laura control were treated as subplots. Each subplot was seeded as a hill with 50 seeds and the hills were spaced on 45 cm centers. One border row, planted with cv. Katepwa, surrounded each experiment. The experiments were planted by hand at a depth of about 6 cm on the North Seed Farm (Dark Brown Chernozem, loam), University of Saskatchewan on May 2, 1994.

3.2.3 Inoculation and disease rating

Prior to seeding, a seed sample of each cultivar was inoculated with each of the six races, by shaking an envelope containing 50 seeds and about 0.8 gram of teliospores. Excess inoculum was removed by shaking the seeds on a fine mesh sieve. Before and after inoculation with each race, the mesh sieve, the sampling spoon and the working counter were all completely cleaned and sterilised using 0.8-1.0% sodium hypochloride solution.

During ripening, the hills were cut and the spikes smashed with a hammer to check for bunt infection; spikes with at least one bunted kernel were rated as susceptible. The ratio of the number of bunted spikes to the total number of spikes in a plot (hill) was recorded as the disease incidence for that plot. Disease incidence was converted to percentage for statistical analysis.

3.2.4 Statistical analysis

Statistical analysis was performed separately for each of the three experiments using the procedure PROC GLM in the statistical package SAS version 6.12 (SAS institute, Cary, NC, USA, 1996). Least significant differences (LSD) were calculated by hand.

3.3 Results

Mean disease incidences for each entry in the three experiments are listed in Tables 3.2, 3.3 and 3.4. The non-inoculated susceptible control (Laura) was not included as it demonstrated very low infection (3.4% on average), indicating that levels of background inoculum in the soil were very low.

Analysis of variance demonstrated that there were highly significant differences among cultivars and bunt races in all three tests (Table A.1). The cultivar x race interaction was highly significant in each experiment, indicating that resistance to common bunt may be race specific (Table A.1). In practice, it is speculated that race specificity exists if there are differences in the virulence of different races and/or there are differences in resistance/susceptibility of different cultivars to the races. Race specific resistance can be seen in the bunt reactions of certain cultivars to different bunt races as there were significant differences among different cultivar-race combinations (Tables 3.2-3.4).

In addition, by comparison of the reaction of the resistant cultivars with the reactions of the known single *Bt*-gene lines, it is postulated that Triple Dirk, RL5407, Katepwa, SK0505, SK0263 and USDA25 may carry the *Bt6*, *Bt8*, or *Bt10* gene. Likewise, cultivar Lake (Table 3.3) and the *Bt2* and *Bt3* single-gene lines (Table 3.4) were highly resistant to

Table 3.2. Mean bunt incidence (% infected heads) for wheat cultivars tested in Experiment 1 using six races of common bunt (Saskatoon, 1994).

Cultivar	Race						Mean
	L7	L16	T1	T6	T13	T19	
Laura	83.2 a[1]	76.3 a	48.8 b	44.8 b	82.6 a	87.5 a	70.5 A
PI414707	78.7 a	67.6 ab	44.6 c	69.6 ab	55.1 bc	63.1 ab	63.1 A
PI428552	56.3 a	62.2 a	31.1 b	22.9 b	56.9 a	71.4 a	50.1 B
PI428536	35.7 b	55.0 a	20.5 c	31.6 bc	45.0 ab	53.8 a	40.3 C
Glaive	30.3 a	30.5 a	17.8 ab	21.9 ab	19.4 ab	13.1 b	22.2 D
Columbus	21.1 ab	26.0 ab	11.1 b	26.8 ab	18.7 ab	27.8 a	21.9 D
Triple Dirk	24.8 a	19.3 a	13.3 a	20.1 a	13.6 a	11.0 a	17.0 DE
RL5407	11.9 a	15.7 a	5.1 a	9.8 a	12.0 a	5.2 a	10.0 EF
Katepwa	6.3 a	2.2 a	2.2 a	6.1 a	7.4 a	8.9 a	5.5 F
Mean	38.7 a	39.4 a	21.6 c	28.2 b	34.5 a	38.0 a	33.4

[1] Means in the same row (except the last column) and means in the last column not followed by the same letter are significantly different at $P = 0.05$ level.

Table 3.3. Mean bunt incidence (% infected heads) for wheat cultivars tested in Experiment 2 using six races of common bunt (Saskatoon, 1994).

Cultivar	Race						Mean
	L7	L16	T1	T6	T13	T19	
Laura	85.5 a[1]	94.7 a	50.0 c	65.4 b	84.6 a	90.9 a	78.5 A
PI469271	54.2 bc	68.7 b	50.2 c	37.9 c	84.5 a	84.3 a	63.3 B
Sun 118A	28.6 c	36.6 bc	35.8 bc	39.2 bc	62.6 a	47.3 ab	41.7 C
Bowie	27.9 ab	33.4 ab	20.9 b	36.0 ab	33.6 ab	38.0 a	31.6 D
Lake	31.6 b	47.2 a	1.4 c	5.5 c	45.6 ab	55.2 a	31.1 D
Columbus	28.3 b	20.5 b	17.7 b	24.1 b	23.5 b	48.6 a	27.1 D
M2145-2-5	29.9 a	17.0 a	26.5 a	31.9 a	22.0 a	28.4 a	26.0 D
Kite	15.3 ab	29.0 a	10.0 b	18.3 ab	15.4 ab	11.7 b	16.6 E
SK0263	11.4 a	20.4 a	16.0 a	16.8 a	15.8 a	12.1 a	15.4 E
Katepwa	13.0 a	10.7 a	7.2 a	6.8 a	11.8 a	20.8 a	11.7 E
SK0505	16.7 a	15.6 a	5.9 a	10.5 a	8.8 a	6.5 a	10.7 EF
USDA 25	0.3 a	0.3 a	2.1 a	7.4 a	3.2 a	3.6 a	2.8 F
Mean	28.6 cd	32.8 bc	20.3 e	25.0 d	34.3 ab	37.3 a	29.7

[1] Means in the same row (except the last column) and means in the last column not followed by the same letter are significantly different at $P = 0.05$ level.

Table 3.4. Mean bunt incidence (% infected heads) for wheat cultivars tested in Experiment 3 using six races of common bunt (Saskatoon, 1994).

Bt-gene line	Race						Mean
	L7	L16	T1	T6	T13	T19	
Laura	86.6 a[1]	92.6 a	31.4 c	54.9 b	90.1 a	93.6 a	74.9 A
Bt7	66.7 b	85.6 a	49.2 c	67.6 b	61.9 bc	89.0 a	70.0 A
Bt1	84.5 a	69.4 b	32.9 c	44.4 c	59.2 b	82.3 a	62.1 B
Bt2	78.5 a	71.3 a	0.0 b	10.0 b	85.5 a	84.2 a	54.9 C
Bt4	48.6 bc	73.4 a	27.1 d	38.2 c	36.0 c	54.6 b	46.3 D
Bt3	45.5 c	51.8 bc	4.1 d	5.4 d	65.0 ab	69.2 a	40.2 E
Columbus	24.4 ab	25.6 ab	12.2 b	17.7 b	13.3 b	33.2 a	21.1 F
Bt9	5.3 ab	2.4 b	19.0 a	8.2 ab	1.0 b	11.0 ab	7.8 G
Bt8	9.2 a	4.6 a	4.7 a	5.5 a	8.8 a	7.7 a	6.8 GH
Bt6	7.6 a	4.2 a	6.5 a	7.0 a	5.7 a	5.1 a	6.0 GH
Bt10	1.5 a	1.1 a	2.5 a	1.4 a	0.3 a	9.7 a	2.8 H
Mean	41.7 bc	43.8 b	17.2 e	23.7 d	38.8 c	49.1 a	35.7

[1] Means in the same row (except the last column) and means in the last column not followed by the same letter are significantly different at $P = 0.05$ level.

races T1 and T6, but susceptible to the other races, suggesting that Lake possibly carries either the *Bt2* or *Bt3* gene. Race-specific resistance was also suggested by differences within the same cultivar in bunt incidence caused by different races (Tables 3.2, 3.3 and 3.4). This significant cultivar x race interaction (Table A.1.) suggested that there may be major resistance gene differences causing the differential reaction of the cultivars to different races (Hovmøller, 1989; Herbers et al., 1992).

In addition, cultivars Triple Dirk, RL5407, Katepwa, SK0263, SK0505 and USDA25 demonstrated uniform resistance to all six races (Tables 3.2 and 3.3). This either suggests that these cultivars possess race-nonspecific resistance or that races to which these cultivars are susceptible were not included in these experiments.

The means for bunt incidence for the different cultivars in the three experiments were compared. In Experiment 1, Katepwa, RL5407, Triple Dirk and Columbus were the most resistant cultivars with average infection rates of 5.5%, 10.0%, 17.0% and 21.9%, respectively (Table 3.2). In Experiment 2, the most resistant varieties included the durum wheat USDA25 (2.8%) followed by two spelt wheats, SK0505 (10.7%) and SK0263 (15.4%), and common wheats Katepwa (11.7%) and Kite (16.6%) (Table 3.3). The durum cultivar USDA25 had the highest level of resistance to all races. Its genetic background and origin are unknown.

Race T1 appeared to be the least virulent race, causing an average infection level of 21.6%, 20.3% and 17.2%, respectively, in the three experiments (Tables 3.2, 3.3 and 3.4). Race T19 was possibly the most virulent race since it induced the highest infection levels in Experiments 2 and 3 (37.3% and 49.1%, respectively) although it did not cause higher disease incidence on all cultivars in Experiment 1. However, in other studies which

included races L16, T1 and T19. Gaudet and Puchalski (1990) found that L16 was the least virulent race. These differences in apparent virulence could be due to different host genotypes tested and/or the different test environments used.

3.4 Discussion

Significant differences for resistance were found among the wheat cultivars tested and for virulence among the bunt races used. Highly significant cultivar x race interactions suggested that some resistances identified are race-specific (Singh et al., 1990; Andrivon and De Vallavieille-Pope, 1992). The resistance of Triple Dirk, Kite, RL5407 and SK0263 was effective against all six races (Tables 3.2 and 3.3). Cultivars Lake, line PI428552 and the *Bt2* and *Bt3* single gene lines exhibited typical race-specific resistance as they showed resistance to some but not all races (Singh et al., 1990). Race-specific resistance is advantageous for resistance breeding since it can provide a high level of resistance against virulence factors and thus is considered to be the simplest way to control fungal diseases by genetic means (Grama et al., 1984; Gerechter-Amitai and Van Silfhout, 1989).

Based on these data (Tables 3.2, 3.3 and 3.4), common wheat cultivars Triple Dirk and Kite, and spelt wheats SK0263, RL5407 and SK0505 were chosen for further genetic study since they were the most resistant cultivars and may possess a major gene for bunt resistance. Laura, which demonstrated the highest disease incidence, was selected as a susceptible parent for making crosses for the genetic study.

It would be more informative to use the race with least virulence than that with the most virulence in the genetic studies. If race 1 is the least virulent race and a host genotype carries *j* genes for resistance to different races of bunt, then race 1 should

detect *j* resistance genes when used as inoculum (Table A.2). However, if the most virulent race (race$_i$) is used instead, then the genetic analysis would identify just one resistance gene, and information on resistance or susceptibility to the other *i*-1 races would still be unknown. Accordingly, the least virulent race, identified as race T1 in these experiments, was used in the genetic studies.

In Experiment 3 which involved the single *Bt* gene lines, the lines carrying *Bt6*, *Bt8* and *Bt10* genes were uniformly resistant to all six races and their resistance level was high (Table 3.4). This agrees with previous results (Gaudet and Puchalski, 1989b) except that they found *Bt6* to be susceptible to L16. This difference in result could be due to environmental difference (year and location) since bunt incidence of a cultivar can vary significantly between years and locations (Reed, 1928). The uniform resistance of *Bt6*, *Bt8* and *Bt10* to the 6 races used here appears to be race non-specific, but in reality, is not since races capable of overcoming these resistances exist but were not included in this experiment (Gaudet and Puchalski, 1989b).

Cultivar Lake possibly carries the *Bt3* gene (Tables 3.3, 3.4) as both Lake and the *Bt3* single-gene line showed similar disease reactions to all six races. Both were highly resistant to races T1 and T6, but susceptible to the other races. Using the same reasoning, RL5407 possibly possesses *Bt6*, *Bt8* or *Bt10* (Tables 3.2, 3.4).

4. INHERITANCE OF RESISTANCE TO COMMON BUNT *(TILLETIA CARIES* AND *T. FOETIDA)* IN WHEAT : I. QUANTITATIVE ANALYSIS

4.1 Introduction

Common bunt, caused by *T. foetida* and *T. caries*, is an important disease of wheat since it can reduce not only grain yield but also grain quality by forming bunt balls containing black toxic fungal chlamydospores (Singh and Chopra, 1986). Fungicide seed treatment is not always effective in controlling this disease and its use can have adverse environmental effects. Therefore, the most satisfactory way to control common bunt is by planting resistant cultivars. So far, 15 major genes conferring resistance to common bunt in wheat have been identified (Table 2.2) for use in breeding programs. Studies on the inheritance of resistance to bunt assist breeding for resistance by providing information on gene action and genetic control of resistance in order to develop appropriate breeding strategies.

Spelt wheat (*T. aestivum* ssp. *spelta* or *T. spelta*), a primitive form of hexaploid wheat, has natural defense against fungal diseases, such as yellow rust (Kema, 1992a, 1992b). In the 1994 bunt tests (Section 3.3), the spelt wheats RL5407, SK0263 and SK0505 and common wheats Kite and Triple Dirk demonstrated high levels of resistance to common bunt, and possibly carry major genes for resistance. The objectives of this

study were (i) to investigate gene action for resistance to common bunt in selected spelt and common wheat cultivars, (ii) to estimate heritability, (iii) to estimate the number of effective factors conferring resistance to common bunt, and (iv) to detect maternal effects on bunt resistance.

4.2 Materials and Methods

4.2.1 Materials

(a) Host materials

On the basis of previous studies (Section 3), the resistant common wheat cultivars Kite, Triple Dirk, the spelt wheat lines RL5407, SK0263, SK0505 and the susceptible common wheat cultivars Laura and Genesis were used as the crossing parents for genetic study (Table 4.1).

The crosses Laura/Triple Dirk, Laura/RL5407 and Genesis/SK0263 were made during winter 1993/1994 and were advanced to F_3 seeds in winter 1994/1995 and F_4 and F_5 seeds in summer 1995 and winter 1995/1996. The cross Laura/Kite was advanced to F_3 in winter 1994/1995 and tested for bunt incidence in 1995. Backcross F_2 progenies were also produced for the crosses Laura/Triple Dirk and Laura/RL5407 in summer 1995 and winter 1995/1996 (Table A.3.).

In 1995, the parental, F_1, F_2 and F_3 generations of the crosses, Laura/Triple Dirk, Laura/RL5407, Laura/Kite and Genesis/SK0263, were studied except that the F_2 generation of the cross Genesis/SK0263 was not included. In 1996, only three crosses, Laura/Triple Dirk, Laura/RL5407 and Genesis/SK0263 were used in the genetic studies. The parental, F_2, F_4, F_5 and BC_1F_2 generations were used for the crosses Laura /Triple Dirk

Table 4.1. Agronomic characteristics of the parental genotypes used in the genetic studies of common bunt resistance.

Variety	Wheat Type	Awnedness	Glume Color	Auricle Color	Bunt Reaction	Origin
Kite	common	non-awned	white	white	resistant	Australia
Triple Dirk	common	non-awned	white	white	resistant	Australia
RL5407	spelt	awned	black	white	resistant	landrace
SK0263	spelt	awned	black	white	resistant	landrace
SK0505	spelt	awned	black	white	resistant	landrace
Laura	common	awned	white	white	susceptible	Canada
Genesis	common	awned	white	red	susceptible	Canada

and Laura/RL5407, and the P_1, P_2, F_2 and F_4 generations were used for cross Genesis/SK0263.

Reciprocal crosses involving Genesis and RL5407 were also made in summer 1995 and advanced to F_2 in winter 1995/1996 to study maternal effects on bunt resistance. The P_1, P_2 and F_2 generations for the crosses Genesis/RL5407 and RL5407/Genesis were studied in 1996.

(b) Pathogen

Race T1 was used as inoculum in all genetic studies based on its virulence in the study of race-specific resistance (Tables 3.2-3.4, Section 3). Similarly, races L7 and T13 were also used to test the P_1, P_2 and F_2 families of the crosses Laura/Triple Dirk and Laura/RL5407, in order to study the allelism of host resistance to these three races.

4.2.2 Experimental design

In 1995, the parental, F_1, F_2 and F_3 generations of each of the four crosses, Laura/Kite, Laura/Triple Dirk, Laura/RL5407 and Genesis/SK0263 were tested separately in a RCBD experiment with two replications. For each cross, 80 to 84 F_3 families and 8 plots for each of the F_1, F_2, P_1 and P_2 generations were randomized in each replicate. Except for the F_3 generation, the plots were hills planted on 46 cm centers. Each plot (hill) contained 20 seeds for the F_2, P_1 and P_2 generations and 4-6 seeds per plot for the F_1 generation. For each F_3 family plot, about 15 seeds were planted separately as a sub-hill at each corner of a 30 cm square, 60 seeds per plot. The experiments were planted on May 11-13th, 1995 at

the North Seed Farm. University of Saskatchewan. Saskatoon. on land where bunt tests had never been grown. The plot map for each experiment is shown in Fig. 4.1.

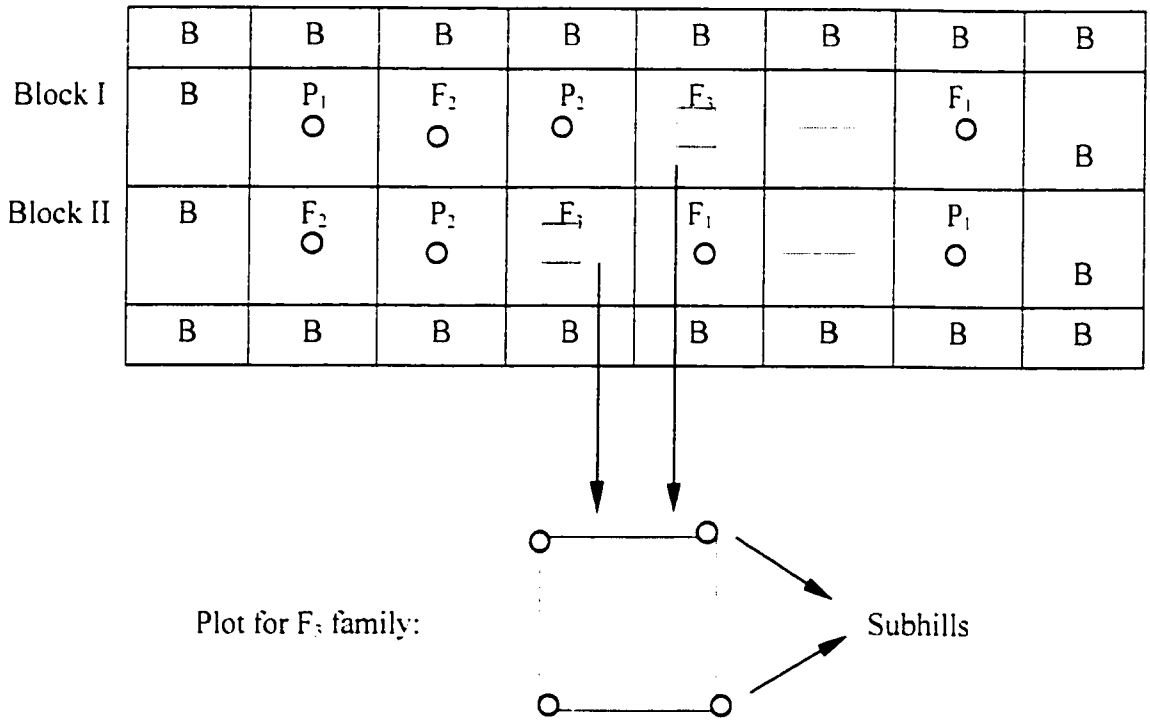

Fig. 4.1. Field map for the randomized complete block designs in 1995.
B = the border. Katepwa.

