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AFLP Markers Linked to *Fusarium* Head Blight Resistance in *Triticum aestivum*

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AFLP Markers Linked to *Fusarium* Head Blight Resistance in *Triticum aestivum*

Tara Potter

Thesis submitted to the Department of Biology

in partial fulfilment of the

requirements for a Master's degree.

Department of Biology

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ABSTRACT

In this study, AFLP technology was used to find markers linked to genes controlling *Fusarium* head blight (FHB) resistance in *Triticum aestivum* L. FHB is a disease of cereal crops that results in reduced wheat yields, discoloured, shrivelled kernels, mycotoxin accumulation, and reduced seed and grain quality. Since resistance mechanisms in wheat are complex, often being confounded by environmental effects, and there is high genotypic variance for resistance, molecular markers closely linked to FHB resistance would help in the screening of resistant germplasm. Candidate markers for resistance that were found among three varieties of wheat, two being susceptible to FHB ('Karena' and 'AC Cartier') and one being resistant to FHB (FHB 148), were followed into double haploid (DH) F_2 from crosses between each of the susceptible varieties and the resistant variety. These DH lines were evaluated for resistance after inoculation with *F. graminearum* and MAXR linear regression was done to determine whether any of the candidate markers could explain the variation in phenotype. In the 'Karena'/FHB 148 DH lines, 67% of the polymorphisms segregated in a 1:1 Mendelian fashion ($p \geq 0.05$), while 50% segregated 1:1 in the 'AC Cartier'/FHB 148 DH lines ($p \geq 0.05$). In the 'Karena'/FHB 148 population, 35% of the variation in the FHB resistance phenotype was explained by two markers ($p \leq 0.05$), while in the 'AC Cartier'/FHB 148 population, two markers explained 29% of the variation in phenotype ($p \leq 0.05$). Cloning and sequencing of these markers would be useful in the development of cultivars resistant to FHB by marker assisted selection.

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LIST OF ABBREVIATIONS

AAFC.....Agriculture and Agri-Food Canada

AFLP.....Amplified restriction fragment polymorphism

APS.....Ammonium persulfate

bp.....Base pairs

CIMMYT.....Centro International para el Mejoramiento de Maíz y Trigo (International Maize and Wheat Improvement Centre)

DH.....Double haploid

DON.....Deoxynivalenol

ECORC.....Eastern Cereal and Oilseed Research Centre

EDTA.....Ethylenediaminetetra-acetic acid

FHB.....*Fusarium* head blight

IRD.....Infrared dye

MAXR.....Maximum R^2 improvement linear regression

MR.....Moderately resistant

MS.....Moderately susceptible

MSE.....Mean squares error

PCR.....Polymerase chain reaction

PR-.....Pathogenesis related

R.....Resistant

RAPD.....Random amplified polymorphic DNA

RFLP.....Restriction fragment length polymorphism

S.....Susceptible

SAS.....Statistical Analysis System

SSR.....Simple sequence repeat (microsatellite)

STS.....Sequence tagged site

TE.....Tris - EDTA

TBE.....Tris-Boric acid-EDTA

TEMED..... N, N, N^{1}, N^{1}-Tetramethylethylene-diamine

QTL.....Quantitative trait loci

ZEN.....Zearalenone

CHAPTER 1. INTRODUCTION

Wheat is the staple food of millions of people, with annual global wheat consumption of 550 million metric tonnes (Fowler, 1998). Wheat is the second most important food grain in the world behind rice, producing over 542 million tonnes on about 222 million hectares (Yen *et al.*, 1996). Canada produces about 5% of the world's wheat and exports more than 75% of its annual production (20% of the world's exports), making wheat production a major source of income for Canadian farmers (Fowler, 1998).

Fusarium head blight (FHB) caused by *Fusarium graminearum* Schw. is a devastating fungal disease of cereal crops, resulting in accumulation of mycotoxins and causing reduced grain quality. The largest loss due to FHB in Ontario occurred in 1996 when the Ontario Wheat Producers Marketing Board estimated a 30% loss. This is equal to 230, 000 tonnes of wheat lost, and at $150 per tonne, the direct loss to producers was $34.5 million. There was a large accumulation of the mycotoxin deoxynivalenol in an additional 750, 000 tonnes, with losses of approximately $45 per tonne. This resulted in a further $33.75 million loss. The wheat was difficult to market, costing an additional $15 per tonne or $11.25 million. The industry lost an additional $20-30 million in buying replacement wheat stocks. The total loss to the industry in 1996 was estimated at more than $100 million (Crop Pest Ontario, 2000).

Research on the resistance to *Fusarium* head blight is required to decrease wheat yield loss, quality loss and to decrease mycotoxin concentration in the grain. Difficulties in screening for FHB resistant germplasm due to confounding environmental effects may be overcome by finding molecular markers linked to resistance genes. These DNA markers

could then be used for marker assisted selection of lines resistant to FHB.

1.1 History of *Fusarium* head blight

Fusarium head blight (FHB) is a disease complex also known as scab, white head and pink mould, is a fungal disease that infects small grain cereal crops such as wheat, barley, corn, oats and rice throughout the world. FHB has become a major problem in the eastern and Midwestern United States, New Zealand, Australia, Poland, Germany, Japan, Canada, Argentina, England, Russia, Sweden, France, Italy, Sicily, Brazil, Holland and Norway.

One of the first descriptions of a *Fusarium* infection was in the 16th century, made by a Franciscan friar in Mexico, who in a native Aztec language, described maize ear rot caused by *F. moniliforme* Sheld. (Booth, 1982). The genus *Fusarium* was first described in 1809 by Link, with *F. roseum* Link being the first species named. *F. graminearum* Schwabe was described in 1838. Scab research began in the Corn Belt of the U.S.A. in 1891 when Chester and Arthur discovered scab in Delaware and Indiana, respectively (Booth, 1982; Placinta, 1999).

The Canadian Plant Disease Survey has reported head blight throughout most of Canada where wheat is grown, although severe outbreaks have been confined to areas in Ontario, Québec, the Maritimes, Manitoba and the Peace River Region of Alberta (Sutton, 1982). In southwestern Ontario, widespread epidemics have occurred approximately once every nine years. A 1980 epidemic was widespread and very destructive, affecting winter and spring wheats in Ontario, Québec and Manitoba. This was the first outbreak to have occurred in Canada since a testing program had been implemented for identifying and

quantifying the mycotoxins deoxynivalenol (DON) and zearalenone (ZEN) in feed (Sutton, 1982). DON was detected at 0.2 - 4.4µg/g of wheat in many winter wheat samples taken in 1980 from farms, grain elevators and flour mills in southwestern Ontario and Québec by Agriculture Canada (Sutton, 1982). In 1980 the loss attributable to DON alone was estimated at $17 million (Teich *et al.*, 1987).

FHB has been considered a major threat to wheat and barley crops since the early years of the last century and in recent years FHB incidence has increased worldwide. CIMMYT has identified FHB as a major factor limiting wheat production in many parts of the world.

1.2 Epidemiology of *Fusarium graminearum*

1.2.1 Description of *F. graminearum*

In a study conducted between 1988 and 1990, *F. graminearum* was the most abundant pathogenic species found in Ontario wheat seed (Clear and Patrick, 1990). *Fusarium* head blight is caused by a number of *Fusarium* species, including *F. graminearum, F. culmorum* and *F. avenaceum* (Atanassov *et al.*, 1994). In Ontario, the most frequently isolated *Fusarium* species from wheat is *F. graminearum*, but *F. sporotrichioides* and *F. poae* are the next most prevalent (Clear and Patrick, 1990).

Fusarium graminearum (Figure 1a, b) is the anamorph or conidial stage of *Gibberella zeae* (Schw.) Petch and is the only common *Fusarium* species that regularly forms its sexual or perfect stage in nature. There are two groups of *F. graminearum*, group I and group II, which are distinguished by their life cycles and ecological requirements (Bai and Shaner, 1996). Group II, which forms perithecia, is the most

prevalent in Eastern Canada, while group I *F. graminearum*, which does not form perithecia, is the most prevalent in the drier climates of North America and Australia (Paulitz, 1996). The fungus has a wide host range, infecting corn, wheat, barley, oats and rice, as well as many species of broad-leaved weeds. The mycelium of *F. graminearum* consists of narrow, branched hyphae, making it naturally fibrous or chewy. Marlow Foods in Britain have exploited this chewy texture in the production of a meat substitute called Quorn®, made from *F. graminearum* mycelium. FHB inoculum may be in the form of ascospores, macroconidia, chlamydospores or hyphal fragments (Sutton, 1982). Ascospores (Figure 2b) are released from purplish-black perithecia (Figure 2a) that arise from stomata on host plant tissue, including debris. They are relatively uniform, ranging from 17-22.5µm x 3-5µm in size, are 3-septate and are hyaline (Sutton, 1982). Macroconidia (Figure 1b) are released from sporodochia (Figure 1a), and are more variable than ascospores, ranging in size from 35-62µm x 2.5-5µm. They are also septate (3-7) but can be distinguished from ascospores by their foot cell that has a distinctive heel (Sutton, 1982). Thick-walled chlamydospores arise from the transformation of macroconidia in the soil. Although ascospores may be the main inoculum in nature, most experimental studies on FHB have used macroconidia as the inoculum (Stack, 1989).

Figure 1a. *F. graminearum* sporodochia on wheat kernels.

(http://www.cgc.ca/Pubs/fusarium/fusarium-e2.htm)

Figure 1b. *F. graminearum* macroconidia. Magnification = 1000X.

(http://www.cgc.ca/Pubs/fusarium/fusarium-e2.htm)


sporodochia

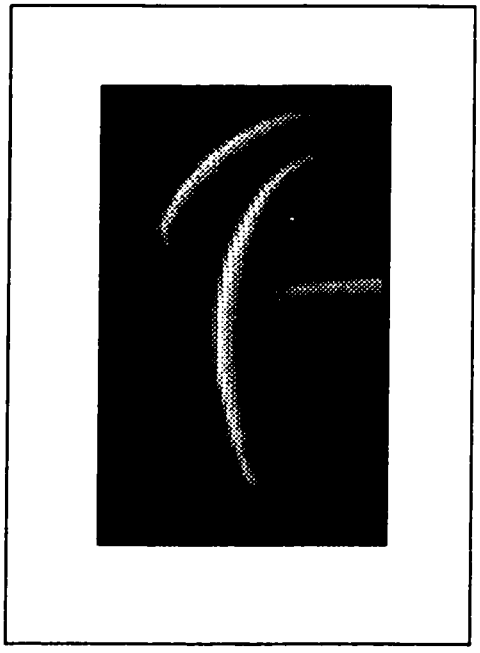
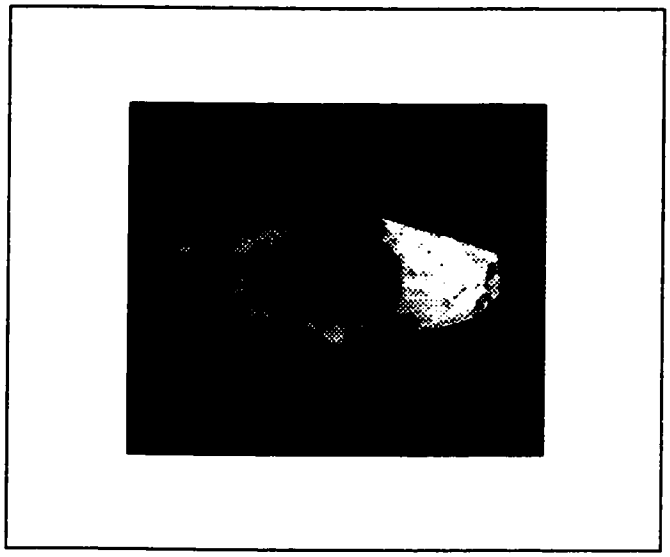


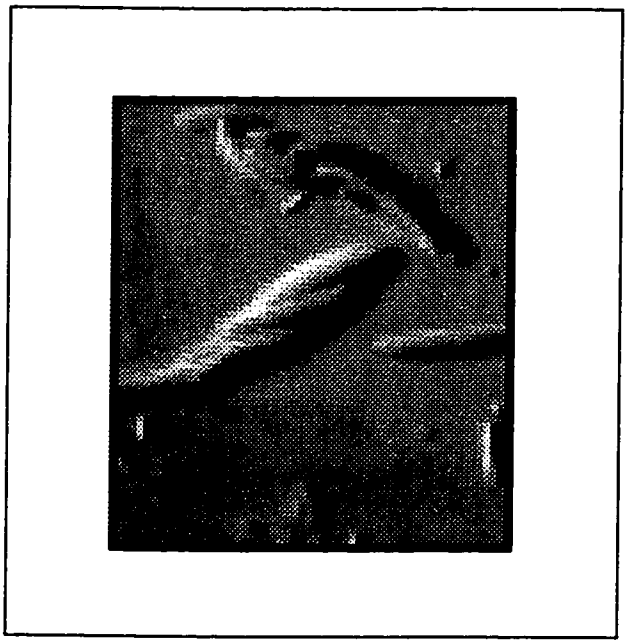
Figure 2a. *Gibberella zeae* perithecia on a wheat kernel.

(http://www.cgc.ca/Pubs/fusarium/fusarium-e2.htm)

Figure 2b. *G. zeae* ascospores. Magnification = 2000X.

(http://www.cgc.ca/Pubs/fusarium/fusarium-e2.htm)





1.2.2 Production of inoculum

Inoculum is produced under warm, moist conditions and as temperature increases, the number of perithecia also increases (Sutton, 1982). The optimum temperature for ascospore production is 25-28°C, although ascospores will still be produced from 13 to 33°C. The optimum temperature for asexual macroconidia production is slightly higher than for ascospores at 32°C, while the range is 16-36°C (Sutton, 1982). In dry weather, conidia are formed in areas where the infection originated, such as at the base of the spikelet where rain drops collect and moisture is held (Atanasoff, 1929). In moist conditions, conidia are formed over the entire surface covered by hyphae (Atanasoff, 1929). Mycelial growth of *G. zeae* in corn kernels is optimal at a relative humidity (RH) of 94%. Although rainfall is important for the formation of perithecia and ascospores, conditions for the release of ascospores may be different (Fernando *et al.*, 2000).

1.2.3 Sources of inoculum

The most important source of inoculum is crop debris left on the soil surface from host crop plants such as wheat, corn and rice on the soil surface (Bai and Shaner, 1994). Planting wheat in the same field within two years of corn crops increases the incidence of FHB (Clear and Abramson, 1986). Alternative hosts such as grass and broad-leaved weeds also provide a source of inoculum (Parry *et al.*, 1995). Gordon (1959) isolated *F. graminearum* from 19 species of grasses and 24 species of weeds. The above suggests possible roles of tillage, crop rotation and weed control in helping to control the disease.

1.2.4 Dispersal of inoculum

Inoculum can be dispersed by air currents or it can be rain-splashed onto developing heads (Fernandez and Fernandes, 1990). Birds, such as swallows and blackbirds, have been reported to carry *Fusarium* spores. They are capable of dispersing the seed along their migration routes between the U. S. and Canada (Warner and French, 1970). *F. graminearum* spores have also been isolated from several species of beetles, flies, leafhoppers and from European corn borer and armyworm (Miller *et al.*, 1998). When there is an increase in RH in the evening, there is an increase in turgor pressure of the asci due to the high osmotic pressure of the vacuoles (Paulitz, 1996). When the ascospores are wetted, they are released from the perithecia in a gelatinous substance. When the gelatinous substance dries, ascospores are discharged (Parry *et al.*, 1995). Ascospores are forcibly shot from the perithecia, while the principal dispersal mechanisms for macroconidia are wind and rain (Fernando *et al.*, 2000; Sutton, 1982). Since macroconidia are not forcibly shot into the air, they need help in dispersing to other plants. This energy comes from heavy rainfall (Fernando *et al.*, 2000).

1.3 Pathology of FHB

1.3.1 Climatic Conditions Favouring FHB Outbreaks

Wheat heads are most susceptible to FHB infections during anthesis (flowering) and during the dough stage of kernel development (Sutton, 1982). The susceptibility declines rapidly following the soft dough stage. Optimum conditions for outbreaks of FHB include a temperature of 25°C and a moist period of 36 to 72 hours. *Fusarium* root rot may develop in the early part of the growing season with warm, dry soil conditions.

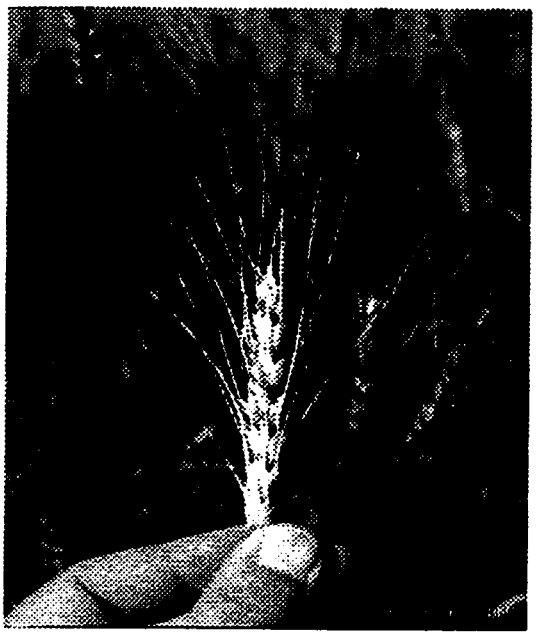
This provides an inoculum on the stem bases which may disperse to developing heads with heavy rainfall at anthesis (Parry *et al.*, 1995).

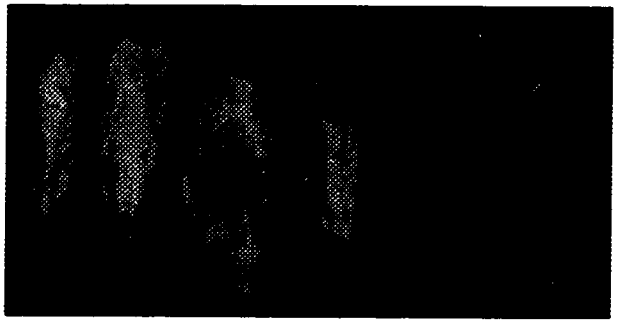
1.3.2 Disease Symptoms

Disease symptoms may appear as early as within two days of infection or up to several weeks later depending on the climate (Teich and Hamilton, 1985). Initially, infections appear as small, water soaked brownish spots at the base to the middle of the glumes (Parry *et al.*, 1995). The area where infected glumes are attached to the rachis then becomes water-soaked (Atanasoff, 1929). Under favourable weather conditions, the size of the water soaked area increases until the whole spikelet is covered (Figure 3a). The infection then spreads to other spikelets (Atanasoff, 1929). The point of infection can be identified by the presence of short, cottony, pinkish fungal growth. Under favourable conditions, the fungal growth grows to cover the infected area. This mycelium becomes the substrate for a layer of conidia (Atanasoff, 1929). As the disease progresses, the infected developing grains shrink and become grey to brown with a discoloured interior (Figure 3b). These smaller, shrivelled, white-pink grains are known as 'tombstone' kernels. The spikelets become bleached in appearance and die prematurely (Parry *et al.*, 1995). Later in the season, purple-black, raised spots called perithecia may develop on spikelets, rachis and seeds (Teich and Hamilton, 1985) (Figure 2a).

Figure 3a. *F. graminearum* infected wheat spike. Infected spikelets are pale in colour, while healthy spikelets are green. (http://www.cgc.ca/Pubs/fusarium/fusarium-e2.htm)

Figure 3b. *F. graminearum* infected wheat kernels. Kernels are infected at progressively later stages in development from left to right. (http://www.cgc.ca/Pubs/fusarium/fusarium-e2.htm)





1.3.3 Infection steps and disease cycle

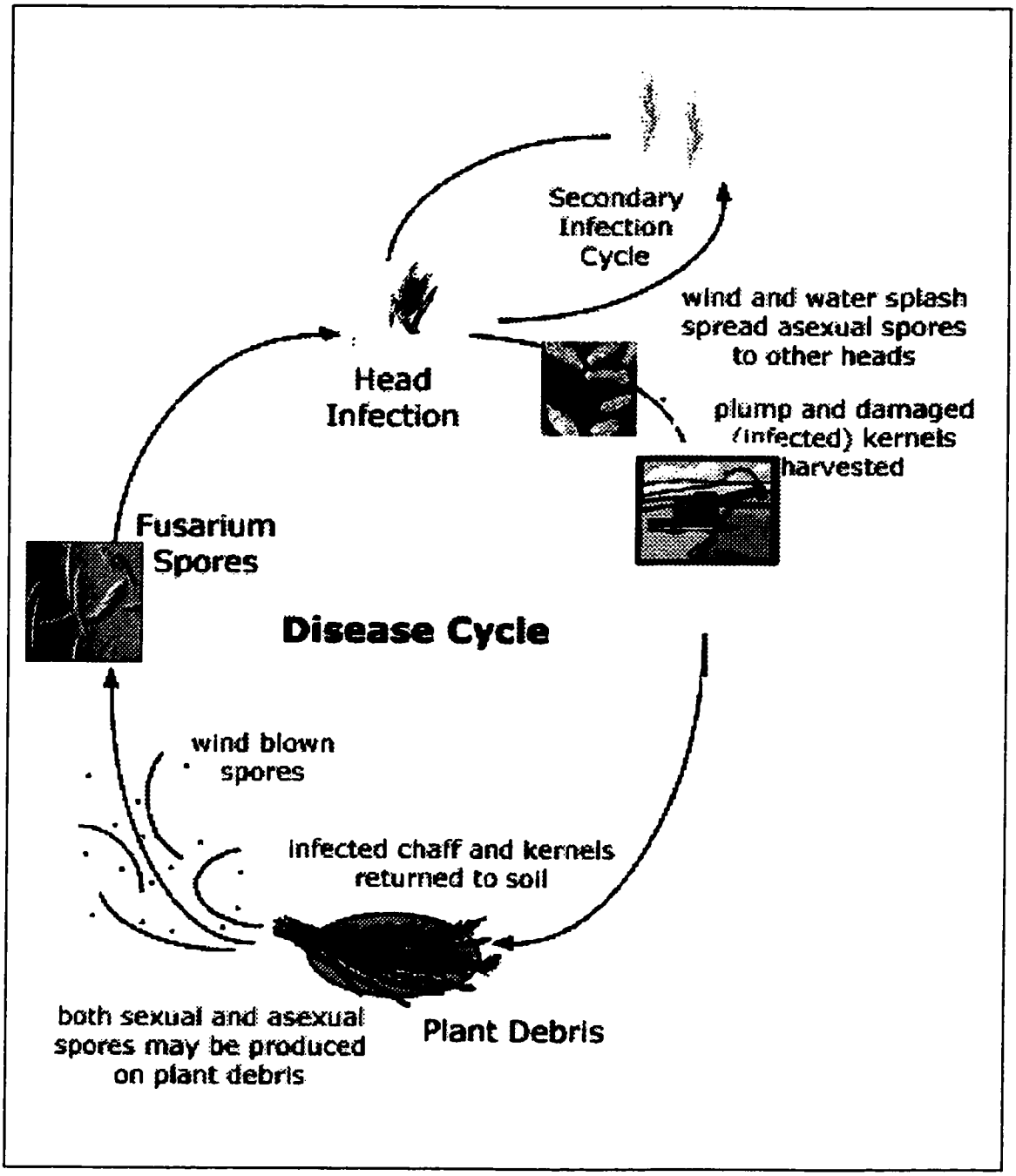
FHB is a monocyclic disease, as it involves only one infection cycle (Sutton, 1982). Since plants are only receptive for 10-20 days, their period of susceptibility is too short for a polycyclic disease which would require the completion of more than one infection cycle (Sutton, 1982).

When anthers are removed from flowers, infection has been shown to occur rarely (Sutton, 1982). When spores are placed on anthers infection occurs, but when placed directly on glumes, infection does not occur (Sutton, 1982). This indicates that anthers may promote infection. Two compounds found in anthers that are responsible for stimulation of *F. graminearum* growth are choline and betaine (Strange *et al.*, 1974). These compounds enhance hyphal growth after germination, but do not stimulate macroconidial germination (Sutton, 1982). The growth of *F. graminearum* on paleas, lemmas, glumes, rachises, grains, leaves, stems and roots seems to be correlated with the choline and betaine content of these organs (Sutton, 1982). Once the anthers are invaded, the fungus spreads through the developing caryopsis, floral bracts, rachis and the ovules. The mycelium invades the parenchyma and clogs the vascular tissue in the rachis. This causes the head to ripen prematurely (Bai and Shaner, 1994).

The *Fusarium* head blight disease cycle (Figure 4) begins when asexual macroconidia are wind blown or rain splashed and the sexual ascospores are forcibly shot from the perithecia from infected crop debris onto developing heads. When this event coincides with anthesis or the early dough stage of kernel development and also coincides with warm moist conditions, the spores germinate. The steps of infection occur on the

developing heads as described above. From the heads that are infected initially, macroconidia may be wind blown or rain splashed to heads of adjacent plants, beginning new disease cycles. The wheat kernels, including *Fusarium*-damaged kernels that are not light enough to be expelled with the chaff are harvested. Infected debris that falls to the ground serves as an overwintering site for the spores which infect the crop the following year.

Figure 4. *Fusarium* head blight disease cycle. Macroconidia and ascospores produced on crop debris are wind blown or rain splashed onto developing wheat heads where they initiate infection. Spores produced on the heads are blown onto heads of adjacent plants where secondary infection cycles begin. Healthy and *Fusarium* damaged kernels are harvested, and in the process, infected debris is returned to the soil where it can cause infection the following year. (Modified from http://www.brewingtechniques.com/bmg/gudmestadsb.html)


Secondary
Infection
Cycle
wind and water splash
spread asexual spores
to other heads
Head
Infection
plump and damaged
(infected) kernels
harvested
Fusarium
Spores
Disease Cycle
wind blown
spores
infected chaff and kernels
returned to soil
both sexual and asexual
spores may be produced
on plant debris
Plant Debris

1.4 Importance of research on FHB

FHB is a devastating disease with the ability to completely destroy a crop (McMullen *et al.*, 1997). Damage from FHB results in reduced yields, discoloured 'tombstone' kernels, mycotoxin contamination and reduced grain and seed quality. There are problems in marketing, exporting, processing and feeding of the infected grain due to reduced test weight and market grade (McMullen *et al.*, 1997).

