GENOTYPIC RESPONSE AND HERITABILITY OF *FUSARIUM OXYSPORUM* RESISTANCE IN TOMATO

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By

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Declaration

I hereby declare that this thesis, prepared for the degree Philosophiae Doctor, which was submitted by me to the University of the Orange Free State, is my own work and has not been submitted to any other university. All sources of materials and financial assistance used for this thesis have been dully acknowledged. I also agree that the University of the Orange Free State has the sole right to publication of this thesis.

Signed on the 05 of November 2003 at the Orange Free State University, Bloemfontein, South Africa.

Signature _____

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Finally, to Him who made all things possible -

THE CREATER OF HEAVEN AND EARTH.

Dedication

This thesis is dedicated to my wife,

Georgia Marina Venter

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LIST OF ABREVIATIONS

Anova	analysis of variance
ARC	Agricultural Research Council
Avrl-2	avirulence I2-gene
ARP	average resistant plant
BC	backcross
bp	base pairs
cAMP	cyclic adenosine mono phospate
cDNA	cyclic deoxyribonucleic acid
CV	coefficient of variance
df	degree of freedom
DNA	deoxyribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
Flo	Flora Dade
Fmk1	*Fusarium* MAP kinase 1
Fusarium wilt	*Fusarium oxysporum* f. sp. *lycopersici*
GCA	general combining ability
h^2_b	broad sense heritability
Hein	Heinz 1370
H_{HP}	highest parent heterose
h^2_n	narrow sense heritabiltity
HR	hypersensitive response
I_1	locus I1
I_2	locus I2
LSD	least significant deviation
MAP	mitogen activated protein
MAPK	mitogen activated protein kinase
min	minutes
µl	microliter
µg	microgram

µM	micromolar
Mon	Moneymaker
MS	mean square
NB-LRR	nucleotide binding leucine-rich repeats
ng	nanogram
Nit	nitrate-nonutilizing
NTP	nucleotide triphospate
P	parental generation
PCR	polymerase chain reaction
PDA	potato dextrose agar
PR-proteins	pathogenesis-related protein
R	resistant
RAPD	random amplified polymorphic DNA
Red K	Red Kaki
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
Rod	Rodade
Ros	Rossal
rpm	revolutions per minute
SCA	specific combining ability
SS	sum of squares
SDS	sodium dodecyl sulphate
SSR	simple sequence repeats
TAE	Tris-Acetic-EDTA buffer
TEMED	N,N,N^1,N^1-tetramethylethylenediamine
t/ha	tonnes per hectare
UV	ultraviolet
VCG	vegetative compatible group
VF	*Verticillium* and *Fusarium* resistant
WA	Watery agar

CHAPTER 1

Introduction

The cultivated tomato (*Lycopersicon esculentum* Mill) is a relatively new addition to the world's important food crops. It is one of the most popular and widely consumed vegetable crops. In 1989 a statistical consumption analysis of 115 countries estimated that 4.55 billion inhabitants, consumed 25.75 million tons of raw tomato per year (Bieche and Covis, 1989). The top four world tomato producers in 2000/01 were the United States (10.1 million tons), Italy (4.7 million tons), Spain (1.45 million tons) and Turkey (1.45 million tons) (USDA/FAS Agricultural Attaché Report, 2002). In South Africa tomato producers are averaging yields between 65 t/ha and 87 t/ha with a total annual production of 200 000 tonnes for the year 2001 (MSN Web Page, 2002). This is a small amount compared to world production, but very important for our own consumption.

Mankind is totally dependent upon agricultural production to provide them with food, but current agricultural outputs are unable to meet basic needs. Shortages lie in parts of Third World countries like Africa, where there is a need for a massive increase in food production. This necessitated a 75% increase in food yields by the end of the year 2000 (Blaxter, 1986). In Africa and especially South Africa tomatoes are mostly consumed raw or combined as a flavor enhancer with other food sources like porridge. Even in rural communities people cultivates tomatoes for own consumption. Protecting crops from losses due to pests, pathogens and weeds could, make a significant difference. Rural people usually don't have any pesticides or any other alternative solutions. Obtaining reliable figures for crop losses is difficult, but most estimates put the total loss of world-wide agriculture production between 20 to 40 % of wasted resources and plant diseases accounts for nearly 12% of these losses (Gatehouse *et al.*, 1992). For the year 1987, Gatehouse *et al.* (1992) discovered that these losses occur despite widespread use of synthetic pesticides, with an end-user value of nearly 20 billion US dollars.

One of the most severe crop-losses incurred in the tomato industry (*Lycopersicon esculentum* Mill.) is due to *Fusarium* wilt disease. *Fusarium o. f. sp. lycopersici* is a vascular wilt pathogen affecting tomatoes and has been recorded in countries like Africa, Asia, North and South America, Australia, New Zealand, Europe and Russia (Walker, 1971). It was first reported in South Africa during 1931 by Doidge and Bottomly (Gorter, 1977). This disease has been, and will continue to be, one of the most feared fungal pathogens in the world, as well as in the nine provinces of South Africa (Jones *et al.*, 1991; Uys, 1996). Visser (1982) recorded losses of up to 23% due to tomato wilt pathogens and controlling these pathogens, could increase yields drastically. Uys (1996) discovered that the average marketable yield for tomatoes was 30% lower in South Africa than the average yield for previous years. This was thought to be due to losses as a result of diseases, pests, weeds and drought in each province. A survey between 1992 and 1995 showed that wilt disease was recorded in all nine regions, and that the dominant cause was *Fusarium o. f. sp. lycopersici* race 2 (Uys, 1996).

Control of *Fusarium* wilt creates many problems, since no method is flawless. Chemical agents are available against this disease, but they are usually expensive and must be used with care not to pollute the environment or to be harmful to humans (Wager, 1981). Incorrect use of toxic chemicals can cause more damage than the actual disease itself. Certain horticultural and other biological techniques can also be used to control this disease, but success depends on training the laborers to carry out exact instructions and on the size of the area under cultivation. In general, it seems that genetic resistance to *Fusarium* wilt, is the most cost effective method and is the only real solution to control this disease.

Breeding can follow two strategies in enhancing tomato crops. Firstly by breeding to improve yield capacity; and secondly by way of defect elimination, where the breeder attempts to improve disease resistance in tomato cultivars. For the purpose of this study it was decided to improve yield production through. Breeding for resistance against a disease involves three basic steps: screening potential sources of resistance; analyzing the inheritance of the resistance in promising genotypes; and the incorporation of the resistance genes into new cultivars. Traditionally, breeders have been reluctant to work with quantitatively inherited resistance due to the fact

that it was easier to transfer single genes. Unfortunately, pathogens were always able to overcome the resistance.

The objective of this study was to investigate the genotypic response and heritability of resistance to *Fusarium* wilt race 2 in tomato. This was done by:

i) evaluating and assessing resistance of selected local South African tomato cultivars;
ii) determining variability of resistance to *Fusarium* wilt in tomato F1 hybrids;
iii) investigating the combining abilities and heritability of *Fusarium* wilt resistance in tomato;
iv) investigating the presence of single gene resistance in local tomato cultivars and their relationship to breeding values for resistance to *Fusarium* wilt.

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CHAPTER 2

General literature review: *Fusarium* wilt in tomatoes

2.1 Historical Background

Hundreds of new cultivars have been developed during the past 60 years to meet the diverse needs of changing situations and climates under which the tomato crop is grown. The recent trend has been towards development of cultivars to meet specific demands, rather than multipurpose cultivars to meet several needs. Disease resistance breeding has made an important contribution to increase tomato yields and current varieties generally now possess resistance to one or more pathogens.

Genetics Cooperative, University of California in Davis, has provided a valuable service to researchers in tomato genetics. By coordinating gene nomenclature, a total of 323 genes have been assigned to their respective chromosomes (Basset, 1986). The extensive genetic information they accumulated from many years of research has permitted the development of genetic maps, showing the relative location of genes controlling a wide variety of traits. These genetic maps have proven useful in the design and planning of breeding programs. Linkage distances can now be used to predict the probability of recombination between these linked genes.

Breeding for resistance against *Fusarium* wilt dates back as early as 1886 when Saccardo first described a *Fusarium* species that was isolated from a tomato (Walker, 1971). G.E. Massee in England first described *Fusarium* wilt in 1895 (Jones *et al.*, 1991). Fusarium crown and root rot diseases, caused by *Fusarium o.* f. sp. *lycopersici* have been found to affect tomato production in both greenhouses as well as in open fields. *Fusarium* wilt outbreaks have been reported in 32 countries (Walker, 1971). *Fusarium* wilt destruction still occurs in South Africa (Uys, 1996).

Fusarium wilt used to be the most common and destructive disease in tomato cultivars in the United States of America before the development of new resistant cultivars. The largest losses have occurred in the 1940's in the United States, in the area east of the Mississippi River and south of the Ohio River (Jones *et al.*, 1991).

Single genes control resistance to many of the common tomato diseases. Dominant resistance has facilitated the development of F1 hybrids with resistance to as many as eight different pathogens. Race 1 has been described as nonpathogenic to tomato plants possessing the *Lycopersicon pimpinellifolium* race 2 resistance factors (Gerdemann and Finley, 1951) and has been transferred by recurrent backcrossing to adapted cultivars of *Lycopersicon esculentum*. Bohn and Tucker (1940) first pioneered work that later identified the dominant allele I_1, which controls resistance to *Fusarium* wilt in *Lycopersicon pimpinellifolium*. It was named Pan America to reflect the North and South American parentage in its pedigree (Basset, 1986). The gene has since been exploited successfully in tomato plants previously affected by *Fusarium* wilt (Alexander and Tucker, 1945).

In 1950, a new race of the pathogen appeared in Florida Gerdemann and Finley (1951) isolated this new strain of *Fusarium*, and designated it as race 2 in order to distinguish it from the common strain referred to as race 1. Alexander and Hoover (1955) discovered resistance to *Fusarium* race 2, in a natural hybrid between *Lycopersicon esculentum* and *Lycopersicon pimpinellifolium*. Resistance to this new race (designated race 2) was soon introduced in a tomato cultivar, Walter, that possessed resistance to both races 1 and 2 of *Fusarium o.* f. sp. *lycopersici.* This cultivar was released in 1969.

Fusarium o. f. sp. *lycopersici* race 3 was first reported in Australia in 1978, then in Florida in 1982, and finally in California in 1987 (Elias and Schneider, 1991). Tolerance has been identified in *Lycopersicon pimpinellifolium* as well as in two other *Lycopersicon esculentum* breeding lines (Basset, 1986). *Fusarium* wilt in tomato, *Fusarium o.* f. sp. *lycopersici* (Sacc.), has now overcome monogenic resistance to race 1 and 2, and 3 (Bournival and Vallejous, 1991).

New hybrid varieties are released each year, because new *Fusarium* vegetative compatible groups, which are susceptible, are still being discovered. Plant breeding for resistance is usually only temporarily. The pathogen is able to overcome the resistance of the host plant in a few years. Today, more than ever, there exists a need for resistant hybrid varieties.

2.2 Classification of tomato *Fusarium oxysporum* species found in South Africa.

Many fungi are known to have septate hyphae and to reproduce by means of conidia. These fungi which apparently lack a sexual phase are known as "imperfect fungi" or "Fungi Imperfecti". They comprise the form class Deuteromycetes of the subdivision Deuteromycotina. Deuteromycetes is divided into different form-orders of which the Moniliales are of particular importance in South Africa. The genus *Fusarium* is the largest in the form-family Tuberculariaceae, as well as the most difficult of the fungal groups to identify. The form genus, *Fusarium,* usually produces two types of conidia that are termed macroconidia and microconidia, which are produced from phialides (Alexopoulos and Mims, 1979).

Van Wyk *et al.* (1986) developed a rose-bengal-glycerine-urea medium to be used exclusively as a selective medium for the isolation of *Fusarium* species from soil or plant debris. Once the *Fusarium* has been isolated, it can be identified according to different morphological characteristics as well as other biochemical and genetic identification criteria (Booth, 1970; Nelson *et al.*, 1983). Rapid growth also occurs on potato dextrose agar (PDA). Aerial mycelia produced on PDA agar are white or light purple. The sclerotia are mostly blue. Colony colors vary from white, to blue-green and pink (Booth, 1970; Nelson *et al.*, 1983). *Fusarium oxysporum* usually produces boat-shaped chlamydospores when first incubated on a potato dextrose agar media. Sterilized distilled water is added later and is left for up to seven days to ensure spore formation (Nelson *et al.*, 1983). According to Nelson *et al.* (1983) and Booth (1970), *Fusarium o.* f. sp. *lycopersici* produces single-celled thin walled, oval to kidney-shaped microconidia (5-12 x 2.2 - 3.5 µm) which are produced on false heads. Sickle-shaped 3-5 septate macroconidia (27 - 46 x 3 - 5 µm) with an

attenuated apical cell and footshaped basal cell are produced under a 12-hour near-ultraviolet white/dark light cycle (Nelson *et al.*, 1983).

Different species of *Fusarium* wilt differ markedly with respect to their phytopathological aspects. The two physiological races of *Fusarium o.* f. sp. *lycopersici,* races 1 and 2, are commonly found all over South Africa. Race 3 of *Fusarium o.* f. sp. *lycopersici,* is found in Northern America and Australia, but has not yet been discovered in South Africa (Grattidge and O'Brien, 1982).

These three races of *Fusarium o.* f. sp. *lycopersici* are the most commonly known *Fusarium* wilt disease pathogens associated with tomato wilt disease. *Fusarium o.* f. sp. *radicus-lycopersici* Javis and Shoemaker, the cause of *Fusarium* crown and root rot, has been reported in Northern America and Japan (Sherf and Macnab, 1986), but has not yet been discovered in South Africa (Uys, 1996). Another *Fusarium* species, *F. equiseti* (Corda) Sacc. was first reported to cause wilt on tomato in Israel (Jöffe and Palti, 1965) and was later associated with wilted tomato plants in South Africa by Visser (1980). The occurrence of other *Fusarium* species likes *F. solani* (Mart.) Appel and Wollenw, *F. compactum* Wollenw. Sensu Gorden, *F. nygami* Burgess & Trimboli and *F. semitectum* (Berk) de Rav. have occasionally been isolated from wilted tomato plants by Uys (1996), but have not yet been found to cause any wilt symptoms.

2.3. Life cycle of *Fusarium oxysporum*

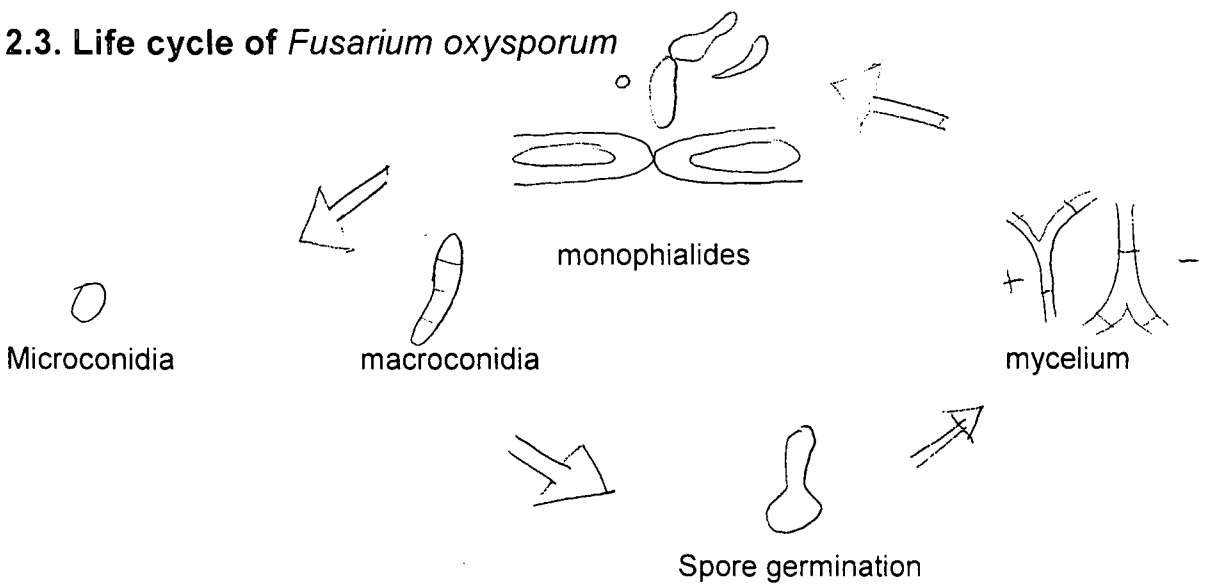

Figure 2.1 Asexual reproductive cycle of *Fusarium oxysporum.*

The Fungi Imperfecti belong to a group of fungi that reproduce only asexually and have a parasexual life cycle (Figure 2.1). A parasexual cycle is defined as a cycle in which plasmogamy, karyogamy and haploidization takes place, but not at a specified time or at any specific point in the life cycle of the organism.

A complete parasexual cycle entails the following sequence of events:

* formation of heterokaryotic mycelia;
* fusion between similar and different nuclei;
* multiplication of diploid and haploid nuclei;
* occasional mitotic cross-over during multiplication of diploid nuclei;
* sorting of diploid nuclei;
* occasional haploidization of diploid nuclei;
* and sorting of new haploid strains (Alexopoulos and Mims, 1979).

Heterokaryosis refers to a condition in which genetically different nuclei are associated in the same protoplast (Alexopoulos and Mims, 1979). In these fungi, the cell body is mostly multinucleate when growth is active. Hyphal fusion, in which nuclei are exchanged between the different mycelia, is a regular occurrence (Allard, 1960). Heterocaryosis improves both the mitotic part of the life cycle and supplements or replaces the meiotic part of the life cycle. It enables the fungus to dispense with meiosis and fertilization, and may well be the reason why so many fungi became imperfect (Allard, 1960). Certain strains carry diploid rather than haploid nuclei in their cells. Haploidization of the diploid nuclei is thought to occur as a result of the failure of regular distribution of the chromosomes during the process of mitosis (Alexopoulos and Mims).

The frequency at which haploidization occurs is usually much higher than in diploid nuclei and it is clear that in this process, the unit of segregation is the chromosomes (Allard, 1960). In the second process of mitotic cross over, the gene is the unit of recombination. The combination of these two processes is usually equal to the sexual cycle, since together they involve diploidization (fertilization), recombination and haploidization (reduction division) (Allard, 1960). The only significant difference is the absence of a precise time sequence in the parasexual cycle. The parasexual

cycle can therefore be regarded as a process of potential importance in the origin of new pathogenic races.

According to Allard (1960), it appears as if a heterocaryon containing different types of nuclei behaves similar to a genetic heterozygote, and the system appears to be capable of providing for a type of " somatic segregation" based on the exchange of the entire nuclei during the hyphal fusion. Thus the genetic composition within a heterocaryotic mycelia could be altered by natural selection. The mating system could also be regarded as a system that promotes outbreeding. Recombination provides genetic variability for the plasticity that is necessary for a response to environmental changes, as well as changes in the frequency of the genes that governs the resistance of the host species (Allard, 1960).

2.4 Symptomatology and disease assessment

The earliest symptoms of *Fusarium* wilt are the yellowing of older leaves, often only on one side of the plant. Leaf yellowing starts at the base of the plant. Most of the foliage is gradually affected, accompanied by the wilting of the plant during the hottest part of the day (Jones *et al.*, 1991). Wilted leaves turn brown and dry, but don't fall off. The wilting then becomes more extensive every day until the plant collapses and dies. Browning of the vascular system is characteristic of wilt disease, and can be used for identification purposes (Jones *et al.*, 1991).

In tomato, symptoms have occurred which are only visual after vascular infections of twigs, petioles and leaves (Gao *et al.*, 1994). Scheffer and Walker (1953) discovered that the infection of the central petiolar bundle of tomato is essential for foliar symptom expression. Infection of one lateral bundle, in addition to the central bundle, produces unilateral symptoms. Infection of both lateral bundles, in addition to the central bundle, produces symptoms in the entire leaf (Gao *et al.*, 1995).

Fusarium wilt disease is mostly identified (Stall and Walter, 1965; Bournival and Vallejous, 1991; Kroon and Elgersma, 1993; Assigbetse *et al.*, 1994) using external symptoms, and its severity is noted according to a disease index from 0 to 5. Usually the disease index stretches from 0 to 5; 0: healthy, 1: epinasty of some leaves, 2:

wilting of some leaves, 3: yellowing and necrosis of some leaves, wilting of all leaves, 4: yellowing and necrosis of most leaves, some leaves fallen, 5: plants dead (Kroon and Elgersma, 1993). Relative rates of tissue colonization can also be determined by plating 2 mm long, surface-sterilized tissue segments on a selective medium, and calculating the percentage of *Fusarium* colonization (Alon *et al.*, 1974).

The defense or susceptibility of tomato varieties to *Fusarium* species is determined by evaluating fungal colonization of the stems and petioles using the modified microslide method of Gao *et al.* (1994). Young shoots are cut and immediately inoculated through the severed ends by the uptake of a suspension of conidia and tracer particles. Cross-sections of each plant are stained with a 75% glycerin solution for examined under a 400x microscopic amplification. Spreading of the fungus from vessel to vessel in the xylem is recorded (Gao *et al.*, 1994). Current research focuses on finding a fast, cheap and easy method for early identification of the disease.

2.5 Vegetative compatibility of *Fusarium oxysporum* species

New races can develop through parasexual recombination within or between existing races of *Fusarium o.* f. sp. *lycopersici*, other former species, nonpathogenic populations of *Fusarium oxysporum*, or any combination of these (Elias and Schneider, 1991). Vegetative compatibility and heterokaryosis are prerequisites for parasexual recombination (Elias and Schneider, 1991). Spontaneous random mutations may also lead to race development.

Fungal plant pathogens have evolved strategies to recognize suitable hosts, to penetrate and invade plant tissue, to overcome host defense, and optimize growth in the plant. To perform these tasks effectively, the fungus must perceive chemical and physical signals from the host and respond with the appropriate metabolic and morpho-genetic changes required for pathogenic development (Pietro *et al.*, 2001). Such changes include direct hyphal growth, adhesion to the plant surface, differentiation of specialized infection structures and secretion of lytic enzymes and phytotoxins (Knogge, 1996). Many of these responses require the synthesis of specific gene products and depend on conserved signal transduction pathways

involving the activation of G proteins (Bölker, 1998) as well as CAMP signaling (Lee and Dean, 1993; Mitchell and Dean, 1995) and mitogen-activated protein kinase (MAPK) cascades (Xu and Hamer, 1996; Xu *et al.*, 1998).

Puhalla (1985) modified a procedure to test for vegetative compatibility in *Fusarium oxysporum* using nitrate-nonutilizing (nit) mutants selected from rapidly growing chlorate-resistant sectors on a chlorate medium (Elmer and Stephens, 1989). Puhalla used this method of forced heterokaryons to place 21 strains of *Fusarium oxysporum* into 16 vegetative compatibility groups (VCG's) (Elmer and Stephens, 1989). Puhalla (1985) proposed that when the sexual stage and meiotic recombination of *Fusarium oxysporum* are lost, the loci that determine vegetative compatibility and pathogenicity become fixed in the same thallus. In this way, distinct VCG's with specific virulence genes due to genetic isolation develop as asexual inbreeding populations. The validity of Puhalla's evolutionary model has been tested in several studies (Correll *et al.*, 1986; Bosland and Williams, 1987; Jacobson and Gordon, 1988; Katan and Katan, 1988). The majority of these studies provide support for this model. A strong correlation between VCG's and pathotype has been found by Elias and Schneider (1991), Ploetz and Correll (1988) and Elmer and Stephens (1989).

Various authors noted diversity occurring within a population strain. Gordon and Okamoto (1991) classified nonpathogenic isolates of *Fusarium oxysporum* from soil into 39 vegetative compatible groups (Gordon and Okamoto, 1991; Gordon and Okamoto, 1992). Elias and Schneider (1991) identified one major, two minor and a large number of single-member VCG's from 115 isolates of *Fusarium o.* f. sp. *lycopersici.* Correlation between VCG's and races, geographical origin or colony morphology was not found suggesting that: a) the development of races occurred before the formation of VCG's; or b) subsequent to the development of VCG's, the races evolved independently in each of the VCG's (Elias and Schneider, 1991). Uys (1996) categorized *Fusarium o.* f. sp. *lycopersici,* found in South Africa, into four distinct VCG's and 10 single incompatible isolates.

These vegetative compatible and non-compatible groups of *Fusarium* can be the reason why plant breeders of tomato varieties could not obtain total resistance

against *Fusarium* wilt disease through resistance breeding programs. Resistance is dependant on the recognition and the interaction between a specific pathogen and a specific antigen or some isolate of the pathogen, but could be completely susceptible to other isolates (Gatehouse *et al.*, 1992).

2.6 Biosystematics of tomato

The commercial tomato belongs to a species most frequently referred to as *Lycopersicon esculentum* Mill. *Lycopersicon* is a relatively small genus within the extremely large and diverse family of Solanaceae. This family consists of 90 different genera divided into two sub-families, the Solanoideae and the Cestroideae. The sub-family Solanoideae is further subdivided into tribes. *Lycopersicon* belongs to the largest tribe, the Solaneae (Atherton and Rudich, 1986). A more meaningful subgenera classification by Rick (1976) divides the genus *Lycopersicon* into six species that are crossed relatively easily with cultivated tomatoes of the *Lycopersicon esculentum* complex and two species that hybridize only with great difficulty with the *Lycopersicon peruvianum* (L.) Mill. complex (Jones *et al.*, 1991).

2.6.1 Species forming the "*Lycopersicon esculentum*-complex"

Species in this group have served as a particularly valuable source of pest resistance for the improvement of cultivated tomatoes (Jones *et al.*, 1991).

2.6.1.1 *Lycopersicon esculentum* Mill.

This species has become widely distributed round the world due to its value as a crop. Modern tomato varieties are closely related to the wild species *Lycopersicon esculentum var. cerasiforme,* and the two groups are intercrossed (Atherton and Rudich, 1986). Most representatives of *Lycopersicon esculentum* are self-compatible and obligate interbreeders (Basset, 1986).

2.6.1.2 *Lycopersicon pimpinellifolium* (Jusl.) Mill.