In 1996, the experiments involved the parental, F_2, F_4, F_5 and BC_1F_2 generations of the crosses Laura/Triple Dirk and Laura/RL5407 and included 30 and 29 F_5 families, 40 and 50 F_4 families, and 11 and 35 BC_1F_2 progenies, respectively. Only the parental, F_2 and F_4 generations (50 F_4 families) were tested for cross Genesis/SK0263. Eight plots of the parental and F_2 generations were included in each experiment as in 1995. All experiments were laid out in a randomized complete block design with two replications and one hill represented one plot. About 40 seeds were seeded in each plot of the F_2, F_4, F_5 and BC_1F_2 generations and 20 seeds for each plot of the parents.

In the tests of crosses Laura/Triple Dirk and Laura/RL5407 inoculated with races L7 and T13, 30 and 29 F_5 families and eight plots of each parent were randomized in each of two blocks. In the experiment to study maternal effects, eight plots of the parental and F_2 generations of crosses Genesis/RL5407 and RL5407/Genesis, were seeded in two randomized blocks using 50 seeds/plot for the F_2 and 30 seeds/plot for the parental generations.

4.2.3 Inoculation and disease rating

Prior to seeding, seeds of each treatment were dusted with spores of the appropriate bunt race by shaking the seeds and bunt spores together in a seed envelope. At maturity, individual plants were pulled and separated, and spikes of each plant smashed to check for bunt infection. Plants with at least one bunted kernel were rated as susceptible. The proportion of infected plants to the total number of plants in a plot was used as the disease infection rate for that plot (McKenzie, 1964; Schmidt et al., 1969; Singh and Chopra, 1986).

4.2.4 Genetic analysis

4.2.4.1 Generation mean analysis

Analyses of variance were conducted to test for differences in disease incidence among all generations and the means and standard errors were calculated for the generations of each cross. Generation mean analysis was used to investigate gene effects and to test for goodness-of-fit of the additive-dominance model (Mather and Jinks, 1982).

Generation mean analyses were carried out by the method of joint scaling tests (Cavalli, 1952). This procedure consists of firstly estimating the parameters m, [d] and [h], based on the means of all available generations, and then comparing the observed generation means with their expected values based on estimates of the three parameters. The parameters m, [d] and [h] were estimated by a weighted least square procedure using the reciprocal of the squared standard error of the mean as the weighting factor.

If the additive-dominance model is assumed to be adequate, then the different generation means should have the following relationships:

$$F_2 = 1/4P_1 + 1/4P_2 + 1/2F_1$$

$$F_3 = 3/8P_1 + 3/8P_2 + 1/4F_1$$

$$F_4 = 7/16P_1 + 7/16P_2 + 1/8F_1$$

$$F_5 = 15/32P_1 + 15/32P_2 + 1/16F_1$$

$$BC_1F_2 = 5/8P_1 + 1/8P_2 + 1/4F_1$$

where P_1, P_2, F_1, F_2, F_3, F_4, F_5 and BC_1F_2 represent generation means. Therefore, the relationships between the generation means and three parameters (m, d and h) could be established as outlined in Table 4.2.

Table 4.2. Coefficients of the three parameters (m, d and h) for the construction of generation means for common bunt incidence.

Generation	m	d	h
P_1	1	-1	0
BC_1F_2	1	-1/2	1/4
F_1	1	0	1
F_2	1	0	1/2
F_3	1	0	1/4
F_4	1	0	1/8
F_5	1	0	1/16
P_2	1	1	0

4.2.4.2 Estimation of heritability

A broad sense heritability for bunt resistance in the F_2-derived i^{th} generations was calculated as the ratio of genetic variance to the phenotypic variance (Falconer, 1989):

$$h^2_{Fi} = V_G/V_P = (V_{Fi}-V_e)/V_{Fi}$$

where V_{Fi} is the phenotypic variance of the generation *i*. The environmental variance (V_e) was estimated using the phenotypic variances of the genetically homogeneous generations (P_1, P_2 and F_1) (Bjarko and Line, 1988; Das and Griffey, 1994). In 1995, V_e was estimated by averaging the phenotypic variances of the P_1, P_2 and F_1 generations using $V_e = (V_{p1} + V_{p2} + V_{F1})/3$. In 1996, the environmental variance was estimated by averaging the variances of two parents because the F_1 generation was not tested.

Estimates of heritability using parent-progeny regression coefficient were also made using the formula (Smith and Kinman, 1965; Camacho-Casas et al., 1995).

$$b = Cov(F_i, F_{i-1})/V_{Fi-1}$$

For the F_2-derived lines, heritability $h^2 = b$ which gives an estimate between broad- and narrow-sense heritability.

4.2.4.3 Estimation of number of effective factors

Estimates were made using the Castle (1921), Wright (1934) and Burton's (1951) method of moments:

$$n_e = D^2/(8*(\sigma_s^2 - \sigma_e^2))$$

where n_e is the estimated number of effective factors, D is the genetic range, σ_e is the standard deviation of environmental variation and σ_s is the standard deviation of the segregating generation (Castle, 1921).

The standard error of the estimated number of effective factors was obtained by the following modification of the formula of Lande (Lande, 1981):

$$S.E. \cong \{n_e^2[(4\sigma_{p1}^2/N_{p1} + 4\sigma_{p2}^2/N_{p2})/(\mu_{p2} - \mu_{p1})^2 + Var(\sigma_g^2)/\sigma_g^4]\}^{1/2}$$

where n_e is the estimated number of effective factors, σ_{p1}^2 and σ_{p2}^2 are the parental variances, μ_{p2} and μ_{p1} are the means of the parental bunt incidence and σ_g^4 is the square of the genetic variance of the segregating generation. $Var(\sigma_g^2)$ is the variance of the genetic variance of the segregating progeny and is estimated by $2\sigma_s^4/N_s + 2/9*(\sigma_{F1}^4/N_{F1} + \sigma_{p1}^4/N_{p1} + \sigma_{p2}^4/N_{p2})$ when the F_1 is present, or $2\sigma_s^4/N_s + \sigma_{p1}^4/(2N_{p1}) + \sigma_{p2}^4/(2N_{p2})$ when the F_1 is absent. N_{p1}, N_{p2}, N_{F1} and N_s are the numbers of plots in the P_1, P_2, F_1 and the segregating generations, and σ_{p1}^4, σ_{p2}^4, σ_{F1}^4 and σ_s^4 are the squares of the variances of the P_1, P_2, F_1 and the segregating generations, respectively.

4.2.5 Determination of maternal effect

Testing for maternal effects was carried out by comparing the F_2 means of bunt incidence for the two reciprocal crosses Genesis/RL5407 and RL5407/Genesis. A difference between them will suggest maternal effects on bunt incidence.

4.3 Results

4.3.1 Examination of disease data

4.3.1.1 The type of distribution and testing normality of the residuals

A plant was rated as "diseased" when at least one bunt ball was found on a spike, otherwise as "resistant" or "healthy". Therefore, data for bunt incidence among plants represent two categories, i.e. 1 and 0, and thus resemble a binomial data set. For each family, the disease rating is based on the proportion of infected plants in the total number of plants rated. Therefore, the disease data for any generation can be either binomially or normally distributed assuming that they do not fit other kinds of distributions.

For a valid analysis of variance in statistical tests and for further genetic analysis, the error terms of individual samples rather than the data themselves must be normally distributed (Sokal and Rohlf, 1981). To test this, the Kolmogorov-Smirnov test can be used (Sokal and Rohlf, 1981; Steel and Torrie, 1997).

The Kolmogorov-Smirnov test (Tables A.4 and A.5) indicated that for most of the samples there was no difference between the observed and the expected distributions for the error terms except in four cases (Triple Dirk in 1995, $F_{4:5}$ lines of Laura/Triple Dirk in 1997, F_3 families of Laura/RL5407 in 1995 and Kite in 1995). In other words, for most of

the disease data, the error terms were found to be normally distributed, indicating that correction for non-normality was not necessary.

4.3.1.2 Analyses of variance

(a) Experiments involving race T1

Analyses of variance were carried out for each experiment (Tables A.6 - A.10). The mean squares for the treatments, among generations and within the different segregating generations (F_3, F_4, F_5, BC_1F_2, $F_{4:5}$, $F_{5:6}$ and $F_{4:6}$) were highly significant for the four crosses tested (Tables A.6 - A.10), except for the mean square for the BC_1F_2 generation in the cross Laura/Triple Dirk in 1996. This last result was probably due to sampling error because of the small number of families (11 families) available for testing in this generation. One exception was the unexpected significant mean square for the F_1 generation of cross Laura/Triple Dirk in 1995 (Table A.7). This was possibly due to the limited number of plants available (about 5 seeds planted per plot) and thus might be more affected by the sampling error than any other generations.

The within-generation mean squares for the F_2, F_1, P_1 and P_2 generations were not significant since the three generations F_1, P_1 and P_2 were genetically homogeneous, and the F_2 generation, although segregating, was seeded in bulk (50 randomly chosen seeds per plot) and therefore, should show only the variation shown by the non-segregating generations.

(b) Experiments involving race T13 and L7

One data point for the resistant parent Triple Dirk showed an unexpectedly high disease incidence of 68.2% as compared to the rest of the parental plots. This outlier data point (r_{11} = 0.908, P = 0.00, Table A.11) was discarded and estimated instead (23.3%) (Sokal and Rohlf, 1981). Analyses of variance were then carried out for crosses Laura/Triple Dirk and Laura/RL5407 (Table A.10). The results showed that the mean squares were highly significant for treatment, among generations and within F_2 progenies for both crosses inoculated with T13 and L7.

4.3.1.3 Detection of heterogeneity of variance

The variances of the non-segregating populations were tested for heterogeneity using Bartlett's test both before and after logarithmic transformation ($\log_{10}$) of data for bunt resistance to race T1 (Table 4.3). No significant heterogeneity among variances was detected before transformation for the four crosses tested in 1996 and 1997. In 1995, however, all four crosses demonstrated highly significant heterogeneity of variances among the non-segregating generations both before and after logarithmic transformation. The larger variances of the F_1 generation may have been inflated due to its small sample size since disease rating data for a small sample could be easily affected by experimental errors and disease escape (Table 4.5). Thus, logarithmic transformation may be ineffective for removing the heterogeneity of variances. In addition, no heterogeneity of variance was detected for the two crosses Laura/Triple Dirk and Laura/RL5407 inoculated with bunt races T13 and L7 (Table 4.4). Therefore, the original data were not transformed in further analyses.

4.3.1.4 Distribution of disease incidence for bunt resistance

The frequency distributions of the number of plots for disease incidence in different crosses inoculated with race T1, T13 and L7 are listed in Tables 4.5 and 4.6, together with the means and standard errors, based on plot means. These distributions indicated that the incidence of bunt infection was continuous for all generations in both 1995 and 1996.

Table 4.3. Bartlett's test of homogeneity of variances for bunt resistance to race T1 in crosses Laura/Kite, Laura/Triple Dirk, Laura/RL5407 and Genesis/SK0263 tested from 1995 to 1997

Cross	Year	Chi-square (χ^2)	
		Before transformation	After transformation[a]
Laura/Kite	1995	21.12**	13.62**
Laura/Triple Dirk	1995	26.17**	18.09**
	1996	4.01	--
	1997	1.18	--
Laura/RL5407	1995	29.45**	23.15**
	1996	2.55	--
	1997	0.00	--
Genesis/SK0263	1995	11.85**	13.89**
	1996	1.19	--
	1997	2.21	--

[a] Log_{10} was used in logarithmic transformation.
** indicates significance at 5% and 1% probability level, respectively.

Table 4.4. Bartlett's test of homogeneity of variances for bunt resistance to race T13 and L7 in the two crosses Laura/Triple Dirk and Laura/RL5407 in 1996.

Cross	Race	Chi-square (χ^2) [a] (without transformation)
Laura/Triple Dirk	T13	0.04
	L7	0.20
Laura/RL5407	T13	2.73
	L7	0.68

[a] All values were not significant at the 5% probability level.

Table 4.5. Distribution of mean plot disease incidence in the experiments on bunt resistance to race T1 in the four crosses Laura/Kite, Laura/Triple Dirk, Laura/RL5407 and Genesis/SK0263 conducted in the field in 1995 and 1996.

	Midpoint value (%)																
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	M ± S.E.	Total
Laura/Kite (1995)																	
P_r[a]	7	1														0.33 ± 0.33	8
F_1	2	0	0	3	0	1	2									15.94 ± 3.10	8
F_2				2	2	1	2	1								23.68 ± 2.67	8
F_3	17	10	11	13	8	9	6	1	2	2	2					15.17 ± 1.42	81
P_s							1	1	2	2	2					42.48 ± 2.21	8
Laura/Triple Dirk (1995-1996)																	
1995																	
P_r	7	1														0.31 ± 0.31	8
F_1		1	2	1	2	0	1	0	1							19.30 ± 4.15	8
F_2			1	1	0	2	2	1	0	1						27.09 ± 3.77	8
F_3	7	11	11	7	8	4	10	11	5	3	4	1	0	1	1	22.89 ± 1.81	84
P_s							1	2	2	2	1					39.51 ± 2.46	8
1996																	
P_r		5	2	1												9.16 ± 1.71	8
F_2						2	1	2	1	0	1	1				36.74 ± 3.73	8
F_4		2	1	3	6	3	4	3	6	5	2	3	2			33.13 ± 2.37	40
F_5	1	1	2	1	3	3	3	3	4	3	2	4				32.86 ± 2.77	30
BC_1F_2						2	1	1	0	0	3	0	2	1	1	46.04 ± 3.55	11
P_s									1	2	2	2	1			47.32 ± 3.54	8

[a] P_r = Kite or Triple Dirk, P_s = Laura.

Table 4.5. (cont'd) Distribution of mean plot disease incidence in the experiments on bunt resistance to race T1 in the four crosses Laura/Kite, Laura/Triple Dirk, Laura/RL5407 and Genesis/SK0263 conducted in the field in 1995 and 1996.

	Midpoint value (%)																
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	M ± S.E.	Total
									Laura/RL5407 (1995-1996)								
1995																	
P_r[a]	8															0.00 ± 0.00	8
F_1	4	0	2	1	0	1										6.98 ± 4.18	8
F_2	1	0	3	1	2	0	0	1								14.56 ± 3.29	8
F_3	15	24	9	11	6	3	2	5	1	0	0	3	0	0	1	13.37 ± 1.64	80
P_s					1	3	1	1	0	1	1					32.62 ± 4.31	8
1996																	
P_r		1	2	4	1											13.55 ± 1.67	8
F_2					1	1	0	3	2	0	1					34.65 ± 2.94	8
F_4	2	4	3	8	1	2	4	7	2	5	3	3	4	2		32.20 ± 2.74	50
F_5	1	1	1	2	1	3	1	2	5	3	2	2	3	2		37.99 ± 3.22	29
BC_1F_2			1	2	2	4	3	4	3	7	4	3	2			38.08 ± 2.26	35
P_s									1	2	1	2	1	1		52.83 ± 3.04	8

[a] P_r = RL5407, P_s = Laura.

Table 4.5. (cont'd) Distribution of mean plot disease incidence in the experiments on bunt resistance to race T1 in the four crosses Laura/Kite, Laura/Triple Dirk, Laura/RL5407 and Genesis/SK0263 conducted in the field in 1995 and 1996.

	Midpoint value (%)																						
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	M ± S.E.	Total
Genesis/SK0263 (1995-1996)																							
1995																							
P_r[a]	6	1	1																			1.41 ± 1.05	8
F_1				1	1	0	0	3	0	2	0	1										35.83 ± 4.54	8
F_3	1	0	4	5	10	5	6	4	10	12	8	3	4	1	4	1	1	1				38.41 ± 2.06	80
P_s														1	2	1	0	1	2	0	1	80.18 ± 4.63	8
1996																							
P_r		2	3	2	0	1																12.46 ± 2.22	8
F_2									2	1	0	3	2									51.24 ± 2.65	8
F_4	1	1	0	0	3	2	1	6	4	12	3	9	4	4								43.56 ± 2.08	50
P_s										1	1	2	2	1	1							56.78 ± 3.39	8

[a] P_r = SK0263, P_s = Genesis.

Table 4.6. Frequency distribution of mean plot disease incidence in the experiments on bunt resistance to race T13 and L7 in the two crosses Laura/Triple Dirk and Laura/RL5407 conducted in the field in 1996.

	Midpoint value (%)																					
	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	M ± S.E.	Total
Laura/Triple Dirk																						
With T13:																						
P_r[a]	1	3	1	2	0	0	1														12.46 ± 2.37	8
F_5					2	2	0	2	2	0	4	8	3	3	1	2	0	1			56.77 ± 2.86	30
P_s														2	1	0	2	2	1		83.51 ± 2.55	8
With L7:																						
P_r	1	1	2	1	2	0	1														19.15 ± 2.84	8
F_5	1	1	0	0	0	1	2	3	3	1	3	4	2	3	2	3	1				54.48 ± 3.54	30
P_s												1	2	2	2	1					69.52 ± 2.38	8
Laura/RL5407																						
With T13:																						
P_r	1	0	4	0	1	0	1	0	1												21.07 ± 4.74	8
F_5			1	0	0	3	1	0	1	4	1	3	6	3	1	5					58.87 ± 3.32	29
P_s															1	0	2	2	1	2	90.28 ± 2.43	8
With L7:																						
P_r			2	1	2	1	2														23.90 ± 2.22	8
F_5	1	0	1	1	1	2	2	1	2	2	1	5	2	0	1	3	3	0	1		54.26 ± 4.36	29
P_s													3	1	0	2	2				75.13 ± 3.08	8

[a] P_r = Triple Dirk or RL5407, P_s = Laura.

4.3.2 Generation means analyses

Using means and variances calculated from individual plot data, joint scaling tests were carried out to test the adequacy of an additive-dominance genetic model for bunt resistance.

The joint scaling tests for crosses Laura/Triple Dirk, Laura/RL5407, Laura/Kite and Genesis/SK0263 showed that there were significant or highly significant additive gene effects for all crosses in both years, except for cross Genesis/SK0263 in 1996 (Table 4.7). Dominance effects were not significant for any cross. The additive-dominance model fit the data for all crosses, except cross Laura/Kite.

Thus, bunt resistance to race T1 was controlled by genes with additive effects. Failure for the additive-dominance model to fit in the cross Laura/Kite suggested that there might be epistatic effects and, thus, more than one gene locus was responsible for bunt resistance in this cross. The presence of epistasis could not be tested for in this cross because an insufficient number of generations were available.