1.4.1 Yield and quality losses

A severe scab epidemic in Minnesota, North, and South Dakota and Manitoba in 1993 resulted in an estimated $1 billion loss due to yield and quality losses. This made it one of the largest single year losses due to any plant disease in North America (McMullen *et al.*, 1997).

In epidemic years where there are tremendous yield losses, the grain that is harvested is often low in test weight, has a high scabby kernel content and high mycotoxin content. For severely infected crops, many producers are forced to decide whether their crop is even worth harvesting. For example in 1993, 18% of wheat acreage in Minnesota was not harvested and the crops were burned to eliminate residues (McMullen *et al.*, 1997). Producers then faced the decision of whether to sell immediately or try to clean the grain on a gravity table and then sell it. Elevator operators were also faced with a dilemma. They were uncertain how much *Fusarium* damage cereal buyers would accept in the wheat, so were reluctant to ship the grain across the country to find that it would not be accepted (McMullen *et al.*, 1997).

There were concerns about the safety of the grain to human and livestock health

due to mycotoxin accumulation (discussed in the following section). Consumers were also concerned about the quality of the wheat flour used in baking. *Fusarium* damage also has a large effect on baking performance of the flour, seen as a decrease in loaf volume. *F. graminearum* invades and destroys starch granules, storage proteins and cell walls (Bechtel *et al.*, 1985). Infection also results in changes in carbohydrate, lipid and protein composition (Boyacioglu and Hettiarachychy, 1995). Following the FHB outbreak in Manitoba in 1994, the ash content and colour were adversely affected and as *Fusarium* damage increased, the dough became more sticky (Dexter *et al.*, 1996).

1.4.2 Mycotoxins

Mycotoxins are secondary metabolites of low molecular weight. These organic compounds are produced during the stationary phase of batch cultures. The production of particular mycotoxins is sensitive to environmental and nutritional conditions (Moss, 1982). Mycotoxins produced by *F. graminearum* include the trichothecenes, zearalenone and moniliformin, the four most important being deoxynivalenol (DON, vomitoxin), nivalenol, T-2 toxin and zearalenone (Pohland and Wood, 1987). Although DON is the mycotoxin most abundantly produced by *F. graminearum* in wheat, zearalenone can also be produced.

1.4.2.1 Deoxynivalenol

Deoxynivalenol (DON, vomitoxin) is a trichothecene, belonging to the sesquiterpenoid compounds and is produced by fungi such as *Fusarium, Trichoderma, Myrothecium* and *Stachybotrys* and also by higher plants such as *Baccharis* (Ueno, 1987). Trichothecenes have a double bond at C-9 ~ C-10, an epoxy ring at C-12 ~ C-13 and are

called 12, 13-epoxy-trichothecene (Figure 5a). The trichothecene ring system arises from cyclization of farnesyl pyrophosphate, followed by 1, 2-methyl group shifts (Ueno, 1987). More than 60 trichothecenes, divided into groups according to their functional groups and hosts have been isolated from fungi and plants. DON belongs to the type B group, characterized by a carbonyl group at C-8. DON is colourless and is an optically active solid (Ueno, 1987). Since DON is soluble in polar solvents such as water, translocation in the phloem as a bulk flow of solution is assumed to occur (Snijders, 1994).

Trichothecenes break down the polyribosomal profile in eukaryotic cells by inhibiting protein synthesis (Hsieh, 1987). Protein synthesis occurs on polysomes that translate mRNA into a polypeptide chain. Ribosomal subunits from the subunit pool join the mRNA at the initiation region, where they undergo elongation. The polypeptide chain is released from the mRNA in response to a terminal codon on the mRNA. DON is an elongation-termination inhibitor (ET-type), preferentially acting on elongation-termination (Wei and McLaughlin, 1974). DON is produced in cells adjacent to the hyphal tips. In response to fungal invasion after penetration, hosts require the synthesis of proteins for their active defence mechanisms. DON prevents protein synthesis and the host cannot prevent further colonization (Snijders, 1994).

Feed contaminated with vomitoxin may result in vomiting and feed refusal in livestock, particularly in swine, which are the most sensitive to this toxin. Vomitoxin may act on the chemoreceptor trigger zone in the medulla oblongata of the central nervous system (Matsuoka and Kubota, 1981). DON also causes reduced feed intake and feed refusal, leading to reduced weight gains and resulting in a longer time for pigs to reach

market weight. DON leads to an increased incidence of disease and infection due to immunosuppression. DON may be the cause of swine reproductive failure, where there are high neonatal mortality rates, increased stillbirths, fetal mummification and high preweaning mortalities.

The first report of human and animal toxicoses caused by ingestion of grain contaminated with *F. graminearum* was in Japan, where epidemics of 'red mould' disease occurred as early as 1914. Between 1946 and 1963, outbreaks occurred in Japan and Korea. They were characterized by nausea, vomiting, diarrhea, abdominal pain, fever and throat irritation (Beardall and Miller, 1994). In China, outbreaks of mycotoxicosis have been reported since 1961. Between July and September 1987 in Kashmir Valley, India, outbreaks of gastrointestinal disorder occurred and affected an estimated 50, 000 people, regardless of age, sex and socioeconomic strata (Bhat *et al.*, 1989). There was an unseasonal rain at harvest time (May) and wheat was left in the field and became mouldy before harvest. Wheat mills mixed it with good wheat, but the quality of flour and bread was poor. Trichothecenes were detected in half the samples tested (Bhat *et al.*, 1989).

1.4.2.2 Zearalenone

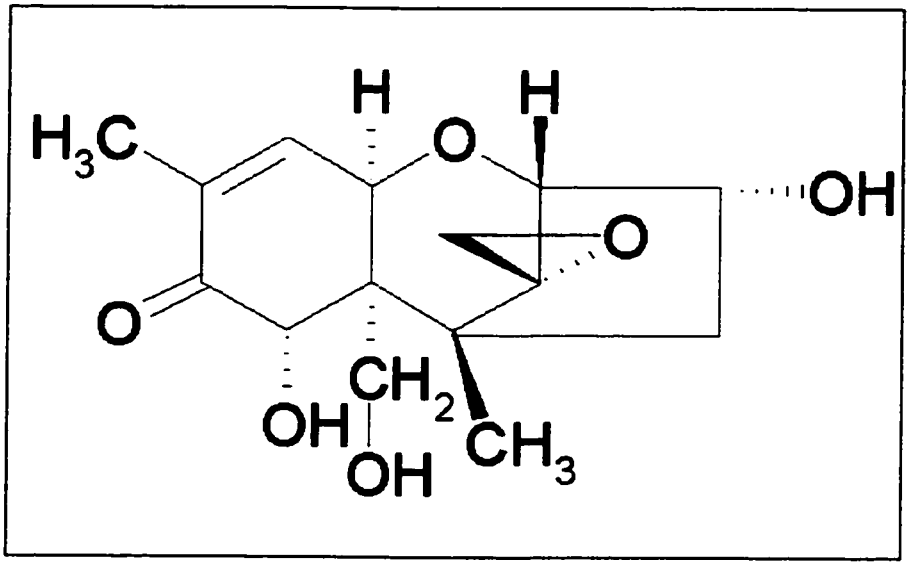
Zearalenone (ZEN) is an oestrogenic toxin that binds with cytosolic receptor proteins and then the oestrogen-receptor complexes are translocated into the nuclei. In mice, ZEN increases the permeability of the uterus to uridine and amino acids and also increases protein and nucleic acid synthesis (Ueno and Yagasaki, 1970 as cited in Hsieh, 1987). In rats, it has been suggested that ZEN affects the oestrogen feedback system through the oestrogen receptor in the brain (Kitagawa *et al.*, 1982 as cited in Hsieh,

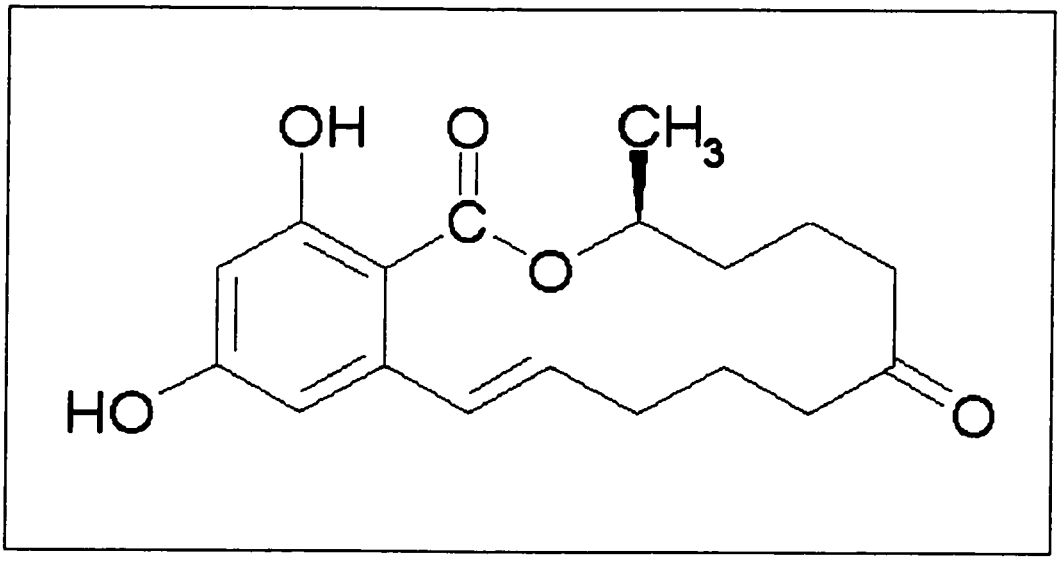
1987). ZEN is a phenolic resorcyclic acid lactone originating from a noneketide (Newberne, 1987) (Figure 5b).

Feed contaminated with ZEN causes uterine enlargement and swelling of the vulva, mammae and nipples in prepubertal female pigs, and causes swollen mammae and testicular atrophy in young male pigs (Mirocha and Christenson, 1974, as cited in Newberne, 1987). It has been reported that ZEN can inhibit fetal development, cause abortion, stillbirths and abnormal gait in newborn animals (Schieffer, 1985). Since ZEN has anabolic properties which result in weight gain, zearalenol ("Ralgro"), a derivative of ZEN is used in beef production (Schieffer, 1985).

Figure 5a. Molecular structure of deoxynivalenol (DON).

Figure 5b. Molecular structure of zearalenone (ZEN).


H
H
H3C
O
OH
O
O
CH2
OH
OH
CH3


OH
O
CH3
C
O
HO
O

1.5 Methods for FHB control

Environmental conditions are the most important variable in contributing to FHB outbreaks, but other factors also may contribute including the tillage regime, short rotation cycles and whether a high percentage of cultivated acreage is planted with crops that are susceptible to FHB (McMullen *et al.*, 1997). To decrease the risk of FHB epidemics, the quantity of inoculum available for dispersal needs to be reduced, dispersal needs to be prevented and if inoculum is present, infection needs to be prevented (Parry *et al.*, 1995). Practices that have been used to reduce the risk of FHB epidemics and to decrease the severity if epidemics do occur, include the tillage of crop debris under the soil surface (Miller *et al.*, 1998; Teich and Hamilton, 1985), rotation of susceptible crops with non-susceptible crops (Teich and Hamilton, 1985), the application of fungicides (Boyacioglu *et al.*, 1992; Milus and Parsons, 1994), weed control (Teich and Hamilton, 1985), and the planting of wheat varieties that are resistant to FHB.

1.5.1 Tilling regime and weed control

Fusarium graminearum survives on debris of host crops such as wheat, corn and barley. Due to concern in the past decade about soil erosion from farmland, there has been a significant increase in the amount of conservation tilling being done. Conservation tillage is a tilling practice where a minimal amount of tilling is done, leaving crop residues on the soil surface. This provides a source of inoculum for FHB outbreaks the following year. It has been shown that *F. graminearum* survives on debris under till and no-till regimes so that infection can occur in the wheat crop planted the next year (Miller *et al.*, 1998). Although tilling of debris in the fall does reduce the prevalence of *Fusarium*,

outbreaks still occur with favourable weather conditions (Miller *et al.*, 1998). This suggests that tilling may not reduce the incidence of FHB and crop rotation and the varieties planted may be more important than tillage practice in the control of FHB. Other studies have also shown that tilling does reduce the prevalence of FHB in wheat crops, although in years where conditions favour *F. graminearum* infection, outbreaks still occur (Dill-Macky and Jones, 2000).

Non-crop plants and weeds such as quackgrass and barnyard grass are hosts for *F. graminearum*, which increases the difficulties in controlling FHB (Martin and Johnston, 1982).

1.5.2 Crop rotation

It has been shown that when wheat is planted following corn that the number of blighted heads is significantly increased over the number when wheat is planted following soybeans or barley (Teich and Hamilton, 1985). In the U. S., the Department of Agriculture has encouraged producers to maintain a high acreage of wheat and discouraged crop rotation because deficiency payments were linked to enrollment in the wheat program (McMullen *et al.*, 1997). Short rotation cycles may occur as a result of economic decisions being made to maximize the use of expensive cereal production equipment (Martin and Johnston, 1982). When wheat is planted following corn, the incidence of FHB is greater than when planted after another cereal crop. This may be because corn debris does not deteriorate as quickly as other crop debris, or corn debris provides more inoculum (Teich and Hamilton, 1985). Since *F. graminearum* has a wide host range, infecting many cereal crops such as corn, rice, barley and wheat, as well as

broad-leaved weeds, it is not possible to control FHB with crop rotation alone (Parry *et al.*, 1995). Crop rotation may be more important than tilling, since FHB severity has been shown to be greater in wheat grown following wheat and corn than after soybeans, regardless of tilling practice (Dill-Macky and Jones, 2000). Crop rotation does not always prevent scab occurrences. For example in Essex county in southwestern Ontario, where wheat is rarely planted following corn, scab was severe in 1980, 1982 and 1986 (Teich *et al.*, 1987).

1.5.3 Chemical control

Fungicides, including Tridemorph, Thiabendazole, Fendropimorph, Triademefon and Propiconazole have been used in attempts to inhibit *F. graminearum* with some success. Tridemorph, Fendropimorph and Thiabendazole inhibit growth of *F. graminearum*, but also stimulate production of mycotoxins. Thiabendazole, Triadimefon and Propiconazole have all been shown to decrease the DON content in the grain, but the timing of application is very important. For example, Thiabendazole needs to be sprayed 2 days before inoculation to reduce DON content by 80% (Boyacioglu *et al.*, 1992). Propiconazole on the other hand will decrease infection by *F. graminearum* when sprayed during and after inoculation, but will have no effect if sprayed before inoculation (Boyacioglu *et al.*, 1992). Although propiconazole has been shown to limit infection, it does not decrease DON in the grain to levels safe for farm use. The decrease in symptom development due to the application of fungicides does not necessarily alter vomitoxin formation. The delay in senescence due to fungicide application may allow a longer time for toxin formation. Triadimefon and propiconazole act by inhibiting ergosterol in fungal

pathogens. A linear relationship between DON and *F. graminearum* with ergosterol, which are both derived from melvonic acid following the lipid biosynthetic pathway, may indicate that ergosterol inhibitors inhibit DON biosynthesis (Boyacioglu, 1991). Due to concern about toxic residues, fungicide treatments are often discouraged (Bhaskara Reddy *et al.*, 1999). Fungicides are also costly in relation to return per acre (McMullen *et al.*, 1997).

1.5.4 Genetic resistance

Since the environment, a factor we cannot control, plays a significant role in the development of FHB outbreaks, the above control methods are not sufficient in limiting the disease every year. Therefore, we must turn to an alternate strategy, that being genetic resistance of wheat plants to FHB. It had been suggested as early as 1929 that "the only effective method of controlling wheat scab (*Fusarium* head blight) is to grow resistant varieties" (Christiansen *et al.*, 1929).

FHB resistance of wheat plants is race-nonspecific, meaning that the resistance of the wheat plants does not depend on the genotype of *F. graminearum* (van Eeuwijk *et al.*, 1995). The study of the mode of inheritance of resistance has been slow due to a lack of its understanding (Ban and Suenaga, 2000). Resistance to FHB is quantitative and there is a large amount of variability in the level of resistance among wheat genotypes (Mesterházy *et al.*, 1999). Between 1977 and 1983 in China, 32 of 17, 000 cultures tested were found to have a high degree of resistance. Of these, most have undesirable agronomic traits, such as being tall landraces and are late maturing with low yielding, small heads (Bai and Shaner, 1994). There have been few superior cultivars and germplasm developed with

improved resistance and also the capacity for high yields and other desired traits (Jiang *et al.*, 1997). There has been difficulty in combining resistance and high qualities due to the expression of resistance along with unfavourable traits such as greater height, late maturing and lower yields (Jiang *et al.*, 1997).

Wheat resistance to head blight can be active, passive and/or a tolerance mechanism (Mesterházy, 1995). Passive resistance includes morphological attributes of the plant such as plant height, presence/absence of awns, spikelet density within the head and escape by flowering in the boot stage (Mesterházy, 1995). Tall plants, plants without awns, plants with low spikelet density or early flowering plants would all have a greater resistance than short plants, plants with awns, plants with very dense spikelets or later flowering plants. Physiological processes are active resistance mechanisms.

There may be as many as five types of resistance to FHB in wheat. Type I and II resistance were proposed by Schroeder and Christensen (1963) and are resistance to primary infection and resistance to spread of colonization after infection respectively. Resistance Types III, IV and V were proposed by Mesterházy (1995). Type III is resistance expressed in the wheat kernels and is seen as a reduction of *Fusarium* damaged kernels. Type IV is resistance to the accumulation of mycotoxins and may be due to the prevention of their production or the ability of the plants to degrade the mycotoxins. Type V resistance is yield tolerance, where the variety is able to produce a crop in the presence of the disease (Mesterházy, 1995).

Fungal mycelia are principally found in the outer coverings of mature grains (Bechtel *et al.*, 1985). This indicates that the infection begins on the outside of the grain

and then moves to the interior and suggests that invasion of the endosperm may be partially limited by the biochemistry of the outer layers (McKeehen *et al.*, 1999). These outer layers of mature grain contain phenolic compounds which have been suggested to be correlated to resistance (Assabgui *et al.*, 1993). Most phenolic acids in wheat kernels are cell wall bound (Mueller-Harvey *et al.*, 1986). The rate and amount of degradation of structural polysaccharides by microorganisms seem to be controlled in part by lignin-carbohydrate complexes in the pericarp (Wallace *et al.*, 1995). Ferulic and *p*-coumaric acids significantly reduce *in vitro* growth of *F. graminearum* (McKeehen *et al.*, 1999). As wheat grains develop, the ratio of ferulic acid to *p*-coumaric acid increases, indicating that these two phenolic compounds may contribute to disease resistance (McKeehen *et al.*, 1999). Phenolic compounds can contribute to fungal resistance by inactivating fungal enzymes or by strengthening plant structural components (Bell, 1981).

When infection occurs, the wheat spike tissues accumulate several defence response proteins encoding peroxidase, PR-1, PR-2, PR-3, PR-4, and PR-5 (Pritsch *et al.*, 2000). PR-4 and PR-5 transcripts appear earlier and to a greater extent in resistant genotypes compared to genotypes susceptible to FHB (Pritsch *et al.*, 2000).

In the past, several methods have been used to select for *Fusarium* resistance. These include the double-layer culture technique and the culture filtrate technique (Ahmed *et al.*, 1991; Ahmed *et al.*, 1996). These techniques involve *in vitro* selection of somaclonal variants that are insensitive to toxic *Fusarium* metabolites. Both techniques proved useful in selecting *Fusarium* resistant calluses, although were disadvantageous in that they produced low output of resistant regenerants (Ahmed *et al.*, 1996).

Resistant wheat germplasm can be divided into three gene pools -- winter wheats from Eastern Europe, spring wheats from China and Japan and spring wheats from Brazil and Italy (Snijders, 1990). Cultivars do exist with resistance levels high enough to withstand severe natural *Fusarium* inoculations. These include the Hungarian cultivar 'Ringó Sztár' (Mesterházy, 1987), the Brazilian spring wheat cultivars 'Frontana' and 'Encruzilhada', the Japanese cultivar 'Nobeokabozu Komugi' and the Chinese cultivars 'Sumai 3' and several 'Ning' selections.

1.6 The use of molecular markers for resistance

In the past, breeders relied on morphological markers such as height or yield. This strategy often does not distinguish between closely related lines differing for a particular trait. The use of traditional, morphological markers is time consuming and costly, often requiring a whole field of a strain of a crop. Plant breeders are now turning away from their traditional approach, toward the development of molecular DNA markers from RFLPs (restriction fragment length polymorphisms), RAPDs (randomly amplified polymorphic DNAs), SSRs (simple sequence repeats) and AFLPs (amplified restriction fragment polymorphism). With the use of DNA markers, environmental effects do not influence the selection of resistant plants (Bai and Shaner, 1994). Breeders are able to use tissue at any growth stage and plants are not destroyed since only a small amount of tissue is necessary (Bai and Shaner, 1994).

AFLP technology is a powerful DNA fingerprinting technique based on selective amplification of restriction fragments from digested DNA (Vos *et al.*, 1995). These polymorphisms are characteristic, heritable, and are seen as the presence or absence of

bands. DNA fingerprints have many uses including the identification of specific DNA samples, a source of DNA markers for genetic linkage maps and the identification of markers linked to specific loci. The use of DNA markers saves considerable time over traditional breeding approaches since plants need only be grown to the seedling stage. The AFLP technology has all the advantages of other DNA markers such as RFLPs, RAPDs, and SSRs, while avoiding their disadvantages. AFLP technology is PCR-based, requiring only small amounts of DNA, while RFLPs require larger amounts. AFLP markers are robust, reliable and reproducible, while RAPDs may not be as reproducible. AFLPs do not require prior sequence knowledge as SSRs do.

The AFLP process involves five steps, including digestion of genomic DNA, ligation of double stranded adapters to the cut site, followed by two PCR (polymerase chain reaction) amplification steps and polyacrylamide gel electrophoresis. Two restriction endonucleases are used for the restriction digestion, the rare cutter EcoRI, with a 6 base pair (bp) recognition sequence and the frequent cutter MseI, with a 4 bp recognition sequence. In the ligation step, double stranded adapters specific for the EcoRI and MseI sites are ligated to the sticky ends of the fragments. This is the template DNA for the first amplification step, known as preamplification. The restriction and adaptor sequences serve as primer binding sites for the preamplification, which employs primers with one selective nucleotide. This PCR reduces the number of fragments 16-fold. The second amplification step, selective amplification, employs primers with three selective nucleotides for a further 256-fold reduction in fragment amplified. Fluorescent labeled EcoRI selective amplification primers are used to enable visualization of the bands when

the products are run on a polyacrylamide gel.

In barley, AFLP markers have been shown to be distributed throughout the genome (Becker *et al.*, 1995), although it has also been shown that markers may cluster in centromeric regions which have a large proportion of repetitive sequences (Qi *et al.*, 1998). Since the AFLP technique can detect polymorphisms as small as one bp in length difference, small variations in repetitive sequences at the centromere can be detected (Qi *et al.*, 1998). If these repetitive sequences in the centromeric regions are being targeted in the AFLP technique, only a small part of the genome is being searched for markers. Although AFLP detects a lower level of polymorphism than RFLPs and microsatellites, the effective multiplex ratio per primer combination is much larger in AFLP, resulting in a highly informative technique compared to other methods (Ridout and Donini, 1999). An effective multiplex ratio is the number of polymorphic loci analysed per experiment in the germplasm tested.

Due to its discriminating power and reliability, AFLP proves valuable in mapping, and varietal identification (Ridout and Donini, 1999). Only a small amount (500ng) of DNA is required for AFLP compared to the amount required for RFLP (~10 000ng) (Ridout and Donini, 1999). RFLP analyses, which are hybridization-based techniques, are labour intensive and time consuming compared to PCR-based markers such as RAPDs, microsatellites and AFLPs (Qi and Lindhout, 1997). RAPDs have been shown to have poor reproducibility and have population specificity (Qi and Lindhout, 1997). Microsatellites require prior sequence knowledge, which is not required of AFLP (Qi and Lindhout, 1997). AFLP also allows the detection of a large number of polymorphisms

from each PCR (VanToai *et al.*, 1997). Although microsatellite markers are known to be highly informative and detect more alleles of a locus than any other marker technology, they are more time consuming and have high development costs. There are also a low percentage of polymorphic microsatellite markers in wheat (Ma *et al.*, 1996).

Although AFLP markers have the disadvantage of being dominant markers, the use of double haploid lines developed from F_1 plants eliminates the problem of working with heterozygous plants. Double haploid lines are homozygous at all loci and therefore the number of replicates can be reduced and dominance effects can be eliminated (Ban and Suenaga, 2000). AFLP markers between parents are inherited in a Mendelian fashion in progenies (Ma and Lapitan, 1998).

In China, most resistant cultivars were derived from 'Sumai 3' and hence all have the same source of resistance. This same source is also being used all over the world. Using the same source of resistance will eventually lead to genetic homogeneity for FHB resistance and vulnerability to *F. graminearum* if it adapts to this source (Bai and Shaner, 1994). For this reason, it is essential that we find new resistant germplasm and develop techniques to combine resistance genes.

1.7 Hypothesis and objectives

1.7.1 Hypothesis

AFLP analysis of candidate markers in double haploid F_2 plants from crosses between two susceptible varieties ('Karena' and 'AC Cartier') and one resistant variety (FHB 148) of *Triticum aestivum* will reveal genetic markers linked to genes that explain the variation in the FHB resistance phenotype.

1.7.2 Objectives

The first objective of this research is to find AFLPs between the two susceptible varieties and the resistant variety and to follow these candidate markers for FHB resistance into the double haploid F_2 generation and determine their segregation ratio. In order to determine whether or not the candidate markers are related to the resistance phenotype, each of the plants on which AFLPs were done are inoculated with *Fusarium graminearum* spores. After completion of classification of the phenotype as resistant or susceptible, each plant will have DNA marker information in the form of presence and absence of candidate markers and also a resistance rating. This phenotype and AFLP genotype will be compared using linear regression to determine whether the variation in resistance phenotype can be explained by one or more of the AFLP markers.

