A HOST-PATHOGEN STUDY OF STRIPE RUST RESISTANCE IN *TRITICUM AESTIVUM*

By

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CONTENTS

ABBREVIATION AND ACRONYMS

A	absorbance
AFLP	amplified fragment length polymorphism
APR	adult plant resistance
ASSV	aborted substomatal vesicle
bp	base pair
BSA	bovine serum agglutinin
CTAB	cetyl trimethyl ammonium bromide
CIMMYT	International Maize and Wheat Improvement Centre
cm	centimeter
cM	centi-Morgan
DNA	deoxyribonucleic acid
dpi	days post-inoculation
et al.	et alii (and others)
e.g.	example
Fig.	Figure
G	germ tube
g	gram
f. sp.	forma specialis
H	haustorium
HMC	haustorium mother cell
HN	haustorium neck
h	hour
hpi	hours post-inoculation
IH	infection hypha
kDA	kilodalton
kV	kilovolts
Lr	leaf rust resistance gene
L	Litre
M	molar
MAS	marker-assisted selection

µg	microgram
µm	micrometer
$\mu molm^{-2}s^{-1}$	micromole per meter square per second
ml	milliliter
mg	milligram
nm	nanometer
PAR	paraxial
PCR	polymerase chain reaction
pH	percentage hydrogen
PIH	primary infection hypha
QTL	quantitative trait loci
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
rpm	revolutions per minute
S	stoma
SCAR	sequenced characterised amplified region
SEM	scanning electron microscope
Sr	stem rust resistance gene
SSV	substomatal vesicle
SSVI	substomatal vesicle initial
STS	sequence tagged site
Taq	*Thermus aquaticus*
TE	Tris/EDTA
U	urediospores
UFS	University of Free State
UV	ultraviolet light
v/v	volume per volume
w/v	weight per volume
Yr	stripe rust resistance gene
%	percentage
^{o}C	degree Celcius

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PREFACE

Understanding the genetics of host-pathogen interactions is essential towards the attainment of durable resistance in wheat cultivars. Knowledge of the mechanisms by which resistance may be conferred is necessary so that effective means of manipulating these interactions can be used in cultivars with poor resistance to pathogens.

Many disciplines aim to determine the interactions between hosts and their pathogens at different levels. Disease screening of cultivars to determine resistance to a pathogen aims to determine host-pathogen interactions at a genetic level while histological techniques have been routinely used to determine the infection process of pathogens and the infection structures they form. The use of molecular techniques aims to determine differences at a DNA level to identify markers to resistance genes that can be used in producing resistant cultivars.

This study sought to use various disciplines towards gaining more insight on the host-pathogen interaction of *Puccinia striiformis* f. sp. *tritici* in wheat. The ability to become an expert in every discipline in order to draw scientifically based conclusions was challenging and very often hindsight indicated better ways of achieving certain objectives. Weaknesses in the study have as far as possible been identified as these have formed an important part of the conclusions drawn. From a personal view, the study has taught me the importance of understanding and integrating information from various disciplines towards host-pathogen interaction of wheat and stripe rust.

Each chapter in this dissertation was prepared as a separate article and therefore some recurrence may occur. As a result, certain adaptations have been made to enhance the readability and integration of information as much as possible.

General Introduction

Plants form a vital part of our existence in the world today. Many believe that the plant kingdom is the most important of the five kingdoms as it meets almost all the basic needs of humans to inhabit the earth. Plants provide oxygen required to sustain life, a variable and nutrient-rich diet and are used to construct shelters against the harsh elements of this world. Following the implementation of early cultivation practises and the further development of these practises, certain crops have become important in terms of the world economy.

Wheat (*Triticum aestivum* L.), is an economically important crops both in terms of its consumption by millions across the world as well as its production and net income to commercial farmers. This prosperous relationship of product demand and supply is however not free of problems. Crops, like all other plants, are affected by many abiotic factors including different environmental conditions and biotic factors including pathogens and viruses. Plants and pathogens have co-evolved to forge both a mutualistic and parasitic relationship. The demise of the host is most often the end result of the relationship with a pathogen's continued existence depending on encountering of another suitable host.

Stripe rust, one of the most important diseases of wheat in the world is caused by *Puccinia striiformis* Westend. f. sp. *tritici*. This pathogen has resulted in major epidemics in almost all the wheat growing regions in the world. Following the introduction of stripe rust in South Africa in the Western Cape in 1996, the pathogen rapidly spread to all the wheat-growing regions of the country and reached epidemic proportions in the eastern Free State in 1998 with only a few commercially produced cultivars found to be resistant. The cultivar, Kariega, was found to display adult-plant resistance to stripe rust although deliberate selection for this trait had occurred.

The development of resistant cultivars and the identification of durable sources of resistance such as Kariega are deemed the most effective means to control wheat

pathogens. Disease management strategies such as the use of chemicals and the implementation of cultural practises aimed at breaking the life cycle of the pathogen can be used to control epidemic outbreaks of a pathogen. The cost of fungicide application and the long-term effects on the environment does not qualify these practises as durable solutions to manage these destructive pathogens. Gene pyramiding (accumulation of resistance genes) could be a method of creating cultivars with durable resistance.

The success of breeding for durable resistance is directly dependant on sound information regarding the genetic variability of both pathogen and host proposed anticipatory breeding for resistance to rust as a disease management strategy. This is defined as the process of predicting future pathotypes and producing resistant germplasm to prevent future losses. Such an approach would be dependant on a national pathotype surveillance program and information (biochemical and genetic) regarding the development of new pathotypes from already existing pathotypes.

The nature of the host-pathogen interaction has been investigated using numerous strategies. Microscopy, long considered an invaluable biological tool has been extensively used to elucidate host-pathogen interactions at a cellular and sub-cellular level. The light microscope was initially the most commonly employed tool used to provide researchers with information regarding the growth pattern of the pathogen on the host's surface. The development of electron microscopy enabled researchers to not only visualise the entire infection process of a pathogen but also to visualise the exact structures formed during the introgression of the pathogen. Scanning and transmission electron microscopy has been extensively used to visualise the infection process of many rust fungi. Although electron microscopy has provided detailed structural studies regarding a pathogens mode of infection, the study of fixed cells does not allow for the visualisation of dynamic chemical and metabolic changes that occur during pathogen infection.

Fluorescence microscopy became more commonly used to elucidate host-pathogen interactions as the developments in fluorescent dyes have allowed greater

discrimination between fungal and plant structures. This type of microscopy is essentially employed to identify the hypersensitive response (HR) of the host cell to the manifestation of the pathogen within the host cell. The HR is characterised by rapidly localised pathogen induced cell death (necrosis) thus restricting the continued infection process of the pathogen and is deemed the most common expression of host resistance to the pathogen. This reaction is described as an incompatible interaction and has been shown to be easily determined with or without the use of fluorescent dyes as necrotic areas produce yellow auto-fluorescence under the conditions for fluorescence microscopy. Fungal development and host HR can thus be determined simultaneously, contributing valuable information to support studies on temporal and spatial aspects of the effect of resistance gene expression during fungal infection. The selection of differential fluorochromes and their application to fungal host-pathogen studies has been a major constraint in the application of fluorescence microscopy. Scanning and transmission electron microscopy has also been extensively employed to study the infection process and the infection structures formed by pathogens.

The most recent development in light microscopy, Confocal laser scanning microscopy (CLSM), holds great potential to further elucidate host-pathogen interactions. This technique combines the discriminatory power of the use of fluorescence together with laser technology and computer imaging in order to follow fungal infection development and the effect of resistance gene expression during the infection process.

The use of DNA markers has become common practise in many first world laboratories in an attempt to produce durably resistant cultivars against stripe rust. However, molecular research has mainly been confined to the host and its reaction to the pathogen while very little research has focussed on determining the genetic basis for pathogenicity.

Molecular markers for stripe, leaf and stem rust resistance genes have been developed using various fingerprinting techniques such as amplified fragment length

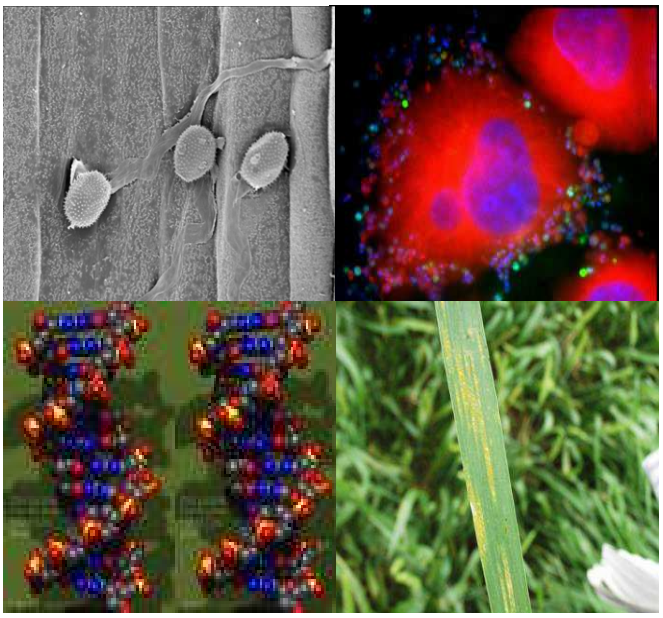
polymorphisms (AFLPs), restriction fragment length polymorphisms (RFLPs), simple sequence repeats (SSRs) and random amplified polymorphic DNA (RAPD). These techniques allow the discrimination of genetic differences at DNA level in their own unique way and hold both advantages and disadvantages. The choice of technique is dependant on the kind of information required and the expertise and equipment available.

It is clear that the concept of durable resistance requires the concerted efforts of researchers across the world from different disciplines to achieve an effective and complete understanding of host-pathogen interactions. The integration of information obtained from microscopy, molecular genetics and other types of investigations such as biochemical studies is most likely to play an increasingly important role in future studies of host-pathogen interactions.

The aim of this study was to introgress the *YrSp* seedling resistance gene into the South African wheat cultivar, Kariega. Secondly, to determine host-pathogen interactions using electron microscopy (SEM and TEM) and assess the applicability of CLSM in host-pathogen interactions. Thirdly, to identify molecular markers for the *YrSp* resistance gene by using amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs) in the F_2 Kariega/*YrSp* population and to test the effectiveness of these markers in the F_3 Kariega/*YrSp* population.

Chapter I

Literature Review



Host-pathogen interactions with an emphasis on wheat (*Triticum aestivum L.*) and stripe rust

1.1 Introduction

The survival of all organisms depends on the continued presence and evolution of specific genetic systems aimed at maintaining diversity in the face of a changing environment (Flor, 1956). Plants are continuously challenged by external stresses that include change in the environment and attack by insects and plant pathogens.

The highly specialised relationship that has evolved between plants and pathogens has been a focal point of research for many years (Heath, 1981; Broers and Lopez-Atilano, 1996). Understanding the nature of the relationship and knowledge of the events that occur at the host-pathogen interface is crucial towards the strategic management of plant pathogens in economically important crops. For millions of years rusts have been co-evolving with their hosts without rendering them extinct. However, the ability of these pathogens to relentlessly reduce yields in domesticated crops with particular emphasis on cereals is a cause for great concern among producers.

1.1.1 Host-pathogen interactions

The host-pathogen interaction generally produces visible responses that are due to the interactions of host and pathogen genotypes as well as the environment prior to and after the onset of infection (Viljanen-Rollinson *et al.*, 2002). Host-pathogen interactions are expressed as an infection type with compatible and incompatible host-pathogen interactions termed as high and low infection types, respectively. Compatibility refers to a successful infection of the host by a pathogen with the display of disease in the plant making it susceptible to the pathogen. Incompatibility refers to an unsuccessful infection where no or little disease is

observed and the plant is referred to as resistant to the pathogen. A hypersensitive response is defined as the rapid death of host mesophyll cells surrounding the infection area while several hypotheses have been developed to explain how plant-hosts and their pathogens have co-evolved (Scott and Chakravorty, 1982). Among these are the gene for gene theory (Flor, 1956) and the Red Queen hypothesis (Van Valen, 1973).

1.1.2 Gene-for-Gene theory

The gene-for-gene hypothesis is used to explain the interaction between a host and a pathogen (Flor, 1956). This theory was described as follows: “For every gene that conditions resistance in the host there is a corresponding gene in the parasite that conditions pathogenicity” (Fig. 1.1). Thus, according to this theory, for every resistance (R) gene in a plant species there is a corresponding avirulence gene (A-) in the pathogen. This theory has been validated in cases where plant resistance is associated with the hypersensitive response. The hypersensitive response mediated by single resistance genes, is considered to be qualitative and race specific resistance. Resistance response that do not involve the hypersensitive response are considered as quantitative resistance and include adult plant, horizontal, slow rusting and other resistances as characterised by Johnson and Lupton (1987).

1.1.3 Red Queen Theory

This hypothesis proposed by Van Valen (1973) assumes that a pathogen becomes progressively specialised to the most common genotypes in a host population. This causes a reduction in fitness of common genotypes in the host population compared to less common genotypes but at the same time resulting in the reduction of fitness to uncommon genotypes as these become more common. Thus, as rare host genotypes become more common, they become more susceptible to pathogens. Such frequency-independent selections have been shown to favour sexual reproduction over asexual reproduction in host populations (Hamilton *et al.*, 1990).

1.2 The host: Wheat (*Triticum aestivum* L.)

Wheat, one of the first domesticated crops, has been the basic staple food of major civilisations in Europe, West Asia and North Africa for 8000 years (Curtis and Rajaram, 2002). Wheat, together with other cereals like barley (*Hordeum vulgare*) form part of the tribe Triticeae Dumort, a festucoid tribe of the family Poaceae (Gramineae), that has long standing economical importance. Although wheat belongs to the genus *Triticum*, only cultivated species of this genus are referred to as wheat (Lupton, 1987). Wheat can be divided into three karyotypic groups: the diploid, or einkorn, containing 14 chromosomes; the tetraploid, or emmer, containing 28 chromosomes; and the hexaploid, containing 42 chromosomes. Today, varieties of common, club, and durum wheat are of commercial importance, however, other species are often grown according to environmental conditions and provide essential genetic stock for breeding programs.

1.2.1 Factors affecting production of *Triticum aestivum* L.

A number of abiotic (non-living elements) and biotic (living elements) factors affect the production of wheat in the world. These factors may affect valuable agronomic traits such as seed set, kernel size, maturation time, yield and baking and milling qualities.

1.2.1.1 Abiotic factors

Wheat requires temperatures between 20 and 23^{o}C to germinate and grow successfully (Curtis and Rajaram, 2002). This requirement is often a limiting factor to the geographical distribution of wheat. Additionally, winter wheat requires a vernalization period or cold period of 4^{o}C before it will produce flowers and mature. Higher temperatures may result in a shorter growing season as the crop develops at a faster rate (Lupton, 1987). This may also reduce the rate of photosynthesis and in combination with the shorter growing season result in decreased wheat yield. Higher than optimum temperatures will additionally affect soil moisture, often increase

bacterial disease, fungal growth, insect infestation as well as decrease the nutrient availability to the plants (Curtis and Rajaram, 2002).

1.2.1.2 Biotic factors

Although wheat is plagued by many biotic factors like insects and viruses, the most pronounced biotic entity to plague wheat based on economical impact are the three kinds of rusts namely stem, leaf and stripe rust. Wheat rusts are all caused by different *Puccinia* species, stem rust by *P. graminis* Pers.:Pers. f. sp. *tritici*, Eriks. & Henn., leaf rust by *P. triticina* (Eriks.) = *P. recondita* Roberge ex Desmaz. f. sp. *tritici* Eriks. & Henn. (D.M. Hend.) and stripe rust by *P. striiformis* Westend f. sp. *tritici* (Fig. 1.2). There are many phenotypic and unique differences between these species. For the purpose of this thesis, stripe rust will be discussed in further detail.

1.3 Classification of rust fungi

Rust fungi are classified in the Kingdom Eumycota. They belong to the phylum Dikaryomycota and subphylum Basidiomycotina. Described in the class Teliomycetes they fall in the order Uredinales. Cereal rust belongs to the family Pucciniaceae and the genus *Puccinia*. The class is composed of a single order of obligate parasites. Common characteristics shared by species in the class Teliomycetes include the basidia being differentially divided and the absence of a basidiocarp. Rust fungi are described as autoecious meaning that all the spore stages are produced on a single host (Knott, 1989). In instances where the spore stages occur on two taxonomically different hosts the fungus is described as being heteroecious (Knott, 1989). However, *P. striiformis* f. sp. *tritici* is considered autoecious as no alternate host has been identified (Stubbs, 1985).

1.3.1 Nomenclature of stripe rust

Stripe rust, commonly known as yellow rust, is a cereal disease caused by *Puccinia striiformis* Westend f. sp. *tritici*. Gadd first described yellow rust in 1777

while Bjerkander described yellow rust as an opportunistic pathogen of rye in Sweden in 1874 (Eriksson and Henning, 1896). G. Westerndorp described yellow rust under the name *Puccinia straminis* (Danial, 1994) while the common names of yellow and stripe rust were given by Humphrey *et al*. (1924) and by Eriksson and Henning (1894) respectively.

1.3.2 Disease symptoms of stripe rust

The phenotypic characteristics of stripe rust include long yellow stripes of pustules on the leaves releasing round to oval, yellow-orange urediospores that have thick and spiny walls and form pustules on the leaf surface. The growth and spread of the pustules are generally restricted by the veins in older leaves while pustule distribution is less restricted on seedling leaves (Knott, 1989). A classification system for stripe rust infection was described by Roelfs (1984) and has been extensively used to rate disease severity (Table 1.2). The fungus only grows on living tissue and survives between seasons on volunteer wheat and barley plants as well as some wild grasses. Although symptoms are more often observed on the leaves, it has been noted that all parts of the plant may be infected (Knott, 1989). Individual pustules often give rise to chlorosis and later necrosis (Kurt, 2002). Symptoms of stripe rust are observed much earlier than the symptoms of any other rusts due to the ability of the fungus to develop at much lower temperatures ranging from 9 to13^{o}C (Roelfs *et al*., 1992).

1.3.3 Life cycle of rust fungi

The different steps of spore formation of fungi in the order Uredinales may be as many as six stages although not all of these stages occur on a single host (Table 1.1). The hosts are termed the main host (e.g. wheat) and the alternate host (e.g. barberry). Using *P. graminis* f. sp. *tritici* as an example, the lifecycle of cereal fungi is described as follows. The fungi produce binucleate urediospores from a bed of tissue (pustules) that erupts through the leaf or stem surfaces. These urediospores can re-infect another wheat plant leading to multiple cycles of infection during the growing

season. Towards the end of the growing season, the pustules produce a different type of spore namely a teliospore that are dormant during spring. In winter, nuclear pairs fuse to form diploid nuclei after which meiosis takes place producing a spore that germinates and gives rise to short hyphae that produces four uninucleate, haploid basidiospores (Hiratsuka and Sato, 1982). The basidiospores can only infect a barberry plant (alternate host). Basidiospores give rise to haploid hyphae of different mating types that produces sexual structures terrmed spermagonia on the upper surface of the barberry leaf. Spermatia (male sexual spores) are formed within the spermagonia and flexuous hyphae (female) project from the neck of the spermagonium. The fertilisation of flexuous hyphae by spermatia is brought about by insects. The nuclei pair in the hyphae forming a dikaryon that gives rise to sporing pustules on the lower surface of the barberry leaf. These spores (aeciospores) can only infect a cereal host, thereby completing the life cycle (Hiratsuka and Sato, 1982). For *P. striiformis* f. sp. *tritici* the life cycle consists of repetitions of the asexual uredinial stage. The sexual stage of *P. striiformis* f. sp. *tritici* has to date not been encountered and no alternate hosts for this fungus have been identified (Stubbs, 1985). However, stripe rust has been found to over-summer on wild grass, *Biomus wildenowii* (Boshoff, 2000).

1.3.4 Pathogen variability

A diversity of races or pathotypes is common in many pathogens. The mechanism by which new variation develops is continually being better understood but mutation from avirulence to virulence is generally considered to occur frequently (Wellings and McIntosh, 1990). The formation of new pathotypes by whole nuclei re-assortment has been observed in studies where host plants were inoculated with two different pathotypes (Little and Manners, 1969; Wright and Lennard, 1980). The ability of rust fungi to rapidly adapt and mutate on different wheat cultivars has made it increasingly difficult for researchers to classify the specific pathotype causing the infection. For this reason, the avirulence and virulence pattern of a pathogen is determined by inoculating a selected group of host plants all with differing genotypes with respect to rust resistance (Roelfs, 1984). Diverse regions in the world use

parameters like environmental conditions, classification systems and differential sets to identify pathotypes (De Vallavieille-Pope and Line, 1990). Due to local differences in host-pathogen interactions, researchers have systematically identified local cultivars as differential hosts that aid the differentiation of pathogenesis. Boshoff (2000) developed such a set for rust fungi in South Africa by combining the World and European differentials supplemented by local testers. Disease ratings are measured as resistant, susceptible and various intermediate infection types such as moderately resistant and moderately susceptible (Fig. 1.3). McIntosh *et al*. (1995) presented a classification system for *P. striiformis* f. sp. *tritici* whereby infection readings can be measured in order to determine pathogenicity. However, the pathotype variability in *P. striiformis* f. sp. *tritici* populations is considered to be low even though there are pathotype differences between regions and host cultivars (Stubbs, 1985; Hovmøller, 2001).

1.4 The pathogen: Stripe rust (*Puccinia striiformis* Westend. f. sp. *tritici*)

Stripe rust was first characterized as a pathogen of wheat in the Western United States in the early 1900's. The pathogen became a problem in the 1950's when the first epidemic of stripe rust destroyed more than 50% of the yield of certain wheat cultivars (McIntosh *et al*., 2001). Stripe rust was first observed in South Africa in 1996 (Pretorius *et al*., 1997). The disease was observed in most of the wheat-producing areas of the Northern, Western and Eastern Cape provinces. The region of the Western Cape was the most severely affected as cool and wet conditions that favour the development of the disease, prevailed in this area. The causal pathotype, later confirmed as pathotype 6E16A-, and the subsequent epidemic by a new pathotype in 1998 indicated the potential of stripe rust becoming an important foliar disease of wheat in South Africa (Boshoff and Pretorius, 1999; Boshoff *et al*., 2002). The new pathotype, 6E22A- was first described in 1997, after a severe epidemic occurred in the Eastern Free State, and was also observed in the province of Kwazulu-Natal (Boshoff and Pretorius, 1999). Various cultivars such as Kariega however exhibited natural resistance to the pathogen and were identified as a source of resistance for further breeding. Following the establishment and geographical

distribution of stripe rust in South Africa, only one additional pathotype, 7E22A-, has been observed in Lesotho but has shown no new virulence on South African wheat cultivars (Komen, 2003).

1.4.1 Economic importance of stripe rust

The introduction of stripe rust to South Africa and subsequent epidemics on susceptible wheat cultivars caused farmers to spend millions of rands in an effort to control the disease through the use of recommended fungicides. Farmers in the Western Cape spent approximately R28 million on fungicides to control the introduced pathotype 6E16A- while farmers in the eastern Free State, spent approximately R18 million on fungicide control of the pathotype 6E22A- in 1997 (Boshoff, 2000). Previously resistant cultivars, Hugenoot and Carina, commonly recorded 100% loss of flag and lower leaves and extensive applications of fungicides were required when pathotype 6E22 was observed in 1997 (Boshoff and Pretorius, 1999). In one study, the control of stripe rust on approximately 42 000 ha of wheat was estimated to cost more than R6 million without the consideration of yield and quality losses (Boshoff, 2000). The rapid spread of stripe rust to major wheat-growing regions of South Africa, the immense cost of chemical control and the severe yield losses have contributed to the implementation of resistance breeding programs, acute monitoring of stripe rust pathotypes and various host-pathogen studies as a management strategy towards durable resistance against the stripe rust pathogen (Boshoff *et al*., 2002).

1.5 Control of stripe rust

1.5.1 Chemical control

The chemical control of stripe rust is an additive measure used in conjunction with resistant cultivars. Several fungicides have been found to be both safe and effective in the control of stripe rust (Knott, 1989). Such fungicides can be divided into two classes namely protectant and eradicant. Protectant fungicides prohibit

pathogen propagules from establishing infections but prove ineffective against already existing infections. Eradicant fungicides penetrate the plants and kill existing infections and latent infections (Manners, 1993). The choice of fungicide is dependent on several factors. The growth stage of the plant, the type of resistance conferred and the disease severity all play significant roles in the choice of fungicide (Viljanen-Rollinson *et al*., 2002). The timing of application of fungicides has been noted to be of great importance as a premature application of fungicides can result in the protectant activity diminishing before high levels of disease pressure is reached, while too late application of eradicant can result in the reach-back activity being insufficient to control disease epidemic (Viljanen-Rollinson *et al*., 2002).

1.5.2 Biological control

Hyperparasitism is the parasitising of a parasite on a particular host. It can serve as a means of biological control of a pathogen if the corresponding parasite of that pathogen is known (Buchenauer, 1982). *Sphaerellopsis filum* is a ubiquitous hyperparasite associated with many species of cereal fungi such as *Puccinia triticina, P. graminis, P. striiformis, P. sorghi* and *P. coronata*. However, biological control has not been used extensively on stripe rust (Buchenauer, 1982).

1.5.3 Resistance mechanisms

At the onset of research into stripe rust, researchers soon identified patterns of infection and noticed that plants were either susceptible or resistant to the pathogen. Differences in some cultivars between seedling and adult plant stage were also observed (Qayoum and Line, 1985). Further studies also revealed the existence of plants that expressed intermediate resistance to stripe rust. This observation gave rise to the idea that resistance to stripe rust is governed by both dominant and recessive genes.

The most effective manner in which to control this disease are resistant cultivars. Many different sources of resistances to stripe rust have been documented

(Qayoum and Line, 1985; Chen and Line, 1993; Milus and Line, 1986b). Thus the aim of breeding for rust resistance is to combine genes in such a way as to obtain durable resistance. Durable resistance has been defined as “resistance that remains effective in a cultivar that is widely grown for a long period of time in an environment favourable to the disease” (Johnson, 1981). Durable resistance is also considered to be a non-specific form of resistance (Milus and Line, 1986a).

1.5.3.1 High temperature adult-plant resistance

High-temperature, adult-plant (HTAP) resistance along with non-specific resistance, field resistance and slow rusting was first described after researchers noticed wheat cultivars that differed with respect to resistance at seedling and adult plant stage (Allen, 1923; Line, 1972; Line *et al*., 1974). Qayoum and Line (1985) reported that the flag leaves of wheat were more resistant than the lower leaves of the same plant. They also noted that cultivars that were resistant at high post-inoculation temperatures became susceptible when transferred to lower temperatures. These results are similar for different races of the pathogen and there is no evidence that this phenomenon is race-specific (Qayoum and Line, 1985). Cultivars exhibiting HTAP resistance remain resistant after exposure to many different pathotypes and are therefore considered to have durable, non-specific resistance to stripe rust (Line, 1980; Milus and Line, 1986a; Line *et al*., 1992). Differences in terms of the infection type were however also observed in cultivars with HTAP resistance. Cultivars with a greater degree of HTAP resistance expressed resistance much sooner than those with a lesser degree of HTAP resistance. This finding was attributed to additive epistatic gene action (Chen and Line, 1995a). Examples of adult plant resistance genes include *Lr34*/*Yr18* complex to leaf and stripe rust as well as *Sr2* to stem rust (Knott, 1968; Suenaga *et al*., 2003).

1.5.3.2 Seedling resistance

Resistance conferred by seedling resistance genes is race specific and has been shown to become susceptible after a short period of time due to the rapid

mutation rate of the pathogen (Chen and Line, 1995b). Early breeding programs exploited simply inherited major genes observed at seedling growth stage that remained effective for the duration of the plants life cycle. The *YrSp* seedling resistance gene first observed in Spaldings Prolific still confers resistance to different stripe rust pathotypes (Table 1.3) (Johnson *et al*., 1972).

1.5.3.3 Prehaustorial and posthaustorial resistance

Prehaustorial resistance has been defined as the prevention of the formation of haustoria by the pathogen. Haustorium mother cells are formed as with normal pathogen infection but haustoria do not develop and papillae are often induced at the site of the attempted cell wall penetration (Heath, 1981; Niks and Dekens, 1986; Jacobs, 1989;). This mechanism of resistance is very common in non-host interactions (Heath, 1981). In genotypes exhibiting posthaustorial resistance, plant cell death (necrosis) occurs after the formation of a haustorium by the pathogen (Heath, 1981). Race-specific hypersensitivity resistance is generally accepted to be posthaustorial resistance (Anker and Niks, 2001).

1.6 Management strategies

1.6.1 Gene pyramiding

Gene pyramiding refers to the combination of resistance genes in a single cultivar towards producing genetic stocks that can be used in a breeding program. Many common crop pathogens, such as rust and bunt in wheat, mutate over time and eventually overcome individual sources of resistance. Gene stacking, or incorporating multiple sources of resistance into one variety, dramatically decreases the chances of that happening (Singh *et al*., 1999).

1.6.2 Boom and bust cycle

The boom and bust cycle was first described by Priestley (1981) with specific reference to cereal varieties. When a new variety with horizontal resistance is developed, it soon becomes popular and the area grown under this cultivar expands and is referred to as the boom stage. The appearance of a new race of the pathogen causing an epidemic on these popular cultivars results in the loss of its popularity and the area under cultivation of that cultivar decreases. This is known as the bust stage. Researchers have warned of the vulnerability of monogenic resistance conferred by major genes to stripe rust (Boshoff and Pretorius, 1999; Bender *et al*., 2000).

1.6.3 Induced resistance

Induced resistance can be described as the immunization of a plant against a particular pathogen resembling the immunization of vertebrates to prevent infectious diseases (Buchenauer, 1982). Cross protection or induced resistance was demonstrated when C. Yarwood inoculated sunflowers with *Uromyces phaseoli* and subsequently with *Puccinia helianthii* (Buchenauer, 1982). The pustules produced on the sunflowers were markedly reduced in comparison to the control plants. Resistance could also be induced in wheat seedlings when virulent and non-virulent strains of *P. striifomis* f. sp. *tritici* were applied simultaneously (Buchenauer, 1982). However, very little is known about the mechanism of infection by *Puccinia striiformis* in *Triticum* species and research of the rust infection process has focussed on leaf and stem rust using microscopy. Microscopy has provided researchers with valuable information regarding the process of rust infection as well as insights into the metabolic and physiological changes the host experiences during the infection process.

1.7 Microscopy as a tool in plant pathology

Microscopy has been utilized extensively in the study of host-pathogen interactions (Heath, 1981; Hu and Rijkenberg, 1998; Adendorff and Rijkenberg,

2000). Various microscopy techniques have allowed the characterization of pathogen infection structures; elucidation of pathogen infection pathways, provided information on cellular changes based on biochemical interactions as well as information on gene transcripts associated with resistance mechanisms.

1.7.1 Light microscopy and its variants

A microscope is a tool used to enlarge the view of objects to see details that otherwise would not be possible with the naked eye. A light microscope typically consists of an eyepiece, light source and a primary and secondary lens. Light is manipulated to pass through an object and is then focused by the primary and secondary lens. Light microscopy does not distinguish between objects that are closer than half the wavelength of light that is 0.55 micrometres. Thus, the images smaller than 0.275 micrometres cannot be resolved using light microscopy (Anonymous, 2004).

1.7.1.1 Bright field, Dark field and Phase contrast Microscopy

The bright field microscope is best known to students and is probably the most well-known and best utilised tool in biology (Heath, 2000). Visible light is focused through a specimen by a condenser lens and then passes through two more lenses placed at both ends of a light-tight tube. The latter two lenses each magnify the image. Limitations to what can be seen in bright field microscopy are not as much related to magnification as they are to resolution, illumination, and contrast. Resolution can be improved using oil immersion lenses, and lighting and contrast can be dramatically improved using modifications such as dark field, phase contrast, and differential interference contrast as well as the use of stains.

The invention of the phase-contrast microscope by Frits Zernika in 1938, won him the Nobel Prize for Physics in 1953. It is based on the ability to differentiate between light rays when passing through different transparent material due to a property of light namely, wavelength phase. Shifts in the wavelength phase may

produce greater contrast of a biological sample. The phase-contrast microscope permits the study of internal cell structure without the need to stain the cells (Anonymous, 2004).

Light microscopy has been extensively used to elucidate the infection process of many fungi including the infection structures they produce (Zhang and Dickinson, 2001). The application of light microscopy to the development of infection-structures of *P. graminis* f. sp. *tritci* dates back to the early 1900's (Allen, 1923). The details of this study have remained significant and accurate almost a century later, only surpassed by studies using electron microscopy (Lennox and Rijkenberg, 1989).

1.7.2 Electron microscopy and its variants

The prototype that forms the basis for the electron microscope was built by Max Knoll and Ernst Ruska in 1931, with the latter being awarded half the Nobel Prize in Physics in 1986 for his contribution (Schamber, 2004). This microscopic technique is based on electron illumination in a vacuum as opposed to illumination by light. The vacuum is required to allow movement of the electrons in the form of a beam. Beams of fast-moving electrons are focused on a sample and are either scattered, known as scanning electron microscopy (SEM) or transmitted and scattered, known as transmission electron microscopy (TEM) by the sample so as to form an image on an electron-sensitive photographic plate such as a television monitor (Robards, 1970).

1.7.2.1 Scanning electron microscopy

The principle of scanning electron microscopy is to scatter electrons off the surface of a sample and forming an image from the reflected electrons. The electron beam illuminating a sample excites a secondary group of electrons on the surface of the sample that forms the image (Schamber, 2004). The primary beam of electrons is scanned across a sample, with the secondary group of electrons emitted in the same scanned pattern, thus the name scanning electron microscopy (Fig 1.4).

Scanning electron microscopy (SEM) techniques are considered of particular value in the study of morphology and ontogeny of infection structures of plant pathogens (Lennox and Rijkenberg, 1989). SEM studies were confined to the descriptions of infection structures arising from urediospore germination on non-host, artificial and host surfaces (Hu and Rijkenberg, 1998). However, the improvement of fracture techniques has greatly advanced research in this area (Lennox and Rijkenberg, 1989).

The general infection process for various cereal rust fungi has been elucidated by means of scanning electron microscopy. Lennox and Rijkenberg (1989) used SEM to investigate the infection pathway of *P. graminis* f. sp. *tritici* on several crops. Other pathogen studies *P. sorghi* (Hughes and Rijkenberg, 1985), *Phakopsora apoda* (Adendorff and Rijkenberg, 2000), *Hemileia vastatrix* (Coutinho *et al*., 1993) and *P. recondita* (Hu and Rijkenberg, 1998). The general infection process and the infection structures formed by *Puccinia* species (Table 1.1) is thought to be the similar to *Puccinia striiformis* f. sp. *tritici* but has never specifically been documented.

1.7.2.2 Transmission electron microscopy

A typical transmission electron microscope (TEM) consists of an electron source, a substage with condenser to focus the electrons onto the specimen, and an objective and projection lens. The projection lens projects the final image onto a fluorescent screen or photographic plate. A number of commercially important pathogens have been studied using the TEM. These include *P. helianthi*, *P. graminis*, *P. triticina* and cowpea rust (Heath, 1974; Wood and Heath, 1986).

TEM microscopy of fixed cells cannot be used to investigate the dynamic chemical processes that take place over time in different regions of the cell when a host is invaded by a pathogen (Zhang and Dickinson, 2001). These limitations in electron microscopy led to the development of fluorochromes and staining techniques to enable researchers to study the chemical processes within a cell through the use of fluorescence microscopy.