All populations of *Lycopersicon pimpinellifolium* are self-compatible, although some populations are uniform morphologically and totally autogamous, while others might show varying degrees of outbreeding (Atherton and Rudich, 1986). The colored fruit resembles that of *Lycopersicon esculentum* but is substantially smaller. *Lycopersicon pimpinellifolium* can be hybridized with *Lycopersicon esculentum*. It is closely related to modern tomatoes (Atherton and Rudich, 1986). *Lycopersicon pimpinellifolium* provides an attractive source of germplasm for plant breeders, and has also been used as a source of resistance against *Fusarium* wilt (Bohn and Tucker, 1940).

2.6.1.3 *Lycopersicon cheesmanii* Riley

This taxon is unique amongst the *Lycopersicon* species. It is found only on the Galapagos Islands, where it has evolved separately due to its extreme geographical isolation from the mainland species (Jones *et al.*, 1991). All forms of *Lycopersicon cheesmanii* are self-compatible and are exclusively interbreeding. In some biotypes, less pigment (β-carotene) is produced, leading to the formation of yellow or yellow-green ripe fruit. There is little doubt that *Lycopersicon cheesmanii* is closely related to *Lycopersicon pimpinellifolium* and *Lycopersicon esculentum* (Atherton and Rudich, 1986). Literature on *Lycopersicon cheesmanii* has not yet shown that there is any useful source of disease resistance genes for plant breeding purposes.

2.6.1.4 *Lycopersicon parviflorum* and *Lycopersicon chmielewskii*

These two closely related species have formerly been known as *Lycopersicon minutum* (Rick, 1976). *Lycopersicon parviflorum* is characterized by small flowers and relatively small simple leaves carried on slender stems. *Lycopersicon chmielewskii* has a more robust plant form, larger fruit and flowers as well as an improved capacity for outbreeding (Atherton and Rudich, 1986). *Lycopersicon chmielewskii* differs from any member of the peruvianum complex in its ability to hybridize with other cultivated tomatoes. Interest has been shown in the high sugar

content of the ripe fruit of both *Lycopersicon parviflorum* and *Lycopersicon chmielewskii* (Atherton and Rudich, 1986).

2.6.1.5 *Lycopersicon hirsutum* Humb. and *Bonpl. f. typicum* and *f. glabratum* Muller

This distinctively green-fruited species is usually found at high elevations. Its typical form is characterized by densely hairy stems, leaves and fruits as well as by large showy flowers which have a much less deeply divided corolla than that found in *Lycopersicon esculentum* and its close relatives (Atherton and Rudich, 1986). Two forms of the species have been recognized. *Lycopersicon hirsutum f. typicum* is an outbreeder with a strong exerted stigma. The majority of this group is self-incompatible, while the alternate form, *Lycopersicon hirsutum f. glabratum* Muller, has been separated from the type-species on the grounds of its less hairy leaves and stems, as well as by the smaller corolla (Jones *et al.*, 1991). It has been noted that wild populations of *Lycopersicon hirsutum* in their natural habitat are remarkably free from insect predators (Rick, 1973). *Lycopersicon hirsutum* appears to be a valuable source of germplasm to enable plant breeders to increase insect tolerance in commercial tomato varieties (Atherton and Rudich, 1986).

2.6.2 The species forming the "*Lycopersicon peruvianum*-complex"

These green-fruited, largely self-incompatible relatives of the cultivated tomato possess a wealth of unique characteristics and pest resistance of potential value to cultivated plants (Jones *et al.*, 1991).

2.6.2.1 *Lycopersicon chilense* Dunal.

Brittle stem and leaves have been found to be associated with *Lycopersicon chilense*. The leaves frequently have as many as 11 or more major leaflets, a character seldom found to the same degree in *Lycopersicon peruvianum*. The long flower truss can be regarded as virtually diagnostic of *Lycopersicon chilense* (Atherton and Rudich, 1986). Severe barriers to intercrossing separate *Lycopersicon*

chilense from the cultivated tomatoes. Alexander and Hoover (1953) found resistance to TMV in 27 lines of *Lycopersicon peruvianum/chilense*.

2.6.2.2 *Lycopersicon peruvianum* (L.) Mill.

The *Lycopersicon peruvianum* representatives have thin wiry stems with short internodes. The leaves are reduced in size and complexity and the inflorescence is unbranched rather than bifurcated as in the remainder of the *peruvianum*-complex, *Lycopersicon peruvianum var. humifusum* C. M. Mull (Atherton and Rudich, 1986). This group has been separated from the typical forms of the species on the basis of its short, dense, non-glandular hairs, its thin procumbent systems, and small-simplified leaves. The leaves typically consist of only two pairs of major lateral leaflets, one terminal leaflet and an almost total absence of minor leaflets. The flower trusses are also simplified, being unbranched, with relatively small bracts (Atherton and Rudich, 1986).

2.7 Mechanism of response and recognition

Resistance to *Fusarium* is thought to involve a specific recognition between a resistant cultivar and a specific pathogen. This interaction then activates a set of responses in an attempt to confine the pathogen. The specificity of this process is often determined by the product of a plant resistance (R) gene and a cognate pathogen avirulence gene (Flor, 1971). According to the gene-for-gene hypothesis, the dominant I-2 gene in tomato would respond to a dominant avirulence gene (Avrl-2), present in race 2 of *Fusarium o.* f. sp. *lycopersici* (Mes *et al.*, 1999a). Thus characterization of a plant's resistance genes is an important step in understanding the initiation of events that lead to the plant defense response mechanisms (Ori *et al.*, 1997).

Resistance responses are usually classified into two groups, namely horizontal and vertical resistance. If an individual displays horizontal resistance to a particular fungal pathogen, it also does so to all other isolates of that species. An individual that displays a vertical resistance will exhibit a stronger resistance to some isolates

of that pathogen (gene) and will be completely susceptible to other isolates (Gatehouse *et al.*, 1992). Hence, vertical resistance is said to be race-specific. Resistance to a particular isolate has been found to be conferred by a single gene called "major genes" or "R genes". The isolates of a particular pathogen are classified into different races on the basis of their interactions with other individuals of host species containing different R genes. The presence of the virulent gene in the pathogen somehow allows the product of the plant R gene to recognize that race, and for a resistance response to occur.

Recent attempts to clone several R genes revealed that despite their origin, these R genes also designated as the nucleotide binding, leucine-rich repeats (NB-LRR) group, usually shared several features (Staskawicz *et al.*, 1995; Boyes *et al.*, 1996). These R genes are all involved in the resistance processes that are characterized by a hypersensitive response (HR). Structurally, a nucleotide binding domain (P loop) and an additional motif of unknown function are conserved near their N-terminal regions as well as on a region of their LRR's of variable length at their C terminus (Ori *et al.*, 1997). The activation of these genes produces physical and biochemical changes in the plant hosts, which allow them to become more resistant to microbial attack. The physical changes taking place include: the accumulation of cell wall hydroxyproline-rich glycoproteins (Esquerre-Tugaye *et al.*, 1979), lignification and suberization (Vance *et al.*, 1980; Espelie *et al.*, 1986), callose deposition (Ride, 1983; Bonhoff *et al.*, 1987), and the accumulation of phenolic compounds (Matta *et al.*, 1969; Hunter, 1974). Among the major biochemical changes taking place are the biosynthesis and accumulation of phytoalexins, as well as secondary metabolites that are toxic to bacteria and fungi (Hahlbrock and Grisebach, 1979; Davill and Albersheim, 1984; Dixon *et al.*, 1983). Other changes include the accumulation of protease inhibitors (Ryan, 1973; Peng and Black, 1976) and the release of oligosaccharide elicitors of plant origin (Bevan *et al.*, 1994).

Plants accumulate a protein termed pathogenesis-related protein (PR-protein) during pathogen attack (Van Loon, 1985). The exact role of PR-proteins is not known, but according to Bevan *et al.* (1994) their presence correlates somehow with disease resistance. The PR-proteins include several hydrolytic enzymes, like chitinase and

β-1,3 glucanase (Bevan *et al.*, 1994). It is now clear that after recognition, a set of genes (termed response genes) are activated. These gene products form the basis of the plant's disease resistance response. Different studies are sometimes contradictory, especially concerning the accumulation of phytoalexins as a possible mechanism in resistance response in monogenetically resistant tomato cultivars (Elgersma and Liem, 1989). Bergey *et al.* (1999) discovered an increase in polygalacturonase levels in extracts from wounded and unwounded tomato leaves. Authors like Sutherland and Pegg (1992) attribute resistance in monogenetically resistant tomato cultivars to the production of phytoalexins that they believe inhibit the growth and spread of certain pathogens. Changes in the plants structure may also retard or prevent the spread of the pathogen in the plant. This is due to a hypersensitive reaction (Elgersma *et al.*, 1972; Alon *et al.*, 1974; Tjamos and Smith, 1974; MacCance and Drysdale, 1975; Hutson and Smith, 1980).

One strategy scientists have explored is to try and engineer durable resistance against fungi in plants that involved the expression of genes encoding proteins able to inhibit fungal growth in vitro. Together with the appearance of resistance the synthesis of a large number of proteins was induced (Bevan *et al.*, 1994). These include chitinase and β-1,3-glucanases, hydrolysis of the sugar polymer chitin and β-1,3-glucan respectively. These polymers are major cell wall components of many fungi (Wessels and Sietsma, 1981). Recent experiments have demonstrated the in vitro antifungal activity on *Fusarium solani* of class I chitinase and β-1,3-glucanases purified from tobacco (Sela-Buurlage *et al.*,1993). These two hydrolases act synergistically and have been found to be very effective inhibitors when applied in combination with each other.

In tomato, the I_2 locus on chromosome 11 has been found to confer resistance against *Fusarium o.* f. sp. *lycopersici* race 2 (Sarfatti *et al.*, 1989). Elgersma and Liem (1989) found that a *Fusarium* wilt resistant cultivar containing the I_1 locus on chromosome 7, contains more of the phytoalexin rishtin than susceptible plants after being inoculated with *Fusarium o.* f. sp. *lycopersici race 1.* Phytoalexin apparently localizes and seals the place of infection by stimulating various mechanisms such as gummosis, tyloses, callose deposition, lignification and suberization of cell walls, as

well as the synthesis of the cell wall degrading enzymes, chitinase and β-1-3-glucanase. β-1-3-glucanase is capable of attacking carbohydrates in fungal cell walls. A series of enzyme catalyzing reactions in the phenylpropanoid pathway have been shown to increase in activity during plant defense responses (Gatehouse *et al.*, 1992). The appearance of chitinase activity probably reflects a capacity for disintegrating fungal cell walls. Endochitinase also shows lysozyme activity that might act against invading microorganisms.

Conway and MacHardy (1978) investigated localized infections of *Fusarium o.* f. sp. *lycopersici* in the root and hypocotyl region. They found that an antifungal compound, α-tomatine, contributes to resistance against *Fusarium* wilt. Some authors (Kroon and Elgersma, 1993; Elgersma and Liem, 1989) demonstrated convincingly that rishitin and the polyacetylenes falcarinol and falcarindiol could not account for the resistance of tomato against *Fusarium o.* f. sp. *lycopersici.* The exact role that the phytoalexins play in retarding the development of tomato wilt is, therefore, still uncertain. According to Sutherland and Pegg (1992), the physical responses to infection appear too complex to be the result of the action of a single R gene.

2.8 Disease control

2.8.1 Management control

Root rot disease results from the use of susceptible cultivars, the lack of crop rotation, poor tillage as well as from cultural practices, soil compaction and lastly the lack of inorganic matter in the soil. Poor soil and cultural environments that favor root rot disease develop over a long period of time. Time and effort are thus required to reverse these conditions and to control root rot diseases.

Wilt pathogens are transmitted in various ways. They are usually soil- or seed-borne diseases, disseminated over long distances, usually by infected seed and/or transplants (Menzies and Jarvis, 1994). It is thus essential to use disease free seed or seedlings. Like most other pathogens, they are also spread locally by contaminated stakes, wire, farm machinery, and even by visiting hawkers (Jones *et*

al., 1991). Thus pathogens can also be disseminated by wind and water (Subramanian, 1970).

2.8.2 Biological control

Many tomato growers use biological control as the primary pest management control method. Others try to integrate pesticides, with few or no harmful effects to the tomato plant, into their breeding program. They may also apply pesticides to localized areas where pest infestations are higher than desired. Some tomato growers try to use biological control for part of the year, only changing to pesticides if pests become to numerous.

Soil is a complex environment that consists of numerous plant, animal and microbial populations, which interact continuously under fluctuating environmental conditions. Some of these organisms form the basis for biological control due to hyperparasitism and the production of toxic metabolites. Competition for nutrients and the available space in the rhizosphere as well as on the rhizoplane also contributes to the process of biological control.

Cultural practices such as altering soil pH, reducing compaction, increasing plant residues and the use of saprophytic microbial growth of antagonists which are promoted (*Bacillus subtilus, fluorescent Pseudomonas, Trichoderma, Gliocladium, Fusarium* and other avirulent organisms) can reduce *Fusarium* wilt disease (Haung, 1992; Tu, 1992). Abdul Wahid *et al.* (2001) discovered that the most effective fungi used for biological control were *Trichoderma pseudokoningii, Paecilomyces variotii, Chaetomium globosum-Emericilla nidulans* and *C. globosum-T. pseudokoningii.*

Non-pathogenic *Fusarium oxysporum* isolates have also been found to suppress *Fusarium. o.* f. sp. *dianthi*, the causal agents of carnation wilt (Postma and Rattink, 1992). Similar results have been found using *Fusarium o.* f. sp. *radicus-lycopersici* in a dual culture together with *F. o. f. sp. lycopersici* (Louter and Edgington, 1990). Peer *et al.* (1990) found that the presence of *Pseudomonas* spp. also reduces

Fusarium wilt of carnation, and Khalil-Gardezi *et al.* (1998) showed that the combination of mycorrhizas and organic matter contributes to the control of *Fusarium. o.* f. sp. *lycopersici* race 3 on tomatoes. Chitosan, a β-1,4-D glucosamine polymer derived from crab-shell chitin, is another biological control method found to increase plant resistance. Benhamou and Theriault (1992) showed that chitosan reduces the number of root lesions caused by *Fusarium o. f. sp. radicis-lycopersici* after foliar application or dipping the roots in this compound. It is also speculated that the enzyme chitinase could be involved in the breaking of fungal walls and in inducing the production of phytoalexins in their hosts. Borges *et al.* (2000) showed that chitosan, as a seed coating agent, was effective in reducing *Fusarium* wilt disease occurrence in emerging roots from tomato seeds.

2.8.3 Chemical control

Various chemicals are available for ridding the soil of *Fusarium* wilt. They include methyl bromide (bromogas, shellfume, curafume, dowfume), Basamid (dazomet), Jeyes Fluid (carbolic acid) and Metham sodium (Wager, 1981; Nel *et al.*, 1993). Methyl bromide and metham sodium are registered as soil fumigants (Nel *et al.*, 1993). Metham sodium fumigation retards the disease development of *Fusarium* wilt against watermelon and triples fruit yield (Gonzalez-Tores *et al.*, 1993). These methods of control are not often used as they are expensive and difficult to apply. The fungus thrives and re-establishes itself quickly in sterilized or fumigated soil due to the absence of any other antagonists. Minuto *et al.* (2000) found in Italy that three weeks of soil solarization plus a half dosage of dazomet were very effective agents at high disease pressure. Chandrasehar *et al.* (2001) showed that calcium at all concentrations tested inhibited toxin production of the pathogen.

Certain herbicides decrease the severity of *Fusarium* wilt in solanaceous crops. The herbicides di-notroaniline, nitralin and trifluralin produce fungitoxic compounds that inhibit the growth and spread of the pathogens. These compounds also activate a defense mechanism in the host responsible for the production of phytoalexins (Grinstein *et al.*, 1976; Grindstein *et al.*, 1984). Another herbicide, diphenamid, inhibits the activity of *Fusarium o.* f. sp. *lycopersici in vitro*. Diphenamid, at high

concentrations, inhibits in vitro spore germination, growth and sporulation of *Fusarium o.* f. sp. *lycopersici.*

2.8.4 Solarization control

Solarization is another method for controlling soil pathogens and consists of a procedure whereby the temperature in the topsoil layers (where the pathogen occurs) is increased. This is usually accomplished by laying a plastic sheeting on top of wet soil. Gonzalez-Tores *et al.* (1993) as well as Martyn and Hartz (1986) showed that a two-month solarization treatment is more effective than fumigation and concluded that solarization treatment of shorter periods would be less effective than fumigation. In soil mulching with polyethylene film, the temperature of solarized soil reached 52°C and 48° C at depths of 10 and 15 cm, respectively, and resulted in 96.3% healthy plants in Egypt (Abdul Wahid *et al.*, 2001).

Solarization would be of value in the warmer parts of South Africa, namely in northern Kwazulu/Natal and the Northern Province, where the summer temperatures are high during the mid summer months for tomato cultivation. Tomato plants grown in solarized soil showed an increase in foliage and root weight, plant height as well as total fruit yield (70%) compared to those grown in non-solarized soil (Wadi, 1999). The only problem with this method is that the soil might be re-contaminated by *Fusarium* wilt.

2.8.5.1 Disease resistance

Prior to breeding for resistance against any disease there is a need to distinguish between qualitative and quantitative resistance. Qualitative genes, for example are those genes within the I_2- loci, which can be identified using molecular markers. To improve the quantitative resistance of tomato the following parameters are important; the variation between available parental lines; the combining ability of the parental lines; and also the heritability of the character involved that needs to be improved.

2.8.5.1.1 The use of molecular markers to identify qualitative resistant genes against *Fusarium o.* f. sp. *lycopersici* in tomato.

Some of the advantages of natural populations are their phenotypic diversity and thus genetic variation that exists as well as the many different phenotypic traits found in most natural populations (Hartl, 2000). The extent of genetic variation within populations or species can be measured directly as the proportion of gene loci that is polymorphic, i.e. that possess more than one allele in the population in frequencies that are not merely the consequence of a mutation (Parkin, 1993). A locus can thus be regarded as being polymorphic if its less common alleles exceed a frequency of 1% (Parkin, 1993). It is thus possible to screen a series of loci in a series of populations, and compare the overall variability of one population with that of another at many loci simultaneously.

Any breeding program designed to produce resistant varieties must start with cultivars that contain resistant-conferring genes. Resistance to *Fusarium o.* f. sp. *lycopersici* race 1 is governed by the gene I1 (Bohn and Tucker, 1939; Bournival *et al.*, 1990; Sarfatti *et al.*, 1991), which originate from accession 160 of *L. pimpinellifolium* and LA716 of *L. pennellii*, respectively (Mes *et al.*, 1999b). The cultivar Pan America and its offspring served as a primary source of resistance against *Fusarium o.* f. sp. *lycopersici* race 1 until 1960. Later the I2 locus was introduced, also from *L. pimpinellifolium,* which confers resistance to race 2 of the pathogen (Stall and Walter, 1965; Cirulli and Alexander, 1966,). Walter served as the source of disease resistance to *Fusarium o.* f. sp. *lycopersici* race 2 (Basset, 1986). In most cases, identifying a good source of resistance does not pose a serious problem due to the fact that many different varieties or strains carrying resistance genes are known and are available.

DNA polymorphisms provide valuable information regarding the degree of variation within and between populations, races and species. In the early years, progress with the analysis of genetic variation between species and cultivars was slow due to the lack of genetic markers, and the only data available was that of backcrossing (BC) and F2 segregation. For use in plant breeding, DNA-based markers need to satisfy several criteria: firstly they have to behave according to Mendel's laws; secondly the

number of individuals must be distinguishable from one another (marker or combination of markers); thirdly an abundance of markers is needed for commercial use (Walton, 1994).

During the late 1960's, the first molecular markers used were allozymes, protein variants detected by differences in migration on starch gel in an electrical field (Lynch and Walsh, 1998). The principle stems from the one-gene-one-enzyme hypothesis also explained as one gene for every one polypeptide chain (Atherton and Rudich, 1986). Under this hypothesis, a gene codes for a polypeptide, or an enzyme that catalyses a certain step in a specific biochemical pathway.

Isozymes are protein-based molecular markers used for purity testing in the seed industry, but since only a few polymorphic isozymes are available, they have limited use (Walton, 1994). Allozymic variants have the advantage of being relatively inexpensive for scoring large numbers of individuals, but often have insufficient protein variation necessary for high-resolution mapping (Lynch and Walsh, 1998).

Today a wide variety of techniques are used to measure DNA variation. One approach is to digest DNA with a number of restriction enzymes. Each enzyme cuts the DNA at a specific sequence and when the digested DNA is run on a gel using an electric current, the DNA fragments separate according to size. Individual bands can be isolated using labeled DNA probes with a base-pair sequence complementarily to particular DNA regions within the genome. This approach forms the basis for assaying restriction fragment length polymorphism (RFLP's). Each RFLP probe is used to score a single-marker locus. Some DNA molecules in the population contain a specific restriction site, whereas others lack this (Hartl, 2000). RFLP's are used to identify polymorphism in DNA sequence variations in individual chromosomes, but the available polymorphic RFLP markers are usually quite limited (Hartl, 2000).

Miller and Tanksley (1990) found that RFLP's are not of much use in tomato cultivars, usually homozygous. Few differences between individual plants can be found using RFLP's. This is also reflected in the observation made by Rus-Kortekaas *et al.* (1994) that the isozyme pattern and RFLPs reveal very little polymorphism.

Thus, resistance loci represent residual regions of foreign DNA, and should be polymorphic in comparison to near isogenic lines (Young *et al.*, 1988). In tomato the RFLP marker, TG105, has been found to be closely linked to the gene I_2 on chromosome 11 which confers resistance to the fungus *Fusarium o.* f. sp. *lycopersici* race 2 (Sarfatti *et al.*, 1989). The main limitations are the need for sufficient genomic DNA from each of a large number of samples to do a Southern blot, the need for a probe and the need for a radioactive label to achieve the most sensitive detection (Hartl, 2000). As a result it has been very difficult to distinguish tomato cultivars at the genetic or molecular level using these methods.

Another molecular marker approach uses short primers for DNA replication via the polymerase chain reaction (PCR). A specific DNA region flanked (in opposite orientation) by primers binding sequences that lie sufficiently close together allows the PCR reaction to replicate this specific region, and thus generate an amplified fragment (Lynch and Walsh, 1998). If the primer binding sites are missing or to far apart, the PCR reaction fails and no fragments are generated for that region. This procedure forms the basis for random amplified polymorphic DNA or as it is known, RAPD's. RAPD's requires no probe DNA and no advance information about the genome, but only uses a set of PCR primers 8 to 10 bases long whose sequence is random (Hartl, 2000). These primers are tried singly or in pairs in the PCR reactions to anneal to the template DNA at multiple sites.

RAPD uses Polymerase Chain Reaction (PCR) technology to overcome some of the technological limitations of RFLP (Walton, 1994). RAPDs require smaller amounts of DNA. RAPDs have an advantage over RFLPs in that a single probe can reveal several loci at once, each corresponding to different regions of the genome with appropriate primer sites. PCR-based technologies inherently have a higher production potential than RFLP and offer a great deal more opportunity to achieve efficiencies, as well as reducing costs, using automation at various steps of the process (Walton, 1994). RAPD's have proven to be less reproducible than RFLP's (Walton, 1994). Williams and St. Clair (1993) discovered that the amount of polymorphism between the accessions from different *Lycopersicon* species that was visualized using RAPDs did indicate that identification with RAPD primers was feasible, particularly using larger numbers of primers.

In this case, microsatellite DNAs, being short arrays of simple repeated sequences, have become the markers of choice. Microsatellites tend to be highly polymorphic, a consequence of their high mutation rate to new alleles. Microsatellite polymorphism is based on a very short core-repeating unit of two to nine base pairs. Microsatellite repeats may be present at many different locations in the genome, each flanked by restriction sites whose distance from the core repeat differs from one location to the next (Lynch and Walsh, 1998). If genomic DNA is cleaved with a restricted enzyme and the resulting fragments are separated by electrophoresis and hybridized in a Southern blot with a probe consisting of core repeats, each location in the genome containing the core repeats yields a separate band in the gel (Hartl, 2000). Since array length is scored, microsatellites are codominant. Heterozygotes show two different lengths, and hence, can be distinguished from homozygotes (Lynch and Walsh, 1998).

A GATA-detecting probe appears to generate quite a lot of polymorphisms between tomato cultivars (Vosman *et al.*, 1992). Microsatellite repeats like GATA or GACA display a high degree of variability that allows for their use in tomato cultivar identification (Vosman and Arens, 1997). Microsatellite DNA is also known as Simple Sequence Repeats (SSR) due to the fact that they are short segments of DNA that consist of a small number of repeated nucleotide sequences (Lynch and Walsh, 1998). Microsatellite markers are multi-allelic and they detect a much higher level of DNA polymorphism than any other known marker system (Rafalski and Tingey, 1993).

The selective restriction fragment amplification (AFLP) positional cloning strategy has been used to identify the I_2 locus in the tomato genome. Genetic complementation analysis in transgenic R1 plants, using a set of overlapping cosmids covering the I_2 locus, revealed three cosmids giving full resistance to *Fusarium o.* f. sp. *lycopersici* race 2 (Simons *et al.*, 1998). They discovered that these cosmids shared a 7-kb fragment containing an open reading frame encoding a protein similar to the nucleotide binding site leucine-rich repeat family of other resistance genes. Members of a new multigene family, complex I2C, were isolated using map-based cloning from the I2 *Fusarium o.* f. sp. *lycopersici* race 2 resistant

locus (Ori *et al.*,1997). Simons *et al.* (1998) discovered that the cosmids containing the I2C-1 or I2C-2 genes can not confer resistance to plants. This indicated that these members are not the functional resistance genes. It has been found that the presence of I2C antisense transgenes terminate race 2, but not race 1 resistance in otherwise normal plants (Ori *et al.*,1997).

Comparison of the leucine-rich repeat region of the I2C gene family members shows variation mainly due to insertions or deletions (Ori *et al.*, 1997). Simons *et al.* (1998) proposed that one or both of these leucine-rich repeats are involved in *Fusarium* wilt resistance with I_2 specificity. Sela-Buurlage *et al.* (2001) has identified six independent *Fusarium* resistant loci in 53 tomato lines conferring varying degrees of resistance to different races of the pathogen. The I loci has been found on chromosome 11, while the I_2 loci is found on chromosome 11, 2, 7 and 10 and loci I_3 on chromosome 7 (Sela-Buurlage *et al.*, 2001).