CHAPTER 2. MATERIALS AND METHODS

2.1 Plant Materials

Two soft winter wheat varieties (*Triticum aestivum* L.) susceptible to FHB and one resistant variety were used (Table 1). Since this project was funded in part by W. G. Thompson and Sons Ltd., an Ontario wheat company, Ontario wheat varieties 'Karena' and 'AC Cartier' were used as the susceptible varieties. The resistant variety used was FHB 148. Seeds of these varieties were retained and are available at ECORC. Double haploids were produced at W. G. Thompson and Sons Ltd. utilizing wheat x maize pollination. In this method, anthers are removed from F_1 hybrid plants and the plants are pollinated with corn pollen. Embryoids are removed from the caryopses and are incubated on plates. Embryoids that form roots and shoots are transplanted to soil and are treated with colchicine to double the chromosomes.

For the parental analysis, 15 individuals of each of the three varieties were used. From the cross 'Karena' x FHB 148, 9 plants from each of 26 lines were grown, while for the 'AC Cartier' x FHB 148 cross, nine plants from each of 36 lines were grown. Plants were either grown in root trainer pots or were grown in larger pots and transplanted into root trainer pots once leaf tissue was harvested. All plants were grown in Conviron growth chambers at 25°C with a 16 hour photoperiod using cool white fluorescent lights (40-60μmol/m^2/s).

Table 1. Description of the three varieties of *Triticum aestivum* L. used.

Variety	Type of Wheat	Varieties in Pedigree	Breeder	Year of Release	Resistance to FHB
'Karena'	soft white winter	'Augusta' 'Harus'	Dr. L. Shugar at W. G. Thompson and Sons Ltd.	1991	Susceptible
'AC Cartier'	soft red winter	'Lennox' 'Fredrick' 'Augusta'	Dr. R. Pandeya at Agriculture and Agri-Food Canada (AAFC)	1996	Susceptible
FHB 148	soft white winter	'Frontana' 'Harus'	Dr. D. Sampson at AAFC	N/A	Resistant

2.2 Plant harvest and DNA extraction

2.2.1 Tissue harvest and grinding

Leaf tissue (2-2.5g) was harvested from each of the double haploid F_2 plants. About 2 inches of leaf tissue was left in the pots to regrow. These plants were later inoculated with *F. graminearum* spore suspension (see Section 2.4). Leaf tissue was ground in liquid nitrogen with a mortar and pestle and then frozen at -80°C until needed for DNA extraction.

2.2.2 DNA extraction

DNA was extracted using the DNeasy™ Plant Mini Kit from Qiagen®, with a few modifications. The mass of starting ground leaf material was increased from 100mg to 200mg and the volumes of lysis and precipitation buffers were increased accordingly. The time for lysis was increased from 10 minutes to 45 minutes to allow more cells to be lysed. Elution was done using 50µl of the elution buffer and the five minute incubation was done in a 65°C water bath. The same 50µl of elution buffer was reapplied to the column membrane for the second elution step, making the final volume 50µl.

2.2.3 DNA quantification and dilutions

The DNA was quantified using a Hoefer DyNaQuant 200 fluorometer. DNA was diluted to 125ng/µl with ddH_2O.

2.3 AFLP reactions

2.3.1 Restriction digestion

Four µl of diluted genomic DNA (125n/µl) were pipetted into tubes, giving 500ng of DNA for restriction digests. Each genomic DNA sample (500ng) was digested with the

restriction endonuclease Tru9I (Roche), a less expensive isoschizomer of MseI, with the four base pair recognition sequence TTAA.

Tru9I Reaction Mix (for DNA samples at 125ng/µl):

	X1	X50
10X buffer M (Roche)	1.0µl	50.0µl
Tru9I 10U/µl (Roche)	0.3µl	15.0µl
ddH_2O	4.7µl	235.0µl
	6.0µl	300.0µl

Tru91 reaction mix (6µl) was added to each 4µl of diluted genomic DNA, incubated at 65°C for 2 hours and then stored on ice. These samples were further digested with 3 units of EcoRI. EcoRI has the six base pair recognition sequence GAATTC.

EcoRI Reaction Mix:

	X1	X50
10X buffer M (Invitrogen)	1.5µl	75.0µl
0.5M Tris pH 7.5	1.2µl	60.0µl
0.5M NaCl	1.5µl	75.0µl
EcoRI 10U/µl (Invitrogen)	0.3µl	15.0µl
ddH_2O	10.5µl	525.0µl
	20.0µl	750.0µl

EcoRI reaction mix (20.0µl) was added to each tube of Tru9I sample on ice and incubated at 37°C for 2 hours. The digestion products were heated to 70°C for 15 minutes and were put on ice.

2.3.2 Ligation of adapters

After restriction digestion, ligation of adapters was done in 25µl of a solution containing 2pMol EcoRI-adapters, 20pMol MseI-adapters, 1U T4 DNA-ligase (Invitrogen), 10mM ATP and ligation buffer (Invitrogen).

EcoRI adapter sequence: 5'-CTCGTAGACTGCGTACC
CATCTGACGCATGGTTAA-5'

MseI adapter sequence: 5'-GACGATGAGTCCTGAG
TACTCAGGACTCAT-5'

Ligation Mix:

	X1	X48
5X ligation buffer (Invitrogen)	3.1µl	148.8µl
10mM ATP	0.4µl	19.2µl
EcoRI adapter (2pMol/µl)	1.0µl	48.0µl
MseI adapter (20pMol/µl)	1.0µl	48.0µl
T4 DNA ligase (Invitrogen) 1U/µl	1.0µl	48.0µl
ddH_2O	18.5µl	888.0µl
	25.0µl	1200.0µl

Ligation mix (25µl) was added to each tube of 25µl digested DNA and was incubated at 20°C for 2 hours. Reactions were heated to 65°C for 10 minutes. After ligation, the reaction mixture was diluted to 1ng/µl (1/10 dilution) with low TE (10mM Tris-HCl, 0.1mM EDTA pH 8.0). This was stored at -20°C until needed for the preamplification reactions.

2.3.3 Preamplification PCR

Preamplification reactions employed two oligonucleotide primers, one corresponding to the EcoRI-ends and the other to the MseI-ends, each with one selective nucleotide. Twenty-five µl preamplification PCRs were performed containing 20µl of a mixture with 15ng MseI-primer, 15ng EcoRI-primer, PCR buffer (Promega), 10mM dNTPs, 25mM $MgCl_2$ and 0.5U Taq DNA polymerase (Promega).

EcoRI preamplification primer sequence: 5'-GACTGCGTACCAATTCA-3'

MseI preamplification primer sequence: 5'-GATGAGTCCTGAGTAAC-3'

Preamplification Mix:

	X1	X48
M-preamp primer mix (30ng/µl)	0.5µl	24.0µl
E-preamp primer mix (30ng/µl)	0.5µl	24.0µl
10X PCR buffer (Promega)	2.5µl	120.0µl
10mM dNTPs	0.5µl	24.0µl
25mM $MgCl_2$ (Promega)	1.5µl	72.0µl
Taq DNA polymerase (Promega)(5U/µl)	0.1µl	4.8µl
ddH_2O	14.4µl	691.2µl
	20.0µl	960.0µl

Diluted ligated DNA (5µl) of each sample was put in tubes for preamplification. Preamplification mix (20µl) was added to each 5µl of DNA ligation dilution and was amplified using the MJ200 DNA Engine. Preamplification PCRs were performed for 20 cycles, with a 30 second denaturation step at 94°C, a 1 minute annealing step at 56°C and a 1 minute extension step at 72°C. Preamplification PCR products were diluted 4-fold with low TE (10mM Tris-HCl, 0.1mM EDTA pH 8.0) and were stored at -20°C until needed for selective amplification PCR.

2.3.4 Selective amplification PCR

Selective amplification reactions also employed two oligonucleotide primers, but each with three selective nucleotides – one corresponding to the EcoRI-ends and one corresponding to the MseI-ends. The EcoRI primer is labelled with an infrared dye that is excited at 700nm (IRD700) (Li-Cor®). Selective amplification PCR reactions were performed in 14.8µl of a reaction mixture containing of 0.48pMol IRD700 labelled EcoRI primer, 30ng MseI-primer, PCR buffer (Promega), 10mM dNTPs, 25mM $MgCl_2$ and 0.5U

of Taq DNA polymerase (Promega).

EcoRI selective amplification primer sequence: 5'-GACTGCGTACCAATTCAGC-3'

MseI selective amplification primer sequence: 5'-GATGAGTCCTGAGTAACTG-3'

The selective amplification primer sequences are the same as the preamplification primer sequences except the last 3 nucleotides which are the selective nucleotides. The sequences above are those for the primer pair G7 (E-AGC X M-CTG).

Selective Amplification Primer Mix:

	X1	X50
IRD700 EcoRI primer (LICOR)(1.6pMol/µl)	0.15µl	7.5µl
MseI primer (30ng/µl) 0.5µl		25.0µl
10X PCR buffer (Promega)	1.0µl	50.0µl
10mM dNTPs	0.2µl	10.0µl
25mM $MgCl_2$ (Promega)	0.6µl	30.0µl
Taq DNA polymerase (Promega) (5U/µl)	0.1µl	5.0µl
ddH_2O	4.85µl	242.5µl
	7.4µl	370.0µl

Preamplification DNA dilution (2.5µl) was added to labelled tubes. Selective amplification reaction mix (7.4µl) was added to each 2.5µl of diluted preamplification DNA. The selective amplification primer pairs used are listed in Table 2. Primer pairs had previously been screened and those with the clearest banding pattern were selected for the experiments.

Selective amplification PCRs were performed for 36 cycles, with a 30s DNA denaturation step at 94°C, a 1 minute annealing step at 65°C (this was decreased by 0.7°C in each of the first 12 cycles, then continued at 56°C for the remaining 23 cycles), followed by a 1 minute extension step at 72°C. Five µl of loading/stop buffer (95% formamide, 20mM EDTA, bromophenol blue) was added after the selective amplification

PCR and products were frozen at -20°C.

Table 2. Primer pair combinations used in selective amplification reactions.

Primer Pair	Code
E*-AGC X M†-CTG	G7
E-AGG X M-CAG	H3
E-ACG X M-CTT	E8
E-ACG X M-CTG	E7
E-ACA X M-CTT	C8
E-AAC X M-CTA	A5

* E denotes EcoRI restriction site primer + 3 selective nucleotides.

† M denotes MseI restriction site primer + 3 selective nucleotides.

2.3.5 Denaturing polyacrylamide gel electrophoresis

Initially, EcoRI selective primers were radiolabelled (P^{33}) and the selective amplification products were run on a 5% denaturing polyacrylamide gel. The gel was then dried for 2 hours and left to expose to Kodak X-O-Matic AR Imaging Film for 3-5 days. LI-COR® sequencers (model 4200) were then used, removing the hazard of working with radioactivity. As well as running the double haploid samples, the parental generation was redone. Gel plates 25cm in length were used with 0.25mm spacers and a 0.25mm thick sharks tooth 48-well comb. Twenty mL of KB^{PLUS} Gel Matrix (6.5%) from LI-COR® was mixed with 15µl of TEMED (N, N, N^1, N^1-tetramethylethylene-diamine) and 150µl of

10% ammonium persulfate (APS). APS provides a source of free radicals necessary for gel polymerization. TEMED stabilizes free radicals and is used promote polymerization. A 1X TBE (Tris-Boric acid-EDTA) running buffer was used. Before loading samples, they were denatured at 94°C for 3 minutes and then put on ice. Each sample (0.8µl) was loaded with a Hamilton® 8-channel syringe. A 50 base pair to 350 base pair weight marker standard (0.5µl) (Li-Cor® IRD700 #4200-44) was added in the first and last lanes. Run conditions were 1500 volts, 40 amperes, 25 watts at a temperature of 45°C. Gels were run using Li-cor® e-Seq DNA Sequencer and analysed using Gene ImagIR® Analysis Software.

2.4 Inoculation experiments

2.4.1 *F. graminearum* spore suspension application and greenhouse conditions

Plants were inoculated with strain DOAM 178148 of a *Fusarium graminearum* spore suspension. The macroconidia were produced from a frozen *F. graminearum* culture at ECORC. The spore suspension of 50,000 spores/ml was sprayed on plants at anthesis. A second inoculation was done one week later. This was done by Dr. Pandeya and his technician Eric Bevis at ECORC. The plants were misted with water to keep them moist for FHB development. Twelve days after the second inoculation, plants were removed from the misting.

2.4.2 FHB susceptibility scoring

Each of the nine heads in each of the 26 and 36 lines of double haploid plants from the crosses 'Karena'/FHB 148 and 'AC Cartier'/FHB 148 were assessed for resistance by Eric Bevis. The scoring was done five days after the plants were removed from the

misting or about 17 days after the second inoculation with *F. graminearum*. In scoring the lines for susceptibility, the number of spikes in each line that showed no signs of *Fusarium* damage were recorded. Nine spikes per line were inoculated (26 lines for 'Karena'/FHB 148 and 36 lines for 'AC Cartier'/FHB 148).

2.5 Analysis of DNA polymorphisms

2.5.1 Scoring of gels

Gels on which parental selective amplification products were run, were analysed for presence and absence of polymorphic bands using GeneImagIR® software. Only intervarietal polymorphisms were recorded. These same polymorphisms were followed into the double haploid F_2 generation from crosses between the two susceptible varieties, 'Karena' and 'AC Cartier', and the resistant variety, FHB 148. The size of the fragments was also recorded. Initially, gels were repeated to be sure the polymorphisms were reproducible. Once it was determined that polymorphisms were reproducible, each sample was run once per primer pair.

2.5.2 χ^2 test for segregation of polymorphic bands in DH lines

Chi-square tests were done to test the data for segregation ratios of 1:1, 1:2, 2:1, 1:3, and 3:1 of polymorphic bands from parent varieties into the double haploid lines, using Microsoft Excel.

2.5.3 MAXR linear regression

MAXR (maximum R^2 improvement) linear regression was done using SAS (statistical analysis system), to determine the amount of variation explained by the markers. The AFLP markers were used as the independent variables while the dependent

variable was the quantitative FHB resistance phenotype. MAXR linear regression determines the best 1-variable model, then the best 2-variable model and so on.

CHAPTER 3. RESULTS

There have been few *T. aestivum* cultivars developed with resistance to *Fusarium* head blight. Traditional breeding approaches are slow because plants have to be grown to flowering and environmental effects confound the selection of resistant plants. The detection of molecular markers linked to resistance genes would aid in the selection of resistant plants.

3.1 Genotypic observations

Three varieties of *Triticum aestivum* had been subjected to AFLP analysis by Mr. André Gauthier. He had used EcoRI selective amplification primers labelled with ^{33}P. Later, a different approach, the use of the Li-Cor® sequencer was employed in order to avoid using hazardous radioactive materials. Using the Li-Cor sequencer resulted in smaller fragments (50-300bp) being better resolved than larger fragments. Since different areas of the gel were resolved depending on whether ^{33}P or IRD labelled primers were being used, the three varieties of *T. aestivum* were run again using the primer pairs listed in Table 2.

Gels using these primer pairs were analysed for presence and absence of bands using GeneImagIR® Software. AFLPs from 15 individuals of the three varieties of wheat ('Karena', 'AC Cartier' and FHB 148) were analysed. This was enough to determine that the polymorphisms were intervarietal rather than intravarietal and 45 samples were a convenient number to fit on gels using a 48-well comb. Table 3 shows polymorphisms detected among the three varieties using six primer pair combinations. This Table summarizes the raw data given in Appendix 1 for all 15 individuals of each variety. Only

those polymorphisms between either of the susceptible varieties ('Karena', 'AC Cartier') and the resistant variety (FHB 148) were recorded since these are candidate markers for resistance to FHB. The number of polymorphisms detected ranged from one per primer pair to eight per primer pair, with an average of four polymorphisms per primer pair. Of the 23 polymorphisms detected, 18 were between the susceptible 'Karena' and the resistant FHB 148, while 22 were between the susceptible 'AC Cartier' and FHB 148. Seventeen of the polymorphic bands were unique to the resistant variety, FHB 148. A unique polymorphism is either present in the resistant variety (FHB 148) and absent from both susceptible varieties ('Karena' and 'AC Cartier') or is present in both susceptible varieties and absent from the resistant variety. These unique polymorphisms are indicated by * in Table 3. One polymorphism was unique to 'Karena' and five were unique to 'AC Cartier'. Polymorphic bands ranged in size from 65bp to 300bp. These polymorphisms were resolved well on the gels and were within the size range of the size standard used (50 - 350bp). An example of a gel showing polymorphisms among the three parental varieties is shown in Figure 6. Polymorphisms at 117 bp, 94 bp and 65 bp are indicated.

The 23 polymorphisms detected among the three varieties were followed into the double haploid F_2 population from crosses between each of the two susceptible varieties and the resistant variety. These crosses will be referred to as 'Karena'/FHB 148 and 'AC Cartier'/ FHB 148. For the 'Karena'/FHB 148 cross, 26 lines with nine heads per line were analysed (234 plants in total). Each of the nine heads per line were products of the same cross. For example for the 'Karena'/FHB 148 double haploid plants, nine seeds from each of 26 crosses were planted. The scoring of these gels is shown in Appendix 2A.

For the second cross, 'AC Cartier'/FHB 148, 36 lines with nine heads (324 plants in total). The scoring of these gels is shown in Appendix 2B. Since the nine plants in each line gave identical results, these results from Appendix 2A and 2B could be summarized as seen in Table 4 and Table 5 respectively. Examples of a gels from the double haploid lines 'Karena'/FHB 148 and 'AC Cartier'/FHB 148 are shown in Figures 7 and 8. Polymorphisms at 117 bp, 94 bp and 65 bp are indicated.

The data were tested for significance against different segregation models (1:1, 1:2, 2:1, 3:1 and 1:3) using SAS (statistical analysis system) to conduct χ^2 tests. The data were tested for these four segregation ratios. For example, in testing the first ratio (1:1), the null hypothesis was that the segregation was 1:1 (i.e. ½ the lines for a cross had a specific band present, ½ did not have the band). Of the subset of data tested for the 1:1 ratio, none of the χ^2 tests were significant, therefore the null hypothesis was not rejected and the null hypothesis that segregation was 1:1 was accepted. Other subsets of the data exhibited segregation ratios of 1:2, 2:1, 1:3 and 3:1. Segregation ratios, χ^2 values and the probability that the deviation from the expected segregation ratios of 1:1, 1:2, 2:1, 1:3 or 3:1 is due to chance alone are summarized in Table 6 for the 'Karena' X FHB 148 cross, and in Table 7 for the 'AC Cartier' X FHB 148 cross. In the 'Karena'/FHB 148 cross, 12 of the 18 polymorphisms appear to segregate in a 1:1 ratio (present:absent), which is expected according to Mendelian genetics. One polymorphism segregates in a 3:1 ratio, 2 segregate in a 1:3 ratio and 3 segregate in a 1:2 ratio (Table 6). In the 'AC Cartier'/FHB 148 cross, eleven of the 22 polymorphisms (50%) appear to segregate in a 1:1 ratio, three segregate in a 3:1 ratio, six segregate in a 1:2 ratio and two displayed 2:1 segregation

ratios (Table 7).

Table 3. AFLPs observed among the three varieties of *T. aestivum*. Primer pairs are written as their codes (see Table 2). The 1's and 0's represent the presence and absence of a particular band, respectively. This Table is a summary of the data presented in Appendix 1 which includes all 15 individuals of each variety. The * indicates polymorphisms unique to FHB 148.

Primer Pair	Band Size (bp)	'Karena'	FHB 148	'AC Cartier'
G7	249*	0	1	0
G7	247*	1	0	1
G7	245*	0	1	0
G7	154	0	0	1
H3	187*	0	1	0
H3	178*	1	0	1
H3	138	1	1	0
E8	225*	1	0	1
E8	216*	1	0	1
E8	187*	1	0	1
E8	120*	0	1	0
E8	068*	1	0	1
E7	300*	1	0	1
E7	208	1	0	0
E7	183	0	0	1
E7	166*	0	1	0
E7	147	0	0	1
E7	117*	1	0	1
E7	094*	0	1	0
E7	065*	1	0	1
C8	271*	1	0	1
C8	145*	1	0	1
A5	091	1	1	0

Figure 6. GeneImagIR image of a gel showing polymorphisms among the three varieties of *T. aestivum* obtained using the primer pair E7. The sizes of the DNA fragments in the Li-Cor IRD700 marker (outer lane on each side) are shown in red and are in base pairs. There are 45 lanes shown, with the first 15 being 'Karena', the next 15 FHB 148 and the last 15 'AC Cartier'. Intervarietal polymorphisms are at 117 bp, 94bp and 65bp. The polymorphism at 117 bp and is present in 'Karena' and 'AC Cartier', but absent from FHB 148. The second, at 94 bp is absent from 'Karena' and 'AC Cartier, but present in FHB 148. The third, at 65 bp is present in 'Karena' and 'AC Cartier', but absent from FHB 148.

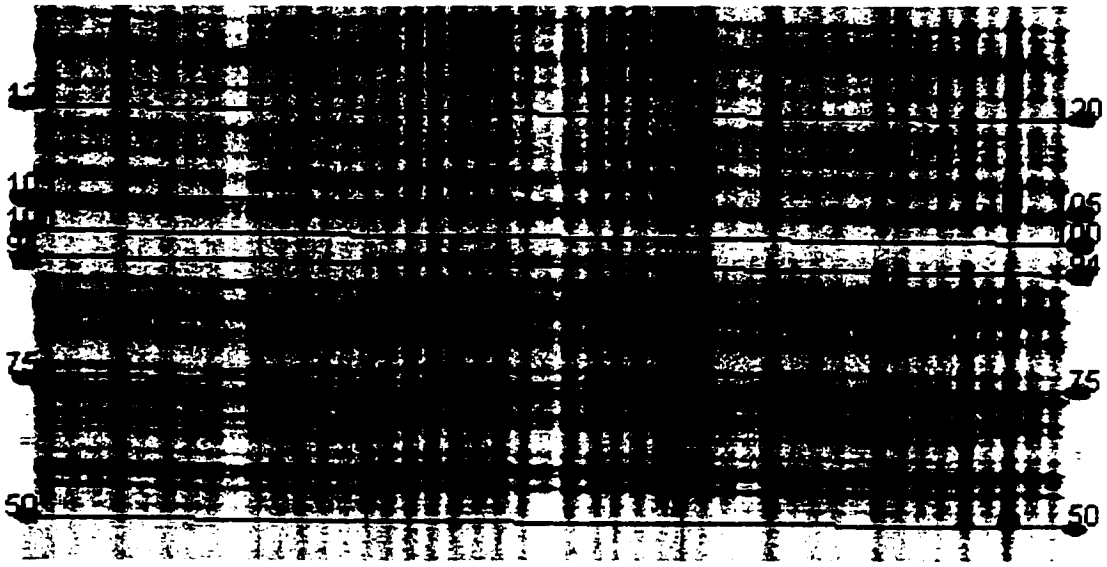

20
75
50

Table 4. Summary of the data presented in Appendix 2A showing the polymorphisms in segregants arising from the double haploid F_2 'Karena'/FHB 148 lines. The 1's and 0's indicate the presence and absence of a band in the replicates of the line, respectively. The primer pair combinations are listed as their codes (ex. G7), and the size of the fragments are in base pairs (bp).

	Primer pair, fragment length (bp)																	
	G7			H3		E8					E7						C8	
LINE	249	247	245	187	178	225	216	187	120	68	300	208	166	117	94	65	271	145
'Karena'	0	1	0	0	1	1	1	1	0	1	1	1	0	1	0	1	1	1
FHB 148	1	0	1	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0
N16-1	1	1	0	0	1	1	1	1	1	0	0	1	1	0	0	1	1	0
N16-2	0	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1
N16-3	0	1	1	0	0	0	0	1	1	1	1	0	0	1	1	0	0	1
N16-4	0	1	1	1	0	0	0	1	0	1	1	0	0	0	0	0	0	0
N16-5	1	0	1	0	0	0	0	1	0	0	1	1	0	0	0	1	0	0
N16-6	1	0	1	1	0	0	0	0	1	0	1	1	1	1	1	1	1	1
N16-7	1	0	1	1	0	1	0	0	1	0	1	0	0	1	1	0	0	1
N16-8	1	0	1	1	0	0	0	0	1	0	1	0	0	1	1	0	0	1
N16-9	1	1	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	1
N16-10	1	1	0	0	0	1	0	1	0	1	0	1	1	0	0	1	1	0
N16-11	0	1	1	1	0	1	0	0	1	0	0	0	1	1	1	0	0	1
N16-12	1	0	1	1	0	0	1	1	0	1	0	1	1	0	1	1	0	0
N16-13	0	1	1	1	0	1	0	1	1	0	1	0	0	0	1	0	1	0
N16-14	1	0	1	0	0	1	1	1	1	1	0	0	1	0	1	0	1	0
N16-15	0	1	1	0	0	0	0	0	0	1	1	0	0	1	1	0	0	1
N16-16	1	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	1
N16-17	1	0	1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	1
N16-18	0	1	1	0	0	1	1	1	1	0	0	0	1	1	1	0	0	1
N16-19	0	1	1	1	0	0	1	0	1	1	1	0	1	0	1	0	0	0
N16-20	1	0	1	0	1	0	0	1	0	1	0	0	1	0	0	0	1	1
N16-21	0	1	1	0	0	1	0	0	1	0	1	0	1	0	0	0	0	1
N16-22	1	0	1	1	0	0	0	0	0	1	0	1	1	0	0	1	0	0
N16-23	1	0	1	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0
N16-24	0	1	1	1	0	0	0	0	1	1	0	0	1	0	0	0	1	0
N16-25	0	1	1	0	0	0	0	0	0	1	1	0	0	1	1	0	0	1
N16-26	0	1	1	1	0	0	0	1	0	0	1	1	0	1	1	1	0	1

Figure 7. GeneImagIR image of a gel showing double haploid 'Karena'/FHB 148 selective amplification products from the primer pair E7. The sizes of the fragments in the IRD700 Li-Cor marker are shown in red and are in base pairs. Polymorphisms at 117 bp, 94 bp and 65 bp are indicated.