4.3.3 Estimation of number of effective factors

The number of effective factors was estimated for the crosses studied in 1995 and in 1996 using the method of Castle (1921) and Wright (1934) (Table 4.8). The estimated number of effective factors ranged from 0.9 to 1.1 for the cross Laura/Triple Dirk and from 0.6 to 1.1 for the cross Laura/RL5407 (Table 4.8), suggesting one gene control for bunt resistance to race T1 in these two crosses. For cross Laura/Kite, the estimate was 1.8 in 1995, implying that two genes for resistance may be involved. However, for cross

Table 4.7. Joint scaling test for gene effects of bunt incidence for race T1 in the four crosses Laura/Triple Dirk, Laura/RL5407, Genesis/SK0263 and Laura/Kite in 1995 and 1996.

Parameter	1995		1996	
	Estimates	p-value	Estimates	p-value
		Laura/Triple Dirk		
Mean (m)	20.66 ± 1.57	<0.01	29.95 ± 1.17	<0.01
Additive effect (d)	-20.32 ± 1.58	<0.01	-20.30 ± 1.37	<0.01
Dominance effect (h)	3.36 ± 5.30	0.59	17.39 ± 6.36	0.07
Goodness of fit	$\chi^2 = 3.84$	0.15	$\chi^2 = 1.84$	0.61
		Laura/RL5407		
Mean (m)	16.02 ± 1.07	<0.01	32.78 ± 2.03	<0.01
Additive effect (d)	-16.02 ± 1.07	<0.01	-18.65 ± 2.26	<0.01
Dominance effect (h)	- 8.03 ± 2.85	0.11	- 1.53 ± 9.67	0.89
Goodness of fit	$\chi^2 = 0.83$	0.66	$\chi^2 = 6.47$	0.09
		Genesis/SK0263		
Mean (m)	40.24 ± 0.73	0.01	36.70 ± 2.99	0.05
Additive effect (d)	-38.88 ± 0.77	0.01	-22.99 ± 3.55	0.09
Dominance effect (h)	- 5.09 ± 1.98	0.24	31.45 ± 11.87	0.23
Goodness of fit	$\chi^2 = 0.15$	0.70	$\chi^2 = 3.24$	0.07
		Laura/Kite		
Mean (m)	20.16 ± 2.56	<0.05	-	-
Additive effect (d)	-19.88 ± 2.59	<0.05	-	-
Dominance effect (h)	-4.76 ± 7.69	0.60	-	-
Goodness of fit	$\chi^2 = 13.32$	0.00	-	-

Table 4.8. Estimates of the number of effective factors conferring bunt resistance to race T1 in the crosses studied in 1995 and 1996.

Cross	Generation and year of study		
	F_3 (1995)	F_4 (1996)	F_5 (1996)
Laura/Kite	1.8 ± 0.7 [a]	--[b]	--
Laura/Triple Dirk	0.9 ± 0.3	1.1 ± 0.6	1.1 ± 0.7
Laura/RL5407	1.1 ± 0.9	0.6 ± 0.2	0.8 ± 0.4
Genesis/SK0263	3.5 ± 2.1	1.6 ± 0.9	--

[a] Mean ± standard error.
[b] "--" indicates no estimate available.

Genesis/SK0263, the estimates were higher in 1995 (3.5) than in 1996 (1.6). This difference might be partly due to the fact that Genesis had much higher disease incidence in 1995 (80.2%) than in 1996 (56.8%). In 1995, the F_1 generation was included for estimating the environmental variance and had a large variance (329.8), possibly a result of small sample size (Table A.9). In 1996, however, only the two parents were used due to lack of F_1 seed. Thus, the environmental variance V_e estimated in 1995 (230.07) was larger than that in 1996 (131.7). In addition, although the higher infection mean of Genesis in F_3 was associated with a higher variance in F_3 than in F_4, the square of the genotypic range (D^2) had a larger impact on the estimated number of effective factors (n_e) than did the variance of the segregating generation (σ_s^2). Therefore, the estimate of effective factors was higher in 1995 than in 1996.

The estimates of the number of effective factors suggested that Triple Dirk and RL5407 possessed one gene for resistance to bunt race T1. Kite possibly carried two genes for resistance. This agrees with the result of the generation mean analysis which suggested

possible epistatic effects. SK0263 might carry two or more genes for bunt resistance, but these estimates could be overestimated because they are probably biased upwards due to the substantial environmental effects (Wright, 1934; Mulitze, 1983).

The estimates of number of effective factors for resistance to races T13 and L7 were higher for race T13 (3.2 and 2.9 for Laura/Triple and Laura/RL5407, respectively) than for race L7 (1.0 and 0.7 for Laura/Triple Dirk and Laura/RL5407, respectively) (Table 4.9). This is partly due to the higher disease ratings obtained for the susceptible parent Laura (causing larger genetic range (D)), when inoculated with race T13 (ratings 83.51 and 90.28) than with race L7 (ratings 69.52 and 75.13) (Table 4.6). However, the larger infection means in the progenies did not cause larger variances. The F_2 variances were actually smaller when inoculated with race T13 (491.2 and 640.3) than with race L7 (752.2 and 1102.0) (Table A.10). The fact that larger means associated with smaller variances could result from sampling error, unequal gene effects in Triple Dirk and RL5407 and/or differences in virulence between the two races, so that the individual plants had unequal infection rates when inoculated with races T13 and L7. Lande (1981) also indicated that the accuracy of the estimates for the number of effective factors depended on the sample sizes of the parental and progeny populations. Therefore, both Triple Dirk and RL5407 may carry a single gene for resistance to race L7, but it is not known at this point whether this gene is the same as the gene conferring resistance to race T1. The estimates of the number of effective factors suggested that Triple Dirk and RL5407 might have more than one gene for resistance to race T13.

Table 4.9. The number of effective factors conferring bunt resistance to race T13 and L7 for the crosses Laura/Triple Dirk and Laura/RL5407 tested in 1996 [a]

Cross	Race	Number of effective factor
Laura/Triple Dirk	T13	3.2 ± 1.4 [b]
	L7	1.0 ± 0.4
Laura/RL5407	T13	2.9 ± 2.1
	L7	0.7 ± 0.3

[a] Estimates were based on F_5 progenies.
[b] Mean ± standard error.

4.3.4 Estimation of heritability

Broad-sense estimates of heritability of bunt resistance were calculated using the among-family variance from different generations for each cross and by $F_5 : F_4$ regression for the crosses Laura/Triple Dirk and Laura/RL5407. The heritability estimates ranged from 0.38 to 0.77 (Table 4.10). For cross Laura/RL5407, the heritability estimated in the F_3 generation was much lower (0.38) than in F_4 (0.77) and F_5 (0.72) generations. This might result from the fact that the environmental variance for the F_3 generation in 1995 was twice as much (192.10) as for the F_4 and F_5 generations (96.05) in 1996. Heritability was estimated as moderate for resistance to race T13 (0.48 and 0.67) and high for resistance to race L7 (0.75 and 0.81) in crosses Laura/Triple Dirk and Laura/RL5407 (Table 4.11).

Table 4.10. Heritability of resistance to common bunt race T1 in different generations in the four crosses tested in 1995 and 1996.

	Method of calculation			
	Variance components [a]			Regression
Cross	F_3	F_4	F_5	F_5 on F_4
Laura/Kite	0.62	--[b]	--	--
Laura/Triple Dirk	0.63	0.57	0.58	0.54
Laura/RL5407	0.38	0.77	0.72	0.72
Genesis/SK0263	0.49	0.53	--	--

[a] Estimates based on variances are broad-sense heritability (on plot mean basis).
[b] "--" = estimates not available.

Table 4.11. Broad-sense heritability estimates of resistance to common bunt races T13 and L7 in crosses Laura/Triple Dirk and Laura/RL5407 tested in 1996

Cross	Race	Heritability [a]
Laura/Triple Dirk	T13	0.67
	L7	0.75
Laura/RL5407	T13	0.48
	L7	0.81

[a] Heritability estimates are based on variance components of bunt incidence in F_5 progenies (on plot mean basis).

4.3.5 Test of maternal effect

The F_2 means for bunt incidence of the cross between Genesis and RL5407 and its reciprocal cross were used to test for maternal effects on bunt resistance. No significant maternal effects were found since there was no significant difference between the F_2 means of the cross Genesis/RL5407 and the cross RL5407/Genesis ($P = 0.09$, Table 4.12).

Table 4.12. Test of cytoplasmic (or maternal) effect on bunt resistance to race T1 by comparing F_2 means of the reciprocal crosses between Genesis and RL5407 in 1996.

Generation	Number of Plots	M ± S.E.[a]	95% Confidence Interval
Genesis	8	55.4 ± 2.2	50.3 - 60.5
Genesis/RL5407 (F_2)	8	52.5 ± 2.8	45.8 - 59.2
RL5407/Genesis (F_2)	8	44.3 ± 3.6	35.8 - 52.7
RL5407	8	6.6 ± 0.8	4.8 - 8.4

Test for the F_2 means: $t_{14} = 1.80$, P-value = 0.09.

[a] M ± S.E. = mean ± standard error.

4.4 Discussion

A test of normality indicated that the residuals for most of the disease data are normally distributed except in four cases (Table A.4, A.5). These four cases involved the resistant parents Triple Dirk and Kite that showed mostly zero infection in 1995 (Table 4.5, A.4). Thus, even logarithmic transformation could not be used due to presence of zero ratings in the data set. In addition, it is believed that "only very skewed distributions would have a marked effect on the significance level of the F-test or on the efficiency of

the design" (Sokal and Rohlf, 1981). Therefore, the majority of normality tests suggested that transformation was not necessary.

Significant additive gene effects and no dominance effects were observed in four crosses, Laura/Triple Dirk, Laura/RL5407, Laura/Kite and Genesis/SK0263 (except for 1996) (Table 4.7). Knox et al (1998) also found incomplete dominance for common bunt resistance to race T19 in the doubled haploid populations (HY377/8021 and HY377/8474). Therefore, selection for bunt resistance in the field should be effective since the selected highly resistant individuals will breed true and will not segregate in the progenies. For Laura/Kite, it did not fit the additive-dominance model, suggesting a non-allelic interaction occurred for bunt resistance, but because of the significant additive gene effect and absence of dominance, the interaction could be due to additive x additive gene action (Mather and Jinks, 1982). Similarly, Cherif and Harrabi (1990) found significant additive and additive x additive epistatic effects in four crosses in the genetic study on resistance to *Pyrenophora teres* in barley. In addition to additive gene action, dominance and epistasis (i.e. additive x dominance and dominance x dominance) were also found to be important genetic effects for the expression of leaf blight (caused by *Alternaria triticina*) and septoria leaf blotch (caused by *Septoria triticia*) resistance in wheat (Camacho-Casas et al., 1995; Sinha et al., 1991). In this study, a model incorporating epistasis could not be tested due to an inadequate number of generations.

Estimates of the number of effective factors for resistance to race T1 ranged from 0.6 to 1.1 in crosses Laura/Triple Dirk and Laura/RL5407. The scaling test (see Section 4.3.2) suggested that Kite possibly carried at least two genes for bunt resistance to race T1, but

the gene estimate was only 1.8. However, these estimates are minimum estimates since the use of the Castle-Wright method of moments to estimate gene number requires certain assumptions, such as (i) no dominance, (ii) all genes have equal effects, (iii) no linkage, (iv) no epistasis, and (v) one parent has all of the positive alleles and the other has none. Violation of these assumptions could bias estimates downwards (Wright, 1934; Lande, 1981; Mulitze, 1983; Mulitze and Baker, 1985b, 1985c). Obviously, for Laura/Kite, assumption (iv) was not met as the scaling test indicated non-allelic interaction for this cross, and thus did not fit the additive-dominance model. Secondly, failure for assumption (v) to be true would also result in downward bias of gene estimates by reducing the genetic ranges. Therefore, if the estimated genetic variance is inflated by epistasis or the genetic range (D) is underestimated, downward bias would definitely occur. Moreover, large environmental variation (σ_e^2) can affect gene number estimates (n_e) by reducing the genetic variances (σ_g^2), further inflating the estimates of the number of effective factors. Thus, not only the estimate for the numerator ($D^2 = [P_1-P_2]^2$) but also that for the denominator (genetic variance) can result in biased gene estimates (see Section 4.2.4.4).

In addition, the estimates of the number of effective factors for resistance to race T1 were not consistent for the cross Genesis/SK0263 between 1995 (3.5) and 1996 (1.6), implying that the inconsistency was due to either overestimation in 1995 or underestimation in 1996. Because a larger environmental variance was estimated in 1995 than in 1996, it is likely that the gene estimate in 1995 was overestimated.

Most of the heritability estimates for bunt resistance to races T1, T13 and L7 were between 0.50 and 0.75 (Tables 4.10, 4.11), implying that selection for bunt resistance

could be effective in breeding program (Goates, 1996). Although a maternal effect was reported for single ear weight in the genetic studies of crosses between common wheat and spelt (Schmid and Winzeler, 1990), no significant maternal effects on bunt resistance were detected in this study, suggesting that cross direction would not affect selection in breeding for bunt resistance.

5. INHERITANCE OF RESISTANCE TO COMMON BUNT *(T. CARIES* AND *T. FOETIDA)* IN WHEAT: II. QUALITATIVE ANALYSIS

5.1 Introduction

There are various procedures in quantitative analysis for estimating the number of effective factors e.g. the method of moments (Castle, 1921), the inbred backcross procedure (Wehrhahn and Allard, 1965), Weber's discriminant analysis (Weber, 1960), genotype assay (Towey and Jinks, 1977; Mulitze and Baker, 1985a) and partitioning method (Powers, 1963). However, all require certain assumptions to be valid in order to provide an accurate estimate of effective factors; these assumptions often can not be met. On the other hand, Mendelian analysis can still provide approximation to the true number of genetic factors as the continuous variation for bunt incidence could result from both major gene segregation and environmental effects. Based on the quantitative analysis of bunt resistance (Section 4), the estimated numbers of effective factors were very low, implying that bunt resistance could possibly be due to major genes.

The objectives of this qualitative analysis were (i) to perform Mendelian analysis to determine the genetic control of bunt resistance in the selected materials, and (ii) to investigate the allelism of resistant genes in spelt wheat and the *Bt*-gene lines and the allelism of resistant genes in Triple Dirk and RL5407 for resistance to different races.

5.2 Materials and Methods

5.2.1 Materials

(a) Host

The materials described in Section 4.2.1 (Table A.3) were used in studies of inheritance of resistance to common bunt in 1995 and 1996.

In addition, crosses RL5407/*Bt* gene lines (*Bt2*, *Bt3*, *Bt6*, *Bt8* and *Bt10*), SK0505/RL5407, SK0505/SK0263 and RL5407/SK0263 were also made in summer 1995 in order to study the allelism of bunt resistance genes in the two parents of each cross. The F_2 seeds for these crosses were obtained during winter 1995/1996. P_1, P_2 and F_2 generations were used in all experiments in 1996.

In 1997, single head derived (SHD) $F_{4:5}$ and $F_{5:6}$ lines were tested for cross Laura/Triple Dirk, $F_{4:5}$ (SHD) lines for cross Genesis/SK0263 and single seed derived (SSD) $F_{4:6}$ lines for cross Laura/RL5407. The corresponding parental generations were also involved in all experiments.

(b) Pathogen

Race T1 was used as inoculum in all experiments conducted from 1995 to 1997. Races L7 and T13 were used as inoculum in additional experiments involving the P_1, P_2 and F_2 generations of the crosses Laura/Triple Dirk and Laura/RL5407.

5.2.2 Experimental design

5.2.2.1 Field experiments

The experiments used for the Mendelian analysis of bunt resistance in 1995 and 1996 were the same as those described in Section 4.2.2. In the allelism studies, eight plots of F_2, P_1 and P_2 generations were used in each of two randomized blocks with 50 seeds/plot for F_2 and 30 seeds/plots for P_1 and P_2.

5.2.2.2 Controlled environment experiments

During the 1996-1997 winter, the parents and 27 F_5 single head-derived F_6 lines of cross Laura/Triple Dirk, inoculated with race T1, were planted for disease evaluation in a CONVIRON growth cabinet (Controlled Environments Ltd., Winnipeg, Canada) using a randomized complete block design with two replications. One pot of each F_6 line, 12 to 15 seeds per pot, plus one pot of each parent was seeded in each block. Plants were grown in 6-inch pots filled with Redi-earth (W.R. Grace & Co. of Canada Ltd., Ajax, Ontario, Canada). About 4 g per pot of the controlled release fertilizer OSMOCOTE PLUS$_{TM}$ 16-8-12 (Scotts-Sierra, Horticultural Products Company, Maryville, Ohio, 1995) was applied after seeding. Plants were grown under a temperature regime modified from that of Gaudet and Puchalski (1989b): 8°C/6°C day/night temperatures for the first 3 weeks, then 14°C and 12°C from the 4th week until heading, and 23°C from heading until mature. An 18 h photoperiod was used.

In a second experiment, 60 $F_{4:5}$ lines and the two parents of cross Laura/RL5407 were planted in a growth cabinet for disease testing during the 1996/1997 winter using the

same design (RCBD) and growth conditions described for the Laura/Triple Dirk cross. Each $F_{4:5}$ line was seeded at 12 to 15 seeds per pot.

5.2.3 Inoculation and disease rating

The inoculation and disease rating methods were the same as described in Section 4.2.3. In the experiments grown under controlled environment conditions, rating was based on individual spikes in each pot instead of individual plants because of the difficulty of separating individual plants. The proportion of the number of diseased spikes to the total number of spikes in a pot was recorded as the disease incidence for that treatment.

5.2.4 Genetic analysis

Since disease incidence was continuously distributed (Tables 4.5, 4.6), no discrete segregation patterns could be observed. Thus, to establish phenotypic groups, the lowest value in the ungrouped distribution of the susceptible parent was used as the cut-point between susceptible and other classes. The cut-point was based on the susceptible parent since there is greater certainty of correspondence between phenotype and genotype for susceptibility than the resistant and intermediate classes. However, if there is an unusual observation (outlier) in the susceptible parent due to disease escape, using the lowest value as the cut-point might inflate the true susceptible grouping in the progenies. To test if an outlier exists, the following criterion

$$r_{11} = (x_2 - x_1)/(x_{n-1} - x_1) \quad (x_1 < x_2 < \ldots < x_{n-1} < x_n)$$

was calculated and compared with the critical value in Table A.11 to see if it was significantly different (Dixon, 1953; Grubbs, 1969).

The breeding scheme, genetic constitution and segregation ratios for different generations are listed in Table 5.1 for a one-gene model and in Table 5.2 for a two-gene model. The proposed segregation ratios were tested using the chi-squared tests for goodness of fit. Since these tests involved only two classes, susceptible (S) versus the rest (R+H), Yate's correction for continuity was used to calculate adjusted chi-square values as follows (Steel and Torrie, 1997):

$$\chi^2_{adjusted} = \{\Sigma(|f_i - f_i'| - 1/2)^2\} / f_i'$$

where f_i is the observed frequency and f_i' is the expected frequency.

5.2.5 Tests for allelism

Tests for allelism of resistance were made by comparing the means of bunt incidence of the parental and F_2 generations for each cross. Differences among the means of disease incidence of the three generations indicated that the resistance genes were not allelic.

Table 5.1. Breeding scheme, genetic expectation and suggested segregation ratios for one gene model based on the grouping of R+H (resistant + heterozygous) : S (susceptible).