1.7.3 Fluorescence microscopy

The fluorescence microscope is based on the phenomenon that certain biological samples emit energy, detected as visible light, when irradiated with light of a specific wavelength. The specimen can either be naturally fluorescing for example chlorophyll or can be made to fluoresce through the use of fluorescent chemicals. The fluorescence microscope consists of two filters. The initial filter allows radiation of the desired wavelength, depending on the fluorescent agent used or the fluorescent capability of the compound itself. The radiation collides with the fluorescent atoms in the sample and the electrons are excited to a higher energy level. The electrons emit light when they return to the lower energy level and an image is formed when the second filter distinguishes between emitted light and excitation light.

Fluorescence microscopy has been primarily employed to study the host necrosis phenomenon as a resistance reaction to pathogen invasion. Fluorescent areas in rust infected leaves have been shown to correspond with cells with the extensive browning characteristics of a hypersensitive reaction (Kuck *et al*., 1981; Goodman and Novacky, 1994). This technique has also been used to study various cereal pathogens such as *P. graminis* (Samborski *et al*., 1977; Kuck *et al*., 1981), *P. recondita* (Bender *et al*., 2000; Zhang and Dickinson, 2001) and *P. striiformis* (Zhang and Dickinson, 2001). Fluorescence microscopy is limited by the one-dimensional view under a microscope and the fluorescence produced is often not differential.

1.7.4 Confocal laser scanning microscopy

Confocal laser scanning microscopy (CLSM) is a new microscopic technique that bridges these constraints by combining microscopy with selective fluorescent probes to produce a powerful new approach to gathering both biochemical and molecular information from living cells (Butt *et al*., 1989). “Confocal” means “having the same foci” and in terms of light microscopy, this can be described as, the section that is imaged will be the same as the section that is illuminated (Czymmek *et al*.,

1994). The result of the confocal technology is that no out of focus light is incorporated into the final image thus enhancing the resolution of the image (Figure 1.4). CLSM is based on a stage-scanning confocal microscope developed by Minsky in 1957 (Minsky 1988). However, commercialisation of the confocal microscope only became apparent with the development in computer and laser technology (Czymmek, *et al*., 1994). This technique has the potential to provide greater insight into the plant infection process and resistance mechanisms as well as intracellular response signals (Heath, 2000).

1.7.4.1 Choice of fluorochromes

Fluorochromes are dyes that fluoresce when illuminated with laser, ultraviolet or visible light. A fluorochrome interacts with specific residues within or on the outside of a cell. It may also conjugate to other agents such as gluten of chitin making their use more specialised for certain cellular components (Butt *et al*., 1989). Even though there are a vast number of fluorochromes currently available, research into the applicability of fluorochromes is still in its infancy. The organisation of microtubules in growing hyphae has been studied and cell wall formation in *Physarum* plasmodia (Salles-Passador *et al*., 1991; Kwon *et al*., 1993).

1.8 Development and application of molecular markers

Prior to the advent of molecular markers, plant breeders used morphological markers, linked to desired traits, to make selections of traits in plants. However, morphological markers are infrequent and even though the potential of genetic markers was recognised more than 60 years ago, the application of such markers was hindered by their lack of availability (Sax, 1923; Melchinger, 1990; Mohan *et al*., 1997).

The development of DNA-based marker technology has contributed to the development of elite hybrids and varieties and has broadened our understanding of the plant genome. Marker assisted selection (MAS) is based on the principle that if a

gene or genes conferring a specific trait of interest is linked to an easily identifiable molecular marker, it is more efficient to select for the marker than the trait itself. The efficiency of a breeding program can be increased by predicting the prospects of crosses for line development before producing and testing lines derived from them (Bohn *et al*., 1999). Furthermore, the accurate identification of germplasm is vital not only to protect the rights of plant breeders but also to accelerate plant breeding programs (William *et al*., 2003).

The main use of marker technology in plants has been in assessing the genetic variability and characterisation of germplasm and identification of different genotypes. The use of molecular technology has mainly been applied to detect monogenic and qualitative trait loci (QTL's), to help with marker-assisted selection of the best cultivars and the identification of sequences of useful candidate genes by means of gene tagging and the construction of cereal genetic maps. A major advantage of molecular markers is the that the expression of DNA type markers is independent of environmental influences and are detectable at all plant growth stages (Melchinger, 1990; Najimi *et al*., 2002). Fingerprinting techniques used on plants and specifically wheat include restriction fragment length polymorphism (RFLPs), random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphism (AFLPs) and simple sequence repeats (SSRs). The development of different fingerprinting techniques has provided researchers with greater choice and the ability to compare techniques and select those suited to their goals (Table 1.4).

1.8.1 Restriction fragment length polymorphism (RFLPs)

Restriction fragment length polymorphism was the first DNA-based marker technique to be used in plants. The foremost RFLP map of a crop plant was produced for tomato by Bernatzky and Tanksley (1986). Restriction fragment length polymorphism is based on the principle that restriction enzymes recognise a specific nucleotide sequence and cut (digest) the DNA at these sites producing restriction fragments of different lengths. Restriction fragments are separated by agarose gel electrophoresis and transferred to a filter by the use of Southern blotting. The

restriction fragments on the filter, is then probed through the complimentary binding of radioactively labelled probe DNA to the restriction fragments. Detection of the fragments is achieved by autoradiography. Base mutations, insertions, deletions, substitutions or sequence rearrangements produces differences in restriction fragment lengths (Avise, 1994). General characteristics of RFLP markers are that they are co-dominant and thus more informative than dominant markers (Tanksley *et al*., 1989). Unfortunately, RFLP technology has some drawbacks including the prior development of probes and the use of radioactive labelling for marker visualization. In addition to this, RFLP analysis is labour intensive and time consuming (Williams *et al*., 1990; Liu and Cordes, 2004).

RFLPs has been used in agronomic crops for linkage analysis and genome mapping (Tanksley *et al*., 1989). The construction of RFLP maps for wheat is considered difficult due to low levels of polymorphism as a result of self-pollination (Chao *et al*., 1989). RFLP-based markers have been utilised to characterise many resistance genes of diseases of wheat such as hessian fly (Najimi *et al*., 2002), leaf rust (Schachermayer *et al*., 1994) and stripe rust for *Yr17,* and *Yr28* and leaf rust for *Lr3* and *Lr35* (Table 1.5) (Robert *et al*., 1999).

1.8.2 Randomly amplified polymorphic DNA (RAPDs)

Randomly amplified polymorphic DNAs (RAPDs) is a PCR (polymerase chain reaction) based marker system developed by Williams *et al*. (1990) and Welsh and McClelland (1990). It is based on the principle that a single PCR primer, an oligonucleotide of 8 to 10 random bases in length, will by chance amplify several different target regions of a genome. Visualization of the set of different sized fragments called amplicons is achieved by gel electrophoresis revealing a unique DNA banding pattern for each primer (Liu and Cordes, 2004).

RAPD markers are dominant and only useful to indicate the presence or absence of a specific marker. RAPDs can be used to generate accurate cultivar specific markers where high levels of genetic variation exists between cultivars, while

almost no genetic variation is found within cultivars (Welsh and McClelland, 1990). Other advantages of RAPDs includes cost effectiveness (requires little genomic DNA, requires no expensive equipment (apart from a thermocycler), is experimentally reasonably simple and does not make use of radioactive detection of markers (Myburg *et al*., 1997). Furthermore, this technique generates a high number of markers without requiring prior sequence information (Williams *et al*., 1990). The reproducibility of RAPD markers is problematic due to among others, the low annealing temperatures used during PCR (Rafalski, 1997).

RAPD markers are often used as species-specific markers in diversity and phylogenic studies (Crowhurst *et al*., 1991; McDermott *et al*., 1992; Smith *et al*., 1992; Chen and Line, 1993). Using RAPDs a marker for stripe rust resistance gene *Yr17*, and *Lr28,* was developed (Table 1.5) (Robert *et al*., 1999).

1.8.3 Amplified fragment length polymorphism (AFLPs)

Amplified fragment length polymorphism was formerly known as selective restriction fragment amplification (SRFA). It is a PCR-based fingerprinting technique and is based on the selective amplification of restriction fragments after the digestion of genomic DNA (Liu and Cordes, 2004). Genomic DNA is typically digested with a rare-cutter (*EcoR*I) and a frequent cutter (*Mse*I) restriction endonucleases. Adapters for each restriction sequence type are ligated to the ends of the restriction fragments. The adapter sequence is such that after ligation, the restriction site is destroyed. Pre-selective amplification is performed on the ligation fragments and is achieved by the complementary binding of primers to the adaptors. During pre-selective PCR, the primers for both types of adptors usually have the addition of a single base, adenine (A), cysteine (C), thymine (T), or guanine (G), reducing the complexity of amplified restriction fragments 16 fold. Selective amplification of the subset of pre-selective product is achieved through the use of primers with three-base extensions, reducing the complexity of restriction fragments 4096-fold. The selective PCR product is resolved according size using polyacrylamide or capillary gel electrophoresis. AFLP

fragments are detected using radioactivity and autoradiography, silver staining or fluorescent detection.

The advantage of AFLPs is that no prior sequence information is required and the technique can be standardized and easily automated for high-throughput applications. It requires less DNA than RFLPs and AFLP markers are robust, reliable and highly reproducible. One disadvantage of AFLP markers is that they are dominant. A number of AFLP markers linked to stripe rust resistance genes *Yr10, YrH52, Yr15 and Yr29* have been published (Table 1.5) (Peng *et al*., 2000; Shoa *et al*., 2001; William *et al*., 2003).

1.8.4 Simple sequence repeats (SSRs)

Simple sequence repeats (SSRs) also known as microsatellite markers is a PCR-based marker technique. SSRs or microsatellites are short, tandem repeats of DNA sequence motifs in eukaryotic (Liu and Cordes, 2004). Typically, these repeats may be dinucleotides, for example $(AC)_n$, trinucleotides $(TCT)_n$ or tetranucleotides $(TATG)_n$, where (n) denotes the number of repeats. Changes in the number of repeats, are occasionally caused by slippage during DNA replication resulting in the loss or the gain of a single repetitive unit (Schlotterer and Tautz, 1992).

SSRs are PCR amplified by using primers complementary to the conserved regions that flank the repeat (Liu and Cordes, 2004). The size of the PCR fragment depends on the amount of tandem repeats the fragment contains and is easily visualised by gel electrophoresis. Roder *et al*. (1998) described the use of SSRs as the method of choice for marker generation in self-pollinating crops. Advantages of this technique include the production of co-dominant markers that distinguish between heterozygote and homozygote genotypes, SSR markers are heritable (Lima *et al*., 2003) and abundant, ubiquitous and distributed throughout the genome (Sun *et al*., 2002). The method to identify SSRs is relatively simple and easily automated once the primers have been developed for a particular crop (Liu and Cordes, 2004).

SSR markers are usually single locus and multi-allelic (Liu and Cordes, 2004). A number of SSR markers have been published for various crops including wheat (Roder *et al*., 1998; Borner *et al*., 2000), sorghum (Brown *et.al*.,1996; Taramino *et al*., 1997), maize (Vigouroux *et al*., 2004) and rice (Biss *et al*., 2003). Approximately 80% of the wheat genome is composed of repetitive DNA and a concerted effort is being made to enrich the repertoire of SSR markers in wheat (Hayden and Sharp, 2001).

Many SSR markers for the resistance genes *Yr5, Yr18 and Yr26* to stripe rust have been identified (Table 1.5) (Ma *et al*., 1999; Sun *et al*., 2002; Wang *et al*., 2002; Suenaga *et al*., 2003). Mapped SSR markers are also used to anchor linkage groups to specific chromosomes in wheat (Roder *et al*., 1998).

1.9 Conclusion

The study of host-pathogen interactions is complex and requires information to be integrated from different disciplines including pathological, histological and molecular components. Still lacking however, is the component of biochemical information needed to understand the complex molecular interaction between plants and pathogens. Even though there is a considerable amount of information available on this topic more studies are required that could better place all existing information into better context.

The aim of this study was to introgress the stripe rust resistance gene, *YrSp*, into a commercial South African wheat variety. Further the host-pathogen interaction of *P. striiformis* f. sp*. tritici* was studied by using histological and molecular techniques.

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Table 1.1 Generic rust spore formation stages and the spores produced during these stages (Karp and Edwards, 1997).

Designation	Rust spore stage	Spore formed
0	Spermagonuim (Pycnium)	Spermatium (Pycniospore)
i	Aecium	Aeciospore
ii	Uredinium (Uredium)	Uredioniospore (Urediospore)
iii	Telium	Teliospore
iv	Basidium	Basidiospore

Table 1.2 Classification of infection types for *Puccinia striiformis* Westend f. sp. *tritici* (Roelfs, 1984).

Class	Infection type[a]	Description of symptoms
Immune	0	No signs of infection to the naked eye but minute flecks may be visible under low magnification
Very resistant	;	No uredia, but distinct flecks of varying sizes, usually a chlorotic yellow but occasionally necrotic
Resistant	1	Small uredia surrounded by yellow chlorotic or necrotic areas
Moderately resistant	2	Small to medium-sized uredia, typically in a dark green island surrounded by a chlorotic area
Moderately susceptible	3	Medium-sized uredia usually surrounded by a light green chlorosis
Susceptible	4	Large uredia with a limited amount of chlorosis; may be diamond-shaped
Mesothetic or Heterogenous	X	A range of infection types from resistant to susceptible scattered randomly on a single leaf; caused by a single isolate
Heterogenous	Z	Large uredia concentrated at the base of the leaf

[a]Pluses and minuses are used to indicate variations in the size of uredia typical of a particular infection type as follows: - uredinia much smaller than typical and at the lower limit for the infection type, + uredia larger than normal and ++/ +++ uredial much larger than typical and at the upper limit for the infection type.

Table 1.3 Seedling infection types produced by the World and European differentials, and supplemental tester lines to the pathotypes 6E16A- and 6E22A- of *Puccinia striiformis* f. sp. *tritici*[a] (Boshoff, 2000).

Cultivar	Yr genes	Chromosome Location	Low infection type	Seedling response pt. 6E16A-	pt. 6E22A-
World Differentials					
Chinese 166	1	2A	0;	0;	;
Clement	2[c] 9 25[f] Cle[e]	7B 1BL 1D 4B	0;	0;	;
Heines Kolben	2 6	7B 7BS	;, n1	4	4
Lee	7	2BL	;n, 1n	4	4
Moro	10 More	1BS 4B	0;	;	;
Strubes Dickkopf	25f Sd	1D	D-	;c, 1cn	;c, 1cn
Suwon 92/Omar	4 Su	6B 2B	;	0;	0;, 1cn
Triticum spelta album	5	2BL	0;, ;	0;	;
Vilmorin 23	3a 4ad	1B 6B	;	;n	;n, 1c
European Differentials					
Carstens V	Cv	D-	D-	;	;c
Compare	8 19[i]	2D 5B	0;, ;	4	4
Heines Peko	2 6 25	7B 7BS 1D	;n, n1	;n	4
Heines VII	2 25[f] HVII[e]	7B 1D 4A	0;, 2	;c	;c, ;1c
Hybrid 46	4bh	6B	;	;	;
Nord Deprez	3a 4b	1B 6B	;	;	;, ;c
Reicherberg 42	7 25[f]	2BL 1D	;n, 1n	;1cn	4

Cultivar	Yr genes	Chromosome Location	Low infection type	Seedling response pt. 6E16A-	pt. 6E22A-
Spalding Prolific	Sp	2B	D-	0;	0;
Supplemental set					
*Yr1/6**AvsS	1	2A	0;	;	;
Kalyansona	2	7B	0;, 2	4	4
*Yr5/6**AvsS	5	2BL	0;, ;	0	0;
*Yr6/6**AvsS	6	7BS	;, ;n1	3	3
*Yr7/6**AvsS	7	2BL	;n, 1n	3	3
*Yr8/6**AvsS	8	2D	0;, ;	3	3
Federation*4/Kavkaz	9	1BL	0;	0;	0;
*Yr9/6**AvsS	9	1BL	0;	0	0;
*Yr10/6**AvsS	10	1BS	0;	;	0;
*Yr11/3**AvsS	11	-	-	3	3
Wembley	14[h]	-	-	3	3
*Yr15/6**AvsS	15	1 BL	0;	0	;
Trident	17	2AS	;c,;1	4	4
*Yr17/3**AvsS	17	2AS	;c,;1	3	3
Jupateco R	18	7DL	-	4	4
*Yr18/3**AvsS	18	7DL	-	4	4
*Yr24/3**AvsS	24	1B	-	;	;
*Yr26/3**AvsS	26	6AS	-	;	;
Selkirk	27d	2BS	-	1cn, 3	1cn, 3
*Yr27/3**AvsS	27	2BS	-	;, 1p=4	;
Avocet R	A	D-	;cn1, 2+	;c, 1c	;c, 1c
YrSp/3*AvsS	Sp[i]	2BS	-	;	;
Avocet S	-	-	-	4	4
Federation 1221	-	-	-	4	4
Japateco S	-	-	-	4	4

[a] Boshoff (2000); [b] McIntosh *et al.,* (1995); [c] Calonnec *et al*. (1997a); [d] McIntosh *et al*., (1998); [e] Chen, *et al*. (1995b); [f] Callonec *et al*.(1997b); [g] not available; [h] Chen *et al*. (1996); [i]Chen *et al*. (1995a); [j]McIntosh *et al*.(2001).

Table 1.4 Table of comparison of the most commonly used molecular marker systems in cereal genetics (Karp and Edwards, 1997).

Feature	RFLP's	RAPD's	AFLP's	SSR's	SNP's
DNA required (µg)	10	0.02	0.5 - 1.0	0.05	0.05
DNA quality	high	High	moderate	moderate	high
PCR-based	no	Yes	yes	yes	yes
Number of polymorphic loci analysed	1.0 - 3.0	1.5 - 50	20 - 100	1.0 - 3.0	1.0
Ease of use	not easy	Easy	easy	easy	easy
Amenable to automation	low	moderate	moderate	high	high
Reproducibility	high	unreliable	high	high	high
Development cost	low	Low	moderate	high	high
Cost per analysis	high	Low	moderate	low	low

Table 1.5 Published DNA based assays for designated and non-designated wheat genes and the marker type for the period of 1996 – 2003 (Koebner, 2004).

Yellow (stripe) rust resistance		Brown (leaf) rust resistance		Black (stem) rust resistance	
Gene	**Marker Type**	**Gene**	**Marker Type**	**Gene**	**Marker Type**
Yr5	SSR[g]	*Lr3*	RFLP	*Sr2*	SSR
Yr7	SSR	*Lr19*	STS from AFLP	*Sr30*	RGA, SSR
Yr9	RGA	*Lr26*	RGA	*Sr31*	RGA
Yr10	AFLP[d]	*Lr28*	STS from RAPD	*Sr36*	SSR
Yr15	AFLP[c] SSR[c]	*Lr34*	SSR[h]	*Sr38*	RGA
Yr17	RFLP[a] SSR[a]	*Lr35*	ISSR[i]	*Sr39*	ISSR[i]
Yr18	SSR[h]	*Lr37*	RGA		
Yr26	SSR[f]	*Lr39*	SSR		
Yr27	RFLP[j]	*Lr46*	AFLP[e]		
Yr28	RFLP[b] RAPD[b]	*Lr47*	STS from RFLP		
Yr29	SSR[e]	*Lr50*	SSR		
YrH52	AFLP[c] SSR[c]	Unspecified	SSR		
YrMoro	STS from AFLP				
YrKat	SSR				

[a]Robert *et al*., 1999, [b]Singh *et al*., 1999, [c]Peng *et al*., 2000, [d]Shoa *et al*., 2001, [e]William 2003, [f]Ma *et al*., 1999, [g]Sun *et al*., 2002, [h]Suenaga *et al*., 2003, [i]Gold *et al*., 1999, [j]McDonald *et al*., 2004

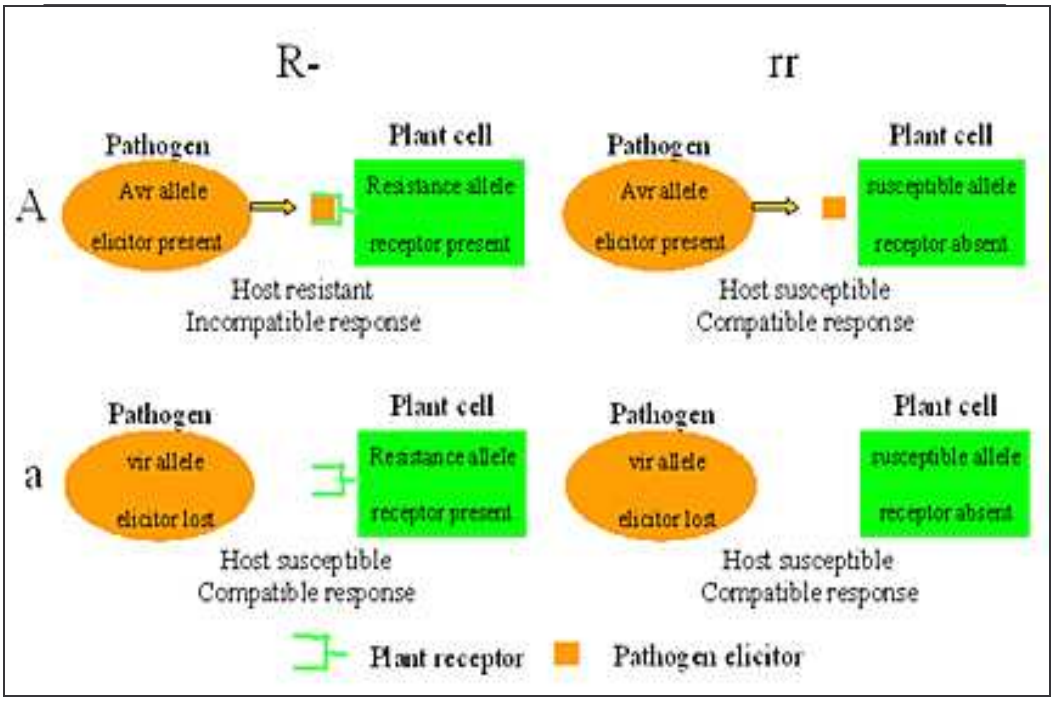

Figure 1.1 Molecular model of the gene-for-gene interaction (McDonald, 2004).

Figure 1.2 The three wheat rusts: stem, leaf and stripe rust from left to right respectively (Malvick, 2002).

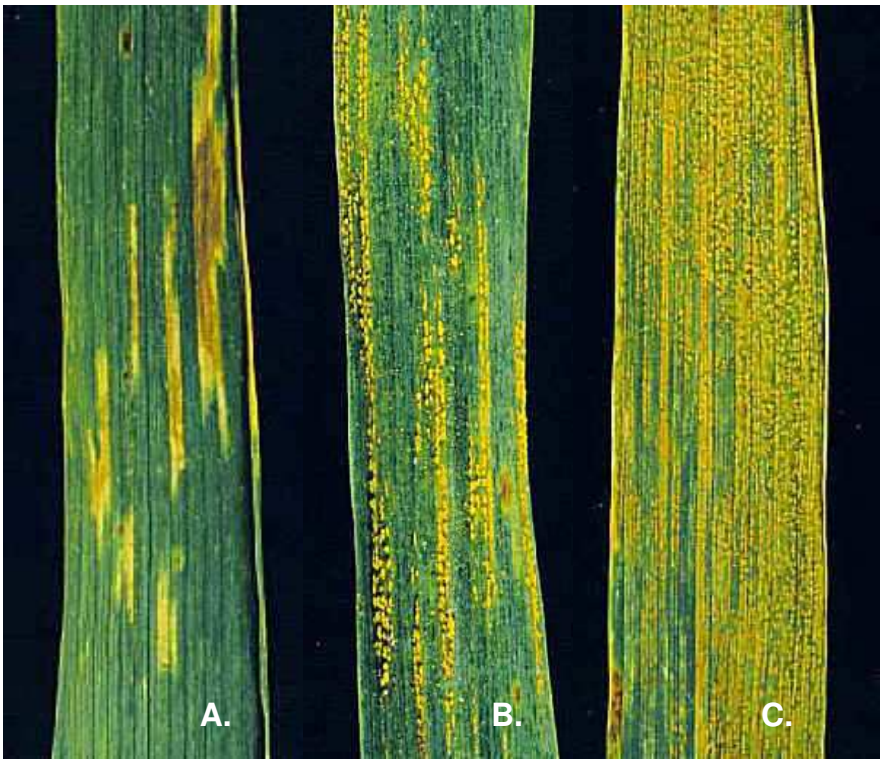

Figure 1.3 Representation of stripe rust severity: A. Moderately resistant B. Moderately susceptible C. Very susceptible (Malvick, 2002).

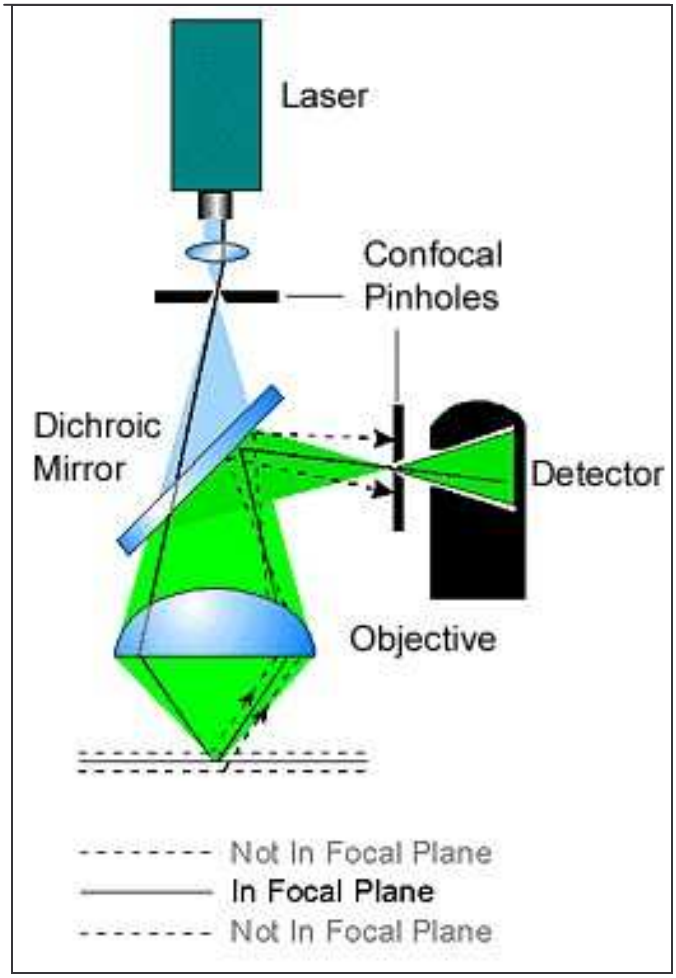

Figure 1.4 Representation of the principles of confocal laser scanning microscopy (Anonymous, 1998).

Chapter II

Pathology

Photograph courtesy of Z. A. Pretorius

Introgression of the stripe rust resistance gene YrSp into Kariega x Avocet/YrSp, a commercial South African wheat cultivar

2.1 Abstract

The introduction of stripe rust caused by *Puccinia striiformis* f. sp. *tritici*, pathotype 6E16A-, to the wheat growing regions of Western Cape, South Africa in 1996 and the subsequent epidemic in 1998 was a great concern amongst wheat farmers. Widely grown cultivars, Hugenoot and Carina, were resistant to the stripe rust pathotype, 6E16A-, but highly susceptible to 6E22A-. The cultivar, Kariega, was resistant to pathotype 6E16A- and remained resistant to 6E22A- but through no deliberate selection for resistance against stripe rust. Kariega exhibits adult-plant resistance to stripe rust with moderate levels of resistance to leaf rust (*P. triticina*) through the *Lr34/Yr18* resistance gene complex and *Lr1* and *Lr3a*. A stripe rust resistance gene, *YrSp*, is a single dominant gene conferring seedling resistance with an immune-type response in Avocet/*YrSp*. In this study a hybrid between Kariega and Avocet/*YrSp* was successfully achieved. A chi-square analysis of 621 F_2 seedlings, screened for resistance, indicated a deviation from the expected ratio for the presence of a single dominant gene. Ten F_3 families were selected from resistant and susceptible F_2 plants, respectively, for with-in family disease screening. The resistance gene was found to segregate within the resistant group while five F_3 families showed complete resistance thus implying that the breeding effort for Kariega x Avs/*YrSp* was highly successful. Only plants from completely resistant F_3 families exhibiting the immune-type resistance response were selected. In two susceptible F_3 families resistant plants were identified, possibly as a result of experimental error, and these were excluded.

2.2 Introduction

Stripe rust caused by *Puccinia striiformis* Westend. f. sp. *tritici* was introduced to the Western Cape, South Africa in 1996, and was initially observed in Palmiet, a South African bread wheat cultivar (*Triticum aestivum*) (Pretorius *et al*., 1997; Boshoff *et al*., 2002). The introduced pathotype was identified as race 6E16- found previously in East and North Africa, the Middle East and Western Asia (Pretorius *et al*., 1997; Boshoff *et al*., 2002). The prolonged cool and wet conditions in the Western Cape favoured the destructive nature of the pathogen and after its first occurrence, periodic surveys indicated the rapid spread of this disease to all major wheat growing regions of Southern Africa (Boshoff, 2000). The introduction of pathotype 6E16A- of *P. striiformis* f. sp. *tritici* caused wheat farmers in the Western Cape to spend an estimated R28 million on fungicides (Boshoff, 2000). From field observations and disease screening it was discovered that commonly grown cultivars, Hugenoot, Carina, Tugela, SST57 and Kariega were resistant to pathotype 6E16A- (Boshoff and Pretorius, 1999; Boshoff *et al*., 2002).

A stripe rust epidemic occurred in the Eastern Free State, in 1998, and was attributed to a new stripe rust pathotype 6E22A- (Boshoff and Pretorius, 1999). Fungicide control of the epidemic in the eastern Free State cost wheat producers an estimated R18 million (Boshoff, 2000). The stripe rust pathotype 6E22A- caused severe losses on previously resistant cultivars (Boshoff and Pretorius, 1999; Boshoff, 2000). The cultivars, Hugenoot and Carina, with resistance to pathotype 6E16A- were all susceptible to pathotype 6E22A- with the exception of Kariega (Boshoff and Pretorius, 1999; Boshoff *et al*., 2002). The pathotype 6E22A- became virulent to *Yr25* through a single step mutation of 6E16A- (Boshoff and Pretorius, 1999). This resulted in Kariega becoming one of the focal points of research to achieve durable resistance to stripe rust (Prins *et al*., 2005; Ramburan *et al*., 2004).

Kariega, a commercial hard-red spring wheat, has a high yield, high protein content and excellent protein quality (Barnard *et al*., 2002). The cultivar is susceptible to stripe rust in the seedling stage but exhibits complete adult plant

resistance (APR) (Prins *et al*., 2005). Kariega exhibits partial resistance to leaf rust (*P. triticina)*, thought to be conferred by the *Lr34*/*Yr18* resistance gene combination and has been shown to contain the seedling leaf rust resistance genes *Lr1* located on chromosome 5D and *Lr3a* on chromosome 6B (Prins *et al*., 2005). The resistance conferred by the resistance gene to stripe rust, *Yr18,* has proven effective for more than 50 years (Singh, 1992; William *et al*., 2003). Kariega also exhibits a morphological marker, leaf tip necrosis, due to the *Ltn* gene located on chromosome 7D (Ramburan *et al*., 2004). Thus the incorporation of seedling resistance to stripe rust in Kariega is of great commercial interest to achieve durable resistance, by means of gene stacking.

Seedling resistance is assumed to follow a gene-for-gene relationship (McIntosh and Wellings, 1986). The *YrSp* seedling resistance gene first described in Spaldings Prolific as a single dominant gene, and described in 1972 (Johnson *et al*., 1972). This gene exhibits an immune-type resistance reaction defined by Tarr (1972) as minute necrotic patches on the upper leaf surface through which the development of the pathogen is inhibited. The *YrSp* gene has been successfully incorporated into the white-seeded Australian spring wheat Avocet, as was proposed by Wellings and McIntosh, (1998) to produce a set of stripe rust Avocet near isogenic lines (NILs). This has resulted in a line containing not only stripe rust resistance but also stem rust resistance due to a translocation from *Thynopyrum elongatum* possibly containing the *Sr26* resistance gene on chromosome 6A in Avocet S although the presence of this gene has not been confirmed (McIntosh *et al*., 1995; Prins *et al*., 2005).

The aim of this study was to introgress the *YrSp* resistance gene from Avocet/*YrSp* into Kariega x Avocet/*YrSp*, towards achieving durable resistance. This was done in different phases that included the following: a) the screening of different cultivars including Kariega with stripe rust pathotype 6E22A- to confirm resistance and compare infection types, b) the screening of Avocet/*YrSp* with stem rust pathotype UVPgt50 and Kariega as a susceptible control to confirm the seed purity and c) the development of an F_1, F_2 and F_3 segregates from Kariega x Avocet/*YrSp*.

2.3 Materials and Methods

2.3.1 Seedling disease screening of different wheat cultivars with *Puccinia striiformis* f. sp*. tritici*

Seedlings of four wheat cultivars, Palmiet, Nantes, Kariega and Marico as well as the control cultivars or lines, Avocet/*YrSp*, Spaldings Prolific, Avocet S and Morocco were grown in a sterile soil-peat mixture (10 cm pots) in a disease-free greenhouse cubicle. Three repetitions of five to ten seeds per cultivar were planted (in clumps) and seven-day old seedlings were fertilised with 3:2:1 N-P-K mix (50 ml per pot of 10 g/l) and light conditioned in a growth chamber at 18^{o}C for 24 h (200 µmolm^{-2}s^{-1} PAR) according to an optimised seedling assessment protocol (Pienaar, 2004). Photosynthetically active radiation (PAR) emitted from cool-white fluorescent tubes (120 µmolm^{-2}s^{-1}) arranged directly above the plants additively supplemented natural daylight. Fertilisation of plants continued weekly until the experiments were terminated. Fresh urediospores of pathotype 6E22A- of *P. striiformis* f. sp. *tritici*, multiplied on the susceptible wheat cultivar, Morocco, were used for inoculation (42 x 10^{4} spores/ml). The spore concentration was determined using a haemocytometer. Urediospores were suspended in a light mineral oil and sprayed (0.8 ml/tray of 4 pots) onto eight day old seedlings using a micro-sprayer to ensure an even distribution of urediospores on the leaf surface. Thereafter, the plants were allowed to dry off in the shade for 1 h before being sprayed with cold sterile reverse osmosis water to produce a thin layer of moisture on the leaf surface. The plants were then placed in the dark, in a dew chamber at 11^{o}C and >96% relative humidity for 48 h.

Upon removal, the seedlings were dried in the shade for approximately 1 h and placed in a greenhouse cubicle at 15^{o}C-19^{o}C with a 14 h day and 10 h night temperature cycle. Disease reactions were scored 14 days after inoculation according to the 0-4 scale (Table 2.1) described by McIntosh *et al*. (1995). The disease screening was repeated in duplicate as previously described. A Bainsvlei (2002) seed source and a second seed source, UFS West Campus (1998), was used for the cultivars Palmiet, Nantes and Marico while a seed source obtained from the Small Grains Institute (SGI) in addition to the Bainsvlei seed source was

used for Kariega to confirm seed purity and the infection types obtained for the different cultivars. This was performed in triplicate as previously described.

