DEVELOPMENT AND PHENOTYPIC CHARACTERIZATION OF WHEAT GERMPLASM RESISTANT TO RUSSIAN WHEAT APHID (*Diuraphis noxia* KURDJUMOV) AND STEM RUST (*Puccinia graminis* Pers. f.sp. *tritici)* RACE 'PgtUg99' IN KENYA

BY:

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DECLARATION AND RECOMMENDATION

Declaration

I hereby declare this thesis as my original work and has not been previously presented to any University for any degree or any other award.

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DEDICATION

To my loving parents Mr. Fanuel P. Oniang'o and Mrs. Lucy A. Oniang'o, you have been very supportive.

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ABSTRACT

Russian wheat aphid (*Diuraphis noxia* Kurdjumov) (RWA) and the emergence of stem rust (*Puccinia graminis* Pers. f.sp. *tritici*) race TTKSK ('Ug99') in Kenya are currently some of the most binding constraints to wheat production in Kenya. Severe infestation by RWA may result in yield losses of up to 90% in commercial wheat cultivars while 'Ug99' infected plants may suffer up to 100% loss. The two pests combined have seriously affected wheat farmers forcing them to heavily rely on pesticides. This has increased the cost of production making wheat an expensive crop to produce. There is therefore great need to come up with cheap and durable solutions to both RWA and 'Ug99'. This research work sought to develop wheat germplasm that is resistant to both RWA and 'Ug99' by pyramiding two major resistance genes. The work was done in a breeding cage and green house at Kenya Agricultural Research Institute (KARI)-Njoro. The RWA and 'Ug99' resistant material were obtained from the breeding and biotechnology departments of KARI-Njoro. Three varieties of wheat were used in this experiment; they include 'Kwale', a Kenyan commercial variety known to be high yielding but susceptible to both RWA and 'Ug99', 'Cook', an Australian variety carrying stem rust resistance gene *Sr36* and is known to confer immunity to 'Ug99' at both seedling and adult plant stages, and 'KRWA9', a Kenyan line known to be resistant to RWA but has poor agronomic attributes. The F_1 of the double cross (DC F_1) was obtained by crossing the F_1 of 'Kwale × Cook' and the F_1 of 'Kwale × KRWA9'. The DC F_1 population was subjected to sequential screening for both RWA and 'Ug99' resistance. The surviving DC F_1 progenies were left to self pollinate in the field in order to obtain the F_2 of the double cross (DC F_2). The DC F_2 progenies were sequentially screened against RWA and 'Ug99' to obtain a population that is resistant to both RWA and 'Ug99'. Genotypic characterization of the DC $F_{2:3}$ families was later done to select only homozygous dominant plants to the two resistance genes. Data collected was subjected to *chi*-square "goodness of fit" using GenSTAT 12th edition to determine the mode of inheritance of RWA and 'Ug99' resistance genes. The results indicated that the RWA resistance gene in 'KRWA9' and 'Ug99' resistance gene *Sr36* were successfully pyramided. These genes proved to be dominant with single crosses exhibiting ratios of 3:1. The genes were simply inherited and easy to transfer into 'Kwale', a Kenyan commercial variety. The fact that the two genes were not linked, they were inherited independently. It was recommended that though races with virulence for *Sr36* have been reported, the gene is immune to the race 'Ug99' and could still be used effectively as a component for 'Ug99' resistance breeding together with other *Sr* genes. This study has clearly demonstrated that it is possible to get one population that is resistant to both RWA and stem rust. This population can be advanced to early generations and selections made within preliminary and advanced yield trials for future variety release.

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LIST OF ACRONYMS

APR	Adult Plant Resistance
BGRI	Borlaug Global Rust Initiative
CAB	Commonwealth Agricultural Bureau
CAN	Calcium Ammonium Nitrate
CIMMYT	International Center for Maize and Wheat Improvement
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DAP	Di-ammonium Phosphate
Dn	*Diuraphis noxia* resistance gene
DNA	Deoxyribonucleic Acid
FAO	Food and Agriculture Organization of the United Nations
GDP	Growth Domestic Product
HYSPLIT	Hybrid Single Particle Lagrangian Integrated Trajectory Model
IRRI	International Rice Research Institute, Philippines
IT	Infection Type
KARI	Kenya Agricultural Research Institute
MR	Moderately Resistant
MS	Moderately Susceptible
R	Resistant
S	Susceptible
Sr	Stem rust resistance gene
SSR	Simple Sequence Repeat
USDA	United States Department of Agriculture
UV	Ultra Violet
WANA	West Asia and North Africa countries

DEFINITION OF TERMS

Adult plant resistance	Resistance expressed near or after heading.
Avirulence	The property of a pathogen by which it cannot cause damage a host plant because of the effectiveness of one or more major genes for resistance.
Biotype	A group of insects having the same fundamental constitution in terms of genetic or hereditary.
Complex race	Race with a wide virulence spectrum.
Culture	A clone of urediniospore that is maintained in a laboratory.
Cultivar	A cultivated variety as opposed to a botanical (taxonomic) variety.
Differential set/host	A set of lines or cultivars by which races/biotypes of a natural enemy can be characterized or distinguished.
Epistasis	The suppression or modification (interallelic interactions) of the effect of a gene by a nonallelic gene.
Fleck	Necrotic or chlorotic spot due to the resistance that results in no sporulation, often assigned the symbol (**;**).
Forma specialis (f. sp.)	Unit within the pathogen that is distinguished by its host range.
Gene pyramid	Accumulation of several genes for resistance to a single disease in a cultivar or line.
Horizontal resistance	Term introduced by Vanderplank (1963), which is synonymous to race-non-specific resistance.
Homologous chromosome	Is a chromosome pair containing two similar alleles, one of paternal origin, the other of maternal origin, that are identical in appearance and pair during meiosis
Homeologous chromosome	Partially homologous. This term is used to describe the relationship of similar chromosomes or parts of chromosomes brought together following inter-species hybridization and allopolyploidization, and whose relationship was completely homologous in an ancestral species.

Infection type	The visible symptoms of disease produced by the interaction of host and pathogen in a specific environment.
Inoculum	The spores or other propagules of the pathogen to which plants are exposed, and from which infection can take place.
Isolate	A sample of a pathogen that is stored alive or maintained in isolation on plants or in nutrient media.
Major gene	Resistance that is easy to measure and due to a single host gene.
Minor gene	Resistance that is difficult to measure and usually thought to be due to several host genes.
Pathogenicity	The ability of a microbial organism to infect a plant species.
Pustule	A uredium in the case of cereal rust.
Race	A group of genotypes within a pathogen species that is distinguished by its virulence.
Resistance	Capacity of a plant to reduce or stop the growth, development and reproduction of the natural enemy after establishment of intimate contact.
Susceptibility	Incapacity of a plant to reduce the growth, development and reproduction of the natural enemy.
Tolerance	Capacity of the host plant to restrict the symptoms or the harmful effects per unit of pathogen otherwise than by restricting the amount of infection.
Vertical resistance	Term introduced by Vanderplank (1963), which is synonymous to race-specific resistance.
Virulence	The capacity of a pathogen to infect a plant with one or more major genes for (hypersensitivity) resistance, because it does not possess any of the corresponding genes for avirulence.
Volunteer plants	Plants that germinate in the field, along the roadside, or elsewhere from seeds that are dropped during harvesting or transportation.

CHAPTER ONE

INTRODUCTION

1.1 Background

Wheat (*Triticum aestivum* L.) was one of the earliest domesticated food crops and for 8,000 years has been the basic staple food of the major civilizations of Europe, West Asia and North Africa (CIMMYT, 2007). Today, wheat is grown on more land area than any other commercial crop and continues to be the most important food grain for humans. It is one of the leading cereal crops produced, consumed and traded in the world. Wheat provides over 20% of the calories for the world population and is a staple food for 35% of the world's population. In 1999, the worldwide area planted with wheat was over 212 million hectares (FAO, 2009). Roughly 90 to 95% of the wheat produced in the world, about 600 million tonnes, is common wheat (*Triticum aestivum*), which is better known as hard or soft wheat, depending on grain hardness. The rest is mostly durum wheat (*Triticum durum*), which is used to produce coarse flour (FAO, 2009).