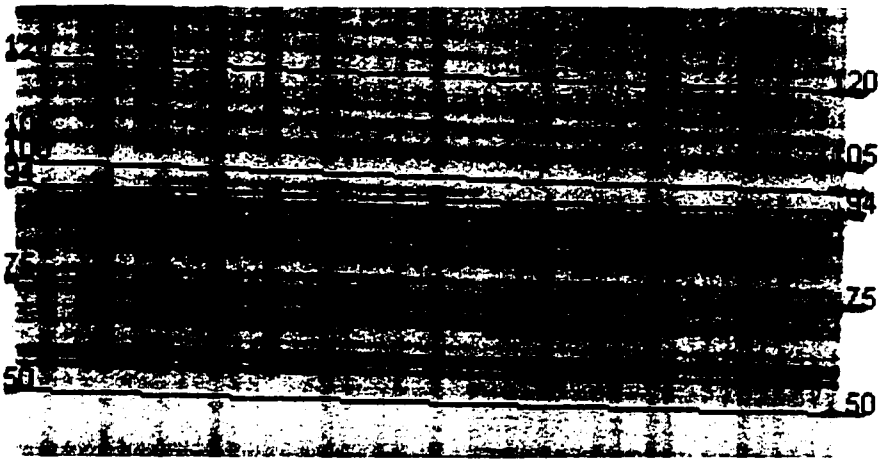

20
05
94
75
50

Table 5. Summary of the data presented in Appendix 2B showing the polymorphisms in segregants arising from the double haploid F_2 'AC Cartier'/FHB 148 lines. The 1's and 0's indicate the presence and absence of a band in the replicates of the line, respectively. The primer pairs are listed as their codes, and the size of the fragments are in base pairs (bp).

	Primer pair, fragment length (bp)																				
	G7				H3			E8					E7							C8		A5
LINE	249	247	245	154	187	178	138	225	216	187	120	068	300	183	166	147	117	094	065	271	145	091
'Cartier'	0	1	0	1	0	1	1	1	1	1	0	1	1	1	0	1	1	0	1	1	1	1
'FHB148'	1	0	1	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0
N15-1	0	1	0	1	1	0	0	1	1	1	0	1	0	1	1	1	1	1	1	0	1	1
N15-2	0	1	1	1	1	0	1	0	1	1	0	0	0	1	1	0	1	1	0	0	1	1
N15-3	0	1	1	1	1	0	1	1	0	0	0	1	1	0	0	1	0	0	1	0	1	1
N15-4	0	1	1	0	0	0	1	0	0	0	0	1	0	1	0	0	0	1	1	0	1	1
N15-6	0	1	1	0	1	0	1	0	1	0	1	0	0	0	0	1	1	1	1	0	0	1
N15-7	0	1	1	1	0	0	1	0	0	1	1	1	1	0	0	0	0	1	1	0	0	1
N15-8	0	1	1	0	0	0	1	0	0	1	0	1	1	1	1	0	0	0	1	0	0	1
N15-9	0	1	1	0	1	1	1	1	1	1	0	1	0	1	1	0	0	0	0	1	0	0
N15-10	0	1	0	0	0	1	0	1	0	0	1	1	0	1	1	1	1	1	1	1	0	1
N15-11	1	0	1	1	0	0	1	1	1	1	0	1	1	0	1	0	0	1	1	1	0	0
N15-12	0	1	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	0	1	0	1	0
N15-13	1	0	1	1	1	1	1	1	1	1	0	1	1	0	1	0	0	1	1	1	0	0
N15-14	1	0	1	0	1	1	1	1	1	0	0	1	0	0	0	1	0	1	0	1	0	1
N15-15	1	0	0	1	1	1	0	1	1	0	1	1	0	1	1	1	0	0	1	1	1	0
N15-16	0	1	1	1	1	0	1	0	1	0	0	0	0	1	1	0	1	0	0	0	1	1
N15-17	1	0	1	1	0	1	1	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0
N15-18	0	1	1	1	1	0	1	0	0	1	0	1	0	0	0	0	1	0	1	0	1	1
N15-19	0	1	0	0	0	1	0	0	1	1	0	1	1	1	1	0	1	0	1	1	1	0
N15-20	1	0	1	1	0	0	1	0	1	0	1	1	0	0	0	0	0	1	1	0	0	0
N15-21	1	0	1	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0
N15-22	0	1	1	1	0	1	0	1	0	0	1	0	0	0	0	1	0	0	1	1	0	1
N15-25	1	0	1	1	1	0	1	0	1	0	0	1	0	0	1	0	0	0	1	0	0	0
N15-26	0	1	0	0	0	1	0	0	1	1	0	1	1	1	1	0	1	0	1	1	1	0
N15-27	0	1	1	1	1	1	0	0	0	0	0	1	0	1	1	1	0	1	1	1	0	1
N15-29	0	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	1	1	1	0	0
N15-30	0	1	1	0	0	0	1	1	1	0	0	0	1	1	1	1	1	0	1	0	1	1
N15-31A-	1	0	0	0	1	0	0	0	1	1	1	1	1	0	1	1	1	0	1	0	0	0
N15-31A+	1	0	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0	1	1	1	1	0
N15-32	1	0	0	1	1	1	0	1	0	0	0	1	0	1	1	0	0	1	1	0	1	0
N15-33	0	1	1	0	0	1	1	0	0	0	0	1	0	1	1	1	0	1	1	0	1	0
N15-34	1	0	1	1	0	0	1	0	1	1	1	0	0	0	0	0	0	1	1	1	0	0
N15-35	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	1
N15-36	0	1	0	1	1	0	0	0	1	1	0	1	0	0	0	0	1	0	1	0	1	1
N15-37	0	1	1	0	1	0	1	0	0	0	0	0	0	0	1	0	0	1	1	1	0	1
N15-38	1	0	1	0	0	0	1	0	1	1	0	1	1	1	1	1	0	1	1	1	0	1
N15-39	0	1	1	0	0	0	0	1	0	1	1	0	0	0	0	1	1	0	0	1	1	1

Figure 8. GeneImagIR image of a gel showing double haploid 'AC Cartier'/FHB 148 selective amplification products from the primer pair E7. The sizes of the fragments in the IRD700 Li-Cor marker are shown in red and are in base pairs. Polymorphisms at 117 bp, 94 bp and 65 bp are indicated.

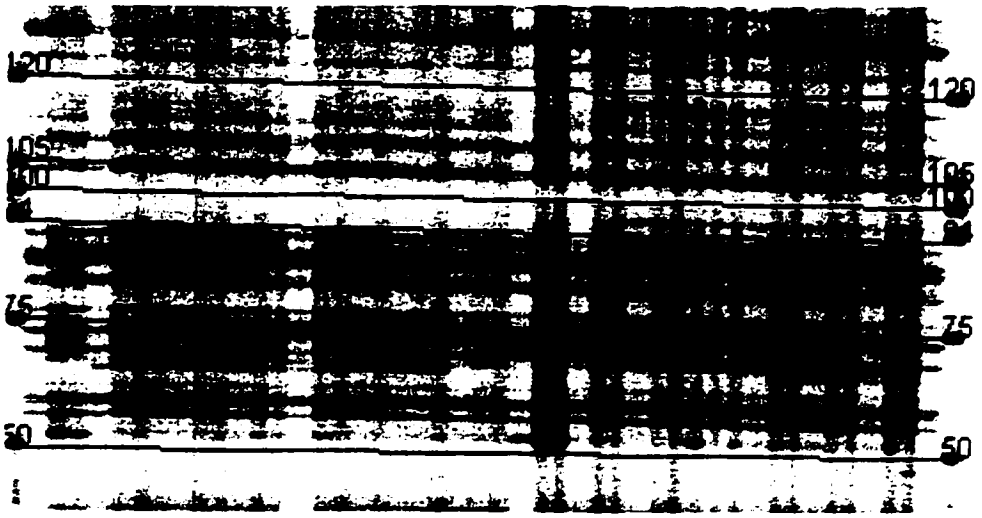

120
105
75
50

Table 6. Total presence and absence of polymorphisms in 26 lines of DH 'Karena'/FHB 148. The segregation ratios tested using the χ^2-test are shown along with the χ^2 and p values. No p-values were significant, indicating that the segregation of the polymorphisms does not deviate from the tested ratios. The size of the fragments are in base pairs (bp).

	Primer pair, fragment length (bp)																
	G7			H3		E8					E7						C8	
	249	247	245	187	178	225	216	187	120	68	300	208	166	117	94	65	271	145
Present	14	15	23	14	3	12	7	13	14	12	14	9	15	12	13	9	8	15
Absent	12	11	3	12	23	14	19	13	12	14	12	17	11	14	13	17	18	11
ratio	1:1	1:1	3:1	1:1	1:3	1:1	1:3	1:1	1:1	1:1	1:1	1:2	1:1	1:1	1:1	1:2	1:2	1:1
χ^2	0.154	0.615	2.513	0.154	2.513	0.154	0.051	0.000	0.154	0.154	0.154	0.019	0.615	0.154	0.000	0.019	0.462	0.615
p*	0.695	0.433	0.113	0.695	0.113	0.695	0.821	1.000	0.695	0.695	0.695	0.891	0.433	0.695	1.000	0.891	0.497	0.433

*p=the probability that the deviation of the observed from that of the expected is due to chance alone.

Table 7. Total presence and absence of polymorphisms in 36 lines of DH 'AC Cartier'/FHB 148. The segregation ratios tested using the χ^2-test are shown along with the χ^2 and p values. No p values were significant, indicating that the segregation of the polymorphisms do not deviate from the tested ratios. The size of the fragments are in base pairs (bp).

	Primer pair, fragment length (bp)																				
	G7				H3			E8					E7							C8		A5
	249	247	245	154	187	178	138	225	216	187	120	68	300	183	166	147	117	94	65	271	145	91
Present	14	22	27	21	18	12	21	16	19	15	11	26	13	18	22	14	13	18	28	17	18	19
Absent	22	14	9	15	18	24	15	20	17	21	25	10	23	18	14	22	23	18	8	19	18	17
ratio	1:2	2:1	3:1	1:1	1:1	1:2	1:1	1:1	1:1	1:1	1:2	3:1	1:2	1:1	2:1	1:2	1:2	1:1	3:1	1:1	1:1	1:1
χ^2	0.500	0.500	0.000	1.000	0.000	0.000	1.000	0.444	0.111	1.000	0.125	0.148	0.125	0.000	0.500	0.500	0.125	0.000	0.148	0.111	0.000	0.111
p*	0.480	0.480	1.000	0.317	1.000	1.000	0.317	0.505	0.739	0.317	0.724	0.700	0.724	1.000	0.480	0.480	0.724	1.000	0.700	0.739	1.000	0.739

*p=the probability that the deviation of the observed from that of the expected is due to chance alone.

3.2 Phenotypic observations

The phenotype of the plants in terms of their susceptibility and resistance to FHB were determined from inoculation experiments done by Dr. R. Pandeya and Eric Bevis. Each of the nine heads per line was classified as resistant or susceptible to FHB and each line was given one of four resistance ratings (S, MS, MR, R), depending on the number of heads per line that were resistant (Table 8). The number of resistant heads per line in each of the 26 and 36 lines of 'Karena'/FHB 148 and 'AC Cartier'/FHB 148 respectively were used as a quantitative description of resistance, where 0 indicated that there were no resistant heads (all susceptible) and increased to 9, where there were 9 resistant heads per line (none susceptible). Error in the phenotypic analysis could arise due to confounding environmental effects in the greenhouse during fungal development. For example, tall plants or plants closer to the mist than other plants would receive more moisture, which is necessary for development of FHB.

Table 8. FHB resistance classification based on the number of heads per line that were resistant to FHB. There is a total of nine plants per line.

No. of FHB Resistant Heads per line	FHB Resistance Classification
< 3	Susceptible
4-5	Moderately Susceptible
6-7	Moderately Resistant
≥ 8	Resistant

3.3 Genotype-phenotype comparisons

SAS was used to run a maximum R^2 improvement (MAXR) regression, using the quantitative phenotype as the dependent variable and the presence and absence of each marker as independent variables. MAXR determines the best 1-variable model, best 2-variable model and so on. It differs from stepwise regression in that many more variables are evaluated with MAXR, which considers all switches before making any switch. The stepwise method may remove the worst variable without considering what the best remaining variable may accomplish, whereas MAXR would consider what the best remaining variable might accomplish.

As variables are added to the model, the R^2, or amount of variation explained by the model increases initially and then levels off. This is shown graphically in Figures 9 and 10. In order to find the model with the most variables in which all parameters are significant at the selected α level of 0.05, it is necessary to start with the combination of variables giving the lowest MSE (mean square error) value or unexplained error. The model with the smallest MSE for 'Karena'/FHB 148 includes seven marker variables, but not all are significant at 0.05. The R^2, p values and variables included in each step of the model are shown in Table 11. The model with all variables significant at the 0.05 level includes two marker variables for 'Karena'/FHB 148. These variables, G7 245 and E7 094 are significant ($p<0.05$) (Table 9). These two variables explain 35.26% of the variation in resistance phenotype. The model is significant ($p<0.05$). The regression equation for this relationship is $FHB_{phenotype} = x(G7245a + E7094b) + intercept$.

The model with the lowest MSE for 'AC Cartier'/FHB 148 includes 12 variables,

but not all are significant at the 0.05 level. The R^2, p values and variables included in each step of the model are shown in Table 12. The model with all variables significant at the 0.05 level includes two marker variables, H3 138 and E7 065 ($p<0.05$) (Table 10). These two variables explain 28.59% of the variation in resistance phenotype. The model is significant ($p<0.05$). The regression equation for this relationship is $FHB_{phenotype}$ = x(H3138a + E7065b) + intercept.

The two bands in each cross that explain a significant amount of variation in FHB resistance phenotype are good candidates for isolation from the gel, cloning and sequencing. For the 'Karena'/FHB 148 DH, the bands are G7 245 and E7 094, while for the 'AC Cartier'/FHB 148 DH, the bands are H3 138 and E7 065.

Figure 9. Effect of increasing the number of polymorphisms included on R^2 in the MAXR linear regression model for 'Karena'/FHB 148 DH. The variables included in each step are shown in Table 11.

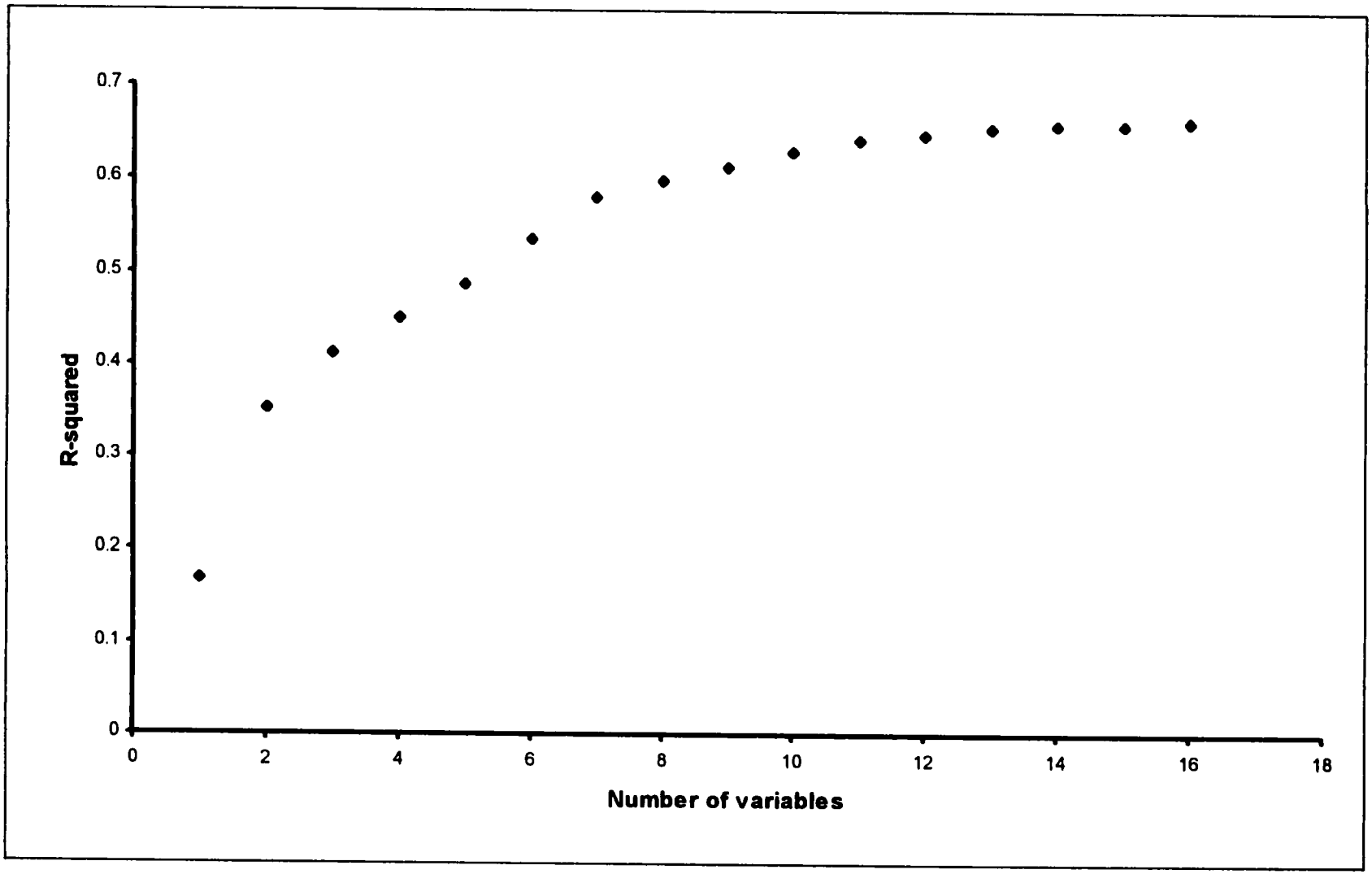

R-squared
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
14
16
18
Number of variables

Figure 10. Effect of increasing the number of polymorphisms included on R^2 in the MAXR linear regression model for 'AC Cartier'/FHB 148 DH. The variables included in each step are shown in Table 12.

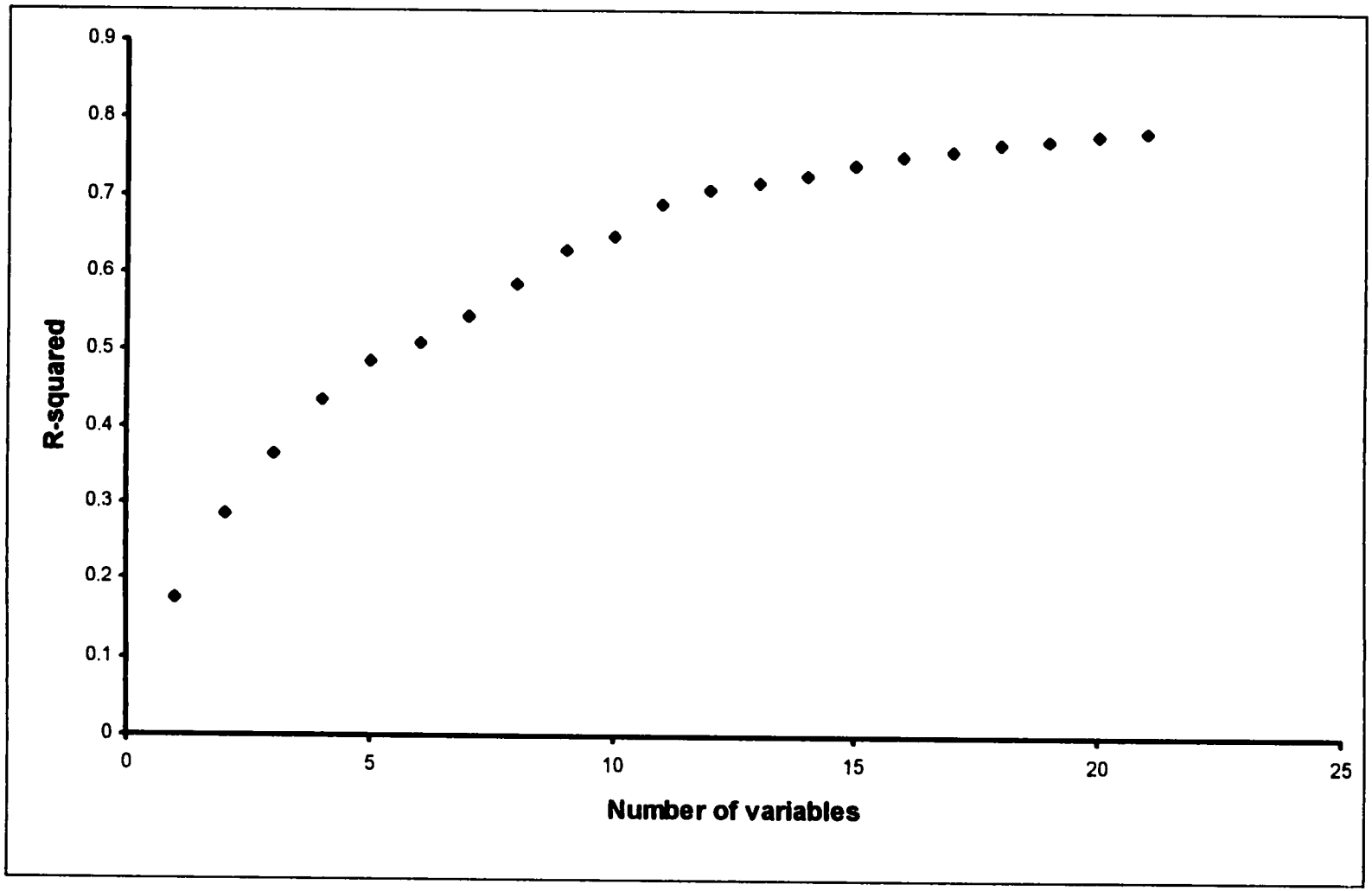

0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
R-squared
0
5
10
15
20
25
Number of variables

Table 9. R^2 and P values for DNA markers significantly associated with FHB resistance ($P \leq 0.05$) in the 'Karena'/FHB 148 DH population as determined by the MAXR linear regression model ($R^2 = 0.3526$, $P = 0.0067$, d.f. = 2).

Marker	Source of marker	R^2 (X100)	*P*
G7 245	FHB 148	17.02	0.0039
E7 094	FHB 148	18.24	0.0181

Table 10. R^2 and P values for DNA markers significantly associated with FHB resistance ($P \leq 0.05$) in the 'AC Cartier'/FHB 148 DH population as determined by the MAXR linear regression model ($R^2 = 0.2859$, $P = 0.0039$, d.f. = 2).

Marker	Source of marker	R^2 (X100)	*P*
H3 138	'AC Cartier'	17.44	0.0100
E7 065	'AC Cartier'	11.15	0.0298

Table 11. Variables included in each step of the MAXR linear regression model for the effect of the candidate markers on the FHB resistance phenotype in the 'Karena'/FHB 148 DH. R^2 and the p-value for each model are also shown. Variables are listed with their primer pair abbreviation (Table 2), followed by the size of the fragment in base pairs.

No. of variables	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
VARIABLES	G7 245	G7 245	G7 245	G7 249	G7 249	G7 249	G7 249	G7 249	G7 249	G7 249	G7 249	G7 249	G7 249	G7 249	G7 249	G7 249
		E7 094	E8 216	G7 245	G7 245	G7 245	G7 245	G7 245	G7 245	G7 245	G7 245	G7 245	G7 245	G7 245	G7 245	G7 245
			E7 094	E8 216	E8 225	E8 216	E8 216	E8 216	E8 216	E8 225	E8 225	E8 225	E8 225	E8 225	H3 178	H3 187
				E7 094	E8 216	E8 187	E8 187	E8 187	E8 187	E8 216	E8 216	E8 216	E8 216	E8 216	E8 225	H3 178
					E7 094	E7 117	E8 120	E8 120	E8 120	E8 120	E8 120	E8 120	E8 187	E8 187	E8 216	E8 225
						E7 094	E7 117	E7166	E8 068	E8 068	E8 068	E8 068	E8 120	E8 120	E8 187	E8 216
							E7 094	E7117	E7 166	E7 208	E7 300	E7 300	E8 068	E8 068	E8 120	E8 187
								E7094	E7 117	E7 166	E7 208	E7 208	E7 300	E7 300	E8 068	E8 120
									E7 094	E7 117	E7 166	E7 166	E7 208	E7 208	E7 300	E8 068
										E7 094	E7 117	E7 117	E7 166	E7 166	E7 208	E7 300
											E7 094	E7 094	E7 117	E7 117	E7 166	E7 208
												C8 145	E7 094	E7 094	E7 117	E7 166
													C8 271	C8 271	E7 094	E7 117
														C8 145	C8 271	E7 094
															C8 145	C8 271
																C8 145
R^2	0.1702	0.3526	0.4125	0.4502	0.4870	0.5350	0.5799	0.5990	0.6125	0.6291	0.6423	0.6477	0.6545	0.6577	0.6593	0.6612
p	0.0362	0.0067	0.0076	0.0107	0.0140	0.0141	0.0142	0.0216	0.0344	0.0500	0.0738	0.1161	0.1709	0.2492	0.3485	0.4608

Table 12. Variables included in the first 12 steps of the MAXR linear regression model for the effect of the candidate markers on the FHB resistance phenotype in the 'AC Cartier'/FHB 148 DH. R^2 and the p-value for each model are also shown. Variables are listed with their primer pair abbreviation (Table 2), followed by the size of the fragment in base pairs. (Continued on following page).