2.3.2 Seedling disease screening of Avocet/*YrSp* and Kariega with *Puccinia graminis* Pers. f. sp. *tritici* Eriks. and Henn.

Seedlings of the parental cultivars, Kariega [pedigree: SST44 {CII3523 (Agent)/3*T4 (Anza)}//K4500.2/Sapsucker S] and Avocet/*YrSp* [pedigree: Avocet S*2/Spaldings Prolific were planted in clumps (10 cm pot) of 5-10 seeds. Seeds were grown in sterile soil-peat mixture in a disease-free greenhouse cubicle at 18-25^{o}C with a 14/10 h day/night temperature cycle. The seedlings were fertilised with 3:2:1 N-P-K mix (50 ml per pot of 10 g/l) once a week until the experiment was terminated. Eight-day old seedlings were inoculated with fresh urediospores of pathotype UVPgt50 of *P. graminis* f. sp. *tritici* suspended in light mineral oil. Following inoculation, seedlings were incubated in the dark at 22^{o}C in a dew chamber for 16 h where after they were placed in greenhouse cubicle maintained at 20-22^{o}C with a 14/10 h temperature cycle. Fluorescent tubes (200 µmolm^{-2}s^{-1} PAR) supplemented natural daylight and were arranged directly above the plants. The infection ratings of *P. graminis* f. sp. *tritici* were scored according to the 0-4 infection scale (Table 2.2) of Stakman *et al*. (1962). The infection scale described by McIntosh (1995) can also be used to rate the infection of stem rust (Table 2.1).

2.3.3 Population development and disease screening of F_2 and F_3 populations

Five seeds of the cultivars Kariega and Avocet/*YrSp* (source of which the purity had been verified) were planted separately in 1-L pots, germinated and cultivated in a disease-free greenhouse cubicle at day/night temperatures of between 18^{o}C-25^{o}C. Seeds were sown for three consecutive weeks to ensure synchronisation of flowering stage for successful crosses. At the onset of flowering the female parent, Avocet/*YrSp* was hand emasculated and three days later fresh pollen from Kariega was used for pollination.

The F_1 seeds were planted in 4-L capacity pots (4 entries per pot), germinated and cultivated in a disease-free greenhouse cubicle as previously described. F_1 plants were allowed to self to obtain an F_2 population. Plants were fertilised with 3:2:1 N-P-K mix (50 ml per pot of 10 g/l) until the experiment was terminated. The F_2 seeds produced from all the F_1 plants were pooled and stored at 4^{o}C for three weeks to ensure good germination. Following the F_2 disease screening, as previously described, 25 resistant and susceptible F_2 plants were selected, respectively, and diseased leaves removed. The diseased leaves were removed so that the plant may grow and produce uninfected leaves for DNA sampling and analysis. These were further cultivated as previously described in 4L pots, with five entries per pot. The plants were allowed to self to obtain F_3 families. Seeds of the F_3 families were collected per family and stored at 4^{o}C. The two parental lines, 10 resistant and 10 susceptible F_3 families were planted and screened as previously described and returned to a greenhouse cubicle at 15^{o}C-19^{o}C with a 14/10 h day/night temperature cycle.

2.4 Results

2.4.1 Disease screening of seedlings with *P. striiformis* f. sp. *tritici*

Stripe rust infection on standard cultivars was as expected and uniform when screened with pathotype 6E22A- of *P. striifomis* f. sp. *tritici*. Avocet/*YrSp* was completely resistant (0), Spaldings Prolific exhibited a resistant reaction and produced a fleck infection type (;). Avocet S (;2^{CN}) and Morocco (3^{++}4) were susceptible to stripe rust (Figure 2.1 and Table 2.3). The infection types produced by the standard cultivars were consistent between repetitions.

Seedlings of the cultivars, Palmiet, Nantes, Kariega and Marico cultivated from Bainsvlei (2002) and UFS West Campus (1998) seed sources exhibited infection types ranging from resistant to susceptible with many intermediate infection types (Table 2.3).

Palmiet was screened using two different seed sources namely, Bainsvlei (2002) and UFS West Campus (1998). Initially, only seedlings from the Bainsvlei

(2002) source was screened and exhibited infection types that ranged from completely resistant (0) to moderately susceptible (;1 and ;2) (Fig. 2.2A). Infection ratings varied between three repeats although the predominant infection types were observed in every repeat (Table 2.3). Following the screening of the initial source (Bainsvlei), Palmiet seedlings from both Bainsvlei (2002) and UFS West Campus (1998) were screened simultaneously in duplicate. Bainsvlei (2002) source seedlings exhibited infection types ranging from resistant (; and ;1) to moderately susceptible (;2) (Table 2.3). The infection types produced by Palmiet seedlings, produced from seeds obtained from the UFS West-Campus (1998), ranged from resistant (0 and ;1) to susceptible (;3). The infection types observed were consistent in a duplicate pot (Table 2.3).

Nantes was screened using two different seed sources namely, Bainsvlei (2002) and UFS West Campus (1998). Firstly, seedlings from the Bainsvlei (2002) source was screened and exhibited resistant infection types ranging from highly resistant (; and $;^{CN}$) to moderately resistant ($;1^{CN}$ and ;2) (Table 2.3). Necrosis and chlorosis were prominent on this cultivar and the infection types produced were consistent between repeats. Secondly, Nantes seedlings, from both Bainsvlei (2002) and UFS West Campus (1998) seed sources were screened in duplicate. Seedlings of Nantes, cultivated from seeds obtained at Bainsvlei (2002), exhibited infection types that ranged from resistant ($;1^{CN}$) to moderately susceptible (;2) (Table 2.3). Nantes seedlings, cultivated from the UFS West-Campus (1998) source, produced infection types ranging from resistant (0, $;^{CN}$ and $;1^{CN}$) to susceptible (;3) but was also characterized by necrosis and chlorosis (Table 2.3). The consistency of the results obtained from both seed sources was reflected in the duplicate pots.

Kariega was screened using two different seed sources namely, Bainsvlei (2002) and SGI (2001). Seedlings from Bainsvlei (2002) seed source was screened initially and produced a highly necrotic infection type with severe chlorosis (Fig. 2.2B and Table 2.3). Contrary, and in addition to the resistant reaction, a highly susceptible ($3^{++}4$) infection type was also produced. The infection types were consistent across three repeats with severe necrosis and chlorosis noted. Following the initial screening, Kariega seedlings, from both

Bainsvlei (2002) and SGI (2001) were screened in duplicate. Seedlings of Kariega, cultivated from seeds of the seed source Bainsvlei (2002), produced a highly necrotic infection type characterised by severe chlorosis. This infection type was also produced by seedlings cultivated from seeds produced by SGI (2001) and these results were consistent in the duplicate pot. This served as a confirmation of the uncharacteristic infection type exhibited by Kariega and the SGI (2002) source was used for crosses and further studies throughout this study.

Marico was screened using two different seed sources namely, Bainsvlei (2002) and UFS West Campus (1998). First the seedlings of the Bainsvlei (2002) seed source was screened and produced consistent infection types between repetitions with the predominant infection types present in each repeat. These ranged from completely resistant (0) to moderately resistant (;1^{CN}) and moderately susceptible (;2) (Table 2.3). Secondly, Marico seedlings, from both Bainsvlei (2002) and UFS West Campus (1998) were screened in duplicate. Seedlings cultivated from the Bainsvlei (2002) source, produced infection types ranging from moderately resistant (;1) to susceptible (;2 and ;3). The seedlings cultivated from the seed produced at UFS West-Campus (1998) produced infection types ranging from resistant (0 and ;) to susceptible (;2 and ;3) with consistent repetition of the infection types in the duplicate pot.

2.4.2 Disease screening of parents Avocet/*YrSp* and Kariega with *Puccinia graminis* f. sp. *tritici*

Following the results of the first or initial seedling screening (Bainsvlei sources), a disease screening of the parental cultivars was performed to screen for resistance to *P. graminis* f. sp. *tritici*. The pathotype UVPgt50 was used to differentiate between susceptibility and resistance to indicate the presence of the *Sr26* resistance gene. The infection types produced by pathotype UVPgt50 of *P. graminis* f. sp. *tritici* on Kariega was a susceptible infection type (3^{c}) and Avocet/*YrSp* exhibited a resistant infection type (;1^{c}) (Fig. 2.5).

2.4.3 Population development and disease screening of F_2 and F_3 generations

The cross between Avocet/*YrSp* (female) and Kariega (male) yielded 45 F_1 seeds. The F_1 seeds were bulked, planted and allowed to self to produce a F_2 seed. The F_2 seed were bulked, planted and used for disease screening for resistance against stripe rust as well as for F_3 development. For F_2 screening, the parental cultivars Kariega and Avocet/*YrSp* along with 621 F_2 seedlings were screened with pathotype 6E22A- of *P. striiformis* f. sp. *tritici*. Kariega produced a susceptible infection type (2 and 3^{CN}) and Avocet/*YrSp* produced a resistant infection type (0) (Fig. 2.3). Of the 621 F_2 seedlings screened, 492 seedlings were resistant (0) and 129 seedlings were susceptible (2^{CN}) (Fig. 2.4 and Table 2.4). Chi-square test analysis performed on the F_2 screening data revealed a Chi-square value of 5.92 for a 3:1 gene model and a Chi-square value of 1.67 for a 13:3 gene model ($p = 0.05$) (Table 2.4).

Following the F_2 screening, resistant and susceptible F_2 seedlings were selected and allowed to self to produce F_3 seeds. Twenty- three F_3 families were derived from resistant F_2 plants, 17 F_3 families were derived from selected susceptible F_2 plants with the rest of the families (25 F_2 plants selected) both not producing enough or too little seed and were discarded. For the F_3 population screening, the parental cultivars Kariega and Avocet/*YrSp* and ten resistant and susceptible F_3 families respectively were randomly selected for screening with pathotype 6E22A- of *P. striiformis* f. sp. *tritici*. Kariega again produced a susceptible infection type (2 and 3^{CN}) with more necrosis and chlorosis noted and Avocet/*YrSp* produced a resistant infection type (0) (Fig. 2.6). Of the ten resistant F_3 families derived from resistant F_2 plants, five resistant F_3 families exhibited complete resistance to stripe rust infection while five F_3 resistant families segregated for the trait (Table 2.4). Of the ten susceptible F_3 families derived from susceptible F_2 plants, seedlings of eight susceptible F_3 families exhibited susceptibility to stripe rust while single plants in two susceptible F_3 families segregated and were discarded (Table 2.4). Infection types ranging from resistant to susceptible with intermediate infection types were observed on F_3 seedlings (Fig. 2.7).

2.5 Discussion

The development of a hybrid between Kariega and Avs/*YrSp* was successfully achieved. Chi-square analysis deviated from the expected 3:1 gene ratio for a single dominant gene being responsible for conferring resistance against stripe rust (Table 2.3). The Chi-square analysis resulted in a Chi-square value of 5.92 for a 3:1 gene model deviating significantly. The 3:1 gene model is however more probable than13:3 as the *YrSp* gene is described as a single dominant gene while the 13:3 gene ratio indicates the presence of a dominant gene and a recessive gene.

The standard cultivars, Avocet/*YrSp*, Spaldings Prolific, Avocet S and Morocco yielded uniform and expected disease infection types to the pathoytype 6E22A- of *P. striiformis* f. sp. *tritici* (Table 2.3). The infection types produced by the cultivars, Palmiet, Nantes, and Marico, when infected with pathotype 6E22A- of *P. striiformis* f sp. *tritici*, varied but were consistent between repeats.

The exhibition of a highly necrotic and chlorotic infection type by Kariega was a cause of great concern as a susceptible infection type (;2 and ;3) is more characteristically associated with Kariega when screened with pathotype 6E22A- of *P. striiformis* f. sp. *tritici* (Boshoff, 2000). This uncharacteristic infection type might be indicative of impure or mixed seed source which would jeopardise the aims of the study. The uncharacteristic infection type enhanced the ability to easily screen for the resistance gene, *YrSp*, when crossed with this cultivar. However, due to the inconsistent infection types produced by the different cultivars and the unexpected infection type of Kariega, an alternative seed source for the four cultivars Palmiet, Nantes, Kariega and Marico, was screened along with the original seed sources in order to confirm the initial disease screening results (Table 2.3). Despite this, there were no significant differences between the infection types observed for the different seed sources of Palmiet, Nantes, Marico and Kariega.

Following the results of the disease screening, Kariega was chosen as the cultivar of choice for the introgression of the *YrSp* resistance gene. The infection

type produced by Kariega allowed for unambiguous selection of resistant individuals, exhibiting the immune-type resistance response in the F_2 population, while the other cultivars, Palmiet, Nantes and Marico produced too many intermediate infection types ranging from resistant to susceptible.

The result of the disease screening of the seedlings and the selection of Kariega as a parent for crosses with Avocet/*YrSp* brought about the disease screening of these lines with pathotype UVPgt50 of *P. graminis* f. sp. *tritici*. The susceptible infection type observed on Kariega was uniform and expected, as this cultivar is susceptible to stem rust (Prins *et al*., 2005). A completely resistant reaction was expected for Avocet/*YrSp* as Avocet S is thought to contain the *Sr26* resistance gene due to a translocated segment from *Thinopyrum elongatum* (Friebe *et al*., 1994; McIntosh *et al*., 1995). A resistant infection type (;1^{CN}) was observed on Avocet/*YrSp* similar to the findings of Prins *et al.* (2004) that served as a confirmation of the observed infection type produced by pathotype UVPgt50 of *P. graminis* f. sp. *tritici* on Avocet/*YrSp*.

Following the disease screening of the ten resistant and ten susceptible F_3 families, it was found that five of the ten resistant F_3 families selected for disease screening displayed segregation with regard to resistance. Most of the F_3 seedlings were resistant exhibiting the stable inheritance of the resistance gene. The resistant F_3 seedling disease screening showed a 1:1 segregation ratio of resistant to susceptible infection types (Table 2.4). A single resistant F_3 family exhibited more susceptible seedlings than resistant seedlings. Single resistant plants were found in two of the ten susceptible F_3 families and these were discarded. The segregation was characterised by two and three resistant seedlings, respectively, in a population of 19 and 21 seedlings, respectively (Table 2.4). The segregation observed in the F_3 families were possibly as a result of heterozygosity and inaccurate F_2 classifications. The highly necrotic and chlorotic phenotype observed in the parent cultivar was once again observed in the F_3 population (Fig. 2.6).

2.6 Conclusion

A hybrid between Avs/*YrSp* and Kariega was successfully achieved and provides a unique research opportunity to study the effect of this gene in a South African wheat cultivar. The addition of seedling resistance conferred by the *YrSp* resistance gene in combination with the durable resistant *Lr*34/*Yr*18 gene complex and *Lr*1 and *Lr*3a seedling resistance genes could potentially provide Kariega with durable resistance to both stripe and leaf rust, and would be of particular academic and commercial interest.

The observation of an uncharacteristic necrotic, chlorotic infection type on Kariega when infected with *P. striiformis* f. sp. *tritici* is a cause of concern as this is indicative of possible impure or mixed seed source. Although Kariega displays leaf tip necrosis, polymorphisms for this trait was observed in a recent study (Ramburan *et al*., 2004) and this may be linked to the unexpected infection type exhibited by Kariega in this study. The uncharacteristic exhibition of this phenotype, however, aided the accuracy with which resistant plants exhibiting the immune-type resistance response could be identified. Kariega may however also be heterogeneous for an additional seedling gene.

The introgression of resistance genes into wheat cultivars with agro-economically important traits such as high yield and good baking and milling qualities is deemed the most cost-effective disease management strategy (Boshoff *et al*., 2002; Chen and Line, 1993a). This strategy is also considered to have the least harmful impact on the environment, as chemical control of rusts is expensive and environmentally hazardous (Singh, *et al*., 2000; Singh, 1992). Understanding the genetics of resistance would be helpful for exploiting resistance, in gene rotation, gene pyramiding and multiline development. This would also greatly increase the understanding of the genetic basis of virulence in stripe rust pathotypes (Chen and Line, 1993b).

Strategic deployment of resistance genes, combining race-specific and non-specific resistance as well as the pyramiding of resistance genes with small

additive effects is proving to be a successful mechanism to increase durable resistance to rust fungi (Rubiales and Niks, 2000).

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Table 2.1 Description of infection types used in classifying the disease reactions to *P. striiformis* f. sp. *tritici* on seedling wheat leaves (McIntosch, 1995).

Seedling infection type	Description
0	No visible uredia
;	Necrotic flecks
;n	Necrotic areas without sporulation
1	Necrotic and chlorotic lesions with restricted sporulation
2	Moderate sporulation with necrosis and chlorosis
3	Sporulation with chlorosis
4	Abundant sporulation without chlorosis
Z	Variable size with larger uredia towards the leaf base

Variations are indicated by the use of - (less than average for the class) and + (more than average for the class).

C and N are used to indicate more than usual degrees of chlorosis and necrosis.

Table 2.2 Description of infection types used in classifying the disease reactions to *P. graminis* f. sp. *tritici* on seedling wheat leaves (Stakman *et al*., 1962).

Class	Infection type[a]	Description of symptoms
Immune	0	No signs of infection to the naked eye but minute flocks may be visible under low magnification
Very resistant	;	No uredia, but distinct flecks of varying sizes, usually a chlorotic yellow but occasionally necrotic
Resistant	1	Small uredia surrounded by yellow chlorotic or necrotic areas
Moderately resistant	2	Small to medium-sized uredia,typically in a dark green island surrounded by a chlorotic area
Moderately susceptible	3	Medium-sized uredia usually surrounded by a light green chlorosis
Susceptible	4	Large uredia with a limited amount of chlorosis; may be diamond-shaped
Mesothetic or Heterogenous	X	A range of infection types from resistant to susceptible scattered randomly on a single leaf; caused by a single isolate
Heterogenous	Z	Large uredia concentrated at the base of the leaf

[a]Pluses and minuses are used to indicate variations from the size of uredia typical of a particular infection type as follows: -- uredia much smaller than typical and at the lower limit for the infection type, - uredia smaller than normal, + uredia larger than normal, ++/ +++ uredia much larger than typical and at the upper limit of the infection type.

Table 2.3 Infection types[a] produced by pathotype 6E22A- of *P. striiformis* f. sp. *tritici* on primary leaves of eight selected cultivars.

Cultivar	1st Seed Source	1st repeat	2nd repeat	3rd repeat
Palmiet	Bainsvlei (2002)	;cn	2p0 / 4p; / 3p;1	3p; / 2p;1 / 2p;2
Nantes	Bainsvlei (2002)	3p; / 9p;cn / 1p;2	4p; / 9p;1cn	;1cn
Kariega	Bainsvlei (2002)	;cn / 1p;3	;cn / 1p3++4	;cn (very necrotic)
Marico	Bainsvlei (2002)	3po / 4p;1cn / 2p;2	4p;2 / 3p; / 4p0	8p;1 / 5p; / 1p;2
Controls				
Avocet/*YrSp*	UFS Greenhouse (2002)	0	0	0
Spaldings Prolific	UFS West Campus (1998)	;	;	;
Avocet S	Australia (1999)	;2cn	;2cn	;2cn
Morocco	Bainsvlei (2002)	3++4	3++4	3++4

Cultivar	2nd Seed source	1st repeat	2nd repeat
Palmiet	Bainsvlei (2002)	1p; / 1p;1 / 8p;2	1p;c / 5p;1 / 4p;2
	UFS West Campus (1998)	11p0 / 9p; / 3p;1	8p0 / 5p;/ 3p;c / 1p cn / 2p;1 / 2p;3
Nantes	Bainsvlei (2002)	13p;1cn / 2p;2	3p;cn / 6p;1cn / 6p;2
	UFS West Campus (1998)	2p0 / 1p; / 4p;cn / 8p;1cn / 5p;2cn / 2p;3	1p; / 1p;cn / 3p;1cn / 2p;1 / 9p;2 / 2p;3
Kariega	Bainsvlei (2002)	13cn	8cn / 2p;1cn
	Small Grain Institute (2001)	4p0 / 2p; / 13p;cn / 3p;1 / 2p;2 / 2p;3	3p0 / 1p; / 7p;cn / 5p;cn / 7p;1 / 1p;2
Marico	Bainsvlei (2002)	3p;1 / 7p;2 / 6p;3	9p;2 / 8p;3
	UFS West Campus (1998)	6p0 / 1p;cn / 11p;1cn / 8p;2	3p0 / 1p;cn / 10p;1 / 6p;2 / 6p;3
Controls			
Avocet/*YrSp*	UFS Greenhouse (2002)	0	0
Spaldings Prolific	UFS West-Campus (1998)	4p0 / 10p;	10p0 / 9p;
Avocet S	Australia (1999)	;2 / 4p;2cn	;2cn
Morocco	Bainsvlei (2002)	3++4	3++4

[a] Infection types according to a 0-4 scale described by McIntosh *et al*. (1995) were recorded 14 days post inoculation on primary leaves of plants inoculated at eight days.

Table 2.4 Infection types[a] produced by pathotype 6E22A- of *P. striiformis* f. sp. *tritici* on primary leaves of parents, F_2 and F_3 populations.

F_2 Population								
Parent Cultivars	ITs[e]	Number of plants	IT[d]	Number of plants	Number of resistant ITs[e]	Number of Susceptible ITs[e]	P-value[b] (3:1)	P-value (13:3)[b]
Avocet/YrSp	0	106	0	314	492	129	5.92[c*]	1.67
Kariega	;1^{CN} ;2^{CN} ;3^{CN}	91	;	174				
			;^{CN}	4				
			2^{CN}	129				
F_3 Population								
Avocet/YrSp	0	33	Resistant Families	Number of resistant plants	Number of susceptible plants	Susceptible Families	Number of resistant plants	Number of susceptible plant
Kariega	;1^{CN} ;2^{CN} ;3^{CN}	42	R 1	15	0	S 1	0	15
			R 2	7	3	S 2	0	12
			R 3	13	4	S 3	0	17
			R 4	12	0	S 4	0	15
			R 5	13	0	S 5	0	14
			R 6	15	0	S 6	2	19
			R 7	7	2	S 7	0	23
			R 8	3	7	S 8	3	21
			R 9	14	0	S 9	0	18
			R 10	9	3	S 10	0	16

[a] Infection types according to 0-4 scale described by McIntosh *et al*. (1995) recorded 14 days post inoculation on primary leaves of plants inoculated at eight days; [b] Chi-square test used to compare F_2 segregation; [c] Df=1; [d] ITs=Infection type; *Significance difference at p=0.05

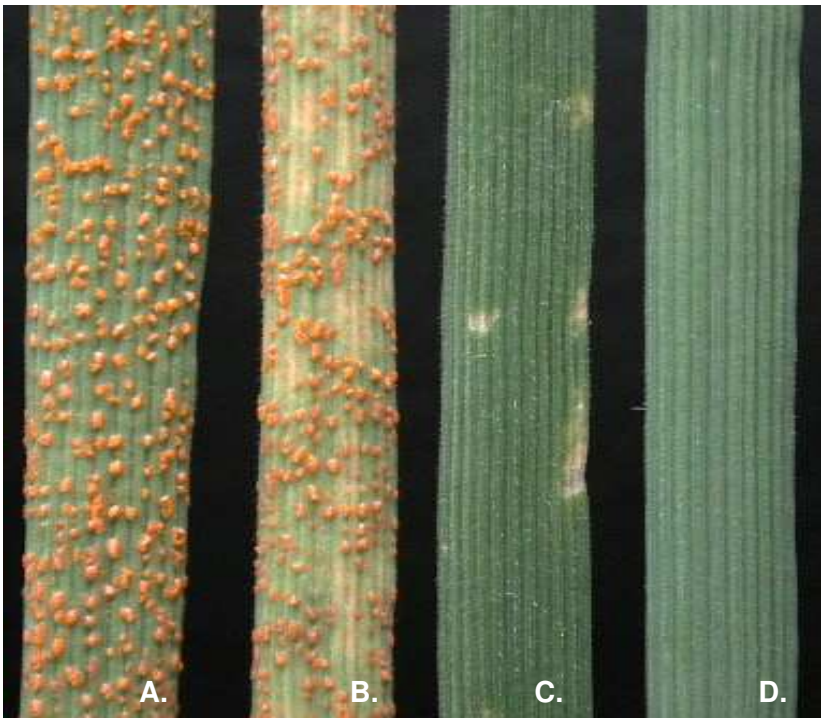


Figure 2.1 The infection types produced by the control cultivars: **A.** Morrocco ($3^{++}4$), **B.** Avocet S ($;2^{CN}$), **C.** Spaldings Prolific (;) and **D.** Avocet/*YrSp* (0) infected with pathotype 6E22A- of *P. striiformis* f. sp. *tritici*.

Figure 2.2 Different infection types produced by (**A**) Palmiet **I.** susceptible (3^{--}), **II.** moderately susceptible ($;2^{CN}$) and **III.** resistant (;) and (**B**) Kariega **I.** susceptible ($3^{++}4$) and **II.** very necrotic ($;^{CN}$) infected with pathoype 6E22A- of *P. striiformis* f. sp. *tritici*.

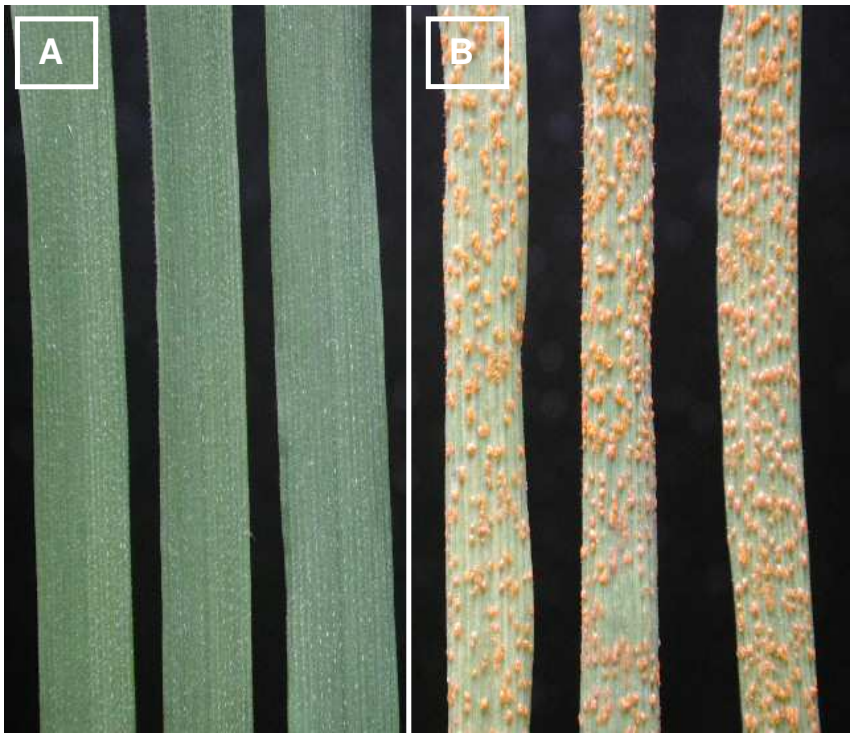


Figure 2.3 The infection types observed on (**A**) Avocet/*YrSp* and (**B**) Kariega when infected with pathotype 6E22A- of *P. striiformis* f. sp. *tritici* in the F_2 screening.

Figure 2.4 Infection types observed on the F_2 individuals when infected with pathotype 6E22A-of *P. striiformis* f. sp. *tritici* ranging from: (**A**) highly resistant (0), (**B**) moderately susceptible (;2^{CN}), (**C**) susceptible ($3^{++}4$).

Figure 2.5 Infection types on Avocet/*YrSp* (**A** and **B**) resistant (;1^{CN}) and Kariega (**C**) susceptible (3^{c}) infected with pathotype UVPgt50 of *P. graminis* f. sp. *tritici*.

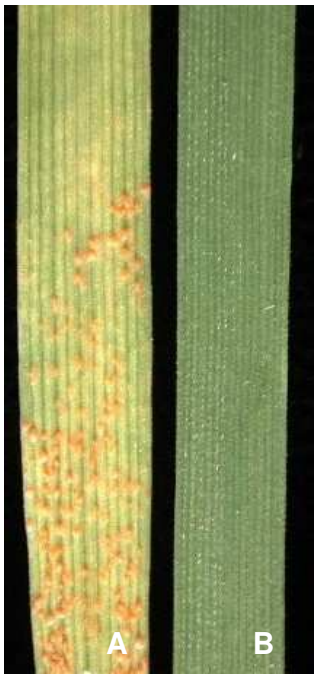


Figure 2.6 The infection types observed on (**A**) Kariega (3^{--C}) and (**B**) Avocet/*YrSp* (0) when infected with pathotype 6E22A- of *P. striiformis* f. sp. *tritici* in the F_3 screening.

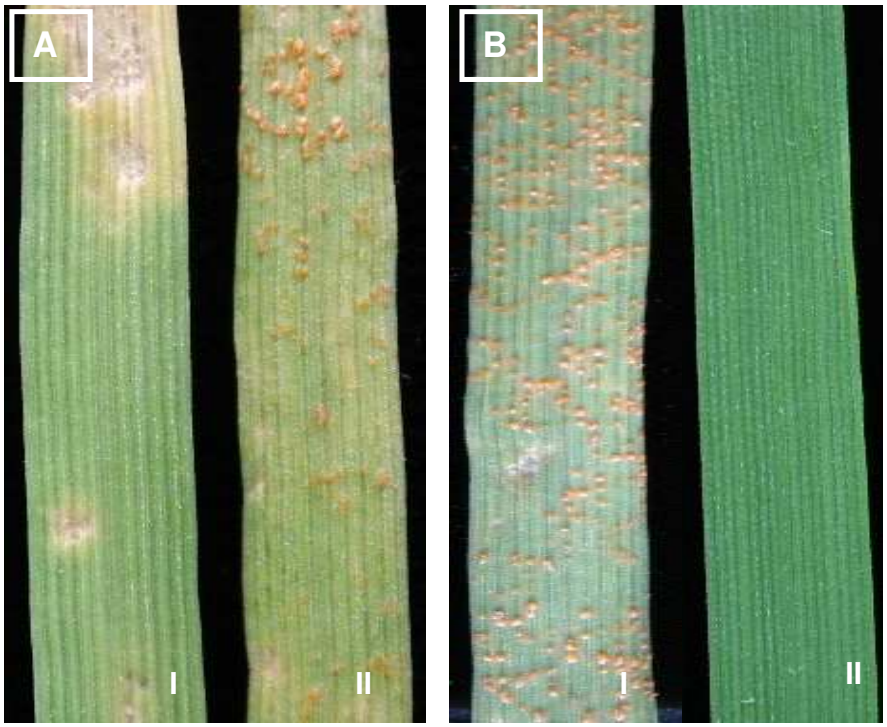
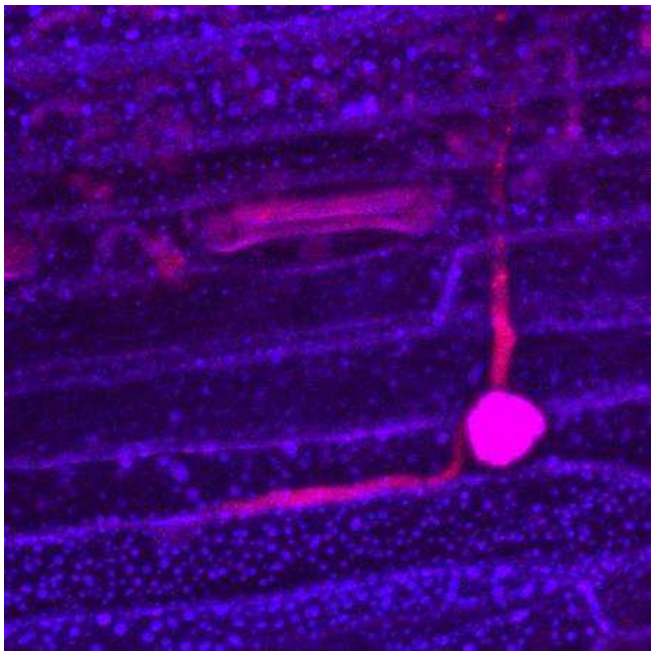


Figure 2.7 Infection types observed on F_3 plants when infected with pathotype 6E22A- of *P. striiformis* f. sp. *tritici* ranging from (**A**) (**I**) necrotic (CN) and (**II**) necrotic susceptible ($;2^{CN}$) to (**B**) (**I**) highly susceptible ($3^{++}4$) and (**II**) resistant (0).

Chapter III

Histology



The use of electron microscopy and the potential of confocal laser scanning microscopy to study the infection of *Puccinia striiformis* Westend. f. sp. *tritici*

3.1 Abstract

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* is a destructive wheat disease. Management strategies are focused on producing resistant cultivars to achieve durable resistance. It is therefore important to understand the underlying basis for the way in which resistance genes function. Fluorescence and electron microscopy has been routinely used to elucidate the infection process of many pathogens. In this study, electron microscopy was used to confirm the infection pathway of *P. striiformis* f. sp. *tritici*. The germination and infection efficiency of *P. striiformis* f. sp. *tritici* was quantified for a resistant cultivar Avocet/*YrSp* and a susceptible cultivar, Kariega at the seedling stage. No significant differences were noted for the structures used in the quantitative analysis between the susceptible and resistant plants suggesting that *YrSp* mediated resistance are posthaustorial. Confocal laser scanning microscopy (CLSM) holds great potential to visualize chemical and cellular events that occur during host-pathogen interactions. A histological study also assessed the applicability of fluorochromes safranin, aniline blue, ethidium bromide, uvitex 2B and the orange G probe for CLSM. The results indicate that the orange G probe and Uvitex 2B are the fluorochromes with the most potential to distinguish between fungal and plant tissue.

3.2 Introduction

Although the production of wheat (*Triticum aestivum* L.) is lower second to that of maize in South Africa it is affected by various biotic factors including viruses and pathogens. Stripe rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* is a destructive pathogen of wheat throughout the world. Following the introduction of pathotype 6E16A- of *P. striiformis* f. sp. *tritici*, wheat farmers in the Western Cape spent an estimated R28 million on fungicides in 1996 and R18 million in 1997 by farmers in the eastern Free State (Boshoff, 2000). Following the epidemic in 1998 in the Eastern Free State caused by 6E22A- a further R6 million in fungicide treatment was required to treat 42 000 ha, not taking into account the loss in yield and quality (Boshoff, 2000). Thus the cost of controlling this disease through fungicides justifies the development of resistant varieties and offers a more durable solution to stripe rust infection. A seedling gene, *YrSp* observed in Spaldings Prolific, was found resistant to stripe rust since and was first characterised in 1972 (Johnson *et al*., 1972). This gene was introgressed into Kariega from Avocet/*YrSp* and presently used to study its effect on the progression of infection (refer to chapter II).

Knowledge of the genetics of the host-pathogen interaction is vital to determine how resistance is conferred and how it can be identified at various growth stages to utilise it more effectively. Microscopy is one of the most useful tools for studying host-pathogen interactions and has been used to study the dynamic interactions between various hosts and pathogens (Heath, 2000). Initially, light microscopy was the most prominent biological tool used, but was replaced by electron microscopy as this technique allows a more detailed visualization of infection structures.

The infection process of *Puccinia striiformis* f. sp. *tritici* has never been documented but is assumed to be the same as *P. triticina* the causal agent of leaf rust, which has been well documented using scanning (SEM) and transmission (TEM) electron microscopy (Hughes and Rijkenberg, 1985; Wood and Heath, 1986; Lennox and Rijkenberg, 1989; Hu and Rijkenberg, 1998).