It has been found that the behavior of the I2 locus shows gene dosage effects which are closer to partial dominance. Mes *et al.* (2000) showed that in resistant plants, fungal growth in the region of the vascular tissue is prevented, suggesting a correlation with the I2 mediated resistance response. The I2-gene has been found to be necessary for resistance against *Fusarium o.* f. sp. *lycopersici* race 2. (Mes *et al.*, 1999b; Xu Zhihao *et al.*, 2000). If a molecular marker could be used to identify the I2-gene, it would be helpful in identifying tomato cultivars resistant against *Fusarium o.* f. sp. *lycopersici* race 2 in a disease resistance breeding program. Molecular markers for identification of the I2C1, I2C2 and I2 gene have already been investigated by Simon *et al.* (1998) who found that the tomato cultivars containing the I2 gene have primer sizes of 310 base pairs, while those containing the I2C1 and the I2C2 have primer sizes of 400 and 240 bp. respectively. PCR-based primers are used for this identification in an agarose gel-stained with ethidium bromide.

According to Walton (1994), microsatellites may be more polymorphic than RFLP markers for a number of crops. Rus-Kortekaas *et al.* (1994) discovered that a probe pWVA 16, which detects GACA-containing microsatellites, can distinguish representatives of *Lycopersicon* species as well as *Lycopersicon esculentum* cultivars. Microsatellite markers can be mapped onto all tomato chromosomes,

except for chromosomes 6 and 7 (Areshchenkova and Ganal, 1999). Sela- Buulage *et al.* (2001) discovered that some of the I2 loci are located on chromosome 7, which might eliminate microsatellite markers for detecting resistance genes against *Fusarium o.* f. sp. *lycopersici* race 2. Areshchenkova and Ganal (1999) discovered that a total of 61 alleles can be identified using 20 analyzed microsatellites, ranging from 1 allele to a maximum of 5 in the genus of *Lycopersicon*. Although microsatellite technology looks very promising, it is unlikely that microsatellites will replace RFLP or RAPD in the near future. All three technologies have unique applications that make them valuable for specific applications in plant breeding of specific crops (Walton, 1994).

2.8.5.2 The use of diallel analysis to improve quantitative resistance against *Fusarium o.* f. sp.*lycopersici* race 2 in tomato.

A frequently used experimental design for crossing inbred lines is the diallel cross in which each parental line is crossed with every other parental line (Griffing, 1956; Falconer and Mackay, 1996). All the possible crosses are not always assayed, for instance reciprocal crosses might be excluded as well as certain crosses within categories. Four types of diallel analysis are possible depending on whether the parental genotypes are viewed as fixed or a random effect (Lynch and Walsh, 1998). A diallel selection mating system are used to simultaneously contribute genes to a gene pool in the F1 generation (Gwanama *et al.*, 2001), but traits with low to moderate heritability are best improved using recurrent selection. Maiero *et al.* (1990) used diallel analysis to investigate the inheritance of collar rot resistance in tomato breeding. The genotypes C1943 and NC EBR-2 were the most resistant to collar rot. He discovered that additive and dominant effects are important in controlling the resistance trait, and collar rot resistance is incompletely recessive to susceptibility (Maiero *et al.*, 1990).

2.8.5.2.1 Genetic variability

Moustafa and Khafagi (1992) classified 11 tomato cultivars screened for infection by *Fusarium oxysporum* into three groups: (1) resistant cultivars, usually contain the I and I2 locus, like the cultivar Walter ; (2) tolerant varieties, include species like Roma VF that only contain the I resistance gene; (3) susceptible varieties that contain no resistance genes like Moneymaker, etc. Disease resistance has made a major contribution in past breeding efforts, and it is today virtually all-important that cultivars possess resistance to one or both *Fusarium o.* f. sp. *lycopersici* races. Ten wild accessions of *Lycopersicon*, belonging to the species *L. hirsutum* subsp. *Glabratum*, *L. peruvianum*, *L. cheesmanii*, *L. chilense* and *L. pimpinellifolium* have been evaluated in field and glasshouse trials and show resistance to *Fusarium o.* f. sp. *lycopersici* race 1 (Malhotra and Vashistha, 1992). Bisht *et al.* (1989) have also discovered that most of the *Lycopersicon pimpinellifolium*, *L. hirsutum* and *L. peruvianum* lines are resistant to *Fusarium oxysporum* f. sp. *lycopersici* and most crosses between *L. pimpinellifolium* X *L. esculentum* were found resistant to *Fusarium* wilt. Haung *et al.* (1997) found wide genetic variation for wilt resistance in wild *Lycopersicon* accessions, but discovered one accession of *L. cheesmanii* and two accessions of *L. chilense* that are highly resistant to races 1 and 2. Every newly discovered resistant accession provides plant breeders with more opportunities for *Fusarium* disease resistance breeding. Unfortunately, there is little genetic variation for disease resistance within elite germplasm, in cultivated tomato, *Lycopersicon esculentum* Mill.

Breeders have turned to primitive cultivars, landraces, or related wild species to find host plant resistance (Hartman and St. Clair, 1999). Unfortunately scientists have worked with polygenic inheritance, rather than with monogenic plant inheritance (Cirulli and Ciccarese, 1982). Plant-to-plant variation for resistance to *Fusarium o.* f. sp. *lycopersici* between the cultivars is great in polygenic resistant tomato cultivars like Marglobe. Gao *et al.* (1995) concluded that the genetic diversity within Marglobe results in different levels of recognition and response to the *Fusarium* pathogen, and thus leads to differences in the degree of localization of *Fusarium* infection within the polygenic population.

2.8.5.2.2 Combining ability

The crossing of a single parental line with several other lines can provide an additional measure of that line, i.e., the mean performance of the line in all its crosses. This mean performance, when expressed as a deviation from the mean of all crosses, is then called the general combining ability of the line. (Griffing, 1956; Falconer and Mackay, 1996). The average value of all the F1's having this line as one parent, can now be used to express this value as a deviation of the overall means of all the crosses. Any particular cross will then have an expected value which is the sum of the general combining abilities of its two parental lines. A F1 cross may deviate from the expected value, called the specific combining ability of the two lines (Falconer and Mackay, 1996).

F2-F4 tomato plants derived from BC2 backcrosses of the trigenomic hybrid (*Lycopersicon esculentum*, *Lycopersicon chilense* and *Lycopersicon peruvianum* var. *humifusum*) with susceptible cultivars have been tested for resistance to *Fusarium o.* f. sp. *lycopersici* race 1 and 2 as well as for various other diseases. It was found that particular lines have various combinations of resistance (Sotirova *et al.*, 1990). Tomato breeders have introgressed numerous single-gene sources of resistance from the wild *Lycopersicon* species (Rick and Chetelat, 1995), but the introgression of polygenic resistance has been less successful (Tanksley and Nelson, 1996).

No combining ability studies have been done for *Fusarium* resistance in tomatoes, but combining studies for *Fusarium* resistance in other plant species were found. The general *Fusarium* resistance level of *Gladiolus* (Straathof *et al.*, 1997), tulips (Van Eijk *et al.*, 1979), daffodils (Bowes *et al.*, 1992) and lilies (Straathof and Löffler, 1994) genotypes could be used to predict *Fusarium* resistance at population levels in their progenies. Straathof *et al.* (1997) studied the inheritance of *Fusarium* resistance in *Gladiolus* seedlings in terms of their general and specific combining ability and discovered that the resistance fits mainly an additive inheritance model. He obtained 37 populations from an incomplete diallel between eight parents with different levels of *Fusarium* resistance and detected significant differences in *Fusarium* infection between and within the populations (Straathof *et al.*, 1997).

2.8.5.2.3 Heritability

Heritability can be defined as the proportion of observed variability due to the additive effects of genes (Allard, 1960). Heritability thus specifies the proportion of the total variability that is due to genetic variance. Falconer (1981) defines heritability as the ratio of additive genetic variance to phenotypic variance. The phenotypic value consists of the genetic value and the environmental deviation. It is possible for the total genetic variation to be entirely additive, but this does not always seem to be the case (Lynch and Walsh, 1998). The genes cannot cause a character to develop unless they have the proper environment. It must be recognized that the variability observed in some characters is caused due to the differences in genes carried by different individuals, and the variability in others might be due to differences in environmental conditions to which those individuals have been exposed.

Two types of heritability are used. The first is based on the ratio of total genetic variation to the total phenotypic variation, and is called the broad sense heritability, h^2_b (Kearsey and Pooni, 1996). The second type of heritability provides a measure of the breeding value of a population, called the narrow sense heritability (h^2_n), and measures the proportion of the variation which is due to additive effects of genes in a specific population (Kearsey and Pooni, 1996). In the narrow-sense, heritability is also thought of as the efficiency of the response to selection (Falconer, 1981). Kozik *et al.* (1991) used the narrow-sense heritability to estimate the inheritance of resistance to *Phytopthora* root rot in tomatoes.

Early breeders and pathologists discovered that single plant selection does not increase resistance beyond a certain point. It has to be assumed on the basis of circumstantial evidence that the resistance in those earlier tomato varieties was also polygenic, and thus influenced more by environmental factors than the resistance in the cultivar Red Currant (Walker, 1971). It was assumed that resistance was polygenic and might be expected to vary to some degree. This point of view was abrubtly changed when Bohn and Tucker (1939, 1940) announced the resistance of Red Currant that was of a distinctly higher caliber and less subjected to environmental conditions.

Stall and Walter (1965) found resistance to both race 1 and race 2, in a selection from accession P.I. 126915. Heritability studies show that the mode of inheritance is similar to that of a single dominant gene. Retig *et al.* (1967) studied the inheritance for *Fusarium* resistance in tomato cultivars. They crossed a highly resistant cultivar Homestead with a susceptible cultivar Marmand and found that the segregation in the F2-generation is incomplete. Giles and Hutton (1958) studied *Fusarium* resistance in the offspring derived from crosses between tomato and *Lycopersicon peruvianum*. It seems as if *Fusarium* wilt resistance is controlled by more than one dominant gene. Sela-Buurlage *et al.* (2001) suggested that at the locus level, multiple active genes might be necessary to express full resistance. He discovered that the I2 locus shows gene dosage effects that are closer to partial dominance than full dominance (Sela-Buurlage *et al.*, 2001).

Malhotra and Vashistha (1993) studied *Fusarium oxysporum* resistance in the F1, F2 and backcrossing generations (BC) derived from crosses between a *Fusarium* resistant line and 2 susceptible parents. More than 90 percent of the F1 plants are resistant. The offspring of the F2 backcross with the susceptible parent co-segregate in a 1: 1 ratio. The offspring of the backcross of the F1 with the resistant parent give mostly resistant plants, indicating monogenic dominance for resistance (Malhotra and Vashistha, 1993). Malhotra *et al.* (1994) concluded that a single gene governs susceptibility to *Fusarium* wilt, and that F2 plants from resistant crosses behave like their resistant parents, while plants from susceptible crosses remain susceptible unless they inherite the resistant gene.

2.9 References

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CHAPTER 3

Assessment of resistance to *Fusarium oxysporum* f. sp. *lycopersici* race 2 in South African tomato cultivars.

3.1 Abstract

Twelve inbred tomato cultivars grown in South Africa were screened for resistance to *Fusarium oxysporum* f. sp. *lycopersici* race 2. Percentage resistant plants, disease and death rates were monitored for intervals of 30 and 60 days respectively. Significant differences were found between cultivars for percentage resistant plants for both intervals as well as for disease progress. Five cultivars, Floradade, Rodade, Heinz 1370, Traffic Jam and Sixpack were associated with resistance after 60 days screening. The cultivar x day interaction was significant for the percentage resistant plants.

3.2 Introduction

The tomato industry in South Africa produces nearly 200 000 tons of raw tomatoes annually. The average yield varies from 65 and 87 t/ha. The largest tomato producing regions in South Africa are Messina (85 000 tonnes); Lutzville (45 000 tonnes); Nelspruit (20 000 tonnes); Duiwelskloof and Pietersburg (both 15 000 tonnes); and Venda (10 000 tonnes) (MSN Web Page, 2002). Most of the tomato production takes place in the Limpopo Province of South Africa.

Tomato yields in each province are reduced every year by various fungal, bacterial and viral diseases (Uys, 1996). One of the major fungal pathogens, causing crop losses is *Fusarium oxysporum* (Jones *et al.*, 1991). Uys (1996) studied *Fusarium* wilt disease and disease reactions of various *Fusarium oxysporum* subspecies. He discovered that *Fusarium o.* f. sp. *lycopersici* race 2 caused the most damage in the far Northern Province. Caesar *et al.* (1998) discovered a significant difference in virulence among different *Fusarium oxysporum* f. sp. *lycopersici* strains of vegetative compatible groups (VCG's). Controll of *Fusarium oxysporum* f. sp. *lycopersici* race 2 can be achieved using various methods (biological, chemical, solarization and management control), but the most effective and convenient means of control is still the use of resistant tomato varieties. Host resistance in tomato has often been derived from wild relatives, and these resistance genes are then incorporated into adapted tomato cultivars using backcross breeding techniques. The problem caused by *Fusarium* wilt has however not yet been solved, despite the fact that resistance genes in seed varieties exist and are freely available.

Traditionally, breeders have chosen to work with qualitatively inherited resistance to *Fusarium* wilt due to the fact that genes are easier to transfer into commercial cultivars. The severity of *Fusarium* wilt disease in tomato plants has been correlated to the extent of fungal spread in the vascular tissue of the stem (Scheffer and Walker, 1954). It is thus possible to evaluate the severity of the wilt disease on external symptoms as well as to classify them accordingly into groups and to evaluate each cultivar's resistance and tolerance against this specific virulent *Fusarium* species. Although different assessment methods are available, screening

for external symptoms using a disease index scale is preferred by most plant breeders to identify the degree of severity of the wilting disease (Grattidge and O' Brien, 1982; Ramsey *et al.*, 1992; Malhotra and Vashistha, 1993). Assessment and evaluation of tomato seedlings against *Fusarium o.* f. sp. *lycopersici* is done to distinguish resistant from susceptible tomato cultivars. Some authors such as Moustafa and Khafagi (1992), Haung *et al.* (1997) distinguished between resistant, tolerant (intermediate resistant) and susceptible tomato cultivars with regard to their reaction to *Fusarium o.* f. sp. *lycopersici.*

Several studies have been conducted to investigate the level of resistance of several tomato cultivars against *Fusarium oxysporum* f. sp. *lycopersici.* The literature indicates that local and international cultivars, like Floradade, Roma, Rodade Heinz and UC82-B are regarded as resistant against *F. oxysporum* f. sp. *lycopersici* (Moustafa and Khafagi, 1992; Storti *et al.*, 1992; Uys, 1996; Awad, 2000), while other cultivars like Moneymaker, Red Kaki are highlly susceptible to Fusarium wilt (Kroon *et al.*, 1991; Uys, 1996). Some tomato cultivars, such as Roma (Moustafa and Khafagi, 1992) are tolerant against *Fusarium* wilt, but eventually the plants are infected. A thorough investigation into the levels of resistance of tomato cultivars against *F. oxysporum* f. sp. *lycopersici* race 1 and race 2 has not yet been conducted.

The main objective of this investigation was 1) to determine: the resistance levels of locally adapted cultivars against *Fusarium oxysporum* f. sp. *lycopersici* and 2) to determine: if there are any real differences between screening for resistance on day 30 and day 60.

3.3 Materials and Methods

Fungal Isolate

An isolate of *Fusarium o.* f. sp. *lycopersici race 2* was obtained from ARC - Roodeplaat. The fungus was first streaked out onto potato dextrose agar (PDA) and tested on a selective Rose-bengal-glycerine-urea agar medium (Van Wyk *et al.*, 1986). The isolate was then grown on a carnation leaf agar medium and identified using the method described by Nelson *et al.* (1983). Race determination was achieved using the methods described by Cirulli and Ciccarese (1982) and Venter (1990). The *Fusarium* culture was cultivated on a PDA agar medium for eight days at 26°C under a twelve-hour fluorescent and black light cycle. Fungal mats containing the micro- and macroconidia were scraped from the agar and washed from the agar with sterile distilled water. Conidia were separated from the mycelium by filtering the mixture through a sterile cheesecloth and the conidia were washed using centrifugation (Alon *et al.* 1974). Density of conidial suspension of each isolate was determined using a haemacytometer and the concentration adjusted to 1 X 10^6 conidia per milliliter (Theron and Holz, 1989; Elias and Schneider, 1991).

Experimental Procedure

The tomato seed used in this study was obtained from different seed companies. Twelve locally adapted inbred tomato cultivars were chosen as parents for this study. They were Red Kaki, Moneymaker, Oxheart, Heinz 1370, Roma, Traffic Jam, Sixpack, Steven, Rossol, Floradade, Rodade and UC 82-B. All the seeds were sterilized using the hot-water method. This process involved placing 120 g of seed of each cultivar in an empty tea-bag, and soaking it in water at 50°C for about 25 minutes (Wager, 1981). This was done in order to prevent the development of inborn diseases. Seeds were subsequently washed with a surface disinfectant (0.5 to 1.0 % sodium hypochlorite) at a concentration of 10 to 20 % (Dodds and Roberts, 1995). This procedure was used to remove any pathogens that might be on the surface of the seeds.

The seedlings were removed after seven days. The roots of the seedlings that represented the control population were washed with sterile distilled water, while the roots of the rest of the seedlings were inoculated by dipping them into an inoculum suspension. Seedlings were then replanted in seedling trays, replicated four times. The trays were put in a greenhouse where the temperature was maintained between 26° C and 30° C. Seedlings were watered daily.

Pathogenicity tests

The seedlings were monitored for any external symptoms of *Fusarium* wilt disease for 60 consecutive days. Microscopic analysis as well as Koch's postulate of isolating the organism, and insuring that it causes the same symptoms when reintroduced into the organism, confirmed the disease. The disease was assessed using the following rating scale: 0, having no wilting, equivalent to the control; 1, slightly stunted, with no wilting or yellowing, <5% leaves affected; 2, slight yellowing and wilting, <25% leaves affected; 3, moderate wilting, between 25 and 50% leaves affected; 4, severe wilting, between 50 and 75% leaves affected; and 5, >75% plant is regarded as being dead (Grattidge and O'Brien, 1982; Ramsey *et al.*, 1992).

Individual seedlings were then grouped in three major classes as not affected (resistance or healthy), affected (diseased) and dead seedlings. Questionable disease symptoms (1) did not really indicate the presence or even the absence of wilt disease. It was thus decided to group ratings 0 and 1 together as resistant seedlings. The affected sick plants were best indicated by scale 2 (limited local symptoms) as well as scale 3 (well-developed symptoms). It was sometimes difficult to distinguish between plants in category 4 (severe wilt) and plants in category 5 (dead plants) and they were therefore decided to combine them into one category for dead plants.

Statistical analysis

The percentage of resistant plants was calculated for each cultivar for both 30 and 60 day intervals. The difference between the two intervals was used to calculate the

disease progress from 30 to 60 days. Analyses of variance were calculated for the percentage resistant plants over the 30 and 60 day intervals as well as for disease progress. A combined analysis of variance (ANOVA), which included all resistant data, was done to test for significant differences between 30 and 60 day intervals. The computer program, Agrobase (2000) was used to conduct these analyses. The analysis provided means, mean squares, and sum of squares, F-values and probability levels of significance, least significant differences (LSD) and coefficient of variation (CV). LSD values were used to test for significant differences between means.

3.4 Results and Discussion

I Disease progress of *Fusarium* wilt race 2 in 12 tomato cultivars grown in South Africa

Red Kaki

The disease progress of *Fusarium* wilt race 2 in Red Kaki is illustrated in Figure 3.1. The number of resistant seedlings in Red Kaki declined after 40 days, from 200 to approximately 120 seedlings. These seedlings remained resistant until day 50. After day 50, the number of resistant seedlings declined further to only 27. These seedlings remained resistant for the rest of the 60 day period. A total of 60 dead seedlings were recorded in Red Kaki after 60 days. Uys (1996) studied seedling resistance against *Fusarium o.* f. sp. *lycopersici* race 2 in Red Kaki and found severe disease symptoms of above 50%. Red Kaki seemed to be mildly tolerant to *Fusarium* wilt race 2 in the early stages of seedling development but became highly susceptible after 50 days.

Moneymaker

Figure 3.2 shows that the number of resistant seedlings in Moneymaker started to decline after 10 days. A total of 55 seedlings remained resistant after 60 days. The number of affected seedlings remained relatively constant for the first 47 days, where after it increased significantly to a total of 50 at the end of the 60 day period. The death rate of the seedlings started to increase drastically after 10 days. Kroon *et*

al. (1991) and Moustafa and Khafagi (1992) found Moneymaker highly susceptible to *Fusarium* wilt race 2.

Oxheart

Figure 3.3 show that the cultivar Oxheart was susceptible to *Fusarium* wilt race 2. After four days the number of resistant seedlings started to decline gradually. Only 14 seedlings remained resistant after 60 days. The number of dead seedlings started to increase after 18 days with a total death rate of 108 seedlings after 60 days. Attitalla *et al.* (2001) found that the disease progress in susceptible tomato cultivars infected with *Fusarium* wilt had a similar pattern to the one found in Oxheart.

Heinz 1370

The number of resistant seedlings in Heinz 1370 (Figure 3.4) remained constant for 30 days before it started to decline to a total of 125 resistant seedlings after 60 days. The number of dead seedlings increased progressively after 30 days with only 33 dead seedlings after 60 days. The cultivar Heinz 1370 could therefore be regarded as relatively resistant to *Fusarium* wilt race 2. These results are in contrast with the findings of Uys (1996) who reported severe wilting symptoms in Heinz 1370. Storti *et al.* (1992) also regarded the cultivar Heinz as highly resistant to *Fusarium* wilt race 2. According to Uys (1996), Heinz 1370 contains the I1 gene for resistance to *Fusarium* wilt race 1.

Roma

In Roma (Fig 3.5), the number of resistant seedlings stayed constant for 33 days. It started to decline progressively with only 15 resistant seedling left after 60 days. The number affected and dead seedlings increased gradually after 33 days. The number of dead seedlings increased to 93 after 60 days. This result is consistent with the findings of Moustafa and Khafagi (1992) who described Roma as a tolerant cultivar to *Fusarium* wilt race 2. According to Wager (1981), Roma carries the I1 gene for resistance against *Fusarium* wilt race 1. According to Jagdish-Singh *et al.* (2002), the disease reaction of Roma is typical of a cultivar having tolerance to *Fusarium* wilt race 2.

Traffic Jam

The disease progress of *Fusarium* wilt in Traffic Jam is illustrated in Figure 3.6. Traffic Jam is a tomato cultivar grown in South Africa. It showed a gradual dicline in the number of resistant seedlings for the first 30 days. It remained stable for the remaining 60 day period, ending with 113 resistant seedlings. The affected seedlings remained relatively low and constant for the entire 60 day period. The number of dead seedlings started to increase after 26 days and 67 dead seedlings after 60 days. No information about Traffic Jam with regard to it's resistance to *Fusarium* wilt race 2 has been published.

Sixpack

The disease progress of *Fusarium* wilt race 2 in Sixpack is illustrated in Figure 3.7. The number of resistant seedlings in Sixpack declined gradually from 200 to 100 after 19 days. It then declined further to 94 seedlings after 60 days. The percentage affected seedlings remained constant. The percentage of dead seedlings increased rapidly after 16 days and reached it's peak of 89 plants after 60 days.

Steven

Steven (Figure 3.8) is a local cultivar with the I1 gene for resistance to *Fusarium* wilt race 1. Steven was derived from a cross between *Lycopersicon esculentum* X *Lycopersicon peruvianum* (Steven *et al.*, 1992). The number of resistant seedlings in Steven declined drastically after six days. Only 74 seedlings remained resistant after 60 days. The number of seedlings affected remained constant, while the number of dead seedlings rose progressively and peaked at 95 after 60 days.

Rossol

The cultivar Rossol (Figure 3.9) contained a resistance gene to *Fusarium* wilt race 1 (Wager, 1981). The number of resistant seedlings declined rapidly over the 60 day period. The number of dead seedlings in Rossol increased sharply after two days and remained high througout the 60 day period. The number of dead seedlings peaked at 130 after 60 days. Rossol can be regarded as the most susceptible cultivar tested.

Flora Dade

The disease progress of the cultivar Flora Dade is illustrated in Figure 3.10. The number of resistant seedlings in Flora Dade remained high until 50 days. It was followed by a gradual decline to end with 97 resistant seedlings after 60 days. The number affected seedlings remained constant and start to increase after 50 days to peak with 96 affected plants after 60 days. The number of dead seedlings remained constant throughout the 60 days. According to Uys (1996) Flora Dade has both the I1 and I2 resistant genes against *Fusarium* wilt. These results were confirmed by Awad (2000) and Jagdish-Singh *et al.* (2002), who found that Flora Dade is resistant to both races 1 and 2 of *Fusarium o.* f. sp. *lycopersici.*

Rodade

Figures 3.11 illustrates the seedling resistance of Rodade to *Fusarium* wilt race 2. The number of resistant seedlings in Rodade declined from 200 to 125 after 60 days. The number of affected seedlings declined to only 70 after 60 days. The number of dead seedlings remained low, with only nine dead after 60 days. Similar results were found by Gao *et al.* (1995) who reported that the majority of resistant plants showed a slight yellowing or wilting of leaves after four weeks.

UC 82B

UC 82B (figure 3.12) is highly susceptible to *Fusarium* wilt. The number of resistant seedlings declined very rapidly after seven days and was reduced to only 67 after 60 days. The number of affected seedlings increased slowly to a total of 52 after 60 days. The number of dead seedlings started to increase after six days. Storti *et al.* (1992) regarded UC 82B as resistant against *Fusarium* wilt race 2, while Iloba and Aschor (1989) described UC 82B as susceptible against *Fusarium* wilt race 2.

The cultivars Heinz 1370, Traffic Jam, Sixpack, Flora Dade and Rodade had the largest number of resistant seedlings after sixty days. Steven and Moneymaker and UC 82B had the second largest number of resistant seedlings and are classified as tolerant. Red Kaki, Oxheart, Roma and Rossol were very susceptible to *Fusarium* wilt race 2 after 60 days.