No. of variables	1	2	3	4	5	6	7	8	9	10	11	12
	H3 138	H3 138	H3 187	H3 187	H3 187	H3 187	H3 187	H3 187	H3 187	H3 187	G7 247	G7 247
V		E7 065	H3 138	H3 138	H3 138	H3 138	H3 138	H3 138	H3 138	H3 138	G7 245	G7 245
A			E7 065	E7 065	E7 065	E8 225	E8 225	E7 300	E8 225	E8 225	G7 154	G7 154
R				C8 271	C8 271	E7 065	E7 065	E7 094	E7 300	E8 187	H3 187	H3 187
I					A5 091	C8 271	C8 271	E7 065	E7 094	E7 300	E8 225	E8 225
A						A5 091	C8 145	C8 271	E7 065	E7 094	E7 300	E8 216
B							A5 091	C8 145	C8 271	E7 065	E7 094	E7 300
L								A5 091	C8 145	C8 271	E7 065	E7 094
E									A5 091	C8 145	C8 271	E7 065
S										A5 091	C8 145	C8 271
											A5 091	C8 145
												A5 091
R^2	0.1744	0.2859	0.3641	0.4361	0.4860	0.5100	0.5447	0.5856	0.6303	0.6483	0.6899	0.7092
p	0.0113	0.0039	0.0021	0.0011	0.0008	0.0012	0.0012	0.0010	0.0007	0.0009	0.0006	0.0008

Table 12 (cont). Variables included in the last 9 steps of the MAXR linear regression model for the effect of the candidate markers on the FHB resistance phenotype in the 'AC Cartier'/FHB 148 DH. (Continued from the previous page)

No. of variables	13	14	15	16	17	18	19	20	21
	G7 247	G7 247	G7 247	G7 247	G7 247	G7 247	G7 247	G7 247	G7 247
	G7 245	G7 245	G7 245	G7 245	G7 245	G7 245	G7 245	G7 245	G7 245
	G7 154	G7 154	G7 154	G7 154	G7 154	G7 154	G7 154	G7 154	G7 154
V	H3 187	H3 187	H3 187	H3 187	H3 187	H3 187	H3 187	H3 187	H3 187
A	E8 225	E8 225	H3 178	H3 178	H3 178	H3 178	H3 178	H3 178	H3 178
R	E8 216	E8 216	E8 225	E8 225	E8 225	E8 225	H3 138	H3 138	H3 138
I	E8 120	E8 120	E8 216	E8 216	E8 216	E8 216	E8 225	E8 225	E8 225
A	E7 300	E7 300	E8 120	E8 120	E8 120	E8 187	E8 216	E8 216	E8 216
B	E7 094	E7 117	E7 300	E7 300	E7 300	E8 120	E8 187	E8 187	E8 187
L	E7 065	E7 094	E7 147	E7 166	E7 166	E8 068	E8 120	E8 120	E8 120
E	C8 271	E7 065	E7 094	E7 147	E7 147	E7 300	E8 068	E8 068	E8 068
S	C8 145	C8 271	E7 065	E7 094	E7 117	E7 166	E7 300	E7 300	E7 300
	A5 091	C8 145	C8 271	E7 065	E7 094	E7 147	E7 166	E7 183	E7 183
		A5 091	C8 145	C8 271	E7 065	E7 094	E7 147	E7 166	E7 166
			A5 091	C8 145	C8 271	E7 065	E7 094	E7 147	E7 147
				A5 091	C8 145	C8 271	E7 065	E7 094	E7 117
					A5 091	C8 145	C8 271	E7 065	E7 094
						A5 091	C8 145	C8 271	E7 065
							A5 091	C8 145	C8 271
								A5 091	C8 145
									A5 091
R^2	0.7178	0.7267	0.7408	0.7513	0.7600	0.7689	0.7734	0.7789	0.7854
p	0.0013	0.0022	0.0031	0.0046	0.0073	0.0112	0.0188	0.0299	0.0452

CHAPTER 4. DISCUSSION AND CONCLUSIONS

This study was designed to find AFLPs between two susceptible varieties ('Karena' and 'AC Cartier') and one resistant variety (FHB 148) of *T. aestivum* and to follow these candidate markers for FHB resistance into the double haploid F_2 generation. By comparing the resistance phenotype from inoculation experiments to the genotype information from AFLP analysis, it can be determined by regression analysis whether any of the AFLP markers may be linked to FHB resistance.

Candidate markers for FHB resistance were identified by searching for intervarietal polymorphisms between each of two susceptible *T. aestivum* varieties ('Karena' and 'AC Cartier') and a resistant variety (FHB 148). Using six primer pairs (Table 2), 23 intervarietal polymorphisms were detected, of which 17 were unique to FHB 148. Since these polymorphisms are either present in both susceptible varieties and absent in the resistant one, or they are absent in both the susceptible varieties and present in the resistant variety, they are considered candidate markers for FHB resistance. These polymorphisms that are candidate markers for FHB resistance are identified by asterisks in Table 3. The intervarietal polymorphisms were followed into the double haploid F_2 from crosses between the two susceptible varieties and resistant variety. In these double haploid plants, the AFLPs should segregate in a Mendelian fashion assuming equal survival of F_1 gametes before doubling occurs.

The AFLP system does not detect codominance, but in double haploids there is no heterozygosity. Since double haploids are homozygous at all loci, the double haploid system is ideal for AFLP analysis. Double haploid lines are ideal for breeding in that they

provide immediate homozygosity thereby reducing the number of generations of inbreeding needed in self pollinating crops (De Buyser *et al.*, 1987). Since double haploid lines are completely homozygous, they are said to be easier to assess and are more precise than segregating material (Wenzeler *et al.*, 1987).

More polymorphisms segregated in a 1:1 (present:absent) ratio in the 'Karena'/FHB 148 lines, than segregated in this Mendelian fashion for the 'AC Cartier'/FHB 148 lines. A number of the markers showed segregation distortion, where observed segregation differed from the expected Mendelian segregation of 1:1 (present:absent) ratio. In 'Karena'/FHB 148, six of the polymorphisms (33%) deviated from the expected 1:1 ratio ($p \leq 0.05$). Two of the polymorphisms showed 1:3 segregation ratios, one showed a 3:1 segregation ratio and three displayed a 1:2 segregation ratio (Table 6). In 'AC Cartier'/FHB 148, eleven of the polymorphisms (50%) deviated from the expected 1:1 ratio ($p \leq 0.05$). Six of the polymorphisms showed 1:2 segregation ratios, two showed 2:1 segregation ratios and three displayed 3:1 segregation ratios (Table 7). This segregation distortion has been seen in *Brassica oleracea* double haploid lines, where 65% of the loci were affected (Voorrips *et al.*, 1997), compared to 5%-12% in F_2 lines (Kianian and Quiros, 1992). The increased segregation distortion in double haploid lines compared to F_2 lines may be caused by an increased selection pressure on deleterious recessive alleles in a DH population (Voorrips *et al.*, 1997). In wheat, double haploids have been produced by the anther culture technique and also maize in wheat (Shugar, 1998). The segregation ratios that deviate from the Mendelian 1:1, could be a result of gamete mortality or selection against deleterious recessive alleles.

AFLP technology has been used successfully to find markers linked to pathogen resistance in a number of plants. For example, an AFLP marker in peach has been correlated with the primary source of root-knot nematode resistance (Lu *et al.*, 1998). This marker was then converted into an STS (sequence tagged site) for use in marker assisted selection (Lu *et al.*, 1998b). STSs are codominant markers that can easily be detected on agarose gels. AFLPs have also been used in tomato to genetically localize the root-knot nematode resistance locus *Mi* (Kaloshian *et al.*, 1998).

As far as genetic markers in *T. aestivum* are concerned, AFLPs, RFLPs, RAPDs and SSRs have been used. RAPDs are not likely to be transferable between laboratories since different patterns result depending on the DNA thermocycler being used (Devos and Gale, 1992). A primer can amplify non-homologous sequences since RAPD products are a result of arbitrary priming. These non-homologous sequences may vary between varieties and it may not be known whether RAPD bands of similar molecular weights represent the same locus in different varieties (Devos and Gale, 1992). A microsatellite (SSR) map of wheat has been done, but the process is tedious and expensive (Röder *et al.*, 1998). Microsatellite primers usually only amplify a single locus from one of the three genomes in wheat, shown by the amplification of 279 microsatellites with 230 primer sets (Röder *et al.*, 1998). Although AFLP technology reveals a lower level of polymorphism than RFLP, SSR, and RAPD, it is the more efficient marker technology because it has the capacity to reveal more than one polymorphic band in a lane (Russell *et al.*, 1997). In scoring AFLP gels, the frequency of erroneous scoring of non-homologous bands is less likely to occur than in RAPDs because of the higher AFLP gel resolution (Powell *et al.*,

1996).

Previous studies have found DNA markers linked to genes controlling FHB resistance. Waldron *et al* (1999) found three RFLP markers derived from the resistant 'Sumai 3' and two from the moderately susceptible 'Stoa' cultivar in a population of F_5-derived recombinant inbred lines. The best single RFLP marker explained 15.4% of the phenotypic variation (Waldron *et al.*, 1999). Waldron *et al* (1999) found two QTL (quantitative trait loci) associated with resistance and RILs with these two QTL had a lower FHB severity. A multiple regression model with three QTL explained 29.5% of the variation in FHB resistance phenotype (Waldron *et al.*, 1999). Anderson *et al* (2001), have found markers linked to genes controlling FHB resistance in two spring wheat recombinant inbred populations which segregate for genes from the resistant 'Sumai 3'. Since the source of the resistance found in most resistant lines is derived from 'Sumai 3' (Bai and Shaner, 1994), there is little allelic variation in resistance. In this study, the resistance source in the resistant line FHB 148 is derived from the spring wheat cultivar 'Frontana'. 'Frontana', a cultivar that has both resistance to initial infection and resistance to hyphal spread, was released in 1943 in Brazil (Singh *et al.*, 1995). It is thought that at least three additive genes and at least one or two minor genes are involved in the scab resistance seen in 'Frontana' (Singh *et al.*, 1995). In a study using a recombinant inbred population with 'Sumai 3' as the resistant source, a multiple regression model developed from three markers significantly associated with resistance explained 54% of the variation for FHB resistance (Anderson *et al.*, 2001). A multiple regression model with three significant marker variables explained 34% of the variation for FHB resistance in a

population with resistance derived from both 'Sumai 3' and 'Wheaton' (Anderson *et al.*, 2001). In a study examining the heritability of resistance, transgressive segregates were observed when a moderately susceptible wheat cultivar 'Capo' was crossed with two resistant breeding lines (Buerstmayr *et al.*, 2000). Transgressive segregates were found not only in the resistant direction, but also in the susceptible direction (Buerstmayr *et al.*, 1998). If two moderately resistant cultivars having different resistant sources were crossed, perhaps the progeny would carry more positive alleles for FHB resistance and would show transgressive segregation towards resistance.

In this study two markers were found to be significantly associated with FHB resistance. In the 'Karena'/FHB 148 population, two markers explained 35% of the variation in FHB resistance phenotype (Table 9), while in the 'AC Cartier'/FHB 148 population, two different markers explained 29% of the variation in FHB resistance phenotype (Table 10). Variation that was not explained by the regression model could result from undetected markers, epistasis or experimental error (Waldron *et al.*, 1999). Only six selective amplification primer combinations were used. If a larger number of primer combinations had been used, more polymorphisms would have been detected and some of these may have explained more of the variation in FHB resistance. Due to time constraints, it was not possible to use all primer combinations. It is possible that these markers would be present in other types of wheat such as spring, red or durham wheats. To determine whether these markers would be useful in these other types of wheat, AFLP analyses employing the same primer pairs would need to be done. If these markers are found, inoculation experiments would need to be done to determine the relationship

between the marker and FHB phenotype. Since grass genomes are highly conserved, it is not unlikely that these markers would be found in other varieties as well.

Natural epidemics are only effective for testing *Fusarium* head blight in environments where the disease occurs regularly, as the environment has a large effect on the severity of the outbreak (Miedaner, 1997). Error in the phenotypic analysis could occur due to differing environmental conditions in the greenhouse. Plants closer to the mist in the greenhouse may have been infected more that plants at a greater distance from the mist. Taller plants may develop more infection than shorter plants that are a greater distance from the moisture. Warm temperatures, from 20°C to 30°C and persistent surface moisture for 48 to 60 hours are necessary for infection when wheat is inoculated with macroconidia (Sutton, 1982). In nature, perithecia of *G. zeae* are often found on infected plants (Sutton, 1982). Ascospores are important for disease development, but most studies on FHB have used *F. graminearum* conidia as opposed to *G. zeae* ascospores (Stack, 1989). Infection differences might be expected, considering the larger size of conidia and hence a greater store of reserves, assuming conidia and ascospores are of equal densities. However this is not the case, as infection of wheat was found to be equivalent using ascospores or macroconidia as inoculum (Stack, 1989). Therefore using conidia as inoculum is a valid indicator even though ascospores may be of greater importance in nature.

Early generation field screening of wheat plants for FHB resistance is time consuming and due to confounding environmental effects, is also unpredictable. Since AFLP analysis is also a time consuming and expensive technology, it would be useful to

convert AFLP fragments of interest into other types of markers such as STS (sequence-tagged-site). In wheat, the development of sequence specific primers from AFLP markers is not efficient. Only six of 26 wheat or barley chromosome-specific AFLP markers retained their specificity after their cloning and conversion into sequence specific primers (Shan *et al.*, 1997). This conversion frequency may be low because primers were generated from sequences that were internal to the AFLP primers. If the nucleotide differences were specific to the AFLP primers, the chromosome specificity would be lost (Shan *et al.*, 1999). If specific PCR primers could be developed, they would be useful in screening germplasm for the markers linked to the FHB resistance genes.

This study has led to the detection of AFLP markers explaining a significant amount of variation in the FHB resistance phenotype of *T. aestivum*. These markers may be used by wheat breeders in marker assisted selection. The use of more selective amplification primer pairs would lead to the discovery of more markers that could potentially explain more of the FHB resistance phenotype.

The restriction enzyme MseI recognizes the sequence 5'-TTAA-3' and will cut frequently in the A+T rich areas such as heterochromatin close to the centromeres (Alonso-Blanco *et al.*, 1998). More polymorphisms may occur in the repetitive sequences around centromeres than in the coding single copy regions, resulting in clustering of AFLP markers around centromeres (Alonso-Blanco *et al.*, 1998). The targeting of pericentromeric regions may reduce the detection of useful markers in other regions of the genome. With the use of a restriction enzyme other than MseI, perhaps markers would be detected that would explain more of the phenotypic variation in FHB resistance.

Further inoculation experiments where all plants would receive the same amount of moisture would result in more reliable FHB resistance phenotype data. Future work includes isolating the bands of interest from silver stained gels and sequencing of the fragments. This work is currently underway.

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APPENDIX 1. Presence and absence of fragments in the three parental cultivars of *Triticum aestivum* L. ('Karena', 'AC Cartier' and FHB 148). 0's represent absence of bands, while 1's represent presence of bands. The sample is named down the left side. The fragment is identified by the short form for the primer pair (Table 2) followed by the size in base pairs.

SAMPLE	GGGGHHHEEEEEEEEEEEECCA 77773338888877777777885 22211112211032111100210 44458732182600864196749 97547885670808367745151
Karena-1	0100011111011100010111
Karena-2	0100011111011100010111
Karena-3	0100011111011100010?11
Karena-4	0100011111011100010111
Karena-5	010001111?11000101?11
Karena-6	0110011111011100010111
Karena-7	011001??10111000101111
Karena-8	0110011111011100010111
Karena-9	01000?1??10111000101??1
Karena-10	011001111101?1000101111
Karena-11	0110011111011100010111
Karena-12	0100011111011100010111
Karena-13	0100011111011100010111
Karena-14	0110011111011100010111
Karena-15	0110011111011100010111
FHB148-1	101010100?1000010010001
FHB148-2	1010101000100001001000 1
FHB148-3	1010101000100001001000 1
FHB148-4	1010101000100001001000 1
FHB148-5	1010101000100001001000 1
FHB148-6	1010101000100001001000 1
FHB148-7	1010101000100001001000 1
FHB148-8	1010101000100001001000 1
FHB148-9	1010101000100001001000 1
FHB148-10	1010101000100001001000 1
FHB148-11	1010101000100001001000 1
FHB148-12	1010101000100001001000 1
FHB148-13	1010101000100001001000 1
FHB148-14	1010101000100001001000 1
FHB148-15	1010101000100001001000 1

APPENDIX 1. (Cont.)

S	GGGGHHHEEEEEEEEEEEEECCA
A	77773338888877777777885
M	22211112211032111100210
P	44458732182600864196749
L	97547885670808367745151
E	
AC Cartier-1	01010101?101?0101101110
AC Cartier-2	01010101?101?0101101110
AC Cartier-3	0101010111010101101110
AC Cartier-4	01010101?101?0101101?10
AC Cartier-5	0101010111010101101110
AC Cartier-6	0101010111010101101110
AC Cartier-7	0101010111010101101110
AC Cartier-8	0101010111010101101110
AC Cartier-9	0101010111010101101110
AC Cartier-10	0101010111010101101110
AC Cartier-11	0101010111010101101110
AC Cartier-12	0101010???0110101101110
AC Cartier-13	0101010111010101101110
AC Cartier-14	0101010111010101101110
AC Cartier-15	0101010?1?0?1010110?110

APPENDIX 2A. Presence (1) and absence (0) of polymorphic bands in 'Karena'/FHB 148 DH. Nine plants in each of 26 lines were analysed. The band size is shown in the top left. Total number of 1's and 0's in each line are also shown (x = no plant).

LINE(G7-249bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N16-1	1	1	1	1	1	1	1	1	1	9	0
N16-2	0	0	0	0	0	0	0	0	0	0	9
N16-3	0	0	0	0	0	0	0	0	0	0	9
N16-4	0	0	0	0	0	0	0	0	0	0	9
N16-5	1	1	1	1	1	1	1	1	1	9	0
N16-6	1	1	1	1	1	1	1	1	1	9	0
N16-7	1	1	1	1	1	1	1	1	1	9	0
N16-8	1	1	1	1	1	1	1	1	1	9	0
N16-9	1	1	1	1	1	1	1	1	1	9	0
N16-10	1	1	1	1	1	1	1	1	X	8	0
N16-11	0	0	0	0	0	0	0	0	X	0	8
N16-12	1	1	1	1	1	1	1	1	1	9	0
N16-13	0	0	0	0	0	0	0	0	0	0	9
N16-14	1	1	1	1	1	1	1	1	X	8	0
N16-15	0	0	0	0	0	0	0	0	X	0	8
N16-16	1	1	1	1	1	1	1	1	1	9	0
N16-17	1	1	1	1	1	1	1	1	1	9	0
N16-18	0	0	0	0	0	0	0	0	0	0	9
N16-19	0	0	0	0	0	0	0	0	0	0	9
N16-20	1	1	1	1	1	1	1	1	1	9	0
N16-21	0	0	0	0	0	0	0	0	0	0	9
N16-22	1	1	1	1	1	1	1	1	1	9	0
N16-23	1	1	1	1	1	1	1	1	1	9	0
N16-24	0	0	0	0	0	0	0	0	X	0	8
N16-25	0	0	0	0	0	0	0	0	X	0	8
N16-26	0	0	0	0	0	0	0	0	0	0	9

APPENDIX 2A (continued).

LINE(G7-247bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N16-1	1	1	1	1	1	1	1	1	1	9	0
N16-2	1	1	1	1	1	1	1	1	1	9	0
N16-3	1	1	1	1	1	1	1	1	1	9	0
N16-4	1	1	1	1	1	1	1	1	1	9	0
N16-5	0	0	0	0	0	0	0	0	0	0	9
N16-6	0	0	0	0	0	0	0	0	0	0	9
N16-7	0	0	0	0	0	0	0	0	0	0	9
N16-8	0	0	0	0	0	0	0	0	0	0	9
N16-9	1	1	1	1	1	1	1	1	1	9	0
N16-10	1	1	1	1	1	1	1	1	X	8	0
N16-11	1	1	1	1	1	1	1	1	X	8	0
N16-12	0	0	0	0	0	0	0	0	0	0	9
N16-13	1	1	1	1	1	1	1	1	1	9	0
N16-14	0	0	0	0	0	0	0	0	X	0	8
N16-15	1	1	1	1	1	1	1	1	X	8	0
N16-16	0	0	0	0	0	0	0	0	0	0	9
N16-17	0	0	0	0	0	0	0	0	0	0	9
N16-18	1	1	1	1	1	1	1	1	1	9	0
N16-19	1	1	1	1	1	1	1	1	1	9	0
N16-20	0	0	0	0	0	0	0	0	0	0	9
N16-21	1	1	1	1	1	1	1	1	1	9	0
N16-22	0	0	0	0	0	0	0	0	0	0	9
N16-23	0	0	0	0	0	0	0	0	0	0	9
N16-24	1	1	1	1	1	1	1	1	X	8	0
N16-25	1	1	1	1	1	1	1	1	X	8	0
N16-26	1	1	1	1	1	1	1	1	1	9	0

APPENDIX 2A (continued).

LINE(G7-245bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N16-1	0	0	0	0	0	0	0	0	0	0	9
N16-2	1	1	1	1	1	1	1	1	1	9	0
N16-3	1	1	1	1	1	1	1	1	1	9	0
N16-4	1	1	1	1	1	1	1	1	1	9	0
N16-5	1	1	1	1	1	1	1	1	1	9	0
N16-6	1	1	1	1	1	1	1	1	1	9	0
N16-7	1	1	1	1	1	1	1	1	1	9	0
N16-8	1	1	1	1	1	1	1	1	1	9	0
N16-9	0	0	0	0	0	0	0	0	0	0	9
N16-10	0	0	0	0	0	0	0	0	X	0	8
N16-11	1	1	1	1	1	1	1	1	X	8	0
N16-12	1	1	1	1	1	1	1	1	1	9	0
N16-13	1	1	1	1	1	1	1	1	1	9	0
N16-14	1	1	1	1	1	1	1	1	X	8	0
N16-15	1	1	1	1	1	1	1	1	X	8	0
N16-16	1	1	1	1	1	1	1	1	1	9	0
N16-17	1	1	1	1	1	1	1	1	1	9	0
N16-18	1	1	1	1	1	1	1	1	1	9	0
N16-19	1	1	1	1	1	1	1	1	0	8	1
N16-20	1	1	1	1	1	1	1	1	1	9	0
N16-21	1	1	1	1	1	1	1	1	1	9	0
N16-22	1	1	1	1	1	1	1	1	1	9	0
N16-23	1	1	1	1	1	1	1	1	1	9	0
N16-24	1	1	1	1	1	1	1	1	X	8	0
N16-25	1	1	1	1	1	1	1	1	X	8	0
N16-26	1	1	1	1	1	1	1	1	1	9	0

APPENDIX 2A (continued).

LINE(H3-187bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N16-1	0	0	0	0	0	0	0	0	0	0	9
N16-2	1	1	1	1	1	1	1	0	0	7	2
N16-3	0	0	0	0	0	0	0	0	0	0	9
N16-4	1	1	1	0	1	1	1	1	1	8	1
N16-5	0	0	0	0	0	0	0	0	0	0	9
N16-6	1	1	1	1	1	1	1	1	1	9	0
N16-7	1	1	1	1	1	1	1	1	1	9	0
N16-8	0	1	1	1	1	1	1	1	1	8	1
N16-9	1	1	1	1	1	1	0	1	1	8	1
N16-10	0	0	0	0	0	0	0	0	X	0	8
N16-11	1	1	1	1	1	1	1	1	X	8	0
N16-12	1	1	1	0	1	0	1	1	1	7	2
N16-13	1	1	1	1	0	1	1	0	0	6	3
N16-14	0	0	0	0	0	0	0	0	X	0	8
N16-15	0	0	0	0	0	0	0	0	X	0	8
N16-16	0	0	0	0	0	0	0	0	0	0	9
N16-17	1	1	1	1	1	1	1	1	1	9	0
N16-18	0	0	0	0	0	0	0	0	0	0	9
N16-19	1	1	1	1	1	1	1	1	1	9	0
N16-20	1	0	0	0	0	0	0	0	0	1	8
N16-21	0	0	0	0	0	0	0	0	0	0	9
N16-22	0	1	1	1	1	1	1	1	1	8	1
N16-23	0	0	0	0	0	0	0	0	0	0	9
N16-24	1	1	1	1	1	1	1	1	X	8	0
N16-25	1	0	0	0	0	0	0	0	X	1	7
N16-26	0	0	0	1	1	1	1	1	1	6	3

APPENDIX 2A (continued).

LINE(H3-178bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N16-1	0	0	1	1	1	0	1	1	1	6	3
N16-2	1	0	0	1	1	1	0	0	1	5	4
N16-3	0	0	0	0	0	0	0	0	0	0	9
N16-4	0	0	0	0	0	0	0	0	0	0	9
N16-5	0	0	0	0	0	0	0	0	0	0	9
N16-6	0	0	0	0	0	0	0	0	0	0	9
N16-7	0	0	0	0	0	0	0	0	1	1	8
N16-8	0	0	0	0	0	1	0	1	1	3	6
N16-9	0	0	0	0	0	0	0	0	0	0	9
N16-10	0	0	1	0	0	1	1	0	X	3	5
N16-11	0	0	0	0	0	0	0	0	X	0	8
N16-12	0	0	0	0	0	0	0	0	0	0	9
N16-13	0	0	0	0	0	0	0	0	0	0	9
N16-14	0	0	0	0	0	0	0	0	X	0	8
N16-15	0	0	0	0	0	0	0	0	X	0	8
N16-16	0	0	0	0	0	0	0	0	0	0	9
N16-17	0	0	0	0	0	0	0	0	0	0	9
N16-18	0	0	0	0	0	0	0	0	0	0	9
N16-19	0	0	0	0	0	0	0	0	0	0	9
N16-20	1	1	1	1	1	0	1	1	1	8	1
N16-21	0	0	0	0	0	0	0	0	0	0	9
N16-22	0	0	0	0	0	0	0	0	0	0	9
N16-23	0	0	0	0	0	0	0	0	0	0	9
N16-24	0	0	0	0	0	1	0	0	X	1	7
N16-25	0	0	0	0	0	0	0	0	X	0	8
N16-26	0	0	0	0	0	0	0	0	1	1	8

APPENDIX 2A (continued).

LINE(E8-225bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N16-1	1	1	1	1	1	1	1	1	1	9	0
N16-2	1	1	1	1	1	1	1	1	1	9	0
N16-3	0	0	0	0	0	0	0	0	0	0	9
N16-4	0	0	0	0	0	0	0	0	0	0	9
N16-5	0	0	0	0	0	0	0	0	0	0	9
N16-6	0	0	0	0	0	0	0	0	0	0	9
N16-7	1	1	0	0	1	1	1	1	0	6	3
N16-8	0	1	0	0	0	0	0	0	1	2	7
N16-9	0	0	1	1	1	1	1	1	1	7	2
N16-10	1	1	1	0	1	0	1	0	X	5	3
N16-11	1	1	1	0	1	1	1	1	X	7	1
N16-12	0	0	0	0	0	0	0	0	0	0	9
N16-13	1	1	1	1	1	1	1	1	1	9	0
N16-14	1	1	1	1	1	1	1	1	X	8	0
N16-15	0	0	0	0	0	0	0	0	X	0	8
N16-16	1	1	1	1	1	1	1	1	1	9	0
N16-17	1	1	1	1	1	1	1	1	1	9	0
N16-18	1	1	1	1	1	1	1	1	1	9	0
N16-19	0	0	0	0	0	0	0	0	1	1	8
N16-20	0	0	0	0	0	0	0	0	0	0	9
N16-21	1	1	1	1	1	1	1	1	1	9	0
N16-22	0	0	0	0	0	0	0	0	0	0	9
N16-23	0	0	0	0	0	0	0	0	0	0	9
N16-24	0	0	0	0	0	0	0	0	X	0	8
N16-25	0	0	0	0	0	0	0	0	X	0	8
N16-26	0	0	0	0	0	0	0	0	0	0	9

APPENDIX 2A (continued).