SEM has been used to show that after germination, a germ tube traverses the leaf surface until it encounters a stomata. The method of penetration is indirect through the stomatal opening. Using fracture techniques it has become possible to determine that after stomatal penetration, a substomatal vesicle (SSV) is formed in the substomatal chamber. The SSV further develops into primary and secondary infection hyphae that when appressed against a host mesophyll cell, form a haustorium mother cell (Hughes and Rijkenberg, 1985; Lennox and Rijkenberg, 1989; Coutinho *et al.*, 1993; Adendorff and Rijkenberg, 2000). TEM has been used to elucidate the development of the haustorium mother cell producing a haustorial neck that penetrates the host mesophyll cell and delimits into an haustorium (Littlefield and Heath, 1979). Although TEM is routinely used to study the cell structure and morphology of sub-cellular biological samples, finding specific structures in sample sections is tedious and time consuming.

The development of fluorescence microscopy made it possible to study host-pathogen interactions using the resolution capacity of light microscopy (Zhang and Dickinson, 2001). This type of microscopy is based on the differential interaction of a fluorescent stain with host and pathogen tissue and can be used to determine the exact stage at which fungal growth is arrested during incompatible reactions and to visualise the infection process in compatible reactions (Bender *et al.*, 2000). A disadvantage of fluorescence microscopy is that it is highly dependent on the discrimination power of the stains and dyes used and does not allow the same resolution of structures as TEM or SEM (Butt *et al.*, 1989; Heath, 2000).

Confocal laser scanning microscopy is a revolutionary advance in microscopy and is based on technology that makes use of laser excitation and detection of fluorescent molecules with different emission wavelengths (Czymmek *et al.*, 1994). Fluorochromes are used to produce differential fluorescence of fungal and plant structures. CLSM works on the principle that all the incident (excitation) illumination is focused to a spot that is scanned across the specimen and only the fluorescence emanating from that area is detected and digitised (Heath, 2000). This results in a high resolution of the illuminated

area only. The ability to collect fluorescent signals from a narrow optical section is ideal for studies of fungal introgression into host plants and for imaging host-pathogen interactions in three dimensions (Howard, 2001; Duncan and Howard, 2000; Matsubara *et al.,* 1999). Confocal laser scanning microscopy holds great potential to visualize chemical and cellular events that occur during host-pathogen interaction.

The aim of this study was to confirm the infection structures formed by pathotype 6E22A- of *P. striiformis* f. sp. *tritici* during infection of cultivars Avocet/*YrSp* and Kariega using SEM and TEM as well as to determine at which stage infection is aborted in resistant plants in order to assess at what level *YrSp* mediated resistance is active. CLSM was also used to determine its application in studying host-pathogen interactions.

3.3 Materials and Methods

3.3.1. Seedling disease screening with *Puccinia striiformis* f. sp*. tritici*

Seven-day old seedlings of Morocco, Avocet/*YrSp* and Kariega were grown in a sterile soil-peat mixture (10 cm pots) in a disease-free greenhouse cubicle. Seedlings were fertilised with a 3:2:1 N-P-K mix (50 ml per pot of 10 g/l) and light conditioned in a growth chamber at 18^{o}C for 24 h (200 µmolm^{-2}s^{-1} photosynthetically active radiation [PAR]) according to an optimised seedling assessment protocol (Pienaar, 2004). Cool-white fluorescent tubes (120 µmolm^{-2}s^{-1}) arranged directly above the plants emitted PAR, additively supplementing natural daylight. Fertilisation of plants continued weekly until the experiments were terminated. Fresh urediospores of pathotype 6E22A- of *P. striiformis* f. sp. *tritici*, multiplied on the susceptible wheat cultivar, Morocco, were used for inoculation (42 x 10^{4} spores/ml). Urediospores were suspended in a light mineral oil and sprayed (0.6 ml/tray of 3 pots) onto eight-day old plants using a micro-sprayer to ensure even distribution of urediospores on the leaf surface. Thereafter, the plants were allowed to dry off in the shade for 1 hour before being sprayed with cold sterile reverse osmosis water to produce a thin layer of

moisture on the leaf surface. The plants were then placed in the dark, in a dew chamber at 11°C and >96% relative humidity for 48 h. Upon removal, the seedlings were dried in the shade for approximately 1 h and placed in a greenhouse cubicle at 15°C-19°C with a 14/10 h day/night temperature cycle. Leaf material collected from Kariega and Avocet/*YrSp* at different times was used for scanning, transmission, light and confocal microscopy while leaf material collected from Morocco was used for confocal laser scanning microscopy only. Different stages and microscopy techniques were used to achieve certain objectives of the study and were determined also by the potential and application of the microscopy used.

3.3.2 Scanning Electron Microscopy (SEM)

Leaf samples of approximately 1 cm were taken from infected wheat seedlings at various time intervals (Table 3.1). These were fixed for 24 h in 3% phosphate-buffered glutaraldehyde. The samples were dehydrated in a graded ethanol series (30%, 50%, 70%, 95% and 100%), critical point dried and mounted onto stubs. The samples were gold/palladium coated and examined using a Jeol Winsem 6400 Scanning Electron Microscope at 5 kV (Centre for Confocal and Electron Microscopy, University of the Free State).

3.3.2.1 Fracture Technique for SEM samples

A modified fracture technique described by Hughes and Rijkenberg (1985) was used to strip the epidermis of leaf samples to allow observation of the infection structures formed at various time intervals. The leaves were fixed in 3% phosphate-buffered gluteraldehyde, dehydrated in a graded ethanol series, as previously described, and critical point dried. The dried leaves were mounted onto stubs with double-sided tape. A second stub with double-sided tape was gently pressed against the mounted leaf sample to remove the leaf epidermis exposing the inner surface of the epidermis. The stripped leaf samples were gold/palladium coated and examined using a Jeol Winsem 6400 Scanning Electron Microscope at 5 kV.

3.3.2.2 Quantitative assessment of the germination and infection process using SEM

A quantitative assessment of the germination process was performed at 24 and 48 h post-inoculation with three repetitions per cultivar per time interval. Germination efficiency was determined by counting approximately 100 urediospores on Avocet/*YrSp* and Kariega leaf segments sampled at 24 and 48 hpi according to the following classes: collapsed urediospores (indentured), collapsed spores with germ tubes (indentured with visible germ tube), non-germinated urediospores (round/oval shaped) and urediospores with germ tubes (Table 3.2). The penetration efficiency of *P. striiformis* f. sp. *tritici* was evaluated by counting 100 germ tubes on Avocet/*YrSp* and Kariega leaf segments at 24 and 48 hpi, respectively, to assess the growth of the germ tube over the stomata compared to stomatal penetration by the germ tube (Table 3.2). These data were analysed for analysis of variance, descriptive statistics and standard deviation using the statistical program NCSS 2004 (Statistical Solutions, Cork, Ireland). The assessment of internal infection structures was performed on stripped leaves as previously described. An average of 350 stomata was studied at 6, 7 and 8 dpi by determining the frequency of infection structures formed by *P. striiformis* f. sp. *tritci* (Table 3.3).

3.3.3 Transmission Electron Microscopy (TEM)

Leaf samples were collected at various time intervals (Table 3.1) and were fixed for 24 h in 3% phosphate-buffered glutaraldehyde, post-fixed in 2% osmium tetroxide, dehydrated in a graded alcohol series and embedded in Spurr's low-viscosity resin. Sections were stained with 5% uranyl acetate followed by lead citrate and examined using a Philips CM100 electron microscope at 60 kV. Sections (3 µm) were also prepared from the resin embedded material and viewed under the light microscope.

3.3.4 Confocal Laser Scanning Microscopy (CLSM)

To study the effect of chlorophyll (naturally fluorescing molecule) on the clarity of confocal images, two fixation protocols were assessed. Fresh leaf material was sampled, with several leaf segments per fixation protocol. For the first fixation protocol, leaf samples were fixed in 100% ethanol for 5 min. In the second protocol, leaf segments were fixed in an ethanol: dichloromethane (3:1 v/v) fixative containing 0.15% trichloroacetic acid for 18 to 24 h until leaves were yellow in colour. The fixed segments from both fixative protocols were rinsed with double distilled water.

Three weeks after inoculation, several leaf sections were fixed and stained according to a modified method of Rohringer *et al*., (1977). The leaf samples were fixed in ethanol: dichloromethane (3:1 v/v) with 0.15% trichloroacetic acid for 18 to 24 h or until leaves turned yellow in colour. The samples were washed twice with 50% ethanol for 15 min followed by two washes with 0.05M NaOH for 15 min or until the leaves turned yellow. The leaf samples were rinsed thrice with double distilled water and soaked in 1M Tris buffer (pH 8.5) for 30 min. The samples were stained using 1% (w/v) safranin (solution), 1% (w/v) aniline blue (in 7% acetic acid), ethidium bromide (10 mg/ml) and 0.1% (w/v) uvitex 2B, in 1M Tris buffer (pH 8.5), respectively, for approximately 5 min. The samples stained with safranin, aniline blue and ethidium bromide were rinsed with 100% ethanol to remove excess dye while sections stained with Uvitex 2B were rinsed with double distilled water. Thereafter, leaf samples were washed for 30 min in 25% aqueous glycerol, mounted and stored in 50% glycerol with a trace of lactophenol. Samples stained for 1 min with the orange G probe was mounted in water. All the leaf samples were examined using a Nikon Confocal Microscope with a 454 to 676 nm Argon-ion, 543 nm green and 633 nm red Helium-Neon laser combination unit (Centre for Confocal and Electron Microscopy, University of the Free State).

3.4 Results and Discussion

3.4.1 Light, scanning and transmission electron microscopy

SEM was successfully used to identify the general morphology of the wheat leaves (Fig. 3.1) and progression of the infection process on the leaf surface (Fig. 3.3 and Fig. 3.4) and stripped leaves (Fig. 3.5 and Fig. 3.6). Light microscopy was used to observe the internal structure of the leaf after preparation for TEM (Fig. 3.2). TEM was used to visualise the infection process in the substomatal chamber (Fig. 3.7 to Fig. 3.10).

3.4.1.1 Infection process of *P. striiformis* f. sp. *tritici*

Echinulate (round to oval shape) urediospores germinated on the leaf surface, giving rise to germ tubes that generally grew perpendicularly to the long axis of the leaf (Fig. 3.3A and Fig. 3.3B). The germ tube was often observed to grow across the stomata and did not necessarily penetrate the first stoma encountered (Fig 3.4A). It was also observed that when the germ tube encountered a stoma, it grew along the stomatal slit (Fig. 3.4A). Many theories for the directional growth patterns of rust fungi on the leaf surface have been posed. Dickinson (1970) suggested that the elongation of a germ tube towards a stoma was due to a curved thigmotropic stimulus due to leaf surface. It has also been suggested that the physical and chemical features of the leaf surface influence the direction of germ tube growth including the presence of cuticular ridges (Wynn, 1976), the pattern of epicuticular wax crystals (Lewis and Day, 1972) and the pH gradient across the leaf surface (Edwards and Bowling, 1986). However, random growth, defined as germ tubes traversing both axes of the leaf surface, was observed in *P. sorghi* by Hughes and Rijkenberg (1985). In this study, the growth of the germ tube always appeared to be directional with the germ tube growing over as many as three stoma.

After stomatal penetration, an appressorium characteristic of all rust fungi is usually formed (Hughes and Rijkenberg, 1985; Lennox and Rijkenberg, 1989;

Hu and Rijkenberg, 1998; Adendorff and Rijkenberg, 2000). However, in *P. striiformis* f. sp. *tritici* an appressorium is not formed and the germ tube directly penetrates the stoma to affect disease infection (Fig. 3.4B). Once the germ tube has penetrated the stomatal chamber, a round to oval shaped substomatal vesicle is formed (Fig. 3.5A). In every instance, the substomatal vesicle was appressed to the stoma and orientated with its long axis parallel to the long axis of the stomatal opening. Although many researchers have described a substomatal vesicle initial (SSVI), the vesicles observed in this study could not be described with certainty as SSVIs (Lennox and Rijkenberg, 1989). Structures appearing to be pseudo-SSVIs were observed in distinct patterns, singly, in pairs on either end of a stoma, in triplicate or quadruplicate evenly distributed across the stomatal slit, in resistant as well as susceptible plants (Fig. 3.5B). Although this has not been described previously it is possible that these “pseudo-SSVIs” may act as a “probe” to determine the conditions within the substomatal chamber before commiting to penetration. Following substomatal vesicle (SSV) formation, the SSV then furcated to produce a characteristic V-shape primary infection hyphae (Fig. 3.6A). Where a primary infection hypha became attached to a mesophyll cell, a septum formed, delimiting a haustorium mother cell (Fig. 3.6B). TEM visualised infection hyphae growing between host mesophyll cells and infection hyphae where a septum delimits a haustorium mother cell (Fig. 3.7A and Fig. 3.7B). The mature haustorium mother cell then penetrated the host cell by means of a haustorial neck extended from the haustorium mother cell (Fig. 3.8A). Host cell penetration was followed by the elongation of the haustorial neck and expansion of the terminal portion into a binucleate haustorium (Fig. 3.8B). The penetration peg used to breach the host wall expands to about twice its width once inside the mesophyll cell. Approximately halfway during the point of penetration a neckband is visible that stained intensively with osmium tetroxide (Fig. 3.8A). An absence of further structure formation or depression at the penetration site suggests that enzymatic action rather than mechanical pressure is responsible for initial penetration of the host cell wall (Ehrlich and Ehrlich, 1971; Bushnell, 1972). The host plasmalemma is invaginated and not penetrated by the developing haustorium. The haustorium consists of a haustorial neck and an

expanded haustorial head, both surrounded by a fungal wall known as the haustorium wall. The haustorium wall is surrounded by an extrahaustorial matrix that is enveloped by an extrahaustorial membrane (Fig. 3.9A). After infection, the intercellular fungal tissue becomes sporogenous and uredial sporogenous cells give rise to urediospore initials that in turn produce a proximal pedicel and a distal immature urediospore (Fig. 3.9B and Fig. 3.10A). Immature urediospores are characterised by a primary as well as a secondary wall and conical spines situated in small, circular depressions in the spore surface (Fig. 3.10B). After further development, secondary wall material is deposited on the inside of the primary wall, which is digested and totally absent in mature urediospores (Fig. 3.10B). Uredia are formed sub-epidermally. As the urediospores form, they press against the host epidermis from the inside causing it to rupture and release the urediospores on the leaf surface (Fig. 3.21).

3.4.1.2 Quantitative analysis of the germination and internal infection structures formed by *P. striiformis* f. sp. *tritici*

Surface observations of the urediospore germination efficiency indicated a small significant difference between the susceptible Kariega and the resistant Avocet/*YrSp* cultivars during three independent repetitions (Table 3.4). However, collapsed urediospores were observed at 24 hpi for Avocet/*YrSp* but were not observed on Kariega at 24 hpi (Fig 3.11 and Table 3.5). The means of the counts for collapsed urediospores at 48 hpi for Avocet/*YrSp* and Kariega were similar to that observed for Avocet/*YrSp* at 24 hpi (Fig. 3.11 and Table 3.5). A marked increase in the means of collapsed urediospores with germ tubes was observed between 24 and 48 hpi for both Avocet/*YrSp* and Kariega (Fig. 3.12 and Table 3.5). The number of collapsed urediospores with germ tubes at 24 hpi was greater than the number of collapsed urediospores in both Avocet/*YrSp* and Kariega at 24 hpi (Fig. 3.11; Fig. 3.12 and Table 3.5). A comparison of non-germinated urediospores between Avocet/*YrSp* and Kariega (Fig. 3.13 and Table 3.5) revealed an increase in the number of urediospores observed on Kariega at 24 and 48 hpi as opposed to Avocet/*YrSp* (Fig 3.13 and Table 3.5). A

decreased number of urediospores with germ tubes were also observed for both Avocet/*YrSp* and Kariega at 24 and 48 hpi although with small differences (Fig. 3.14 and Table 3.5). These results indicate that there is a small significant difference in the germination efficiency of urediospores on the resistant cultivar Avocet/*YrSp* and the susceptible cultivar Kariega (Table 3.4).

Variation for germ tubes growing over stomata was bigger for Avocet/*YrSp* when compared to Kariega at both 24 and 48 hpi (Fig. 3.15; Fig 3.16 and Table 3.5), however the variation for germ tubes penetrating stomata between Avocet/*YrSp* and Kariega was very small if not similar at both 24 and 48 hpi. A greater number of germ tubes were observed growing over stomata on Avocet/*YrSp* at 48 hpi when compared to Kariega at the same time interval (Fig. 3.16). The highest incidence of germ tubes growing over stomata on Avocet/*YrSp* at 24 hpi was higher than germ tube penetration (Fig. 3.15). The number of observations of germ tube growth over stomata was far greater than the incidences of a germ tube penetrating a stoma (Fig. 3.15; Fig 3.16 and Table 3.5). The incidence of observed stomatal penetration by *P. striiformis* f. sp*. tritici* was low (Figure 3.25 and Table 3.2) and is similar to results by Kotze (1999), Broers and Lopez-Atilano (1996) and Mares and Cousen (1977). As much as 67% of the germ tubes observed grew over stomata with only 5% seemingly penetrating a stoma (Figure 3.15, Fig. 3.16 and Table 3.2). However, the low penetration efficiency did not result in a low infection rate as observed by pustule formation on the leaf surface of susceptible plants. In contrast to other rusts, a previous study by Rubiales and Niks (1992) on *Hordeum chilense* also found that less than 60% of *P. striiformis* f. sp. *tritici* urediospores germinated on the leaf surface. Of those that germinated, more than 70% did not penetrate a stoma. This suggests that this pathogen differs from other cereal rusts in its recognition sensitivity of stomata. Niks (1990) observed a negative correlation between germ tube length and infection rate of *P. hordei* on *Hordeum vulgare*. It was suggested that the formation of long germ tubes and exploratory branches reduced the amount of energy available to infect the plant (Ferreira and Rijkenberg, 1989).

The fracture technique was used to quantify the internal infection structures formed by the pathogen. Although oval to round shaped vesicles were observed, these could not be conclusively identified as substomatal vesicle initials (SSVIs) according to published descriptions (Lennox and Rijkenberg, 1989; Hu and Rijkenberg, 1998). This is due to the observation of more than one such pseudo-ISSV and its specific arrangement along the stomatal slit that has not been described previously (Fig.3.5B and Fig. 3.17). These vesicles were orientated along the long axis of the stoma and occurred in pairs on either side of a guard cell or in numbers of three or four evenly spaced along the stomatal slit (Fig.3.5A and Table 3.3). The number of infection structures formed by Kariega when compared to Avocet/*YrSp* is higher at 6 and 7 dpi and a marked increase in the incidence of infection structures for Kariega was observed at 8 dpi when compared to Avocet/*YrSp*. (Fig. 3.18). The presence of haustorium mother cells and haustoriums in Avocet/*YrSp* suggests that the resistance conferred by *YrSp* is post-haustorium.

3.4.2 Confocal laser scanning microscopy (CLSM)

The confocal images produced from leaf samples not cleared of chlorophyll and fixed in 100% ethanol, produced an immense amount of auto-fluorescence (Fig. 3.19). Germ tubes were however easily distinguishable from plant material and produced green fluorescence under a combination of green and red fluorescence (Fig. 3.19). Stomata were also clearly visible and fluoresced green under a combination of green and red fluorescence (Fig. 3.19). Leaf samples prepared using the modified method of Rohringer *et al*. (1977) produced clear confocal images of the urediospores, germ tubes and stomata (Fig. 3.20). The elimination of the auto-fluorescence allowed laser sectioning of the material. This resulted in the visualisation of the substomatal vesicle (infection structure formed when a germ tube penetrates a stoma) appressed underneath the stoma (Fig. 3.20). From these results it can be seen that cleared leaf material (containing no chlorophyll) allowed a greater depth of visualisation.

Leaf samples stained with safranin allowed the visualization of early pustule formation using a combination of transmitted laser light and red fluorescence and red fluorescence only (Fig. 3.22). Safranin was found to be the least successful in staining the fungal material on the leaf surface only permitting the visualisation of early pustule formation.

Aniline blue was effective in identifying pustules and urediospores. The stain did not effectively discriminate between plant and fungal tissue and it cannot be useful to elucidate internal infection structures (Fig. 3.23). Emerging urediospores were visualised by red fluorescence when stained with aniline blue under a combination of transmitted laser light and red fluorescence and red fluorescence only (Fig. 3.23).

Although ethidium bromide is a useful fluorochrome in the characterisation of fungal structures that include haustorial necks and haustoria under fluorescence microscopy (Kuck *et al*., 1981), the fluorochrome was also effective in identifying pustules and urediospores with CLSM (Fig. 3.24). A combination of red fluorescence and transmitted laser light produced a less distinct image of the emerging hyphae when stained with ethidium bromide (Fig. 3.24) in comparison to the image produced under red fluorescence only. However, it could not discriminate between plant and fungal tissue and therefore would not be useful in elucidating such interactions.

Uvitex 2B proved effective in identifying pustules. Leaf sections stained with uvitex 2B appeared to allow discrimination between fungal material emitting green fluorescence and infected leaf tissue emitting red fluorescence (Fig. 3.25). Pustules stained with uvitex 2B could be clearly distinguished by blue fluorescence (Fig. 3.25), whereas a combination of green and red fluorescence resulted in less distinct visualisation of the pustule (Fig. 3.25). This stain is routinely used to elucidate host-pathogen interactions under fluorescence microscopy (Jacobs *et al*., 1996; Bender *et al*., 2000). Uvitex 2B has been successfully utilised to study the infection structures formed by *P. striiformis* f.

sp. *tritici* using CLSM where the microscope is equipped with a UV laser (J. Moldenhauer- personal communication).

Germinating urediospores stained with the orange G probe allowed visual differentiation between the emerging germ tube and the spore coat (Fig. 3.26). The germ tube was clearly distinguishable from the leaf surface (Fig. 3.26). Although orange G was effective in distinguishing between the plant epidermis and germ tubes it did not differentially stain germ tubes and stomata (Fig. 3.26). A possible reason for this is that orange G detects living tissue. Optical sectioning allowed the visualisation of a substomatal vesicle appressed beneath a stoma in the substomatal chamber (Fig. 3.26). Progressive optical sectioning yielded no fluorescence indicating the probe could not further penetrate the epidermis. This result is however indicative of the discriminatory power of CLSM to distinguish between plant and fungal material and its potential to visualise fungal infection structures below the epidermis.

Although a large number of different fluorochromes are available, these need to be evaluated for their applicability in CLSM in terms of their power of discrimination between plant and fungal material. Fluorochromes that have been used, such as DiO, DAPI have been utilised to visualise nuclei (Czymmek *et al*., 1994). Calcofluor is specifically formulated to bind to chitin found in fungi, while the fluorochrome uvitex 2B has also shown great potential for confocal microscopy with a UV laser component and green-fluorescent protein has been used in the sequenced plant *Arabidopsis thaliana* with great success (Li *et al*., 1999). Thus the potential of a fluorochrome is dependent on the chemical composition of the stain and its interaction with host-pathogen structures.

3.5 Conclusion

The infection pathway of *P. striiformis* f. sp. *tritici* was successfully confirmed using SEM, TEM and light microscopy. The infection structures observed for *P. striiformis* f. sp. *tritici* are similar to that observed for other rust fungi. The most significant findings in the infection process are the absence of

an appressorium formed by *P. striiformis* f. sp*. tritici* and the presence of multiple pseudo-SSVIs.

The quantification of the infection process did not determine any significant differences between resistant and susceptible lines up to haustorium formation. Despite this, there was a very clear distinction between resistant and susceptible plants after 7 dpi. Thus, the resistance mediated by the *YrSp* gene is posthaustorial. This study indicated a significant difference between resistant and susceptible cultivars after seven days of inoculation, when the infection structure incidence in susceptible cultivars continued to increase while the infection structure incidence in the resistant cultivar decreased. The reduction of infection structure incidence is not generally expected and may be due to sampling error or the distribution of pathogen density during inoculation. In another study, the incidence of infection structures in resistant cultivars was found to reach a plateau while the incidence of infection structures in susceptible cultivars increased (J. Moldenhauer- personal communication-). Heath (1981) proposes that the fungus has been inhibited rather than killed by the resistance mechanism. It may then be deduced that even though the germination efficiency is good but the penetration efficiency of *P. striiformis f. sp. tritici* is poor, the infection efficiency of this pathogen would very high. Therefore, once the pathogen has penetrated a stoma, prolific growth of the pathogen occurs.

The results from this study determined that uvitex 2B and the orange G probe hold the best potential of the fluorochromes evaluated for use with the CLSM. Unfortunately, the stains used in this study were not effective in studying the subcellular structures formed by the pathogen after stoma penetration. This highlights the need for further research into the development of fluorochromes for specific application.

Confocal laser scanning microscopy holds great potential to elucidate many uncertain and unexplored facets of host-pathogen interactions. The biggest constraint on the potential impact of confocal microscopy in host-pathogen interactions is the difficulty of selecting or developing fluorochromes

with the ability to differentiate between plant and fungal material (Heath, 2000). The multitude of technological advances in fluorescent chemistry, optical, computer and laser technology will continue to expand the potential of confocal microscopy imaging for resolving biological processes.

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Table 3.1 Collection times of leaf samples, application of the data and microscope technique used.

Time collected	Application	Microscopy Technique
24 hpi	Quantification Identify infection process	SEM SEM, TEM & CLSM
48 hpi	Quantification Identify infection process	SEM SEM, TEM & CLSM
72 hpi	Fluorochrome assessment	CLSM
6 dpi	Quantification Identify infection process	SEM & TEM
7 dpi	Quantification Identify infection process	SEM
8 dpi	Quantification Identify infection process	SEM
21 dpi	Fluorochrome assessment	CLSM

Hpi- Hours post-inoculation; dpi- days post-inoculation; SEM- Scanning Electron Microscopy; CLSM- Confocal Laser Scanning Microscopy; TEM- Transmission Electron Microscopy

Table 3.2 Total number of urediospore germination, germ tube growth and stomatal penetration of *Puccinia striiformis* f. sp. *tritici.*

	1st Repeat				2nd Repeat				3rd Repeat			
Spore Description	Avocet/*YrSp* (24 hpi)	Kariega (24 hpi)	Avocet/*YrSp* (48 hpi)	Kariega (48 hpi)	Avocet/*YrSp* (24 hpi)	Kariega (24 hpi)	Avocet/*YrSp* (48 hpi)	Kariega (48 hpi)	Avocet/*YrSp* (24 hpi)	Kariega (24 hpi)	Avocet/*YrSp* (48 hpi)	Kariega (48 hpi)
Collapsed urediospores	11	0	3	6(5.6)	1	0	3	7(6.8)	0	0	2	0
Collapsed urediospores with germ tubes	7	4(3.7)	9	25(23.1)	2	6(5.8)	18(17.8)	20(19.4)	6(5.9)	4(3.8)	12	6
Non-germinated urediospores	33	68(63)	24	29(26.9)	26	30(29.1)	24(23.8)	46(44.7)	30(29.7)	49(47.1)	24	29
Urediospores with germ tubes	49	36(33.3)	64	48(44.4)	71	67(65.0)	56(55.5)	30(29.1)	65(64.4)	51(49.0)	62	65
Total number of urediospores	100	108(100)	100	108(100)	100	103(100)	101(100)	103(100)	101(100)	104(100)	100	100
Germ tubes growing over stomata	11	14	67	25	42	31	26	10	25	20	30	26
Germ tubes penetrating stomata	5	4	0	3	0	0	0	0	0	2	0	0
Percentage germination	7.49	4.36	9.64	25.48	2.71	6.67	18.56	20.3	6.65	4.51	12.62	6.65

() = Raw counts were converted to values out of 100 spores where more than 100 spores were observed and these values were used for data analysis using NCSS 2001 (Statistical Solutions, Corkland, Ireland).

Table 3.3 Total number infection structures formed by *P.striiformis* f. sp. *tritici* per 100 stomata within the susceptible cultivar Kariega and resistant line Avocet/*YrSp.*

Infection stuctures	Avocet/*YrSp* 6 dpi	Avocet/*YrSp* 7 dpi	Avocet/*YrSp* 8 dpi	Kariega 6 dpi	Kariega 7 dpi	Kariega 8 dpi
V:						
1 per stoma	(10) 10	(4) 4	(8) 8	(14) 14	(11) 11	(9) 9
2 per stoma	(17) 34	0	(4) 8	(5) 10	(1) 2	(12) 24
3 per stoma	(6) 18	(1) 3	0	(10) 30	(6) 18	(4) 12
Total	**62**	**7**	**16**	**54**	**31**	**45**
Percentage	17.714	2.000	4.571	15.428	8.857	12.857
[a]SSV	5	3	1	4	7	10
[a]PH	4	3	1	4	7	10
[a]HMC	2	1	1	0	3	4

V- Vesicle; SSV- Substomatal vesicle; PH- Primary hyphae; HMC- Haustorium mother cell; [a]Observations made per 350 stomata; dpi- days post-inoculation; ( )- Number of incidences observed

Table 3.4 Analysis of variance for the germination efficiency of urediospores and germ tube penetration of stomata by *P. striiformis* f. sp. *triciti*.

Term	Degrees of freedom	Sum of squares	Mean of square	F-Ratio	Probability Level	Power (alpha=0.05)
Germination efficiency						
A: Treatment[a]	3	3.95E-03	1.32E-03	0	1	0.05
B: Classes	3	19950.25	6650.08	83.3	0*	1
AB	9	1709.22	189.91	2.38	0.0345*	0.831
S	32	2554.63	79.83			
Total	47	24214.11				
Germ tube penetration efficiency						
A: Treatment[a]	3	347.46	115.82	1	0.42	0.22
B: Classes	1	4082.04	4082.04	35.42	0*	0.99
AB	3	468.46	156.15	1.35	0.29	0.29
S	16	1844	115.25			
Total	23	6741.96				

[a] Different treatments: Avocet/*YrSp* and Kariega 24 h and 48 h, respectively.

* Significant at alpha = 0.05.

Table 3.5 Mean, standard deviation and standard error of urediospore and germ tube classes.

Classes	Treatments	Mean	Standard deviation	Standard error
Collapsed urediospores	Avs/*YrSp* 24 h	4	6.1	3.5
	Kar 24 h	0	0	0
	Avs/*YrSp* 48 h	2.7	0.6	0.3
	Kar 48h	4.1	3.6	2.1
Collapsed urediospores with germ tubes	Avs/*YrSp* 24 h	4.9	2.6	1.5
	Kar 24 h	4.4	1.2	0.7
	Avs/*YrSp* 48 h	12.9	4.8	2.6
	Kar 48h	16.2	8.9	5.2
Non-germinated urediospores	Avs/*YrSp* 24 h	29.6	3.5	2
	Kar 24 h	46.4	16.9	9.8
	Avs/*YrSp* 48 h	23.9	0.1	6.7
	Kar 48h	33.5	9.7	5.6
Urediospores with germ tubes	Avs/YrSp 24 h	61.5	11.3	6.5
	Kar 24 h	49.1	15.6	9.2
	Avs/*YrSp* 48 h	60.5	4.4	2.6
	Kar 48h	46.2	18	10.4
Germ tubes growing over stomata	Avs/*YrSp* 24 h	26	15.5	9
	Kar 24 h	21.7	8.6	5
	Avs/*YrSp* 48 h	41	22.6	13.1
	Kar 48h	20.3	8.9	5.2
Germ tubes penetrating stomata	Avs/*YrSp* 24 h	1.7	2.9	1.7
	Kar 24 h	2	2	1.2
	Avs/*YrSp* 48 h	0	0	0
	Kar 48h	1	1.7	1

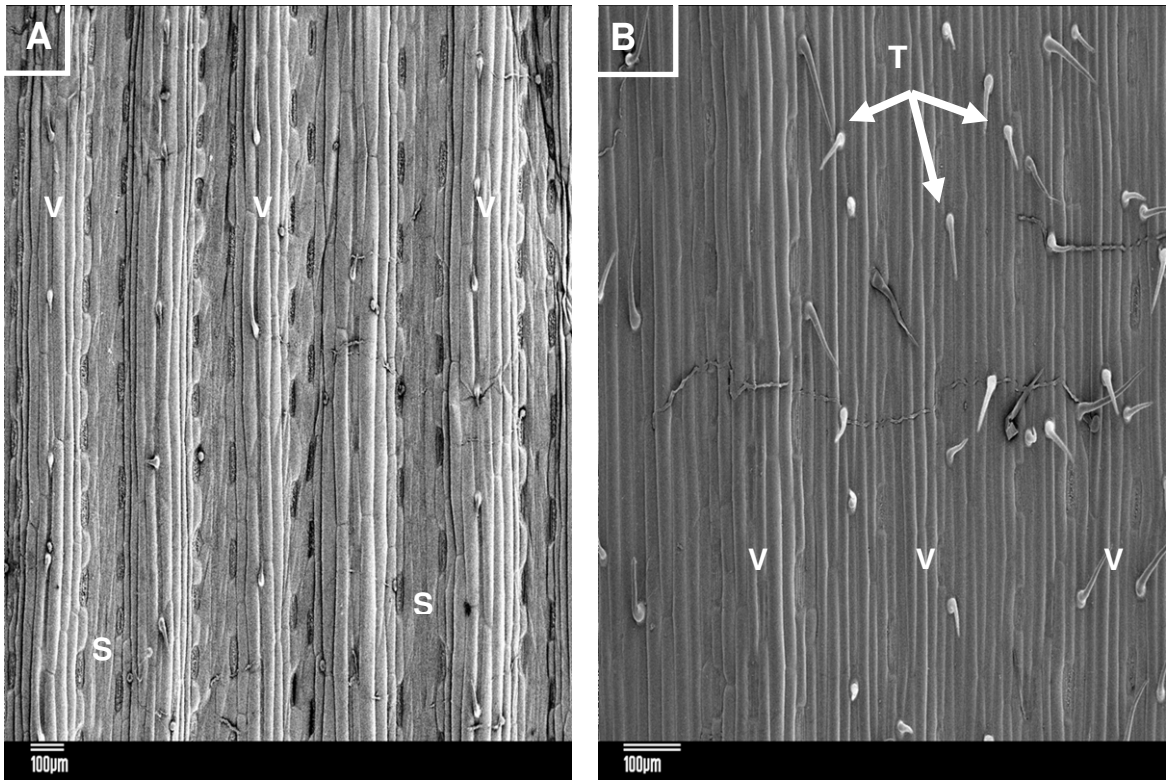


Figure 3.1 **A.** Adaxial (upper) surface of Avocet/*YrSp* leaf showing veins (V), stomata (S) and trichomes (T). **B.** Abaxial (lower) surface of leaf showing veins (V), stomata and trichomes (T).