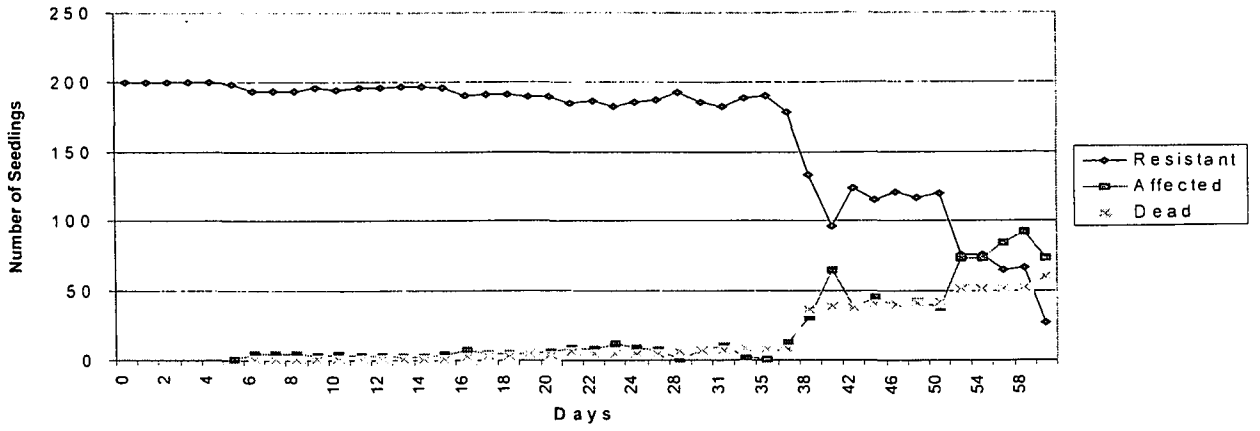

Figure 3.1. Disease progress of *Fusarium* wilt race 2 in Red Kaki

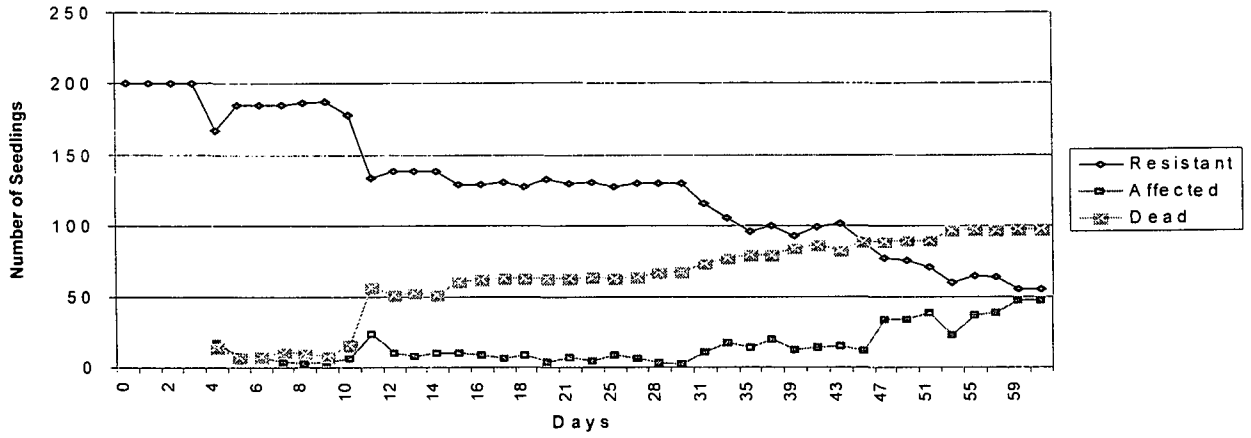

Figure 3.2. Disease progress of *Fusarium* wilt race 2 in Moneymaker

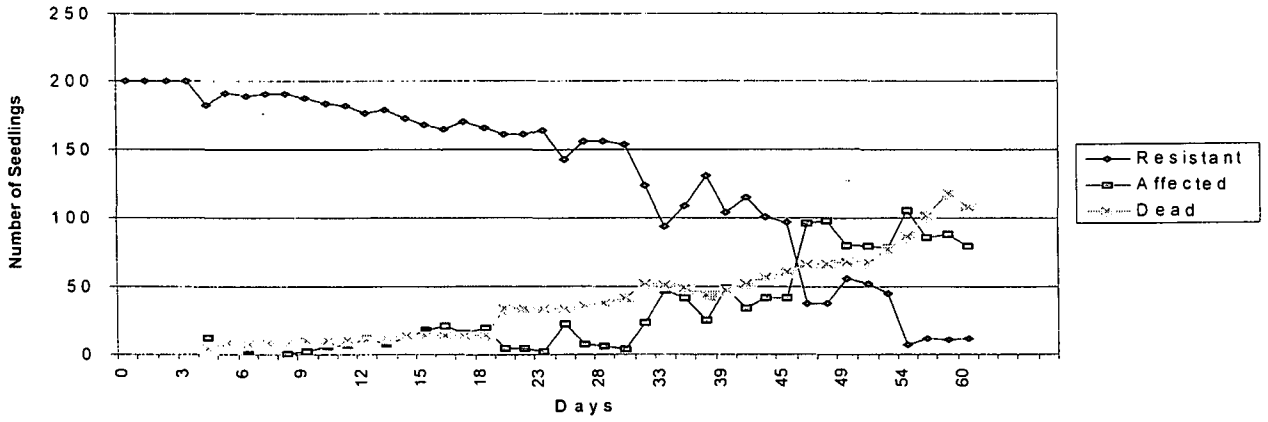

Figure 3.3. Disease progress of *Fusarium* wilt race 2 in Oxheart

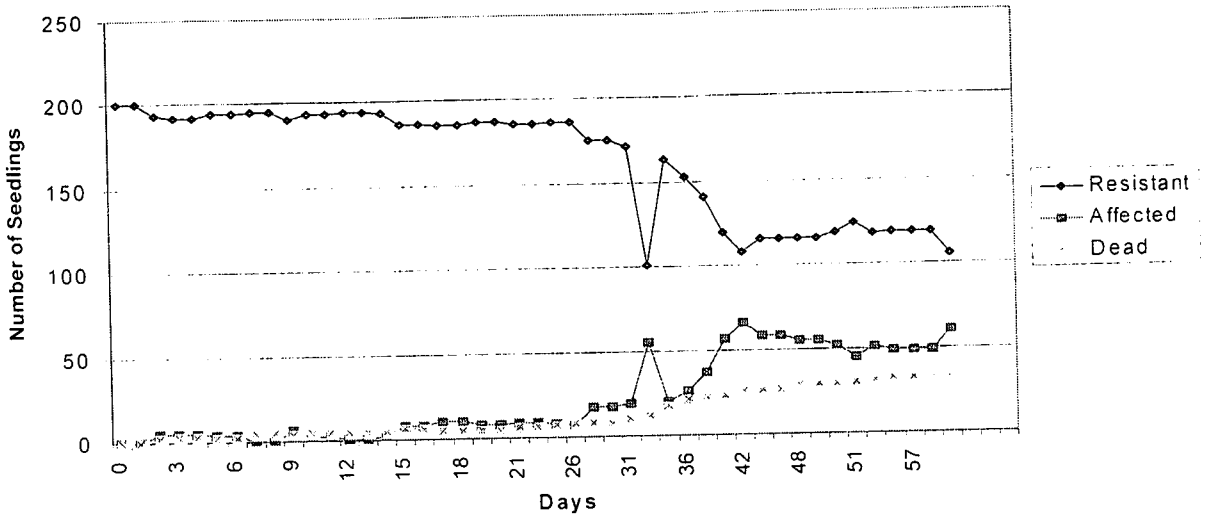

Figure 3.4. Disease progress of *Fusarium* wilt race 2 in Heinz 1370

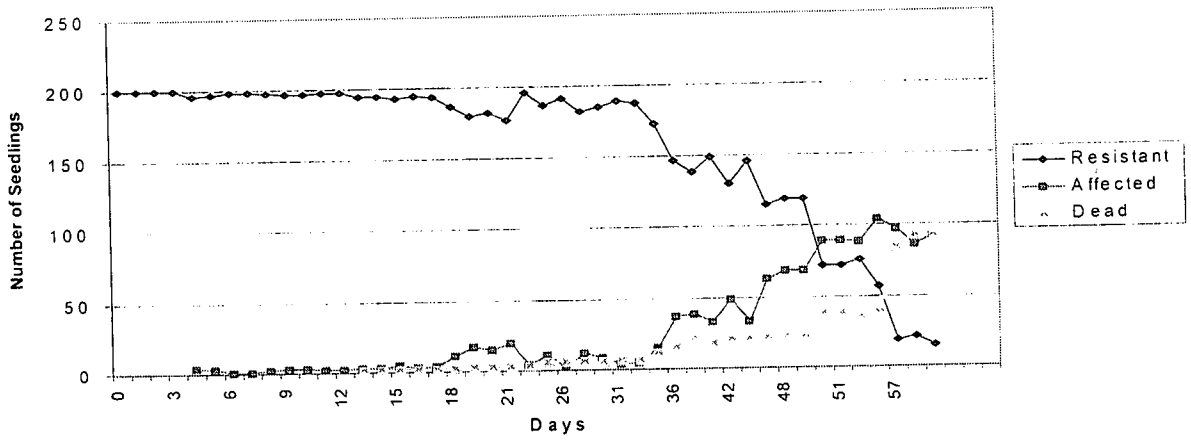

Figure 3.5. Disease progress of *Fusarium* wilt race 2 in Roma

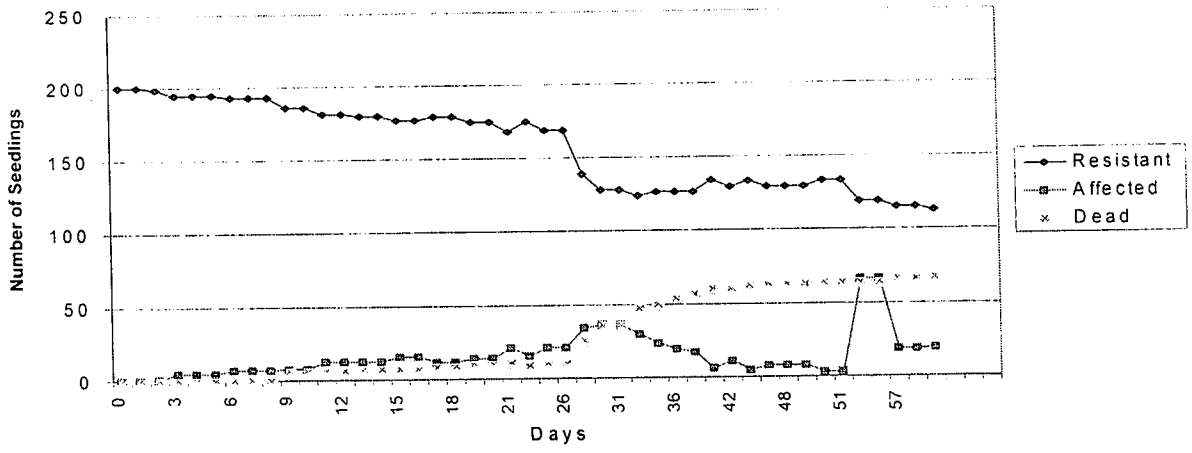

Figure 3.6. Disease progress of *Fusarium* wilt race 2 in Traffic Jam

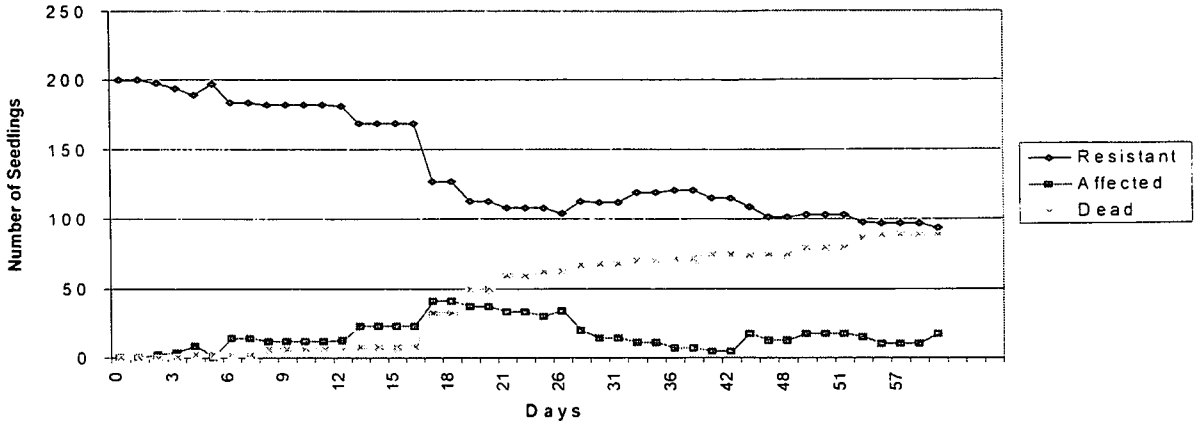

Figure 3.7. Disease progress of *Fusarium* wilt race 2 in Sixpack

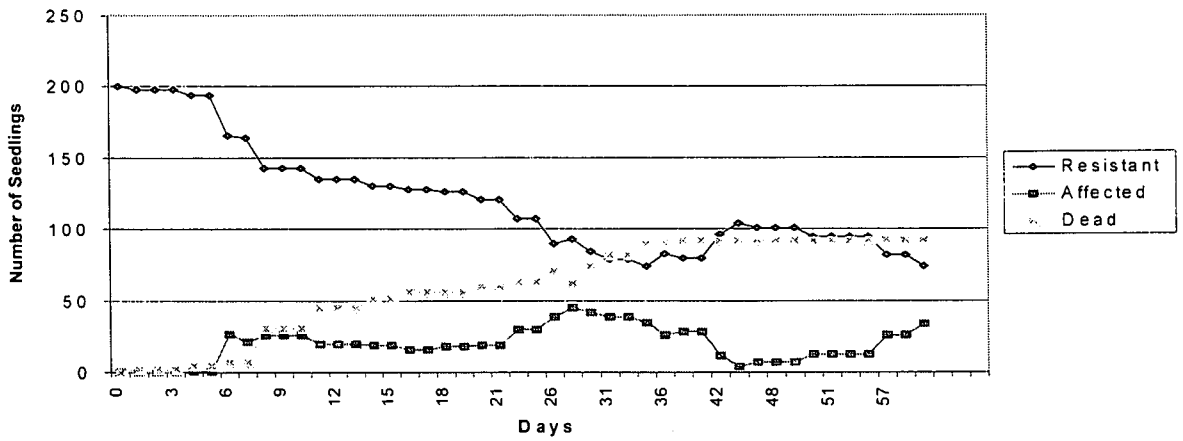

Figure 3.8. Disease progress of *Fusarium* wilt race 2 in Steven

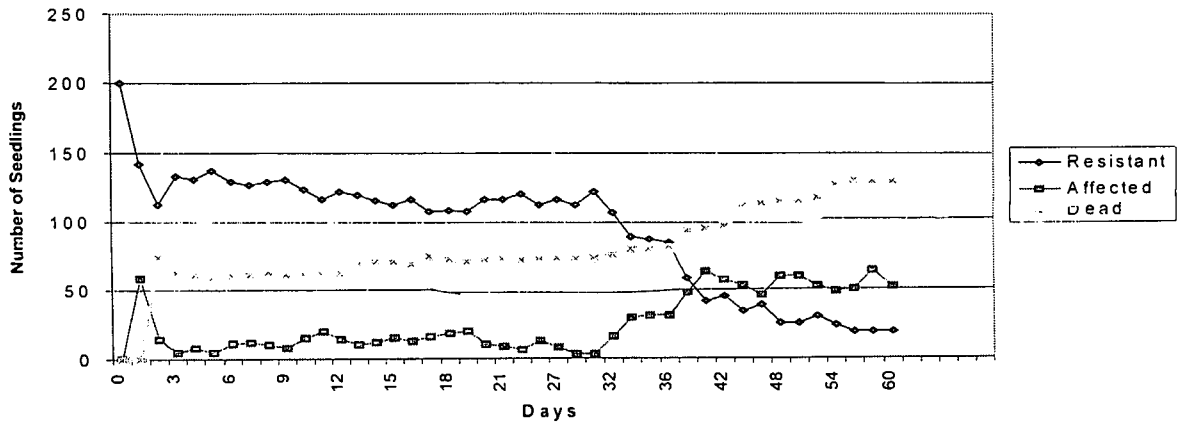

Figure 3.9. Disease progress of *Fusarium* wilt race 2 in Rossol.

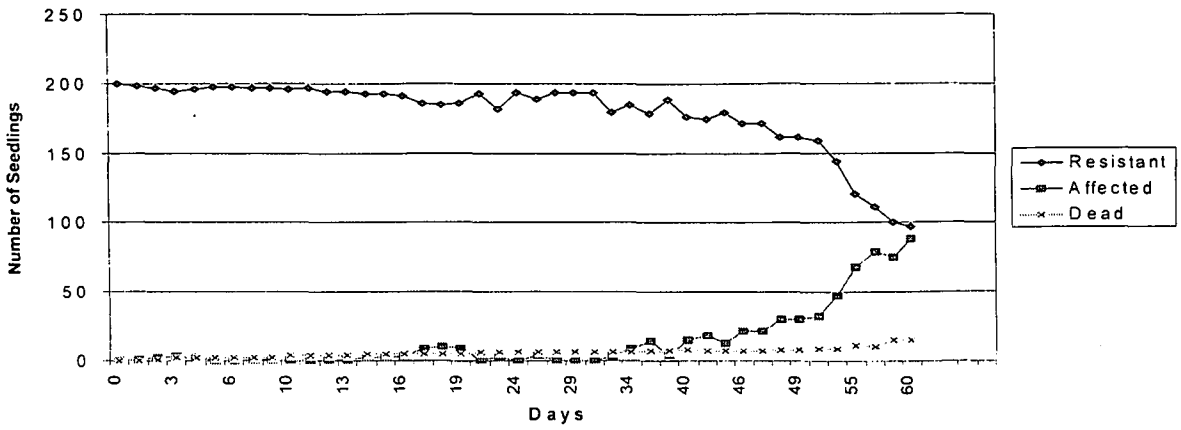

Figure 3.10. Disease progress of *Fusarium* wilt race 2 in Flora Dade

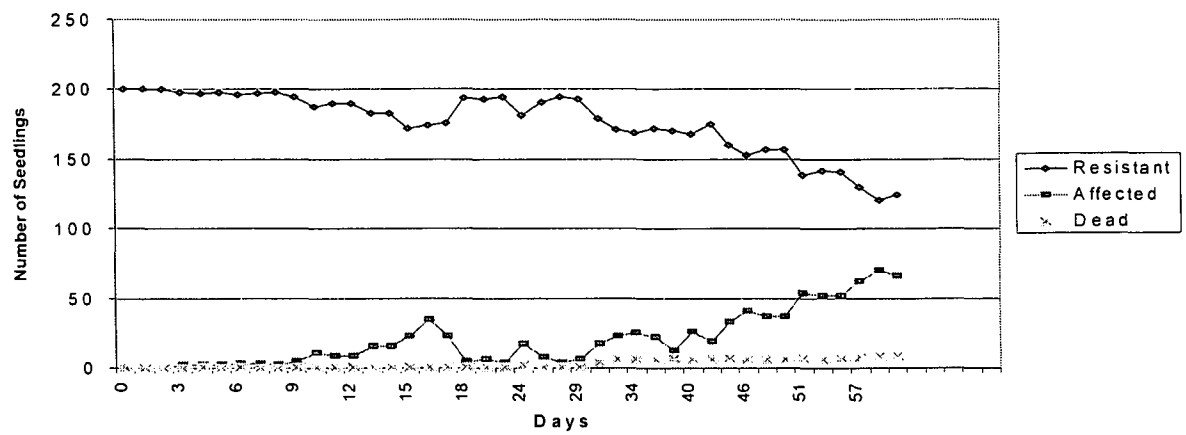

Figure 3.11. Disease progress of *Fusarium* wilt race 2 in Rodade

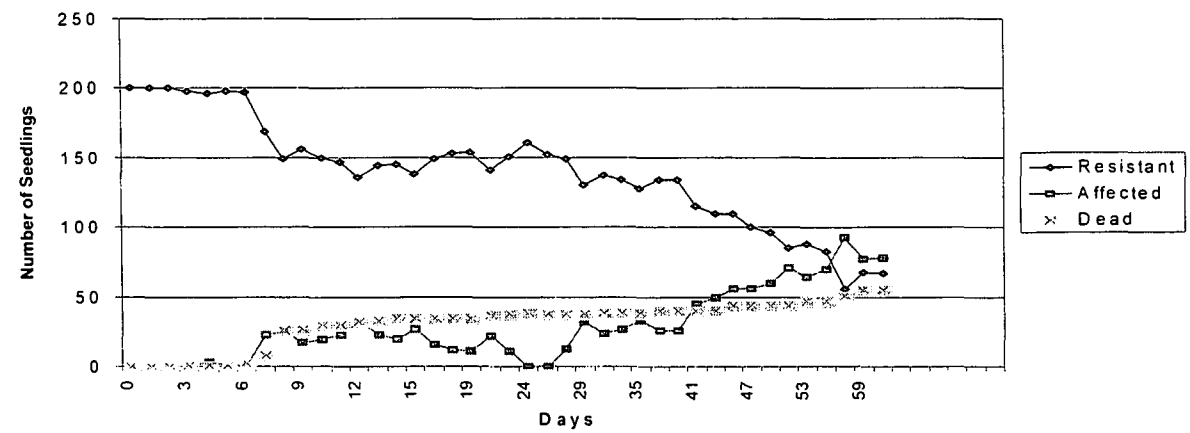

Figure 3.12. Disease progress of *Fusarium* wilt race 2 in UC 82B

II Resistant plants for two time intervals.

The percentage of resistant seedlings in each cultivar was subjected to an ANOVA to study the resistance of the cultivars against *Fusarium* wilt race 2. Analysis was conducted for 30 and 60 day intervals and disease progress (difference between 30 and 60 day intervals). The mean square and F-values are listed in Table 3.1. Significant differences were found between cultivars for the percentage of resistant seedlings for both 30 and 60 day intervals as well as for disease progress.

A combined ANOVA (Table 3.2) was conducted on the percentage resistant seedlings in each cultivar. It was done to study the effect of time on disease development and on the interaction between time of screening and cultivars. There was a significant reduction in the number of resistant seedlings from 30 to 60 days. The interaction between time of screening and cultivars was highly significant. It indicates a significant change in the disease ranking of cultivars over time.

30 day interval

The percentage of resistant seedlings for each cultivar after 30 days are illustrated in Figure 3.13. The cultivars Red Kaki, Heinz 1370, Roma, Flora Dade and Rodade had the largest number of resistant seedlings. The average level of resistance after 30 days exceeds 80 percent and was significantly higher than the rest of the cultivars. Red Kaki carries no known single resistance gene against *Fusarium* wilt race 2. Its resistance is probably caused by the accumulation of polygenes after years of selection. The percentage resistant plants in Rossol, Oxheart, UC 82B and Moneymaker exceeded 60% and was significantly greater than the percentage of resistant plants in Steven. The percentage resistant plants in Oxheart and UC 82B was significantly larger than that of Sixpack. The percentage of resistant plants in Steven and Sixpack was less than 60 percent. Only two of the cultivars Heinz 1370 and Roma carried the I1 resistance gene.

Table 3.1: Analysis of variance for percentage resistant seedlings to *Fusarium* wilt race 2 in local tomato cultivars.

Source	DF		SS			MS			F-value	
		30days	60days	Disease progress	30days	60days	Disease progress	30days	60days	Disease progress
Total	47	21197	21519.67	38309						
Cultivars	11	15559	18239.67	34499	1414.46	1658.15	3136.27	8.69**	17.38**	28.68**
Blocks	3	267	131	201.67	89.00	43.67	67.22	0.55	0.46	0.61
Residual	33	5371	3149	3608.33	162.76	95.42	109.34			

*Significant

**Highly Significant

Table 3.2: Combined analysis of variance for percentage resistance seedlings to *Fusarium* wilt race 2.

Source	DF	Sum of Squares	Mean Square	F-ratio
Total	95	83662.96		
A: Cultivars	11	16934.46	1539.496	12.71**
B: Day	1	39935.04	39935.04	329.63**
AB combined	11	18070.46	1642.769	13.56**
Error	72	8723	121.1528	

*Significant

**Highly Significant

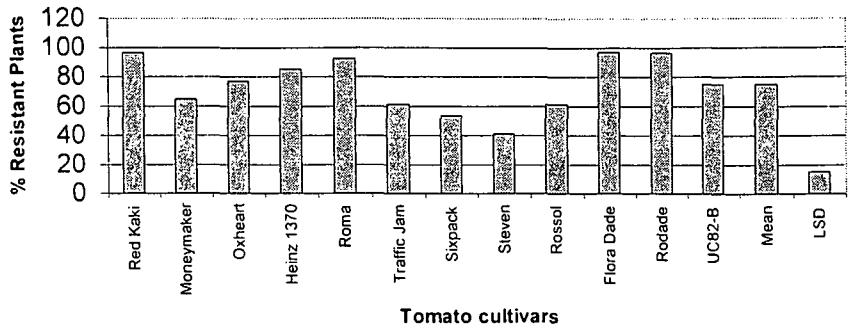

Figure 3.13. Percentage resistant seedlings on 30 day interval.

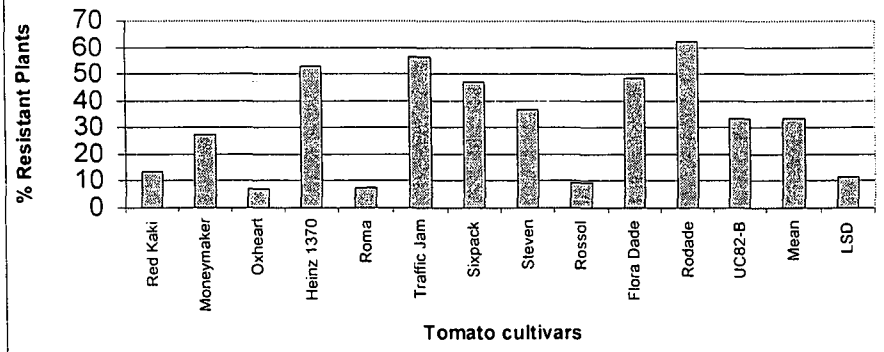

Figure 3.14. Percentage resistant seedlings on 60 day interval.