LINE(E8-216bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N16-1	1	1	1	1	1	1	1	1	1	9	0
N16-2	0	0	0	0	0	0	0	0	0	0	9
N16-3	0	0	0	0	0	0	0	0	0	0	9
N16-4	0	0	0	0	0	0	0	0	0	0	9
N16-5	0	0	0	0	0	0	0	0	0	0	9
N16-6	0	0	0	0	0	0	0	0	0	0	9
N16-7	0	0	0	0	0	0	0	0	0	0	9
N16-8	0	0	0	0	0	0	0	0	0	0	9
N16-9	0	0	0	0	0	0	0	0	0	0	9
N16-10	0	0	0	0	0	0	0	0	X	0	8
N16-11	0	0	0	0	0	0	0	0	X	0	8
N16-12	1	1	1	1	1	1	1	1	1	9	0
N16-13	0	0	0	0	0	0	0	0	0	0	9
N16-14	1	1	1	1	1	1	1	1	X	8	0
N16-15	0	0	0	0	0	0	0	0	X	0	8
N16-16	1	1	1	1	1	1	1	1	1	9	0
N16-17	1	1	1	1	1	1	1	1	1	9	0
N16-18	1	1	1	1	1	1	1	1	1	9	0
N16-19	1	1	1	1	0	1	1	1	1	8	1
N16-20	0	0	0	0	0	0	0	0	0	0	9
N16-21	0	0	0	0	0	0	0	0	0	0	9
N16-22	0	0	0	0	0	0	0	0	0	0	9
N16-23	0	0	0	0	0	0	0	0	0	0	9
N16-24	0	0	0	0	0	0	0	0	X	0	8
N16-25	0	0	0	0	0	0	0	0	X	0	8
N16-26	0	0	0	0	0	0	0	0	0	0	9

APPENDIX 2A (continued).

LINE(E8-187bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N16-1	1	1	1	1	1	1	1	1	1	9	0
N16-2	1	1	1	1	1	1	1	1	1	9	0
N16-3	1	1	1	1	1	1	1	1	1	9	0
N16-4	1	1	1	1	1	1	1	1	1	9	0
N16-5	1	1	1	1	1	1	1	1	1	9	0
N16-6	0	0	0	0	0	0	0	0	0	0	9
N16-7	0	0	0	0	0	0	0	0	0	0	9
N16-8	0	0	0	0	0	0	0	0	0	0	9
N16-9	0	0	0	0	0	0	0	0	0	0	9
N16-10	1	1	1	1	1	0	1	1	X	7	1
N16-11	0	0	0	0	0	0	0	0	X	0	8
N16-12	1	1	1	1	1	1	1	1	1	9	0
N16-13	1	1	1	1	1	1	1	1	1	9	0
N16-14	1	1	1	1	1	1	1	1	X	8	0
N16-15	0	0	0	0	0	0	0	0	X	0	8
N16-16	1	1	1	1	1	1	1	1	1	9	0
N16-17	0	0	0	0	0	0	0	0	0	0	9
N16-18	1	1	1	1	1	1	1	1	1	9	0
N16-19	0	0	0	0	0	1	1	1	1	4	5
N16-20	1	1	1	1	1	1	1	1	1	9	0
N16-21	0	0	0	0	0	0	0	0	0	0	9
N16-22	0	0	0	0	0	0	0	0	0	0	9
N16-23	0	0	0	0	0	0	0	0	0	0	9
N16-24	0	0	0	0	0	0	0	0	X	0	8
N16-25	0	0	0	0	1	1	1	0	X	3	5
N16-26	1	1	0	1	1	0	1	1	0	6	3

APPENDIX 2A (continued).

LINE(E8-120bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N16-1	1	1	1	1	1	1	1	1	1	9	0
N16-2	1	1	1	1	1	1	1	1	1	9	0
N16-3	1	1	1	1	1	1	1	1	1	9	0
N16-4	0	0	0	0	0	0	0	0	0	0	9
N16-5	0	0	0	0	0	0	0	0	0	0	9
N16-6	1	1	1	1	1	1	1	1	1	9	0
N16-7	1	1	1	1	1	1	1	1	1	9	0
N16-8	1	1	1	1	1	1	1	1	1	9	0
N16-9	0	0	0	0	0	0	0	0	0	0	9
N16-10	0	0	0	0	0	0	0	0	X	0	8
N16-11	1	1	1	1	1	1	1	1	X	8	0
N16-12	0	0	0	0	0	0	0	0	0	0	9
N16-13	1	1	1	1	1	1	1	1	1	9	0
N16-14	1	1	1	1	1	1	1	1	X	8	0
N16-15	0	0	0	0	0	0	0	0	X	0	8
N16-16	0	0	0	0	0	0	0	0	0	0	9
N16-17	0	0	0	0	0	0	0	0	0	0	9
N16-18	1	1	1	1	1	1	1	1	1	9	0
N16-19	0	1	1	1	1	1	1	1	0	7	2
N16-20	0	0	0	0	0	0	0	0	0	0	9
N16-21	1	1	1	1	1	1	1	1	1	9	0
N16-22	0	0	0	0	0	0	0	0	0	0	9
N16-23	0	0	0	0	0	0	0	0	0	0	9
N16-24	1	1	1	1	1	1	0	1	X	7	1
N16-25	0	0	0	0	0	0	0	0	X	0	8
N16-26	0	0	0	0	0	0	0	0	0	0	9

APPENDIX 2A (continued).

LINE(E8-068bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N16-1	0	0	0	0	0	0	0	0	0	0	9
N16-2	1	1	1	1	1	1	1	1	1	9	0
N16-3	1	1	1	1	1	1	1	1	1	9	0
N16-4	1	1	1	1	1	1	1	1	1	9	0
N16-5	0	0	0	0	0	0	0	0	0	0	9
N16-6	0	0	0	0	0	0	0	0	0	0	9
N16-7	0	0	0	0	0	0	0	0	0	0	9
N16-8	0	0	0	0	0	0	0	0	0	0	9
N16-9	0	0	0	0	0	0	0	0	0	0	9
N16-10	1	1	1	1	1	1	1	1	X	8	0
N16-11	1	0	0	0	0	0	0	0	X	1	7
N16-12	1	1	1	1	1	1	1	1	1	9	0
N16-13	1	0	0	0	0	0	0	0	0	1	8
N16-14	1	1	1	1	1	1	1	1	X	8	0
N16-15	1	1	1	1	1	1	1	1	X	8	0
N16-16	0	0	0	0	0	0	0	0	0	0	9
N16-17	0	0	0	0	1	0	0	1	0	2	7
N16-18	0	0	0	0	0	0	0	0	0	0	9
N16-19	0	1	0	1	0	1	1	1	1	6	3
N16-20	1	1	1	1	1	1	1	1	1	9	0
N16-21	1	1	0	1	1	0	0	0	0	4	5
N16-22	1	1	1	1	1	1	1	1	1	9	0
N16-23	0	0	0	0	0	0	0	0	0	0	9
N16-24	1	1	1	1	1	1	1	1	X	8	0
N16-25	1	1	1	1	1	1	1	1	X	8	0
N16-26	0	0	0	0	0	0	0	0	0	0	9

APPENDIX 2A (continued).

LINE(E7-300bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N16-1	0	0	0	0	0	0	0	0	0	0	9
N16-2	1	1	1	1	1	1	1	1	1	9	0
N16-3	0	0	0	0	0	0	0	0	0	0	9
N16-4	1	1	1	1	1	1	1	1	1	9	0
N16-5	1	1	1	1	1	1	1	1	1	9	0
N16-6	1	1	1	1	1	1	1	1	1	9	0
N16-7	1	1	1	1	1	1	1	1	1	9	0
N16-8	1	1	1	1	1	1	1	1	1	9	0
N16-9	1	1	1	1	1	1	1	1	1	9	0
N16-10	1	1	1	1	1	1	1	1	X	8	0
N16-11	0	0	0	0	0	0	0	0	X	0	8
N16-12	0	0	0	0	0	0	0	0	0	0	9
N16-13	0	0	0	0	0	0	0	0	0	0	9
N16-14	0	0	0	0	0	0	0	0	X	0	8
N16-15	1	1	1	1	1	1	1	1	X	8	0
N16-16	0	0	0	0	0	0	0	0	0	0	9
N16-17	1	1	1	1	1	1	1	1	1	9	0
N16-18	0	0	0	0	0	0	0	0	0	0	9
N16-19	0	0	0	0	0	0	0	0	0	0	9
N16-20	0	0	0	0	0	0	0	0	0	0	9
N16-21	1	1	1	1	1	1	1	1	1	9	0
N16-22	0	0	0	0	0	0	0	0	0	0	9
N16-23	1	1	1	1	1	1	1	1	1	9	0
N16-24	0	0	0	0	0	0	0	0	X	0	8
N16-25	1	1	1	1	1	1	1	1	X	8	0
N16-26	1	1	1	1	1	1	1	1	1	9	0

APPENDIX 2A (continued).

LINE(E7-208bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N16-1	1	1	1	1	1	1	1	1	1	9	0
N16-2	1	1	1	1	1	1	1	1	1	9	0
N16-3	0	0	0	0	0	0	0	0	0	0	9
N16-4	0	0	0	0	0	0	0	0	0	0	9
N16-5	1	1	1	1	1	1	1	1	1	9	0
N16-6	1	1	0	1	1	0	1	1	0	6	3
N16-7	0	0	0	0	0	0	0	0	1	1	8
N16-8	0	0	1	0	0	1	0	0	0	2	7
N16-9	0	0	0	0	0	0	0	0	0	0	9
N16-10	1	1	1	1	1	1	1	1	X	8	0
N16-11	0	0	0	0	0	0	0	0	X	0	8
N16-12	1	1	1	1	1	1	1	1	1	9	0
N16-13	0	0	0	0	0	0	0	0	0	0	9
N16-14	0	0	0	0	0	0	0	0	X	0	8
N16-15	0	0	0	0	0	0	0	0	X	0	8
N16-16	0	0	0	0	0	0	0	0	0	0	9
N16-17	0	0	0	0	0	0	0	0	0	0	9
N16-18	0	0	0	0	0	0	0	0	0	0	9
N16-19	0	0	0	0	0	0	0	0	0	0	9
N16-20	0	0	0	0	0	0	0	0	0	0	9
N16-21	0	0	0	0	0	0	0	0	0	0	9
N16-22	1	1	1	1	1	1	1	1	1	9	0
N16-23	1	1	1	1	1	1	1	1	1	9	0
N16-24	0	0	0	0	0	0	0	0	X	0	8
N16-25	0	0	0	0	0	0	0	0	X	0	8
N16-26	1	1	1	1	1	1	1	1	1	9	0

APPENDIX 2A (continued).

LINE(E7-166bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N16-1	1	1	1	1	1	1	1	1	1	9	0
N16-2	1	1	1	1	1	1	1	1	1	9	0
N16-3	0	0	0	0	0	0	0	0	0	0	9
N16-4	0	0	0	0	0	0	0	0	0	0	9
N16-5	0	0	0	0	0	0	0	0	0	0	9
N16-6	1	1	1	1	1	1	1	1	1	9	0
N16-7	0	0	0	0	0	0	0	0	0	0	9
N16-8	0	0	0	0	0	0	0	0	0	0	9
N16-9	1	1	1	1	1	1	1	1	1	9	0
N16-10	1	1	1	1	1	1	1	1	X	8	0
N16-11	1	1	1	1	1	1	1	1	X	8	0
N16-12	1	1	1	1	1	1	1	1	1	9	0
N16-13	0	0	0	0	0	0	0	0	0	0	9
N16-14	1	1	1	1	1	1	1	1	X	8	0
N16-15	0	0	0	0	0	0	0	0	X	0	8
N16-16	1	1	1	1	0	0	0	0	0	4	5
N16-17	1	1	1	1	1	1	1	1	1	9	0
N16-18	1	1	1	1	1	1	1	1	1	9	0
N16-19	1	1	1	1	1	1	1	1	1	9	0
N16-20	1	1	1	1	1	1	1	1	1	9	0
N16-21	1	1	1	1	1	1	1	1	1	9	0
N16-22	1	1	1	1	1	1	1	1	1	9	0
N16-23	0	0	0	0	0	0	0	0	0	0	9
N16-24	1	1	1	1	1	1	1	1	X	8	0
N16-25	0	0	0	1	1	1	0	0	X	3	5
N16-26	0	0	0	0	0	0	0	0	0	0	9

APPENDIX 2A (continued).

LINE(E7-117bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N16-1	0	0	0	0	0	0	0	0	0	0	9
N16-2	1	1	1	1	1	1	1	1	1	9	0
N16-3	1	1	1	1	1	1	1	1	1	9	0
N16-4	0	0	0	0	0	0	0	0	0	0	9
N16-5	0	0	0	0	0	0	0	0	0	0	9
N16-6	1	1	1	1	1	1	1	1	1	9	0
N16-7	1	1	1	1	1	1	1	1	1	9	0
N16-8	1	1	1	1	1	1	1	1	1	9	0
N16-9	1	1	1	1	1	1	1	1	1	9	0
N16-10	0	0	0	0	0	0	0	0	X	0	8
N16-11	1	1	1	1	1	1	1	1	X	8	0
N16-12	0	0	0	0	0	0	0	0	0	0	9
N16-13	0	0	0	0	0	0	0	0	0	0	9
N16-14	0	0	0	0	0	0	0	0	X	0	8
N16-15	1	1	1	1	1	1	1	1	X	8	0
N16-16	0	0	0	0	0	0	0	0	0	0	9
N16-17	1	1	1	1	1	1	1	1	1	9	0
N16-18	1	1	1	1	1	1	1	1	1	9	0
N16-19	0	0	0	0	0	0	0	0	0	0	9
N16-20	0	0	0	0	0	0	0	0	0	0	9
N16-21	0	0	0	0	0	0	0	0	0	0	9
N16-22	0	0	0	0	0	0	0	0	0	0	9
N16-23	0	0	0	0	0	0	0	0	0	0	9
N16-24	0	0	0	0	0	0	0	0	X	0	8
N16-25	1	1	1	1	1	1	1	1	X	8	0
N16-26	1	1	1	1	1	1	1	1	1	9	0

APPENDIX 2A (continued).

LINE(E7-094bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N16-1	1	1	0	1	0	0	0	0	0	3	6
N16-2	0	0	0	0	0	0	0	0	0	0	9
N16-3	1	1	1	1	1	1	1	1	1	9	0
N16-4	0	0	0	0	0	0	0	0	0	0	9
N16-5	0	0	0	0	0	1	0	0	0	1	8
N16-6	1	1	0	1	1	0	1	1	1	7	2
N16-7	1	1	1	1	1	1	1	1	1	9	0
N16-8	1	1	1	1	1	0	1	1	1	8	1
N16-9	0	0	0	0	0	0	0	0	0	0	9
N16-10	0	0	0	0	0	0	0	0	X	0	8
N16-11	1	0	1	1	1	1	1	1	X	7	1
N16-12	1	1	1	1	1	0	1	1	1	8	1
N16-13	1	1	1	1	1	1	1	1	1	9	0
N16-14	1	1	1	1	1	1	1	1	X	8	0
N16-15	1	1	1	1	1	1	1	1	X	8	0
N16-16	0	0	0	0	0	0	0	0	0	0	9
N16-17	0	0	0	0	0	0	0	0	0	0	9
N16-18	1	1	1	1	1	1	1	1	1	9	0
N16-19	1	1	1	1	1	1	1	1	1	9	0
N16-20	0	0	0	0	0	0	0	0	0	0	9
N16-21	0	0	0	0	0	0	0	0	0	0	9
N16-22	0	0	0	0	0	0	0	0	0	0	9
N16-23	0	0	0	0	0	0	0	0	0	0	9
N16-24	0	0	0	0	0	1	0	0	X	1	7
N16-25	1	1	1	1	1	1	1	1	X	8	0
N16-26	1	1	1	1	1	1	1	1	1	9	0

APPENDIX 2A (continued).

LINE(E7-065bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N16-1	1	1	1	1	0	1	1	1	1	8	1
N16-2	1	1	1	0	1	1	1	1	1	8	1
N16-3	0	0	0	0	0	0	0	0	0	0	9
N16-4	0	0	0	0	0	0	0	1	1	2	7
N16-5	1	1	1	1	1	1	1	1	1	9	0
N16-6	1	1	0	1	1	0	1	1	0	6	3
N16-7	0	0	0	0	0	0	0	0	1	1	8
N16-8	0	0	0	0	0	0	0	0	0	0	9
N16-9	0	0	0	0	0	0	0	0	0	0	9
N16-10	1	1	1	1	1	1	1	1	X	8	0
N16-11	0	0	0	0	0	0	0	0	X	0	8
N16-12	1	1	1	1	1	1	1	1	1	9	0
N16-13	0	0	0	0	0	0	0	0	0	0	9
N16-14	0	0	0	0	0	0	0	0	X	0	8
N16-15	0	0	0	0	0	0	0	0	X	0	8
N16-16	0	0	0	0	0	0	0	0	0	0	9
N16-17	0	0	0	0	0	0	0	0	0	0	9
N16-18	0	0	0	0	0	0	0	0	0	0	9
N16-19	0	0	0	0	0	0	0	0	0	0	9
N16-20	0	0	0	0	0	0	0	0	0	0	9
N16-21	0	0	0	0	0	0	0	0	0	0	9
N16-22	1	1	1	1	1	1	1	1	1	9	0
N16-23	1	1	1	1	1	1	1	1	1	9	0
N16-24	0	0	0	0	0	0	0	0	X	0	8
N16-25	0	0	0	0	0	0	0	0	X	0	8
N16-26	1	1	1	1	1	1	1	1	1	9	0

APPENDIX 2A (continued).

LINE(C8-271bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N16-1	1	1	1	1	1	1	1	1	1	9	0
N16-2	1	1	1	1	1	1	1	1	1	9	0
N16-3	0	0	0	0	0	0	0	0	0	0	9
N16-4	0	0	0	0	0	0	0	0	0	0	9
N16-5	0	0	0	0	0	0	0	0	0	0	9
N16-6	1	1	0	1	0	0	1	1	0	5	4
N16-7	0	0	0	0	0	0	0	0	0	0	9
N16-8	0	0	0	0	0	0	0	0	0	0	9
N16-9	0	0	0	0	0	0	0	0	0	0	9
N16-10	0	1	1	1	1	1	1	1	X	7	1
N16-11	0	0	0	0	0	0	0	0	X	0	8
N16-12	0	0	0	0	0	0	0	0	0	0	9
N16-13	1	1	1	1	1	1	1	1	1	9	0
N16-14	1	1	1	1	1	1	1	0	X	7	1
N16-15	0	0	0	0	0	0	0	0	X	0	8
N16-16	0	0	0	0	0	0	0	0	0	0	9
N16-17	0	0	0	0	0	0	0	0	0	0	9
N16-18	0	0	0	0	0	0	0	0	0	0	9
N16-19	0	0	0	0	0	0	0	0	1	1	8
N16-20	1	1	1	1	1	1	1	1	1	9	0
N16-21	0	0	0	0	0	0	0	0	0	0	9
N16-22	0	0	0	0	0	0	0	0	0	0	9
N16-23	0	0	0	0	0	0	0	0	0	0	9
N16-24	1	1	1	1	1	0	1	1	X	7	1
N16-25	0	0	0	0	0	0	0	0	X	0	8
N16-26	0	0	0	0	0	0	0	0	0	0	9

APPENDIX 2A (continued).

LINE(C8-145bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N16-1	0	0	0	0	0	0	0	0	0	0	9
N16-2	1	0	1	1	1	1	1	1	1	8	1
N16-3	1	1	1	1	1	1	1	1	1	9	0
N16-4	1	0	0	0	0	0	0	0	1	2	7
N16-5	0	0	1	1	0	1	1	0	0	4	5
N16-6	1	1	1	1	1	1	1	1	1	9	0
N16-7	1	1	1	1	1	1	1	1	1	9	0
N16-8	1	1	1	1	1	1	1	1	1	9	0
N16-9	1	1	1	1	1	1	1	1	1	9	0
N16-10	0	1	0	0	0	0	1	0	X	2	6
N16-11	1	1	1	1	1	1	1	1	X	8	0
N16-12	0	0	0	0	0	0	0	0	0	0	9
N16-13	0	0	0	0	0	0	0	0	0	0	9
N16-14	0	0	0	0	0	0	0	0	X	0	8
N16-15	1	1	1	1	1	1	1	1	X	8	0
N16-16	1	1	1	1	1	1	1	1	1	9	0
N16-17	1	1	1	1	1	1	1	1	1	9	0
N16-18	1	1	1	1	1	1	1	1	1	9	0
N16-19	0	0	0	0	0	0	0	0	0	0	9
N16-20	1	1	1	1	1	1	1	1	1	9	0
N16-21	1	1	1	1	1	1	1	1	1	9	0
N16-22	0	0	0	0	0	0	0	0	0	0	9
N16-23	0	0	0	0	0	0	0	0	0	0	9
N16-24	0	0	0	0	0	0	0	0	X	0	8
N16-25	1	1	1	1	1	1	1	1	X	8	0
N16-26	1	1	1	1	1	1	1	1	1	9	0

APPENDIX 2B. Presence (1) and absence (0) of polymorphic bands in 'AC Cartier'/FHB 148 DH (x = no plant)

LINE (G7-249)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N15-1	0	0	0	0	0	0	0	0	0	0	9
N15-2	0	0	0	0	0	0	0	0	0	0	9
N15-3	0	0	0	0	0	0	0	0	0	0	9
N15-4	0	0	0	0	0	0	0	0	0	0	9
N15-6	0	0	0	0	0	0	0	0	0	0	9
N15-7	0	0	0	0	0	0	0	0	0	0	9
N15-8	0	0	0	0	0	0	0	0	0	0	9
N15-9	0	0	0	0	0	0	0	0	0	0	9
N15-10	0	0	0	0	0	0	0	0	X	0	8
N15-11	1	1	1	1	1	1	1	1	1	9	0
N15-12	1	0	0	0	0	0	0	0	0	1	8
N15-13	1	1	1	1	1	1	1	1	X	8	0
N15-14	1	1	1	1	1	1	1	1	1	9	0
N15-15	1	1	1	1	1	1	1	1	1	9	0
N15-16	0	0	0	0	0	0	0	0	0	0	9
N15-17	1	1	1	1	1	1	1	1	1	9	0
N15-18	0	0	0	0	0	0	0	0	0	0	9
N15-19	0	0	0	0	0	0	0	0	0	0	9
N15-20	1	1	1	1	1	1	1	1	1	9	0
N15-21	1	1	1	1	1	1	1	1	1	9	0
N15-22	0	0	0	0	0	0	0	0	0	0	9
N15-25	1	1	1	1	1	1	1	1	1	9	0
N15-26	0	0	0	0	0	0	0	0	0	0	9
N15-27	0	0	0	0	0	0	0	0	0	0	9
N15-29	0	0	0	0	0	0	0	0	0	0	9
N15-30	0	0	0	0	0	0	0	0	0	0	9
N15-31A-	1	1	1	1	1	1	1	1	1	9	0
N15-31A+	1	1	1	1	1	1	1	1	1	9	0
N15-32	1	1	1	1	1	1	1	1	1	9	0
N15-33	0	0	0	0	0	0	0	0	0	0	9
N15-34	1	1	1	1	1	1	1	1	1	9	0
N15-35	1	1	1	1	1	1	1	1	1	9	0
N15-36	0	0	0	0	0	0	0	0	X	0	8
N15-37	0	0	0	0	0	0	0	0	0	0	9
N15-38	1	1	1	1	1	1	1	1	1	9	0
N15-39	0	0	0	0	0	0	0	0	0	0	9

APPENDIX 2B (continued).

LINE (G7-247bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N15-1	1	1	1	1	1	1	1	1	1	9	0
N15-2	1	1	1	1	1	1	1	1	1	9	0
N15-3	1	1	1	1	1	1	1	1	1	9	0
N15-4	1	1	1	1	1	1	1	1	1	9	0
N15-6	1	1	1	1	1	1	1	1	1	9	0
N15-7	1	1	1	1	1	1	1	1	1	9	0
N15-8	1	1	1	1	1	1	1	1	1	9	0
N15-9	1	1	1	1	1	1	1	1	1	9	0
N15-10	1	1	1	1	1	1	1	1	X	8	0
N15-11	0	0	0	0	0	0	0	0	0	0	9
N15-12	1	1	1	1	1	1	1	1	1	9	0
N15-13	0	0	0	0	0	0	0	0	X	0	8
N15-14	0	0	0	0	0	0	0	0	0	0	9
N15-15	0	0	0	0	0	0	0	0	0	0	9
N15-16	1	1	1	1	1	1	1	1	1	9	0
N15-17	0	0	0	0	0	0	0	0	0	0	9
N15-18	1	1	1	1	1	1	1	1	1	9	0
N15-19	1	1	1	1	1	1	1	1	1	9	0
N15-20	0	0	0	0	0	0	0	0	0	0	9
N15-21	0	0	0	0	0	0	0	0	0	0	9
N15-22	1	1	1	1	1	1	1	1	1	9	0
N15-25	0	0	0	0	0	0	0	0	0	0	9
N15-26	1	1	1	1	1	1	1	1	1	9	0
N15-27	1	1	1	1	1	1	1	1	1	9	0
N15-29	1	1	1	1	1	1	1	1	1	9	0
N15-30	1	1	1	1	1	1	1	1	1	9	0
N15-31A-	0	0	0	0	0	0	0	0	0	0	9
N15-31A+	0	0	0	0	0	0	0	0	0	0	9
N15-32	0	0	0	0	0	0	0	0	0	0	9
N15-33	1	1	1	1	1	1	1	1	1	9	0
N15-34	0	0	0	0	0	0	0	0	0	0	9
N15-35	0	0	0	0	0	0	0	0	0	0	9
N15-36	1	1	1	1	1	1	1	1	X	8	0
N15-37	1	1	1	1	1	1	1	1	1	9	0
N15-38	0	0	0	0	0	0	0	0	0	0	9
N15-39	1	1	1	1	1	1	1	1	1	9	0

APPENDIX 2B (continued).