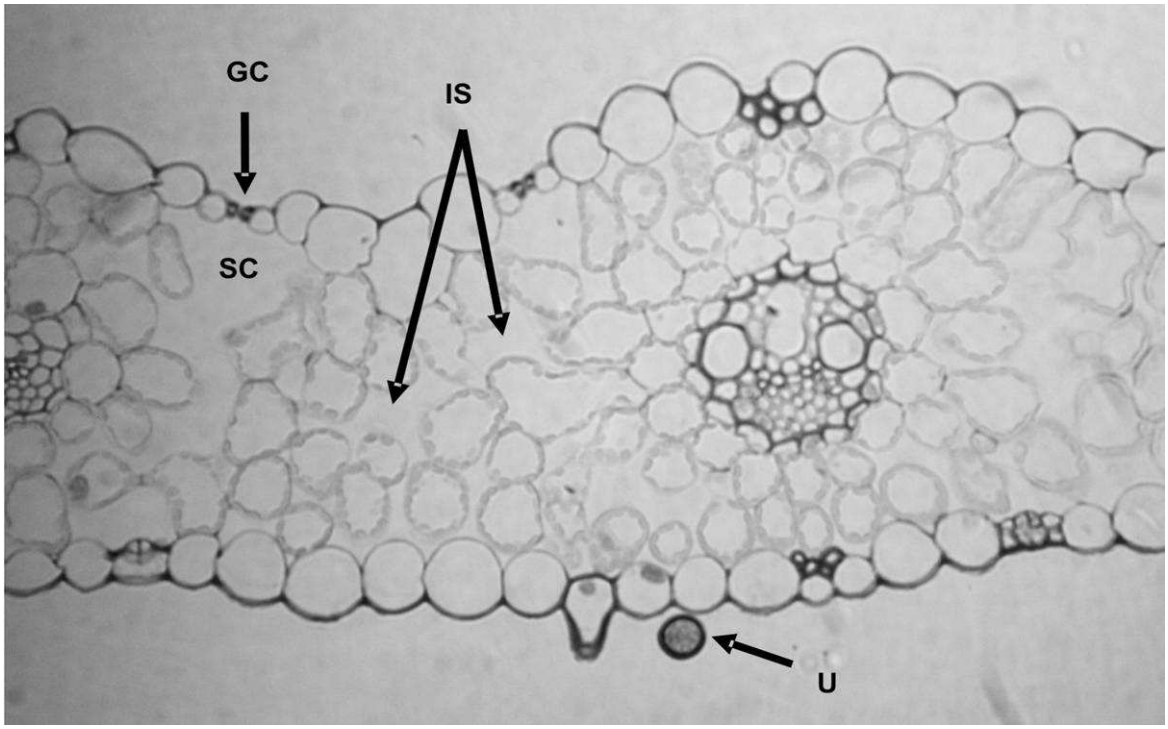


Figure 3.2 Light micrograph of a cross section of a Kariega leaf showing leaf structure. Guard cells (GC), intercellular spaces (IS), Substomatal chamber (SC), urediospore (U).

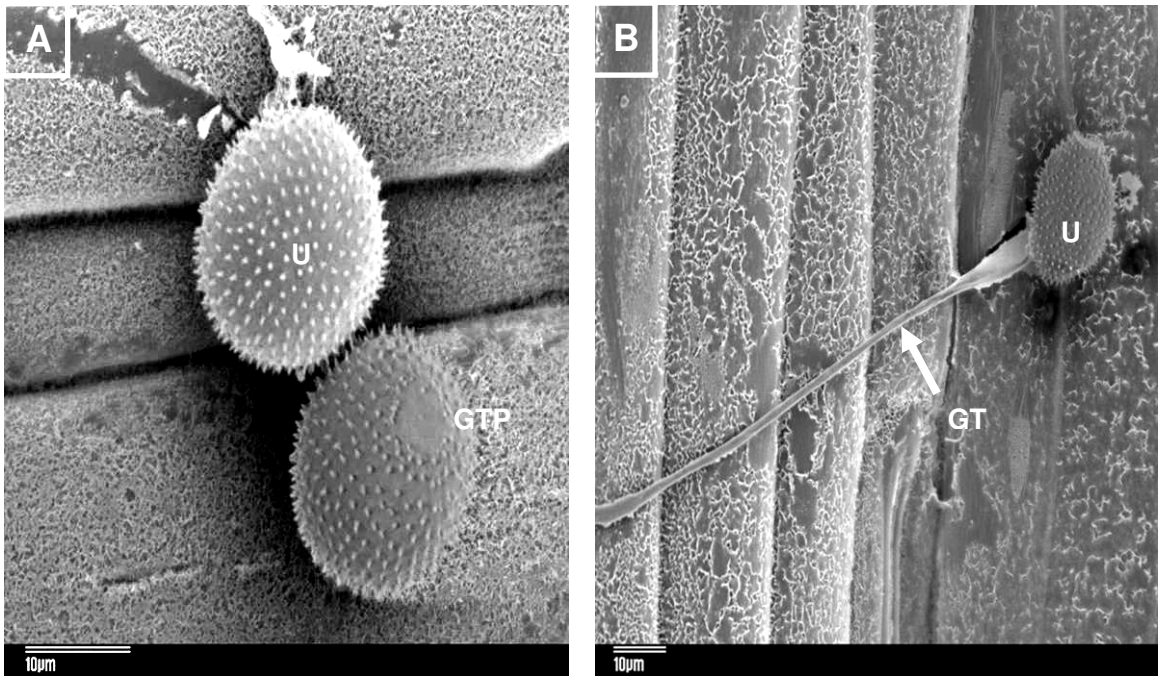


Figure 3.3 **A.**Urediospores (U) of *P. striiformis* f. sp. *tritici* on Avocet/*YrSp* leaf showing germ tube pore (GTP). **B.** A germinated urediospore (U) with germ tube (GT) growing perpendicularly to the long axis of an Avocet/*YrSp* leaf.

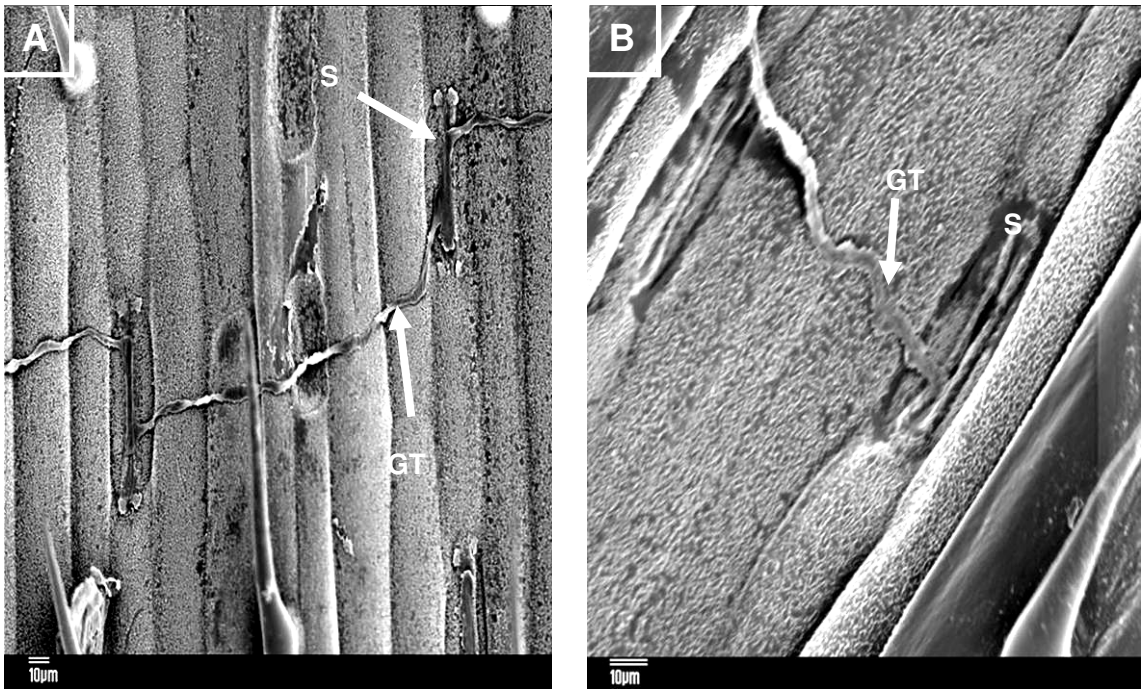


Figure 3.4 **A.** Continuous growth of germ tube (GT) of *P. striiformis* f. sp. *tritici* across stoma (S) on an Avocet/*YrSp* leaf. **B.** The penetration of a stomata by a germ tube (GT) on a Kariega leaf surface.

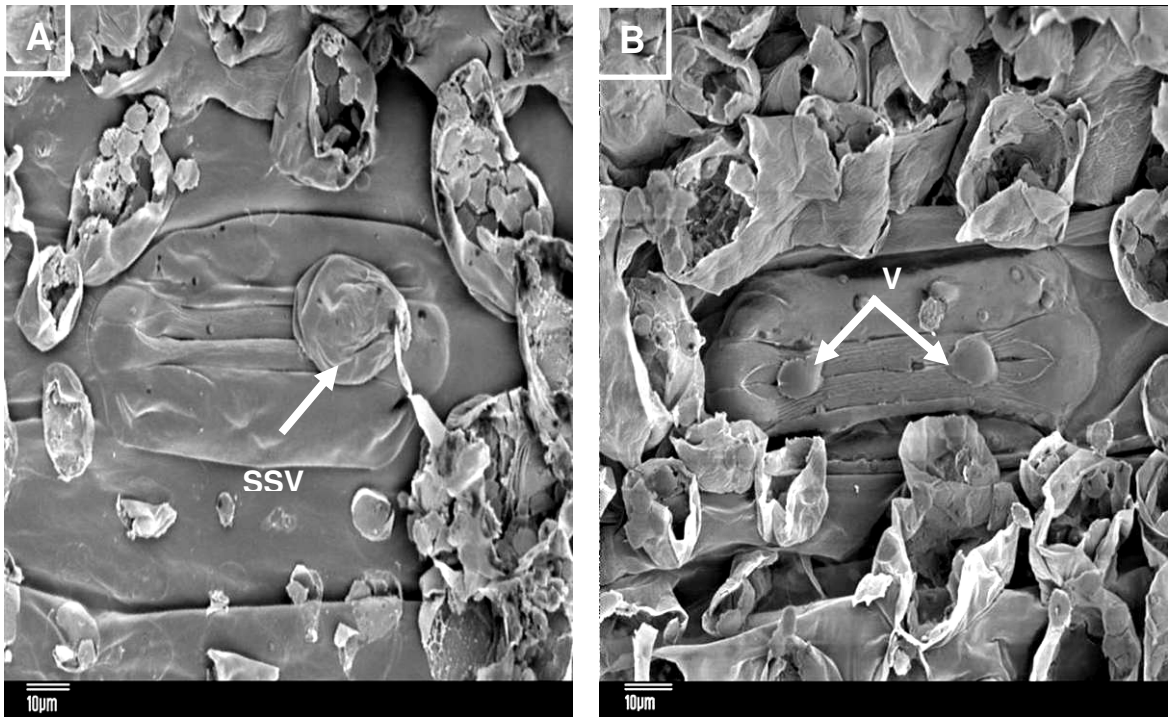


Figure 3.5 **A.** Stripped leaf epidermis of Avocet/*YrSp* (6 dpi) showing a substomatal vesicle (SSV). **B.** Stripped leaf epidermis of Avocet/*YrSp* (6 dpi) showing vesicles (V) on stomatal slit.

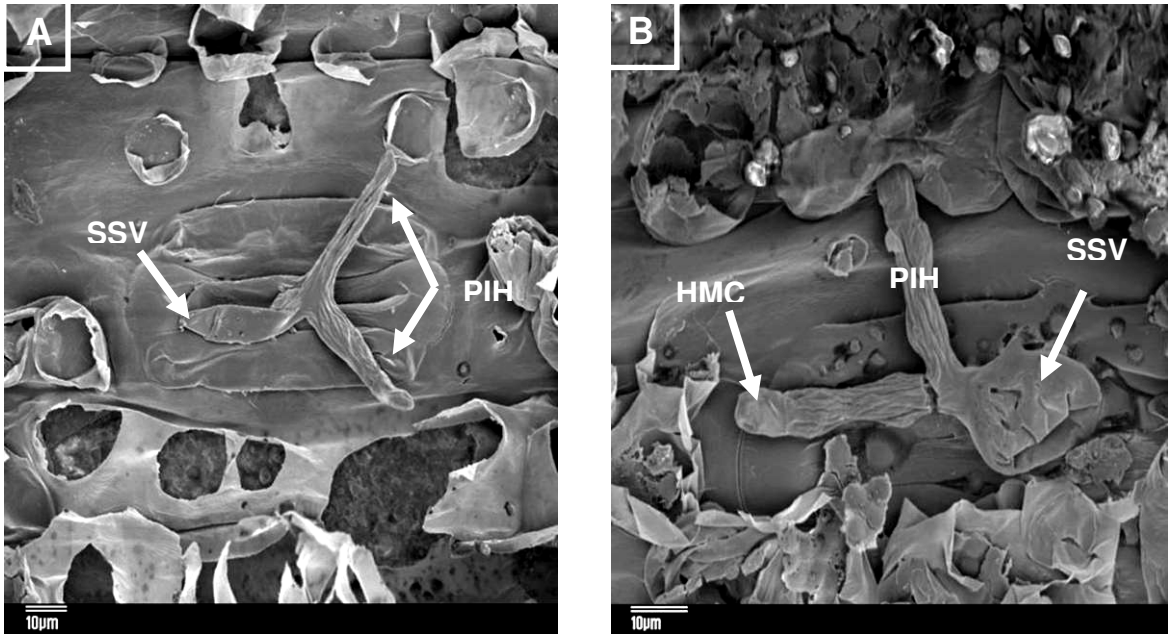


Figure 3.6 **A.** Stripped leaf epidermis of Kariega (7 dpi) showing substomatal vesicle (SSV) with bifurcated primary hyphae (PH). **B.** Stripped leaf epidermis of Kariega (7 dpi) showing a substomatal vesicle (SSV), bifurcated primary infection hyphae (PH) and haustorium mother cells (HMC).

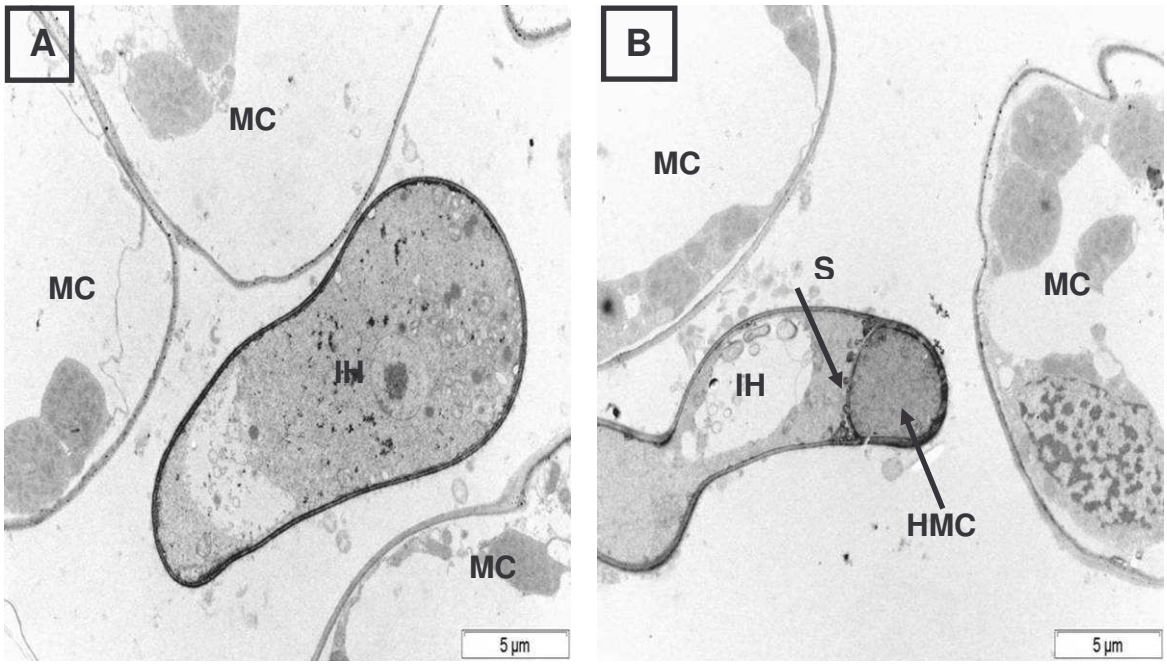


Figure 3.7 **A.** An oblique cross section of an intercellular infection hypha (IH) in a Kariega leaf . **B.** Longitudinal section of intercellular infection hypha (IH) showing haustorium mother cell (HMC). MC=Mesophyll cell S=Septum

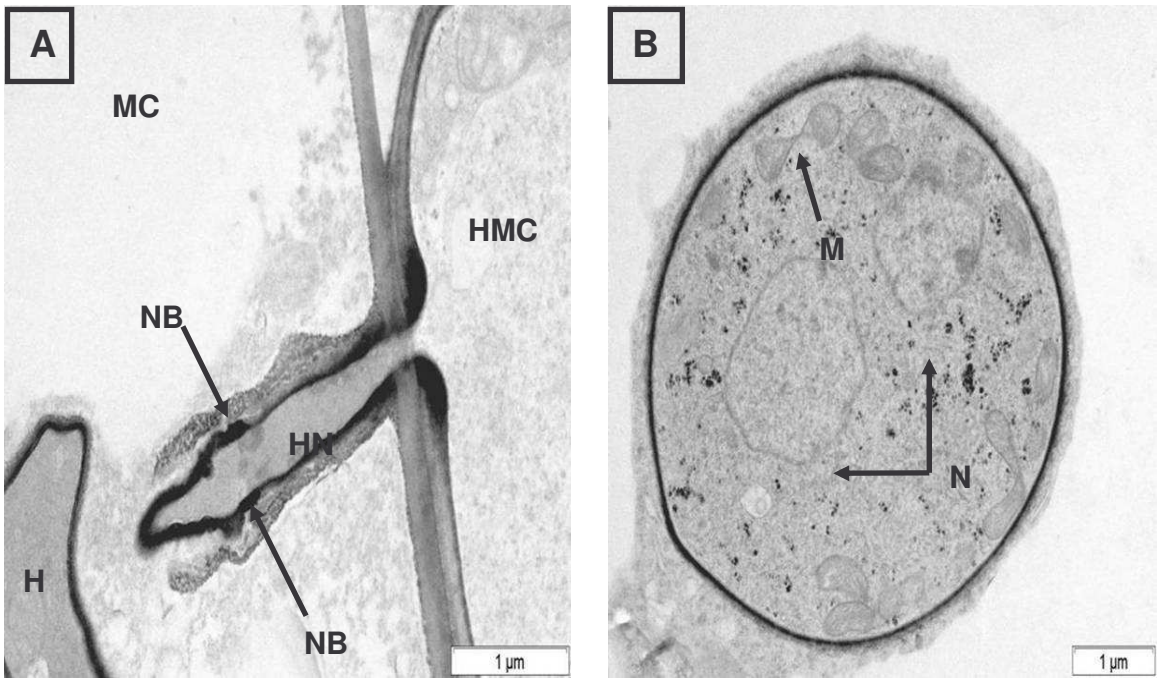


Figure 3.8 **A.** TEM micrograph showing part of a haustorium mother cell (HMC), haustorial neck (HN) with neckband (NB) and a part of a haustorium (H). **B.** A cross section of a haustorium showing mitochondria (M) and the two nuclei (N).

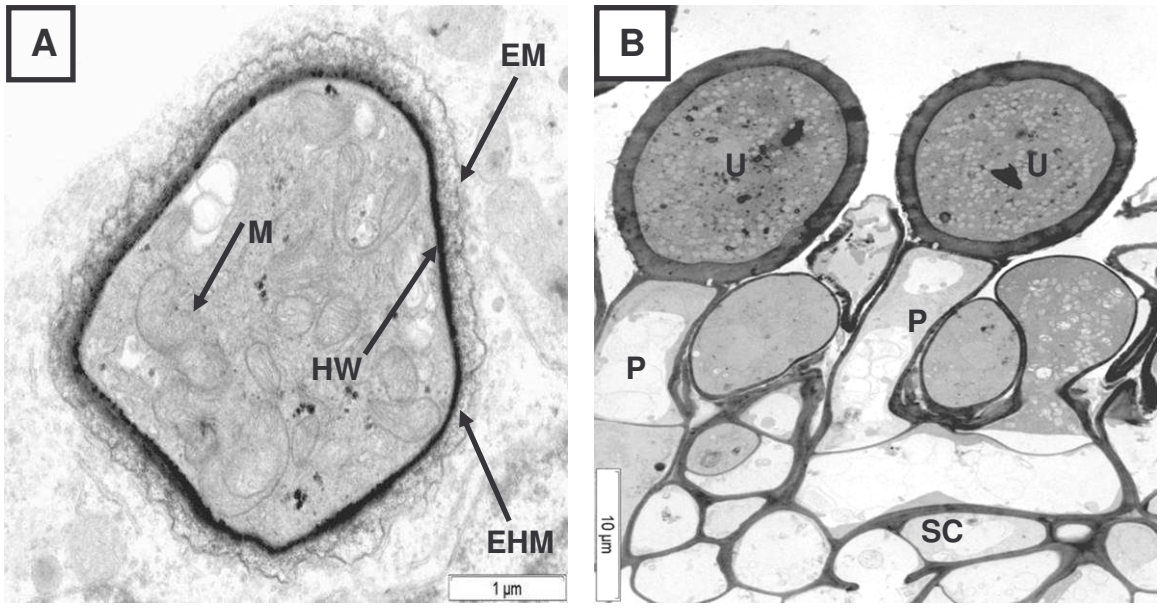


Figure 3.9 **A.** TEM micrograph of a cross section of a haustorium showing the haustorium wall (HW), extrahaustorial matrix (EHM) and extrahaustorial membrane (EM). M=Mitochondria B. TEM micrograph of a part of a uredium showing pedicelled (P) urediospores (U) and sporogenous cells (SC).

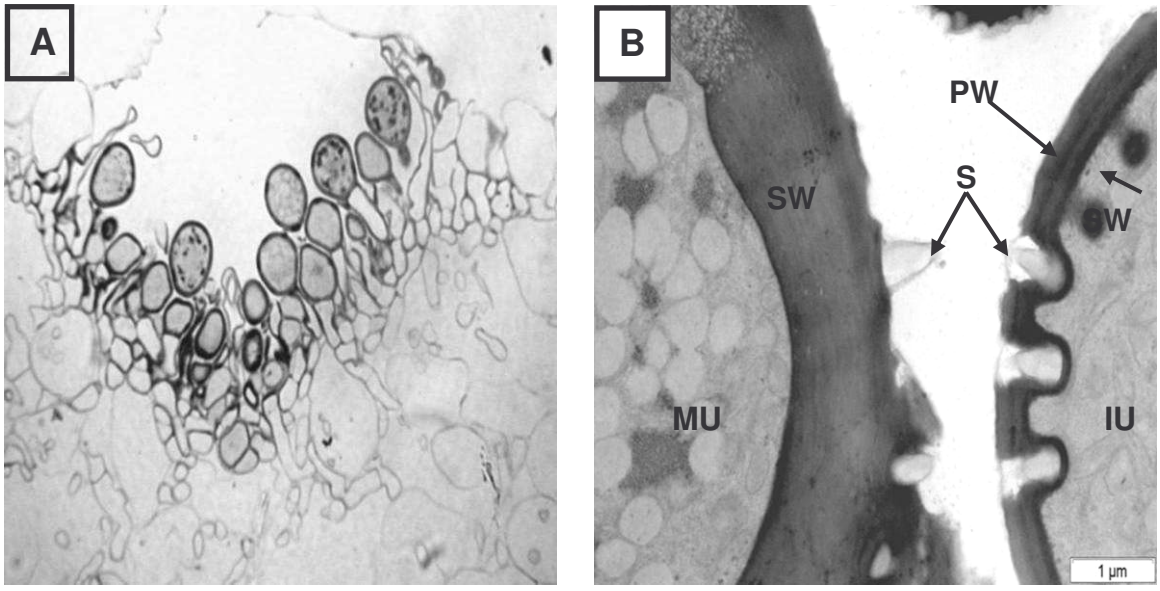


Figure 3.10 **A.** Light micrograph of uredium showing sporogenous tissue and pedicelled urediospores. **B.** TEM micrograph of a part of the immature (IU) and mature urediospore (MU) wall, Primary wall (PW), S=Spines, Secondary wall (SW).

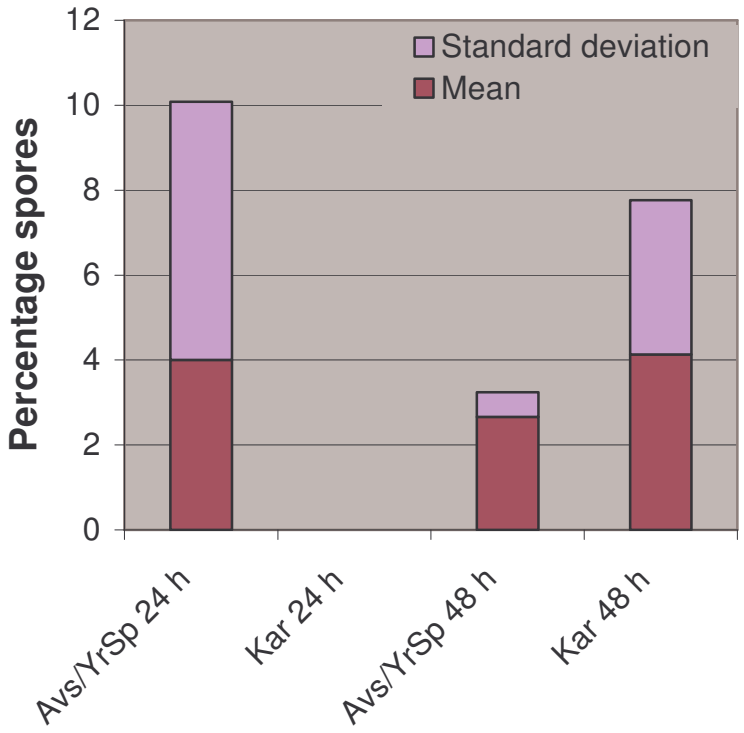


Figure 3.11 Mean and standard deviation of collapsed urediospores on Avocet/*YrSp* and Kariega at 24 and 48 hpi.

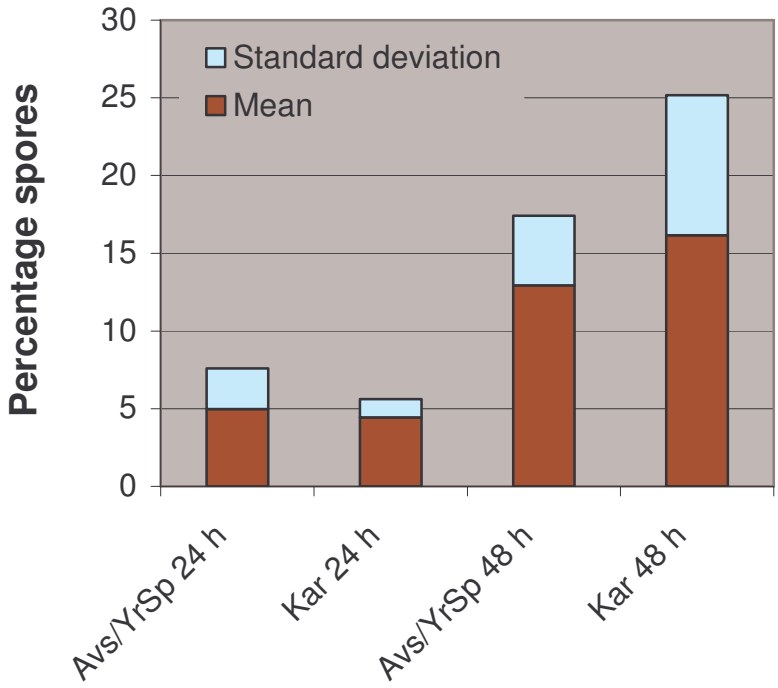


Figure 3.12 Mean and standard deviation of collapsed urediospores with germ tubes on Avocet/*YrSp* and Kariega at 24 and 48 hpi.

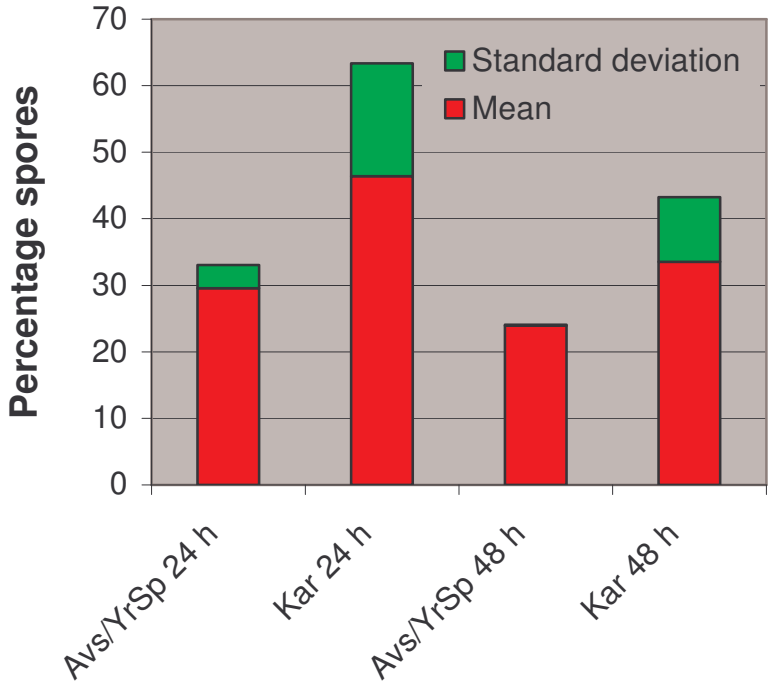


Figure 3.13 Mean and standard deviation of non-germinated urediospores on Avocet/*YrSp* and Kariega at 24 and 48 hpi.

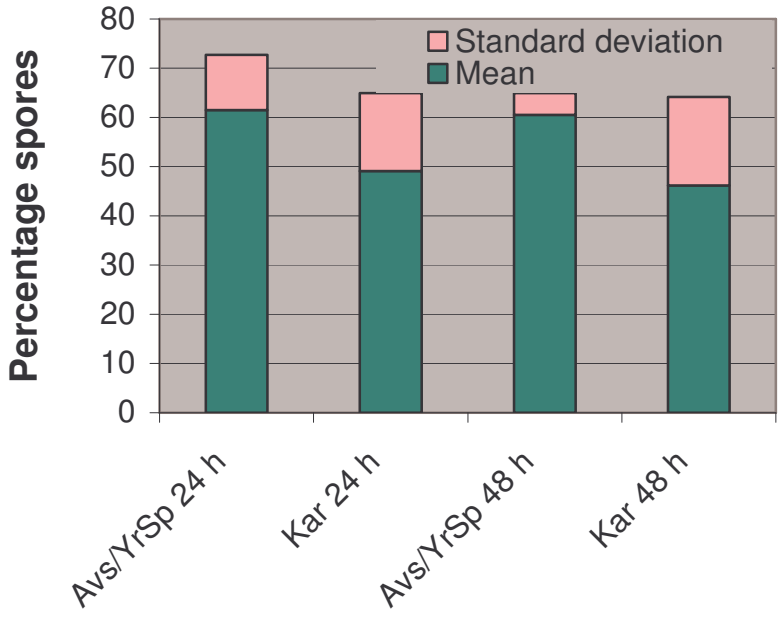


Figure 3.14 Mean and standard deviation of urediospores with germ tubes on Avocet/*YrSp* and Kariega at 24 and 48 hpi.

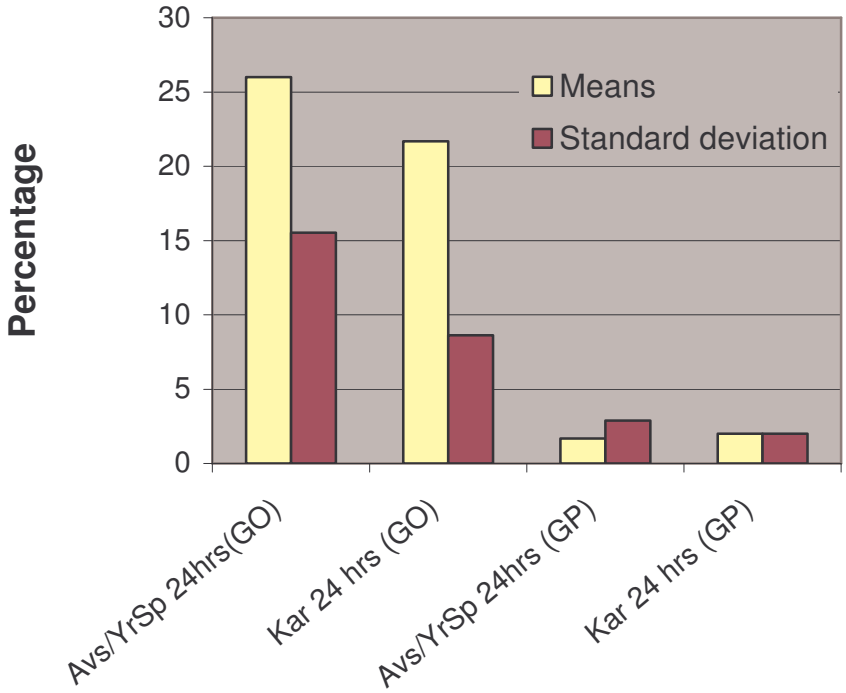


Figure 3.15 Mean and standard deviation of germ tube growth over (GO) and germ tube penetrating (GP) stoma on Avocet/*YrSp* and Kariega at 24 hpi.

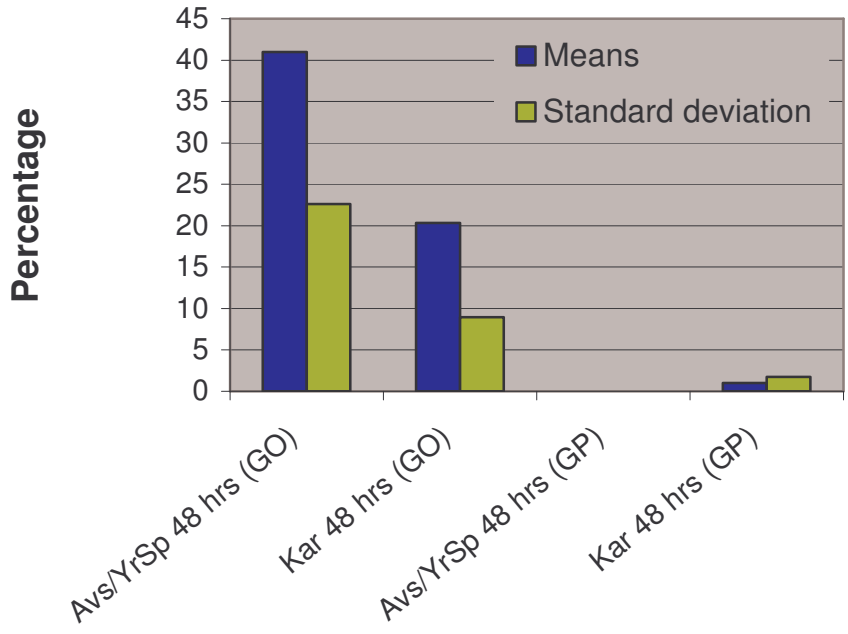


Figure 3.16 Mean and standard deviation of germ tube growth over (GO) and germ tube penetrating (GP) stoma on Avocet/*YrSp* and Kariega at 48 hpi.