Generation	Breeding scheme	Genetic expectation	Segregation ratio
$P_1 \times P_2$	Crossing	rr x RR	N/A[a]
F_1	Bulked hybrids	Rr	N/A
F_2	Bulk from F_1	1/4RR : 1/2Rr : 1/4rr	N/A
F_3	F_2 single plant derived	1/4RR:1/2(1/4RR:1/2Rr:1/4rr):1/4rr	3(R+H) : 1S
F_4	Bulk from F_3 family	1/4RR:1/2(3/8RR:1/4Rr:3/8rr):1/4rr	3(R+H) : 1S
F_{5a}	Bulk from F_4 family	1/4RR:1/2(7/16RR:1/8Rr:7/16rr):1/4rr	3(R+H) : 1S
F_{5b}	Single head derived ($F_{4.5}$)	7/16RR:1/8(1/4RR:1/2Rr:1/4rr):7/16rr	9(R+H) : 7S
F_{6a}	Single head derived ($F_{5a.6}$)	15/32RR:1/16(¼RR:½Rr:¼rr):15/32rr	17(R+H) : 15S
F_{6b}	Single seed descent ($F_{4.6}$)	15/32RR:1/16(¼RR:½Rr:¼rr):15/32rr	17(R+H) : 15S

[a] N/A = not applicable.

Table 5.2. Breeding scheme, genetic expectation and suggested segregation ratios for two gene model based on the grouping of R+H (resistant + heterozygous) : S (susceptible).

Generation	Breeding scheme	Genetic expectation	Segregation ratio
$P_1 \times P_2$	Crossing	$r_1r_1r_2r_2 \times R_1R_1R_2R_2$	N/A[a]
F_1	Bulked hybrids	$R_1r_1R_2r_2$	N/A
F_2	Bulk from F_1	$9/16R_1\_R2\_ : 3/16R_1\_\ r_2r_2 :$ $3/16r_1r_1R_2\_ : 1/16\ r_1r_1r_2r_2$	N/A
F_3	F_2 single plant derived	$15/16(R_1\_R_2\_+R_1\_\ r_2r_2+r_1r_1R_2\_) : 1/16\ r_1r_1r_2r_2$	15(R+H) : 1S
F_4	Bulk from F_3 family	$15/16(R_1\_R_2\_+R_1\_\ r_2r_2+r_1r_1R_2\_) : 1/16\ r_1r_1r_2r_2$	15(R+H) : 1S
F_5	Single head derived ($F_{4:5}$)	$207/256(R_1\_R_2\_+R_1\_\ r_2r_2+r_1r_1R_2\_) : 49/256\ r_1r_1r_2r_2$	207(R+H) : 49(S) $[\approx 4.2(R+H) : 1(S)]$

[a] N/A = not applicable

5.3 Results

5.3.1 Determination of the criterion for phenotypic classification

If an agronomic trait is controlled by major genes and the progenies of a cross between two inbred lines show discrete segregation, the number of genes involved can be readily determined by Mendelian analysis. However, because of large environmental variation among progenies and sampling error among the individuals within a plot, the disease rating could appear to be continuous (Kim et al., 1989; Elsidaig and Zwer, 1993; Kornegay et al., 1993; Singh et al., 1995a, 1995b). Thus, other approaches must be sought in order to classify the segregating progenies into different groups to permit Mendelian analysis.

The segregating generations of the crosses were classified into two groups, susceptible versus the rest (resistant and heterozygous), based on the ungrouped distributions of the susceptible parents using the lowest infection rate as the cut point. Prior to phenotypic grouping, the lowest values in the susceptible parent distributions were tested for extreme observations (or outliers) due to disease escape or error in bunt rating and data recording (Dixon, 1953; Grubbs, 1969). The tests indicated that the *P*-values for testing outliers were high (>0.10) for each cross, suggesting that there were no significant outlying data points. Therefore, the range of bunt incidence shown by the susceptible parent could be used to determine the cutoff point to classify the progenies into the two different groups (Tables 5.3 and 5.4).

Table 5.3. Means and ranges of disease incidence for race T1 and testing of outliers of plot data for the susceptible parent in the four crosses from 1995 to 1997.

Susceptible Parent	Year	# Plot	Mean (%)	Range of infection (%)	Criterion [a]	*P*-value [b]
			Laura/Triple Dirk			
Laura	1995	8	39.5	30.2 - 50.0	0.089	>0.30
	1996	8	47.3	40.8 - 67.1	0.015	>0.30
	1997	8	55.0	37.5 - 75.0	0.084	>0.30
			Laura/RL5407			
Laura	1995	8	32.6	18.3 - 54.3	0.131	>0.30
	1996	8	52.8	41.4 - 66.7	0.139	>0.30
	1997	8	46.5	32.6 - 55.0	0.470	>0.10
			Laura/Kite			
Laura	1995	8	42.5	32.5 - 50.0	0.203	>0.30
			Genesis/SK0263			
Genesis	1995	8	80.2	62.5 - 100.0	0.186	>0.30
	1996	8	56.8	44.2 - 67.1	0.077	>0.30
	1997	8	55.8	41.0 - 76.7	0.124	>0.30

[a] The criteria were calculated using the equation $r_{11} = (x_2 - x_1)/(x_n - x_1)$ where x_1 was the smallest value (Dixon, 1953; Grubbs, 1969).

[b] *P*-values were obtained by comparing the criteria with the critical values in Table A.11.

Table 5.4. Means and ranges of disease incidence for races T13 and L7 and testing of outliers of plot data for the susceptible parent in the two crosses in 1996.

Susceptible Parent	Race	# Plot	Mean (%)	Range of infection (%)	Criterion [a]	*P*-value [b]
			Laura/Triple Dirk			
Laura	T13	8	83.5	70.2 - 94. 2	0.190	>0.30
	L7	8	69.5	61.0 - 81.8	0.230	>0.30
			Laura/RL5407			
Laura	T13	8	90.3	72.8 - 100.0	0.259	>0.30
	L7	8	75.1	64.9 - 86.8	0.074	>0.30

[a] The criteria were calculated using the equation $r_{11} = (x_2 - x_1)/(x_{-} - x_1)$ where x_1 was the smallest value (Dixon, 1953; Grubbs, 1969).
[b] *P*-values were obtained by comparing the criteria with the critical values in Table A.11.

5.3.2 Genetic analyses for the F_2 derived lines

5.3.2.1 Resistance to race T1

A) Laura/Triple Dirk

The distributions of disease incidence ratings for the different generations in all crosses are listed in Tables 4.5 and 4.6. Based on the susceptible parental distribution, the progenies were classified into two classes, susceptible and resistant + heterozygous (Table 5.5). The segregation ratios in the F_3, F_4 and F_5 generations fit into a 3 (resistant + heterozygous) : 1 (susceptible) ratio (P=0.09-0.26) (Table 5.5), which was expected for single gene segregation for bunt resistance (Table 5.1). The backcross also gave good fit to a 1 heterozygous : 1 susceptible ratio (P=0.55), confirming a one gene model, although the number of families tested was small. Because there were two replications in each experiment, the disease data from individual lines in the susceptible or resistant +

heterozygous groups can be tested for homogeneity. The results of the homogeneity tests of individuals within the two groups are indicated by the *P*-values in the brackets beside the observed numbers (Table 5.5). All the *P*-values for the susceptible group were large (*P*=0.37-0.95), suggesting that the individual lines in the susceptible group were homogeneous, but the *P*-values for the R+H group were small (*P*=0.00-0.05) due to the presence of the two genotypes RR and Rr in this group.

Table 5.5. Goodness-of-fit test for the segregation ratio for common bunt resistance to race T1 in the cross Laura/Triple Dirk in 1995 and 1996.

Generation	Observed #			Ratio tested	χ^2_{adj} [b]	*P*-value
	(R + H)[a]	S	Total			
F_3 (1995)	58 (0.00)[c]	26 (0.37)	84	3 : 1	1.29	0.26
F_4 (1996)	26 (0.05)	14 (0.85)	40	3 : 1	1.63	0.20
F_5 (1996)	18 (0.04)	12 (0.95)	30	3 : 1	2.84	0.09
BC_1F_2 (1996)	4 (0.48)	7 (0.94)	11	1 : 1	0.36	0.55

[a] Progenies within the susceptible parent range were classified as susceptible (S) and the rest were classified as resistant (R) + heterozygous (H).
[b] The corrected χ^2 using Yate's correction for continuity (Steel and Torrie, 1997).
[c] Inside the bracket is the *P*-value used to test homogeneity of individuals within the genotypic class, namely R+H and S.

B) Laura/RL5407

The segregation ratio tests for the different generations in the Laura/RL5407 cross showed that the F_3, F_4 and F_5 progenies segregated 3 (resistant + heterozygous) : 1 susceptible and the backcross F_2 segregated 1 heterozygous : 1 susceptible, suggesting single gene control of bunt resistance (Tables 5.1 and 5.6). The *P*-values for the homogeneity tests also indicated that these genotypic groupings were appropriate. The low

P-values ($P<0.05$) for the homogeneity tests of the R+H group suggested that the individuals within this group were genetically different, providing further support for the suggestion that resistance to bunt is neither dominant nor recessive.

Table 5.6. Goodness-of-fit test for segregation ratio for common bunt resistance to race T1 in Laura/RL5407 in 1995 and 1996.

Generation	Observed #			Ratio tested	χ^2_{adj} [b]	*P*-value
	(R + H)[a]	S	Total			
F_3 (1995)	59 (0.05)[c]	21 (0.14)	80	3 : 1	0.02	0.89
F_4 (1996)	33 (0.01)	17 (0.71)	50	3 : 1	1.71	0.19
F_5 (1996)	17 (0.00)	12 (0.74)	29	3 : 1	3.32	0.07
BC_1F_2 (1996)	19 (0.79)(H)	16 (0.99)	35	1 : 1	0.11	0.74

[a] Progenies within the susceptible parent range were classified as susceptible (S) and the rest were classified as resistant (R) + heterozygous (H).
[b] The corrected χ^2 using Yate's correction for continuity (Steel and Torrie, 1997).
[c] Inside the bracket is the *P*-value used to test homogeneity of individuals within the genotypic class, namely R+H and S.

C) Laura/Kite

Only the F_3 generation of cross Laura/Kite was available for genetic analysis. The F_3 segregation data fit a 15 : 1 ratio (Table 5.7), suggesting two-gene control of bunt resistance to race T1 (Table 5.2). This agreed with the quantitative analysis where detection of epistatic effects suggested more than one gene for resistance to race T1 in the cross Laura/Kite (Section 4).

Table 5.7. Goodness-of-fit test for the segregation ratio for common bunt resistance to race T1 in Laura/Kite in 1995

Generation	Observed #			Ratio tested	χ^2_{adj} [b]	*P*-value
	(R + H)[a]	S	Total			
F_3 (1995)	74 (0.00)[c]	7 (0.12)	81	15 : 1	0.44	0.51

[a] Progenies within the susceptible parent range were classified as susceptible (S); the rest were classified as resistant (R) + heterozygous (H).
[b] The corrected χ^2 using Yate's correction for continuity (Steel and Torrie, 1997).
[c] Inside the bracket is the *P*-value used to test homogeneity of progenies within the genotypic class, namely R+H and S.

D) Genesis/SK0263

The segregation ratio for F_3 families tested in 1995 fit a two gene model (15:1 ratio) (*P*=0.25, Table 5.8), but the F_4 segregation ratio obtained in 1996 did not fit either a one gene model (3:1) (Table 5.1), or a two gene model (15:1) (Table 5.2) because of an excess of susceptible lines.

Table 5.8. Goodness-of-fit test for the segregation ratio for common bunt resistance to race T1 in the cross Genesis/SK0263 in 1995 and 1996.

Generation	Observed #			Ratio tested	χ^2_{adj} [b]	p-value
	(R + H)[a]	S	Total			
F_3 (1995)	72 (0.04)[c]	8 (0.76)	80	15 : 1	1.33	0.25
F_4 (1996)	26 (0.05)	24 (0.92)	50	15 : 1	141.48	0.00
				3 : 1	12.91	0.00

[a] Progenies within the susceptible parent range were classified as susceptible (S) and the rest were classified as resistant (R) + heterozygous (H).
[b] The corrected χ^2 using Yate's correction for continuity (Steel and Torrie, 1997).
[c] Inside the bracket is the *P*-value used to test homogeneity of individuals within the genotypic class, namely R+H and S.

5.3.2.2 Resistance to race T13

Using the same procedure as for crosses tested with race T1, the F_5 families in crosses Laura/Triple Dirk and Laura/RL5407 tested with race T13 were also classified into two groups. The progenies in both crosses Laura/Triple Dirk and Laura/RL5407 fit a 3 (resistant + heterozygous) : 1 (susceptible) segregation ratio ($P = 0.21$ and 0.45, respectively) (Table 5.9), suggesting monogenic inheritance of resistance to race T13. However, the segregation data for cross Laura/Triple Dirk also fit a two-gene segregation ratio (15 : 1) ($P = 0.11$). However, the number of families available (30) in the cross Laura/Triple Dirk was not sufficient to differentiate between a 3:1 and 15:1 segregation ratios. Thus, in conclusion, the simpler model, i.e. a one-gene model ($P = 0.21$), would likely be accepted although the two-gene model could not be completely excluded.

Table 5.9. Goodness-of-fit test for the segregation ratio for common bunt resistance to race T13 in F_5 in the two crosses Laura/Triple Dirk and Laura/RL5407 in 1996.

Cross	Observed #			Ratio tested	χ^2_{adj} [b]	*P*-value
	(R + H)[a]	S	Total			
Laura/Triple Dirk	26 (0.01)[c]	4 (0.13)	30	3 : 1	1.60	0.21
				15 : 1	2.55	0.11
Laura/RL5407	24 (0.02)	5 (1.00)	29	3 : 1	0.58	0.45

[a] Progenies within the susceptible parent range were classified as susceptible (S) and the rest were classified as resistant (R) + heterozygous (H).
[b] The corrected χ^2 using Yate's correction for continuity (Steel and Torrie, 1997).
[c] Inside the bracket is the *P*-value used to test homogeneity of individuals within the genotypic class, namely R+H and S.

5.3.2.3 Resistance to race L7

The F_5 family segregation data from both crosses, Laura/Triple Dirk and Laura/RL5407, fit a 3 (resistant + heterozygous) : 1 susceptible ratio (P = 0.09 and 0.34, respectively), suggesting that one gene controls resistance to race L7 (Tables 5.1 and 5.10). Moreover, the P-values for homogeneity tests were both large for the susceptible group in the two crosses (P = 0.68 and 0.38 respectively), indicating that the individuals within each group were homogeneous and that these classifications of the progenies were appropriate. However, the progenies in the (R+H) group for cross Laura/Triple Dirk also demonstrated homogeneity (P = 0.09) rather than the expected heterogeneity. This might be due to the presence of dominance for resistance to race L7 or to disease escape of the heterozygous lines.

Table 5.10. Goodness-of-fit tests for the segregation ratio for bunt resistance to race L7 in F_5 in the two crosses Laura/Triple Dirk and Laura/RL5407 in 1996

Cross	Observed #			Ratio tested	χ^2_{adj} [b]	P-value
	(R + H)[a]	S	Total			
Laura/Triple Dirk	18 (0.09)[c]	12 (0.68)	30	3 : 1	2.84	0.09
Laura/RL5407	19 (0.00)	10 (0.38)	29	3 : 1	0.91	0.34

[a] Progenies within the susceptible parent range were classified as susceptible (S); the rest were classified as resistant (R) + heterozygous (H).
[b] The corrected χ^2 using Yate's correction for continuity (Steel and Torrie,1997).
[c] Inside the bracket is the P-value used to test homogeneity of individuals within the genotypic class, namely R+H and S.

5.3.3 Mendelian analysis for single head and single seed derived lines

5.3.3.1 Experiments under controlled environment

The $F_{5:6}$ lines of Laura/Triple Dirk and $F_{4:5}$ lines of Laura/RL5407 were both tested for resistance to race T1 in a growth chamber during the 1996 - 1997 winter. The results showed that the segregation ratio for resistance fit a 17 : 15 and 9 : 7 ratios in Laura/Triple Dirk and Laura/RL5407, respectively (Table 5.11). Based on the single gene model illustrated in Table 5.1, these results suggest one gene was responsible for resistance in both Triple Dirk and RL5407.

Table 5.11. Goodness-of-fit tests for segregation ratio for bunt resistance to race T1 in $F_{4:5}$ and $F_{5:6}$ lines under controlled environment in the two crosses (1996-1997 winter)

Cross	Observed #			Ratio tested	χ^2_{adj} [b]	*P*-value
	(R + H)[a]	S	Total			
Laura/Triple Dirk ($F_{5:6}$)	18 (0.30)[c]	9 (0.14)	27	17 : 15	1.48	0.22
Laura/RL5407 ($F_{4:5}$)	41	19	60	9 : 7	3.09	0.08

[a] Progenies within the susceptible parent range were classified as susceptible (S); the rest were classified as resistant (R) + heterozygous (H).
[b] The corrected χ^2 using Yate's correction for continuity (Steel and Torrie, 1997).
[c] Inside the bracket is the *P*-value used to test homogeneity of individuals within the genotypic class, namely R+H and S. This *P*-value was not available for Laura/RL5407 ($F_{4:5}$) as only one replication was available.

If resistance is controlled by a major gene, the distribution of disease incidence, on a single plant basis, in advanced generations such as F_5 and F_6 should show bimodality since the proportions for the two extreme genotypes RR and rr are gradually increasing (Table 5.1 and Fig. A.1). The distributions of disease incidence data from the growth chamber

experiments exhibited bimodality both in the $F_{4:5}$ generation for Laura/RL5407 (Fig. 5.1) and in the $F_{5:6}$ generation for Laura/Triple Dirk (Fig. 5.2). In particular, the distribution of the $F_{5:6}$ generation for Laura/Triple Dirk showed discrete separation.

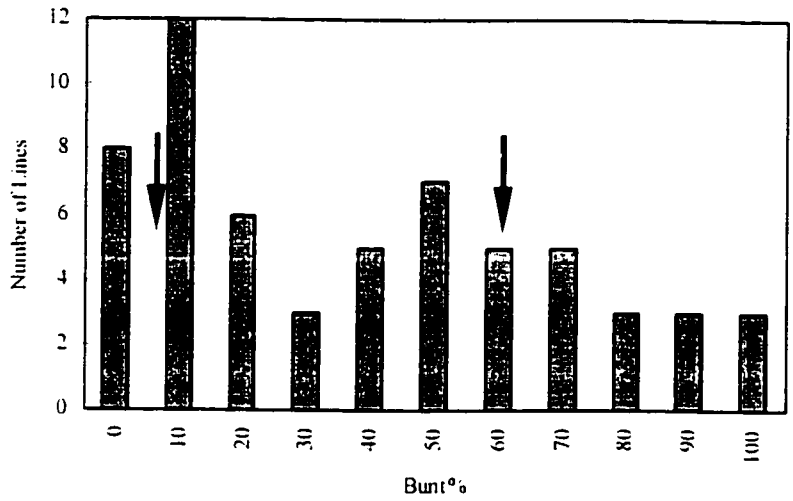

Fig. 5.1. Distribution of disease incidence for bunt resistance to race T1 for the F_4 single head-derived F_5 lines tested in growth cabinet for the cross Laura/RL5407 (Left and right arrows indicate the resistant and susceptible parents respectively).