In Kenya, wheat is the second most important cereal crop after maize (*Zea mays*) and it is increasingly becoming an important source of food for both man and livestock. Wheat was introduced in Kenya towards the end of 19th century and has since been grown on an increasing scale in the highland areas (Kiplangat, 2005). Wheat is grown under rain-fed conditions, in small and large farms. The large scale farmers dominate wheat production with a share of about 75% of the wheat acreage and 80% of production (FAO, 2009). All the wheat is spring wheat and several varieties of both hard and soft wheat are grown. More than 100 varieties suited to various agro-ecological zones have been released by KARI National Plant Breeding Centre Njoro. Wheat growing areas in Kenya include the Rift Valley regions of Uasin Gishu, Narok, Marakwet, Elgeyo, Londiani, Molo, Nakuru and Timau areas. These areas have altitudes ranging between 1200m and 1,500m above sea level, with annual rainfall varying between 800 mm and 2,000 mm, with up to 2,500 mm on higher grounds. The area under wheat production in Kenya increased from 144,000 ha in 2007 to 150,000 ha in 2008. The demand for wheat and wheat products is growing at a rate of 7% per annum. Even though the national production is increasing only about 35% of domestic consumption requirement are realized, the remaining is met through imports (FAO, 2009).

1.2 Russian Wheat Aphid

Russian wheat aphid (RWA), *Diuraphis noxia* (Kurdjumov), is one of the most noxious pests of cereal crops throughout the world (Kovalev *et al*., 1991). Since its introduction in the USA in 1986, the economic losses were in excess of US$ 900 million by 1994 (Webster *et al*., 1994). In South Africa, yield losses due to RWA were reported to be 21% to 92% (Hewitt, 1988), and above 90% (Du Toit and Walters, 1984). RWA is a recent pest in Kenya as it was first identified in farmers' field in 1995 (Macharia *et al*., 1999). It then spread quickly to all the wheat growing areas of the country and became evident that most of the commercial wheat varieties in the country were susceptible to RWA (Malinga, 2007). In Kenya it has been reported to cause yield losses of up to 90% (Kinyua *et al*., 2002).

The Russian wheat aphid is pale to light green in colour with an elongated, spindle shaped body and grows up to 2 mm long. It has short antennae with rounded, very short, nearly invisible cornicles. The feature that easily distinguishes it from other cereal aphids is the presence of an appendage (supra-caudal process) above the cauda, giving the aphid the appearance of having two tails (Michaud and Sloderbeck, 2005). It feeds on wheat until the plant is mature and can often be found in developing heads. RWA feeds on the newest growth on the plant and effectively cause cessation of chlorophyll production in those leaves. It is believed that RWA injects toxins into the plants during feeding, which prevents the production of chlorophyll and causes leaf curling (Tolmay, 2006). They feed by probing their stylets intercellulaly until they reach the phloem (Fouché *et al*, 1984). As it feeds, the RWA causes the leaf to curl and creates an enclosure that protects the insect from harsh weather, natural enemies, and insecticides. Symptoms of damage include reduced plant height, sterile heads, low kernel weight, white, yellow or purple longitudinal streaks on the leaf and in the most severe condition, death (Walters, 1984).

There are at least two RWA biotypes reported in South Africa and USA (Nora *et al.,* 2007) and at least two biotypes exists in Kenya (Maling'a *et al.,* 2007). These biotypes appear different from those ones found in South Africa and USA. Two wheat genotypes 'KRWA9' and 'KRWA16' have been found to be resistant to the local RWA biotypes conferred by two non-allelic RWA resistance genes (Pathak *et al.*, 2007; Kenduiwa, 2009). Therefore, there is still need to develop wheat cultivars resistant to the local RWA biotypes in Kenya.

1.3 Stem Rust of Wheat (Race TTKSK or 'Ug99')

Stem rust or black rust of wheat is caused by the fungal pathogen, *Puccinia graminis* Pers. f.sp. *tritici*. The host range of this form of *Puccinia graminis* is inconsistently reported in the literature but it is fairly wide (up to 28 species) with its main asexual host being wheat (*Triticum spp.*); other cereals and a range of grasses can also become infected (Kurt, 2001). The fungus completes its sexual cycle on the broad-leaved hosts' barberry (*Berberis* spp.) and *Mahonia* spp. Its distribution is worldwide. Infected areas are rough to the touch. The red rust or summer spore stage appears on leaves and stems as elongate pustules (uredia) containing reddish brown spore masses. The black rust or autumn spore stage (teliospores) is similar except for color (Kurt, 2001).

Historically, stem rust has caused massive yield losses of wheat wherever it occurred, but in the last 50 years the disease has not been of great concern because it has been effectively controlled through selection and breeding for stem rust resistance genes known as *Sr* genes. There are at least 50 *Sr* genes which confer resistance to different races of stem rust (CIMMYT, 2007). In Uganda, in 1999, a new virulent stem rust race known as 'Ug99' was found on wheat lines known to have the stem rust resistance gene *Sr31*, a gene for which no virulence had been reported previously anywhere in the world (CIMMYT, 2007). Similar virulence was observed in 2001 in Kenya and in 2003 in Ethiopia (Wanyera *et al*., 2006). The new race ('Ug99') blocks the vascular tissues in cereal grains including wheat, oats (*Avena sativa*) and barley (*Hordeum vulgare* L.). It is highly damaging to wheat production and according to experiments done in affected areas it is reported to have caused yield losses of up to 71% (CIMMYT, 2007). Unlike leaf or stripe rusts that may reduce crop yields, 'Ug99'-infected plants may suffer up to 100% loss (Hildebrant, 2008). According to FAO, an estimated 80% of the wheat varieties currently being grown in East African region are considered susceptible to 'Ug99'.

By overcoming the main sources of host resistance in the varieties of wheat that are commonly grown in Africa and Asia, 'Ug99' has spread from Uganda to Kenya, Ethiopia, Sudan, Yemen and Iran (Singh *et al.*, 2006). It is now predicted that it may also be in Pakistan. The wind models predicted that if the fungus crossed from Eastern Africa to the Arabian Peninsula it

could easily spread to the vast wheat-growing areas of North Africa, the Middle East, Pakistan and India. There is every reason to believe the new 'Ug99' strain of stem rust represents a much greater risk to world wheat production. Annual losses of as much as US$ 3 billion in Africa, the Middle East and south Asia alone are possible (CIMMYT, 2007). According to the FAO (2009), countries in the predicted, immediate pathway grow more than 65 million hectares of wheat, accounting for 25% of the global wheat harvest. If not controlled, stem rust race Ug99 will have a major impact on food security, especially since global wheat stocks are at a historic low. Most commercial wheat varieties in Kenya are susceptible to race 'Ug99'. Over 60,000 accessions were recently screened at KARI-Njoro and a few have been found to possess acceptable levels of resistance against 'Ug99' (Macharia, 2009).

1.4 Statement of the Problem

Russian wheat aphid and the emergence of stem rust race 'Ug99' in Kenya are some of the most binding constraints to wheat production in Kenya. Severe infestation by RWA may result in yield losses of up to 90% in commercial wheat cultivars and 'Ug99' infected wheat plants may suffer up to 100% loss. The two pests combined have seriously affected farmers forcing them to heavily rely on chemicals. Control of RWA and stem rust ('Ug99') using chemicals is neither environmentally friendly nor economically feasible; this has led to low farm incomes in the country. It is therefore vital to develop resistant varieties to both RWA and 'Ug99'. The use of resistant cultivars is a safe, effective and economical management option to protect wheat from RWA and 'Ug99' while minimizing the use of chemicals.