LINE (G7-245bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N15-1	0	0	0	0	0	0	0	0	0	0	9
N15-2	1	1	1	1	1	1	1	1	1	9	0
N15-3	1	1	1	1	1	1	1	1	1	9	0
N15-4	1	1	1	1	1	1	1	1	1	9	0
N15-6	1	1	1	1	1	1	1	1	1	9	0
N15-7	1	1	1	1	1	1	1	1	1	9	0
N15-8	1	1	1	1	1	1	1	1	1	9	0
N15-9	1	1	1	1	1	1	1	1	1	9	0
N15-10	0	0	0	0	0	0	0	0	X	0	8
N15-11	1	1	1	1	1	1	1	1	1	9	0
N15-12	1	1	1	1	1	1	1	1	1	9	0
N15-13	1	1	1	1	1	1	1	1	X	8	0
N15-14	1	1	1	1	1	1	1	1	1	9	0
N15-15	0	0	0	0	0	0	0	0	0	0	9
N15-16	1	1	1	1	1	1	1	1	1	9	0
N15-17	1	1	1	1	1	1	1	1	1	9	0
N15-18	1	1	1	1	1	1	1	1	1	9	0
N15-19	0	0	0	0	0	0	0	0	0	0	9
N15-20	1	1	1	1	1	1	1	1	1	9	0
N15-21	1	1	0	1	1	0	1	1	0	6	3
N15-22	1	1	1	1	1	1	1	1	1	9	0
N15-25	1	1	1	1	1	1	1	1	1	9	0
N15-26	0	0	0	0	0	0	0	0	0	0	9
N15-27	1	1	1	1	1	1	1	1	1	9	0
N15-29	1	1	1	1	1	1	1	1	1	9	0
N15-30	1	1	1	1	1	1	1	1	1	9	0
N15-31A-	0	0	0	0	0	0	0	0	0	0	9
N15-31A+	0	0	0	0	0	0	0	0	0	0	9
N15-32	0	0	0	0	0	0	0	0	0	0	9
N15-33	1	1	1	1	1	1	1	1	1	9	0
N15-34	1	1	1	1	1	1	1	1	1	9	0
N15-35	1	1	1	1	1	1	1	1	1	9	0
N15-36	0	0	0	0	0	0	0	0	X	0	8
N15-37	1	1	1	1	1	1	1	1	1	9	0
N15-38	1	1	1	1	1	1	1	1	1	9	0
N15-39	1	1	1	1	1	1	1	1	1	9	0

APPENDIX 2B (continued).

LINE (G7-154bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N15-1	1	1	1	1	1	1	1	1	1	9	0
N15-2	1	1	1	1	1	1	1	1	1	9	0
N15-3	1	1	1	1	1	1	1	1	1	9	0
N15-4	0	0	0	0	0	0	0	0	0	0	9
N15-6	0	0	0	0	0	0	0	0	0	0	9
N15-7	1	1	1	1	1	1	1	1	1	9	0
N15-8	0	0	0	0	0	0	0	0	0	0	9
N15-9	0	0	0	0	0	0	0	0	0	0	9
N15-10	0	0	0	0	0	0	0	0	X	0	8
N15-11	1	1	1	1	1	1	1	1	1	9	0
N15-12	1	1	1	1	1	1	1	1	1	9	0
N15-13	1	1	1	1	1	1	1	1	X	8	0
N15-14	0	0	0	0	0	0	0	0	0	0	9
N15-15	1	1	1	1	1	1	1	1	1	9	0
N15-16	1	1	1	1	1	1	1	1	1	9	0
N15-17	1	1	1	1	1	1	1	1	1	9	0
N15-18	1	1	1	1	1	1	1	1	1	9	0
N15-19	0	0	0	0	0	0	0	0	0	0	9
N15-20	1	1	1	1	1	1	1	1	1	9	0
N15-21	1	1	1	1	1	1	1	1	1	9	0
N15-22	1	1	1	1	1	1	1	1	1	9	0
N15-25	1	1	1	1	1	1	1	1	1	9	0
N15-26	0	0	0	0	0	0	0	0	0	0	9
N15-27	1	1	1	1	1	1	1	1	1	9	0
N15-29	1	1	1	1	1	1	1	0	0	7	2
N15-30	0	0	0	0	0	0	0	0	0	0	9
N15-31A-	0	0	0	0	0	0	0	0	0	0	9
N15-31A+	1	1	1	1	1	1	1	1	1	9	0
N15-32	1	1	1	1	1	1	1	1	1	9	0
N15-33	0	0	0	0	0	0	0	0	0	0	9
N15-34	1	1	1	1	1	1	1	1	1	9	0
N15-35	0	0	0	0	0	0	0	0	1	1	8
N15-36	1	1	1	1	1	1	1	1	X	8	0
N15-37	0	0	0	0	0	0	0	0	0	0	9
N15-38	0	0	0	0	0	0	0	0	0	0	9
N15-39	1	0	0	0	0	0	0	0	0	1	8

APPENDIX 2B (continued).

LINE (H3-187bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N15-1	1	1	1	1	1	1	1	1	1	9	0
N15-2	1	1	1	1	1	1	1	1	1	9	0
N15-3	1	1	1	1	1	1	1	1	0	8	1
N15-4	0	0	0	0	0	0	0	0	0	0	9
N15-6	1	1	1	1	1	1	1	1	1	9	0
N15-7	0	0	0	0	0	0	0	0	0	0	9
N15-8	0	0	0	0	0	0	0	0	0	0	9
N15-9	1	1	1	1	1	1	0	1	0	7	2
N15-10	0	0	0	0	0	0	0	0	X	0	8
N15-11	0	0	0	0	0	0	0	0	0	0	9
N15-12	0	0	0	0	0	0	0	0	0	0	9
N15-13	1	1	1	1	1	1	1	1	X	8	0
N15-14	1	1	1	1	1	1	1	1	1	9	0
N15-15	1	1	1	1	1	1	1	1	1	9	0
N15-16	1	1	1	1	1	1	1	1	1	9	0
N15-17	0	0	0	0	0	0	0	0	0	0	9
N15-18	1	1	1	1	1	1	1	1	1	9	0
N15-19	0	0	0	0	0	0	0	0	0	0	9
N15-20	0	0	0	0	0	0	0	0	0	0	9
N15-21	0	0	0	0	0	0	0	0	0	0	9
N15-22	0	0	0	0	0	0	0	0	0	0	9
N15-25	1	1	1	1	1	1	1	0	1	8	1
N15-26	0	0	0	0	0	0	0	0	0	0	9
N15-27	1	1	1	1	1	1	1	1	1	9	0
N15-29	1	1	1	1	1	1	1	1	1	9	0
N15-30	1	0	0	0	0	0	0	1	1	3	6
N15-31A-	1	1	1	1	1	1	1	1	1	9	0
N15-31A+	1	1	1	1	1	1	1	1	1	9	0
N15-32	1	1	1	1	1	1	1	1	1	9	0
N15-33	0	0	0	0	0	0	0	0	0	0	9
N15-34	0	0	0	0	0	0	0	0	0	0	9
N15-35	0	0	0	0	0	0	0	0	0	0	9
N15-36	1	1	1	1	1	1	1	1	X	8	0
N15-37	1	1	1	1	1	1	1	1	1	9	0
N15-38	0	0	0	0	0	0	0	0	0	0	9
N15-39	0	0	0	0	0	0	0	0	0	0	9

APPENDIX 2B (continued).

LINE (H3-178bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N16-1	0	0	0	0	0	0	0	0	0	0	9
N16-2	0	0	0	0	0	0	0	0	0	0	9
N16-3	0	0	0	0	0	0	0	0	0	0	9
N16-4	0	0	0	0	0	0	0	0	0	0	9
N16-6	0	0	0	0	0	0	0	0	0	0	9
N16-7	0	0	0	0	0	0	0	0	0	0	9
N16-8	0	0	0	0	0	0	0	0	0	0	9
N16-9	1	1	0	1	1	1	0	0	0	5	4
N16-10	1	1	1	1	1	1	0	1	X	7	1
N16-11	0	0	0	1	0	0	1	0	1	3	6
N16-12	0	0	0	0	0	0	0	0	0	0	9
N16-13	1	1	1	1	1	1	1	1	X	8	0
N16-14	1	1	1	1	0	1	1	1	1	8	1
N16-15	1	1	1	1	1	1	1	1	1	9	0
N16-16	0	0	0	0	0	0	0	0	0	0	9
N16-17	1	1	0	1	1	1	0	0	1	6	3
N16-18	0	0	0	0	0	0	0	0	0	0	9
N16-19	1	1	1	1	1	1	1	1	1	9	0
N16-20	0	0	0	0	0	0	0	0	0	0	9
N16-21	0	0	0	0	0	0	0	0	0	0	9
N16-22	1	1	1	1	1	1	1	1	1	9	0
N16-25	0	0	0	0	0	0	0	0	1	1	8
N16-26	1	0	1	1	1	1	1	1	1	8	1
N16-27	1	1	1	1	1	1	1	1	1	9	0
N16-29	0	0	0	0	0	0	0	0	0	0	9
N16-30	0	0	0	0	0	0	0	0	0	0	9
N16-31A-	0	0	0	0	0	0	0	0	0	0	9
N16-31A+	0	0	0	0	0	0	0	0	0	0	9
N16-32	0	1	1	1	1	1	1	1	1	8	1
N16-33	1	1	1	1	1	1	1	1	1	9	0
N16-34	0	0	0	0	0	0	0	0	0	0	9
N16-35	0	0	0	0	0	0	0	0	0	0	9
N16-36	0	0	0	0	0	0	0	0	X	0	8
N16-37	0	0	0	0	0	0	0	0	0	0	9
N16-38	0	0	0	0	0	0	0	0	0	0	9
N16-39	0	0	0	0	0	0	0	0	0	0	9

APPENDIX 2B (continued).

LINE (H3-138bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N15-1	0	0	0	0	0	0	0	0	0	0	9
N15-2	1	1	1	1	1	1	1	1	1	9	0
N15-3	1	1	1	1	1	1	1	1	1	9	0
N15-4	1	1	1	1	1	1	1	1	1	9	0
N15-6	1	1	1	1	1	1	1	1	1	9	0
N15-7	1	1	1	1	1	1	1	1	1	9	0
N15-8	1	1	1	1	1	1	1	1	1	9	0
N15-9	1	1	1	1	1	1	1	1	1	9	0
N15-10	0	0	0	0	0	0	0	0	X	0	8
N15-11	1	1	1	1	1	1	1	1	1	9	0
N15-12	0	0	0	0	0	0	0	0	0	0	9
N15-13	1	1	1	1	1	1	1	1	X	8	0
N15-14	1	1	1	1	1	1	1	1	1	9	0
N15-15	0	0	0	0	0	0	0	0	0	0	9
N15-16	1	1	1	1	1	1	1	1	1	9	0
N15-17	1	1	1	1	1	1	1	1	1	9	0
N15-18	1	1	1	1	1	1	1	1	1	9	0
N15-19	0	0	0	0	0	0	0	0	0	0	9
N15-20	1	1	1	1	1	1	1	1	1	9	0
N15-21	0	0	0	0	0	0	0	0	0	0	9
N15-22	0	0	0	0	0	0	0	0	0	0	9
N15-25	1	1	1	1	1	1	1	1	1	9	0
N15-26	0	0	0	0	0	0	0	0	0	0	9
N15-27	0	0	0	0	0	0	0	0	0	0	9
N15-29	1	1	1	1	1	1	1	1	1	9	0
N15-30	0	0	1	1	1	1	1	1	1	7	2
N15-31A-	0	0	0	0	0	0	0	0	0	0	9
N15-31A+	0	0	0	0	0	0	0	0	0	0	9
N15-32	0	0	0	0	0	0	0	0	0	0	9
N15-33	1	1	1	1	1	1	1	1	1	9	0
N15-34	1	1	1	1	1	1	1	1	1	9	0
N15-35	0	0	0	0	0	0	0	0	0	0	9
N15-36	0	0	0	0	0	0	0	0	X	0	8
N15-37	1	1	1	1	1	1	1	1	1	9	0
N15-38	1	1	1	1	1	1	1	1	1	9	0
N15-39	0	0	0	0	0	0	0	0	0	0	9

APPENDIX 2B (continued).

LINE (E8-225bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N15-1	1	1	1	1	1	1	1	1	1	9	0
N15-2	0	0	0	0	0	0	0	0	0	0	9
N15-3	1	1	1	1	1	1	1	1	1	9	0
N15-4	0	0	0	0	0	0	0	0	0	0	9
N15-6	0	0	0	0	0	0	0	0	0	0	9
N15-7	0	0	0	0	0	0	0	0	0	0	9
N15-8	0	0	0	0	0	0	0	0	0	0	9
N15-9	1	1	1	1	1	1	1	1	1	9	0
N15-10	1	1	1	1	1	1	1	1	X	8	0
N15-11	1	1	1	1	1	1	1	1	1	9	0
N15-12	0	0	0	0	0	0	0	0	0	0	9
N15-13	1	1	1	1	1	1	1	1	X	8	0
N15-14	1	1	1	1	1	1	1	1	1	9	0
N15-15	1	1	1	1	1	1	1	1	1	9	0
N15-16	0	0	0	0	0	0	0	0	0	0	9
N15-17	1	1	1	1	1	1	1	1	1	9	0
N15-18	0	0	0	0	0	0	0	0	0	0	9
N15-19	0	0	0	0	0	0	0	0	0	0	9
N15-20	0	0	0	0	0	0	0	0	0	0	9
N15-21	0	0	0	0	0	0	0	0	0	0	9
N15-22	1	1	1	1	1	1	1	1	1	9	0
N15-25	0	0	0	0	0	0	0	0	0	0	9
N15-26	0	0	0	0	0	0	0	0	0	0	9
N15-27	0	0	0	0	0	0	0	0	0	0	9
N15-29	1	1	1	1	1	1	1	1	1	9	0
N15-30	1	0	1	1	1	1	1	1	1	8	1
N15-31A-	0	0	0	0	0	0	0	0	0	0	9
N15-31A+	1	1	1	1	1	1	1	1	1	9	0
N15-32	1	1	1	1	1	1	1	1	1	9	0
N15-33	0	0	0	0	0	0	0	0	0	0	9
N15-34	0	0	0	0	0	0	0	0	0	0	9
N15-35	1	1	1	1	1	1	1	1	1	9	0
N15-36	0	0	0	0	0	0	0	0	X	0	8
N15-37	0	0	0	0	0	0	0	0	0	0	9
N15-38	0	0	0	0	0	0	0	0	0	0	9
N15-39	1	1	1	1	1	1	1	1	1	9	0

APPENDIX 2B (continued).

LINE (E8-216bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N15-1	1	1	1	1	1	1	1	1	1	9	0
N15-2	1	1	1	1	1	1	0	1	1	8	1
N15-3	0	0	0	0	0	0	0	0	0	0	9
N15-4	0	0	0	0	0	0	0	0	0	0	9
N15-6	1	1	1	1	1	1	1	1	1	9	0
N15-7	0	0	0	0	0	0	0	0	0	0	9
N15-8	0	0	0	0	0	0	0	0	0	0	9
N15-9	1	1	1	1	1	1	1	1	1	9	0
N15-10	0	0	0	0	0	0	0	0	X	0	8
N15-11	1	1	1	1	1	1	1	1	1	9	0
N15-12	0	0	0	0	0	0	0	0	0	0	9
N15-13	1	1	1	1	1	1	1	1	X	8	0
N15-14	1	1	1	1	1	1	1	1	1	9	0
N15-15	1	1	1	1	1	1	1	1	1	9	0
N15-16	1	1	1	1	1	1	1	1	1	9	0
N15-17	0	0	0	0	0	0	0	0	0	0	9
N15-18	0	0	0	0	0	0	0	0	0	0	9
N15-19	1	1	1	1	1	1	1	1	1	9	0
N15-20	1	1	1	1	1	1	1	1	1	9	0
N15-21	0	0	0	0	0	0	0	0	0	0	9
N15-22	0	0	0	0	0	0	0	0	0	0	9
N15-25	1	1	1	1	1	1	1	1	1	9	0
N15-26	1	1	1	1	1	1	1	1	1	9	0
N15-27	0	0	0	0	0	0	0	0	0	0	9
N15-29	1	1	1	1	1	1	1	1	1	9	0
N15-30	1	1	1	1	1	1	1	1	1	9	0
N15-31A-	1	1	1	1	1	1	1	1	1	9	0
N15-31A+	0	0	0	0	0	0	0	0	0	0	9
N15-32	0	0	0	0	0	0	0	0	0	0	9
N15-33	0	0	0	0	0	0	0	0	0	0	9
N15-34	1	1	1	1	1	1	1	1	1	9	0
N15-35	0	0	0	0	0	0	0	0	0	0	9
N15-36	1	1	1	1	1	1	1	0	X	7	1
N15-37	0	0	0	0	0	0	0	0	0	0	9
N15-38	1	1	1	1	1	1	1	1	1	9	0
N15-39	0	1	1	1	1	1	1	1	1	8	1

APPENDIX 2B (continued).

LINE (E8-187bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N15-1	1	1	1	1	1	1	1	1	1	9	0
N15-2	1	1	1	1	1	1	1	1	1	9	0
N15-3	0	0	0	0	0	0	0	0	0	0	9
N15-4	0	0	0	1	0	0	0	0	0	1	8
N15-6	0	0	0	0	0	0	0	0	0	0	9
N15-7	1	1	1	1	1	1	1	1	1	9	0
N15-8	1	1	1	1	1	1	1	1	1	9	0
N15-9	1	1	1	1	1	1	1	1	1	9	0
N15-10	0	0	0	0	0	0	0	0	X	0	8
N15-11	1	1	1	1	1	1	1	1	1	9	0
N15-12	0	0	0	0	0	0	0	0	1	1	8
N15-13	1	1	1	1	1	1	1	1	X	8	0
N15-14	0	0	0	0	0	0	0	0	0	0	9
N15-15	0	0	0	0	0	0	0	0	0	0	9
N15-16	0	0	0	0	0	0	0	0	0	0	9
N15-17	0	0	0	0	0	0	0	0	0	0	9
N15-18	1	1	1	1	1	1	1	1	1	9	0
N15-19	1	1	1	1	1	1	1	1	1	9	0
N15-20	0	0	0	0	0	0	0	0	0	0	9
N15-21	0	0	0	0	0	0	0	0	0	0	9
N15-22	0	0	0	0	0	0	0	0	0	0	9
N15-25	0	0	0	0	0	0	0	0	0	0	9
N15-26	1	1	1	1	1	1	1	1	1	9	0
N15-27	0	0	0	0	0	0	0	0	0	0	9
N15-29	0	0	0	0	0	0	0	0	0	0	9
N15-30	0	0	1	1	1	1	1	1	1	7	2
N15-31A-	1	1	1	1	1	1	1	1	1	9	0
N15-31A+	0	0	0	0	0	0	0	0	0	0	9
N15-32	0	0	0	0	0	0	0	0	0	0	9
N15-33	0	0	0	0	0	0	0	0	0	0	9
N15-34	1	1	1	1	1	1	1	1	1	9	0
N15-35	0	0	0	0	0	0	0	0	0	0	9
N15-36	1	1	1	1	1	1	1	1	X	8	0
N15-37	0	0	0	0	0	0	0	0	0	0	9
N15-38	1	1	1	1	1	1	1	1	1	9	0
N15-39	1	0	0	0	0	0	0	0	0	1	8

APPENDIX 2B (continued).

LINE (E8-120bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N15-1	0	0	0	0	0	0	0	0	0	0	9
N15-2	0	0	0	0	0	0	0	0	0	0	9
N15-3	0	0	0	0	0	0	0	0	0	0	9
N15-4	0	0	0	0	0	0	0	0	0	0	9
N15-6	1	1	1	1	1	1	1	1	1	9	0
N15-7	1	1	1	1	1	1	1	1	1	9	0
N15-8	0	0	0	0	0	0	0	0	0	0	9
N15-9	0	0	0	0	0	0	0	0	0	0	9
N15-10	1	1	1	1	1	1	1	1	X	8	0
N15-11	0	0	0	0	0	0	0	0	0	0	9
N15-12	0	0	0	0	0	0	0	0	0	0	9
N15-13	0	0	0	0	0	0	0	0	X	0	8
N15-14	0	0	0	0	0	0	0	0	0	0	9
N15-15	1	1	1	1	1	1	1	1	1	9	0
N15-16	0	0	0	0	0	0	0	0	0	0	9
N15-17	0	0	0	0	0	0	0	0	0	0	9
N15-18	0	0	0	0	0	0	0	0	0	0	9
N15-19	0	0	0	0	0	0	0	0	0	0	9
N15-20	1	1	1	1	1	1	1	1	1	9	0
N15-21	1	1	1	1	1	1	1	1	1	9	0
N15-22	1	1	1	1	1	1	1	1	1	9	0
N15-25	0	0	0	0	0	0	0	0	0	0	9
N15-26	0	0	0	0	0	0	0	0	0	0	9
N15-27	0	0	0	0	0	0	0	0	0	0	9
N15-29	1	1	1	1	1	1	1	1	1	9	0
N15-30	0	0	0	0	0	0	0	0	0	0	9
N15-31A-	1	1	1	1	1	1	1	1	1	9	0
N15-31A+	0	0	0	0	0	0	0	0	0	0	9
N15-32	0	0	0	0	0	0	0	0	0	0	9
N15-33	0	0	0	0	0	0	0	0	0	0	9
N15-34	1	1	1	1	1	1	1	1	1	9	0
N15-35	1	0	0	0	1	0	0	0	0	2	7
N15-36	0	0	0	0	0	0	0	0	X	0	8
N15-37	0	0	0	0	0	0	0	0	0	0	9
N15-38	0	0	0	0	0	0	0	0	0	0	9
N15-39	0	1	1	1	1	1	1	1	1	8	1

APPENDIX 2B (continued).

LINE (E8-068bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N15-1	0	1	1	1	1	1	1	1	1	8	1
N15-2	0	0	0	0	0	0	0	0	0	0	9
N15-3	1	1	1	1	1	1	1	1	1	9	0
N15-4	1	1	1	1	1	1	1	1	1	9	0
N15-6	0	0	0	0	0	0	0	0	0	0	9
N15-7	1	1	1	1	1	1	1	1	1	9	0
N15-8	1	1	1	1	1	1	1	1	1	9	0
N15-9	1	1	1	1	1	1	1	1	1	9	0
N15-10	1	1	1	1	1	1	1	1	X	8	0
N15-11	1	1	1	1	1	1	1	1	1	9	0
N15-12	0	0	0	0	0	0	0	0	1	1	8
N15-13	1	1	1	1	1	1	1	1	X	8	0
N15-14	1	1	1	1	1	1	1	1	1	9	0
N15-15	1	1	1	1	1	1	1	1	1	9	0
N15-16	0	0	0	0	0	0	0	0	0	0	9
N15-17	1	1	1	1	1	1	1	1	1	9	0
N15-18	1	1	1	1	1	1	1	1	1	9	0
N15-19	1	1	1	1	1	1	1	1	1	9	0
N15-20	1	1	1	1	1	1	1	1	1	9	0
N15-21	1	1	1	1	1	0	1	1	0	7	2
N15-22	1	0	0	0	0	0	0	0	0	1	8
N15-25	1	1	1	1	1	1	1	1	1	9	0
N15-26	1	1	1	1	1	1	1	1	1	9	0
N15-27	1	1	1	1	1	1	1	1	1	9	0
N15-29	1	1	1	1	1	1	1	1	1	9	0
N15-30	1	0	0	0	0	0	0	0	0	1	8
N15-31A-	1	1	1	1	1	1	1	1	1	9	0
N15-31A+	1	1	1	1	1	1	1	1	1	9	0
N15-32	1	1	1	1	1	1	1	1	1	9	0
N15-33	1	1	1	1	1	1	1	1	1	9	0
N15-34	1	0	0	0	0	0	0	0	0	1	8
N15-35	0	0	0	0	0	0	0	0	0	0	9
N15-36	1	1	1	1	1	1	1	1	X	8	0
N15-37	0	0	0	0	0	0	0	0	0	0	9
N15-38	1	1	1	1	1	1	1	1	1	9	0
N15-39	1	0	0	0	0	0	0	0	0	1	8

APPENDIX 2B (continued).

LINE (E7-300bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N15-1	0	0	0	0	0	0	0	0	0	0	9
N15-2	0	0	0	0	0	0	0	0	0	0	9
N15-3	1	1	1	1	1	1	1	1	1	9	0
N15-4	0	0	0	0	0	0	0	0	0	0	9
N15-6	0	0	0	0	0	0	0	0	0	0	9
N15-7	1	1	1	1	1	1	1	1	1	9	0
N15-8	1	1	1	1	1	1	1	1	1	9	0
N15-9	0	0	0	0	0	0	0	0	0	0	9
N15-10	0	0	0	0	0	0	0	0	X	0	8
N15-11	1	1	1	1	1	1	1	1	1	9	0
N15-12	1	1	1	1	1	1	1	1	1	9	0
N15-13	1	1	1	1	1	1	1	1	X	8	0
N15-14	0	0	0	0	0	0	0	0	0	0	9
N15-15	0	0	0	0	0	0	0	0	0	0	9
N15-16	0	0	0	0	0	0	0	0	0	0	9
N15-17	0	0	0	0	0	0	0	0	0	0	9
N15-18	0	0	0	0	0	0	0	0	0	0	9
N15-19	1	1	1	1	1	1	1	1	1	9	0
N15-20	0	0	0	0	0	0	0	0	0	0	9
N15-21	1	1	1	1	1	1	1	1	1	9	0
N15-22	0	0	0	0	0	0	0	0	0	0	9
N15-25	0	0	0	0	0	0	0	0	0	0	9
N15-26	1	1	1	1	1	1	1	1	1	9	0
N15-27	0	0	0	0	0	0	0	0	0	0	9
N15-29	1	1	1	1	1	1	1	1	1	9	0
N15-30	1	1	1	1	1	1	1	1	1	9	0
N15-31A-	1	1	1	1	1	1	1	1	1	9	0
N15-31A+	0	0	0	0	0	0	0	0	0	0	9
N15-32	0	0	0	0	0	0	0	0	0	0	9
N15-33	0	0	0	0	0	0	0	0	0	0	9
N15-34	0	0	0	0	0	0	0	0	0	0	9
N15-35	0	0	0	0	0	0	0	0	0	0	9
N15-36	0	0	0	0	0	0	0	0	X	0	8
N15-37	0	0	0	0	0	0	0	0	0	0	9
N15-38	1	1	1	1	1	1	1	1	1	9	0
N15-39	0	0	0	0	0	0	0	0	0	0	9

APPENDIX 2B (continued).