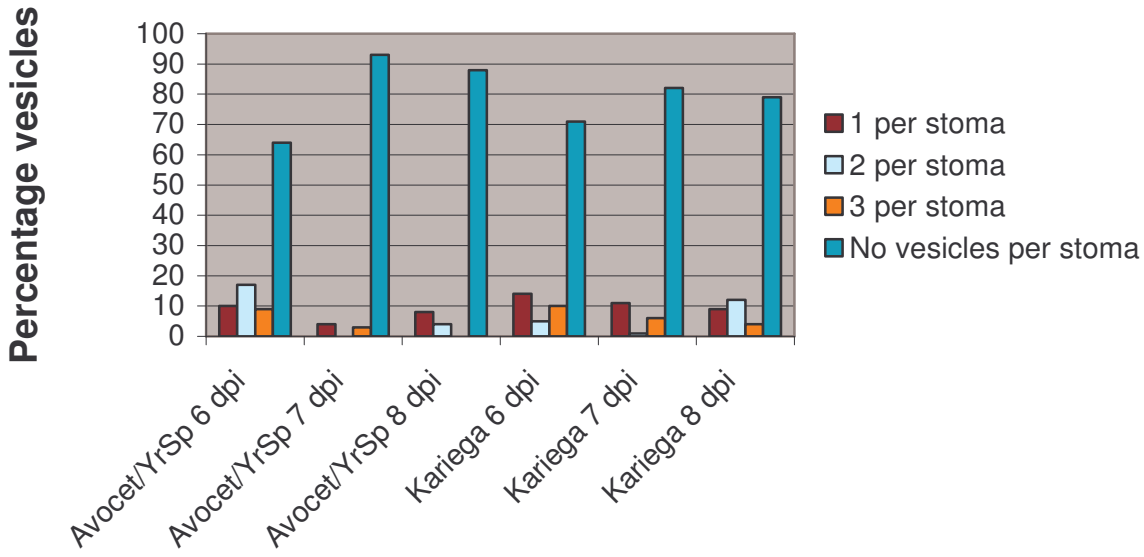


Figure 3.17 Histogram depicting the number of vesicles observed per 100 stomata observed on Avocet/*YrSp* and Kariega when infected with *P. striiformis* f. sp. *tritici*.

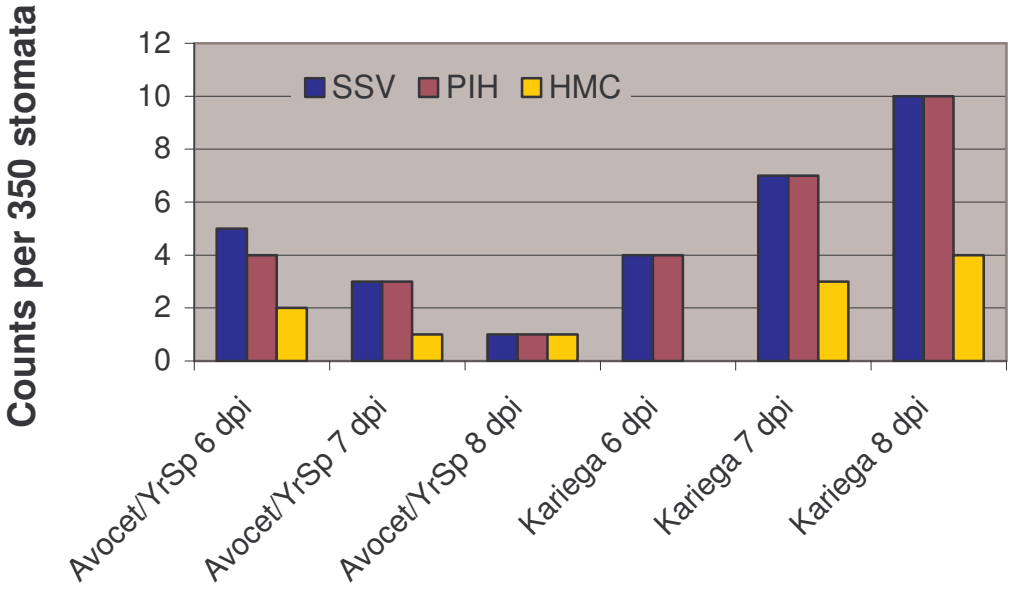


Figure 3.18 Histogram depicting the number of infection structures formed per 350 stomata in Avocet/*YrSp* and Kariega when infected with *P. striifomis* f. sp. *tritici*.

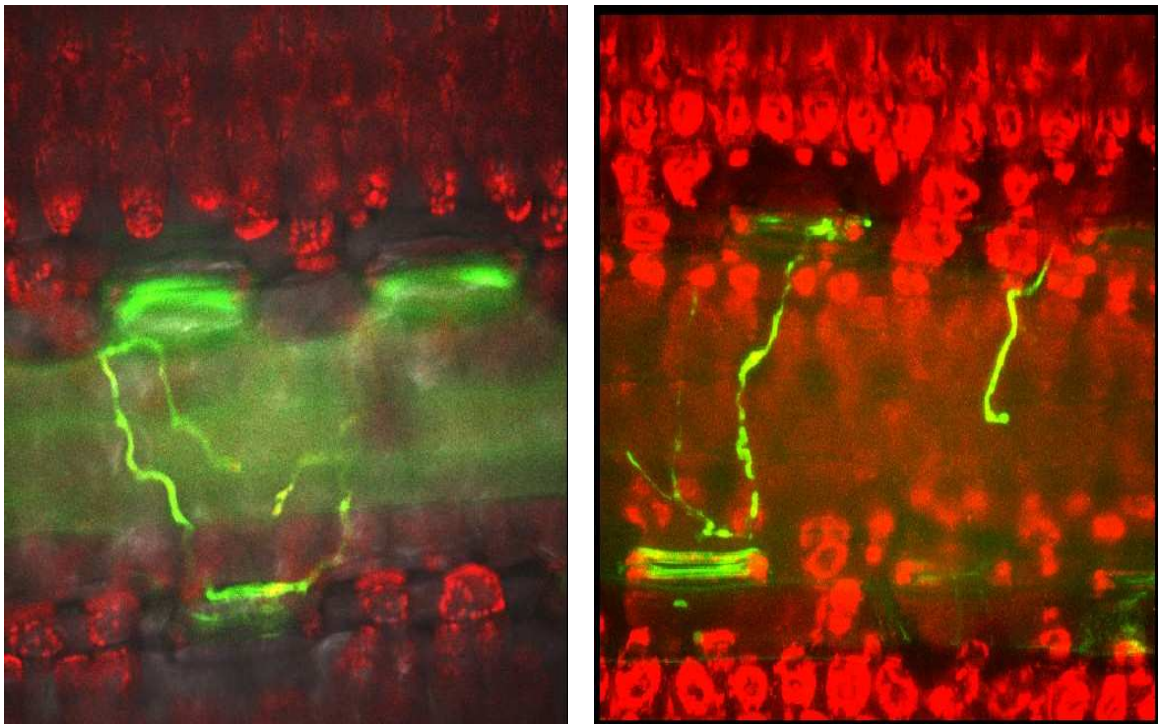


Figure 3.19 Germ tubes associated with stomata stained with the orange G probe and visualised using a combination of green and red fluorescence (not cleared).

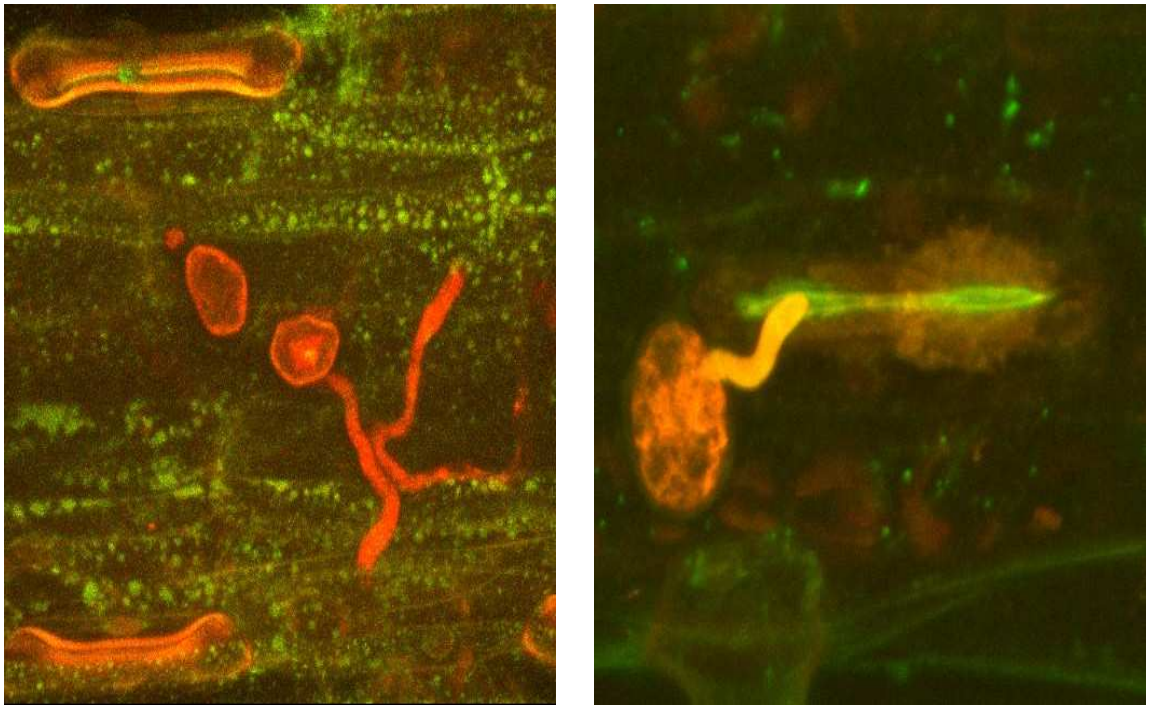


Figure 3.20 Urediospores stained with the orange G probe and visualised using a combination of green and red fluorescence (Cleared).

Figure 3.21 Susceptible Morocco cultivar with uredia rupturing the leaf epidermis to form characteristic stripes on the leaf surface (photograph courtesy of F.J. Kloppers).

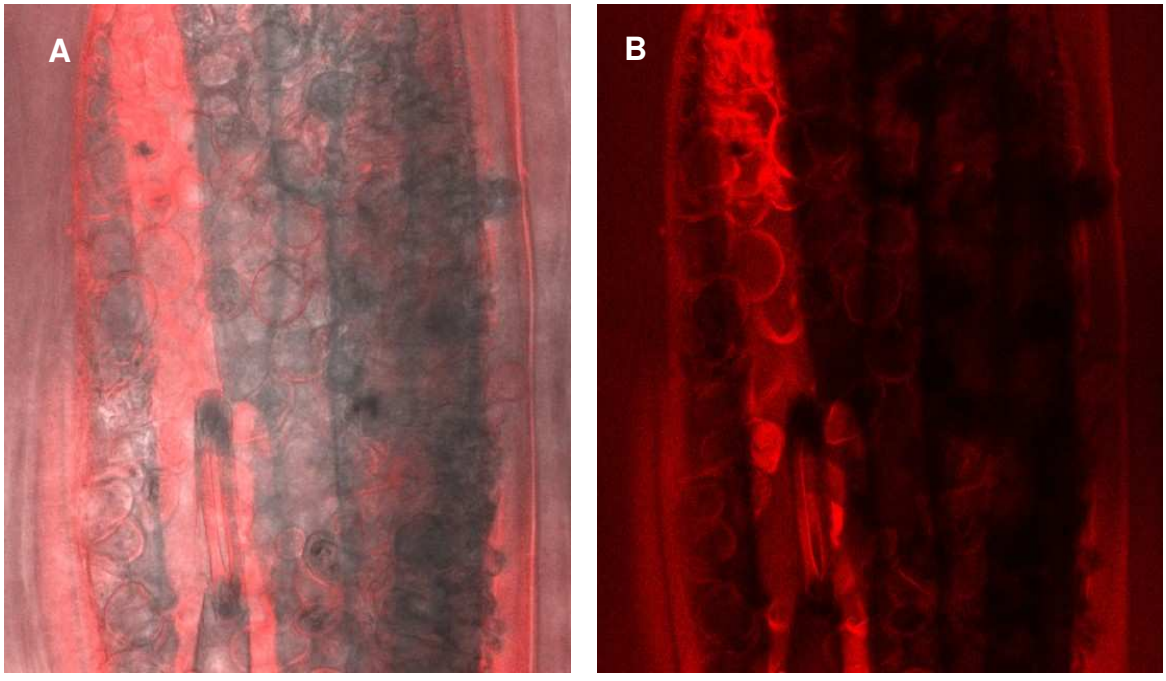


Figure 3.22 Leaf of Morocco showing early pustule formation stained with safranin: (**A**) Combination of transmitted laser light and red fluorescence and (**B**) red fluorescence only.

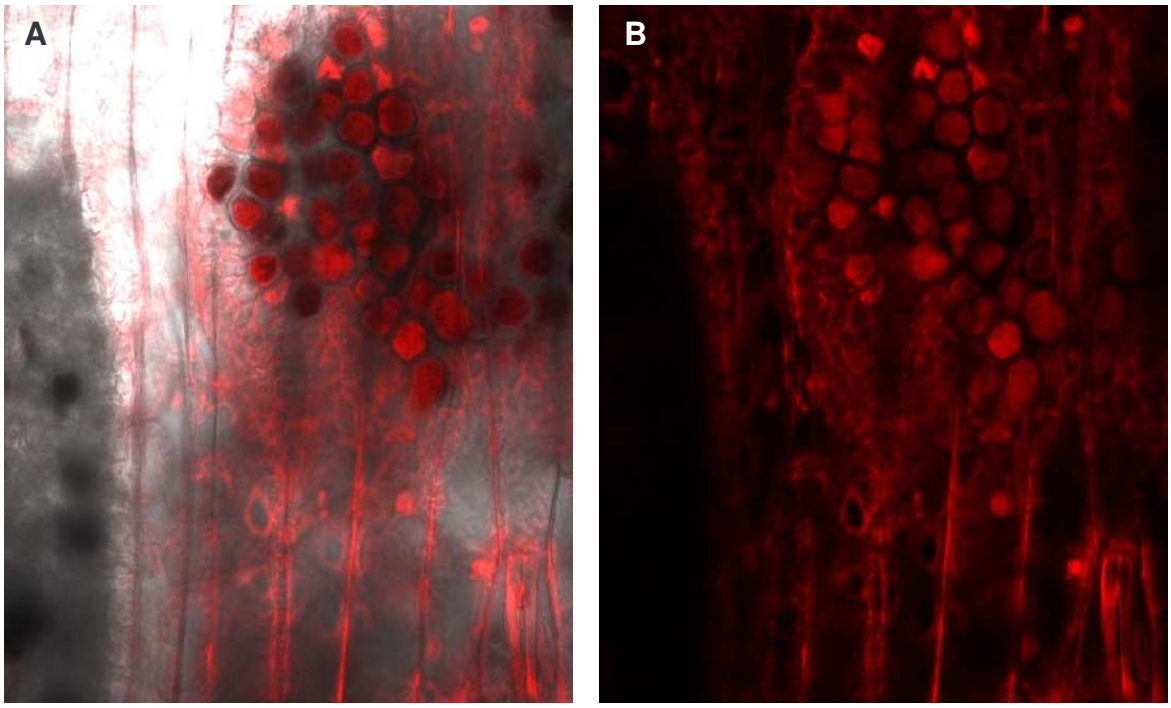


Figure 3.23 Leaf of Morocco showing emerging urediospores stained with analine blue: (**A**) Combination of transmitted laser light and red fluorescence and (**B**) red fluorescence only.

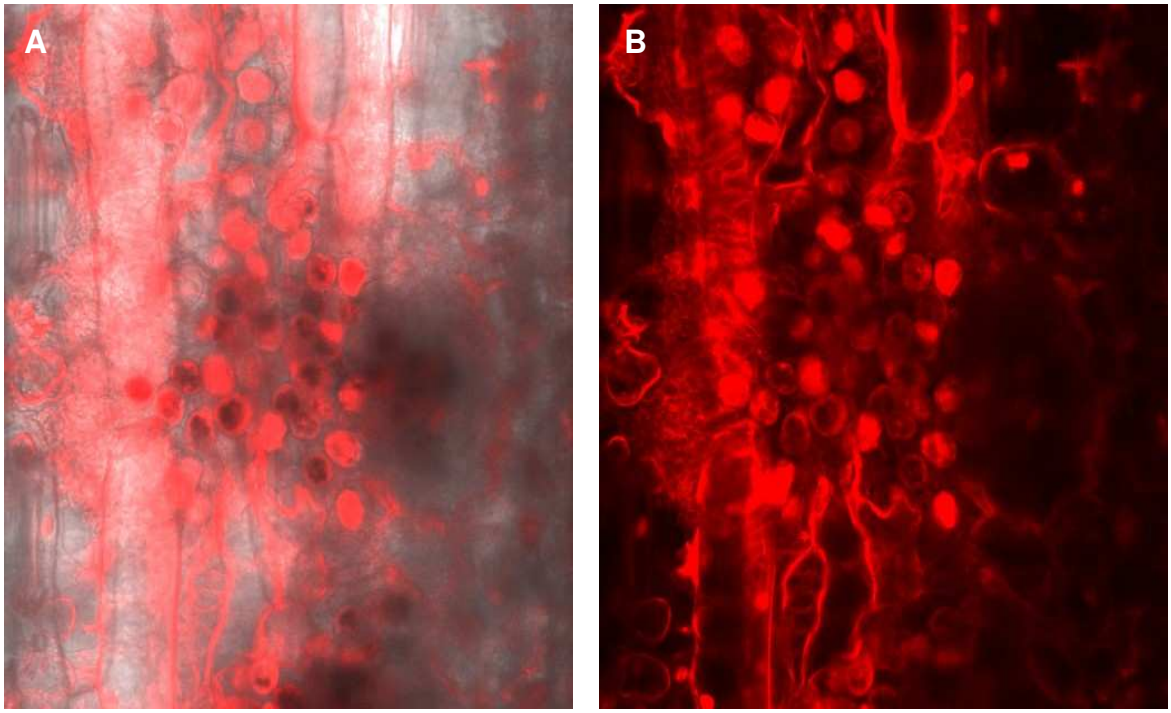


Figure 3.24 Leaf of Morocco showing emerging urediospores stained with ethidium bromide: (**A**) Combination of transmitted laser light and red fluorescence and (**B**) red fluorescence only.

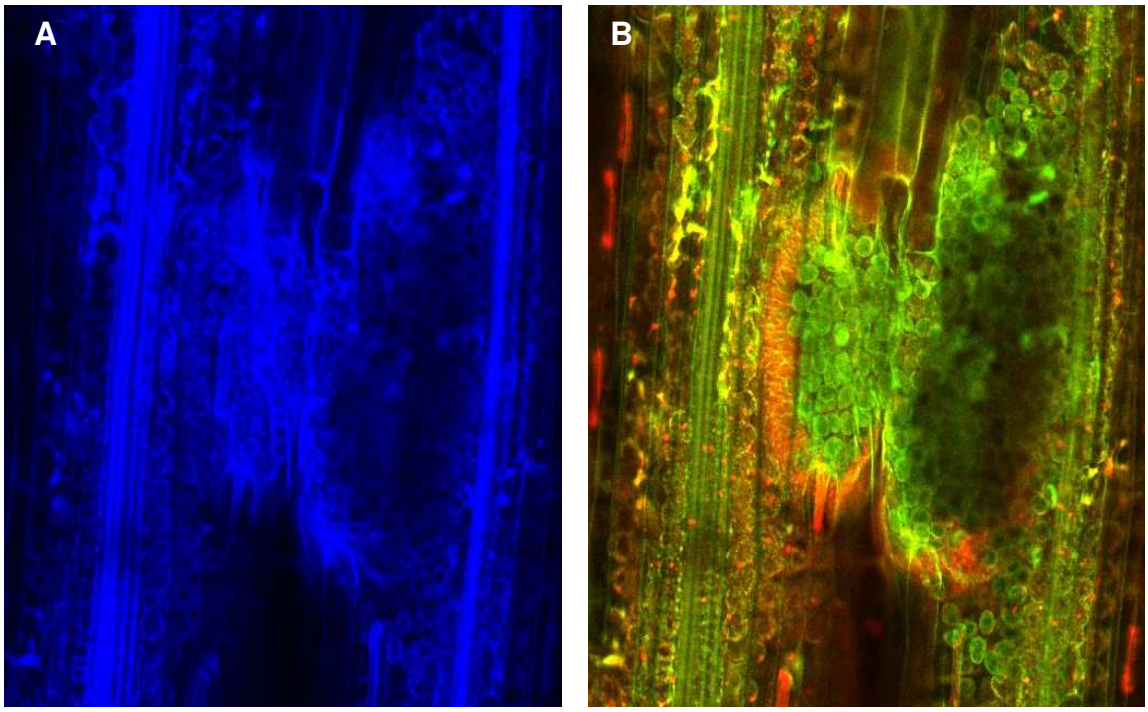


Figure 3.25 Leaf of Morocco showing a pustule stained with uvitex 2B. (**A**) Blue fluorescence and (**B**) Combination of green and red fluorescence.

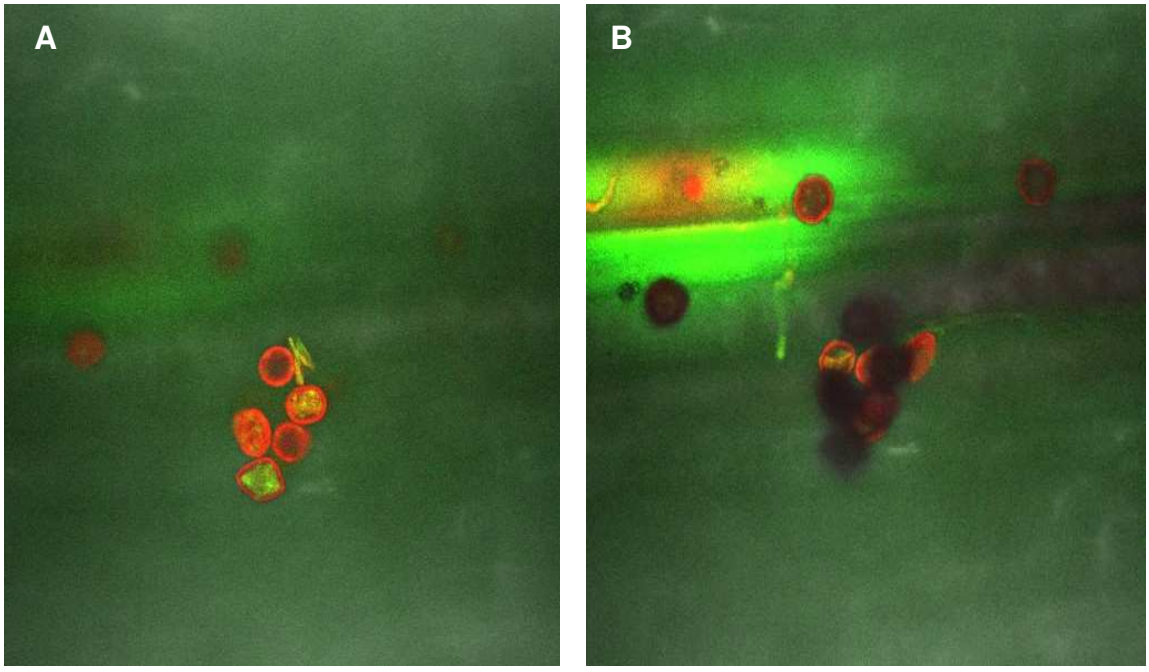
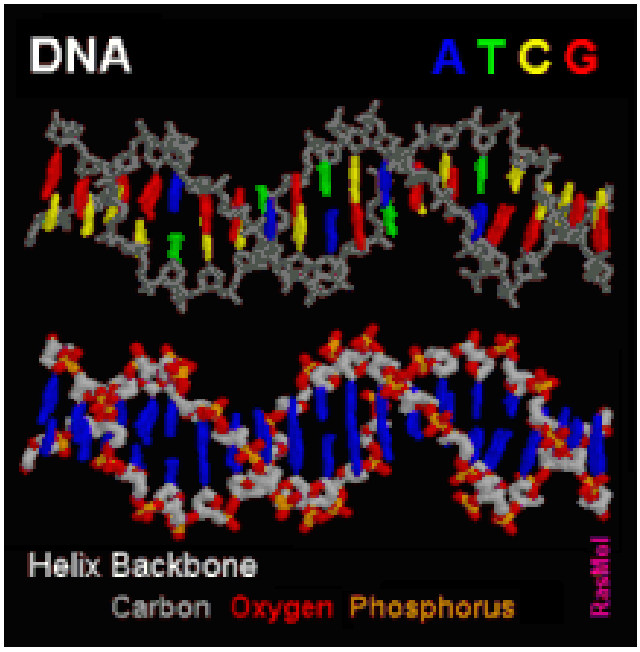


Figure 3.26 Leaf of Morocco showing urediospores and germ tubes stained with the orange G probe. (**A** and **B**) Combination of red and green fluorescence.

Chapter IV

Molecular Characterisation



Molecular characterisation of the *YrSp* resistance gene by means of SSR and AFLP fingerprinting

4.1 Abstract

Fingerprinting techniques such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs), simple sequence repeats (SSRs) and amplified fragment length polymorphisms (AFLPs) have allowed molecular characterisation of various pathogens and mapping of genes and quantitative trait loci (QTLs) in economically important crops like wheat (*Triticum aestivum*). Molecular characterisation has promoted efforts of wheat growers globally in producing quality crops with resistance against pathogens while gaining a better understanding of host-pathogen interactions at a DNA level. The polymerase chain reaction has fast-tracked the development and application of molecular marker technology as a tool for marker-assisted breeding (MAS). The technology has advanced breeding programs through the detection of markers for desirable traits in cultivar development. SSRs and AFLPs, both PCR based methods, were used in this study to identify markers for the *YrSp* gene introgressed into Kariega x Avocet/*YrSp*. A SSR marker (Xgwm148) was used to confirm the location of the gene on chromosome 2B. Further, AFLP markers for the *YrSp* gene were identified in a recombinant F_2 population and confirmed in the F_3. In addition to this, QTLs, *QYrSgi*-2B.1 and *QYrSgi*-7D for major genes conferring additive adult-plant resistance (APR) in Kariega, was also confirmed in Kariega x Avocet/*YrSp*. Approximately 800 AFLP fragments were generated using eight AFLP primer sets, 76 of which were polymorphic between the parents with segregation in the F_2 population. Fifty markers fitted a 3:1 ratio while 21 markers fitted a 1:1 ratio. A total number of 40 markers mapped to the short arm of chromosome 2B with the *YrSp* gene flanked by two AFLP markers L15 and L68 at map distances of 19.0 and 24.4 cM, respectively. A total map area coverage of 382.5 cM was achieved.

4.2 Introduction

The invention of the polymerase chain reaction (PCR) by Kerry Mullis in 1983 revolutionised the ability of researchers to detect differences at DNA level in plants and pathogens. PCR employs DNA polymerase that amplifies a short, targeted sequence of DNA to produce millions of copies of that specific sequence. PCR has become a routine component of almost every molecular biology laboratory and is a constantly changing tool whose potential shows no signs of levelling off (Mullis, 1994).

Prior to the development of PCR, hybridisation fingerprinting techniques such as restriction fragment length polymorphisms (RFLPs) were developed. This technique employs cloned DNA sequences as a probe to detect restriction fragments (Beckmann and Soller, 1986). Although RFLPs have been used extensively in fingerprinting studies of plants, the technique requires the prior development of probes which is tedious and time consuming (Choa *et al*., 1989; Tanksley *et al*., 1989; Liu and Cordes, 2004). Subsequent to the development of PCR, a number of different PCR-based fingerprinting techniques have been developed namely RAPDs, AFLPs, RFLPs and SSRs. Random amplified polymorphic DNA (RAPDs) uses a single short oligonucleotide primer of arbitrary DNA sequence to PCR amplify anonymous target segments of DNA (Joshi and Nguyen, 1993; Williams *et al*., 1990)). Amplified fragment length polymorphisms (AFLPs) are considered as a combination between RFLPs and PCR. Anonymous fragments are PCR amplified using primers and adaptors after the production of restriction fragments and adapter ligation (Vos *et al*., 1995). AFLPs provide new opportunities for gene tagging and mapping in plants with large genomes (Gupta *et al*., 1999; Ridout and Donini, 1999). Simple sequence repeats (SSRs) refer to tandem repeats of a basic repeated motif of less than six base pairs (Taramino, et al., 1997; Lima *et al*., 2003). The technique employs primers complimentary to the flanking regions of a locus specific SSR (Ma *et al*., 1996).

The potential application of genetic markers as a tool for plant breeders was first recognised over 70 years ago (Sax, 1923). Molecular marker

technology has become a powerful tool for identifying quantitative traits and quantitative trait loci (QTLs) (William *et al*., 2003). The use of molecular markers has become an essential tool in plant breeding (Gupta *et al*., 1999; Staub *et al*., 1996; Mohan *et al*., 1997; Suenaga *et al*., 2003).

In wheat, DNA markers have been successfully applied in mapping agronomically important genes for insect and disease resistance. For example, RFLP markers have been developed for rust resistance genes *Yr27*, *Yr17, Sr39, Lr35*, RAPD markers for *Yr17, YrH52*, AFLP markers for *Yr10*, *Yr29* and SSR markers for *Lr39, Yr15*, *Yr18*, *Yr26*, *Yr28* (Gold *et al*., 1999; Peng *et al*., 2000; Robert *et al*., 1999; Singh *et al*., 2000; Raupp *et al*., 2001; Suenaga *et al*., 2003; William *et al*., 2003). Marker biotechnology has been useful in identifying markers linked to disease resistance facilitating the introgression of these genes into different cultivars (Melchinger, 1990).

The *YrSp* gene for seedling resistance to stripe rust was introgressed into Kariega x Avocet/*YrSp* during this study. Kariega is a bread wheat with a high yield and excellent bread baking qualities. Recently, two major and two minor QTLs for adult plant stripe rust resistance were identified in Kariega on chromosome 2B (*QYrSgi*-2B.1) and 7D (*QYrSgi*-7D). (Ramburan *et al*., 2004)). This cultivar is also known to carry seedling leaf rust resistance genes *Lr1* and *Lr3a* and is thought to contain the adult plant resistance complex *Lr34*/*Yr18* based on pedigree analysis and the presence of leaf tip necrosis, associated with this gene complex (Dyck, 1991; Singh, 1992; William *et al*., 2003).

The *YrSp* seedling resistance gene was first identified in Spaldings Prolific (Johnson *et al*., 1972). This dominant gene confers an immune-type resistance characterised by minute chlorotic patches on the leaf surface (Tarr, 1972). *YrSp* was also incorporated into the Australian wheat cultivar Avocet in order to develop a set of stripe rust near isogenic lines (NILs) (Wellings and McIntosh, 1998). The *YrSp* was mapped to the short arm of chromosome 2B in Avocet/*YrSp* (McIntosh *et al*., 2001). The Avocet/*YrSp* line is thought to contain an introgressed segment from *Thynopyrum elongatum* on chromosome 6A that is known to confer stem rust resistance due to the presence of *Sr26*. The

presence of the introgressed segment in Avocet S was confirmed the molecular evidence indicates a highly polymorphic region on chromosome 6A, as found by Prins *et al.* (2005).

The aim of this study was to identify potential markers for the *YrSp* seedling resistance gene introgressed into Kariega x Avocet/*YrSp* using SSRs and AFLPs as well as to confirm the presence of markers for APR. SSR markers were specifically selected for chromosome 2A, 2B, 2D, 6A, 7A, 7B and 7D). The parental cultivars, Avocet/*YrSp* and Kariega were analysed for polymorphisms and the segregation of these markers were determined in an F_2 Kariega x Avs/*YrSp* population. Subsequently, the markers were confirmed in an F_3 Kariega x Avs/*YrSp* population.

4.3 Materials and Methods

4.3.1 Plant material

Leaves of ten Kariega and Avocet/*YrSp* plants, respectively, and 20 resistant and susceptible F_2 plants after crosses, respectively, were selected for DNA extraction. For the F_3 families, two leaves from ten plants per family, with ten resistant and susceptible families, respectively, were bulked for DNA extraction. DNA was extracted from the leaves at the two-leaf growth stage. All the plants were cultivated in 4-L pots with four or five entries per pot. These were fertilies from seven-day old seedlings with 3:2:1 N-P-K mix (50 ml per pot of 10 g/L) once a week until the experiment was terminated. Leaf samples were collected on ice followed by DNA extraction.

4.3.2 DNA extraction

Fresh leaf material was homogenised in liquid nitrogen using a mortar and pestle. Finely ground powder was transferred to 50 ml tubes and extraction buffer (10ml) containing CTAB buffer (1ml) and 5 M $NaCl_2$ (2 ml) was added. Samples were placed in a waterbath at 65^{o}C for 2 h and inverted every 10 to 20

minutes. Chloroform/isoamylalcohol (24:1) (v/v) was added to each sample homogenate, mixed and centrifuged at 10k rpm for 15 min. The supernatant was transferred to a new 50 ml tube, and the chloroform/isoamylalcohol step repeated until the interface was clear. The supernatant was transferred to a new 50 ml tube and two volumes of cold absolute ethanol added and the nucleic acid allowed to precipitate overnight at 4°C. The DNA precipitate was pooled using a modified pasteur pipette and transferred to a 1.5 ml eppendorf tube and rinsed thrice with 1 ml of 70% ethanol (v/v). The DNA precipitate was transferred to a new 1.5 ml eppendorf tube and the excess 70% ethanol was allowed to drain off after which the DNA was re-suspended in 250 µl sterile double distilled water (Sabax). The DNA concentration was determined and samples diluted to 100 ng/µl. This was done to ensure that the DNA concentration was correct and uniform. The DNA was stored at 4°C. The parent DNA was bulked by combining 100 ng of ten plants each, respectively. Extracted stock DNA was stored at -20°C.

4.3.2.1 DNA quantification

The extracted DNA was quantified at 260 nm using a spectrophotometer (Cary 3 varian UV-visible) (Table 4.1 and Table 4.2). DNA quantification was performed in duplicate for each sample and the average optical density (OD) used to calculate the DNA concentration according to the following formula:

$$[\text{DNA}] = \frac{\text{OD x volume (500 µl) x C}}{\text{Total volume of DNA (µl)}}$$

where C is a constant (50 µg/ml).

4.3.3 DNA fingerprinting

4.3.3.1 Simple Sequence Repeats (SSRs)

The polymerase chain reaction (PCR) was used to detect SSRs on chromosome 2 namely Xgwm636, Xgwm526, Xgwm261, Xgwm148, chromosome 6 namely Xgwm169, Xgwm219 and chromosome 7 namely Xgwm276, Xgwm43, Xgwm428, Xgwm295 (Table 4.5). PCR reactions were performed in a final volume of 25 µl containing 200 ng DNA, 1.0 µl 25mM $MgCl_2$ (Promega), 2.5 µl 10X PCR reaction buffer (100 mM Tris-HCl [pH 8.4] and 500 mM KCl) (Promega), forward and reverse primer (60 µM) and 0.5 U *Taq polymerase* (Promega). Temperature cycling was performed in a Hybaid Thermocycler and the amplification profile comprised an initial denaturation at 94^{o}C for 3 minutes followed by 45 cycles of 95^{o}C for 1 minute, 50^{o}C, 55^{o}C or 60^{o}C annealing temperature for 1 minute (depending on the primer pair) and 72^{o}C for 2 minutes followed by a final elongation step of 72^{o}C for 10 minutes (Table 4.5) (Roder *et al*., 1998).

4.3.3.1.1 SSR visualisation

SSR products were visualised on a 2.5% MS agarose gel (Inqaba Biotech) at 80V for 2 h in 0.5X TAE (0.438 g/L Tris, 0.09 ml/L Acetic acid and 0.022 g/L EDTA). SSR gels were stained with ethidium bromide (100 ul of 150 mg/ml) in 1 L double distilled water) and visualised under UV light using the Gel Doc 1000^{TM} image analysis system (Biorad). SSR fragments were sized using a 100 basepair size marker (Inqaba Biotech).