1.5 Justification

The most economic problems that have consistently affected Kenya include food insecurity despite the fact that the economy is agricultural based. Wheat is one of the seven major crops that are central to achieving development of agriculture and the second most important cereal crop after maize in Kenya. Unfortunately, Kenya's wheat production is constrained by abiotic and biotic stresses. Among the biotic stresses include pests and diseases. Russian wheat aphid and the emergence of *Puccinia graminis* f.sp. *tritici* race 'Ug99' in Kenya, are some of the most binding constraint to wheat production. Under severe infestation RWA can cause yield losses of up to 90% (Maling'a, 2007) while 'Ug99' can cause up to 100% yield loss

(Hildebrant, 2008). Control of the RWA and 'Ug99' with pesticides is neither environmentally safe nor economically effective because majority of famers in Kenya are low resource endowed and cannot afford this resources. Therefore there is great need to come up with cheap and durable solutions to both RWA and 'Ug99'. One of the most effective ways to increase local wheat production in an economically and ecologically sound manner would be to breed for combined resistance to both RWA and 'Ug99'. The use of resistant cultivars is a safe, effective and economical management option to protect wheat from RWA and 'Ug99' while minimizing the use of chemicals.

1.6 Objectives

1.6.1 Broad Objective

To contribute to increased wheat yield through effective management of Russian wheat aphid and wheat stem rust in Kenya.

1.6.2 Specific Objectives

1. To develop wheat germplasm resistant to both Russian wheat aphid and stem rust race 'Ug99'.

2. To phenotypically characterize wheat germplasm resistant to Russian wheat aphid and stem rust race 'Ug99'.

1.7 Null Hypotheses

1. It is not possible to develop wheat germplasm with resistance to Russian wheat aphid and stem rust race 'Ug99'.

2. The Double Cross F_2 (DC F_2) segregants will not follow monohybrid segregation ratios of 3:1 for each of the two resistance genes.

CHAPTER TWO

LITERATURE REVIEW

2.1 Wheat Production in Kenya

Wheat was introduced in Kenya towards the end of 19th century and has since been grown on an increasing scale in the highland areas (Kiplangat, 2005). Wheat is the second most important cereal grain in Kenya, after maize. The crop is produced by both small scale and large scale farmers. The large scale farmers dominate wheat production with a share of about 75% of the wheat area and 80% of production. The crop is grown largely for commercial purposes (FAO, 2009). All the wheat is spring wheat and several varieties of both hard and soft wheat are grown. More than 100 varieties suited to various agro-ecological zones have been released by Kenya Agricultural Research Institute (KARI) which is the National Plant Breeding Centre (Appendix I).

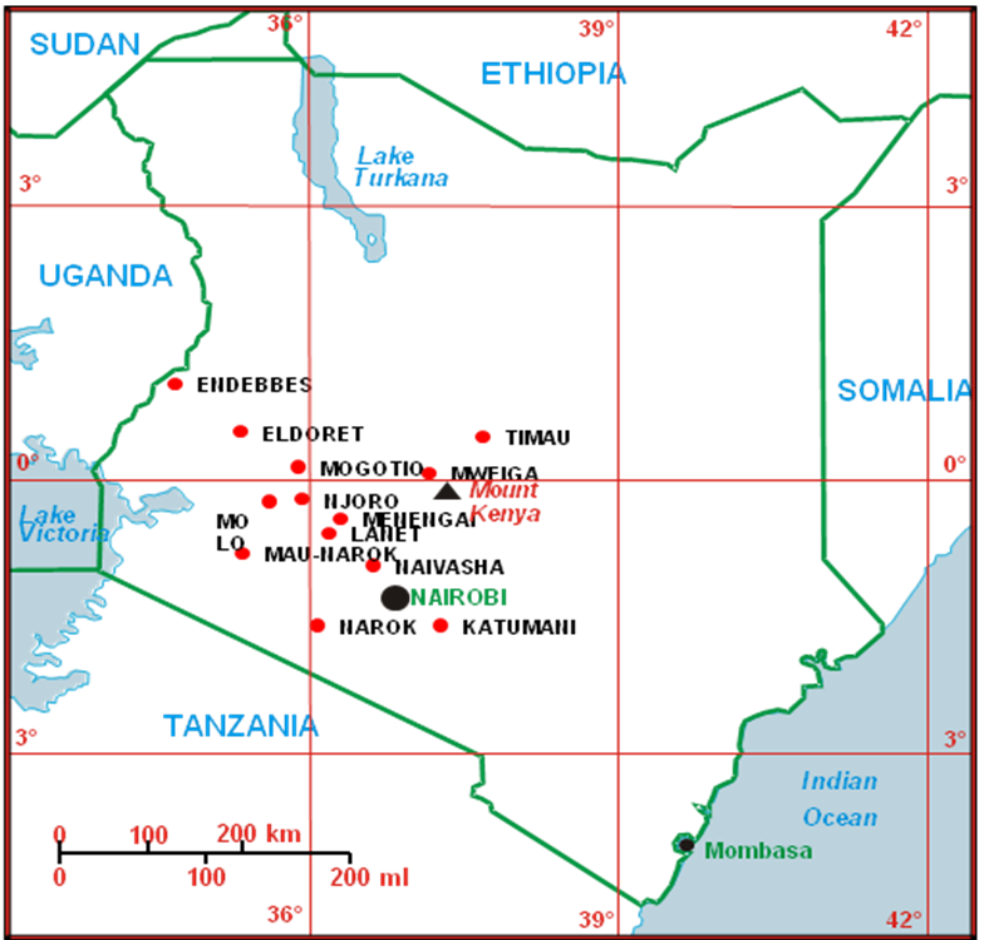

Figure 1: A map showing wheat growing areas in Kenya (Source: http://www.cimmyt.org)

Wheat growing areas in Kenya include the Rift Valley regions of Uasin Gishu, Narok, Marakwet, Elgeyo, Londiani, Molo, Nakuru and Timau areas (Figure 1). These areas have altitudes ranging between 1,200m and 1,500m above the sea level, with annual rainfall varying between 800 mm and 2,000 mm, with up to 2,500 mm on higher grounds. The area under wheat production in Kenya increased from 144,000 ha in 2002 to 150,000 ha in 2008. The demand for wheat and wheat products is growing at a rate of 7% per annum. Even though the national production is increasing only about 35% of domestic consumption requirement are realized the remaining 70% is met through imports (FAO, 2009).

2.2 Genetics of Wheat

The term 'wheat' is used to refer to the cultivated species of the genus *Triticum.* The genus *Triticum* is complex and it is classified into three ploidy groups; diploids (2n=2x=14), tetraploids (2n=4x=28) and hexaploids (2n=6x=42). The polyploidy series is believed to have been formed by closely related species which combined in nature. There are 13 diploid, 12 tetraploid and 5 hexaploid species of *Triticum.* The bread wheat *Triticum aestivum* L. (AABBDD) is hexaploid. It originated as an alloploid combining the tetraploid species *Triticum turgidum* (AABB) and the diploid species *Triticum tauschii* (DD). Each of the three contributing genomes has 7 pairs of homologous chromosomes. However, each homologous group A, B and D genome are partially homologous (homeologous). Chromosomes within homologous groups carry many loci in common, even though the chromosomes originated within a different genome. The loci in common indicate that the genomes were probably derived from a common ancestor. In nature hexaploid wheats perform as a diploid species (n=21, and 2n=42). The hexaploid wheat acquired this property of diploid pairing (21 pairs) from a dominant gene Ph in the long arm of chromosome 5B which inhibits pairing between homeologous chromosomes. In the absence of the Ph gene, homologous chromosomes may pair in a disorganized manner i.e. multivalent formation (Sears *et al.,* 1974).