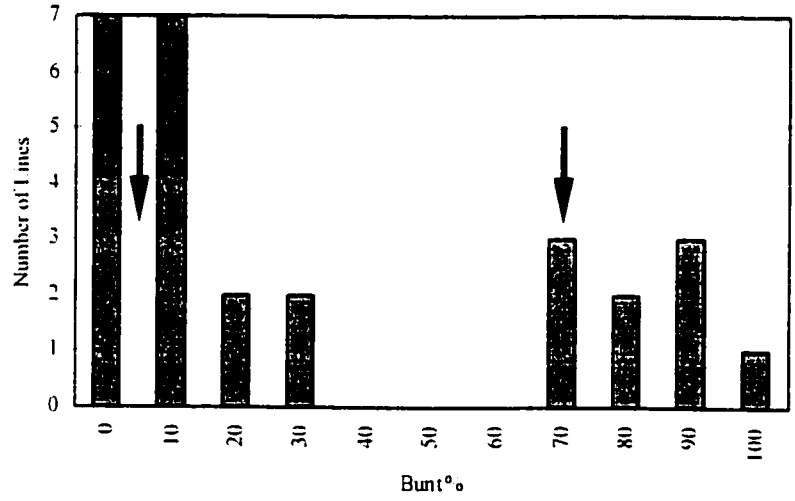

Fig. 5.2. Distribution of disease incidence for bunt resistance to race T1 for F_5 single head-derived F_6 lines tested in growth cabinet for the cross Laura/Triple Dirk (Left and right arrows indicate the resistant and susceptible parents respectively).

5.3.3.2 Field experiments

The frequency distribution for the mean disease incidence of the $F_{4:5}$ (SHD), $F_{5:6}$ (SHD) and $F_{4:6}$ (SSD) lines when tested with race T1 are shown in Table 5.12. For cross Laura/Triple Dirk, both the $F_{4:5}$ and $F_{5:6}$ generations exhibited bimodal segregation. In the Laura/RL5407 and Genesis/SK0263 crosses, the $F_{4:6}$ (SSD) and $F_{4:5}$ (SHD) generations also demonstrated bimodal distributions although the bimodality was not as apparent for Laura/Triple Dirk.

The single head-derived lines were classified into two groups, R+H and S, based on the distribution range of the susceptible parents (Table 5.13). The segregation ratios obtained were tested for goodness-of-fit to the segregation ratio expected for a one- or two-gene model (Tables 5.1 and 5.2). A good fit to the ratio was obtained for crosses Laura/Triple Dirk and Laura/RL5407 expected for one-gene segregation, but not for the two-gene model (Table 5.13). These results were consistent with those for the F_2-derived lines tested in 1995 and 1996 (Tables 5.5 and 5.6). However, the data for the $F_{4:5}$ lines of Genesis/SK0263 did not fit either one gene (9 : 7) or two gene (207 : 49) model ($P<0.05$, Table 5.13).

Table 5.12. Distribution of mean plot disease incidence in the experiments on bunt resistance to race T1 in the single head/seed derived lines in three crosses in 1997

Generation	Midpoint value (%) 0	10	20	30	40	50	60	70	80	90	M ± S.E.	Total
Laura/Triple Dirk												
Triple Dirk	1	5	1	1							12.54 ± 3.45	8
$F_{5:6}$ (SHD)	13	3	0	1	3	3	2	0	1		20.09 ± 4.76	26
$F_{4:5}$ (SHD)	22	9	6	2	8	12	7	3	0	1	26.03 ± 2.93	70
Laura					3	1	2	0	2		54.96 ± 5.31	8
Laura/RL5407												
RL5407		1	4	2	1						23.85 ± 2.83	8
$F_{4:6}$ (SSD)	11	8	8	9	11	6	3	3			27.54 ± 2.65	59
Laura				1	1	5	1				46.88 ± 2.47	8
Genesis/SK0263												
SK0263			3	4	1						26.50 ± 3.32	8
$F_{4:5}$ (SHD)	3	3	4	18	9	15	11	7	7	3	45.80 ± 2.44	80
Genesis					4	0	1	1	2		55.77 ± 6.03	8

Table 5.13. Goodness-of-fit tests for one- and two-gene segregation ratios for bunt resistance to race T1 in the single head/seed derived lines of three crosses in 1997.

Generation	Observed number (R + H)[a]	S	Total	Ratio tested	χ^2_{adj} [b]	*P*-value
			Laura/Triple Dirk			
$F_{4:5}$ (SHD)	41 (0.05)[c]	29 (0.98)	70	9 : 7	0.07	0.79
$F_{5:6}$ (SHD)	17 (0.57)	9 (0.12)	26	17 : 15	1.32	0.25
			Laura/RL5407			
$F_{4:6}$ (SSD)	35 (0.21)	24 (0.12)	59	17 : 15	0.70	0.40
			Genesis/SK0263			
$F_{4:5}$ (SHD)	35 (0.99)	45 (0.96)	80	9 : 7	4.59	0.03
				207 : 49	71.11	0.00

[a] Progenies within the susceptible parent range were classified as susceptible (S) and the rest were classified as resistant (R) + heterozygous (H).
[b] The corrected χ^2 using Yate's correction for continuity (Steel and Torrie, 1997).
[c] Inside the bracket is the *P*-value used to test homogeneity of individuals within the genotypic class, namely R+H and S.

5.3.4 Determination of allelism

5.3.4.1 Allelism of bunt resistance in spelt wheat and the *Bt*-gene lines

Crosses between resistant lines were made in 1995 to investigate allelism of genes controlling bunt resistance. The crosses were evaluated for disease incidence in 1996.

If two parents of a cross carry the same gene for resistance, their progenies should not segregate and all three populations should have the same mean and variance for disease incidence. The F_2 population means of the RL5407/*Bt*-gene line crosses were higher than either of the parents in all crosses, indicating possible non-allelic relationships due to independent segregation of the resistant genes (Table 5.14). This is because some of the F_2

Table 5.14. Means and 95% confidence intervals for disease incidence for bunt race T1 in the crosses of RL5407 x *Bt*-gene lines in 1996

Cross	Generation [a]	M ± S.E. [b]	95% C.I. [c]
RL5407/*Bt10*	P_1	11.03 ± 2.02	6.25 - 15.81
	F_2	17.31 ± 1.80	13.05 - 21.57
	P_2	2.46 ± 0.72	0.76 - 4.16
RL5407/*Bt8*	P_1	11.65 ± 1.83	7.32 - 15.98
	F_2	24.88 ± 2.01	20.13 - 29.63
	P_2	7.52 ± 1.54	3.88 - 11.16
RL5407/*Bt6*	P_1	13.55 ± 1.97	8.89 - 18.21
	F_2	37.11 ± 2.34	31.58 - 42.64
	P_2	11.65 ± 2.67	5.34 - 17.96
RL5407/*Bt3*	P_1	14.93 ± 1.40	11.62 - 18.24
	F_2	20.65 ± 1.95	16.04 - 25.26
	P_2	9.41 ± 1.09	6.83 - 11.99
RL5407/*Bt2*	P_1	16.05 ± 2.69	9.69 - 22.41
	F_2	16.22 ± 0.77	14.40 - 18.04
	P_2	2.38 ± 0.56	1.06 - 3.70

[a] P_1 = RL5407 and P_2 = the *Bt*-gene line, i.e. *Bt2*, *Bt3*, *Bt6*, *Bt8*, or *Bt10* gene.

[b] M ± S.E. = mean ± standard error.

[c] 95% C.I. = 95% confidence interval.

plants are susceptible segregants, lacking a resistant gene from either parent and resulting in a higher average bunt infection for this generation. This is also suggested by the fact that the 95% confidence intervals for all three means did not overlap in any of the crosses (Table 5.14).

Similarly, F_2 means of the crosses involving the three spelt wheats, SK0505, RL5407 and SK0263 were higher than the parental means and the 95% confidence intervals of the three population means did not overlap in the crosses, SK0505/RL5407 and SK0505/SK0263 (Table 5.15). This suggests that the resistance genes were not allelic between SK0505 and RL5407, or between SK0505 and SK0263. However, in the third cross RL5407/SK0263 the means of the parental and F_2 generations did overlap, suggesting that cultivars RL5407 and SK0263 might carry the same gene for resistance.

Table 5.15. Means and 95% confidence intervals for disease incidence for bunt race T1 in the three spelt wheat crosses in 1996

Cross	Generation	M ± S.E. [a]	95% C.I. [b]
SK0505/RL5407	SK0505	7.06 ± 1.07	4.53 - 9.59
	F_2	11.86 ± 0.83	9.90 - 13.82
	RL5407	7.08 ± 1.09	4.50 - 9.66
SK0505/SK0263	SK0505	9.99 ± 1.69	5.99 - 13.99
	F_2	20.19 ± 3.16	12.72 - 27.66
	SK0263	8.49 ± 1.34	5.32 - 11.66
RL5407/SK0263	RL5407	32.91 ± 3.86	23.78 - 42.04
	F_2	36.92 ± 2.94	29.97 - 43.87
	SK0263	25.41 ± 3.82	16.38 - 34.44

[a] M ± S.E. = mean ± standard error.
[b] 95% C.I. = 95% confidence interval.

5.3.4.2 Allelism of genes resistant to different bunt races

Allelism of *R*-genes carried by the resistant parent conferring resistance to two races can be tested by testing if the resistant (or susceptible) progenies are simultaneously resistant (or susceptible) to the two races. In other words, if disease reactions of the progenies to one race are independent from the reactions to the other race, then the *R*-genes governing resistance to these two races are not allelic. For each race, the progenies were classified, based on the disease reaction of the susceptible parent, into two groups and a 2 x 2 contingency table could be obtained to identify the relationship of the *R*-genes conferring resistance to the two races in question.

The data for bunt resistance to races T1, T13 and L7 were used to test allelism of genes for resistance to these three races for the F_2 progenies in cross Laura/Triple Dirk (Tables 5.16, 5.17 and 5.18). The results showed that bunt reactions of the *R*-genes in the F_2 progenies were different (or independent) from one race to another because of the large *P*-values. Thus, the genes conferring resistance to races T1, T13 and L7 are not allelic in Triple Dirk.

Tests of allelism of genes resistant to races L7, T1 and T13 for the F_2 progenies in cross Laura/RL5407 demonstrated that the genes carried by RL5407 for resistance to races T13 and L7 were possibly allelic as the disease reactions of the progenies to these two races were not independent ($P = 0.00$) (Table 5.19), in other words, the progenies demonstrating resistance (or susceptibility) to race T13 are also resistant (susceptible) to race L7. But, this gene was not allelic to the gene conferring resistance to race T1 (Tables 5.20 and 5.21).

Table 5.16. Test of allelism of resistance to races T13 and L7 of common bunt of wheat for the F_5 progenies in the cross Laura/Triple Dirk in 1997.

	Response to race T13 [a]		
Response to race L7	R + H	S	Total
R + H	17	3	20
S	9	1	10
Total	26	4	30
	χ^2_{adj} [b] = 0.04	$P = 0.84$	

[a] R+H = resistant + heterozygous lines and S = susceptible lines.
[b] χ^2_{adj} = corrected Chi-square using Yate's correction for continuity (Steel and Torrie, 1997).

Table 5.17. Test of allelism of resistance to races T1 and L7 of common bunt of wheat for the F_5 progenies in the cross Laura/Triple Dirk in 1997.

	Response to race L7 [a]		
Response to race T1	R + H	S	Total
R + H	10	8	18
S	10	2	12
Total	20	10	30
	χ^2_{adj} [b] = 1.41	$P = 0.24$	

[a] R+H = resistant + heterozygous lines and S = susceptible lines.
[b] χ^2_{adj} = corrected Chi-square using Yate's correction for continuity (Steel and Torrie, 1997).

Table 5.18. Test of allelism of resistance to races T1 and T13 of common bunt of wheat for the F_5 progenies in the cross Laura/Triple Dirk in 1997.

	Response to race T13 [a]		
Response to race T1	R + H	S	Total
R + H	16	2	18
S	10	2	12
Total	26	4	30
	χ^2_{adj} [b] = 0.01	$P = 0.92$	

[a] R+H = resistant + heterozygous lines and S = susceptible lines.
[b] χ^2_{adj} = corrected Chi-square using Yate's correction for continuity (Steel and Torrie, 1997).

Table 5.19. Test of allelism of resistance to races L7 and T13 of common bunt of wheat for the F_5 progenies in the cross Laura/RL5407 in 1997.

	Response to race T13 [a]		
Response to race L7	R + H	S	Total
R + H	19	0	19
S	5	5	10
Total	24	5	29
	χ^2_{adj} [b] = 8.24	$P = 0.00$	

[a] R+H = resistant + heterozygous lines and S = susceptible lines.
[b] χ^2_{adj} = corrected Chi-square using Yate's correction for continuity (Steel and Torrie, 1997).

Table 5.20. Test of allelism of resistance to races T1 and T13 of common bunt of wheat for the F_5 progenies in the cross Laura/RL5407 in 1997.

	Response to race T13 [a]		
Response to race T1	R + H	S	Total
R + H	15	3	18
S	9	2	11
Total	24	5	29
	χ^2_{adj} [b] = 0.16	$P = 0.69$	

[a] R+H = resistant + heterozygous lines and S = susceptible lines.
[b] χ^2_{adj} = corrected Chi-square using Yate's correction for continuity (Steel and Torrie, 1997).

Table 5.21. Test of allelism of resistance to races T1 and L7 of common bunt of wheat for the F_5 progenies in the cross Laura/RL5407 in 1997.

	Response to race L7 [a]		
Response to race T1	R + H	S	Total
R + H	11	7	18
S	8	3	11
Total	19	10	29
	χ^2_{adj} [b] = 0.06	$P = 0.81$	

[a] R+H = resistant + heterozygous lines and S = susceptible lines.
[b] χ^2_{adj} = corrected Chi-square using Yate's correction for continuity (Steel and Torrie, 1997).

Therefore, in conclusion, Triple Dirk possibly carries three different loci, each conferring resistance to one of the three races T1, T13 and L7 (Table 5.22). In RL5407, one gene governed resistance to races L7 and T13 and an additional gene conferred resistance to race T1 (Table 5.22).

Table 5.22. Allelic relationship of resistance genes carried by the two resistant parents Triple Dirk and RL5407 for bunt resistance to the three races T1, T13 and L7 in wheat.

		Race		
Cross	R-gene	T1	T13	L7
Tripe Dirk	R_1	R_1	S	S
	R_2	S	R_2	S
	R_3	S	S	R_3
RL5407	R_1	R_1	S	S
	R_2	S	R_2	R_2

5.4 Discussion

In this qualitative analysis, no discontinuous frequency distributions of plot disease incidence were observed except for the single head derived $F_{5:6}$ lines in Laura/Triple Dirk (Table 5.12, Fig. 5.2). Because of the higher degree of certainty of identifying susceptible individuals, the progenies of each cross were classified into two groups, susceptible (S) and resistant+segregating (R+H). However, while there is no guarantee that this grouping is absolutely accurate for every single progeny, the homogeneity tests (Tables 5.5-5.11, 5.13) suggested that this method of grouping was, in general, appropriate.

The results indicated that segregation for resistance to bunt races T1, T13 and L7 fit the suggested ratios ($p>0.05$) for crosses Laura/Kite, Laura/Triple Dirk and Laura/RL5407. The segregation data for resistance to race T1 in crosses Laura/Triple Dirk and Laura/RL5407 fit expected ratios in all generations, e.g. 3:1 for the F_2-derived generations (F_3, F_4 and F_5), 1:1 for the backcross generation (BC_1F_2), 9:7 (SHD $F_{4:5}$) and 17:15 (SHD $F_{5:6}$ / SSD $F_{4:6}$) for the single head/seed derived generations, either in the field or in testing under controlled environmental conditions. This consistency in Mendelian analyses strongly supports monogenic control of resistance to race T1 in cultivars Triple Dirk and RL5407.

However, in the test of resistance to race T13, the disease data for cross Laura/Triple Dirk fit both a 3:1 (P=0.21) and a 15:1 (P=0.11) ratio (Table 5.9). To differentiate these two ratios, at least 50 lines are required (Mather, 1938; Hanson, 1959), but only 30 $F_{2:5}$ lines were available for testing. In addition, the estimate of the number of effective factors (3.2 ± 1.4) suggested that resistance to race T13 might range from one to a few genes. However, the number of effective factors for resistance to race T13 may be overestimated because of high estimation of genetic range but low estimation of the genetic variance (see Section 4.3.3). Therefore, it seems appropriate to propose that resistance to race T13 in Triple Dirk is likely due to one or two major genes.

Segregation for resistance to race T1 in Genesis/SK0263 fit a two gene ratio (15:1) in the F_3 but not in the F_4 generation (Table 5.8), nor in the single head derived $F_{4:5}$ (207:49) generation (Table 5.13). This poor fit might be due to unusual disease development (Parker and Hooker, 1993). In this study, Genesis did not have good disease development in 1996 (56.78%) and 1997 (55.77%) compared with 1995 (80.18%). In contrast, disease

infection of the resistant parent SK0263 was much higher in 1997 (26.50%) and 1996 (12.46%) than in 1995 (1.41%). This inconsistent disease reaction in the parental generations could result from genotype x environment interaction, differences in inoculum load of bunt spores or unknown reasons. Genotype x environment interaction could result in change of the relative degree of disease infection among the different genotypes (Reed, 1928; Gaudet and Puchalski, 1989b), and difference in inoculum load could affect the chances of exposure of seeds to disease infection. Consequently, the infection distribution of Genesis could be shifted towards the resistance in 1996 and 1997 and could cause an inappropriate cut-off point for Mendelian analysis. This did not occur in the F_4 and F_5 segregating progenies because of their different genetic background from both parents.

The poor fit might also be due to misclassification of genotypes (Clarke et al., 1994). In a study of the inheritance of glaucousness and epicuticular wax in durum wheat, Clarke et al (1994) reported inconsistent fitting of segregation data to Mendelian ratios in different generations, even when planted in the same year. They attributed misclassification as the cause of backcross segregation data fitting a 1:1 ratio, but F_2 data not fitting a 1:2:1 ratio. However, in the present study, misclassification due to disease escape was less likely in the cross Genesis/SK0263, since an excess number of progenies were rated as susceptible in both the F_4 generation (Table 5.8) and the single head derived $F_{4:5}$ generation (Table 5.13). The higher than expected frequency of susceptible lines could be caused by sampling error and/or natural selection against the resistance allele if this allele is closely linked to an unfavourable allele or it has detrimental pleiotropic effects.

Similarly, in a study of net blotch resistance in barley, Douiyssi et al (1996) found that segregation of $F_{2:5}$ families in the cross Heartland x Arig 8 did not fit any expected

ratio and the number of susceptible lines exceeded the expectation for a monogenic segregation. Krasichynska (1997) also observed a higher than expected frequency of susceptible plants in the progenies for true loose smut resistance. She attributed this unexplained ratio to chromosomal rearrangements and unconscious selection during the breeding process.

In previous studies on common bunt resistance, arbitrary grouping for segregating progenies was used in many genetic studies. Metzger et al (1979) grouped F_3 progenies with less than 6% infected plants as resistant and progenies with more than 50% infected plants as susceptible in the study of *Bt9* gene. Likewise, Metzger and Silbaugh (1971) considered F_4 progenies with less than 10% as resistant and those with 40% or more diseased plants as susceptible. Since phenotypic grouping in this study was based on the susceptible parent, this method could exclude the error of grouping due to the chance of disease escape (Metzger et al., 1979), but inconsistent bunt reaction in the susceptible parent could also affect the grouping of the segregating progenies.

Therefore, although the segregation of F_4 families (Table 5.8) and $F_{4:5}$ lines (Table 5.13) for the cross Genesis/SK0263 did not fit expected ratios due to excess of susceptible lines, the good fit of a 15 : 1 ratio in 1995 and the bimodal distribution of bunt infection in 1997 (Table 5.12) still suggested possible digenic inheritance.