4.3.3.2 AFLP fingerprinting

4.3.3.2.1 Restriction enzyme digest

Restriction enzyme digests were performed on each DNA sample using the frequent cutter restriction enzyme, *MseI* (T*TAA) and the rare cutter, *EcoRI*

(G*AATTC) (Table 4.6). DNA samples (1µg) were digested in a reaction mixture containing 0.4 µl (10 U/ul) *MseI* and 5 µl of 10x *MseI* buffer in a final volume of 39 µl and incubated at 37°C for 5 h. Thereafter, 0.5 µl (8 U/µl) *EcoRI*, was added to the samples after the addition of 0.5 µl 50 mM $NaCl_2$. The samples were further incubated at 37°C overnight.

4.3.3.2.2 Adapter and primer preparation for AFLPs

The primers were reconstituted by adding 0.1X TE buffer to obtain a primer concentration of 1µg/µl. These were mixed and placed at 4°C for 48 hours after which the primers were diluted to 30 ng/µl final concentration in 0.1X TE buffer. The adapters (50 pmol/µl final concentration) for *MseI* and *EcoRI,* respectively were heated at 65°C for 10 min and allowed to cool overnight in the waterbath.

4.3.3.2.3 Ligation of adaptors

Ligation reactions were performed by adding 10 µl of a reaction mixture containing 1 µl 10X ligase buffer, 1.2 µl 20 mM ATP, 1 µl *MseI*-adapter, 1 µl *EcoRI*-adapter and 1 µl (1 U/µl) T4 *Ligase* to the 50 µl restriction digest and incubated at 16°C overnight in a waterbath.

4.3.3.2.4 Pre-selective amplification

Pre-selective amplification was performed in a 50 µl reaction mixture containing 5.0 µl of the ligation solution for each DNA sample, 5 µl of 10X PCR buffer (100 mM Tris-HCl [pH 8.4] and 500 mM KCl) (Promega), 4 µl of 25 mM $MgCl_2$ (Promega), 8 µl of 1.25 mM dNTPs, 1 µl of primer Mse-C, 1 µl of primer Eco-A and 0.2 µl (5 U/µl) *Taq polymerase* (Promega) (Table 4.7). Temperature cycling was performed in a Hybaid Thermal Cycler at 94°C for 1 minute to denature DNA followed by 30 cycles of 94°C for 30 seconds, 56°C annealing for 60 seconds and 72°C for 60 seconds with a final elongation step of 72°C. Pre-selective fragments were separated using a 1.5% agarose gel in 0.5X TAE (0.438 g/L Tris, 0.09 ml/L Acetic acid and 0.022 g/L EDTA) and visualized after

staining with ethidium bromide (100 µl of 150 mg/ml) in 1 L double distilled water under UV light using the Gel Doc 1000^{TM} image analysis system (Biorad). Pre-selective fragments were sized using molecular marker III (lambda DNA digested with *Hind*III).

4.3.3.2.5 Selective amplification

Pre-selective amplification product was diluted 1:20 or 1:30 using 0.1X TE buffer based on the amplification of pre-selective fragments. Fluorescently labelled *EcoRI*-primer in combination with an *MseI*-primer was used for selective AFLP reactions (Table 4.7). Selective PCR was performed by the addition of 5 µl of *MseI*-reaction mixture containing 3.2 µl 1.25mM dNTPs, 1 µl *MseI*-selective primer and 10 µl of *EcoRI*-reaction mixture containing 2.0 µl 10X PCR buffer (100 mM Tris-HCl [pH 8.4] and 500 mM KCl) (Promega), 1.6 µl 25mM $MgCl_2$, 0.2 µl 100 mg/ml BSA, 1.0 µl *EcoRI*-selective primer, 0.15 µl (5 U/µl) *Taq polymerase* (Promega) and 5 µl of the diluted pre-selective amplification product. Primer combinations were as follows: M-CAT with E-ACT, M-CAT with E-ACC, M-CTG with E-ACT, M-CTG with E-ACC, M-CAG with E-ACT, M-CAG with E-ACC, M-CTT with E-ACA and M-CTT with E-AAC. Selective amplification was performed for 35 cycles at 94^{o}C for 30 seconds, 65^{o}C for 60 seconds (with a temperature reduction of 0.7^{o}C per cycle for 12 cycles) and 72^{o}C for 2 minutes with a final elongation step of 72^{o}C for 10 minutes on a Hybaid Touchdown Thermocycler.

4.3.3.2.6 AFLP electrophoresis

Following selective amplification, 5 µl of PCR product was added to 24.0 µl deionized formamide and 0.75 µl 1000 Rox^{TM} size standard marker (Applied Biosystems). The mixture was denatured at 94^{o}C for 5 minutes and quickly cooled on ice. The AFLP fragments were resolved using an ABI Prism 310 Automated capillary sequencer (Applied Biosystems).

4.3.4 Data Analysis

SSR and AFLP markers were scored for the presence "1" or absence "0". (Appendices A to I). AFLP profiles were analysed from 40 bp to approximately 400 bp and peak heights of greater and equal to 20 fluorescent units were scored. Parental cultivars were initially fingerprinted and AFLP fragments were scored for segregation in the F_2 population while possible markers were confirmed and scored in the F_3 population. SSRs and AFLPs markers were analysed for a 3:1 and 1:1 segregation ratio using Chi-square test procedure in Windows Excel (Table 4.7). The frequency for the presence of each marker in the resistant and susceptible F_2 and F_3 populations, respectively, was also calculated (Table 4.7).

Linkage estimation was based on the recombination frequency defined as the summed frequency of recombination types among the total progeny. The recombination frequency was transformed to map units using the Kosambi function (Kosambi, 1944). Map Manager QTX version 0.30 was used to map the markers at a minimum LOD of 4.0 (Manly *et al*., 2001). Marker positions were optimised using the "Ripple" and "Distribute" command. The map distances and markers loci were confirmed using Mapmaker/Exp version 3.0 (Lander *et al*., 1987).

4.4 Results and Discussion

4.4.1 Simple sequence repeats (SSRs)

Of the SSR markers specific for chromosome 2 and 7, Xgwm636 (2A-L), Xgwm526 (2B-L), Xgwm261 (2D-L), Xgwm276 (7A-S), Xgwm43 (7B-S) Xgwm428 (7D-L) and Xgwm295 (7D-L) were present in both parents. Only Xgwm148 (2B-S) was polymorphic in the parental cultivars. The presence of the Avocet/*YrSp* allele in Kariega x Avs/*YrSp* was a further indication of the introgression. Markers specific to chromosome 6, Xgwm169 (6A-S) and Xgwm219 (6B-S), were not polymorphic in the parental cultivars (data not

shown). A study including the previously reported introgression of a segment in chromosome 6A in Avocet S showed no other significant clustering of AFLP markers on chromosomes other than on chromosome 6A (Prins *et al*., 2005).

SSR marker, Xgwm148 (2B-S), specific to chromosome 2B was present in both parents and produced differential alleles for Avocet/*YrSp* (200 bp) and Kariega (175 bp). The 175 bp allele in Kariega is the expected size of $(CA)_{22}$ as reported by Roder *et al*. (1998). Therefore a 200 bp fragment could be classified as a $(CA)_{35}$ which was only present in Avocet/*YrSp* and resistant F_2 and F_3 progeny. The primer Xgwm148 was also found linked to major QTLs *QYrSgi*-2B.1 for adult-plant stripe rust resistance (Ramburan *et al*., 2004). The QTLs found by Ramburan *et al*. (2004) explained approximately 33-46% host reaction type and 17-50% leaf area infected and was characterised by significant chlorosis and necrosis and 27-29% proportion of the variance in percentage leaf area as opposed to the 9-18% in the host reaction type respectively.

4.4.2 Amplified fragment length polymorphisms (AFLPs)

A total of eight AFLP primer combinations were used to amplify 818 fragments (Appendices A - I). The number of fragments per primer combination ranged from 80 (M-CTT/E-AAC) to 120 (M-CAG/E-ACC). Of these, 76 fragments were polymorphic for the parents with 50 markers fitting a 3:1 segregation ratio and 21 markers a 1:1 segregation ratio in the F_2 population (Table 4.7). Approximately 12% of AFLP fragments exhibited non-Mendelian segregation in the F_2 population and were not used in data analysis (Appendices A - I).

A total of 40 markers and the *YrSp* gene mapped to the short arm of chromosome 2B with a map size of 382.5 cM (P=0.0001) (Table 4.8). The *YrSp* gene was flanked by a coupling- phase marker, L15 (LOD 4.9) at 19.0 cM, and a repulsion phase marker, L68 (LOD 7.5) at 24.4 cM (Table 4.8 and Fig. 4.1). SSR marker Xgwm148, used as a chromosome anchor, was flanked by a repulsion-phase marker L13 (LOD 7.1) and L60 (LOD 6.3) at 20.6 and 20.1 cM,

respectively. Two clusters of AFLP markers were found on chromosome 2B with one cluster consisting of nine markers within a distance of 7.2 cM while the other was composed of five markers within a distance of 9.6 cM (Table 4.8).

Bulk segregation analysis (BSA) was used in this study. It aimed to bulk different plants in each parent group to ensure that the presence of possible deviant individual plants for each parent genotype did not contribute to the false identification of polymorphisms. Using individual plants from the F_2 population gave an exact reflection of marker segregation, while using bulked F_3 families proved a cost effective method to confirm the presence of markers as individual plants did not have to be tested. BSA, a method described as pooling entries from the two extremes for a segregating trait, has been the method of choice for identifying molecular markers associated with disease resistance genes and has enabled identification of molecular markers associated with resistance genes to leaf and stripe rust (Michelmore, *et al*., 1991; William *et al*., 1997; Chague *et al*., 1999;).

4.5 Conclusion

The mapping of the *YrSp* gene to the short arm of chromosome 2B as found by McIntosh *et al*. (2001) was confirmed in this study using molecular markers. Furthermore, two markers were found to flank the *YrSp* gene in Kariega x Avocet/*YrSp* on chromosome 2B, L15 (M-CAT/E-ACC: 290 bp) and L68 (M-CTG/E-ACC: 59 bp) at 19.5 and 21.4 cM, respectively. The SSR marker Xgwm148 was used to anchor the linkage group as it is specific to chromosome 2B and it could then be assumed that the group that links with the SSR marker is located on chromosome 2B. In addition, Kariega/*YrSp* was also found to carry QTL markers *QYrSgi*-2B.1 and *QYrSgi*-7D for adult plant stripe rust resistance on chromosome 2B and 7D (Ramburan *et al*., 2004). Even though not all the resistant plants contained the markers Xgwm148, QYrSgi-2B.1 and QYrSgi-7D there were individuals that contained all these markers that can be utilised in further studies on the Kariega x Avs/*YrSp* hybrid.

The successful introgression of *YrSp* into Kariega x Avs/*YrSp* has been achieved as an ongoing process to produce a durable resistant cultivar.

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Table 4.1 Concentration of DNA extracted from ten Kariega and Avocet/*YrSp* plants, respectively.

Parent	Plant	1st OD reading	2nd OD reading	Average OD	[DNA] ng/ul
Kariega	1	1.34	0.97	1.155	1443.75
	2	0.34	0.35	0.345	431.25
	3	0.84	0.81	0.825	1031.25
	4	0.99	1.56	1.275	1593.75
	5	1.02	1.07	1.045	1306.25
	6	1.00	1.04	1.02	1275.0
	7	1.02	1.74	1.38	1725.0
	8	1.00	1.02	1.01	1262.5
	9	1.94	1.05	1.495	1868.75
	10	0.19	0.41	0.30	375.0
Avocet/*YrSp*	1	1.25	1.36	1.305	1631.25
	2	1.80	1.80	1.80	2250
	3	0.92	1.40	1.16	1450.0
	4	1.62	1.64	1.63	2037.5
	5	0.87	0.93	0.90	1125.0
	6	1.97	1.83	1.90	2375.0
	7	0.95	0.98	0.965	1206.25
	8	1.91	1.08	1.495	1868.75
	9	0.29	0.30	0.295	368.75
	10	0.79	1.00	0.895	1118.75

Table 4.2 Concentration of DNA extracted from 20 resistant and susceptible F_2 plants, respectively.

Resistant F_2 Population					Susceptible F_2 Population				
Plant	**1st OD reading**	**2nd OD reading**	**Average OD**	**[DNA] ng/ul**	**Plant**	**1st OD reading**	**2nd OD reading**	**Average OD**	**[DNA] ng/ul**
1	0.58	0.63	0.61	1512.5	1	0.73	0.83	0.78	1950.0
2	0.98	1.01	0.99	2487.5	2	0.85	0.75	0.8	2000.0
3	0.55	0.56	0.55	1387.5	3	0.52	0.52	0.52	1300.0
4	0.74	0.78	0.76	1900.0	4	0.88	0.83	0.855	2137.5
5	1.07	0.97	1.02	2550.0	5	0.88	0.93	0.905	2262.5
6	0.99	0.99	0.99	2475.0	6	0.76	0.75	0.755	1887.5
7	0.42	0.44	0.43	1075.0	7	0.82	0.92	0.87	2175.0
8	0.81	0.76	0.78	1962.5	8	0.56	0.57	0.565	1412.5
9	1.08	1.11	1.09	2737.5	9	0.33	0.32	0.325	812.5
10	0.94	0.93	0.93	2337.5	10	0.70	0.71	0.705	1762.5
11	0.91	0.97	0.94	2350.0	11	0.81	0.93	0.87	2175.0
12	0.96	0.97	0.96	2412.5	12	0.65	0.65	0.65	1625.0
13	0.88	0.81	0.84	2112.5	13	0.55	0.54	0.545	1362.5
14	0.62	0.59	0.60	1512.5	14	0.49	0.44	0.465	1162.5
15	0.85	0.74	0.79	1987.5	15	0.38	0.26	0.32	800.0
16	0.94	0.88	0.91	2275.0	16	0.34	0.54	0.44	1100.0
17	0.75	0.74	0.74	1862.5	17	0.19	0.15	0.17	425.0
18	1.1	0.98	1.04	2600.0	18	0.15	0.15	0.15	375.0
19	0.92	0.82	0.87	2175.0	19	0.13	0.13	0.13	325.
20	0.73	0.71	0.72	1800.0	20	0.49	0.46	0.475	1187.5

Table 4.3 Concentration of DNA extracted from ten resistant and susceptible F_3 families, respectively.

Family	1st OD reading	2nd OD reading	Average OD	[DNA] ng/ul
Resistance families				
RR1	1.58	1.72	1.65	2063.0
RR2	1.55	1.62	1.59	1988.6
RR3	1.64	1.61	1.63	2038.9
RR4	1.57	1.52	1.54	1933.0
RR5	1.67	1.57	1.62	2029.0
RR6	1.53	1.52	1.52	1911.5
RR7	1.46	1.51	1.48	1860.3
RR8A	1.52	1.67	1.59	1994.7
RR8B	1.59	1.76	1.67	2098.0
RR9	1.72	1.77	1.75	2190.4
RR10	1.67	1.52	1.59	1994.6
Susceptible families				
SS1	1.67	1.59	1.63	2040.9
SS2	1.65	1.64	1.65	2066.5
SS3	1.43	1.46	1.44	1811.6
SS4	1.66	1.63	1.65	2067.2
SS5	1.74	1.70	1.72	2157.5
SS6	1.50	1.63	1.57	1962.6
SS7	0.92	0.87	0.90	1130.6
SS8	1.63	1.59	1.61	2017.5
SS9	1.46	1.49	1.47	1849.5
SS10	1.68	1.73	1.70	2135.4

Table 4.4 SSR primer sequences (Roder *et al*., 1998).

Chromosome Location	SSR Name	Sequence	Repeat Motif
2A-L	Xgwm636	F-CGG TAG TTT TTA GCA AAG AG R-CCT TAC CGT TCT TGG CAG AA	(GA)28 imp
2B-L	Xgwm526	F-CAA TAG TTC TGT GAG AGC TGC G R-CCA ACC CAA ATA CAC ATT CTC A	(CT)16
2B-S	Xgwm148	F-GTG AGG CAG CAA GAG AGA AA R-CAA AGG TTG ACT CAG ACC AAA	(CA)22
2D-L	Xgwm261	F-CTC CCT GTA CGC CTA AGG C R-CTC GCG CTA GCC ATT G	(CT)21
6A-S	Xgwm169	F-ACC ACT GCA GAG AAC ACA TAC G R-GTG CTC TGC TCT AAG TGT GGG	(GA)23
6B-S	Xgwm219	F-GAT GAG CGA CAC CTA GCC TC R-GGG GTC CGA GTC CAC ACC	(GA)35imp
7A-S	Xgwm276	F-ATT TGC CTG AAG AAA ATA TT R-AAT TTC ACT GCA TAC ACA AG	(CT)24
7B-S	Xgwm43	F-CAC CGA CGG TTT CCC TAG AGT R-GGT GAG TGC AAA TGT CAT GTG	(CA)22
7D-L	Xgwm295	F-GTG AGG CAG ACC CAC AAC AC R-GAC GGC TGC GAC GTA GAG	(GA)25
7D-L	Xgwm428	F-CGA GGC AGC GAG GAT TT R-TTC TCC ACT AGC CCC GC	(GA)22

Table 4.5 AFLP adapter and primer sequences (Vos *et al*., 1995).

Adapter	Sequence (5' to 3')
EcoRI	F-CTC GTA GAC TGC GTA CC R-AAT TGG TAC GCA GTC TAC
MseI	F-GAC GAT GAG TCC TGA G R-TAC TCA GGA CTC AT
	Pre-selective primer
EcoRI	GAT CTG CGT ACC AAT TC
MseI	GAT GAG TCC TGA GTA A
	Selective primers ***MseI* selective primer**
M-CAT	GAT GAG TCC TGA GTA A CAA
M-CTG	GAT GAG TCC TGA GTA A CTG
M-CTT	GAT GAG TCC TGA GTA A CTT
M-CAG	GAT GAG TCC TGA GTA A CAG
	***EcoRI* selective primer**
E-ACA	GAT CTG CGT ACC AAT TC ACA (Fam labelled)
E-AAC	GAT CTG CGT ACC AAT TC AAC (Ned labelled)
E-ACC	GAT CTG CGT ACC AAT TC ACC (Fam labelled)
E-ACT	GAT CTG CGT ACC AAT TC ACT (Ned labelled)

Table 4.6 AFLP adapter and primer concentration.

Adapters	**nMoles**	**mg**	**pMoles**	**ug**	**1ug/ul Stock**
EcoRI-F	21.7	0.11	21710	110.0	110 TE
EcoRI-R	9.6	0.05	9620	50.0	50.0
MseI-F	13.6	0.07	13670	70.0	70.0
MseI-R	32.0	0.14	32090	140.0	140.0
Pre-selective primers					
EcoRI-	21.2	0.11	21260	110.0	110.0
MseI	22.7	0.12	22700	120.0	120.0
Selective primers					
MseI-selective primers					
M-CAA	21.7	0.13	21760	130.0	130.0
M-CAC	25.0	0.15	25080	150.0	150.0
M-CAG	12.3	0.07	12300	70.0	70.0
M-CAT	23.3	0.14	23320	140.0	140.0
M-CTA	23.6	0.14	23670	140.0	140.0
M-CTC	25.1	0.15	25120	150.0	150.0
M-CTG	12.6	0.07	12640	70.0	70.0
M-CTT	26.1	0.15	26110	150.0	150
EcoRI-selective primers					
E-ACC	N/A	N/A	N/A	0.30*	N/A
E-ACA	N/A	N/A	N/A	0.30*	N/A
E-ACT	N/A	N/A	N/A	0.30*	N/A
E-AAC	N/A	N/A	N/A	0.30*	N/A

* Commercial primer used per manufacturers instructions

Table 4.7 AFLP marker sizes, Chi-square values for a 3:1 and 1:1 segregation ratio and marker frequencies in F_2 and F_3 individuals.

	Segregation						Frequency	
Marker	**AFLP Primer Combination**	**Polymorphic Parent**	**Size bp**	**Observed ratio F2**	**Expected ratio F2**	**X^2**	**F_2 Resistant**	**F_2 Susceptible**
L2	CAT/ACT	Kar	45	21:19	1:1	0.1[a]	0.22	0.30
L4	CAT/ACT	Avs/*YrSp*	72	39:1	3:1	10.8	0.48	0.50
L6	CAT/ACT	Kar	196	38:2	3:1	8.5	0.45	0.50
L7	CAT/ACT	Avs/*YrSp*	280	30:10	3:1	0[a]	0.32	0.42
L8	CAT/ACC	Kar	48	39:1	3:1	10.8	0.5	0.48
L9	CAT/ACC	Kar	50	33:7	3:1	1.2[a]	0.4	0.42
L10	CAT/ACC	Kar	89	38:2	3:1	8.5	0.48	0.48
L11	CAT/ACC	Avs/*YrSp*	102	31:9	3:1	0.23[a]	0.42	0.35
L12	CAT/ACC	Avs/*YrSp*	154	39:1	3:1	10.8	0.5	0.48
L13	CAT/ACC	Kar	240	32:8	3:1	0.5[a]	0.48	0.32
L14	CAT/ACC	Kar	288	33:7	3:1	1.2[a]	0.42	0.40
L15	CAT/ACC	Avs/*YrSp*	290	10:30	1:1	10	0.25	0.00
L17	CTT/ACA	Avs/*YrSp*	46	21:19	1:1	0.1[a]	0.22	0.30
L18	CTT/ACA	Avs/*YrSp*	94	26:14	3:1	2.13[a]	0.25	0.40
L19	CTT/ACA	Avs/*YrSp*	104	34:6	3:1	2.13[a]	0.42	0.42
L20	CTT/ACA	Kar	105	12:28	1:1	6.4	0.1	0.20
L21	CTT/ACA	Avs/*YrSp*	193	38:2	1:1	0.1[a]	0.2	0.32
L22	CTT/ACA	Kar	227	38:2	3:1	8.5	0.45	0.50
L23	CTT/ACA	Kar	244	13:27	1:1	4.9	0.05	0.28
L24	CTT/ACA	Avs/*YrSp*	251	13:27	1:1	4.9	0.22	0.10
L25	CTT/ACA	Avs/*YrSp*	304	31:9	3:1	0.1[a]	0.35	0.42

L26	CTT/ACA	Kar	305	10:30	1:1	10	0.13	0.13
L27	CTT/AAC	Kar	40	23:17	1:1	0.9[a]	0.3	0.28
L28	CTT/AAC	Kar	42	32:8	3:1	0.5[a]	0.42	0.38
L30	CTT/AAC	Kar	102	37:3	3:1	6.5[c]	0.42	0.50
L31	CTT/AAC	Kar	107	38:2	3:1	8.5	0.48	0.48
L32	CTT/AAC	Kar	113	37:3	3:1	6.5	0.42	0.50
L33	CTT/AAC	Kar	132	37:3	3:1	6.5	0.42	0.50
L35	CTT/AAC	Kar	163	35:5	3:1	3.3[a]	0.4	0.48
L36	CTT/AAC	Avs/*YrSp*	168	11:29	1:1	8.1	0.25	0.03
L37	CTT/AAC	Kar	172	18:22	1:1	0.4[a]	0.25	0.20
L38	CTT/AAC	Avs/*YrSp*	192	25:15	1:1	2.5[a]	0.42	0.20
L39	CTT/AAC	Kar	211	36:4	3:1	4.8	0.42	0.48
L40	CTT/AAC	Kar	469	33:7	3:1	1.2[a]	0.38	0.45
L41	CAG/ACT	Avs/*YrSp*	132	34:6	3:1	2.1[a]	0.45	0.40
L42	CAG/ACT	Kar	134	32:8	3:1	0.5[a]	0.45	0.35
L43	CAG/ACT	Avs/*YrSp*	196	32:8	3:1	0.5[a]	0.35	0.45
L44	CAG/ACT	Avs/*YrSp*	242	38:2	3:1	8.5	0.48	0.48
L45	CAG/ACT	Kar	409	20:20	1:1	0[a]	0.35	0.15
L46	CAG/ACC	Kar	46	30:10	3:1	0[a]	0.3	0.45
L47	CAG/ACC	Avs/*YrSp*	56	35:5	3:1	3.3[a]	0.48	0.40
L48	CAG/ACC	Kar	67	39:1	3:1	10.8	0.48	0.50
L50	CAG/ACC	Avs/*YrSp*	106	17:23	1:1	0.9[a]	0.3	0.12
L51	CAG/ACC	Avs/*YrSp*	120	36:4	3:1	4.8	0.42	0.48
L52	CAG/ACC	Kar	137	24:16	1:1	1.6[a]	0.35	0.25
L53	CAG/ACC	Kar	189	25:15	1:1	2.5[a]	0.32	0.30
L54	CAG/ACC	Kar	252	34:6	3:1	2.1[a]	0.42	0.42
L55	CAG/ACC	Kar	272	26:14	3:1	2.1[a]	0.28	0.38

L56	CAG/ACC	Kar	376	29:11	3:1	0.27[a]	0.35	0.38
L57	CAG/ACC	Avs/*YrSp*	379	29:11	3:1	0.27[a]	0.38	0.35
L58	Xgwm148	Avs/*YrSp*	200	9:31	1:1	12.1	0.125	0.10
L59	CTG/ACT	Kar	50	38:2	3:1	8.5	0.95	0.95
L60	CTG/ACT	Kar	146	38:2	3:1	8.5	1	0.90
L62	CTG/ACT	Kar	241	23:17	1:1	0.9[a]	0.4	0.75
L63	CTG/ACT	Kar	418	35:5	3:1	3.3[a]	0.8	0.95
L64	CTG/ACT	Kar	425	33:7	3:1	1.2[a]	0.65	1.00
L65	CTG/ACC	Kar	47	35:5	3:1	3.3[a]	0.85	0.90
L66	CTG/ACC	Kar	49	36:4	3:1	4.8	0.85	0.95
L67	CTG/ACC	Kar	51	33:7	3:1	1.2[a]	0.7	0.95
L68	CTG/ACC	Kar	59	21:19	1:1	0.1[a]	0.25	0.80
L69	CTG/ACC	Kar	67	39:1	3:1	10.8	1.0	0.95
L70	CTG/ACC	Kar	92	39:1	3:1	10.8	1.0	0.95
L71	CTG/ACC	Kar	139	38:2	3:1	8.5	1.0	0.90
L72	CTG/ACC	Kar	151	38:2	3:1	8.5	1.0	0.90
L73	CTG/ACC	Kar	190	39:1	3:1	10.8	1.0	0.95
L75	CTG/ACC	Kar	272	18:22	1:1	0.4[a]	0.4	0.50
L76	CTG/ACC	Kar	329	38:2	3:1	8.5	0.9	1.00
L77	CTG/ACC	Kar	406	32:8	3:1	0.5[a]	0.65	0.95

[a]$X_{0.05}$ =3.84 (df=1)

Table 4.8 Linkage group of markers mapped.

Marker code	locus	M	P	X	N	Map	SE	Low	Hi	LOD
L38	M4E1N192	26	16							
				18	42	21.4	5.2	14.2	31.8	6.3
L68	M3E2N59	21	21							
				16	42	19	4.9	12.3	29.1	7.5
YrSp	YrSp	21	21							
				20	41	24.4	5.6	16.6	35.2	4.9
L15	M2E2N290	11	30							
				20	41	24.4	5.6	16.6	35.2	4.9
L46	M6E2N46	11	31							
				14	42	16.7	4.5	10.4	26.4	8.8
L67	M3E2N51	8	34							
				8	42	9.5	3.4	5	17.9	13.8
L65	M3E2N47	6	36							
				2	42	2.4	1.7	0.7	8.4	21.2
L66	M3E2N49	5	37							
				14	41	17.1	4.6	10.6	27	8.4
L13	M2E2N240	8	33							
				16	41	19.5	5	12.6	29.8	7.1
L58	Xgwm148	10	32							
				18	42	21.4	5.2	14.2	31.8	6.3
L60	M3E2F146	3	39							
				4	42	4.8	2.4	2	11.8	18.3
L71	M3E2N139	3	39							
				2	42	2.4	1.7	0.7	8.4	21.2
L70	M3E2N92	2	40							
				0	42	0	0	0	4.3	25.3
L73	M3E2N190	2	40							
				0	42	0	0	0	4.3	25.3
L69	M3E2N67	2	40							
				2	42	2.4	1.7	0.7	8.4	21.2
L72	M3E2N151	3	39							
				4	42	4.8	2.4	2	11.8	18.3
L31	M4E1N107	3	39							
				4	42	4.8	2.4	2	11.8	18.3
L59	M3E2F50	3	39							
				16	41	19.5	5	12.6	29.8	7.1
L9	M2E2N50	7	34							
				14	41	17.1	4.6	10.6	27	8.4
L22	M4E1F227	4	38							
				4	42	4.8	2.4	2	11.8	18.3
L10	M2E2N89	2	40							
				2	41	2.4	1.7	0.8	8.5	20.6
L8	M2E2N48	1	40							

				0	41	0	0	0	4.4	24.7
L61	M3E2F207	1	41							
				0	42	0	0	0	4.3	25.3
L1	M2E2F42	1	41							
				0	42	0	0	0	4.3	25.3
L74	M3E2N269	1	41							
				0	42	0	0	0	4.3	25.3
L3	M2E2F66	1	41							
				0	42	0	0	0	4.3	25.3
L5	M2E2F81	1	41							
				2	42	2.4	1.7	0.7	8.4	21.2
L48	M6E2N67	2	40							
				2	42	2.4	1.7	0.7	8.4	21.2
L6	M2E2F196	3	39							
				6	42	7.1	2.9	3.4	14.9	15.9
L16	M4E1F42	4	38							
				4	42	4.8	2.4	2	11.8	18.3
L30	M4E1N102	4	38							
				0	42	0	0	0	4.3	25.3
L32	M4E1N113	4	38							
				0	42	0	0	0	4.3	25.3
L33	M4E1N132	4	38							
				4	42	4.8	2.4	2	11.8	18.3
L35	M4E1N163	6	36							
				10	42	11.9	3.8	6.7	20.8	12
L39	M4E1N211	5	37							
				20	42	23.8	5.5	16.2	34.4	5.3
L42	M6E2F134	9	33							
				20	42	23.8	5.5	16.2	34.4	5.3
L54	M6E2N252	7	35							
				6	42	7.1	2.9	3.4	14.9	15.9
L63	M3E2F418	6	36							
				12	42	14.3	4.2	8.5	23.7	10.3
L40	M4E1N469	8	34							
				14	41	17.1	4.6	10.6	27	8.4
L14	M2E2N288	7	34							
				20	41	24.4	5.6	16.6	35.2	4.9
L28	M4E1N42	9	33							

M- Maternal Genotype, P-Paternal genotype, X- Number of cross overs for this interval, N- Number of informative loci for this interval, SE- Standard error of the map distance for this interval, Low- Lower limit of the 95% confidence interval for the map distance, Hi- Higher limit of the 95% confidence interval for the map distance, LOD-Linkage of the markers flanking this interval.

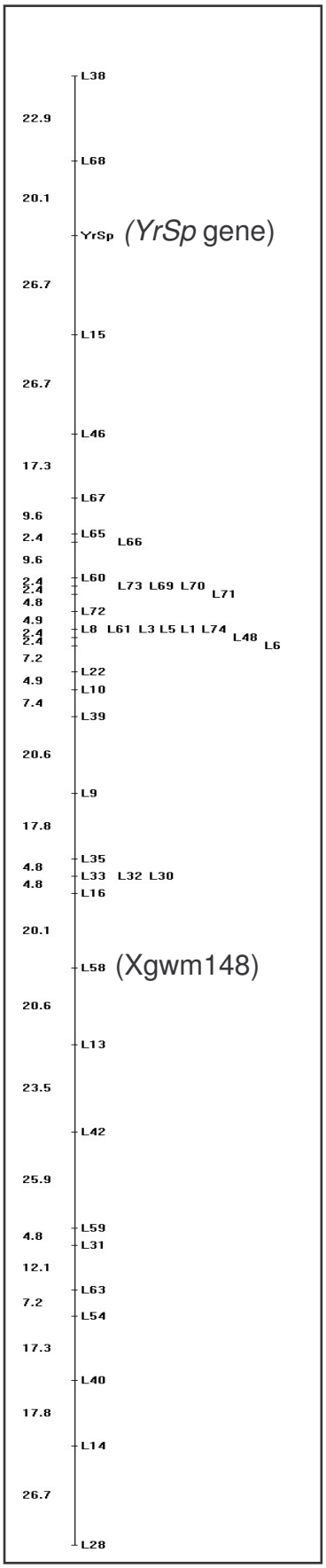

Figure 4.1 Map of chromosome 2B with AFLP markers and SSR anchor (L58).

Summary

The successful introgression of the stripe rust seedling resistance gene *YrSp* into Kariega/ Avs/*YrSp* is towards the continued pursuit of durable resistance in this cultivar.

Following the development of Kariega/ Avs/*YrSp* hybrid, an F_2 and F_3 Kariega/*YrSp* population was obtained and screened for virulence to pathotype 6E22A- of *P. striiformis* f. sp. *tritici*. The disease screening of the F_2 population deviated from the expected single dominant gene ratio and did not fit a 3:1 gene segregation ratio determined by Chi-square analysis. Disease screening of the resistant F_3 families exhibited segregation while susceptible F_3 families was not considered to segregate with the observation of singular resistant plants in two susceptible F_3 families.

Histological studies firstly confirmed the infection pathway of *P. striiformis* f. sp. *tritici* as is described for other cereal rusts with the absence of the formation of appressoria and the observation of pseudo-SSVIs the most marked differences. The pseudo-SSVIs are thought to be due to the unsuccessful stomatal penetration by germ tubes as successful penetration results in a single SSVI that is generally larger in size than the vesicles observed but this requires further study. Secondly, quantitative analysis indicated that urediospores of *P. striiformis* f. sp. *tritici* have good germination efficiency but germ tubes were rarely observed to penetrate stomata. After penetration, the infection efficiency of the pathogen is high in susceptible cultivars due to systemic colonisation. The resistance conferred by the *YrSp* gene is considered posthaustorial as no significant difference in infection efficiency could be determined before haustorium formation. An increase in the number of internal infection structures after 7 dpi in the susceptible cultivar was observed while the resistant line reflected a decrease. Thirdly, fluorochrome assessment indicated uvitex 2B and the orange G probe hold the best potential of the fluorochromes tested for use with the confocal laser scanning microscopy (CLSM). Furthermore, the CLSM holds great potential to elucidate host-pathogen interactions if more research into fluorochromes selection and development occurs.

Using molecular markers obtained from AFLPs and SSRs the *YrSp* gene was mapped to the short arm of chromosome 2B. The *YrSp* gene was located on the short arm of chromosome 2B in the Kariega/ Avs/*YrSp* hybrid. Two AFLP markers were found to flank the gene namely L15 and L68 at 19.5 and 21.4 cM respectively. In addition to this, 2 QTL markers, Q*Yr*Sgi-2B.1and Q*Yr*Sgi-7D for adult plant resistance was present in the Kariega /*YrSp* population. The previous report of an introgression on chromosome 6A into Avocet/*YrSp* was not found to contribute to the introgressed trait.

The integration of various disciplines provided insight into the host-pathogen interaction of *P. striiformis* f. sp. *tritici* on wheat at different levels towards a holistic understanding of such interactions and the mechanisms involved in conditioning resistance.