2.3 Russian Wheat Aphid

The Russian wheat aphid (RWA) is a significant pest of wheat and barley. This aphid occurs throughout the major wheat producing areas of the world except Australia. Its origin is thought to be in the wheat producing region of central Asia. The RWA was not considered as a serious

pest of cereal crops until 1978, when it was discovered causing extensive damage to wheat in South Africa (Walters, 1984). The RWA gained international pest status after being discovered in Mexico infesting wheat in 1980. From there it moved into the US via Texas in 1986 and rapidly spread throughout the primary wheat producing states in the western half of the US by 1987 to 1988 (Morrison, 1988). The RWA continues to be one of the most important pests of dry land wheat and barley in the USA and South Africa, and now in Kenya (Maling'a, 2007).

Nearly 1 million hectares of the total 27 million hectares of wheat planted in the western US was treated for RWA at a cost of $17 million in 1987. Insecticide costs in combination with wheat yield losses caused by RWA damage exceeded $53 million, with about one-half of this total incurred by the state of Colorado alone (Webster *et al.,* 1994). Losses to cereal crops totaled $893 million from 1987 to 1993. RWA is a recent pest in Kenya as it was first identified in farmers' field in 1995 (Macharia *et al*., 1999). It then spread quickly to all the wheat growing areas of the country and became evident that all the commercial wheat varieties in the country were susceptible to RWA (Malinga, 2007). In Kenya it has been reported to cause yield losses of up to 90% (Kinyua *et al*., 2002). The impact of RWA on wheat production became negligible after 1994, following the release of 'Halt', a RWA resistant wheat cultivar which carried the *Dn4* resistance gene (Saidi and Quick, 1996).

Wheat and barley are the main cultivated hosts for RWA. Triticale and oat also can serve as hosts. However, the RWA must utilize volunteer cereal growth and other Graminaceous hosts to survive between grain harvest and the next planting event. Just as important to RWA ecology are the wild grass hosts that include *Agropyran* spp., *Elymas* spp., *Pascopyrum* spp., and *Aegilops* spp. (Armstrong *et al.,* 1996). With its broad host range, RWA can exist without the presence of wheat and barley. However, cultivated hosts provide RWA with the opportunity to exploit a host crop monoculture and thus become economically significant.

RWA is small in size usually 2 mm long, lime-green in colour with a distinctive spindle-shaped body. The legs, antennae and cornicles are short compared to most other aphids (Figure 2). Viewed from the side, the terminal segment of the abdomen has a supracaudal structure that appears as a double tail (Michaud and Sloderbeck, 2005).

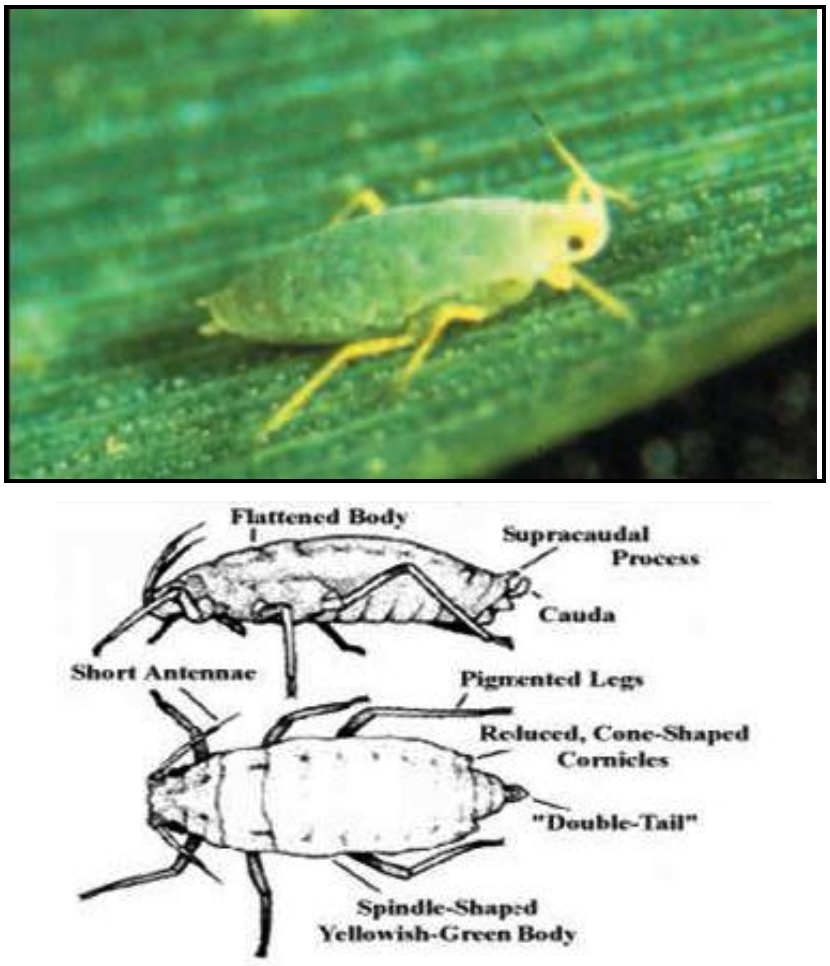

Figure 2: The Russian Wheat Aphid. (Source: Tillage handbook - http://www.pnwsteep.wsu.)

The RWA feeds on wheat until the plant is mature and can often be found in developing heads. When wheat plants die in response to heavy aphid feeding, the third and forth instar aphids develop wings (Berner, 2006). Russian wheat aphids feed on the newest growth on the plant and effectively cause cessation of chlorophyll production in those leaves. They feed by probing their stylets intercellulaly until they reach the phloem (Fouché *et al*, 1984). As it feeds, the Russian wheat aphid causes the leaf to curl and creates an enclosure that protects the insect from harsh weather, natural enemies, and insecticides (Figure 3a). It is also believed that RWA secretes a phytotoxin during feeding, which results in the early breakdown of chloroplasts in susceptible cultivars (Tolmay, 2006). Symptoms of damage include reduced plant height, sterile heads, low kernel weight, white, yellow or purple longitudinal streaks on the leaf and in the most severe condition, death (Figure 3a, 3b, 3c, 3d, 3e 3f and 3g) (Walters, 1984).