In the allelic study (Section 5.3.4.1), allelism of resistance genes was tested by comparing the means of three generations, P_1, P_2 and F_2. The usual approach to detect allelism is to use individual F_2 plants from a cross between two resistant parents for disease evaluation (Singh and Reddy, 1989; Singh et al., 1993; Singh et al., 1995a, 1995b). But, for common bunt resistance, the use of F_2 plants is not informative since the

resistance genotype can not be reliably determined on a single plant basis (Stanford, 1941; Metzger et al., 1979). Use of F_3 families is adequate but involves more time, labour and expense.

Results of the allelism tests (Section 5.3.4.1) showed that RL5407 might carry a resistance gene not allelic to *Bt2*, *Bt3*, *Bt6*, *Bt8* or *Bt10* for resistance to race T1. In addition, the gene conditioning resistance to race L7 was not independent of the gene for resistance to race T13 in cross Laura/RL5407 ($P<0.01$, Table 5.19). It was concluded that the genes for resistance to these two races are allelic. An alternative explanation is that the two genes are not allelic but are closely linked. For the purpose of disease resistance breeding, a gene or a cluster of genes conferring resistance to two or more races is a valuable genetic resource.

6. IDENTIFICATION OF RAPD MARKERS LINKED TO COMMON BUNT RESISTANCE

6.1 Introduction

Breeding for bunt resistance is not only time consuming and labour intensive, but also frequently inconclusive due to large environmental effects. Because of this, host materials should be screened over several years for accurate evaluation of bunt reaction. Identification of molecular markers associated with a disease resistance gene could facilitate the selection of breeding lines carrying that resistance gene.

Random amplified polymorphic DNA (RAPD) (Williams et al., 1990) has been widely used for the identification of molecular markers linked to disease resistance genes in wheat (Devos and Gale, 1992) and in many other crops (Uphoff and Wricke, 1992; Haley et al., 1993; Kutcher et al., 1996). Molecular markers have been identified for the powdery mildew resistance genes *Pm18* (Hartl et al., 1995) and *Pm21* (Qi et al., 1996) and the leaf rust resistance gene *Lr24* (Schachermayr et al., 1995). For common bunt, one RAPD marker associated with *Bt10* has been identified (Demeke et al., 1996). Since this marker was identified by using a series of resistant cultivars possessing this gene rather than using progenies derived from a cross between two inbred lines, no information on linkage of this marker with *Bt10* could be obtained. Therefore, the value of this 1.0-kb fragment marker is unknown. Since spelt wheat cultivar RL5407 possibly carries a single

gene for bunt resistance which was not allelic to genes *Bt1* to *Bt10* (Sections 4 and 5), this study was conducted to identify RAPD markers linked to bunt resistance in RL5407. Such marker(s) could be used to assist selection for the resistance possessed by RL5407.

6.2 Materials and methods

6.2.1 Plant materials

The population used for identification of RAPD markers was developed from the cross Laura/RL5407. A single embryo from each of 60 F_4 plants was seeded to raise a plant from which one tiller was used for extraction of DNA and then was grown to maturity to produce F_n seed for disease testing (Fig. A.1). To ensure accuracy of the disease data, the choice of individual lines for bulked-segregant analysis was based on the disease reaction of the F_4 single head-derived F_5 lines which were tested in a growth cabinet, and on the F_4 single seed-derived F_n lines tested in the field in 1997. The individual lines chosen for bulked-segregant analysis had similar bunt reactions in both the $F_{4:5}$ (SHD) and $F_{4:n}$ (SSD) generations. calculation of linkage between the marker and bunt resistance was based on the disease reaction of the F_4 single seed-derived F_n progenies (Table A.14).

6.2.2 DNA extraction

DNA samples were harvested from the F_4-derived F_5 plants of the cross Laura x RL5407. The procedure for DNA isolation was based on the method of Procunier et al. (1991). From each $F_{4:5}$ plant, one tiller was taken at the three to four leaf stage for DNA

extraction. From this tiller about 0.2 g fresh tissue was cut, placed in a 1.5 ml microfuge tube and liquid nitrogen added. After the fresh tissue was ground to powder, 0.5 ml 2 x CTAB buffer (Table A.12) at 65°C was added to each tube and mixed well. The tubes were then placed in a 65°C water bath for 5 min before adding 0.5 ml chloroform/isoamyl alcohol (24:1) to each tube. After microfuging at 13,000 RPM for 5 to 10 min, the upper aqueous phase (about 450 µl) was transferred to a new tube. The tube was then added a 0.1 volume of 10% CTAB (45µl at room temperature) and placed in 65°C water bath for 15 min followed by adding one volume of 24:1 chloroform/isoamyl alcohol. Each tube was mixed well, microfuged at 13,000 RPM for 5 to 10 min and the supernatant (about 300 µl) was transferred to a new tube. Two volumes of cold (-20°C) 95% ethanol (about 600 ml) were added to each tube. The tubes were placed on ice for 5 min to precipitate the DNA which was pelleted by centrifugation (13,000 RPM for 10 min). The alcohol was then poured off and 500 ml of cold (-20°C) ethanol was added again to the tube followed by microfuging for 5 min to purify the DNA pellet. The alcohol was removed, the DNA pellet air dried at room temperature, resuspended in 200 µl of 1x TE (10 mM Tris, 1 mM EDTA, pH 8.0) and kept at 4°C overnight for rehydration. Finally, the isolated DNA was quantified on the basis of UV absorption at 260 nm (GeneQuant, Pharmacia LKB Biochrom Ltd), diluted to a final concentration of 25 ng/µl and stored at -20°C for future use.

6.2.3 Random primers

The oligonucleotide primers (10 mer) used for the PCR reactions were purchased from the Biotechnology Laboratory, University of British Columbia, Canada. Two sets of primers, UBC201 to UBC300 and UBC501 to UBC600, were used in this study.

6.2.4 Bulked-segregant analysis

An equal amount of DNA from each of the five most resistant and the five most susceptible $F_{4:5}$ plants was bulked to form a resistant and a susceptible bulk, respectively. Disease rating data for both $F_{4:5}$ (SHD) and $F_{4:6}$ (SSD) lines were used to confirm that the individual lines chosen for the two bulks were truly resistant or susceptible (Table 6.1).

Table 6.1. Bunt incidence (%) of the individual lines of cross Laura/RL5407 used in the bulked-segregant analysis.

Resistant bulk			Susceptible bulk		
Line	$F_{4:5}$ (SHD)[a]	$F_{4:6}$ (SSD)	Line	$F_{4:5}$ (SHD)	$F_{4:6}$ (SSD)
17	0.0	0.0	5	95.5	44.4
22	5.3	0.0	6	100.0	65.9
33	0.0	21.8	44	66.7	43.6
40	5.9	8.3	45	84.2	41.5
46	5.0	0.0	62	71.4	67.5

[a] SHD = single head derived and SSD = single seed descent. The $F_{4:5}$ lines were tested in the growth cabinet in winter 1996/1997 and the $F_{4:6}$ lines were tested in the field in 1997.

Only these two bulks were used during the initial screening of primers, but once a polymorphism was found, the primer was retested using the two bulks and the two parents. If the polymorphism was reproduced, testing was repeated again using the

individual DNA samples which composed the two bulks. Primers producing repeated polymorphisms were used to screen the rest of the $F_{4:5}$ (SHD) lines to determine the linkage between the marker and bunt resistance based on the disease data of $F_{4:6}$ (SSD) lines. The determination of genotypes for bunt reaction was based on the distribution of bunt incidence for the susceptible parent Laura (Table A.14).

6.2.5 Polymerase chain reaction and DNA amplification

The PCR reaction was based on the methods of Schachermayr et al. (1995) and Demeke et al. (1996) with some modifications. The final volume of 25 µl mixture was composed of 50 mM KCl, 2.5 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.3, including 200 µM of each of dTTP, dATP, dCTP, and dGTP, 0.4 µM primer, 1.0 unit of *Taq* DNA polymerase and 20 ng of genomic DNA. The mixture was covered by 20 µl of light mineral oil. The PCR reaction was run in a RoboCycler® Gradient 40 thermocycler (Stratagene®, La Jolla, California, USA) programmed for 1 cycle at 94°C for 6 min followed by 45 cycles of 1 min at 92°C, 1 min at 36°C and 1 min at 72 °C, and finally 1 cycle at 72°C for 5 min for extension and then maintained at 6°C.

6.2.6 Electrophoresis

After amplification, 4 µl of loading buffer was added to each tube and the PCR products were separated through electrophoresis by running on a 1.5% (w/v) horizontal agarose gel in TAE buffer at 70 V to 80 V electric voltage for three to four hours. Ethidium bromide (10 mg/ml) was added to each gel for staining and a 1kb DNA ladder was used as marker for the comparison of molecular sizes of the amplified products. The

separated DNA fragments were visualized under UV light 312nm (Cole-Parmer Instrument Co., Chicago, France) and photographed using the gel documentation system UVP ImageStore 7500 (DiaMed Lab Supplies Inc., Mississauga, Ontario).

6.2.7 Statistical analysis

After a potential marker was identified by bulked segregant analysis, the DNA samples from all available individual lines were tested to detect the linkage between the RAPD marker and bunt resistance and between the two markers. A Chi-square (χ^2) test of independence was used to test the association of a marker with bunt resistance and between the two RAPD markers. The linkage distance and sequence among different loci were obtained using the computer program MAPMAKER/EXP V3.0b (Lincoln et al., 1993).

6.3 Results

6.3.1 RAPD markers

Six primers, UBC217, UBC248, UBC266, UBC274, UBC543 and UBC548, produced repeatable polymorphisms in the bulks and parents. However, only two RAPD markers were found to be linked to bunt resistance when DNA samples from the individual lines were tested. One was generated by primer UBC548 (GTA CAT GGG C) (Fig. 6.1, 6.2) and the other by UBC274 (GAG TAA GCG G) (Fig. 6.1). The markers generated by UBC548 and UBC274 were linked to susceptibility and resistance, respectively. Both markers flanked the resistance gene. Primer UBC548

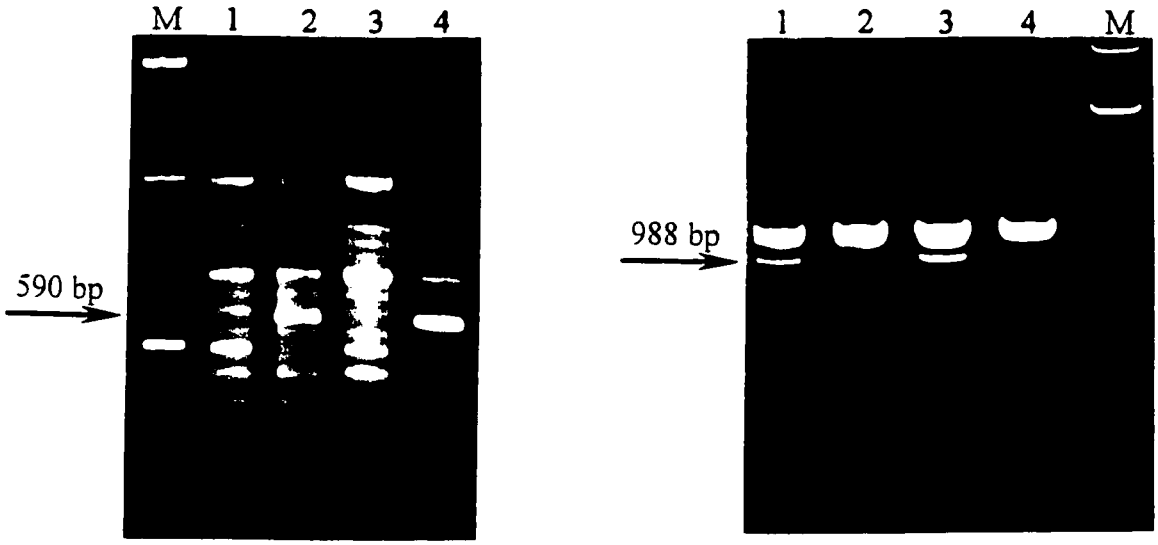

Fig. 6.2 Polymorphisms generated by the two primers UBC548 (left picture, repulsion linkage) and UBC274 (right picture, coupling linkage). 1 and 3 = the resistant parent (RL5407) and the resistant bulk, respectively, 2 and 4 = the susceptible parent (Laura) and the susceptible bulk, respectively. M = 1kb DNA size marker, ladder.

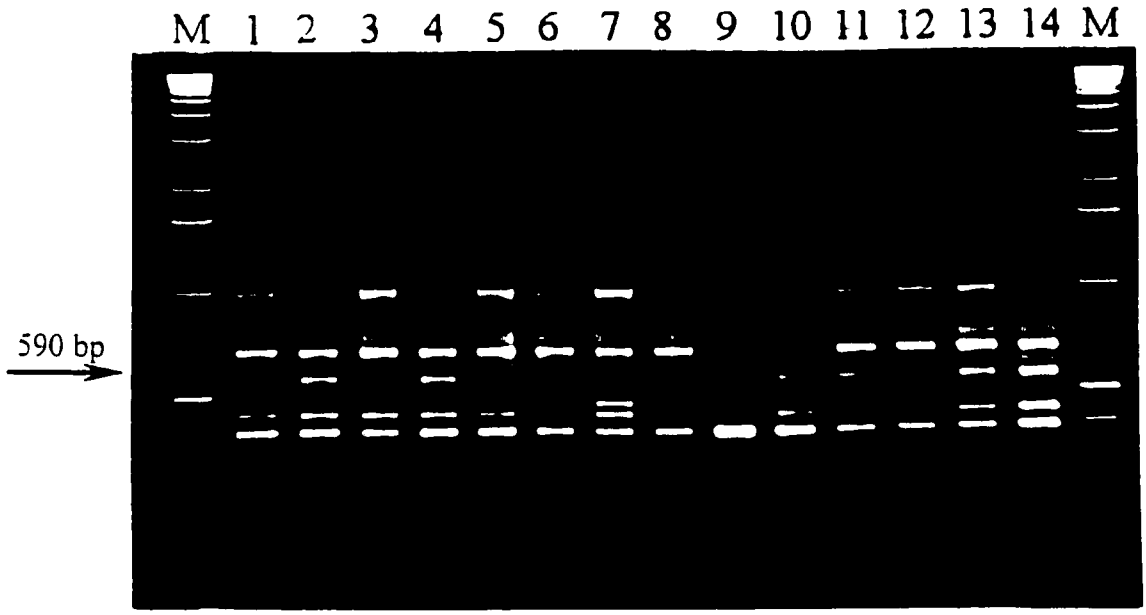

Fig. 6.3 Polymorphism (generated by UBC548) displayed between the parents, 2 bulks and the 10 individual lines from the 2 bulks. The resistant parent RL5407 (lane 1), resistant bulk (lane 3) and resistant lines (lanes 5-9) did not show the DNA fragment, while the susceptible parent Laura (lane 2), susceptible bulk (lane 4) and susceptible lines (10-14) had the DNA fragment (590 bp).

generated a DNA fragment of 590 bp and UBC274 generated a DNA fragment of 988 bp.

6.3.2 Linkages

a) $UBC548_{590}$ and bunt resistance

F_5 plants homozygous for resistance or susceptibility were identified by the disease reaction of the F_5 single seed derived F_6 families. DNA samples from 51 homozygous plants were used to determine the linkage between the marker and bunt resistance. The banding pattern data for the 51 random recombinant inbred lines were used for linkage analysis using the computer program Mapmaker/Exp. This analysis showed that the marker produced by UBC548 was linked to susceptibility at a map distance of 9.1 cM (Table 6.2).

Table 6.2. Detection of linkage between the marker and bunt resistance based on the banding patterns generated by primer UBC548.

	Banding pattern		
Disease reaction	+ [a]	-	Total
Resistant	3	19	22
Susceptible	27	2	29
Total	30	21	51
Test of linkage	χ^2_{adj} [b] = 29.42.	P – value = 0.00	
Map distance [c] = 9.1 cM			

[a] "+" and "-" indicate the presence and absence of DNA fragments, respectively;
[b] χ^2_{adj} = corrected Chi-square using Yate's correction for continuity (Steel and Torrie, 1997).
[c] Map distance was calculated by the computer program Mapmaker/Exp.

b) UBC274$_{988}$ and bunt resistance

DNA samples from the F_4 single seed-derived F_5 lines and the disease reaction of the F_4 single seed-derived F_6 lines were used to determine the linkage of marker UBC274$_{988}$ with bunt resistance. Forty-seven DNA samples were randomly chosen (excluding a few with inferior DNA quality) and used for screening the RAPD marker. After these 47 DNA samples were screened with the primer UBC274, it was found that this marker was only loosely linked to bunt resistance at a map distance of 18.2 cM (Table 6.3). Therefore, no further screening using this primer was done.

Table 6.3. Detection of linkage between the marker and bunt resistance based on the banding patterns generated by primer UBC274.

	Banding pattern		
Disease reaction	+ [a]	-	Total
Resistant	20	5	25
Susceptible	6	16	22
Total	26	21	47
Test of linkage	χ^2_{adj} [b] = 11.12,	*P* - value = 0.00	
Map distance [c] = 18.2 cM			

[a] "+" "-" indicate the presence and absence of DNA fragments, respectively.
[b] χ^2_{adj} = corrected Chi-square using Yate's correction for continuity (Steel and Torrie, 1997).
[c] Map distance was calculated by the computer program Mapmaker/Exp.

c) UBC548$_{590}$ and UBC274$_{988}$

The banding patterns produced by primers UBC548 and UBC274 were used to calculate linkage between the two markers (Table 6.4). It was found that both markers were linked at a map distance of 28.8 cM, a distance which was a little larger than the

sum of the two map distances between the markers and the resistance gene (9.1 + 18.2 = 27.3 cM). This close agreement (28.8 cM vs 27.3 cM) further suggested that these two markers were flanking the bunt resistance gene.

Table 6.4. Detection of linkage between the two markers based on the banding patterns generated by the primers UBC548 and UBC274.

		$UBC548_{590}$		Total
		-[a]	+	
$UBC274_{988}$	+	16	8	24
	-	6	17	23
Total		22	25	47
Test of linkage		χ^2_{adj}[b] = 6.22,	P - value = 0.01	
Map distance[c] = 28.8 cM				

[a] "+" "-" indicate the presence and absence of DNA fragments, respectively.
[b] χ^2_{adj} = corrected Chi-square using Yate's correction for continuity (Steel and Torrie, 1997).
[c] Map distance was calculated by the computer program Mapmaker/Exp.

6.4 Discussion

For the identification of RAPD markers, F_4-derived F_5 progenies from the cross Laura/RL5407 were used. After testing 200 primers, six primers, UBC548, UBC248, UBC217, UBC274, UBC543 and UBC266, were found to produce strong, repeatable polymorphisms in the bulks and parents, but only UBC548 and UBC274 were found to be linked to bunt resistance. The success of achieving the polymorphisms is believed due in part to the fact that RL5407 is a spelt wheat, and thus has a relatively higher divergence, in terms of DNA sequence, compared to common wheat. The probability of identifying a RAPD marker depends not only on the number of primers screened, but also

on the degree of divergence of DNA sequence in the genome (Martin et al., 1991). The chance of detecting a polymorphism would be much lower if the DNA sequences in the two parents were very similar. In the cross Laura/RL5407, the two parents certainly have higher degree of divergence than if both were common wheat or spelt wheat. A second reason was that RL5407 possessed a high level of resistance (Section 3), whereas Laura was shown to be a true susceptible parent with a high level of disease incidence (Gaudet et al., 1993). This large phenotypic range for bunt infection between these two parents assisted identification of true resistant and susceptible lines.