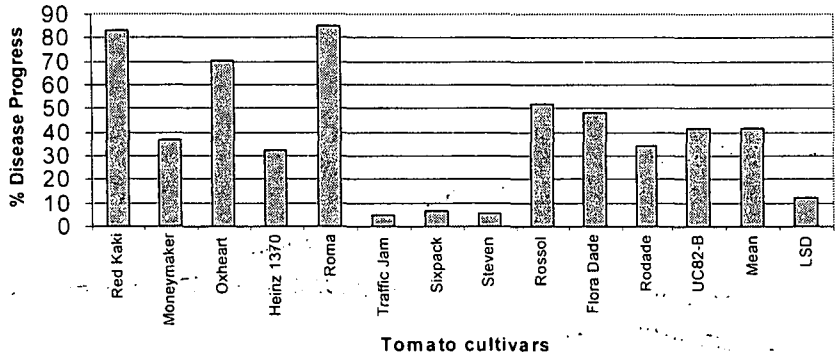

Figure 3.15. Disease progress

60 day interval

Significant differences were found (Figure 3.14) between cultivars after sixty days. The percentage resistant seedlings were significantly higher in Rodade, Heinz 1370 and Traffic Jam exceeded 50%. Resistant seedlings Flora Dade and Sixpack exceeded 40%. The percentage of resistant seedlings in Flora Dade and Sixpack was significantly higher than the rest of the cultures. The only exception was Steven. The percentage resistant plants in UC 82B, Moneymaker and Stevens was significantly larger than of Rossol, Oxheart, Red Kaki and Roma. The latter cultivars are highly susceptible with less than 20% resistant seedlings.

Disease progress

The disease progress (between 30 and 60 day intervals) of the different cultivars is illustrated in Figure 3.15. The disease progress of Steven, Traffic Jam and Sixpack was significantly lower than for the rest of the cultivars. The low disease progress in Steven is the result of its high level of susceptibility at the thirty day interval. The disease progress in Flora Dade, Rodade, UC 82B, Moneymaker and Heinz 1370 was significantly lower than in Red Kaki and Roma. Both these cultivars are very susceptible to *Fusarium* wilt race 2.

3.5 Conclusions

The variability of *Fusarium* wilt within the 12 tomato cultivars ranged from high levels of resistance to highly susceptible which is typical for a quantitative parameter. Falconer (1981) describes these kinds of characters as a threshold character and defined it as one with two scales, namely, a visual scale with discrete classes like resistance, affected and dead with thresholds between the different classes. The second scale is referred to as the underlying scale, which fits a normal distribution with two extremes at the end of the scale. In this case, the extreme were dead and resistant. Since the variability between seedlings within cultivars is larger than the

variability between cultivars, selection should rather be applied between seedlings within cultivars. This can be done by selecting all the seedlings above the threshold that separates the affected from resistant seedlings. It is thus possible to improve the mean level of resistance in existing cultivars through selection of the resistant seedlings.

The time of screening is very important. Literature shows that most researchers screen the seedlings 30 days after inoculation. The results found in this study have shown that the number of resistant seedlings is significantly lower after 60 days. This is further complicated by a significant interaction between the time of screening and the percentage resistant seedlings. Red Kaki is a good example of such a cultivar with a high number of resistant seedlings after 30 days which became very susceptible after 60 days. The cultivar x time of screening interaction makes it very difficult to select resistant seedlings in the early stages of seedling development since it tends to change the ranking of cultivars. Therefore, it is recommend that the time of screening be postponed for at least 60 days after inoculation.

The present study showed significant genetic variability for *Fusarium* wilt resistance within and between the 12 cultivars assessed. The cultivars Flora Dade, Rodade, Heinz 1370 and Traffic Jam showed resistance to *Fusarium* wilt race 2 after 60 days of screening. The percentage resistant seedlings in Sixpack was slightly lower. Disease development was also slow in Traffic Jam and Sixpack. The relationship between the disease progress of a cultivar and its number of resistant seedlings should be studied. It would enable breeders to select the cultivars on the basis of their disease progress. The results obtained in this study showed very little correlation between the disease progress of a cultivar and its percentage resistant seedlings.

The following recommendations can be made:

1.Screening for the I-2 resistant gene in tomatoes should not be made too early in the growth stage of development, even though some studies indicated early screening for poligenic resistance was possible due to the fact that disease symptoms develop later than 30 days in many cultivars.

2. A possible solution to *Fusarium* wilt disease might be to develop "horizontal" resistance in newly developed variety or hybrid species, and test them for resistance against more than one vegetative compatible group of a specific *Fusarium o.* f. sp. *lycopersici* race.

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CHAPTER 4

Genetic variability for resistance to *Fusarium wilt* in F1-Tomato offspring.

4.1 Abstract

Fusarium wilt resistance was studied in six inbred tomato cultivars viz., Rossol, Red Kaki, Moneymaker, Rodade, Flora Dade and Heinz 1370, and their F1-hybrids. The percentage resistant seedlings for each cultivar and F1-hybrid were determined after 30 and 60 days. Disease progress was also calculated. Variance analysis indicated a significant genotype x day interaction for *Fusarium* wilt. Significant differences were found between entries after 30 and 60-days assessments as well as for disease progress. The most *Fusarium* wilt resistance was found in the cultivars Moneymaker, Rodade and Flora Dade. Moneymaker had the slowest disease progress. The disease progress in five of the crosses Flo x Mon, Flo x Ros, Rod x Ros and Mon x Ros were less than 10%. Disease progress in F1-hybrids were significantly lower than in inbred lines, emphasing the importance of tomato hybrids to efficiently reduce levels of *Fusarium* wilt.

4.2 Introduction

One of the most important pathogens causing disease in tomato crops which results in significant crop losses is *Fusarium oxysporum* f. sp. *lycopersici* (Jones *et al.*, 1991). South Africa processes nearly 200 000 tons of raw tomatoes annually (MSN Web Page, 2002) but in every province the tomato yields are reduced each year due to diseases, pests, and drought. Uys (1996) discovered that *Fusarium o.* f. sp. *lycopersici* race 1 was dominant in all the tomato-growing regions, while race 2 was confined to the northern parts of South Africa.

Various studies have indicated the presence of genetic variability between and within different tomato breeding lines, showing different levels of resistance to *Fusarium* wilt. Gao *et al.* (1995) found significant differences in the expression of disease resistance in tomato cultivars. Genetic diversity results in different levels of recognition and response to the pathogen which leads to differences in the degree of localization of *Fusarium* infections (Gao *et al.*, 1995). Rodeva and Stancheva (1998) showed that resistance to *Fusarium o.* f. sp. *radicus-lycopersici* was directly related to genotype and was only slightly affected by the isolate used. New vegetative compatibility groups of different *Fusarium* wilt races are still discovered every year (Elias and Schneider, 1991; Gordon and Okamoto 1991; Gordon and Okamoto, 1992; Uys, 1996) and breeding for resistance to these newly found strains is needed to prevent crop losses.

Most breeders have been reluctant to use quantitative or polygenic breeding methods and have rather turned to single gene inherited breeding techniques. The maintenance of multiple resistance in tomato to the fungal pathogen *Fusarium* wilt, must be addressed both at the single locus level containing more than one active gene copy (Bohn and Tucker, 1939; Stall and Walter 1965; Cirulli and Alexander, 1966, McGrath *et al.*, 1987)

as well as at the whole genome level, in which genes display overlapping functions (Sela-Buurlage *et al.*, 2001). At the locus level, Sela-Buurlage *et al.* (2001) discovered that multiple active genes might be necessary to express full resistance to *Fusarium* wilt race 2.

The appearance of new races of *Fusarium o.* f. sp. *lycopersici* could necessitate the use of polygenic resistant tomato germplasm or the combination of polygenic and monogenic resistance. Cirulli and Ciccareese (1982) discovered that screening for polygenically resistant tomato plants against *Fusarium* wilt could be done in a greenhouse using an inoculum dilution of 10^6 conidia/ml and a growth temperature of 26° C with an average screening time of 26 days. Variation for resistance to *Fusarium* wilt between polygenic tomato cultivars, like Marglobe is usually very high and Gao *et al.* (1995) concluded that this was due to different levels of recognition and response to the *Fusarium* wilt.

Any breeding program designed to produce resistant varieties must start with cultivars that contain genes conferring resistance. Some tomato cultivars are more resistant than other cultivars to a specific *Fusarium* wilt race. Cultivars with no resistance are classified as susceptible. Breeders have made considerable efforts towards the development of resistant cultivars.

The objective of the study was to investigate the genetic variability in the F1 hybrid progenies of tomato cultivars with different levels of resistance to *Fusarium* wilt race 2.

4.3 Materials and Methods

Parental lines

The six tomato cultivars used in this diallel (Griffing, 1956) study were Flora Dade, Rodade, Heinz 1370, Rossol, Moneymaker and Red Kaki. They were chosen as parental plants based on their variable resistance to *Fusarium* wilt race 2. Details of the disease resistance of these cultivars are listed in Table 4.1.

Table 4.1. *Fusarium* wilt resistance of six parental cultivars.

Cultivar	Disease rating	Company
Flora Dade	Resistant	Hygrotech
Rodade	Resistant	Hygrotech
Heinz 1370	Resistant	Kirshoff's
Rossol	Susceptible	Hygrotech
Moneymaker	Susceptible	Kirshoff's
Red Kaki	Susceptible	Kirshoff's

Development of F1-hybrids

The six cultivars were planted in seedling trays in the greenhouse at the University of Venda during March 2001. They were replanted in pots after four weeks. To synchronize crosses, two plantings were made, the first planting three weeks prior to the second. Plants used as females were emasculated during the late budding stage. Pollen from the male line was removed with a pinset and transferred to the stigma of the female plant. The stigma was then covered with a small bag to prevent any foreign pollen from entering. The pinset was cleaned before each transfer with an ethyl alcohol (90%) solution.

The six parents were then crossed in all possible combinations (Griffing, 1956). The fruit of the respective parental lines and the F1-hybrids were harvested after ripening. Their surfaces were disinfected with an ethyl (70%) alcohol solution. Fruit were then crushed to remove the seeds. Seeds were dried on clean sterilized filter paper and then sterilized using the hot water method described by Wager (1981). Seeds were washed with a surface disinfectant (0.5 to 1.0% sodium hypochlorite) at a concentration of 10 to 20% (Dodds and Roberts, 1995). The six parental lines and their 15 F1-hybrids were used as experimental material in this diallel study.

Fungal isolate

An isolate of *Fusarium* wilt race 2 was obtained from the Roodeplaat, Vegetable and Ornamental Plant Institute (ARC). The *Fusarium* isolate were first streaked out on potato dextrose agar (PDA) and then tested on a selective rosebengal-glycerine-urea agar medium, specially developed for isolation of *Fusarium* (Van Wyk *et al.*, 1986). A carnation leaf agar medium described by Nelson *et al.* (1983) was used for species identification. Race determination and verification were conducted according to methods described by Cirulli and Ciccareese (1982) and Venter (1990).

The *Fusarium* culture was cultivated on a PDA medium for eight days at 26° C under fluorescent and black - light cycles. Fungal mats containing micro- and macro-conidia were scraped and washed from the agar with sterile distilled water. Conidia were separated from the mycelium slurry by filtering the mixture through sterile cheesecloth and then washing the conidia using centrifugation (Alon *et al.*, 1974). The density of the conidial suspension of each isolate was determined using a haemocytometer. The concentration of the suspension was adjusted to 1 x 10^6 conidia per milliliter (Theron and Holtz, 1989; Elias and Schneider, 1991).

Disease assessment

Sterile seeds of the six parental lines and their 15 F1-hybrids were placed on a watery agar (WA) medium and incubated for seven days at 26°C (Pleban and Strobel, 1998). Viable seedlings were transplanted in new sterile trays containing 200 wells filled with sterilized soil. The parental and F1-hybrid seedlings were inoculated by dipping their roots in the fungal isolation medium. Seedlings were removed from the trays after seven days and replanted in trays in a randomize block design with 21 entries and four replications. The cultivar Red Kaki was planted as a control. Seedlings were watered daily and the temperature was maintained between 26 and 30°C.

The 200 seedlings in each entry are screened for symptoms. The following scale was used for disease assessment: 0, = no wilt (control); 1, slightly stunted, no wilting or yellowing, < 5% leaves affected; 2, slight yellowing and wilting, < 25% leaves affected; 3, moderate wilting, 25 to < 50% leaves affected; 4, severe wilting, 50 to < 75% leaves affected; 5 > 75% leaves wilted (Ramsey et al., 1992; Grattidge and O'Brien, 1982). The percentage resistant tomato seedlings (disease rating 0 and 1) were calculated for each parental line and the 15 F1-hybrid progenies.

Analysis of variance

An analysis of variance (ANOVA) was conducted on the percentage resistant plants 30 and 60 days after inoculation. The 30 and 60 day disease data were subjected to a combined analysis of variance to study the variability between entries and time intervals. The disease progress (difference in percentage resistant plants between 30 and 60 days) was also subjected to an analysis of variance.

4.4 Results and Discussion

Combined Analysis of variance

The data showed significant differences for *Fusarium* wilt resistance between the cultivars and F1-hybrids after 30 and 60 days (Table 4.2). Significant differences for percentage resistant plants were found between 30- and 60 day intervals. The entry x day interaction was significant which is an indication that genotypes reacted differently to *Fusarium* wilt at different time intervals.

A separate analysis of variance was also conducted on the disease data at the 30 and 60 days intervals and for disease progress. Significant differences were found between entries at both 30 and 60 day intervals and for disease progress (Table 4.3).

30 Day assessment. (Screening for early resistance on day 30)

The percentage of resistant plants in Red Kaki, Flore Dade, Rodade and Heinz 1370 exceeded 85% and were significantly greater than in Rossol and Moneymaker which had the lowest number of resistant plants after 30 days (Fig 4.1). The percentage resistant plants in Rossol (75%) exceed those in Moneymaker. Flora Dade and Rodade contained both I_1 and I_2 resistance loci to *Fusarium oxysporum* f. sp. *lycopersici* race 1 and 2, while Heinz 1370 and Rossol contained resistance loci I_1 to *Fusarium oxysporum* f. sp. *lycopersici* race 1 only (Wager 1981; Uys, 1996). No indication of single resistant genes against *Fusarium o.* f. sp. *lycopersici race* 1 or race 2 could be found in Red Kaki and Moneymaker. Both Uys (1996) and Wager (1981) described the tomato cultivar Red Kaki as having no known resistant genes to *Fusarium* wilt race 2. Moneymaker is known to be extremely susceptible to *Fusarium* wilt (Kroon *et al.*, 1991; Moustafa and Khafagi, 1992).

Table 4.2: Combined ANOVA of parental inbred lines and their F1 progenies for resistance to *Fusarium* wilt.

Source	DF	Sum of Squares	Mean Square	F-ratio
Total	167	113480.48		
A: Entries	20	75261.23	3763.06	42.24**
B: Day	1	13933.93	13933.93	156.39**
AB combined	20	12356.82	617.84	6.93*
Replication	3	969.62	323.21	3.63**
Error	123	113480.48	89.10	

*Significant

**Highly Significant

Table 4.3: Variance analysis of parental inbred lines and their F1 progenies for resistance to *Fusarium* wilt.

Source	DF		SS			MS			F-values	
		30days	60days	Disease progress	30days	60days	Disease progress	30days	60days	Disease progress
Total	83	48353.67	49710.99	27622.99						
Entries	20	43494.67	43172.24	23072.24	2174.73	2158.61	1153.61	28.66**	22.22*	15.39**
Blocks	3	306.05	710.61	54.61	102.02	236.87	18.20	1.34	2.44**	00.24
Residual	60	4552.95	5828.14	4496.14	75.88	97.14	74.94			

*Significant

**Highly Significant

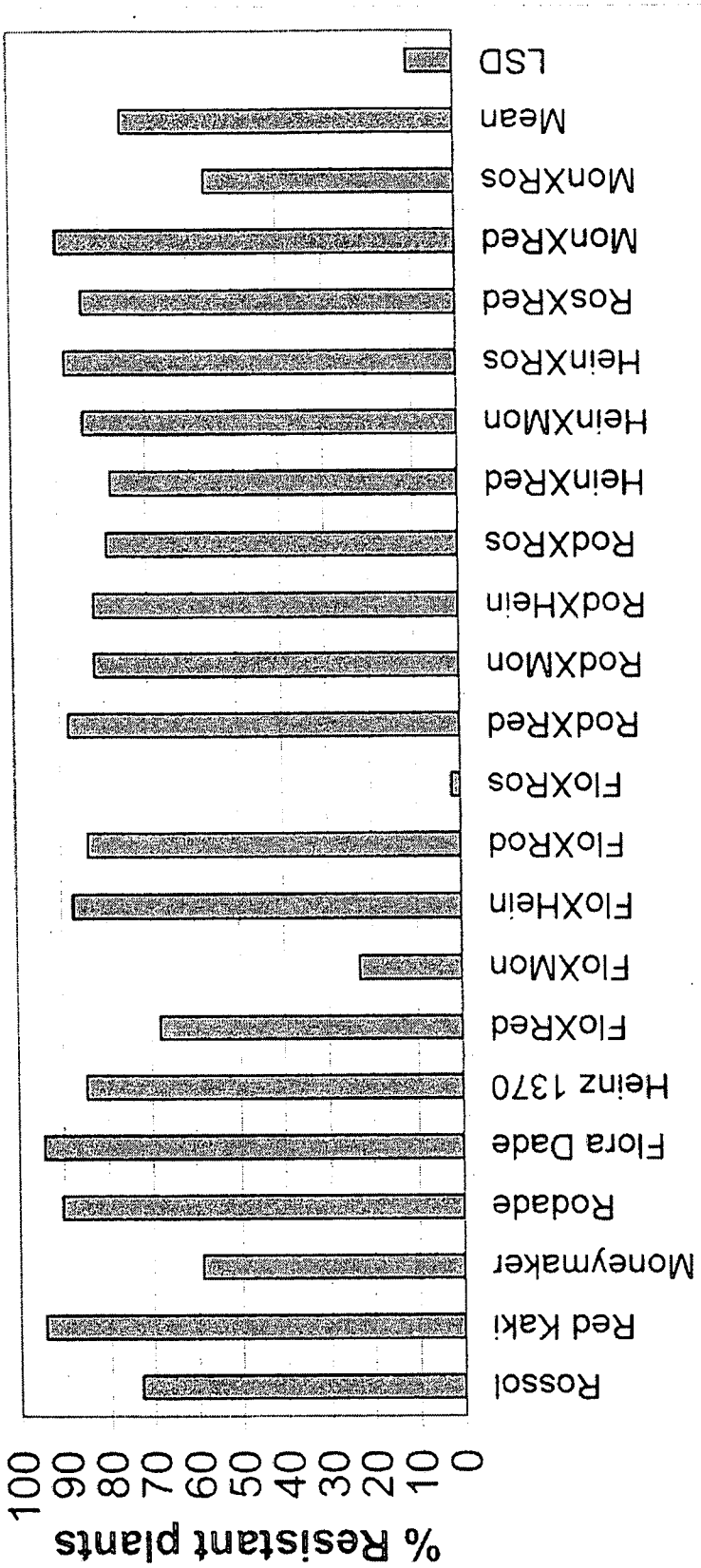

Figure 4.1. Percentage resistant plants against *F.o. lycopersici* of parental inbred lines and their F1 hybrids on day 30.

Eleven of the F1-hybrids shared high levels of resistance against the *Fusarium* wilt isolate. The percentage of resistant plants in these F1-hybrids, Flo X Hein, Flo X Rod, Rod X Red, Rod X Mon, Rod X Hein, Rod X Red, Rod X Ros, Hein X Mon, Hein X Ros, Ros X Red, Mon X Red exceeded 70%. The number of resistant plants in these crosses were significantly higher than in the four remaining crosses, Flo X Red K, Flo X Mon, Flo X Ros and Mon X Ros. Resistant plants in Flo X Red K significantly exceeded those in Flo X Mon, Flo X Ros and Mon X Ros. Mon X Ross responded significantly better than Flo X Ros and Flo X Mon. The latter crosses had the lowest percentage of resistant plants (<25%). Heinz 1370 and Rodade inherited some resistance genes. Although Red Kaki did not have any major resistance genes to *Fusarium* wilt, their F1 hybrids have shown good resistance to *Fusarium* wilt race 2.

60 days assessment

The number of resistant plants in Rodade, Flora Dade and Heinz exceeded 60% and was significantly higher than in Moneymaker, Red Kaki and Rossol (Fig 4.2). The percentage resistant plants in Moneymaker and Red Kaki exceeded that of Rossol, which were extremely low (12 %) after 60 days.

The percentage resistant plants in the F1 crosses Flo X Heinz, Rod X Red K, Rod X Mon, Rod X Ros, Hein X Mon, Hein X Ros, Mon X Red K were close to or exceeded 80% and were significantly higher than Flo X Red K, Flo X Rod and Mon X Ros. Resistant plants in Mon X Ros was significantly higher than the crosses Flo X Mon and Flo X Ros. Flo X Mon with 20% resistant plants had significantly more resistant plants than Flo X Ros, which was extremely susceptible after 60 days.

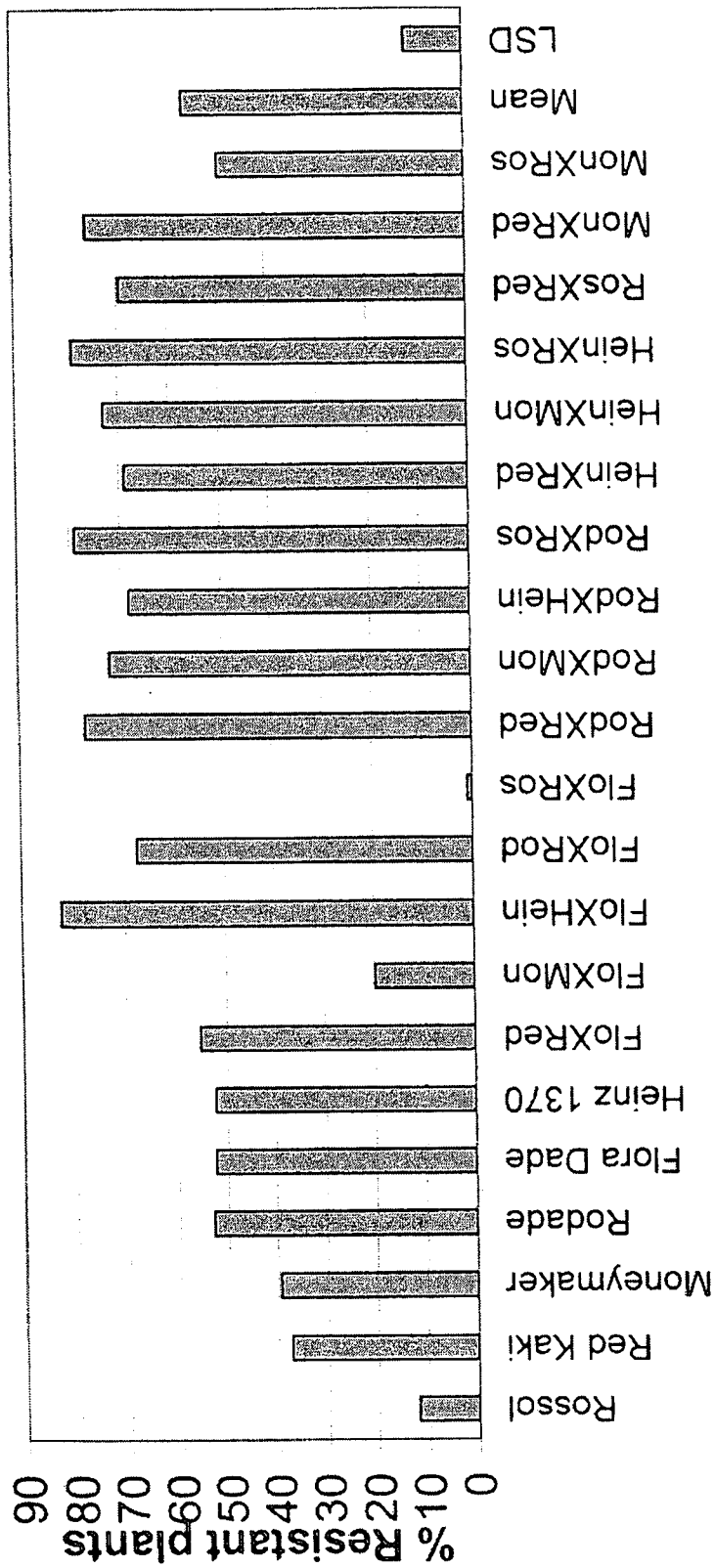

Figure 4.2. Percentage resistant plants against *F.o. lycopersici* of parental inbred lines and their F1 hybrids on day 60.

The parental cultivars Heinz 1370, Moneymaker, Rossol and Red Kaki produced F1 hybrid combinations between each other that were highly resistant to *Fusarium* wilt race 2. None of these cultivars are known to have the I2 resistant gene. Sela-Buurlage (2001) discovered that different resistant loci on different chromosomes display overlapping resistant functions against different *Fusarium* races. The most susceptible cultivar Rossol transferred it's susceptibility to two of the hybrids namely, Flo X Ros and Mon X Ros. Moneymaker with a low level of resistance to *Fusarium* wilt, transferred its susceptibility to two of its crosses, Mon X Flo and Mon X Ros. Although Red Kaki was classified as susceptible, due to the absence of major resistance genes to *Fusarium* wilt, it combined extremely well in most crosses with other cultivars. It was evident that most of the F1's inherited resistance from their parents made them more resistant than their parents (Falconer and Mackay, 1996).

Disease progress

The disease progress between 30 and 60-day intervals was calculated for each entry (Fig 4.3). The disease development in the cultivars Rossol and Red Kaki was significantly higher than the rest of the cultivars. This was due to a very sharp reduction in the percentage resistant plants between 30 and 60 days. In the case of Rossol, the percentage resistant seedlings decreased from 73% to 11%. The disease progress in Rodade, Flora Dade and Heinz 1370 exceeds 30% and was significantly higher than in Moneymaker which had the lowest disease progress of all the cultivars (Kroon *et al.*, 1991). Moneymaker displayed some horizontal resistance with a disease progress of only 20%.