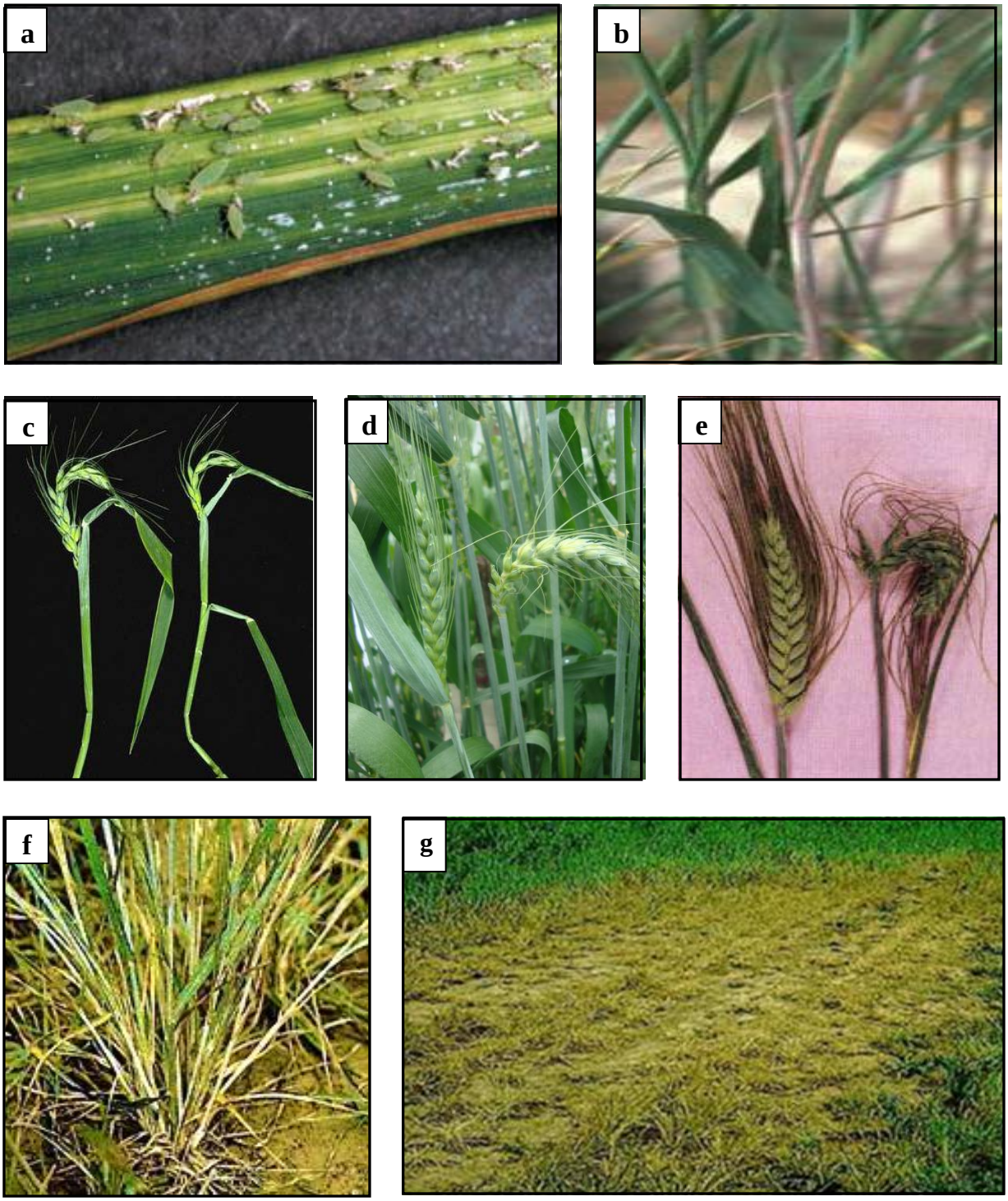

Figure 3:(a) Unrolled leaf section showing yellow and white longitudinal streaks. **(b)** Purpling of the plant. **(c)** Trapped heads. **(d)** One of the heads (right) exhibiting fish-hook deformation. **(e)** Sterile head (right). **(f)** Symptoms of chlorosis and Stunted growth. **(g)** A wiped out field by RWA. (Source: Tillage handbook - http://www.pnwsteep.wsu.)

2.3.1 Life cycle and biology of the RWA

The life cycle of the RWA is typical of most aphids, whereby it reproduces parthenogenically as female viviparae under warm temperatures. This life cycle is called holocyclic (Figure 4). However under cold temperatures the life cycle is anholocyclic (Figure 4) (Purteka *et al.,* 1992). Generation times range from 8 to 42 days and females can produce 13 to 46 nymphs per generation. Reproductive rates increase and generation time is reduced as air temperature increases (Aalbersberg *et al.,* 1989).

The aphids feed on the plant until the plant dies down as it matures or in response to heavy aphid feeding. In such instances an increased proportion of the immature aphids (nymphs), in response to unfavorable conditions such as food shortage or overcrowding, develop wings that look like shoulder pads on third and fourth instar nymphs. These grow into alate female adults which fly away to colonize new wheat fields (Gibson and Rice, 1989; Nyaanga, 2002; Maling'a, 2007).

The alatae (winged) female adults differ in biology and appearance from their apterous (wingless) sisters. They give rise to few young ones because most of their stored food reserve is used during flight (Michaud and Sloderbeck, 2005; Dagg, 2002). They may feed for several days on the plant where they were born, but they do not begin reproducing until they fly away and find a fresh suitable host. They take to flight in response to the blue ultraviolet light from the sky and fly upwards (Gibson and Rice, 1989). They are carried on wind currents for long distances. Their function is to seek a suitable host plant and initiate a new colony (Blackman and Eastop, 2000). When descending from the sky, the Russian wheat aphid is attracted to the orange -yellow-green light reflected from leaves of plants. The size, shape and contrast of plants against its background affect its attractiveness to the alatae (Gibson and Rice, 1989). Infestations often begin along field borders where the contrast between young plants and bare soil is greatest (Michaud and Sloderbeck, 2005). The alate (winged) aphids have well developed wing muscles and fat bodies that store energy for flight, they have smaller reproductive organs and are thus less fecund than their apterous (wingless) (Dagg, 2002). The daughters born to the colonizing alate aphids invariably develop into wingless adults which give birth to more daughters thus accelerating colony growth in the second generation (Le Trionnai re *et al* . , 2008).