Prior to this study, Demeke et al (1996) identified one DNA marker linked to the *Bt10* bunt resistance gene in wheat using two near-isogenic lines (BW553 and Neepawa). This marker was then further tested in 38 cultivars of winter and spring wheat instead of testing a number of segregating individuals from a cross. The linkage between the marker and bunt resistance gene is unknown, thus the value of this RAPD marker is very limited (Williams et al., 1990). In this study, the marker generated by UBC548 showed tighter linkage (9.1 cM) than the other marker produced by UBC274 (18.2 cM), and thus it could possibly be used for indirect selection for bunt resistance. In order to make it more useful in bunt resistance breeding, this marker (generated by UBC548) could be further converted to a SCAR (sequence-characterized amplified region) marker since SCAR markers have advantages over RAPD markers (Paran and Michelmore, 1993; McDermott et al., 1994). In addition, since these RAPD markers are not completely linked to the resistance locus, it is suggested that they not be used beyond this cross. However, if more primers were screened, a more closely linked RAPD marker might be obtained, but would involve more time, labour and cost as well.

The use of two molecular markers flanking the resistance gene could enhance the efficiency of indirect selection for bunt resistance (Fig. 6.3). Although these two markers showed loose linkage to bunt resistance, the error rate (due to double crossovers) would be much lower when both markers were used together. The marker ($UBC548_{590}$) linked to susceptibility would be more useful than the one ($UBC274_{988}$) linked to bunt resistance since it can differentiate homozygous resistant genotypes from heterozygous and homozygous susceptible genotypes.

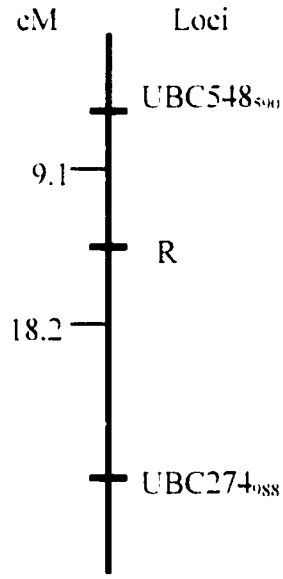

Fig. 6.3. Linkage map for the two RAPD markers and the bunt resistance gene.

The RAPD markers linked to disease resistance in this spelt cross could facilitate incorporation of resistance gene from spelt to common wheat, promote gene pyramiding in breeding for bunt resistance, and speed up breeding procedure by eliminating the disease evaluation which is particularly time-consuming for bunt disease (Rafalski et al., 1991).

7. GENERAL DISCUSSION

The main objectives of this study were to determine the genetic control of resistance to common bunt in both common and spelt wheats and to identify RAPD markers linked to common bunt resistance. Most of the disease data in this study showed continuous variation, suggesting that the genetic control was due either to polygenes or to major genes affected substantially by environmental effects (Stanford, 1941; Smeltzer, 1952; Russell, 1992; Comstock, 1996). The bunt disease data in this study were collected primarily from field experiments; only two disease tests were conducted in a growth chamber where bimodal (Fig 5.1) and discontinuous (Fig 5.2) distributions were observed. Thus, it is speculated that the environment had a great impact on common bunt infection.

It is not known whether a continuous distribution could result also from the methodology used to rate disease, i.e. disease incidence versus disease severity. Disease incidence was used in this study and was evaluated on a whole-head basis since a plant was considered susceptible as long as at least one bunt ball was observed on a head. Disease severity is determined on the floret level of a plant since it is calculated as the percentage of infected florets on a plant. The infection process of common bunt consists of two main stages: a) penetration of the coleoptile of a germinating seed by the secondary sporidia produced from the fungal spores; and b) progress of the fungal mycelia to the terminal meristematic tissue. Obviously, disease incidence stresses the importance of the

initial infection (penetration) of the fungus while disease severity directly relates to both penetration and the development of fungal mycelia within the plant tissue. In genetic studies of karnal bunt (*Tilletia indica* Mitra), disease severity was used and discrete distributions were observed for this disease (Fuentes-Davila et al., 1995; Singh et al., 1995a, 1995b). However, for common bunt, only disease incidence has been used and the disease distributions were continuous in most studies (Stanford, 1941; Smeltzer, 1952; McKenzie, 1964; Luthra and Chandra, 1983; Singh and Chopra, 1986).

Continuous variation makes it extremely difficult to identify the different host resistance genotypes and has been observed for most disease reaction data except for the few cases where immune parental materials were involved (Gasser, 1970; 1972). Since no unique approach has yet been developed to analyse this type of disease data (Mo, 1993), the experimenter has to establish a basis for grouping the observed individuals. Various methods have been used to achieve this. The first method most people have used is to arbitrarily set a cut-off point based primarily on standard disease rating scales (Rodenhiser and Holton, 1937; Kendrick, 1961; Waud and Metzger, 1970; Metzger and Silbaugh, 1971; Luthra and Chandra, 1983; Singh and Reddy, 1989; Potts, 1990; Elsidaig and Zwer, 1993; Ma, 1993; Singh et al., 1993). However, the criteria for deciding the cut-off point has varied when the same rating scale (such as 1 to 9) was used for different diseases (Singh and Reddy, 1989; Singh et al., 1993; Elsidaig and Zwer, 1993) and even for different generations when studying the same disease (Singh et al., 1993). Another approach is to base the cut-off point exactly on the parental distributions in the same disease test (Kornegay et al, 1993; Parker and Hooker, 1993). Parker and Hooker (1993), studying resistance to *Erwinia stewartii* in dent corn, based their cut-point on the

distribution of the resistant parent. However, due to the possibility of disease escape, a more legitimate approach would seem to be use of the susceptible parental distribution since there is greater certainty in identifying true susceptible than resistant individuals. Griffey and Das (1994) and Das and Griffey (1994) used the parental means plus or minus one standard deviation ($\mu \pm \sigma$) for genotypic grouping. However, the validity of such a method is questionable on both a genetic and a statistical basis. This method actually only includes about 84% (68%+32%/2) of the individuals from a parental distribution. This method would give a good fit to Mendelian ratios when the standard deviation is large, but when the standard deviation is small, it would give a poorer fit than would be expected from using the 95% confidence interval method.

Although different criteria have been used for grouping resistant individuals, arbitrary criteria of 40% to 100% infection has commonly been used to establish a susceptible class for common bunt (Rodenhiser and Holton, 1937; Kendrick, 1961; Waud and Metzger, 1970; Metzger and Silbaugh, 1971). Hoffmann and Metzger (1976) and Luthra and Chandra (1983) used the criterion of <10% infection rate to identify lines as resistant. Singh and Chopra (1986) used different criteria to group individuals in different populations. Cultivars with up to 10% infection were considered resistant and those with above 10% infection as susceptible. But for F_3 families, they classified those with less than 6% bunted plants as resistant, those having 6%-50% bunted plants as segregating and the progenies with more than 50% bunted plants as susceptible. Such arbitrary groupings may not be practical for genetic studies of bunt resistance since the level of bunt infection for any genotype can change from one year to another due to environmental effects (Reed, 1928; Gaudet et al., 1993).

Besides the controversial bases for the grouping of genotypes among different studies, additional questions may arise as "are these groupings of a continuous distribution legitimate in order to use Mendelian analysis?" "Are the continuous variations due to polygenic control and thus should not they be analysed using quantitative approaches?"

Mendelian analysis was originally developed for analyzing qualitative data and thus, strictly speaking, might not be appropriate for analysis of disease data showing continuous variation. In considering a trait showing continuous distribution, Crow (1966) stated that "these characters are all distinguished by the fact that many genes are involved". Mo (1993) also equated quantitative variation with polygenic inheritance. However, other quantitative geneticists have used a different definition. Allard (1960) indicated that quantitative traits are usually distributed continuously in progenies derived from a cross and can be measured on a decimal measuring system. Likewise, Falconer (1989) used a similar definition and stated that variation "without natural discontinuities is called continuous variation, and characters that exhibit it are called quantitative characters or metric characters, because their study depends on measurement instead of on counting". In these definitions, the term "quantitative trait" refers not to genetic control but to a decimal measurement. Others make reference to genetic control but do not suggest the number of genes. For example, Comstock (1996) stated:

> The difference between quantitative and qualitative traits resides in the relative magnitude of allele substitution effects. If the effect of substituting one allele for another is large relative to total phenotypic variation, the trait is qualitative. If such substitution effects are small relative to total phenotypic variation, the trait is quantitative. If allele substitution effects are small it is because the trait is affected by numerous genes and/or because a substantial portion of the total variation of the trait is nongenetic in origin.

Therefore, as stated earlier, continuous variation could be due to environmental effects even though the trait is actually controlled by major gene(s).

In addition to qualitative and quantitative traits, Mo (1993) defined a third type of agronomic trait, called qualitative-quantitative traits (Q-Q traits). Such traits, e.g. resistance, fertility and height, are controlled by a few major genes and many minor genes. So far, there is no accurate method for genetic analysis of such traits, and consequently studies have been based either on qualitative analysis (Waud and Metzger, 1970; Metzger and Silbaugh, 1971; Luthra and Chandra, 1983; Singh and Reddy, 1989; Elsidaig and Zwer, 1993; Kornegay et al, 1993; Barker et al., 1994; Singh et al., 1995) or on quantitative analysis (Kim et al., 1989; Cherif and Harrabi, 1990; Campbell and White, 1995; Pecchioni et al., 1996) or on both (Dey and Singh, 1993; Parker and Hooker, 1993; Das and Griffey, 1994). Mo (1993) indicated that both these types of analyses were not appropriate for qualitative-quantitative traits. He proposed use of F_2-derived F_3 families to permit calculation of the mean and variance for each individual family and then to plot family means versus variances for each generation (Table A.13). If the trait was under major gene control, Mo (1993) anticipated that three distinct groups should be evident. If three distinct groups could not be observed, then he considered it unlikely that major genes were controlling the trait.

However, I strongly suspect that Mo's expectation of three distinct groupings would never be achieved, as suggested in Table A.13. Theoretically speaking, this method of grouping may work if the environmental variation is small enough to be neglected. But the experiments of this study were conducted under field conditions, and bunt infection was found to be very sensitive to the environment such as soil moisture and temperature. The

plot of means versus variances might not produce groups since random environmental variation can not be predicted. Even if environmental effects are minimized, such as through use of controlled environments, this method (Table A.13) could only work with one gene model. When two or three genes are involved, the situation would become very complicated.

Despite the foregoing discussion, Mendelian analysis for bunt reaction, a quantitative trait, was attempted in this study since it was felt it could still provide an approximation of the true number of effective factors controlling resistance. The probability of making a wrong decision was considered low and the conclusions developed could be confirmed through other research (Sections 3 and 6). In this Mendelian analysis, the cut-off point was initially set at the lowest value of the susceptible parent. This lowest value was then tested for outlier in the susceptible parental distribution prior to phenotypic grouping (Dixon, 1953; Grubbs, 1969). If it was an outlier, due to disease escape or sampling error, the grouping could be adjusted to minimize bias. The results of the Mendelian analysis demonstrated that the disease data fit a two gene ratio in the cross Laura/Kite and one gene ratio in crosses Laura/Triple Dirk and Laura/RL5407. The segregation data in Genesis/SK0263 did not fit the expected Mendelian ratios in 1996 and 1997.

Estimates of effective factors in the quantitative analysis also suggested that Triple Dirk and RL5407 possibly carry one gene and Kite may carry two genes conditioning resistance to race T1 (Tables 4.7, 4.8), indicating agreement with the qualitative analysis (Tables 5.5-5.7, 5.11). For the cross Genesis/SK0263, the F_3 data, both in the quantitative analysis (Table 4.8) and the Mendelian analysis (Table 5.8), suggested at least two gene control of resistance to race T1, but the F_4 data did not fit a two gene segregation model

due to an excess number of susceptible lines. This failure could be due to misclassification (Clarke et al., 1994), unusual disease development (Parker and Hooker, 1993) or genotype-environment interaction since different genotypes could have different bunt ratings and different ranks in different years (Reed, 1928).

Both the quantitative and the qualitative analyses were attempted in this study. The results obtained by both methods largely agreed with each other in terms of genetic control except for bunt resistance to race T1 in the cross Genesis/SK0263. Both analyses suggested that Triple Dirk and RL5407 carry a single gene conferring resistance to race L7 (Tables 4.9, 5.10). However, qualitative analysis suggested only one gene for resistance to race T13 in Triple Dirk and RL5407 (Table 5.9), whereas the gene number estimates suggested more than one gene (Table 4.9). This might be the result of overestimation of gene number as environmental variation can bias gene estimates upwards (Mulitze, 1983).

Based on the data obtained in this study, it was found that the single seed/head derived populations in later generations were more useful than F_2-derived populations for genetic analysis (Section 5.3.3) and could be as effective as doubled haploid populations (Knox et al., 1998). Disease data obtained in these populations can demonstrate discrete or bimodal distributions (Table 5.12, Fig. 5.1, 5.2) and all generations studied fit a one gene segregation ratio except for the $F_{4:5}$ data of the cross Genesis/SK0263 (Table 5.13). Since these single seed/head derived materials have a higher degree of homozygosity for resistance and susceptibility, disease rating on a plant basis in these populations tends to be "all" or "nothing". Thus, there is great advantage for selection for bunt resistance in the single seed/head derived populations.

Generation mean analysis indicated no significant dominance effects for common bunt resistance (Table 4.7), which agrees with many of the previous studies (Gaines, 1920; Briggs, 1940; Stanford, 1941; Smeltzer, 1952; McKenzie, 1964, Knox et al., 1998). However, Singh and Chopra (1986) reported a single dominant gene governing resistance to bunt (*Tilletia foetida*) in HB 501 and Kalyansona. Metzger et al (1979) found two dominant genes for resistance to race T14 in C.I. 7090. The lack of dominance found for the resistance in the materials of this study can facilitate breeding for bunt resistance by simply selecting the highly resistant individuals. In addition, the estimated heritabilities of resistance were moderate to high (Table 4.10, 4.11), further suggesting that selection for resistance should be effective (Goates, 1996).

The successful identification of RAPD markers also confirmed that the resistant parent RL5407 possibly carried a single gene for resistance to race T1 (Sections 4 and 5). A closely linked molecular marker can not only assist selection for bunt resistance, but also facilitate isolating the resistance genes. An alternative approach for determining genetic control is to use genome map-based analysis (Nieto-Lopez and Blake, 1994). This method can be used to detect the resistance gene(s) using existing molecular markers (such as RFLP markers) distributed across all chromosomes of the wheat genome. It can provide information on the location of the chromosome for the resistance genes by detecting association between the markers and disease resistance (Newcombe et al., 1996; Wilcox et al., 1996). In a study on the inheritance of resistance to Russian wheat aphid (RWA) in two barley lines PI366453 and PI366444, Nieto-Lopez and Blake (1994) found two genes responsible for resistance to RWA while molecular studies detected two different regions in barley genome (Chromosomes 2 and 5) associated with RWA

resistance genes, indicating agreement between these two analyses. However, the molecular approach is more costly than conventional Mendelian analysis.

Moreover, the results of analyses provided sufficient evidence for race-specific resistance to common bunt in this study (Tables A.1, 3.2-3.4). It suggests that one should be very cautious when choosing a genotype to make crosses for incorporating resistance genes into a breeding program for gene deployment, or introducing new cultivars for wheat production to a particular area where corresponding virulent bunt race(s) are present.

Based on this study, a number of research areas are suggested for further study.

Disease rating for bunt infection based on disease incidence (presence or absence of a single bunt ball on a plant) might not reflect disease development thus may distort disease data. Because of this limitation, a histological study is suggested to study disease development and to provide valuable information for investigation of the mechanism of resistance. A comparison of disease severity and disease incidence should also be carried out to evaluate these different rating systems and possibly propose a new disease rating system for common bunt.

According to the allelic study, RL5407 might not carry any of the known *Bt*-genes used in this study, i.e. *Bt2, Bt3, Bt6, Bt8, Bt10*. However, further allelism studies between RL5407 and the rest of the known *Bt*-genes must be conducted to determine if the gene conferring resistance to race T1 in RL5407 is a new gene. Similarly, allelic studies involving Triple Dirk and SK0263 and the *Bt*-genes should be done.

In this genetic study, only three races T1, T13 and L7 were used, and it was found that RL5407 possibly carries a single gene for resistance to race T1 and an additional gene

conferring resistance to both T13 and L7 (Tables 5.19, 5.22). However, there are over 40 races in western Canada and the six prevalent races, L-7, L-16, T-1, T-6, T-13, and T-19, are considered the most important (Gaudet and Puchalski, 1989a). Thus, to understand more thoroughly the spectrum of resistance conferred by the genes in the resistant spelt lines, and further to provide guidance for pyramiding the resistance genes, additional genetic studies using races L16, T6 and T19 should be conducted.

In order to facilitate marker-assisted selection for bunt resistance, RAPD markers $UBC548_{590}$ and $UBC274_{988}$ could be converted to SCAR (sequence-characterized amplified regions) markers. SCAR markers have advantages over RAPD markers in that only a single locus is detected and their amplification is less sensitive to reaction conditions due to the use of longer primers and higher annealing temperatures. The single band produced by a SCAR marker can facilitate rapid screening of segregating progenies in breeding programs in selection for bunt resistance.

8. CONCLUSIONS

1) Significant differences were found in resistance to common bunt among cultivars and in virulence among different races. Race T1 was found to be the least virulent and T19 was possibly the most virulent. Cultivars possessing race-specific resistance were identified.

2) Common wheat cultivars Kite and Triple Dirk and spelt wheat cultivars RL5407, SK0263 and SK0505 were found to be highly resistant and were considered to possibly carry major genes for bunt resistance.

3) Generation mean analyses indicated that additive effects were the major genetic effects in all crosses and dominant effects were not significant. Epistatic gene action in the cross Laura/Kite was suggested.

4) Qualitative genetic analysis suggested that Triple Dirk carries a single gene controlling bunt resistance to each of the races T1 and L7, and one or two genes for resistance to race T13. RL5407 carries a gene conferring resistance to both races T13 and L7, plus a single gene for resistance to race T1. Kite possibly carries two genes and SK0263 carries at least two genes for resistance to race T1.

5) Heritability estimates ranged from 0.38 to 0.77 for bunt resistance to race T1, from 0.48 to 0.67 for resistance to race T13 and from 0.75 to 0.81 for resistance to race L7.

6) No evidence of maternal effects on common bunt resistance to race T1 was detected.

7) Allelism studies suggested that the RL5407 gene for resistance to race T1 was not allelic to *Bt2, Bt3, Bt6, Bt8* or *Bt10*. The genes carried by RL5407 and SK0263 are likely allelic, but are different to a gene carried by SK0505.

8) Two RAPD markers $UBC548_{590}$ and $UBC274_{988}$ linked to common bunt resistance to race T1 were identified in the cross Laura/RL5407 at map distances of 9.1 cM and 18.2 cM, respectively.

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10. APPENDICES

Table A.1. Analyses of variance for bunt incidence in cultivars tested for race-specific resistance to common bunt of wheat at Saskatoon in 1994.