This disease progress of the 15 F1-hybrids was extremely low. It was less than 20% indicating a much slower disease development in hybrids. The disease progress in four of the crosses Flo x Mon, Flo x Ros, Rod x Ros and Mon x Ross were less than 10%. The cultivars with the lowest disease progress were

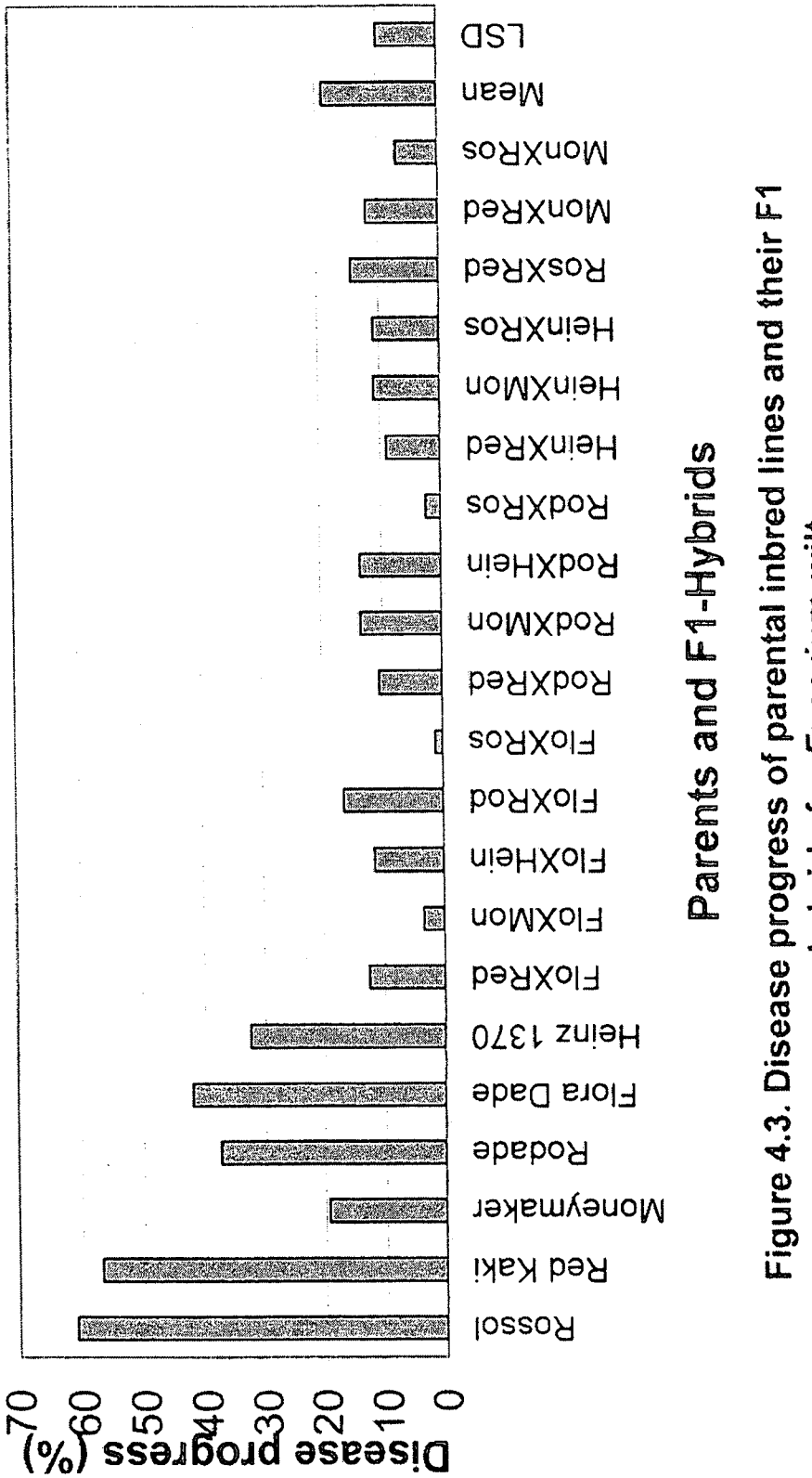

Figure 4.3. Disease progress of parental inbred lines and their F1 hybrids for *Fusarium* wilt.

not necessarily involved in these crosses. The cultivar Moneymaker with the best disease progress was only involved in two of the crosses, while Rossol with the highest disease progress was involved in four of the crosses. The good performance of Rossol could only be due to large heterotic effects in combination with other cultivars, which resulted in strong vigorous growing plants that were less susceptible to *Fusarium* wilt.

The disease progress of the F1-hybrids was significantly lower than the inbred cultivars. This is probably due to hybrid vigour caused by heterotic effects in the F1-generation. This result is consistent with Straathof *et al.* (1997), Van Eijk and Eijkelboom (1983) who also found an increase in the number of resistant seedlings in F1-hybrids after infection with *Fusarium* wilt. Mes *et al.*, (2000) reported similar results.

4.5 Conclusions

The entry x day interaction for resistant plants was highly significant in the combined analysis of variance. It indicates an interaction between genotypes and time of disease assessment. *Fusarium* wilt assessment should therefore be postponed until at least 60 days after inoculation.

Significant differences for *Fusarium* wilt resistance was found between entries after 30 and 60 days. The variability after 60 days was significantly larger than after 30 days, indicating larger differences between resistant and susceptible entries. Sufficient levels of variability were demonstrated which can be utilized by plant breeders to develop tomato cultivars with high levels of *Fusarium* wilt resistance.

The cultivars Red Kaki, Flora Dade, Rodade and Heinz 1370 had the highest level of resistance 30 days after inoculation, while Rodade, Flora Dade and Heinz 1370 had the best resistance after 60 days. These cultivars could be used as parental lines in a crossing block to enhance *Fusarium* wilt resistance in local breeding material.

Eleven of the F1-hybrids had good levels of resistance after 30 days, while only seven of the F1-hybrids Flo x Heinz, Rod x Red K, Rod x Mon, Rod x Ros, Hein x Mon, Hein x Ros and Mon x Red K showed good levels of resistance after 60 days. These crosses possessed sufficient resistance, which can be successfully utilized in an early generation selection program.

Moneymaker, Rodade, Flora Dade and Heinz 1370 had the best disease progress after 30 and 60 days assessment. The disease progress in five of the F1-hybrids was less than 10%. The parental lines with the best disease progress did not necessarily transfer its superiority to its entire offspring. Therefore, additional information about the combining abilities of the parents is necessary to decide which offspring will yield the best *Fusarium* wilt resistance. The low level of disease progress in the F1-hybrids emphasizes the important contribution, which F1-hybrids can make to increase *Fusarium* wilt resistance in tomato hybrids.

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CHAPTER 5

Combining and heritability studies for resistance to *Fusarium* wilt in Tomato.

5.1 Abstract

The objective of this study was to determine the heritability of *Fusarium* wilt resistance in tomato. Six inbred tomato cultivars Rossol, Red Kaki, Moneymaker, Rodade, Flora Dade and Heinz 1370 were crossed in a diallel configuration to produce 15 F1-hybrids. Assessment for *Fusarium* wilt resistance were done after 30 and 60 days and for disease progress. Significant differences were found for GCA- and SCA- effects. The GCA: SCA ratio's for 30 and 60 days assessments indicates, both additive and non-additive gene action. Disease progress was mainly control by dominant effects. The narrow sense heritabilities for *Fusarium* wilt resistance were respectively 12% and 8% after the 30 and 60 days. Significant levels of heterosis were found in crosses for *Fusarium* wilt resistance. The cultivar Heinz 1370 have the largest GCA-effects while the cross Rod x Ross possessed the largest SCA-effects.

5.2 Introduction

Until 1940 inbred tomato cultivars were used by farmers for commercial production. Since then the tomato industry suffered heavy losses due to the dreaded wilt disease (Basset, 1986). The survival of the tomato industry depends on the selection and use of resistant genes against *Fusarium* wilt. Alleles which shows resistance to *Fusarium* wilt race 2 was found on the I2 locus (Simons *et al.*, 1998; Sela-Buurlagen *et al.*, 2001). Back-crossing methods were used to transfer the resistance genes originated from wild tomato relatives to adopted local cultivars. Therefore, most current tomato varieties possess a single resistant gene against *Fusarium* wilt race 1 and 2. The problem with this strategy is that new vegetative compatible groups of *Fusarium* wilt races, which could overcome the resistance may appear at any time after the introduction of new resistant cultivars. For this reason, breeders should investigate other breeding strategies which will include polygenic resistant genes.

Identifying parental lines with superior breeding values (combining abilities) for *Fusarium* wilt resistance, will be a good starting point to enhance the polygenetic control of the disease. Several researchers (Garretsen and Eijkelboom, 1979; Sing *et al.*, 1986) determine the combining ability of the parents for *Fusarium* resistance in various crops. Van Eijk and Eijkelboom (1983) found that resistance to *Fusarium oxysporum* in tulip bulbs appears to be largely based on additive gene action and most of the resistant parental cultivars show high general combining ability values. Hartman and St. Clair (1999) investigated the combining ability for Beet Armyworm (BWA) in tomato and observed significant male and female General Combining Ability (GCA) values for BWA, but detected no significant Specific Combining Ability (SCA) values for BWA resistance.

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Little information is available on the heritability of *Fusarium* wilt resistance in tomato. The importance of heritabilities lies in their predictive function as well as the expression of reliability of the phenotypic value as a guide to the breeding value. The heritability of a polygenetic character has a large effect on the response to selection when selection is applied for *Fusarium* wilt resistance in early generation populations.

The aim with hybridization is to obtain new lines or varieties that is superior to the best current commercial cultivars available (Yonezawa *et al.*, 1999). Crossing the inbred parental cultivars usually show a more vigorous hybrid offspring than either of the parental strains separately. Hybrids are mostly superior to inbred lines, due to an increase in fitness (e.g. size, fertility, vigor, longevity and resistance to diseases), referred to as heterosis or hybrid vigor (Falconer, 1981). Tomato hybrids generally do not show yield advantages compared to other inbred cultivars (Basset, 1986). The advantage to tomato hybrids appear to be derived from improved earlyness as well as better consistency in performance, particularly under less than optimal growing conditions (Yordanov, 1983). The objective of this study was to:

i) calculate the combining abilities of locally grown tomato cultivars

ii) determine the heritability of *Fusarium* wilt resistance in tomato

iii) investigate the possible use of hybrid vigor as a means to improve *Fusarium* wilt resistance in F1-hybrids.

5.3 Materials and Methods

Parental lines

The six tomato cultivars used in this diallel study were Flora Dade, Rodade, Heinz 1370, Rossol, Moneymaker and Red Kaki. They were chosen as parental plants on because of their variability in *Fusarium* wilt race 2 resistance. Detail of the disease resistance of these cultivars were listed in Table 5.1.

Table 5.1. *Fusarium* wilt resistance of six parental cultivars.

Cultivar	Disease rating	Company
Flora Dade	Resistant	Hygrotech
Rodade	Resistant	Hygrotech
Heinz 1370	Resistant	Kirchoffs
Rossol	Susceptible	Hygrotech
Moneymaker	Susceptible	Kirchoffs
Red Kaki	Susceptible	Kirchoffs

Development of F1-hybrids

The six cultivars were planted in seedling trays in the greenhouse at the University of Venda during March 2001. It was replanted in pots after four weeks. To sinchronize the crosses two plantings were made, the first planting three weeks prior to the second. The plants used as females were emasculated during the late budding stage. The pollen from the male line was removed with a pinset and transferred to the stigma of the female plant.

The stigma was then covered with a small bag to prevent any foreign pollen from entering. The pinset was cleaned before each transfer with 90% ethyl alcohol

solution. The six parents were then crossed in all combinations. The fruit of the respective parental lines and the F1 hybrids were harvested after ripening. They were surface sterilized with a 70 % ethyl alcohol solution to remove all pathogens on the surface. The fruit were chrushed to remove the seeds. The seeds were dried on clean sterilized filter paper. It was then sterilized using the hot water method described by Wager (1981). Afterwards, the seeds were washed with a surface disinfectant (0.5 to 1.0% sodium hypochlorite) at a concentration of 10 to 20% (Dodds and Roberts, 1995). The six parental lines with their 15 F1-hybrids were used as experimental material in this diallel study.

Fungal Isolate

An isolate of *Fusarium* wilt race 2 was obtained from the Roodeplaat, Vegetable and Ornamental Plant Institute (ARC). The *Fusarium* isolate was first streaked out on Potato Dextrose Agar (PDA) and then tested on a selective Rosebengal-glycerine-urea agar medium, specially developed for isolation of *Fusarium* (Van Wyk *et al.*, 1986). A carnation leave agar medium described by Nelson *et al.* (1983) was used for species identification. Race determination and verification was done according to methods described by Cirulli and Ciccarese (1982) and Venter (1990).

The *Fusarium* culture was cultivated on a PDA medium for eight days at 26° C under fluorescent, black and light cycles. Fungal mats containing the micro- and macro-conidia were scraped and washed from the agar with sterile distilled water. Conidia were separated from the mycelium by filtering the mixture through sterile cheesecloth and the conidia washed using centrifugation (Alon *et al.*, 1974). The density of the conidia suspension of each isolate was determined using a haemaytometer. The suspension concentration was adjusted to 1×10^6 conidia per milliliter (Theron and Holz, 1989; Elias and Schneider, 1991).

Disease assessment

The sterile seeds of the six parental lines and their 15 F1-hybrids were place in aseptic petri dishes containing a Watery Agar (WA) medium and incubated for seven days at 26° C (Pleban and Strobel, 1998). Only viable seedlings were transplanted in new sterile trays containing 200 wells. These trays were filled with sterilized soil. The parental and F1-hybrid seedlings were inoculated by dipping their roots in the fungal isolation medium. Seedlings were removed from the trays after seven days and replanted in another set of trays in a randomize block design with 21 entries and four replications. The cultivar Red Kaki was planted as a susceptible control. Seedlings were watered daily and the temperature maintained between 26 and 30 ° C.

Each of the 200 seedlings within each entry was screened for disease resistance against *Fusarium* wilt race 2. The following scale were used for disease assessment: 0, indicating no wilt; equivalent to the control; 1, slightly stunted, no wilting or yellowing, < 5% leaves affected; 2, slight yellowing and wilting, < 25% leaves affected; 3, moderate wilting, 25 to < 50% leaves affected; 4, severe wilting, 50 to < 75% leaves affected; 5 > 75% leaves wilted (Ramsey *et al.*, 1992; Grattidge and O'Brien, 1982). The percentage resistant tomato seedlings (disease rating 0 and 1) were calculated for each parental line as well as for the 15 F1-hybrid progenies.

Diallel analysis

In any diallel experiment the same set of parents are used on both axes, with the same parental inbred lines serving as both male and female donors. In this experiment a fixed-effect interpretation were used, and the parents were the only genotype that varied. The goal with the diallel analysis was to estimate the

genetic variability of the specific cultivars and their hybrid offspring to *Fusarium* wilt race 2 and thus to identify higher yielding parental combinations.

An incomplete diallel cross with no reciprocal crosses as well as no crosses within the parental lines were used to determine the general combining abilities of the parental inbred lines as well as the specific combining abilities of their F1 progenies. The difference between the mean performance of the progeny of a given male and the mean of the progeny for all males within that population are called the general combining ability (Kearsey and Pooni, 1996). In this study no disthingtion was made as to which plant served as paternal or maternal parents. This reflects how well the genes of male's combine on average with those of females. The assumption were made that there would be no paternal effects (aside from direct inheritance) as well as no significant sex-linked effects, and the fact that reciprocal crosses are expected to yield equal progeny, phenotypic distribution (Lynch and Walsh, 1998).

In the absence of dominance or epistasis, it should be possible to predict the mean performance of the progeny as a cross between the specific male and female as the GCA value (Kearsey and Pooni, 1996). Any significant deviation from this GCA value has to be due to either dominance or epistatic effects. These deviations, specific for each individual cross, are referred to as specific combining ability values and are measured by the mean squares (MS) illustrated in Table 5.2 of the diallel cross experiment.

Combining ability

The percentage resistant plants was calculated for each cultivar after 30 and 60 days. The difference between the two intervals were used to calculate the disease progress from 30 to 60 days. The general combining ability (GCA) and specific combining ability (SCA) were then calculated using the average

Table 5.2: Anova for combining ability analysis (Method 2, Model 1 of Griffing, 1956).

Source	Degree of freedom (DF)	Sum of squares (SS)	Mean square (MS)	E (MS)	Variance component estimates
General combining ability (G.C.A.)	n-1	Sg	Mg = s1/n-1	$\delta^2e + \delta^2 s + (n+2)\delta^2g$	$\delta^2g = \frac{1}{n+2}(Mg - Ms)$
Specific combining ability (S.C.A.)	n(n-1)/2	Ss	$Ms = \frac{s2}{n(n-1)/2}$	$\delta^2e + \delta^2s$	$\delta^2s = Ms - Me$
Error	rb-1	Se	Me = s3/rb-1	δ^2e	$\delta^2e = Me$

This table was derived from Singh and Chaudhry (1979), and figures in columns for DF and SS substituted with appropriate symbols and formulae.

resistant plant (ARPs) values of the six homozygous inbred lines and their 15 F1 hybrid progeny populations.

The results of the diallel experiment were analyzed using the ACB statistics command from analysis of variance sub-menu of Agrobase (2000). The analysis provided means, mean squares, F-value and probability levels of significance, least significant differences (LSD) and coefficient of variation (CVs).

Method 2, Model 1, of Griffing (1956) was used for this diallel analysis.

Where;

$$Y\ddot{y} = m + gi + gj + sij + \frac{1}{bc}\Sigma\Sigma eijkl$$

i,j = 1,2 ....., n

k = 1,2 ....., b

l = 1,2 .....,c

Where m is the population mean
Yij is the mean of ixjlt genotype over k and l
gi is the general combining ability (g.c.a.) effect of ilt parent
gj is the general combining ability (g.c.a.) effect of jlt parent
sij is the interaction, i.e. specific combining ability (s.c.a) effect
1/bcΣΣeijkl is the mean error effect.

The calculation of the different parameters is shown in Table 5.2.

General combining ability effects were estimated using the formula:

$$gi = 1/n+2 [(Yi + Yii) - 2Y/n..]$$

Specific combining ability effects were estimated using the formula:

$$Sij = Yij - 1/n+2 (Yi + Yii + Yj + Yjj) + 2Y/(n+1)(n+2)..$$

Heritability

i) Narrow sense heritability was calculated from the formula:

$$h^2 = \delta^2A/\delta^2P$$

where δ^2A = Additive genetic variance = $2\delta^2$g.c.a.

δ^2G = Total genetic variance = 2 δ^2g.c.a. + δ^2s.c.a.

δ^2P = Phenotypic variance = δ^2 g + δ^2e

thus:
$$h^2 = \frac{2 \text{ g.c.a.} + \delta^2\text{s.c.a.}}{2 \text{ g.c.a.} + \text{sca} + \delta^2 e}$$

ii) Broad sense heritability was calculated as follow

$$h^2_b = \frac{2\delta^2 \text{ g.c.a.} + \delta^2 \text{ s.c.a.}}{2\delta^2 \text{ g.c.a.} + \delta^2 \text{ s.c.a.} + \delta^2 s_e}$$

The highest parent heterosis (H_{HP}) was calculated as follow :

$$H_{HP} = \frac{F1 - P_{HP}}{P_{HP}} \times \frac{100}{1}$$

where H_{HP} = highest parent heterosis

P_{HP} = highest parent value

5.4 Results and Discussion

I Diallel analysis

The diallel analysis (Table 5.3) revealed significant GCA effects between the parental lines at 30 and 60 day assessments as well as for disease progress. Significant SCA effects were found between crosses at 30 and 60 day assessments and for disease progress.

The GCA: SCA ratio's can be used to determine if the trait under investigation is controlled by additive or non-additive gene action (Sing *et al.*, 1986). A large GCA: SCA ratio indicates additive gene action that prevails over dominance and it also imply the presence of heterosis (Quick, 1978; Sayed, 1978).

The GCA: SCA ratio for the 30 and 60 day assessments was respectively 1.5 : 1.0 and 1.9 : 1.0 indicating larger additive gene action. The GCA ratio for disease progress was 0.32 : 1 indicating larger non-additive gene effects. Therefore, disease progress is mainly under the control of dominant genes.

II General and specific combining abilities

General combining ability

The general combining ability (GCA) is also equal to the breeding value for a specific individual (Lynch and Walsh, 1998). General combining ability describes the average performance of a parent line in hybrid combination with other similar genotypes (Lynch and Walsh, 1998). The GCA values for the individual cultivars for 30 and 60 day assessments and disease progress are listed in Table 5.4. The cultivars Rodade, Heinz 1370 and Red Kaki have large

Table 5.3: Diallel analysis based on average disease ratings (ADR) obtained after testing F1 hybrid seedlings infected with *F. o.* f. sp. *Lycopersici* race 2(P<0.001).

Source	DF		SS			MS			F-values	
		30days	60days	Disease progress	30days	60days	Disease progress	30days	60days	Disease progress
Total	20	10873.67	10729.24	5768.07						
GCA	5	3709.85	3326.23	570.89	741.97	665.25	114.18	39.11	27.42	6.09
SCA	15	7163.81	7403.01	5197.17	477.59	493.53	346.48	25.18	20.34	18.49
Residual	60	4552.95	5822.86	4496.14	18.97	24.26	18.73			

Table 5.4 General combining ability (GCA) effects for *Fusarium* wilt resistance.

Cultivar	30 days	60 days	Disease Progress
Flora Dade	-8.79	-7.98	-0.48
Rodade	9.96	9.24	-0.20
Heinz 1370	8.21	10.37	-1.51
Rossol	-8.60	-11.67	3.15
Moneymaker	-8.92	-3.39	-5.89
Red Kaki	8.15	3.43	4.93
LSD	4.36	4.93	4.33

Table 5.5 Specific combining ability (SCA) effects for *Fusarium* wilt resistance.

Crosses	30 days	60 days	Disease Progress
Flo X Rod	8.00	9.80	-1.80
Flo X Heinz	13.25	23.67	-5.5
Flo X Ros	-55.44	-36.04	-20.40
Flo X Mon	-34.13	-25.08	-9.20
Flo X Red K	-6.19	3.61	-11
Rod X Hein	-11.00	-7.54	-3.80
Rod X Ros	2.81	24.99	-19.50
Rod X Mon	13.63	8.21	0.57
Rod X Red K	-4.94	8.39	13.20
Hein X Ros	13.56	23.86	-9.70
Hein X Mon	9.88	9.58	-0.62
Hein X Red K	-13.19	-1.23	-13.4
Ros X Mon	-1.31	8.11	-9.27
Ros X Red K	9.63	21.30	-12.60
Mon X Red K	7.94	19.52	-6.05
LSD	4.36	4.93	4.33

positive GCA values for *Fusarium* wilt resistance after 30 day assessment. Their GCA effects were significantly larger than the remaining three cultivars, Flora Dade, Rossol and Moneymaker.

The cultivars Rodade and Heinz 1370 had larger positive GCA effects for *Fusarium* wilt resistance after 60 days assessment. Their GCA effects were significantly larger than the rest of the cultivars. Red Kaki had the third largest GCA effect, which was significantly larger than Flora Dade, Moneymaker and Rossol. Moneymaker had by far the lowest GCA-effect for disease progress, which was significantly lower than the GCA-effects of the remaining cultivars. The cultivars Heinz 1370, Flora Dade and Rodade had the second lowest GCA-effects with no significant differences between them. Their disease progress were significantly lower than the cultivars Red Kaki and Rossol which possess the poorest disease progress.

The rankings of the cultivars GCA-effects differs largely with regard to the 30 and 60 day assessment and disease progress. The rankings for Flora Dade were fifth, fifth and third respectively, for Moneymaker it was six, fourth and first and for Red Kaki it was third, third and sixth, respectively. These changes in the rankings of the GCA-effects was caused by genotype x disease assessment interaction and will complicate the task of breeders to decide which set of combining abilities to use.

Specific combining abilities

Specific combining ability describes the degree to which specific parental combinations could lead to a deviation in progeny phenotypes from the expectations based on their average parental performance (Lynch and Walsh, 1998). The combining abilities of the 15 F1 hybrid crosses for *Fusarium* wilt resistance are listed in Table 5.5.

The crosses with the largest positive specific combining abilities at the 30 day assessment were Rod x Mon, Hein x Ros, Flo x Hein. Their combining abilities were significantly larger than the remaining crosses. The only exceptions were Hein x Mon and Ros x Red K. The two crosses Flo x Rod and Mon x Red K had the second largest positive SCA-effects, which were significant larger than the remaining crosses which possessed either very low positive or negative SCA-effects.

The crosses with the largest positive SCA-effects at the 60 day assessment were Rod x Mon, Hein x Ros, Flo x Hein, Ros x Red K and Mon x Ros. Their SCA-effects were significantly larger than the rest of the crosses. The two crosses with the best SCA-effects for disease progress were Flo x Ros and Rod x Ros. The SCA-effects were significantly lower than the rest of the crosses. It seems that the cultivar Rossol which is very susceptible, carries some specific genes which slow down the disease progress when it's used in combination with Flo and Rod. The other crosses which possessed good disease progress were Hein x Ros, Ros x Mon, Ros x Red K, Hein x Red K and Flo x Mon. The cultivar Ros was involved in three of the crosses. The GCA-effects of the rest of the crosses indicate poor disease progress.

III) Heritability of the *Fusarium* wilt resistance in tomato

Heritability indicates the proportion of total variation due to genetic variance (Falconer, 1981) and is formulated in different ways according to the selection procedure adopted (Yonezawa *et al.*, 1999). The broad sense heritability measures the ratio of total genetic variation to the total phenotypic variation. Under controlled environmental conditions narrow sense heritability measures the proportion of the variation that is due to additive effects of the genes in a specific population (Kearsey and Pooni, 1996). The broad and narrow sense

heritabilities for *Fusarium* wilt were given in Table 5.6. The narrow sense heritabilities after 30 and 60 days were 12,15% and 8.00% respectively. These extremely low heritabilities will slow down the accumulation of additive genes when selection is applied for *Fusarium* wilt resistance.

Table 5.6. Narrow and Broad sense heritabilities for *Fusarium* wilt resistance in tomato.

Heritabilties	30 days	60days	Disease Progress
H_n	12.15	8.00	___
H_b	96.51	95.47	___

The broad sense heritabilities after 30 and 60 days was 96.5% and 95.4% respectively. The large differences between the narrow and broad sense heritabilities was due to the large expected variance component for dominance. The heritability for disease progress was negative, caused by the negative expected variance component for additive effects.