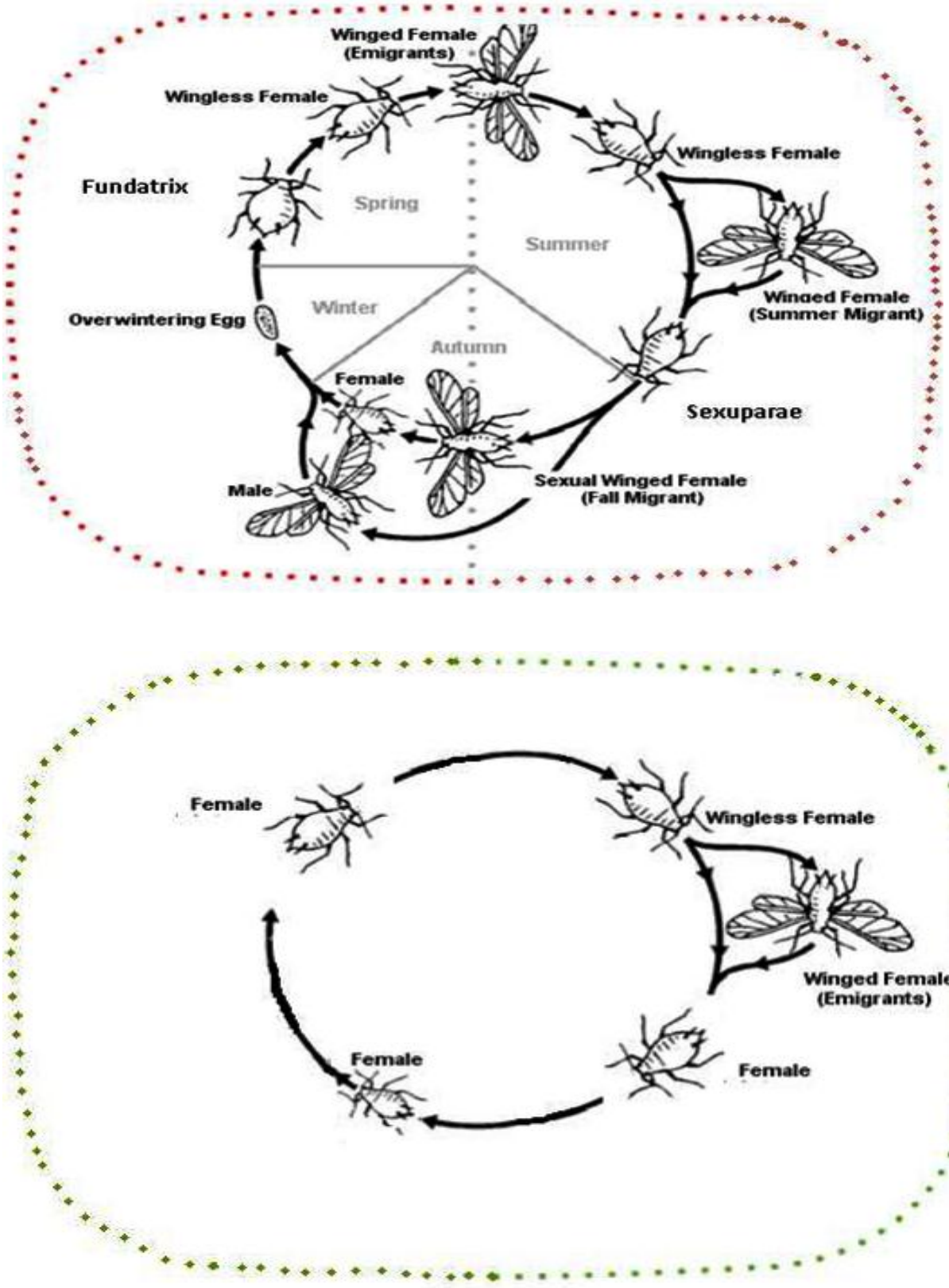

Figure 4: Holocyclic Lifecycle (Above) Anholocyclic lifecycle of the RWA. (Source: http://www.pnwsteep.wsu)

2.3.2 RWA biotypes

A RWA population can contain biotypes that have the ability to damage previously resistant cultivars. The occurrence can have a serious impact on managing RWA with host plant resistance. Biotypes were first reported in RWA populations from the former Soviet Union, Europe, and the Middle East in 1989. The RWA from Syria and Kirghiz regions were shown to severely damage wheat with the *Dn4* gene (Puterka *et al.,* 1992). Table 1 summarizes the reactions of all sources of resistance genes in wheat to Russian wheat aphid biotypes. In 2003, a new biotype of RWA appeared in Colorado which seriously damaged wheat that carried the *Dn4* gene. The biotype designated RWA2, could acutely damage wheat with anyone of eight of the eleven *Dn* resistance genes with the exception of the *Dn7* (Haley *et al.,* 2004). Biotypes RWA3, RWA4 and RWA5 were soon discovered which differentially damaged *Dn1* to *Dn9* resistance genes in wheat (Burd *et al.,* 2006). By 2005, RWA2 had already dominated the biotype complex in the western US (Puterka *et al.,* 2007). The extensive distribution and predominance of RWA2 indicated that wheat cultivars containing the *Dn4* gene would have little value in managing RWA. Fortunately the primary sources of RWA1 resistance in barley, STARS 9301B and STARS 9577B, have remained resistant to all known RWA biotypes (Puterka *et al.,* 2006). At least two biotypes appear to exist in Kenya (Kiplangat, 2005; Maling'a *et al.,* 2007). These biotypes appear different from those ones found in South Africa and USA (Liu *et al*., 2010). More research is still needed to determine the number of biotypes in Kenya and their virulence spectrum.

The origin of the new biotypes critically remains unknown in Kenya although they have been characterized in USA. Wheat breeders are currently focusing efforts to move *Dn7* resistance into wheat and find new sources of resistance to these biotypes. The threat of new RWA biotypes to wheat production is not limited to the US but also has become a problem in South Africa (Tolmay *et al.,* 2007). Effective deployment of RWA resistance in cereals will rest on a thorough characterization of biotypic diversity and testing of candidate resistance genes in the field, as well as vigilantly monitoring RWA biotype frequency after resistance gene deployment.

Table 1: Russian wheat aphid resistance genes and biotypic interactions in wheat in USA (Haley *et al.*, 2004)

Resistance Gene	Russian Wheat Aphid Biotype				
	RWA1	RWA2	RWA3	RWA4	RWA5
	Reaction to biotype				
Dn1	S	S	S	S	S
Dn2	R	S	S	S	S
dn3	R	S	S	S	S
Dn4	R	S	S	R	R
Dn5	R	S	S	S	R
Dn6	R	S	S	R	R
Dn7	R	R	S	S	R
Dn8	S	S	S	S	S
Dn9	S	S	S	S	S

R and S indicate Resistant and Susceptible reactions respectively

2.3.3 Control of the Russian Wheat Aphid

2.3.3.1 Cultural Control

Although resistant varieties and insecticides provide the most effective RWA control, several other practices can provide additional control of the aphid. These practices should also help with other pest problems and make good agronomic sense as well. This involves practices such as use of correct seed rate to ensure good plant density. Planting should also be done as early as possible and crops should be well-fertilized to make them more tolerant to aphid attack. All volunteer plants and grasses should be removed because they act as the aphid's hosts even before the main crop has been planted. Grazing of volunteer plants after harvesting can be done (Karren, 1993). RWA being polyphagous becomes difficult to control because some alternate hosts are actually important crops i.e. rice and barley (Marasas, 1999).

2.3.3.2 Biological Control

Studies of natural enemy-aphid and plant-habitat interactions have substantially increased since the mid-1980s, resulting in classical biological control programs. Predators and parasitoids that attack other grain aphids also feed on the Russian wheat aphid, although not all are effective at reaching them in rolled leaves. Several studies have been carried out to identify the most effective natural enemies of RWA. Exclusion cage studies in Western Kansas indicate that the convergent lady beetle *Hippodamia convergens*, also the key predator of greenbug in the

region, is one of the most important natural controls. The seven-spotted lady beetle, *Coccinella sepempuncata*, is common in wheat fields in early spring and may play a role in reducing RWA numbers and *Coleomegilla maculata* DeGeer is also good (Mitchels *et al*, 2001). Flower fly larvae (*Syrphidae*) prey on aphids, whereas adults feed on nectar, pollen, and aphid honeydew. The host ranges of these species can include some use of non-aphid prey, but they broadly can be classified as aphid specialists, at least when compared to spiders, carabids, and staphylinids. In the USA, a few species of green lacewings (Chrysopidae) inhabit cereal fields with the common green lacewing, *Chrysoperla plorabunda* F., being the most common and widespread species. Green lacewings that inhabit cereals are predaceous only as larvae. Adults feed on aphid honeydew, nectar, and pollen. Brown lacewings (*Hemerobiidae*) sometimes also occur in cereals and feed on aphids but are generally less abundant than chrysopids. Predatory Heteroptera (*Nabidae* and *Anthocoridae*) feed on aphids in cereals. The common damasel bug, *N. americoferus* are winged and highly mobile. They effectively prey upon aphids but lack high prey specificity (Elliot *et al*, 1998b).

Various entomopathogenic fungi can cause diseases in Russian wheat aphids, but most require substantial humidity to be effective, which makes them a less likely cause of death in arid regions where the aphid is most prevalent (Michaud and Sloderbeck, 2005). A fungal biological control agent, *Beauveria bassiana* GHA (Botanigard), is recommended for Russian wheat aphid control on oats (Alston and Reding, 1996).

2.3.3.3 Chemical Control

Systemic insecticides are the most effective pesticides for the control of the RWA. Gaucho 350FS, Cruiser and Carbofuran 350 ST and foliar sprays i.e. disulfoton (Di-Syston), Thunder & Dimethoate (Cygon) are some of the most effective insecticides (Table 2). In Kenya, some of these insecticides have been tested to determine their effectiveness against RWA. Seed dressing with Gaucho 350FS, Carbofuran 350 ST or foliar spraying with Brigade increased yields by 175%, 147% and 123% respectively over the untreated control. Similarly, foliar sprays with 120 ml/ha of Decis 100EC (Deltamethrin 100g/L) and 40 tablets /ha of Decistab (Deltamethrin 0.25 g/tablet) resulted in significant yield increases of 21.8% and 16.8% respectively (KARI, 2008). Yield losses due to RWA are however still high since most farmers still use non-dressed seeds or fail to use effective sprays due to the high costs of systemic insecticides.

Table 2: Chemicals used to control RWA (KARI, 2008).