Source of variation	DF	Mean square	*F*-value	*P*-value
		Experiment 1		
Block	3	2319.44	10.44	0.00
Cultivar	8	13323.79	59.96	0.00
Block x Cultivar	24	222.21	1.60	0.05
Race	5	1823.22	13.16	0.00
Cultivar x Race	40	360.97	2.61	0.00
Error	135	138.51		
		Experiment 2		
Block	3	2815.73	14.80	0.00
Cultivar	11	11925.39	62.69	0.00
Block x Cultivar	33	190.23	1.56	0.04
Race	5	1922.20	15.77	0.00
Cultivar x Race	55	415.56	3.41	0.00
Error	180	121.89		
		Experiment 3		
Block	3	75.73	1.07	0.38
Cultivar	10	18502.76	260.65	0.00
Block x Cultivar	30	70.99	0.67	0.90
Race	5	6817.75	64.48	0.00
Cultivar x Race	50	946.67	8.95	0.00
Error	165	105.73		

Table A.2. Relationships between the number of detectable resistance (R) genes and their reaction types to races of different virulence.

R-gene	$Race_1$ (Least virulent)	$Race_2$	$Race_3$	.....	$Race_n$ (Most virulent)
R_1	R_1	R_1	R_1	.....	R_1
R_2	R_2	R_2	R_2	.....	S
.	.	.	.	.....	.
.	.	.	.	.....	.
.	.	.	.	.....	.
R_{j-1}	R_{j-1}	R_{j-1}	S	.....	S
R_j	R_j	S	S	.....	S
Number of *R*-genes detectable	j	j-1	j-2	.....	1

Table A.3. Crosses and generations used for genetic studies, studies of maternal effects and allelism of bunt resistance genes from 1995 to 1997.

Cross and purpose	Inoculated with T-1				Inoculated with L7 and T13
	Summer 1995	Summer 1996	Winter 1996-1997	Summer 1997[a]	Summer 1996
Genetic Studies					
Laura/Triple Dirk	F_1, F_2, F_3	F_2,F_4,F_5, BC_1F_2	$F_{5:6}$ (SHD)	$F_{4:5}$, $F_{5:6}$[c] (SHD)	F_5
Laura/RL5407	F_1, F_2, F_3	F_2,F_4,F_5, BC_1F_2	$F_{4:5}$ (SHD)	$F_{4:6}$ (SSD)	F_5
Laura/Kite	F_1, F_2, F_3	---	---	---	
Genesis/SK0263	F_1, F_3	F_2, F_4	---	$F_{4:5}$ (SHD)	
Maternal Effect					
Genesis/RL5407	---	F_2			
RL5407/Genesis	---	F_2			
Allelic Studies					
RL5407/*Bt*-genes (*Bt2, 3, 6, 8, 10*)	---	F_2			
SK0505/RL5407	---	F_2			
SK0505/SK0263	---	F_2			
RL5407/SK0263	---	---	---	F_2	

[a] SHD = single head derived (lines), SSD = single seed derived (lines).

[b] W = winter, S = summer.

[c] $F_{5:6}$ for 1997 is a different set of SHD from that for 1996-1997 winter as two heads from each line were randomly harvested in F_5 generation in 1996.

Table A.4. Kolmogorov-Smirnov test for the normality of the error terms of the different samples for the resistance to race T1 from 1995 to 1997.

Year	Generation	# Line (plot)	Difference [a]		Critical value ($P = 0.05$)
			d^-	d^-	
			Laura/Triple Dirk		
1995	Laura	8	0.222	0.148	0.454
	F_1	8	0.178	0.150	0.454
	F_2	8	0.154	0.172	0.454
	F_3	84	0.111	0.099	0.148
	Triple Dirk	8	0.513*	0.388	0.454
1996	Laura	8	0.217	0.162	0.454
	F_2	8	0.219	0.157	0.454
	BC_1F_2	11	0.150	0.219	0.391
	F_4	40	0.088	0.079	0.210
	F_5	30	0.062	0.064	0.242
	Triple Dirk	8	0.252	0.170	0.454
1997	Laura	8	0.187	0.159	0.454
	$F_{5:6}$	26	0.258	0.219	0.259
	$F_{4:5}$	70	0.165*	0.151	0.162
	Triple Dirk	8	0.263	0.158	0.454
			Laura/RL5407		
1995	Laura	8	0.188	0.122	0.454
	F_1	8	0.348	0.277	0.454
	F_2	8	0.231	0.230	0.454
	F_3	80	0.169*	0.181*	0.152
	RL5407	8	- [b]	-	0.454
1996	Laura	8	0.114	0.092	0.454
	F_2	8	0.186	0.193	0.454
	BC_1F_2	35	0.083	0.098	0.224
	F_4	50	0.122	0.102	0.192
	F_5	29	0.083	0.115	0.246
	RL5407	8	0.187	0.146	0.454
1997	Laura	8	0.143	0.261	0.454
	$F_{5:6}$	59	0.111	0.094	0.177
	RL5407	8	0.181	0.133	0.454

[a] d is the maximum of difference between two cumulative relative frequency distributions, an observed (F) and an expected (F') distribution. $d^- = |F_i - F_i'|$, $d^- = |F_{i-1} - F_i'|$.

[b] - indicates not available.

* indicates significant at $P = 0.05$.

Table A.4. (continued) Kolmogorov-Smirnov test for the normality of the error terms of the different samples for the resistance to race T1 from 1995 to 1997.

Year	Generation	# Line (plot)	Difference[a]		Critical value ($P = 0.05$)
			d^-	d^+	
			Genesis/SK0263		
1995	Genesis	8	0.185	0.193	0.454
	F_1	8	0.141	0.151	0.454
	F_2	80	0.074	0.061	0.152
	SK0263	8	0.432	0.318	0.454
1996	Genesis	8	0.164	0.151	0.454
	F_2	8	0.176	0.301	0.454
	F_4	50	0.104	0.124	0.192
	SK0263	8	0.211	0.250	0.454
1997	Genesis	8	0.260	0.225	0.454
	$F_{4:5}$	80	0.050	0.045	0.152
	SK0263	8	0.188	0.143	0.454
			Laura/Kite		
1995	Laura	8	0.114	0.157	0.454
	F_1	8	0.342	0.249	0.454
	F_2	8	0.161	0.169	0.454
	F_3	81	0.105	0.117	0.151
	Kite	8	0.513*	0.388	0.454

[a] d is the maximum of difference between two cumulative relative frequency distributions, an observed (F) and an expected (F') distribution. $d^- = |F_i - F_i'|$, $d^+ = |F_{i-1} - F_i'|$.
* indicates significant at $P = 0.05$.

Table A.5. Kolmogorov-Smirnov test for the normality of the error terms of the different samples for the resistance to races T13 and L7 in 1996.

Race	Generation	# Line (plot)	Difference[a]		Critical value (P=0.05)
			d^-	d^+	
			Laura/RL5407		
T13	Laura	8	0.094	0.146	0.454
	F_5	29	0.106	0.141	0.246
	RL5407	8	0.256	0.143	0.454
L7	Laura	8	0.212	0.202	0.454
	F_5	29	0.072	0.097	0.246
	RL5407	8	0.165	0.146	0.454
			Laura/Triple Dirk		
T13	Laura	8	0.152	0.204	0.454
	F_5	30	0.117	0.150	0.242
	Triple Dirk	8	0.315	0.267	0.454
L7	Laura	8	0.150	0.103	0.454
	F_5	30	0.068	0.100	0.242
	Triple Dirk	8	0.164	0.133	0.454

[a] d is the maximum of difference between two cumulative relative frequency distributions, an observed (F) and an expected (F') distribution. $d^- = |F_i - F_i'|$, $d^+ = |F_{i-1} - F_i'|$.

Table A.6. Analysis of variance for common bunt incidence for race T1 in the cross Laura/Kite in 1995.

Source of Variation	DF	MS
Block	1	343.4
Treatment	112	398.0**
Among generations	4	4022.6**
Within F_3	80	325.8**
Within F_2	7	114.0
Within F_1	7	153.3
Within P_1	7	77.9
Within P_2	7	1.7
Error	112	116.7
Total	225	

*, ** indicate significance at 5% and 1% probability level, respectively.

Table A.7. Analysis of variance for bunt incidence for race T1 in the cross Laura/Triple Dirk from 1995 to 1997.

Source of Variation	DF	MS
	1995	
Block	1	646.4*
Treatment	115	547.9**
Among generations	4	3258.6**
Within F_3	83	551.5**
Within F_2	7	226.8
Within F_1	7	275.5*
Within P_1	7	97.0
Within P_2	7	1.6
Error	115	127.0
Total	231	
	1996	
Block	1	1122.6**
Treatment	104	509.8**
Among generations	5	3306.0**
Within F_5	29	461.2**
Within F_4	39	447.5**
Within BC_1F_2	10	237.1
Within F_2	7	222.7
Within P_1	7	200.3
Within P_2	7	46.8
Error	104	143.1
Total	209	
	1997	
Block	1	549.1
Treatment	111	1213.7**
Among generations	3	6040.2**
Within $F_{5,6}$	25	1176.6**
Within $F_{4,5}$	69	1198.4**
Within P_1	7	451.4
Within P_2	7	190.8
Error	111	282.0
Total	223	

*, ** indicate significance at 5% and 1% probability level, respectively.

Table A.8. Analysis of variance for bunt incidence for race T1 in the cross Laura/RL5407 from 1995 to 1997.

Source of Variation	DF	MS
	1995	
Block	1	34.7
Treatment	111	439.2**
Among generations	4	2366.0**
Within F_3	79	431.0**
Within F_2	7	172.9
Within F_1	7	279.3
Within P_1	7	297.0
Within P_2	7	0.0
Error	111	228.1
Total	223	
	1996	
Block	1	636.7
Treatment	137	602.7**
Among generations	5	2878.9**
Within F_3	28	601.5**
Within F_4	49	751.4**
Within BC_1F_2	34	358.7**
Within F_2	7	138.7
Within P_1	7	147.7
Within P_2	7	44.4
Error	137	184.0
Total	275	
	1997	
Block	1	105.9
Treatment	74	746.3**
Among generations	2	2771.2**
Within $F_{3:6}$	58	825.7**
Within P_1	7	128.6
Within P_2	7	127.7
Error	74	168.2
Total	149	

*, ** indicate significance at 5% and 1% probability level, respectively.

Table A.9. Analysis of variance for bunt incidence for race T1 in the cross Genesis/SK0263 from 1995 to 1997.

Source of Variation	DF	MS
	1995	
Block	1	867.4
Treatment	103	1051.3**
Among generations	3	16641.0**
Within F_3	79	677.7**
Within F_1	7	329.8
Within P_1	7	342.6
Within P_2	7	17.8
Error	103	351.2
Total	207	
	1996	
Block	1	5038.9**
Treatment	73	587.5**
Among generations	3	6343.0**
Within F_4	49	433.3**
Within F_2	7	112.4
Within P_1	7	79.2
Within P_2	7	184.2
Error	73	190.6
Total	147	
	1997	
Block	1	97.8
Treatment	95	924.8**
Among generations	2	3716.0**
Within $F_{4:5}$	79	950.8**
Within P_1	7	582.5
Within P_2	7	176.1
Error	95	549.5
Total	191	

*, ** indicate significance at 5% and 1% probability level, respectively.

Table A.10. Analysis of variance for bunt incidence for the two crosses Laura/Triple Dirk and Laura/RL5407 inoculated with races T13 and L7 in 1996.

Source of Variation	DF	MS	
		T-13[a]	L-7
		Laura/Triple Dirk	
Block	1	395.3	4441.5**
Treatment	45	1279.1**	1017.5**
Among generations	2	20980.1**	11219.6**
Within F_5	29	491.2**	752.2**
Within P_1	7	103.9	90.4
Within P_2	7	89.6	128.8
Error	45 (44[b])	145.0	234.4
Total	91 (90[b])		
		Laura/RL5407	
Block	1	1493.1*	4334.3**
Treatment	44	1355.3**	1225.8**
Among generations	2	19266.0**	10729.5**
Within F_5	28	640.3**	1102.0**
Within P_1	7	94.3	152.0
Within P_2	7	359.2	79.2
Error	44	260.0	125.4
Total	89		

*, ** indicate significance at 0.05 and 0.01 level, respectively.

[a] The adjusted analysis of variance for bunt resistance to race T13 after using the estimated outlier.

[b] The degree of freedom is 1 less for the error term in the experiment on resistance to race T13 due to the use of an estimated data point (Sokal and Rohlf, 1969; Steel and Torrie, 1997).

Table A.11. Critical values and criteria for testing for extreme values (Dixon, 1953).

N	*P*-value							Criterion[a]
	0.30	0.20	0.10	0.05	0.02	0.01	0.005	
3	0.684	0.781	0.886	0.941	0.976	0.988	0.994	$r_{10} = (x_2 - x_1)/ (x_n - x_1)$ if smallest value is suspect; or $r_{10} = (x_n - x_{n-1})/ (x_n - x_1)$ if largest value is suspect.
4	0.471	0.560	0.679	0.765	0.846	0.889	0.926	
5	0.373	0.451	0.557	0.642	0.729	0.780	0.821	
6	0.318	0.386	0.482	0.560	0.644	0.698	0.740	
7	0.281	0.344	0.434	0.507	0.586	0.637	0.680	
8	0.318	0.385	0.479	0.554	0.631	0.683	0.725	$r_{11} = (x_2 - x_1)/ (x_{n-1} - x_1)$ if smallest value is suspect; or $r_{11} = (x_n - x_{n-1})/ (x_n - x_2)$ if largest value is suspect.
9	0.288	0.352	0.441	0.512	0.587	0.635	0.677	
10	0.265	0.325	0.409	0.477	0.551	0.597	0.639	

[a] $x_1, x_2, x_3, \ldots, x_{n-1}, x_n$ represent the order of smallest values to largest values.

Table A.12. Buffers and solutions used for DNA extraction for the identification of RAPD markers.

Solution	Composition	Amount
2 x CTAB buffer	2% CTAB (w/v)	20g
	1% PVP (w/v)	10g
	100mM Tris pH8.0	100ml of 1 M Tris pH8.0
	20mM EDTA pH8.0	40ml of 0.5 M stock
	2.8M NaCl	163.6g
10% CTAB solution	10% CTAB (w/v)	100g
	1.4M NaCl	81.8g
	DdH_2O	818.2g
1M Tris (pH8.0)	Tris	121.1g
	HCl	42ml
Gel loading buffer/EDTA stock mixture (for 1 ml)	20% SDS	37.5µl
	2% Bromophenol Blue	37.5µl
	2% Xyene Cyanol	37.5µl
	0.5M EDTA	150.0µl
	30% Ficoll 400	625.0µl
	dd H_2O	113.0µl
	Electrophoresis Buffers	
TAE (Tris-Acetate)	50x:	
	Tris base	242.0g
	Glacial acetic acid	57.1ml
	0.5M EDTA (pH8.0)	100.0ml
TBE(Tris-Borate)	5x:	
	Tris base	54.0g
	Boric acid	27.5g
	0.5M EDTA (pH8.0)	20.0ml
TE buffer	1M Tris pH8.0	10.0ml
	0.5M EDTA pH8.0	2ml
	dd H_2O	988ml

Table A.13. Genetic constitution and the magnitude of means and variances in the progenies from a cross of two parents differing one major gene (G) and one minor gene (A) (modified from Mo, 1993).

Parental	Progeny	Genetic Constitution[a]	Mean	Variance
P_1 (large value)		GGAA, or GGaa		
P_2 (small value)		ggaa, or ggAA		
F_1		GgAa		
F_1	F_2 (1)	1/4 GG (1/4AA : 1/2Aa : 1/4aa)		
	F_2 (2)	1/2Gg (1/4AA : 1/2Aa : 1/4aa)		
	F_2 (3)	1/4gg (1/4AA : 1/2Aa : 1/4aa)		
F_2 (1)	F_3 (1)	1/4 GG (3/8AA:1/4Aa:3/8aa)	large	small
F_2 (2)	F_3 (2)	1/2 (1/4Gg : 1/2Gg : 1/4gg) (3/8AA : 1/4Aa : 3/8aa)	varied	large
F_2 (3)	F_3 (3)	1/4gg (3/8AA : 1/4Aa : 3/8aa)	small	small
B_1 (F_1xP_1)	B_1(1)-S_1[b]	1/2GG (1/2AA : 1/2Aa)	large	small
	B_1(2)-S_1	1/2Gg (1/2AA : 1/2Aa)	varied	large
B_2 (F_1xP_2)	B_2(1)-S_1	1/2gg (1/2aa : 1/2Aa)	small	small
	B_2(2)-S_1	1/2Gg (1/2aa : 1/2Aa)	varied	large

[a] GG is the major gene, and AA is the minor gene.
[b] B_1(1)-S_1 indicates that B_1(1) is selfed.

Table A.14. Bunt incidence (%) for $F_{4:5}$ (SHD) progenies of the cross Laura/RL5407 tested in the growth cabinet in winter 1996/1997 and for $F_{4:6}$ (SSD) progenies tested in the field in 1997[a].

	$F_{4:5}$ (SHD)[b]		$F_{4:6}$ (SSD)[c]	
Line	Bunt %	Putative genotype[d]	Bunt %	Putative genotype
1	70.6	S	46.7	S
2	72.2	S	29.0	S
5	95.5	S	44.4	S
6	100.0	S	65.9	S
9	6.7	R	7.2	R
11	18.2	H	43.8	S
14	52.9	H	3.9	R
15	90.9	S	48.7	S
16	21.1	R/H	0.0	R
17	0.0	R	0.0	R
19	43.3	H	46.3	S
20	62.5	S	59.9	S
22	5.3	R	0.0	R
25	10.0	R	7.2	R
26	50.0	H/S	34.5	S
27	10.5	R	16.7	R
28	68.4	S	34.4	S
29	35.3	R/H	27.7	R
30	36.8	H	55.0	S
31	23.5	H	48.5	S
32	47.4	H/S	32.3	S
33	0.0	R	21.8	R
34	13.3	H	51.2	S
35	35.7	H	39.2	S
37	15.4	R	13.8	R
39	14.3	R	22.2	R
40	5.9	R	8.3	R
41	80.0	S	30.8	S
42	38.9	H	33.3	S
43	85.0	S	44.1	S
44	66.7	S	43.6	S
45	84.2	S	41.5	S
46	5.0	R	0.0	R
47	53.3	H/S	40.7	S
48	21.4	R/H	4.4	R
49	10.0	R	9.4	R
50	64.3	S	39.9	S
52	45.0	R/H	26.3	R
54	5.3	R	10.0	R
55	52.9	H/S	37.2	S
56	84.2	S	39.2	S
58	40.0	R/H	25.9	R
59	94.4	S	63.3	S
60	50.0	H/S	47.2	S
61	37.5	H	41.7	S
62	71.4	S	67.5	S
63	55.6	H/S	66.9	S
64	56.3	H/S	41.7	S
65	0.0	R	0.0	R
66	0.0	R	19.6	R
69	20.0	R/H	0.0	R

[a] From one spike harvested at random from each F_4 family, one seed was used to produce a $F_{4:6}$ (SSD) line and the remaining seed used to produce $F_{4:5}$ (SHD) lines.

[b] Bunt incidence (infected spikes %) for the susceptible parent Laura was 58% based on the single pot, thus the determination of genotypes in $F_{4:5}$ (SHD) was only approximate.

[c] Bunt incidence (infected plants %) for the susceptible parent Laura ranged from 29% to 55% based on the mean bunt ratings of 8 plots in two replicates.

[d] S = homozygous susceptible, R = homozygous resistant and H = heterozygous.