IV) Heterosis

When inbred cultivars were crossed, the progeny show an increase in the characters that previously suffered a reduction due to inbreeding, and the fitness lost, tends to be restored. The amount of heterosis can also be viewed as the difference between the crossbred and inbred means (Falconer and Mackay, 1996). The amount of heterosis produced by the F1 hybrid crosses were measured as the deviation from the mid-parent value. In order to do this every mean of the parental population combination were divided by two and this mid-parent value were deducted from the specific F1 hybrid mean. Heterosis thus depends for its occurrence on the existence of phenotypically expressed dominance. The amount of heterosis of a cross between two particular populations also depends on the square of the difference of the gene frequency

between the populations and if the populations crossed did not differ in gene frequencies there would be no heterosis (Falconer and Mackay, 1996).

The percentage highest-parent heterosis (HP) for *Fusarium* wilt race 2 resistance after 30 and 60 days were listed in Table 5.7. The percentage heterosis found for *Fusarium* wilt resistance after 30 days were negative in fourteen of the fifteen crosses. Hein x Ros was the only exception with a positive heterotic effect. The percentage heterosis after 60 days were highly positive for most of the crosses. The only exceptions were Flo x Mon (-61,9%) and Flo x Ros (-89,6%) with high negative heterotic effects. The F1-hybrid Mon x Red K had an extremely high heterotic effect for *Fusarium* wilt resistance after 60 days. The result indicates a significant increase in heterotic effect for *Fusarium* wilt resistance in the F1 hybrids from 30 to 60 days. This increase in heterotic effects is probably the result of an increase in hybrid vigor during the first 60 days of plant development.

The heterotic effects for disease progress were mainly large and negative. It's an indication that the disease progress in the F1-hybrids were much lower than in the parental population.

5.5 Conclusions

Significant variability was found between the GCA-effects of the cultivars and the SCA-effects of the F1-hybrids. The GCA: SCA ratio's for 30 and 60 days assessments accentuate the importance of both additive and non-additive gene action. Disease progress is mainly the control of non-additive gene action indicating the involvement of specific gene combinations. The narrow sense heritabilities for *Fusarium* wilt were to low to gain any response with selection in a short time. Significant levels of heterosis were found after 60 days and for disease progress.

Table 5.7: Highest parent heterosis for *Fusarium* wilt race 2 resistance in 15 F1 tomato hybrids.

Cultivars	30 Days	60 Days	Disease Progress
Flo X Red K	-28.04	5.71	-78.07
Flo X Mon	-75.70	-61.91	-91.67
Flo X Hein	- 7.41	57.14	-72.62
Flo X Rod	- 0.11	1.89	-60.71
Flo X Ross	- 97.8	-89.57	-97.95
Rod X RedK	-6.88	46.23	-81.58
Rod X Mon	-9.39	36.79	-64.00
Rod X Hein	-89.47	29.25	-64.00
Rod X Ross	-12.71	49.06	-95.90
Hein X Red K	-17.46	31.43	-84.21
Hein X Mon	-1.18	39.05	-66.15
Hein X Ross	3.53	50.48	-81.97
Ross X Red K	-11.11	84.00	-76.23
Mon X Red K	-5.29	92.40	-80.33
Mon X Ross	-5.09	25.32	-88.53

Although significant variability exists among the cultivars with regard to their GCA-effects, the heritability of *Fusarium* wilt resistance was to low to make any progress by means of accumulating additive resistant genes. Since *Fusarium* wilt resistance is mainly controlled by non-additive gene action, breeders should rather exploid the development of hybrids as a means to solve the problem of *Fusarium* wilt in tomato. The significant levels of heterosis for *Fusarium* wilt obtained in this study, also confirm the importance of tomato hybrids to cope with this disease. The hybrid which possessed the best resistance in the study was Rod x Ross.

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CHAPTER 6

Investigating the presence of I_2 loci resistance genes in local tomatoes, using PCR marker technology.

6.1 Abstract

PCR technology with specific DNA primers and tomato leaf cDNA was used to investigate the presence of different single genes in six local tomato cultivars. These cultivars were Flora Dade, Rodade, Moneymaker, Heinz 1370, Rossol and Red Kaki. The presence of the single genes I2C1, I2C2, I2, I2C3 and I2C5 were tested for. These genes are suspected of being responsible for *Fusarium* wilt race 2 resistance. The I2C1 and I2C2 genes were identified in all cultures except for Rossol which did not have the I2C1 gene. No I2C3 or I2C5 genes were identified. I2 genes were only identified in Flora Dade and Rodade, which was mainly responsible for it's *Fusarium* wilt resistance. No significant relationship could be found between I2 resistance alleles of the cultivars and their breeding values.

6.2 Introduction

Fusarium oxysporum f. sp. lycopersici race 2 is a soil-borne fungal pathogen that causes wilting disease in tomatoes by infecting their roots via direct penetration or wound infections. After penetrating the xylem vascular tissue of the plants the fungal spores colonize and block off the water passage to the leaves (Pietro *et al.*, 2001). Genetic resistance to *Fusarium* wilt race 2 was first discovered in *L. pimpinellifolium* and then the I_2 locus was transferred to other *L. esculentum* cultivars (Stall and Walter, 1965; Cirulli and Alexander, 1966). Resistance against a particular fungal isolate are caused by a single resistance gene called a "major or R-gene" and race-specific resistance usually exhibit a stronger resistance against some fungal isolates than towards other isolates (Gatehouse *et al.*, 1992). The presence of the virulent gene in *Fusarium* allows the product of the plant R gene to recognize the specific fungal pathogen race and a resistant response occur. According to the gene-for-gene hypothesis, the dominant I2 gene in resistant tomatoes cultivars resulted in a dominant avirulence (Avrl-2) gene, present in *Fusarium* wilt race 2 species (Mes *et al.*, 1999). Mes *et al.* (1999) also discovered a difference in resistance to vegetative groups of *Fusarium* wilt race 1 and 2 due to the presence or absence of the I_1 or I_2 resistance loci. Sela-Buurlage *et al.* (2001) predicted that the I_1 and I_2 loci reside on different arms of chromosome 11.

Simons *et al.* (1998) discovered that the I_2 locus was composed of seven homologous, each spanning a region of 90 kb with intergenic regions separating homologous of 8 to 10 kb. The functional I2 gene in the I_2 locus on chromosome 11, is flanked on one side by the I2C-2 gene and on the other side by the I2C-1 gene, separated by an additional gene copy (Simons *et al.*, 1998). Ori *et al.* (1997) claimed that the I2C-1 and I2C-2 genes confer partial resistance to *Fusarium* wilt race 2, while Simons *et al.* (1998) discovered that these genes were not essential for resistance to *Fusarium* wilt race 2. Mes *et al.* (2000)

suggested a correlation between the tomato resistant I-2 gene, and the I-2 mediated resistance response to *Fusarium* wilt race 2. This fact was also confirmed by Xu Zhihao *et al.* (2000) who suggested that the I2 gene was responsible for resistance to *Fusarium* wilt race 2.

Sarfatti *et al.* (1991) described resistance as the ability of the tomato cultivar to suppress or restricted the activity of the *Fusarium* wilt pathogen, a quantitative trait often determined by the activity of a single gene. Segal *et al.* (1992) referred to resistance as a dominant character that suppresses the susceptible character thought to be recessive. Simons *et al.* (1998) proposed that it was the difference in nucleotide binding leucine-rich repeats (NBS) within the I2 genes of the different I_2-complex locus families that caused the different degree or level of resistance to *Fusarium* wilt race 2. Ori *et al.* (1997) compared the LRR regions of the various resistant-type members of the I2C family and sequenced two partial cDNA clones from this family, designated as I2C-3 and I2C-5, of 1200 and 1600 bp, respectively. Sela-Buurlage *et al.* (2001) discovered no significant homology between the NBS of the I_2 complex locus families and suggests this to be the reason for the different levels of resistance to *Fusarium* wilt race 2.

In this study short primers for cDNA replication via the polymerase chain reaction (PCR) were used to delimit fragment sizes. A DNA region is flanked in opposite direction by a forward and reverse primer that bind to a specific DNA sequence. If the primer binding sites are sufficiently close together, it will allow the PCR reaction to replicate this DNA region, thus generating amplified specific fragments. If the primer binding sites are missing or to far apart, the PCR reaction will fail and no fragments will be generated for that region (Lynch and Walsh, 1998). PCR fragments are then visualized as bands on an agarose gel using ethidium bromide under UV light (Hartl, 2000).

The objective of this study was:

i) to identify single inherited resistance genes to *Fusarium* wilt race 2 in local tomato cultivars using PCR polymorphic band fragmentation techniques;

ii) secondly to study the relationship between the I2 alleles of the cultivars and their general combining ability values;

iii) and lastly to study the possible relationship between the I2 alleles and the specific combining abilities of the crosses.

6.3 Materials and Methods

Plant materials

Six tomato cultivars, Red Kaki, Moneymaker, Heinz 1370, Rossol, Flora Dade and Rodade were used as sources of cDNA which were used to screen for the presence of *Fusarium* wilt resistant alleles on the I_2 locus. The cultivars used in this study carries different *Fusarium* resistant genes. Red Kaki and Moneymaker carry no resistant alleles for *Fusarium* wilt on the I_1 and I_2 loci (Uys, 1996; Sela-Buurlage *et al.*, 2001). Heinz 1370 and Rossol possess some resistance alleles on the I_1 locus. According to the literature Flora Dade and Rodade possess of resistance alleles on both the I_1 and I_2 loci (Wager, 1981).

DNA extraction and isolation

Mature tomato leaves were harvested and put into liquid nitrogen. A 100 mg aliquot of each sample was dissolved in 610 µl extraction buffer (containing 2% hexadecyl trimetyl ammonium, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris pH 8.0, 2% polyvinyl-pyrrolidone, 2% bovine serum albimin and 0.2% mercaptoethanol) after which 20 µl 5M NaCl and 70 µl 20% SDS were added. The mixture was incubated for 1 hour at 65°C.Two µg RNAse A was added to each sample and the mixture was incubated at 37°C for 10 min.

The mixture was extracted twice with 700 µl chloroform/isoamylalcohol (24:1), followed by centrifugation at 13000 rpm for 15 min. The aqueous phase was collected and mixed with 0.6 volume of isopropanol and DNA precipitated at -20°C overnight. The samples were centrifuged at 13000 rpm for 15 min and the pellet was washed with 1 ml 70% ethanol. The pellet was vacuum dried and re-suspended in 50 µl of sterile distilled water.

PCR gel electrophoresis

PCR products were size-fractionated by 2% agarose (Sigma) gel electrophoreses (0.5 x TAE, 5 V/cm) and visualized by UV illumination (317nm) of ethidium bromide stained fragments.

Design of primers and PCR conditions

Prior to the testing of the different primer set combinations the viability of the cDNA of the six tomato cultivars were first tested for the presence of RNAse activity. The primers NS7 and NS 8 both yeast primers with known product sizes were used for this purpose and the base composition of these primers are illustrated in table 6.1.

New primers designed were first aligned using Clustal Co from cDNA sequences for the different I2C family protein complexes downloaded from the NCBI-network as sequenced by Ori *et al.* (1997) and then designed using the program Primer 3 (Rozen and Skatetsky, 1998). Primers were purchased from Whitehead Scientific. The Primers TFi2c1/2F (21 mer), and TFi2c1/2R (20 mer) were used to amplify the I2C1 and I2C2 genes of the I_2 resistance locus. TFi2/c3/5F (20 mer) and Tfi2c3/5R (19 mer) were used to amplify the I2C family member variation of the LRR, I2C3 and I2C5. Tfi2/3c/5F (20 mer) and Tfi2R (19 mer) primers were used to amplify the I2 genes of the I_2 resistance locus. Primers

Table 6.1 Primers used in PCR amplification

Primer	Sequence (5' - 3')	cDNA position
TFI2c1/2F Forward	5'-CAGTTTGGAGATACCG/CATCCA-3'	I2C1 - 2942 I2C2 - 2819
TFI2c1/2R Reverse	5'-GCTGTTGGGAGAAACTCAGG-3'	I2C1 - 3175 I2C2 - 3133
TFi2/c3/5F Forward	5'-GCCAGCTTTCCTCCTTTTCT-3'	I2C3 - 632 I2C5 - 3773 I2 - 3440
TFi2c3/5R Reverse	5'-GGCCAGTATTCCCCCTTTA/C-3'	I2C3 - 1073 I2C5 - 4145
TFi2R Reverse	5'-GGCCAGTATTCCCCCTTGT-3'	I2 - 3752
TFi2R Reverse	5'-GGCCAGTATTCCCCCTTGT-3'	I2 - 3752
TFI21RREC1 Forward	5'-CCTCCTTTTCTCACCTCACTTCGC-3'	I2 - Simon *et al.*, (1998)
TFI21RREC2 Reverse	5'-ATT TGT GGC CAG TAT TCC CC-3'	I2 - Simon *et al.*, (1998)

TFI21RRE C1 and TFI21RRE C2 were used to confirm the presence of the I2 gene at polymeric position 310 according to Simons *et al.* (1998). Primer sequences and respective cDNA positions are listed in Table 6.1.

PCR reactions of tomato DNA were performed in a 20 µl volume using a Perkin Elmer PCR System 9700. The reaction mixture contained 2µl of 1x Ex Taq buffer (TaKaRa Biotechnology), 0.25 mM d NTP, 0.3 µM of each primer, 0.05 µl Ex-Taq units TaKaRa Polymerase and 10 ng genomic DNA sample of each tomato specimen. The PCR reaction started with an initial denaturating step at 94°C for 5 min; followed by annealing that was touched down (0.7°C/cycle) from 57°C to 47°C for 15 cycles followed by 30 cycles at 47°C. PCR reactions were also conducted at 57° C for the newly designed primer set combinations.

Confirmation of the fragment sizes of the PCR reaction using the primers of Simons *et al.* (1998), were first tested in a non-denaturing polyacrylamide gel electrophoresis for the separation of large amounts of small DNA fragments (<1000bp). A 6% acrylamide gel were then used consisting of 1 ml of 1 X TAE, 2 ml of 29:1 acrylamide/bisacrylamide and 7 ml of sterile distilled water. Ten µl TEMED were added as well as 10 % ammonium persulfate. DNA samples were run in a horisontal gel at 5 V/cm^2 (Ausubel *et al.*, 1987).

The following product fragment sizes are indicated in Table 6.2, as expected for the different primer set combinations used in the PCR reactions under different conditions, using tomato cDNA sample. Visual different band fragmentation with different product sizes could then be used to identify the different I2C resistant genes of the I_2 loci in the six different local tomato cultivars.

Table 6.2. Fragment size of polymorphic bands expected for each primer combination used on the cDNA samples.

Primer	I2C1-gene	I2C2-gene	I2C3-gene	I2C5-gene	I2-gene
I2C1/2F+R	234	315	315	315	315
I2/C3/5F+R	----	---	442	373	---
I2F+R	244	244	244	244	313
RREC1/2F+R	400	240	----	-----	310

Standard graph

A standard graph Figure 6.1 was drawn for each PCR reaction visualized with ethidium bromide using the markers Lamda Hind III and the phage ϕ 174 DNA runned simultaniously with the tested cultivar cDNA in a 2% agarose gel. A graph was then created using the log molecular mass or fragmentation size of the known marker DNA against the distance migrated (in millimeters) in two hours when run in an agarose gel. The migration distance of each unknown fragmentation bands were plotted on the graph and their respective fragmentation sizes determined.

6.4 Results and Discussion

I) PCR polymorphic band formation and gene identification

First the tomato cultivars were tested for RNAse activity, using primers NS 7 and NS 8, with known product formation fragmentation size of 370bp. This only proved that the DNA was viable for testing with other experimental primers.

Simons *et al.* (1998) first described primers for the identification of the presence of the I2 gene found in resistant cultivars against *Fusarium* wilt race 2. The I2 resistance gene was found and formed a fragmention primer product size of 310 bp (Simons *et al.*, 1998). These primers TFI21RREC1 and TFI21RREC2 used by Simons *et al.* (1998) were first tested in a non-denaturing 6% polyacrylamide gel, using touchdown PCR reaction conditions. According to Ausubel *et al.* (1987), a 5% acrylamide gel could be used to separate large amounts of small DNA fragments, between 100 to 500 bp and results obtained confirmed the product fragmentation size to be as expected 310 bp.

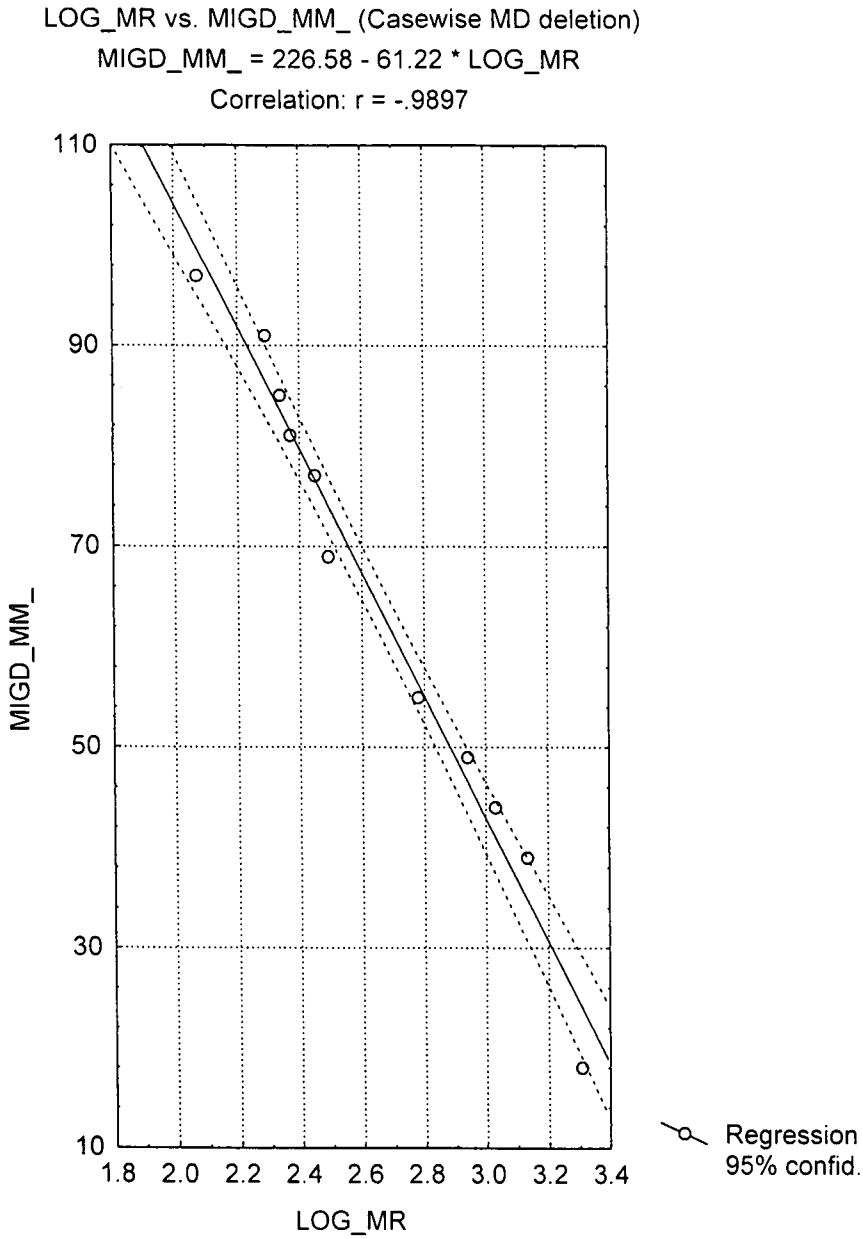

Figure 6.1 Standard graph of migration distance in mm to log molecular mass of the fragment size of the molecular markers using PCR primers Tfi2/c3/5F, Tfi2R, Tfi2c1/2F and Tfi2c1/2R.

Table 6.3: Comparison of migration distances of tomato cultivar DNA using PCR-primers for I2 resistance genes with the migration distances of the molecular markers with known fragment sizes.

No	Fragment size	Log MR.	Migration distance in mm.	Red K	Money	Heinz	Rossol	Flora Dade	Rodade	H_2O
1	2027	3.307	18							
2	1353	3.131	39							
3	1073	3.031	44							
4	872	2.941	49							
5	603	2.780	61	61mm	61mm	61mm	61mm	61mm	61mm	
6	564		58							
7	310	2.491	74					74mm	74mm	
8	281	2.449	77							
9	234	2.369	81					80mm	80mm	
10	217	2.337								
11	194	2.288	91							
12	125	2.097								
13	118	2.072	97							

Table 6.4: Comparison of migration distances of tomato cultivar DNA using PCR-primers for C2I2 & C1I2 resistance genes with the migration distances of the molecular markers with known fragment sizes.

No	Fragment size	Log MR.	Migration distance in mm.	Red K	Money	Heinz	Rossol	Flora Dade	Rodade	H_2O
1	2027	3.307	18							
2	1353	3.131	39							
3	1073	3.031	43							
4	872	2.941	48	49mm	49mm	49mm	49mm	49mm	49mm	
5	603	2.780	55					55mm	55mm	
6	310	2.491	69	68mm	68mm	68mm	68mm	68mm	68mm	
7	281	2.449	72							
8	234	2.369	74	74mm	74mm	74mm		74mm	74mm	
9	217	2.337	78							
10	194	2.288	84							
11	125	2.097	77							
12	118	2.072	90							
13										

Then the primers Tfi2/C3/5F and Tfi2R (Table 6.2) with determined product fragmentation sizes of 244 and 315 bp respectively were tested for the identification of the I2 genes in local tomato cultivars. Only the cultivars Flora Dade and Rodade contained known inbred resistance genes against *Fusarium* wilt race 2. Figure 6.2 clearly indicate the formation of two sets of mono-morphic bands found in the cultivars Flora Dade and Rodade that separate these tomato cultivars containing the inbred I2 resistance genes from the other tomato cultivars.

A standard graph Figure 6.1 was used to convert the fragmented bands for each respective PCR band formed to known Lamda Hind III fragmented bands with known product sizes. Table 6.3 was determined using this standard graph and it revealed mono-morphic bands correlating to Lamda markers with product sizes of 310 and 234bp. These results were in agreement with the 310 bp fragment bands found by Simons *et al.* (1998). None of the other cultivars possessed any identifiable I2 genes. Using the primers Tfi21RREC 1 & 2 described and used by Simons *et al.* (1998) as well as touchdown PCR reaction conditions did not produced the same results in local cultivar cDNA. Fragmented bands were formed in tomato cDNA that had no known inherited resistance to *Fusarium* wilt race 2 and thus created doubtful results.

Screening for the presence of the I2C1 and I2C2 resistance genes in local tomato cultivars the primers Tfi2c1/2F and Tfi2c1/2R were used. Figure 6.3 illustrates a touchdown PCR reaction visualized on a 2% agarose gel stained with etidium bromide. Simons *et al.* (1998) discovered that the two genes I2C1

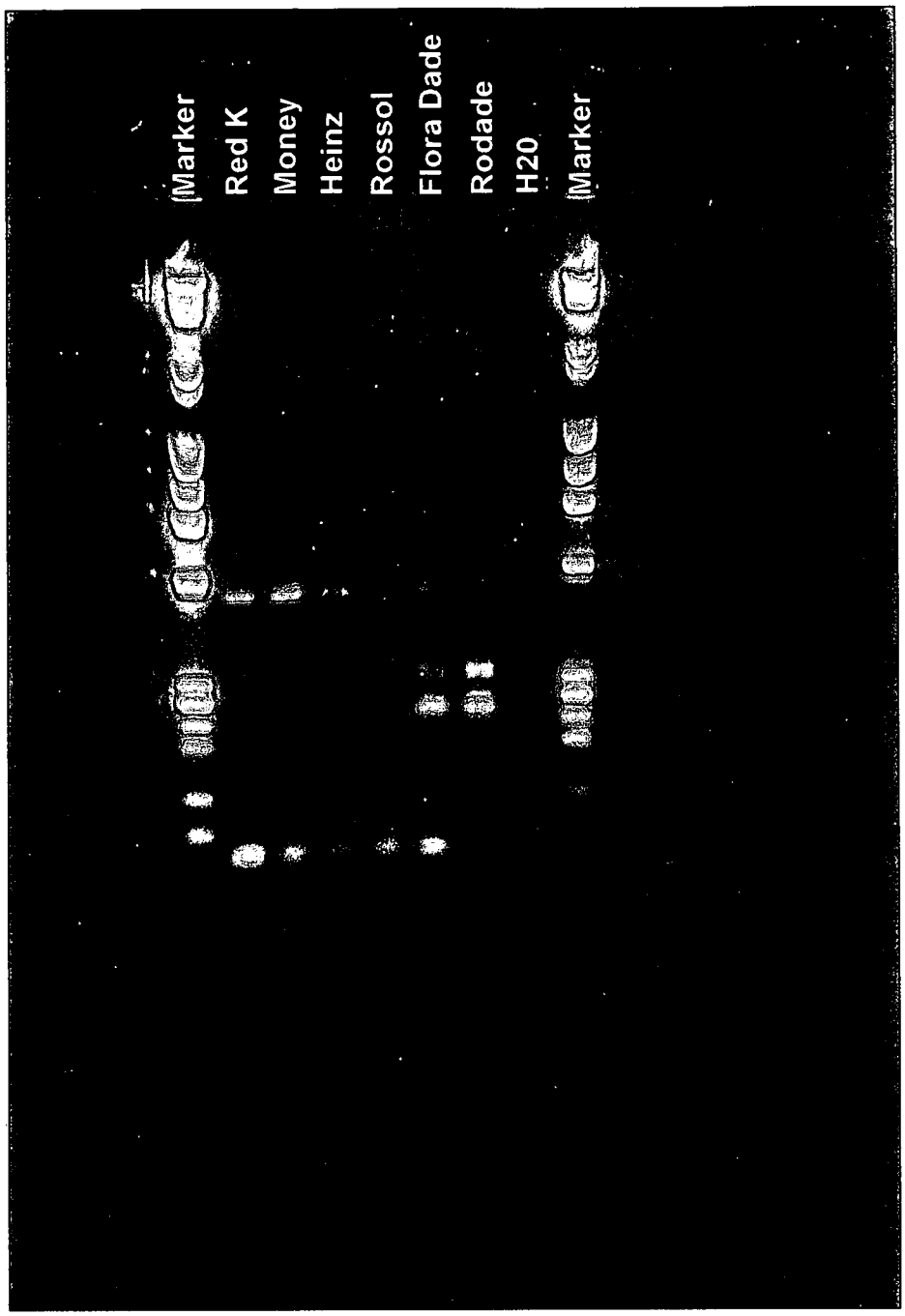

Fig 6.2 Primers Tfi2/c3/5F and Tfi2R for detecting the I2 gene in tomato cDNA sequences at PCR touch-down reaction conditions.