Chemicals applied as foliar insecticides		
Chemical	**Rate/ha + 200 L of water**	**Rate/20L sprayer**
Bulldock© Star (Beta-cyfluthrin 12.5g/L + chlorpyrifos 250g/L)	450 - 600ml	450ml - 600 ml
Metasystox (Oxydemeton-methyl 250g/L)	500 ml	50 ml
Cyclone© 505 EC (cypermethrin10% + chlorpyriphos35%)	300 ml	30 ml
Chemicals applied with seed at planting		
Chemical	**Rate/100kg of seed**	
Gaucho© 350 FS (Imidacloprid 350g/L)	200 ml	
Redigo Deter (50 g/L prothioconazole + 250 g/L clothianidin)	200 ml	
Furadan© 350 ST (Carbofuran 350g/L)	740 ml	

2.3.3.4 Host Plant Resistance

Wheat plants resistant to the RWA are able to maintain their yield under infestation conditions whereas the susceptible lines show decreases in yield. This resistance is governed by three mechanisms; antixenosis, antibiosis and tolerance. Antixenosis involves non preference of host for feeding, oviposition and resting. Antibiosis refers to all adverse effects exerted by the plant on insect biology, for example, survival, development and reproduction. Tolerance includes all plant responses resulting in the ability to withstand infestation and to support an insect population that would severely damage susceptible plants (Painter, 1951).

In Kenya, all commercial varieties are susceptible to the local RWA biotypes (Maling'a, 2007). However, several lines have been tested by different scientists and have shown good levels of resistance to the local RWA biotypes. Kiplagat (2005) reported that two PI-294994 derived lines, designated P1 and P2 showed very high levels of resistance. KRWA8 was reported to be moderate to high resistant, the resistance being governed by a single dominant gene (Maling'a *et al.,* 2004; Pathak *et al.,* 2004). KRWA9 was also found to be highly resistant to the local RWA biotypes and this resistance is governed by a single dominant gene (Pathak *et al.,* 2007; Maling'a, 2007). This was later confirmed by Kenduiwa (2009). Another line which showed some good levels of resistance was KRWA16 (Maling'a *et al.,* 2004; Pathak *et al.,* 2004;

Pathak *et. al.,* 2007; Kenduiwa, 2009), where the resistance is conferred by two dominant genes with recessive epistatis (Pathak *et al*., 2007).

2.3.3.5 Resistance Genes for the Control of RWA

The spread of the RWA to the USA and South Africa during the 1980's intensified the search for resistance genes to the RWA. These genes were introduced into lines with more acceptable agronomic characteristics by means of the backcrossing technique (Berner, 2006). Since then, 11 *Diuraphis noxia* (*Dn*) genes conferring resistance to Russian wheat aphid found in bread wheat and its relatives have been identified and described. They include *Dn1, Dn2, dn3, Dn4, Dn5, Dn6, Dn7, Dn8, Dn9, Dnx,* and *Dny*.

Dn1 was found to be in common wheat accession PI 137739 and was located on chromosome 7D, and *Dn2* in PI 262660 located on chromosome 7DL (Du Toit, 1988). A recessive gene *dn3* was found in the goat-grass (*Aegilops tauschii*) line SQ24 (Nkongolo *et al.,* 1991a). A dominant gene *Dn4* was identified in PI 372129 located on 1DS (Ma *et al*., 1998). *Dn5* was placed on chromosome 7DL and was identified in PI 294994(Du Toit, 1987). *Dn6* (un-located) was found in PI 243781(Saidi and Quick, 1996), *Dn7* was derived from rye and transferred to the 1RS/1BL translocation in 'Gamtoos' wheat (Marais *et al*., 1998). *Dn8* and *Dn9* (7D and 1D respectively) were identified in PI 294994. A single dominant gene *Dnx* was found in PI 220127 (Liu *et al*., 2001) and *Dny* placed on 1DL was found in a variety developed by Kansas State University known as 'Stanton' derived from PI 220350 (Michaud and Sloderbeck, 2005). Appendix II shows a summary of the known RWA genes, sources and their chromosomal locations.

2.4 Wheat Stem Rust

Stem rust was once the most feared disease in most wheat growing regions of the world. Several references in the Bible relate to epidemics of cereal rusts and smut which inflicted Israelites as punishment for their sins (Chester, 1946). Fragments of wheat stem rust from the Bronze age have been discovered in Israel (Kislev, 1982).

In recent history, several authors have documented losses associated with stem rust as exemplified in Table 3.

Table 3: Examples of recorded losses due to stem rust

Year	Region	Estimated Yield losses (%)	Source
1932	Eastern and central Europe	5-20	Zadoks,1963
1951	Scandinavia	9-33	Zadoks,1963
1974	Southern Australia	-	Watson,1981
1948,1951,1952,1956	North China and inner Mongolia	-	Roelfs,1978
1935	North Dakota and Minnesota	50	Leonard,2001
1950-55	North America and Canada	40	Roelfs,1978

Stem rust or black rust of wheat is caused by the fungal pathogen, *Puccinia graminis* Pers. f.sp. *tritici*. The host range of this form of *Puccinia graminis* is inconsistently reported in the literature but it is fairly wide (up to 28 species) with its main asexual host being wheat (*Triticum spp.*); other cereals and a range of grasses can also become infected. The fungus completes its sexual cycle on the broad-leaved hosts barberry (*Berberis* spp.) and *Mahonia spp*. Its distribution is worldwide. Infected areas are rough to the touch. The red rust, or summer spore stage appears on leaves and stems as elongate pustules (uredia) containing reddish brown spore masses (Figure 5). The black rust or autumn spore stage (teliospores) is similar except for colour (Kurt, 2001).

Figure 5: Close-up of stem rust on wheat. (Source: USDA, Photo by Cereal Disease Lab)

The pustules of stem rust that are seen on wheat during most of its growing cycle are called uredia and produce urediospores. Urediospores contain two genetically different nuclei, that is are dikaryotic, oblong in shape and reddish brown in color. A tremendous number of spores are produced by each individual uredium for several weeks. In the absence of strong wind current, most spores remain within the crop canopy and cause re-infection. On the contrary, wind carries spores sometimes to long distances. Luig (1985) observed that spores have been carried from southern Africa to Australia in at least three occasions. The more common phenomena involve movement of spores from field to field over short distances.