Die *YrSp* geen is suksesvol van Avocet/*YrSp* na Kariega, ‘n kommersiële Suid-Afrikaanse kultivar oorgedra. Na inkorporering van die geen is die F_2 en F_3 Kariega/*YrSp* populasies vir weerstand teen die patotipe 6E22A- van *Puccinia striiformis* f. sp. *tritici* geëvalueer. Resultate van die siekteweerstands-evalueering van die F_2 populasie het op die teenwoordigheid van ‘n enkel dominante geen gedui en het nie voldoen aan ‘n 3:1 segregasie verhouding. Verdere evaluasie van weerstandbiedende F_3 families het segregasie getoon in teenstelling met die vatbare F_3 families. Die ontwikkeling van Kariega/*YrSp* is die volgende stap in die voortdurende strewe na volhoubare weerstand in hierdie kultivar.

Histologiese studies, met behulp van transmissie-elektronmikroskopie (TEM) en skandeer-elektronmikroskopie (SEM) het bevestig dat die infeksieproses by *P. striiformis* f. sp. *tritici* dieselfde is as wat beskryf is vir ander graanroese, behalwe dat geen appressoria gevorm het nie. Verder is die inisiële fase van die pseudo-substomatale vesikel (SSVIs) van streeproes gedurende hierdie studie waargeneem en beskryf. Die vesikels vorm waarskynlik as gevolg van onsuksesvolle penetrasie van die stomata deur die kiembuis aangesien suksesvolle penetrasie ‘n enkele vesikel vorm en oor die algemeen groter is. Hierdie aspek benodig verdere navorsing. ‘n Kwantitatiewe analise van streeproesinfeksies, het aangetoon dat ten spyte van swak kiembuispenetrasie, die infeksietempo van *P. striiformis* f. sp. *tritici* hoog was in vatbare kultivars waarskynlik as gevolg van sistemiese kolonisasie. Daar is gevind dat die weerstand, oorgedra deur die *YrSp*-geen, na-haustoriaal is, omdat geen verskil in infeksievlak of struktuur tussen Avs/*YrSp* en Kariega voor haustoriumontwikkeling opgemerk is nie. Die aantal interne infeksiestrukture in die vatbare kultivars het 7 dae na infeksie toegeneem, terwyl die aantal strukture in die weerstandbiedende kultivars afgeneem het. ‘n Vergelyking van verskillende fluorochrome, het bevind dat vir die bestudering van swaminfeksiestrukture, uvitex 2B en oranje G die doeltreffendste fluorochrome was vir gebruik met konfokale-laser-skandeermikroskopie (KLSM). Die KLSM kan potensieel help om gasheer-patogeen interaksies op te klaar, maar verdere navorsing in fluorochroom seleksie en ontwikkeling is nodig.

Die *YrSp* geen is suksesvol op die kort arm van chromosoom 2B gekarteer deur gebruik te maak van ‘n AFLP merker, geanker deur ‘n chromosoom spesifieke SSR merker. Die studie het die posisie van *YrSp* op chromosoom 2B met behulp van molekulêre merkers, asook die kombineering van die geen in Kariega/ Avs/*YrSp*, bevestig. Twee aangrensende AFLP merkers (L15 en L19, 19.5 en 21.4 cM onderskeidelik vanaf die geen) is gevind. Die teenwoordigheid van twee QTL merkers, Q*YR*Sgi-2B.1 en Q*YR*Sgi-7D, vir volwasse weerstand in Kariega/*YrSp,* is bevestig. In teenstelling met ‘n vorige studie, is gevind dat die inkorporering van ‘n gedeelte op chromosoom 6A in Kariega, nie bydra tot die oorgedraagde kenmerk nie.

Die integrasie van verskeie dissiplines het tot beter insig in die gasheer-patogeen interaksie van *P. striiformis* f. sp. *tritici* in koring gelei, en hoop om by te dra tot ‘n meer holistiese begrip van die interaksies en meganismes betrokke by streeproesweerstand.

Appendix A AFLP fragments for primer combination M-CAT/E-ACT

M-CAT/E-ACT																									
Cultivar	**42**	**43**	**45**	**46**	**48**	**49**	**51**	**52**	**53**	**56**	**59**	**62**	**63**	**64**	**65**	**66**	**68**	**69**	**71**	**72**	**74**	**75**	**76**	**78**	**79**
Avs	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Kar	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1
R1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
R2	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
R3	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
R4	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
5	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1
6	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	0	1	1	1
7	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
8	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
9	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	0	1	1	1
10	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1	1	1	0	1	1	1
11	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1
12	0	1	0	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1
13	0	1	0	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1
14	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1
15	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
16	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
17	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
18	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1
19	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1
20	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1
S1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1	1	1	1	1	1	1
S2	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
S3	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1
S4	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1
5	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	0	1	1	1
6	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1
7	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	0	1	1	1
8	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1
9	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1
10	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1
11	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1
12	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1
13	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1
14	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1
15	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1
16	0	1	0	1	1	1	1	1	0	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1
17	0	1	0	1	1	1	1	0	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1
18	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1
19	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1
20	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	0	1	1	1

M-CAT/E-ACT																									
Cultivar	**81**	**83**	**84**	**86**	**88**	**90**	**93**	**94**	**95**	**98**	**105**	**107**	**109**	**110**	**111**	**112**	**117**	**118**	**123**	**127**	**130**	**133**	**135**	**143**	**145**
Avs	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Kar	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R2	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
R3	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
R4	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
7	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S2	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S3	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S4	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	0	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	0	1	1	1	1	1	0
8	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

M-CAT/E-ACT																									
Cultivar	**148**	**149**	**150**	**153**	**155**	**156**	**159**	**162**	**166**	**170**	**173**	**176**	**180**	**190**	**191**	**196**	**199**	**210**	**217**	**219**	**230**	**232**	**234**	**237**	**242**
Avs	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
Kar	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R2	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R3	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
R4	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
14	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
15	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
17	1	1	0	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1
18	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
19	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
S1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S2	1	1	0	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1
S3	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S4	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	0	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1
6	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
7	1	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1
8	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
9	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
10	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
11	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
12	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
13	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
15	1	1	0	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1
16	1	1	0	1	0	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1
17	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
18	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
20	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1

M-CAT/E-ACT																										
Cultivar	**246**	**249**	**252**	**256**	**260**	**263**	**268**	**271**	**280**	**283**	**289**	**292**	**298**	**309**	**313**	**323**	**326**	**344**	**346**	**355**	**360**	**379**	**394**	**413**	**429**	**438**
Avs	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Kar	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R2	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
R3	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R4	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	0	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	0	1	0	1	1	1	0	1	0	1	1
10	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	0	1	1	1	0	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	0	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S2	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
S3	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S4	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	0	1	1	1	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0
8	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
15	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	0	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1

Appendix B AFLP fragments for primer combination M-CAT/E-ACC

	M-CAT/E-ACC																								
Cultivar	**42**	**44**	**46**	**48**	**50**	**51**	**53**	**56**	**61**	**61**	**66**	**67**	**68**	**70**	**73**	**75**	**78**	**81**	**83**	**87**	**89**	**91**	**92**	**95**	**99**
Avs	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
Kar	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R3	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
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19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
S3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
S4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
17	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

M-CAT/E-ACC																									
Cultivar	**100**	**102**	**105**	**107**	**108**	**109**	**111**	**115**	**119**	**123**	**125**	**126**	**128**	**130**	**132**	**133**	**137**	**138**	**140**	**141**	**142**	**147**	**150**	**153**	**154**
Avs	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Kar	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
R1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	1
R3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1
R4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	0	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
15	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
17	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1
S1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1
S2	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
S3	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1
S4	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	0	1
5	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	0	1
6	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
7	0	1	1	1	1	1	0	1	1	0	1	1	1	1	0	1	0	0	0	1	1	1	0	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	0	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	0	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1

M-CAT/E-ACC																									
Cultivar	**156**	**159**	**162**	**166**	**172**	**174**	**177**	**180**	**185**	**189**	**191**	**194**	**196**	**200**	**205**	**207**	**213**	**216**	**220**	**226**	**240**	**245**	**252**	**260**	**267**
Avs	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
Kar	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R2	1	1	0	1	0	0	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0
R3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
S1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
S2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
S3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
S4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

M-CAT/E-ACC																						
Cultivar	**270**	**274**	**281**	**283**	**288**	**290**	**293**	**302**	**316**	**324**	**327**	**344**	**349**	**363**	**367**	**388**	**392**	**397**	**419**	**445**	**452**	**477**
Avs	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Kar	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R2	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	0	0	1	0	1	1
R3	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
7	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
11	1	1	1	1	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S2	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1
S3	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1
S4	1	1	1	1	0	0	1	0	1	1	1	1	0	1	1	1	0	1	0	0	1	1
5	0	1	1	1	0	0	1	0	1	1	1	1	0	1	1	1	0	1	0	0	1	1
6	1	1	1	1	1	0	1	0	1	1	1	1	0	1	1	1	1	1	0	0	1	1
7	1	1	1	0	0	0	1	0	1	1	1	1	1	0	0	1	0	0	0	0	0	0
8	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	0
17	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
20	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1

Appendix C AFLP fragments for primer combination M-CTG/E-ACT

M-CTG/E-ACT																									
Cultivar	**42**	**43**	**46**	**48**	**50**	**52**	**55**	**57**	**59**	**61**	**64**	**65**	**66**	**69**	**71**	**74**	**78**	**79**	**84**	**85**	**88**	**91**	**117**	**123**	**125**	**127**
Avs	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Kar	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R2	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
S2	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S3	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
S4	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
6	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

M-CTG/E-ACT																									
Cultivar	**131**	**133**	**134**	**136**	**142**	**144**	**146**	**148**	**155**	**158**	**161**	**167**	**172**	**176**	**179**	**185**	**190**	**191**	**194**	**202**	**204**	**207**	**209**	**211**	**217**
Avs	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1
Kar	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
R1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
R2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
R3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
R4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1
7	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	0	1	0	1	1	0	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1
10	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
11	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
17	1	0	1	0	1	0	1	1	0	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
20	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
S1	1	1	0	1	1	1	0	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
S2	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
S3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
S4	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
6	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
7	1	1	1	0	0	0	1	1	0	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
15	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
20	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

M-CTG/E-ACT																									
Cultivar	**221**	**223**	**226**	**228**	**230**	**232**	**236**	**241**	**246**	**249**	**255**	**263**	**273**	**276**	**293**	**301**	**303**	**318**	**323**	**330**	**339**	**393**	**418**	**425**
Avs	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0
Kar	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0
R1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
R2	1	1	0	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	0	1	0	0
R3	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0
R4	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0
6	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1
7	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0
8	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1
9	1	1	0	0	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	0	0	0	0	1
10	1	1	1	0	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1
11	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
12	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	0
13	1	1	1	0	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
15	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
16	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
17	0	0	0	1	1	1	0	0	1	1	1	1	1	0	1	1	1	1	1	0	0	1	0	1
18	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
19	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
S1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
S2	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
S3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
S4	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
5	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
6	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	0	0	0	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	0	0	0	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
13	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
14	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
15	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
16	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Appendix D AFLP fragments for primer combination M-CTG/E-ACC

M-CTG/E-ACC																										
Cultivar	**41**	**42**	**43**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**	**54**	**57**	**58**	**59**	**64**	**67**	**69**	**71**	**73**	**74**	**77**	**79**	**80**	**83**	**86**
Avs	1	1	1	1	1	0	1	0	1	0	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1
Kar	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R2	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
R4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	0
10	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	0
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
12	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	0	1	1
14	1	1	0	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0
16	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0
17	1	1	0	1	1	0	1	0	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0
18	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
19	1	1	1	1	1	0	1	0	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	0	1	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0
S1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S2	1	1	1	1	1	0	1	0	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0
S3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1
S4	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0
6	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	0	0	1	1	1	0	1	0	1	1	0	0	1	1	0	1	1	1	1	1	1	0
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
11	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
13	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1

M-CTG/E-ACC																										
Cultivar	**87**	**89**	**90**	**92**	**96**	**98**	**101**	**102**	**105**	**106**	**111**	**114**	**118**	**120**	**127**	**130**	**134**	**136**	**137**	**138**	**139**	**144**	**146**	**151**	**154**	**156**
Avs	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1	1
Kar	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
R1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
R2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
R3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
R4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
9	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
10	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
12	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	0	1	0	0
13	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	x	x	x	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
17	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
S1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	0	0	1	1	1
S2	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1	1
S3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
S4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
9	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
20	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0

M-CTG/E-ACC																										
Cultivar	**160**	**163**	**167**	**171**	**173**	**174**	**181**	**184**	**187**	**190**	**191**	**193**	**195**	**197**	**199**	**204**	**206**	**207**	**212**	**214**	**221**	**223**	**227**	**232**	**235**	**238**
Avs	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	0
Kar	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
R1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	0	1	1	1
R2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	0
R3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
R4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	0	0	0	1	0	1	1	1	1	0
10	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	0	0	1	1	1	1	0	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	0	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	0	1	1	0
15	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	0	0	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	0	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	0	1	1	1	1	1	1	1	1
18	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	0	0	1	1	1	1	1	1	1	1
19	1	1	1	1	0	0	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S2	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
S4	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	0	1	1	1	1	1	1	1	1	1
7	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	0	1	1	1	1	1	1	0	1	1	0	1	0	0	1	1	1	1	1	1	1	1

M-CTG/E-ACC																												
Cultivar	**241**	**243**	**246**	**249**	**254**	**256**	**261**	**262**	**263**	**269**	**272**	**276**	**285**	**291**	**300**	**305**	**319**	**329**	**334**	**342**	**362**	**366**	**377**	**390**	**406**	**409**	**416**
Avs	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
Kar	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
R1	1	1	1	1	1	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
R2	1	1	0	1	0	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
R3	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
R4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
7	1	1	1	1	1	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	0	0	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	0	1	1	0	1	1
10	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
12	0	0	0	0	1	1	1	0	0	1	0	0	1	1	1	0	1	0	0	1	0	0	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	0	1	1	1	0	1	1
14	1	1	1	1	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
15	1	1	0	1	1	1	1	0	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
17	1	0	0	0	1	1	1	0	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1
18	1	1	0	1	0	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1
19	1	1	0	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
20	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S2	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S3	1	1	1	1	1	1	1	1	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
S4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	0	0	0	0	0	1	1	1	0	1	0	0	1	1	0	0	1	1	0	0	0	0	1	1	1	1	1
8	1	1	1	1	0	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1

Appendix E AFLP fragments for primer combination M-CTT/E-ACA

M-CTT/E-ACA																									
Cultivar	**41**	**42**	**45**	**46**	**47**	**48**	**50**	**51**	**54**	**55**	**58**	**59**	**60**	**62**	**64**	**65**	**66**	**67**	**70**	**73**	**74**	**75**	**76**	**78**	**81**
Avs	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Kar	1	0	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R1	1	1	1	0	1	0	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1
R2	1	0	1	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R3	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R4	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	0	1	0	1	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
7	1	0	0	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
8	1	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	0	0	0	0	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1
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16	1	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
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20	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S2	1	0	0	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S3	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S4	1	0	0	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
5	1	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
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12	1	0	0	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
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17	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
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20	1	0	1	0	1	0	1	1	1	1	1	0	0	1	1	1	1	1	1	0	1	0	1	1	1

M-CTT/E-ACA																										
Cultivar	**84**	**85**	**87**	**88**	**90**	**92**	**94**	**96**	**98**	**102**	**103**	**104**	**105**	**106**	**109**	**111**	**113**	**115**	**117**	**121**	**123**	**125**	**128**	**129**	**131**	**132**
Avs	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	0
Kar	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0
R1	1	0	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	0	1	0
R2	1	0	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
R3	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	0
R4	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	0
5	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	0
6	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	0
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12	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	0
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S1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0
S2	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	0
S3	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0
S4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0
5	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0
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15	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	0
16	1	1	0	1	1	1	0	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	0
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0
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19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0
20	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	0

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Cultivar	**134**	**136**	**137**	**140**	**141**	**148**	**151**	**152**	**154**	**157**	**159**	**163**	**165**	**167**	**169**	**171**	**172**	**176**	**178**	**181**	**183**	**184**	**188**	**189**	**191**	**193**	**194**
Avs	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
Kar	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1
R1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1
R2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1
R3	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1
R4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1
9	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	0
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
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Cultivar	**197**	**200**	**205**	**207**	**210**	**212**	**215**	**221**	**224**	**226**	**227**	**230**	**232**	**233**	**239**	**242**	**244**	**247**	**250**	**251**	**256**	**261**	**264**	**268**	**270**	**274**
Avs	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
Kar	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
R1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0	1	1	0	1	0	0	1	1	0
R2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	0	1	1	1	0
R3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1
R4	1	1	1	1	1	1	1	1	0	1	0	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	0
7	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0
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9	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	0	1	1	1	1	0	1	1	1	0
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
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12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	x	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	x	1	1	1
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16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	x	0	1	1
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19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1
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Cultivar	**278**	**284**	**292**	**297**	**300**	**304**	**305**	**306**	**307**	**311**	**312**	**328**	**331**	**333**	**336**	**340**	**349**	**370**	**375**	**412**	**416**	**424**	**428**	**435**	**477**
Avs	1	1	1	1	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Kar	1	1	1	1	1	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R1	1	1	0	0	1	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R2	1	1	1	1	1	1	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R3	1	1	1	1	1	1	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R4	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
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7	1	1	1	0	1	1	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	0	1	1	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	0	1	0	1	1	1	0	0	0	1	0	1	1	1	1	1	1	0	0	0	1	1	1	1	1
10	1	1	1	1	1	1	0	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	1	0	1	1	1	1	0	1	1
12	1	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	1	0	1	1	1	1	0	1	1
13	1	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	1	0	1	1	1	1	0	1	1
14	1	1	1	1	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	0	1	0	0	0	1	0	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1
16	1	1	1	1	1	1	0	0	1	0	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1
17	0	1	1	0	1	0	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
18	1	1	1	0	1	0	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	0	1	0	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	0	1	0	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S1	1	1	1	0	1	0	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S2	1	1	1	0	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
S3	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S4	1	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	0	1	1	1	1	0	0	0	1	0	0	0	0	1	1	0	1	0	0	1	0	1	1	1	0
8	1	1	1	1	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	1	1	1	0
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	0	1	1	0	0	1	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	0	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	0	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	0	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Appendix F AFLP fragments for primer combination M-CTT/E-AAC

M-CTT/E-AAC																														
Cultivar	**40**	**41**	**42**	**45**	**47**	**48**	**50**	**52**	**58**	**61**	**64**	**65**	**66**	**67**	**69**	**71**	**75**	**78**	**81**	**82**	**83**	**86**	**88**	**89**	**90**	**92**	**94**	**98**	**96**	**102**
Avs	0	1	0	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	0	1	1	0	1	1	1	0	1	0
Kar	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	0	1	1	0	1	1	1	0	1	1
R1	0	1	0	1	0	1	1	1	1	1	1	0	1	1	1	1	0	1	0	1	0	1	1	0	1	1	1	0	1	0
R2	0	1	0	1	0	1	1	1	1	1	1	0	1	1	1	1	0	1	0	1	0	1	1	0	1	1	1	0	1	0
R3	0	1	0	1	0	1	1	1	1	1	1	0	1	1	1	1	0	1	0	1	0	1	1	0	1	1	1	0	1	0
R4	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
5	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
6	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
7	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
8	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
12	0	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1
13	0	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1
14	0	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1
15	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1
16	0	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1
17	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1
18	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
20	0	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
S1	0	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
S2	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
S3	0	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
S4	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1	1	0	1	1	1	1	1
5	0	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1
7	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1
8	0	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1	1	0	1	1	1	1	1
9	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
10	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
11	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
12	0	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
13	0	1	0	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1
14	0	1	0	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1
15	0	1	0	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1
16	0	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1
17	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1
18	1	0	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1
19	1	1	0	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1	0	0	1	1	1	1	1
20	1	1	0	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	0	1	1	0	0	1	1	1	1	1

M-CTT/E-AAC																										
Cultivar	**104**	**105**	**106**	**107**	**108**	**109**	**110**	**113**	**120**	**121**	**122**	**124**	**128**	**132**	**135**	**136**	**138**	**146**	**152**	**163**	**168**	**171**	**172**	**174**	**176**	**177**
Avs	1	1	1	0	1	1	1	0	1	1	0	1	1	0	0	1	1	1	1	0	1	1	0	1	1	1
Kar	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
R1	0	1	1	1	1	1	1	0	1	1	0	1	1	0	0	1	1	1	1	0	1	1	x	1	0	1
R2	0	1	1	1	1	1	1	0	1	1	0	1	1	0	0	1	1	1	1	0	1	1	x	1	1	1
R3	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	1	1	1	1	0	1	1	x	1	0	1
R4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	0
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1	0
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1	0
12	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0
13	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	0	0	1	1	1	1	0
14	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	0	1	0	1	0	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	0
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	0	1	0	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	0
18	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	0	1	1	1	1	0
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1	0
20	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	0	1	0	1	1	0
S1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1
S2	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1	1
S3	1	0	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	0	1	0	1	0	0
S4	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1	0
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	0	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1	0
7	1	1	0	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1	0
8	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	0	1	1	1	0	1
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12	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	0	1	0	1	1	0
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	0	1
14	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	0	1	0	1
15	1	0	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	0	1	1	1	0	1
16	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	0	1	0	1
17	1	0	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	0	0	1	1	1	0	1
18	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	0	1	1	1	0	1
19	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	0	1
20	1	1	1	1	1	0	0	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1	0

M-CTT/E-AAC																						
Cultivar	**183**	**186**	**192**	**196**	**197**	**211**	**213**	**216**	**221**	**229**	**235**	**241**	**246**	**257**	**265**	**303**	**308**	**309**	**318**	**422**	**436**	**469**
Avs	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	0
Kar	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1
R1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	0
R2	1	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0	0	1	1	0
R3	1	0	0	0	1	0	0	1	1	1	1	1	1	1	0	1	0	0	0	1	1	0
R4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
9	1	1	1	1	1	0	1	1	0	0	1	1	1	1	1	1	1	0	0	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
11	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	0	1	1	1
12	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	0	1	1	1
13	1	1	1	1	1	0	1	1	0	0	1	1	1	1	1	1	1	0	0	1	1	1
14	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	0	1	0	1
15	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	0	1	0	0
16	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	0	1	1	1
17	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	0	1	1	0
18	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	0	1	1	1
19	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	0	1	0	1	1	1
20	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	0	1	0	1	1	1
S1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	0	1	1	1	1	1
S2	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	0	1	0	1	0	1
S3	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	0	1	0	1	1	1
S4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	0
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
9	1	1	0	1	1	1	1	1	0	0	1	1	1	1	1	1	0	1	0	1	1	1
10	1	1	0	1	1	1	1	1	0	0	1	1	1	1	1	1	0	1	0	1	1	1
11	1	0	0	0	1	0	0	1	0	0	1	1	1	0	0	1	0	0	0	1	0	0
12	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
13	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
14	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	0	1	1	1
15	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	0	1	1	1
16	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
17	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1
18	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
19	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
20	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	0	1	0	1

Appendix G AFLP fragments for primer combination M-CAG/E-ACC

M-CAG/E-ACC																									
Cultivar	**41**	**42**	**45**	**46**	**48**	**49**	**50**	**53**	**56**	**57**	**59**	**61**	**62**	**63**	**65**	**67**	**68**	**69**	**70**	**73**	**75**	**77**	**78**	**79**	**81**
Avs	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1
Kar	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
R2	0	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1
R3	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
R4	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	0	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
7	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
8	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
9	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
10	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1
11	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1
12	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1
13	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1
14	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
15	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
16	0	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
17	1	1	1	0	1	0	0	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1
18	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
19	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1
20	1	1	1	0	1	0	0	1	1	1	1	0	1	1	1	1	1	0	1	0	0	1	1	1	1
S1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
S2	0	0	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
S3	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
S4	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
5	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	0	1	0	0	1	1	1	1	0	1	1	1	1	1	0	1	1	1	0	1	1	1
8	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
9	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
10	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
11	0	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
12	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
13	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
14	0	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
15	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
16	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
18	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
19	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
20	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1

M-CAG/E-ACC																										
Cultivar	**82**	**83**	**85**	**88**	**89**	**92**	**93**	**95**	**97**	**98**	**103**	**105**	**106**	**107**	**109**	**110**	**111**	**113**	**116**	**120**	**124**	**125**	**129**	**130**	**131**	**132**
Avs	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Kar	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1
R1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
R2	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
R4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
5	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1
7	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	0	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1
12	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	0	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S2	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1
S3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
S4	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1
13	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
18	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1

M-CAG/E-ACC																									
Cultivar	**134**	**136**	**137**	**140**	**143**	**145**	**146**	**147**	**149**	**152**	**154**	**156**	**157**	**160**	**161**	**166**	**168**	**174**	**176**	**180**	**184**	**189**	**190**	**193**	**196**
Avs	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
Kar	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R2	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1
R3	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
R4	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
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12	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
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S1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S2	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	0	1	1	0	0	0	1	1	1	0
S3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
S4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
5	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
9	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
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16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1

M-CAG/E-ACC																									
Cultivar	**200**	**205**	**210**	**212**	**217**	**219**	**223**	**226**	**235**	**238**	**241**	**251**	**252**	**256**	**259**	**266**	**272**	**281**	**289**	**292**	**295**	**302**	**313**	**316**	**318**
Avs	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1
Kar	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
R2	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	0	0	1	1
R3	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
R4	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1
5	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
6	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1
8	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1
9	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	0	1	1	1	1	0	1	1	1
10	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
11	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
12	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
13	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1
14	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
15	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
16	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1
18	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0	1	0	1	1	1
20	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1
S1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
S2	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	0	1	1	1
S3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
S4	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	0	1	1	1
5	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	0	0	1	0
8	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1
9	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
11	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	0	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

M-CAG/E-ACC																				
Cultivar	**337**	**346**	**353**	**355**	**359**	**367**	**376**	**379**	**386**	**397**	**399**	**403**	**417**	**421**	**428**	**432**	**447**	**453**	**460**	**476**
Avs	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
Kar	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
R1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R2	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
R3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	0	1	1	1
9	1	1	1	1	1	1	1	0	0	1	0	1	1	1	1	1	0	0	1	1
10	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	0	1	1	1	0	1	1	0	1	0	1	1	1
17	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
S1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S2	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	0	1	1	1	1
S3	1	1	1	1	1	1	1	0	1	1	1	0	1	1	0	1	0	1	1	1
S4	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	0	1	0	0	1	0	1	1	1	0	1	0	0	1	1
8	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	0	1	0	0	0	1	0	1	0	0	1	1

Appendix H AFLP fragments for primer combination M-CAG/E-ACT

M-CAG/E-ACT																									
Cultivar	**41**	**43**	**45**	**49**	**50**	**52**	**53**	**55**	**56**	**58**	**60**	**61**	**62**	**63**	**66**	**68**	**69**	**72**	**73**	**76**	**77**	**79**	**81**	**84**
Avs	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
Kar	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
R1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1
R2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
R3	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1	1
R4	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1
6	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
10	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	0	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1
13	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1
17	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
S1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
S2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
S3	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
S4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1
10	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
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16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
17	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1

M-CAG/E-ACT																									
Cultivar	**85**	**88**	**89**	**90**	**91**	**93**	**94**	**96**	**97**	**99**	**103**	**105**	**109**	**111**	**112**	**116**	**120**	**122**	**123**	**125**	**129**	**132**	**134**	**135**	**144**
Avs	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
Kar	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
R1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R2	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	0	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1
13	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
14	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S2	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1
S4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
6	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
7	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	0	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
20	0	0	0	0	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1

M-CAG/E-ACT																									
Cultivar	**146**	**150**	**153**	**156**	**157**	**159**	**163**	**165**	**166**	**169**	**174**	**175**	**179**	**182**	**188**	**191**	**193**	**196**	**198**	**200**	**202**	**210**	**217**	**221**	**225**
Avs	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1
Kar	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	0	1	1	1	1
R1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1
R2	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	0	1	1	1	1
R3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
R4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1
6	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
9	1	1	1	1	1	1	1	0	1	1	0	0	1	1	1	1	1	0	1	1	1	1	1	1	1
10	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	0	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	0	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	0	0	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1
14	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	0	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	0	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1
S2	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S3	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1
S4	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
10	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
12	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	0	1	1	1	1	0	0	1	1	1	1	1	1	0	1	1	0	1	1	1	1

M-CAG/E-ACT																									
Cultivar	**237**	**241**	**242**	**249**	**251**	**256**	**259**	**264**	**267**	**271**	**274**	**281**	**288**	**296**	**303**	**308**	**310**	**315**	**317**	**321**	**335**	**344**	**347**	**354**	**359**
Avs	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Kar	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
R4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
9	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	0	0	1	1	1	0	1	1	1
10	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
17	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
18	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
19	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S3	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	0	0	1	1	1	1	0	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1
14	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
20	0	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

M-CAG/E-ACT													
Cultivar	**367**	**377**	**385**	**396**	**400**	**409**	**413**	**417**	**421**	**427**	**432**	**436**	**SSR**
Avs	1	1	1	1	1	0	1	1	1	1	1	1	1
Kar	1	1	1	1	1	1	1	1	1	1	1	1	0
R1	1	1	1	1	1	1	1	1	1	1	1	1	0
R2	1	1	0	1	1	0	1	1	1	1	1	1	0
R3	1	1	1	1	1	1	1	1	1	1	1	1	1
R4	1	1	1	1	1	0	1	1	1	1	1	1	1
5	1	1	1	1	1	0	1	1	1	1	1	1	0
6	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	0	1	1	1	1	1	1	0
8	1	1	1	1	1	1	1	1	1	1	1	1	0
9	1	1	1	1	1	0	1	1	0	0	0	1	0
10	1	1	1	1	1	1	1	1	1	1	1	1	0
11	1	1	1	1	1	1	1	1	1	1	1	1	0
12	1	1	1	1	1	1	1	1	1	1	1	1	0
13	1	1	1	1	1	1	1	1	1	1	1	1	0
14	1	1	1	1	1	0	1	1	1	1	1	1	0
15	1	1	1	1	1	1	1	1	1	1	1	1	0
16	1	1	1	1	1	1	1	1	1	1	1	1	0
17	1	1	1	1	1	1	1	1	1	1	1	1	0
18	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1	1	0
S1	1	1	1	1	1	0	1	1	1	1	1	1	1
S2	1	1	1	1	1	0	1	1	1	1	1	1	0
S3	1	1	1	1	1	0	1	1	1	0	1	1	1
S4	1	1	1	1	1	0	1	1	1	1	1	1	1
5	1	1	1	1	1	0	1	1	1	1	1	1	0
6	1	1	1	1	1	0	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	0	1	1	1	0
8	1	1	1	1	1	0	1	1	1	1	1	1	0
9	1	1	1	1	1	1	1	1	1	1	1	1	0
10	1	1	1	1	1	1	1	1	1	1	1	1	0
11	1	1	1	1	1	1	1	1	1	1	1	1	0
12	1	1	1	1	1	0	1	1	1	1	1	1	0
13	1	1	1	1	1	0	1	1	1	1	1	1	0
14	1	1	1	1	1	0	1	1	1	1	1	1	0
15	1	1	1	1	1	0	1	1	1	1	1	1	0
16	1	1	1	1	1	0	1	1	1	1	1	1	0
17	1	1	1	1	1	0	1	1	1	1	1	1	0
18	1	1	1	1	1	1	1	1	1	1	1	1	0
19	1	1	1	1	1	1	1	1	1	1	1	1	0
20	1	1	0	0	1	0	1	1	0	0	1	1	0

Appendix I AFLP fragments for primer combination M-CAT/E-ACT and Xgwm148

Polymorphic fragment	Avs	Kar	RR1	RR2	RR3	RR4	RR5	RR6	RR7	RR8A	RR8B	RR9	RR10	SS1	SS2	SS3	SS4	SS5	SS6	SS7	SS8	SS9	SS10
M-CAT/E-ACT:**42**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M-CAT/E-ACT:**45**	0	1	0	1	0	1	1	0	1	0	0	1	1	1	1	1	0	1	1	1	1	0	0
M-CAT/E-ACT:**66**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M-CAT/E-ACT:**72**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M-CAT/E-ACT:**81**	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
M-CAT/E-ACT:**280**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M-CAT/E-ACC:**48**	1	0	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
M-CAT/E-ACC:**89**	1	0	1	0	1	0	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1
M-CAT/E-ACC:**102**	1	0	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1
M-CAT/E-ACC:**153**	1	0	1	0	0	0	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1
M-CAT/E-ACC:**240**	1	0	1	1	1	0	0	1	0	1	0	0	1	1	1	1	1	1	1	1	1	1	1
M-CTT/4E-ACA:**42**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M-CTT/4E-ACA:**46**	1	0	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0
M-CTT/4E-ACA:**94**	1	0	1	1	0	0	1	1	1	1	0	1	0	0	1	1	1	1	1	1	1	0	0
M-CTT/4E-ACA:**104**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M-CTT/4E-ACA:**105**	0	1	0	1	1	1	1	1	0	1	0	1	0	1	0	1	0	1	0	1	1	0	0
M-CTT/4E-ACA:**227**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M-CTT/4E-ACA:**244**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M-CTT/4E-ACA:**251**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M-CTT/4E-ACA:**304**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M-CTT/4E-ACA:**305**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M-CTT/E-AAC:**40**	1	0	1	1	0	0	1	0	1	1	0	1	0	0	1	1	0	0	1	1	1	1	1
M-CTT/E-AAC:**107**	1	0	1	1	0	0	1	0	1	0	1	1	0	0	1	1	0	0	1	0	1	1	0
M-CTT/E-AAC:**192**	1	0	1	1	0	0	0	0	0	1	0	1	1	1	1	0	0	0	1	1	1	1	0
M-CTT/E-AAC:**211**	1	0	1	1	1	1	1	0	0	1	1	0	1	1	1	0	0	1	1	1	1	0	0
M-CAG/E-ACT:**132**	1	0	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1	1	1	0	0
M-CAG/E-ACT:**134**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M-CAG/E-ACT:**196**	1	0	1	1	1	0	0	0	1	1	0	1	1	0	1	0	0	0	1	1	1	0	0
M-CAG/E-ACT:**242**	1	0	1	0	1	0	0	0	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1
M-CAG/E-ACT:**409**	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M-CAG/E-ACC:**46**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M-CAG/E-ACC:**56**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M-CAG/E-ACC:**67**	0	1	1	1	1	1	1	0	1	1	0	1	1	0	1	1	0	1	1	1	1	0	1
M-CAG/E-ACC:**69**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

M-CAG/E-ACC:**272**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M-CAG/E-ACC:**376**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M-CAG/E-ACC:**379**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M-CTG/E-ACT:**50**	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M-CTG/E-ACT:**146**	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M-CTG/E-ACT:**207**	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M-CTG/E-ACT:**241**	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M-CTG/E-ACT:**418**	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M-CTG/E-ACT:**425**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M-CTG/E-ACC:**47**	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M-CTG/E-ACC:**49**	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M-CTG/E-ACC:**51**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M-CTG/E-ACC:**59**	0	1	1	1	1	1	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0
M-CTG/E-ACC:**67**	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M-CTG/E-ACC:**92**	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M-CTG/E-ACC:**139**	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M-CTG/E-ACC:**151**	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M-CTG/E-ACC:**190**	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M-CTG/E-ACC:**269**	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M-CTG/E-ACC:**272**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M-CTG/E-ACC:**329**	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M-CTG/E-ACC:**406**	0	1	1	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Xgwm148	1	0	1	0	1	1	0	0	0	1	X	0	1	0	0	0	0	0	0	0	0	0	0	0