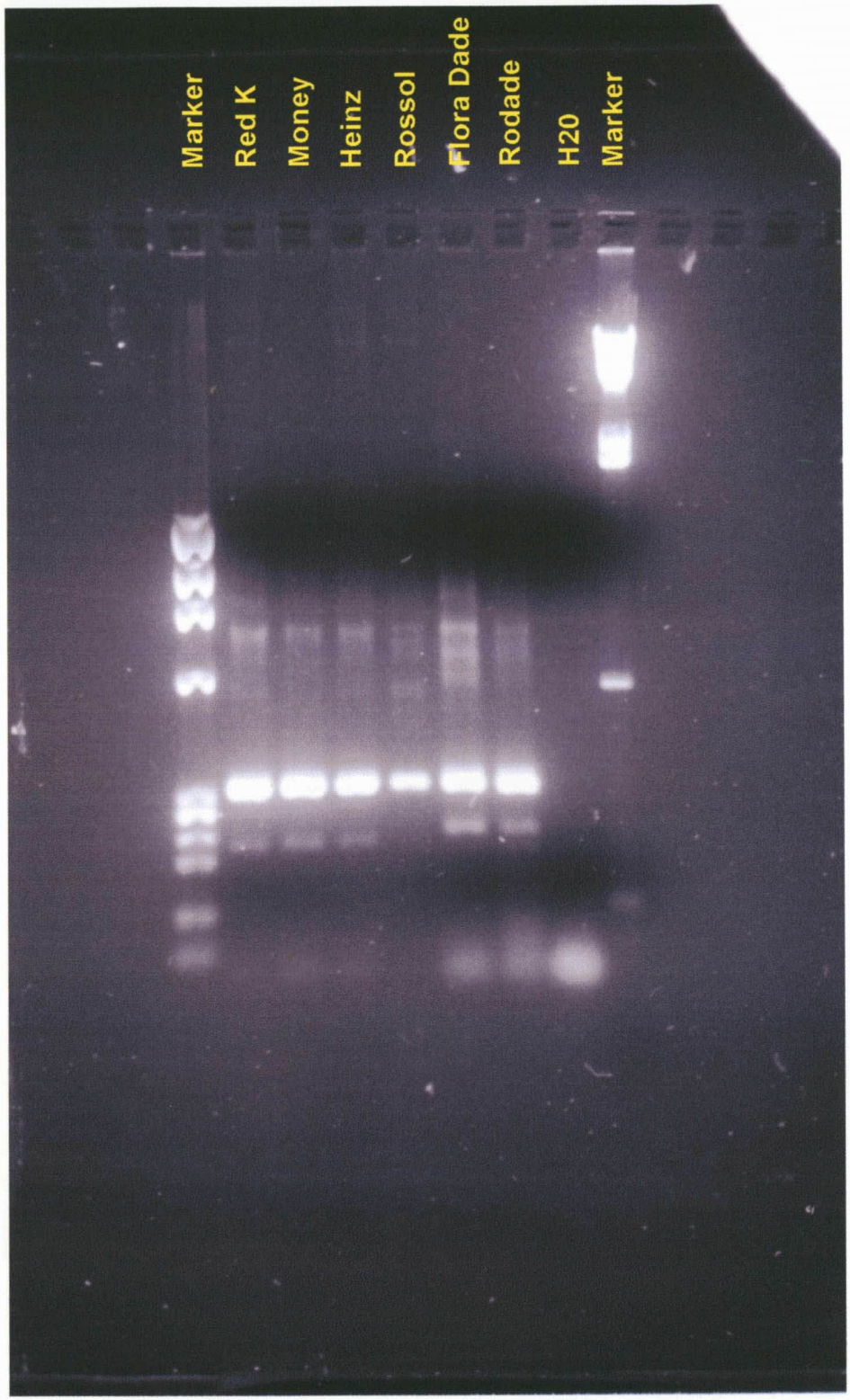

Fig 6.3 Primers Tfi2c1/2F and Tfi2c1/2R for detecting the I2C1 and I2C2 genes in tomato cDNA sequences during touch-down PCR reaction conditions.

and I2C2 were reasonably similar in nucleotide sequence composition. Due to several overlapping similarities in their nucleotide composition, one set of primers could be designed to screen for both genes simultaneously. Expected primer product sizes for the primers Tfi2c1/2F and Tfi2c1/2R were determined as 234 and 315bp respectively (Table 6.2).

A standard graph similar to Figure 6.1 was used to convert the fragmented bands for each respective PCR band formed, to known Lamda Hind III fragmented bands with known product sizes. Table 6.4, was determined using a standard graph (Figure 6.1) and revealed polymorphic bands correlating with the Lamda Hind III markers with product sizes of 310 and 234bp. All the cultivars screened had the same 310 bp fragmented band that represented the I2C2 gene (Table 6.2). The 234 bp fragment product size represented the I2C1 gene were found in all the cultivars except for Rossol that did not seem to have this single gene.

Screening for the presence of the I2C3 and I2C5 resistance genes the primers Tfi2C3/5F and Tfi2C3/5R were used and expected to give band fragment product sizes of 442 and 373bp respectively (Table 6.2). Figure 6.4 show mono-morphic bands formed in the cultivars Red Kaki, Heinz 1370 and Rossol. Red Kaki and Heinz 1370 both formed one mono-morphic band that lay between 1353 and 1072bp and another band with a fragment product size of more than 1072bp. Rossol formed two not so clearly separated fragmentation bands, one between 1353 and 1072bp and a second band closer too 2000bp. These fragmented mono-morphic bands did not correlated to the expected product sizes determined in Table 6.2. Unfortunately the I2C3 and I2C5 genes could not be detected using the designed primers. This might have been due to a lack of the respective I2C3 or I2C5 genes present in any of the local tomato cultivars or it could have been that the primer binding sites were to close together and that other primers will have to be redesigned.

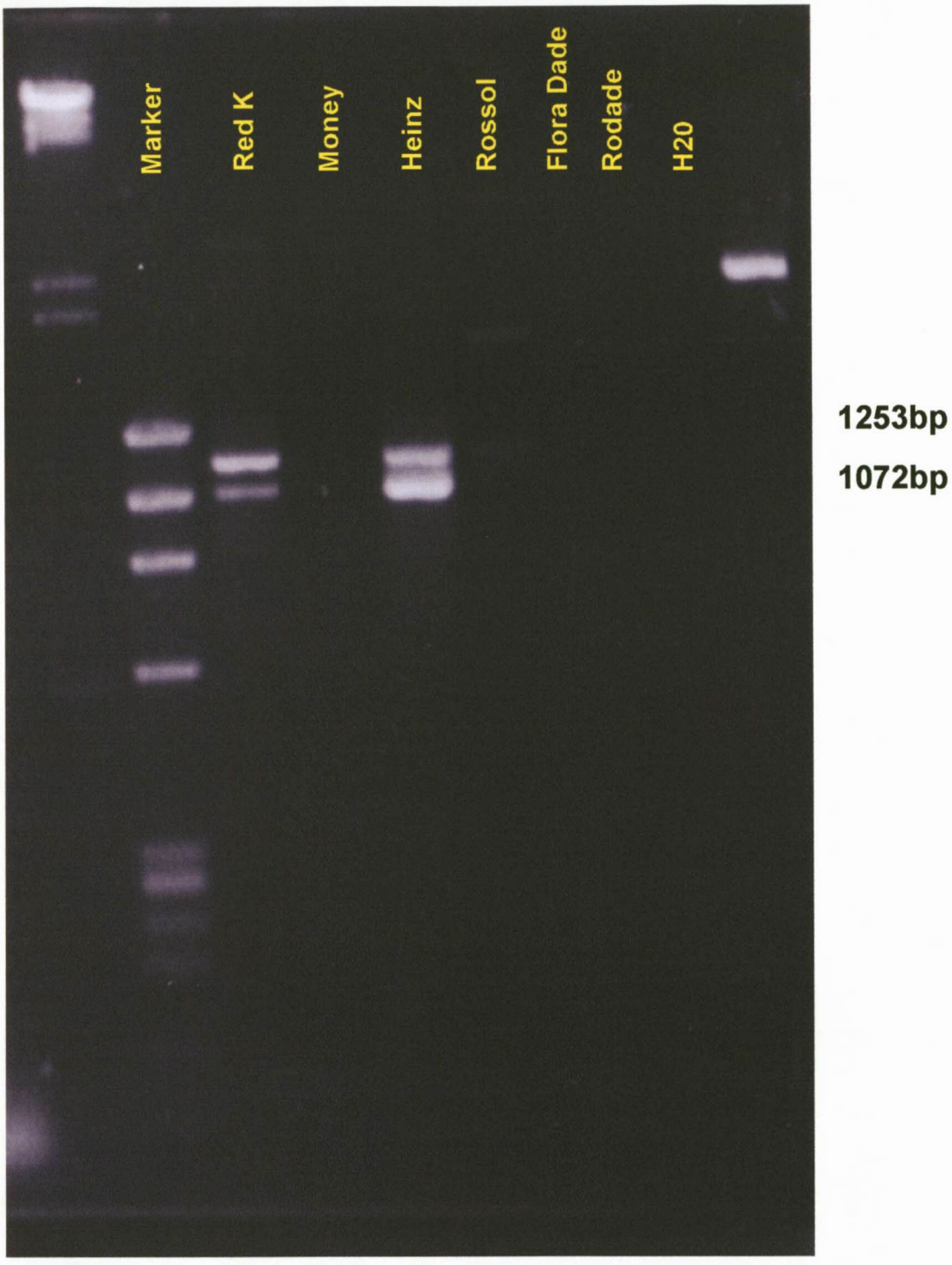

Fig 6.4 Primers Tfi2c3/5F and Tfi2/c3/2R for detecting the I2C3 and I2C5 genes in tomato cDNA sequences during PCR touch-down reaction conditions.

II) Relationship between general combining ability (GCA) values and the I2 resistant alleles.

The relationship between the GCA-values of the parental cultivars and their I2 resistant alleles were studied. The GCA-values of the cultivars and their resistant alleles were listed in Table 6.5. The GCA-values were discussed in Chapter 5. The two cultivars Heinz 1370 and Rodade possessed the best GCA-values for resistance to *Fusarium* wilt race 2. Heinz 1370 possessed both the I2C1 and I2C2 alleles while Rodade carries the I2C1, I2C2 and I2C2 alleles. Flora Dade and Rossol possess the lowest GCA-values for *Fusarium* wilt resistance. Flora Dade carries the I2C1, I2I2 and I2C2 alleles. The difference between the breeding values of Rodade and Flora Dade is propably cause by homozygous (I2I2) and heterozygous (I2i2) loci.

The loci in Rodade and Heinz 1370 are propable homozygous, while the loci in Flora Dade are propably heterozygous which accounts for it's lower breeding value. These results is in agreement with the findings of Sarfatti *et al.* (1989) who found a three fold increase in disease rate in heterozygous (I2i2) versus homozygous (I2I2) plants. The low breeding values of Rossol for *Fusarium* wilt resistance is expected since it carries only the I2C2 allele. No association could be found between the breeding values of Moneymaker and Red Kaki and the I2C1 and I2C2 alleles carried by these two cultivars.

III) Relationship between specific combining ability (SCA) values and I2 resistant alleles.

The relationship between the SCA values of the different crosses and their resistance alleles to *Fusarium* wilt were studied in Chapter 5. The SCA-values of the different crosses and their expected resistance alleles were listed in Table 6.6.

Table 6.5 Relationship between general combining ability values and single gene resistance against *Fusarium* wilt.

Cultivar genotypes	GCA value 30 days	GCA value 60 days	GCA value Disease Progress	I2C1 Gene	I2C2 Gene	I2 Gene	I2C3 Gene	I2C5 Gene
Flora Dade	-8.79	-7.98	-0.48	Present	Present	Present	Absent	Absent
Rodade	9.96	9.24	-0.20	Present	Present	Present	Absent	Absent
Heinz 1370	8.21	10.37	-1.51	Present	Present	Absent	Absent	Absent
Rossol	-8.60	-11.67	-3.15	Absent	Present	Absent	Absent	Absent
Moneymaker	-8.92	-3.39	-5.89	Present	Present	Absent	Absent	Absent
Red Kaki	8.15	3.43	4.93	Present	Present	Absent	Absent	Absent

Table 6.6 Possible relationship between specific combining ability values and single gene inherited resistance to *Fusarium* wilt.

Parental Genotype	Parental Genotype	SCA value 30 day	SCA value 60 day	SCA value Disease Progress	Possible Gene combination
Flora Dade C1I2C2	Rossol C2	-55.44	-36.04	-20.40	C1I2C2
Flora Dade C1I2C2	Moneymaker C1_C2	-34.13	-25.08	-9.20	C1I2C2
Heinz 1370 C1_C2	Rodade C1I2C2	-11.00	-7.54	-3.80	C1I2C2
Heinz 1370 C1_C2	Red Kaki C1_C2	-13.19	-1.23	-13.40	C1-C2
Red Kaki C1_C2	Flora Dade C1I2C2	-6.19	3.61	-11.00	C1I2C2
Rossol C2	Moneymaker C1_C2	-1.31	8.11	-9.29	*C1* C2
Rodade C1I2C2	Moneymaker C1_C2	13.63	8.21	0.57	C1I2C2
Rodade C1I2C2	Red Kaki C1_C2	-4.94	8.39	13.20	C1I2C2
Heinz 1370 C1_C2	Moneymaker C1_C2	9.88	9.58	-0.62	C1_C2
Rodade C1I2C2	Flora Dade C1I2C2	8.00	9.80	-1.80	C1I2C2
Red Kaki C1_C2	Moneymaker C1_C2	7.94	19.52	-6.05	C1_C2
Red Kaki C1_C2	Rossol C2	9.63	21.30	-12.60	C1_C2
Heinz 1370 C1_C2	Flora Dade C1 I2 C2	13.25	23.67	-5.5	C1I2C2
Heinz 1370 C1_C2	Rossol C2	13.56	23.86	-9.70	C1_C2
Rodade C1_C2	Rossol C2	2.81	24.99	-19.50	C1I2C2

Rodate x Moneymaker, Heinz 1370 x Moneymaker, Rodade x Flora Dade, Red Kaki x Moneymaker, Red Kaki x Rossol, Heinz 1370 x Flora Dade, Heinz 1370 x Rossol and Rodade x Rossol gave relatively high SCA-values for *Fusarium* wilt resistance. The two cultivars, Rodade and Heinz 1370 who possess the best resistance were involved in six of the eight best crosses. The two remaining crosses Red Kaki x Moneymaker and Red Kaki x Rossol possess the I2C1 and I2C2 alleles. In the light of the strong heterotic effect already found in the F1-hybrids for *Fusarium* wilt resistance, the performance of these two crosses could easily be explained by an interaction between the C1C2 alleles provided that the parents are homozygous for these alleles.

The five crosses with the lowest SCA-values were Flora Dade x Rossol, Flora Dade x Moneymaker, Heinz 1370 x Rodade, Heinz 1370 x Red Kaki and Red Kaki x Flora Dade. The susceptible parent Flora Dade is involved in three of these crosses despite the fact that it carries at least three resistance genes. There is strong evidence that Flora Dade is highly heterozygous. The two most susceptible cultivars, Flora Dade and Rossol also produced the cross with the lowest SCA value for *Fusarium* wilt resistance. The rest of the crosses showed no clear relationship between the single genes involved and their SCA-values.

6.5 Conclusions

The relationship between the GCA-values of the parental cultivars and their single *Fusarium* wilt resistance genes were studied. With a few assumptions, it was possible to find associations between the GCA-values and their single resistance genes for three of the parental cultivars. The problem with PCR-technology is that it can't distinguish between homozygous (I2I2) and heterozygous (I2i2) loci. As long as this restriction prevails, it will be difficult to

predict the GCA-values of tomato cultivars for *Fusarium* wilt resistance using PCR technology.

In some of the crosses it was possible to establish a relationship between the SCA-values of some of the crosses and the genes they carry for *Fusarium* wilt. There was a strong indication that there were interactions between the C1C2 alleles in some of the crosses. This finding makes it very difficult to predict the SCA-values of crosses for *Fusarium* wilt resistance in tomato with the help of PCR technology.

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CHAPTER 7

Recommendations and conclusions

Significant differences were found for resistance to *Fusarium* wilt race 2 in twelve inbred tomato cultivars. Variability of resistance to *Fusarium* wilt within the tomato cultivars ranged from high levels of resistance to highly susceptible. ANOVA indicated that the variability between seedlings within cultivars was larger than the variability between the cultivars. The level of resistance in existing cultivars could be improved through selection between seedlings within cultivars.

The entry x day interaction for resistant plants and their F1-offspring in the combined analysis of variance was highly significant after 30 and 60 days and for disease progress. The cultivar x day of screening interaction made it very difficult to select resistant seedlings in the early stages of seedling development. It is recommended that screening be postponed for at least 60 days after inoculation.

Significant variability was found between the GCA-effects of the cultivars and the SCA-effects of the F1-hybrids for 30 and 60 days assessment. It was concluded that disease progress was mainly controlled by non-additive gene action or that many genes were involved, indicating the involvement of specific gene combinations. Heritability for *Fusarium* wilt resistance was too low to gain any significant response with pedigree breeding methods. Due to the significant levels of heterosis for *Fusarium* wilt resistance obtained in this study, breeders should rather use hybrid methods to increase *Fusarium* wilt resistance in tomato.

It was possible to find associations between the GCA-values of the cultivars and their I2 resistance genes in three of the cultivars. The problem experienced with PCR-technology was that it could not distinguish between homozygous (I2I2) and heterozygous (I2i2) *Fusarium* wilt resistance alleles.

Neither could it distinguish between the presence or absence of interactions between the different alleles at the I2 loci. In order to identify superior *Fusarium* wilt resistant F1-hybrids, breeders should still test for general combining ability.

CHAPTER 8

Summary

This study reviewed principal aspects relating to *Fusarium* wilt race 2 resistance in tomatoes, caused by the fungus *Fusarium oxysporum* f. sp. *lycopersici*. The classification and life cycle of *Fusarium* wilt, the symptomatology, disease assessment and host-pathogen interaction was reviewed. Different methods of disease management were investigated, with emphasis on genetic control. Twelve inbred tomato cultivars grown in South Africa were screened for resistance to *Fusarium oxysporum* f. sp. *lycopersici* race 2. Percentage resistant plants, and disease progress were monitored over intervals of 30 and 60 days respectively. Significant differences were found between cultivars for percentage resistant plants for both intervals as well as for disease progress. Five cultivars, Floradade, Rodade, Heinz 1370, Traffic Jam and Sixpack were associated with resistance after 60 days screening, while Rossol, Oxheart, Red Kaki and Roma were found to be highly susceptible after 60 days. The cultivar x day interaction was significant for the percentage resistant plants.

Fusarium wilt resistance was then studied in six of the inbred tomato cultivars viz., Rossol, Red Kaki, Moneymaker, Rodade, Flora Dade and Heinz 1370 and their F1-hybrids. Percentage resistant seedlings for each cultivar and their F1-hybrids were determined after 30 and 60 days assessment. Disease progress was also calculated. Variance analysis indicated significant genotype x day interaction for *Fusarium* wilt. Significant differences were found between entries after 30 and 60-days assessments as well as for disease progress. The best *Fusarium* wilt resistance was found in the cultivars Moneymaker, Rodade and Flora Dade. Moneymaker also had the best disease progress. Disease progress in five of the crosses Flo x Mon, Flo x Ros, Rod x Ros and Mon x Ros were

less than 10%. Disease progress in the F1-hybrids were significantly lower than in the inbred lines, which emphasis the efficiency of tomato hybrids to reduce the levels of *Fusarium* wilt in tomato.

Another objective of this study was to determine the combining ability and heritability of *Fusarium* wilt resistance in tomato. Six inbred tomato cultivars Rossol, Red Kaki, Moneymaker, Rodade, Flora Dade and Heinz 1370 were crossed in a diallelic manner to produce 15 F1-hybrids. Assessment for *Fusarium* wilt resistance was done after 30 and 60 days and for disease progress. Significant differences were found for GCA- and SCA- effects. The GCA: SCA ratio's for 30 and 60 days assessments indicating both additive and non-additive gene actions. Disease progress was mainly controlled by dominant effects. The narrow sense heritabilities for *Fusarium* wilt resistance were respectively 12% and 8% after 30 and 60 days assessments. Significant levels of heterosis were observed in crosses for *Fusarium* wilt resistance. The cultivar Heinz 1370 had the largest GCA-effect while the cross Rod x Ross possessed the largest SCA-effect.

PCR technology with specific DNA primers and tomato leave cDNA was used to investigate the presence of different single genes in six local tomato cultivars. These cultivars included Flora Dade, Rodade, Moneymaker, Heinz 1370, Rossol and Red Kaki. The presence of the single genes I2C1, I2C2, I2, I2C3 and I2C5 were tested for as they were suspected of being responsible for *Fusarium* wilt race 2 resistance. The I2C1 and I2C2 genes were identified in all cultivars except for Rossol that did not possess the I2C1 gene. No I2C3 or I2C5 genes were identified in any cultivars. The I2 gene was only identified in Flora Dade and Rodade. In three of the cultivars significant relationships could be found between the I2 resistance alleles and their breeding values.

CHAPTER 8

Opsomming

Ondersoek is ingestel na sekere aspekte met betrekking tot *Fusarium* wilt ras 2 weerstand in tamaties. *Fusarium* wilt word veroorsaak deur die fungus *Fusarium oxysporum* f. sp. *lycopersici*. Die klassifikasie en lewenssiklus van *Fusarium* wilt, die simtome, siekte ontwikkeling en gasheer interaksie is ondersoek. Verskeie metodes van siekte beheer is ondersoek met spesiale nadruk op genetiese beheer. Twaalf ingeteelde tamatie kultivars beskikbaar in Suid Afrika is ondersoek vir weerstand teen *Fusarium oxysporum* f. sp. *lycopersici* ras 2. Die persentasie weerstandbiedende plante, en tempo van siekte ontwikkeling is gemonitor met tydsintervalle van 30 en 60 dae onderskeidelik. Betekenisvolle verskille is gevind tussen die kultivars vir persentasie weerstandbiedende plante en siekte ontwikkeling oor beide tydsintervalle. Vyf kultivars, Flora Dade , Rodade, Heinz 1370, Traffic Jam en Sixpack word geassosieer met weerstandbiedenheid na 60 dae, terwyl Rossol, Oxheart, Red Kaki en Roma hoogs vatbaar is vir verwelkingsiekte na 60 dae. Die kultivar x dag interaksie was betekenisvol vir persentasie bestande plante.

Fusarium wilt bestandheid is in ses ingeteelde tamatie lyne ondersoek, nl. Rossol, Red Kaki, Moneymaker, Rodade, Flora Dade en Heinz 1370 sowel as hul F1-basters. Die persentasie weerstandbiedende saailinge vir elke kultivar en F1-basters is bepaal na 30 en 60 dae. Siekte ontwikkeling is bereken. Variansie analise toon ń betekenisvolle genotipe x dag interaksie vir *Fusarium* wilt. Betekenisvolle verskille is gevind tussen inskrywings na 30 en 60 dae vir die tempo van siekte ontwikkeling. Die beste *Fusarium* wilt weerstandbiedenheid is gevind in die kultivars Moneymaker, Heinz 1370, Rodade en Flora Dade. Moneymaker het die laagste tempo van siekte ontwikkeling getoon. Siekte ontwikkeling in vier van die F1-basters nl. Flo x Mon, Flo x Ros, Rod x Ros en Mon x Ros was minder as 10 persent. Die tempo van siekte ontwikkeling in

die F1-basters was betekenisvol laer as by die ingeteelde lyne. Dit beklemtoon die doeltreffendheid van tamatie basters om die voorkoms van *Fusarium* wilt in tamaties te verlaag.

ń Studie is gedoen om die kombineervermoeë en die oorerwing van *Fusarium* wilt weerstand in tamaties te bepaal. Ses ingeteelde tamatie kultivars Rossol, Red Kaki, Moneymaker, Rodade, Flora Dade en Heinz 1370 is gekruis as n dialleel om 15 F1-basters te produseer. Monitering van *Fusarium* wilt bestandheid en die siekte ontwikkeling is gedoen na 30 en 60 dae. Betekenisvolle verskille is gevind vir die GCA- en die SCA-effekte. Die GCA : SCA verhouding vir 30 en 60 dae monitering impliseer beide additiewe en nie-additiewe geen aksies. Die tempo van siekte ontwikkeling is hoofsaaklik beheer deur dominante effekte. Oorerwing in die eng sin vir *Fusarium* wilt weerstand was onderskeidelik 12% en 8% na 30 en 60 dae. Die kultivar Heinz 1370 het die grootste GCA-effekte getoon, terwyl die kruising Rod x Ross oor die grootste SCA effek gewys.

PCR tegnologie met spesifieke DNA merkers en tamatie blaar cDNA is gebruik om ondersoek in te stel na die teenwoordigheid van verskillende enkel gene in die ses tamatie kultivars. Die kultivars het ingesluit Flora Dade, Rodade, Moneymaker, Heinz 1370, Rossol en Red Kaki. Daar is getoets vir die teenwoordigheid van die enkel gene I2C1, I2C2, I2, I2C3 and I2C5 wat verantwoordelik blyk te wees vir *Fusarium* wilt ras 2 weerstand. Die I2C1 en I2C2 gene is geidentifiseer by alle kultivars met die uitsondering van Rossol. Geen I2C3 of I2C5 gene is nie in die kultivars geidentifiseer nie. Die I2 geen was slegs teenwoordig in Flora Dade and Rodade. Slegs in drie gevalle is betekenisvolle assosiasies gevind tussen die (I2) weerstands gene van die kultivars en hul kombineervermoeë.