LINE (E7-183bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N15-1	1	1	1	1	1	1	1	1	1	9	0
N15-2	1	1	1	1	1	1	1	1	1	9	0
N15-3	0	0	0	0	0	0	0	0	0	0	9
N15-4	1	1	1	1	1	1	1	1	1	9	0
N15-6	0	0	0	0	0	0	0	0	0	0	9
N15-7	0	0	0	0	0	0	0	0	0	0	9
N15-8	1	1	1	1	1	1	1	1	1	9	0
N15-9	1	1	1	1	1	1	1	1	1	9	0
N15-10	1	1	1	1	1	1	1	1	X	8	0
N15-11	0	0	0	0	0	0	0	0	0	0	9
N15-12	1	1	1	1	1	1	1	1	1	9	0
N15-13	0	0	0	0	0	0	0	0	X	0	8
N15-14	0	0	0	0	0	0	0	0	0	0	9
N15-15	1	1	1	1	1	1	1	1	1	9	0
N15-16	1	1	1	1	1	1	1	1	1	9	0
N15-17	0	0	0	0	0	0	0	0	0	0	9
N15-18	0	0	0	0	0	0	0	0	0	0	9
N15-19	1	1	1	1	1	1	1	1	1	9	0
N15-20	0	0	0	0	0	0	0	0	0	0	9
N15-21	0	0	0	0	0	0	0	0	0	0	9
N15-22	0	0	0	0	0	0	0	0	0	0	9
N15-25	0	0	0	0	0	0	0	0	0	0	9
N15-26	1	1	1	1	1	1	1	1	1	9	0
N15-27	1	1	1	1	1	1	1	1	1	9	0
N15-29	1	1	1	1	1	1	1	1	1	9	0
N15-30	1	1	1	1	1	1	1	1	1	9	0
N15-31A-	0	0	0	0	0	0	0	0	0	0	9
N15-31A+	1	1	1	1	1	1	1	1	1	9	0
N15-32	1	1	1	1	1	1	1	1	1	9	0
N15-33	1	1	1	1	1	1	1	1	1	9	0
N15-34	0	0	0	0	0	0	0	0	0	0	9
N15-35	0	0	0	0	0	0	0	0	0	0	9
N15-36	0	0	0	0	0	0	0	0	X	0	8
N15-37	0	0	0	0	0	0	0	0	0	0	9
N15-38	1	1	1	1	1	1	1	1	1	9	0
N15-39	0	0	0	0	0	0	0	0	0	0	9

APPENDIX 2B (continued).

LINE (E7-166bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N15-1	1	1	1	1	1	1	1	1	1	9	0
N15-2	1	1	1	1	1	1	1	1	1	9	0
N15-3	0	0	0	0	0	0	0	0	0	0	9
N15-4	0	0	0	0	0	0	0	0	0	0	9
N15-6	0	0	0	0	0	0	0	0	0	0	9
N15-7	0	0	0	0	0	0	0	0	0	0	9
N15-8	1	1	1	1	1	1	1	1	1	9	0
N15-9	1	1	1	1	1	1	1	1	1	9	0
N15-10	1	1	1	1	1	1	1	1	X	8	0
N15-11	0	1	1	1	1	1	1	1	1	8	1
N15-12	1	1	1	1	1	1	1	1	1	9	0
N15-13	1	1	1	1	1	1	1	1	X	8	0
N15-14	1	1	0	0	0	0	0	0	0	2	7
N15-15	1	1	1	1	1	1	1	1	1	9	0
N15-16	1	1	1	1	1	1	1	1	1	9	0
N15-17	0	0	0	0	0	0	0	0	0	0	9
N15-18	0	0	0	0	0	0	0	0	0	0	9
N15-19	1	1	1	1	1	1	1	1	1	9	0
N15-20	0	0	0	0	0	0	0	0	0	0	9
N15-21	0	0	0	0	0	0	0	0	0	0	9
N15-22	0	0	0	0	0	0	0	0	0	0	9
N15-25	1	1	1	1	1	1	1	1	1	9	0
N15-26	1	1	1	1	1	1	1	1	1	9	0
N15-27	1	1	1	1	1	1	1	1	1	9	0
N15-29	1	1	1	1	1	1	1	1	1	9	0
N15-30	1	1	1	1	1	1	1	1	1	9	0
N15-31A-	1	1	1	1	1	1	1	1	1	9	0
N15-31A+	1	1	1	1	1	1	1	1	1	9	0
N15-32	1	1	1	1	1	1	1	1	1	9	0
N15-33	1	1	1	1	1	1	1	1	1	9	0
N15-34	1	0	0	0	0	0	0	0	0	1	8
N15-35	0	0	0	0	0	0	0	0	0	0	9
N15-36	0	0	0	0	0	0	0	0	X	0	8
N15-37	1	1	1	1	1	1	1	1	1	9	0
N15-38	1	1	1	1	1	1	1	1	1	9	0
N15-39	1	0	0	0	0	0	0	0	0	1	8

APPENDIX 2B (continued).

LINE (E7-147bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N15-1	1	1	1	1	1	1	1	1	1	9	0
N15-2	0	0	0	0	0	0		0	0	0	9
N15-3	1	1	1	1	1	1	1	1	1	9	0
N15-4	0	0	0	0	0	0	0	0	0	0	9
N15-6	1	1	1	1	1	1	1	1	1	9	0
N15-7	0	0	0	0	0	0	0	0	0	0	9
N15-8	0	0	0	0	0	0	0	0	0	0	9
N15-9	0	0	0	0	0	0	0	0	0	0	9
N15-10	1	1	1	1	1	1	1	1	X	8	0
N15-11	0	0	0	0	0	0	0	0	0	0	9
N15-12	0	0	0	0	0	0	0	0	0	0	9
N15-13	0	0	0	0	0	0	0	0	X	0	8
N15-14	1	1	1	1	1	1	1	1	1	9	0
N15-15	1	1	1	1	1	1	1	1	1	9	0
N15-16	0	0	0	0	0	0	0	0	0	0	9
N15-17	0	0	0	0	0	0	0	0	0	0	9
N15-18	0	0	0	0	0	0	0	0	0	0	9
N15-19	0	0	0	0	0	0	0	0	0	0	9
N15-20	0	0	0	0	0	0	0	0	0	0	9
N15-21	0	0	0	0	0	0	0	0	0	0	9
N15-22	1	1	1	1	1	1	1	1	1	9	0
N15-25	0	0	0	0	0	0	0	0	0	0	9
N15-26	0	0	0	0	0	0	0	0	0	0	9
N15-27	1	1	1	1	1	1	1	1	1	9	0
N15-29	0	0	0	0	1	1	1	1	1	5	4
N15-30	1	0	1	1	1	1	1	1	1	8	1
N15-31A-	1	1	1	1	1	1	1	1	1	9	0
N15-31A+	0	0	0	0	0	0	0	0	0	0	9
N15-32	0	0	0	0	0	0	1	1	1	3	6
N15-33	1	1	1	1	1	1	1	1	1	9	0
N15-34	0	0	0	0	0	0	0	0	0	0	9
N15-35	1	1	1	1	1	1	1	1	1	9	0
N15-36	0	0	0	0	0	0	0	0	X	0	8
N15-37	0	0	0	0	0	0	0	0	0	0	9
N15-38	1	1	1	1	1	1	1	1	1	9	0
N15-39	1	1	1	1	1	1	1	1	1	9	0

APPENDIX 2B (continued).

LINE (E7-117bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N15-1	1	1	1	1	1	1	1	1	1	9	0
N15-2	1	1	1	1	1	1	1	1	1	9	0
N15-3	0	0	0	0	0	0	0	0	0	0	9
N15-4	0	0	0	0	0	0	0	0	0	0	9
N15-6	1	1	1	1	1	1	1	1	1	9	0
N15-7	0	0	0	0	0	0	0	0	0	0	9
N15-8	0	0	0	0	0	0	0	0	0	0	9
N15-9	0	0	0	0	0	0	0	0	0	0	9
N15-10	1	1	1	1	1	1	1	1	X	8	0
N15-11	0	0	0	0	0	0	0	0	0	0	9
N15-12	1	1	1	1	1	1	1	1	1	9	0
N15-13	0	0	0	0	0	0	0	0	X	0	8
N15-14	0	0	0	0	0	0	0	0	0	0	9
N15-15	0	0	0	0	0	0	0	0	0	0	9
N15-16	1	1	1	1	1	1	1	1	1	9	0
N15-17	0	0	0	0	0	0	0	0	0	0	9
N15-18	1	1	1	1	1	1	1	1	1	9	0
N15-19	1	1	1	1	1	1	1	1	1	9	0
N15-20	0	0	0	0	0	0	0	0	0	0	9
N15-21	0	0	0	0	0	0	0	0	0	0	9
N15-22	0	0	0	0	0	0	0	0	0	0	9
N15-25	0	0	0	0	0	0	0	0	0	0	9
N15-26	1	1	1	1	1	1	1	1	1	9	0
N15-27	0	0	0	0	0	0	0	0	0	0	9
N15-29	0	0	0	0	0	0	0	0	0	0	9
N15-30	1	1	1	1	1	1	1	1	1	9	0
N15-31A-	1	1	1	1	1	1	1	1	1	9	0
N15-31A+	0	0	0	0	0	0	0	0	0	0	9
N15-32	0	0	0	0	0	0	0	0	0	0	9
N15-33	0	0	0	0	0	0	0	0	0	0	9
N15-34	0	0	0	0	0	0	0	0	0	0	9
N15-35	0	0	0	0	0	0	0	0	0	0	9
N15-36	1	1	1	1	1	1	1	1	X	8	0
N15-37	0	0	0	0	0	0	0	0	0	0	9
N15-38	0	0	0	0	0	0	0	0	0	0	9
N15-39	1	1	1	1	1	1	1	1	1	9	0

APPENDIX 2B (continued).

LINE (E7-094bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N15-1	1	1	1	1	1	1	1	1	1	9	0
N15-2	1	1	1	1	1	1	1	1	1	9	0
N15-3	0	0	0	0	0	0	0	0	0	0	9
N15-4	1	1	1	1	1	1	1	1	1	9	0
N15-6	1	1	1	1	1	1	1	1	1	9	0
N15-7	1	1	1	1	1	1	1	1	1	9	0
N15-8	0	0	0	0	0	0	0	0	0	0	9
N15-9	0	0	0	0	0	0	0	0	0	0	9
N15-10	1	1	1	1	1	1	1	1	X	8	0
N15-11	1	1	1	1	1	1	1	1	1	9	0
N15-12	0	0	0	0	0	0	0	0	0	0	9
N15-13	1	1	1	1	1	1	1	1	X	8	0
N15-14	1	1	1	1	1	1	1	1	1	9	0
N15-15	0	0	0	0	0	0	0	0	0	0	9
N15-16	0	0	0	0	0	0	0	0	0	0	9
N15-17	0	0	0	0	0	0	0	0	0	0	9
N15-18	0	0	0	0	0	0	0	0	0	0	9
N15-19	0	0	0	0	0	0	0	0	0	0	9
N15-20	1	1	1	1	1	1	1	1	1	9	0
N15-21	0	0	0	0	0	0	0	0	0	0	9
N15-22	0	0	0	0	0	0	0	0	0	0	9
N15-25	0	0	0	0	0	0	0	0	0	0	9
N15-26	0	0	0	0	0	0	0	0	0	0	9
N15-27	1	1	1	1	1	1	1	1	1	9	0
N15-29	1	1	1	1	1	1	1	1	1	9	0
N15-30	0	0	0	0	0	0	0	0	0	0	9
N15-31A-	1	1	0	0	1	0	0	0	0	3	6
N15-31A+	1	1	1	1	1	1	1	1	1	9	0
N15-32	0	0	0	1	1	1	1	1	1	6	3
N15-33	1	1	1	1	1	1	1	1	1	9	0
N15-34	1	1	1	1	1	1	1	1	1	9	0
N15-35	0	0	0	0	0	0	0	0	0	0	9
N15-36	0	0	0	0	0	0	0	0	X	0	8
N15-37	1	1	1	1	1	1	1	1	1	9	0
N15-38	1	1	0	1	1	1	1	1	1	8	1
N15-39	0	0	0	0	0	0	0	0	0	0	9

APPENDIX 2B (continued).

LINE (E7-066bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N15-1	1	1	1	1	1	1	1	1	1	9	0
N15-2	0	0	0	0	0	0	0	0	0	0	9
N15-3	1	1	1	1	1	1	1	1	1	9	0
N15-4	1	1	1	1	1	1	1	1	1	9	0
N15-6	1	1	1	1	1	1	1	1	1	9	0
N15-7	1	1	1	1	1	1	1	1	1	9	0
N15-8	1	1	1	1	1	1	1	1	1	9	0
N15-9	0	0	0	0	0	0	0	0	0	0	9
N15-10	1	1	1	1	1	1	1	1	X	8	0
N15-11	1	1	1	1	1	1	1	1	1	9	0
N15-12	1	1	1	1	1	1	1	1	1	9	0
N15-13	1	1	1	1	1	1	1	1	X	8	0
N15-14	0	0	0	0	0	0	0	0	0	0	9
N15-15	1	1	1	1	1	1	1	1	1	9	0
N15-16	0	0	0	0	0	0	0	0	0	0	9
N15-17	0	0	0	0	0	0	0	0	0	0	9
N15-18	1	1	1	1	1	1	1	1	1	9	0
N15-19	1	1	1	1	1	1	1	1	1	9	0
N15-20	1	1	1	1	1	1	1	1	1	9	0
N15-21	0	0	0	0	0	0	0	0	0	0	9
N15-22	1	1	1	1	1	1	1	1	1	9	0
N15-25	1	1	1	1	1	1	1	1	1	9	0
N15-26	1	1	1	1	1	1	1	1	1	9	0
N15-27	1	1	1	1	1	1	1	1	1	9	0
N15-29	1	1	1	1	1	1	1	1	1	9	0
N15-30	1	1	1	1	1	1	1	1	1	9	0
N15-31A-	1	1	1	1	1	1	1	1	1	9	0
N15-31A+	1	1	1	1	1	1	1	1	1	9	0
N15-32	1	1	1	1	1	1	1	1	1	9	0
N15-33	1	1	1	1	1	1	1	1	1	9	0
N15-34	1	1	1	1	1	1	1	1	1	9	0
N15-35	0	0	0	0	0	0	0	0	0	0	9
N15-36	1	1	1	1	1	1	1	1	X	8	0
N15-37	1	1	1	1	1	1	1	1	1	9	0
N15-38	1	1	1	1	1	1	1	1	1	9	0
N15-39	0	0	0	0	0	0	0	0	0	0	9

APPENDIX 2B (continued).

LINE (C8-271bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N15-1	0	0	0	0	0	0	0	0	0	0	9
N15-2	0	0	0	0	0	0	0	0	0	0	9
N15-3	0	0	0	0	0	0	0	0	0	0	9
N15-4	0	0	0	0	0	0	0	0	0	0	9
N15-6	0	0	0	0	0	0	0	0	0	0	9
N15-7	0	0	0	0	0	0	0	0	0	0	9
N15-8	0	0	0	0	0	0	0	0	0	0	9
N15-9	1	0	1	1	1	1	1	1	1	8	1
N15-10	1	1	1	1	1	1	1	1	X	8	0
N15-11	1	1	1	1	1	1	1	1	1	9	0
N15-12	0	0	0	0	0	0	0	0	0	0	9
N15-13	1	1	1	1	1	1	1	1	X	8	0
N15-14	1	1	1	1	1	1	1	1	1	9	0
N15-15	1	1	1	1	0	0	1	1	1	7	2
N15-16	0	0	0	0	0	0	0	0	0	0	9
N15-17	1	1	1	1	1	1	1	1	1	9	0
N15-18	0	0	0	0	0	0	0	0	0	0	9
N15-19	1	1	1	1	1	1	1	1	1	9	0
N15-20	0	0	0	0	0	0	0	0	0	0	9
N15-21	0	0	0	0	0	0	0	0	0	0	9
N15-22	1	1	1	1	1	1	1	1	1	9	0
N15-25	0	0	0	0	0	0	0	0	0	0	9
N15-26	1	1	1	1	1	0	1	1	1	8	1
N15-27	1	1	1	1	1	1	1	1	1	9	0
N15-29	1	1	1	1	1	1	1	1	1	9	0
N15-30	1	0	0	0	0	0	0	0	0	1	8
N15-31A-	0	0	0	0	0	0	0	0	0	0	9
N15-31A+	1	1	1	1	1	1	1	1	1	9	0
N15-32	0	0	0	0	0	0	0	0	0	0	9
N15-33	0	0	0	0	0	0	0	0	0	0	9
N15-34	1	1	1	1	1	1	1	1	1	9	0
N15-35	0	0	0	0	0	0	0	0	0	0	9
N15-36	0	0	0	0	0	0	0	0	X	0	8
N15-37	1	1	1	1	1	1	0	1	1	8	1
N15-38	1	1	1	1	1	1	1	1	1	9	0
N15-39	1	1	1	1	1	1	1	1	1	9	0

APPENDIX 2B (continued).

LINE (C8-146bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N15-1	1	1	1	1	1	1	1	1	1	9	0
N15-2	1	1	1	1	1	1	1	1	1	9	0
N15-3	1	1	1	1	1	1	1	1	1	9	0
N15-4	1	1	1	1	1	1	1	1	1	9	0
N15-6	0	0	0	0	0	0	0	0	0	0	9
N15-7	0	0	0	0	0	0	0	0	0	0	9
N15-8	0	0	0	0	0	0	0	0	0	0	9
N15-9	0	0	0	0	0	0	0	0	0	0	9
N15-10	0	0	0	0	0	0	0	0	X	0	8
N15-11	0	0	0	0	0	0	0	0	0	0	9
N15-12	0	1	1	1	1	1	1	1	1	8	1
N15-13	0	0	0	0	0	0	0	0	X	0	8
N15-14	0	0	0	0	0	0	0	0	0	0	9
N15-15	1	1	1	1	1	1	1	1	1	9	0
N15-16	1	1	1	1	1	1	1	1	1	9	0
N15-17	1	1	1	1	1	1	1	1	1	9	0
N15-18	1	1	1	1	1	1	1	1	1	9	0
N15-19	1	1	1	1	1	1	1	1	1	9	0
N15-20	0	0	0	0	0	0	0	0	0	0	9
N15-21	0	0	0	0	0	0	0	0	0	0	9
N15-22	0	0	0	0	0	0	0	0	0	0	9
N15-25	0	0	0	0	0	0	0	0	0	0	9
N15-26	1	1	1	1	1	0	1	1	1	8	1
N15-27	0	0	0	0	0	0	0	0	0	0	9
N15-29	0	0	0	0	0	0	0	0	0	0	9
N15-30	0	0	1	1	1	1	1	1	1	7	2
N15-31A-	0	0	0	0	0	0	0	0	0	0	9
N15-31A+	1	1	1	1	1	1	1	1	1	9	0
N15-32	1	1	1	1	1	1	1	1	1	9	0
N15-33	1	1	1	1	1	1	1	1	1	9	0
N15-34	1	0	0	0	0	0	0	0	0	1	8
N15-35	1	1	1	1	1	1	1	1	1	9	0
N15-36	1	1	1	1	1	1	1	1	X	8	0
N15-37	0	0	0	0	0	0	0	0	0	0	9
N15-38	0	0	0	0	0	0	0	0	0	0	9
N15-39	1	1	1	1	1	1	1	1	1	9	0

APPENDIX 2B (continued).

LINE (A6-091bp)	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9	Total no. of 1's	Total no. of 0's
N15-1	1	1	1	1	1	1	1	1	1	9	0
N15-2	1	1	1	1	1	1	1	1	1	9	0
N15-3	1	1	1	1	1	1	1	1	1	9	0
N15-4	0	1	0	0	1	1	0	1	1	5	4
N15-6	0	1	1	1	1	1	1	1	1	8	1
N15-7	1	1	1	1	1	1	1	1	1	9	0
N15-8	1	1	1	1	1	1	1	1	1	9	0
N15-9	0	0	0	0	0	0	0	0	0	0	9
N15-10	1	1	1	1	1	1	1	1	X	8	0
N15-11	0	0	0	0	0	0	0	0	0	0	9
N15-12	0	0	0	0	0	0	0	0	1	1	8
N15-13	0	0	0	0	0	0	0	0	X	0	8
N15-14	1	1	1	1	1	1	1	1	1	9	0
N15-15	0	0	0	0	0	0	0	0	0	0	9
N15-16	1	1	1	1	1	1	1	1	1	9	0
N15-17	0	0	0	0	0	0	0	0	0	0	9
N15-18	1	1	1	1	1	1	1	1	1	9	0
N15-19	0	0	0	0	0	0	0	0	0	0	9
N15-20	0	0	0	0	0	0	0	0	0	0	9
N15-21	0	0	0	0	0	0	0	0	0	0	9
N15-22	1	1	1	1	1	1	1	1	1	9	0
N15-25	1	0	0	0	0	0	0	0	0	1	8
N15-26	0	0	1	0	0	0	0	0	0	1	8
N15-27	0	1	1	1	1	1	1	1	1	8	1
N15-29	0	0	0	0	0	0	0	0	0	0	9
N15-30	1	1	1	1	1	1	1	1	1	9	0
N15-31A-	0	0	0	0	0	0	0	0	0	0	9
N15-31A+	0	0	0	0	0	0	0	0	0	0	9
N15-32	0	0	0	0	0	0	0	0	0	0	9
N15-33	0	0	0	0	0	0	0	0	0	0	9
N15-34	0	0	0	0	0	0	0	0	0	0	9
N15-35	1	1	1	1	1	1	1	1	1	9	0
N15-36	1	1	1	1	1	1	1	1	X	8	0
N15-37	1	1	1	1	1	1	1	1	1	9	0
N15-38	1	1	1	1	1	1	1	1	1	9	0
N15-39	1	1	1	1	1	1	1	1	1	9	0

APPENDIX 3A. Susceptibility of the double haploid 'Karena'/FHB 148 plants as determined by inoculation experiments. S = susceptible, R = resistant, X = plant did not flower.

LINE	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9
N16-1	S	S	S	S	S	S	S	S	S
N16-2	S	S	S	S	S	S	R	R	S
N16-3	S	R	S	S	S	S	S	S	S
N16-4	R	R	R	R	S	R	R	R	R
N16-5	S	S	S	S	R	S	R	X	S
N16-6	R	S	R	R	S	S	S	S	S
N16-7	S	S	S	S	S	S	S	S	S
N16-8	S	S	S	S	S	S	S	S	S
N16-9	S	S	S	S	S	S	S	S	S
N16-10	S	S	S	S	S	S	S	S	S
N16-11	S	S	S	S	S	S	S	S	S
N16-12	S	S	S	R	R	R	R	R	R
N16-13	R	R	S	S	R	R	R	R	R
N16-14	S	S	S	S	S	S	S	S	X
N16-15	S	S	S	S	S	S	S	S	X
N16-16	S	S	S	S	R	R	R	R	R
N16-17	R	R	R	R	R	R	R	R	R
N16-18	R	R	R	R	R	R	R	R	R
N16-19	R	R	R	R	R	R	R	R	S
N16-20	R	R	R	R	R	R	R	R	R
N16-21	R	R	R	R	R	S	R	R	R
N16-22	R	R	R	R	R	R	S	R	R
N16-23	R	R	R	R	R	R	R	R	R
N16-24	R	R	X	R	R	R	R	R	X
N16-25	R	R	R	R	R	R	R	R	X
N16-26	X	S	S	S	S	S	S	R	X

APPENDIX 3B. Susceptibility of the double haploid 'AC Carter'/FHB 148 plants as determined by inoculation experiments.

S = susceptible, R = resistant, X = plant did not flower, NR = no plant.

LINE	HEAD 1	HEAD 2	HEAD 3	HEAD 4	HEAD 5	HEAD 6	HEAD 7	HEAD 8	HEAD 9
N15-1	S	S	S	S	S	S	R	S	S
N15-2	S	R	R	S	R	S	R	R	R
N15-3	S	S	R	R	R	S	NR	R	R
N15-4	R	NR	NR	R	R	R	R	X	R
N15-6	R	S	X	R	S	R	R	S	X
N15-7	S	S	X	S	R	S	S	S	R
N15-8	S	S	S	S	S	S	S	S	S
N15-9	R	R	S	S	S	R	S	R	R
N15-10	S	S	S	S	S	S	S	S	X
N15-11	S	R	R	S	S	S	S	S	S
N15-12	S	S	S	S	S	S	S	S	S
N15-13	R	R	R	R	R	R	R	R	X
N15-14	R	R	R	R	S	R	R	R	S
N15-15	S	S	R	S	S	S	S	R	R
N15-16	R	R	R	R	R	R	R	R	R
N15-17	S	R	S	R	S	S	R	S	S
N15-18	R	X	S	S	S	X	NR	X	X
N15-19	X	S	X	X	X	X	X	R	R
N15-20	NR	X	S	S	R	X	R	R	X
N15-21	S	S	S	S	R	S	S	S	R
N15-22	S	S	S	S	S	S	S	S	S
N15-25	S	S	S	R	S	S	S	S	S
N15-26	S	S	R	S	S	S	S	R	S
N15-27	R	S	S	R	R	R	S	NR	R
N15-29	R	R	X	R	S	S	S	NR	S
N15-30	S	S	R	NR	R	R	R	S	S
N15-31A-	S	R	X	R	X	X	X	S	S
N15-31A+	X	X	R	X	R	R	X	X	X
N15-32	X	X	R	X	X	X	R	X	X
N15-33	S	R	R	X	X	X	S	R	X
N15-34	S	X	R	S	R	X	X	R	R
N15-35	S	S	S	S	S	S	S	S	R
N15-36	S	S	S	S	R	S	R	S	S
N15-37	R	R	R	R	R	R	X	S	R
N15-38	R	S	R	R	R	R	R	R	R
N15-39	R	R	R	R	S	R	S	R	R