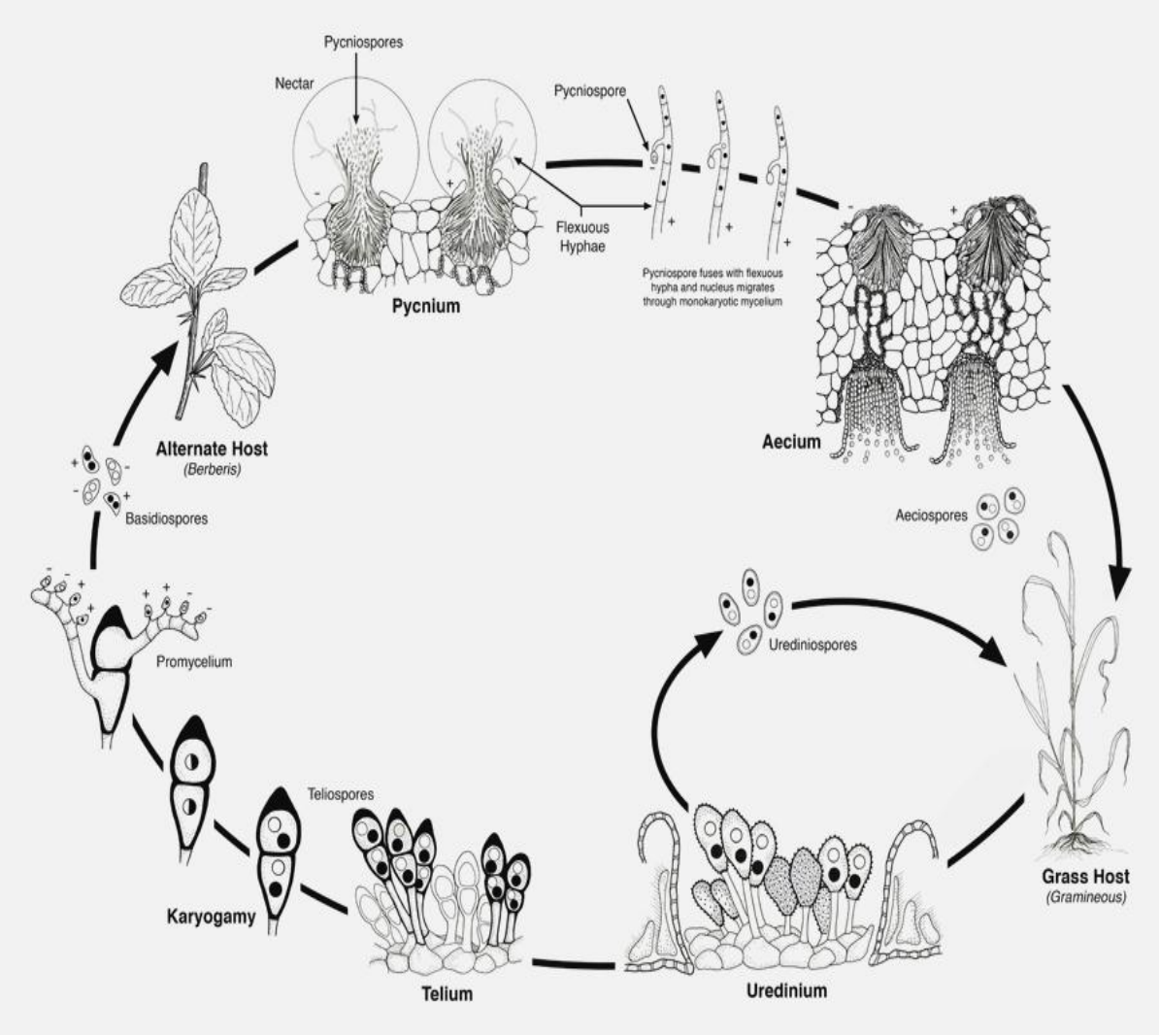

Figure 6: Reproduction cycle of *Puccinia graminis* (Kurt and Szabo, 2005)

2.4.2 Wheat Stem Rust in Kenya

Progress in the breeding of wheat in Kenya has been on the background of past achievements vis-à-vis set-backs from changing rust races (Dixon, 1960). Two stem rust physiologic races were differentiated in 1927 (Green *et al.,* 1968). Later another form appeared on wheat variety reliance at Njoro in 1930 and a fourth one was recognized in 1931 (Anonymous, 1933). By 1947, eight stem rust races (on the basis of Kenya differentials) had been identified (Green *et al.,* 1968, Payne *et al.,* 2000). Four more races appeared three years later with little or no stable resistance available in the Kenyan gene pool (Table 4). Based on international race differentiation system, the races K1, K5 and K12 were identified as American 'race 21'. K8, K10 and K11 as American race 24 (Thorpe, 1958).

The absence of winter coupled with an abundance of wild and cultivated hosts for survival is thought to be the key factors in the broad virulence spectrum of wheat stem rust in Kenya (Payne *et al.,* 2000). Leppik (1970) draws attention to *Berberis holstii* which is endemic to Kenya and at times prone to heavy rust infection, as the probable alternate host for wheat stem rust in East Africa. However earlier observations revealed that Aecidial cultures taken from this species of barberry failed to infect wheat building on the conclusion that *Berberis holstii* is non-functional in the stem rust cycle and that there is no known alternate host of the rust in Kenya (Thorpe, 1958).

Studies done on 164 collections of wheat stem rust on local wild and exotic grasses produced no positive hosts either (Harder *et al.,* 1972). Surveys to date indicate that grass species are not important in the epidemiology of wheat stem rust in East Africa. Certainly however, since large areas of grassland are yet to be surveyed, it is possible that grass species may play some role (Harder *et al.,* 1972).

The close relationship of the earlier wheat varieties caused them to be destroyed as a group by new rust races (Thorpe, 1958). The year 1953 marked the beginning of a new era in the Kenya wheat industry (Dixon, 1960). There was a large scale introduction and scrutiny of diverse germplasm through the 'International spring wheat nursery'-funded by the Rockfeller Foundation programme in Mexico and 'Near East nurseries' -organized by FAO (Payne *et al.,* 2000). The opportunity derived from these nurseries was to fold: (a) the evaluation of the

material on the broadest possible basis and (b) the chance to select new sources of resistance based not only on Kenya observations but on world-wide basis year by year (Thorpe, 1958; Dixon, 1960).

Table 4: Races of stem rust recorded in Kenya from 1928 to 2007 (CIMMYT, 2007)

Race		
Kenya No.	**International No.**	**Year Identified**
K1	21	1928
K2	17	1928
K3	34	1930
K4	116	1931
K5	21	1936
K6	107	1940
K7	-	1943
K8	24	1948
K9,10,11	122,24,24	1950
K12	21	1951
K13,14	-	1953
K15	-	1954
K16	-	1955
K17,18,19	-	1956/57
K20	-	1958
PgtUg99	TTKSK	2001
Ug99 + *Sr24*	TTKST	2006
Ug99 + *Sr36*	TTTSK	2007

Following the global influence on the Kenyan wheat breeding programme, large number of crosses, on average three hundred thirty five per year were made and cultivar releases were numerous averaging nearly four per year from 1960 – 1968 (Pinto and Hurd, 1970). Despite the progress achieved through the introduced sources of resistance, in 1968 losses due to stem rust and leaf rust were substantial, reaching over £500,000 (Hurd *et al.,* 1969). There was an

attempt to spread the risk of huge losses by growing a large number of varieties with different but unidentified sources of resistance (Evans *et al.,* 1969). In the mid 1970's the government of Kenya provided facilities at the National Plant Breeding Research Centre, Njoro with logistical support from CIMMYT to allow screening and off-season generation advancement of breeder's material from West Asia and North Africa (WANA) and other countries.

Wheat is planted in Kenya at times chosen to make maximum use of rainfall while the crop is growing, yet have it ready for harvest during a dry period. In most of Kenya's wheat growing areas ranging from 1800 – 2800m meters above sea level (Payne *et al.,* 2000), the long rains usually occur between March and August, the short rains during October and November. Dry conditions usually prevail from December to March (Green *et al.,* 1968). Most wheat is planted from March to June and harvested from August to January (Figure 6). Markedly, only a small proportion of the crop is grown above 2400m and on a small acreage. On this land rust inoculum can be produced, and remains standing in January and February as a reservoir for rust in the main wheat growing season. Urediospores produced on this wheat could be in the air after the new crop at lower altitudes has emerged. The wheat growing season has temperature characteristically ranging from 18-30^{o}C with days uniformly about 12.5 hours long. Dews are heavy and precipitation occurs frequently as showers during the main growing season.

As at First World War, only 10,000 acres of wheat were grown in Kenya (Thorpe, 1958). By the year 1940 production had rose steadily to some 200,000 acres. Production subsequently rose to 340,000 acres in 1955 following the great stimulus provided by World War II. The Kenya development plan of 1970-1974 estimated that 500,000 ha of land were suitable for wheat production (Payne *et al.,* 2000). Only about a third of this land was on wheat by 1994. Presently, wheat is grown in many agro-ecological zones that have different planting dates (Ralph and Helmut, 1983; Kamidi, 1995). This provides the ever present reservoir of rust inocolum often termed the 'green- bridge' (Roelfs *et al.,* 1992).

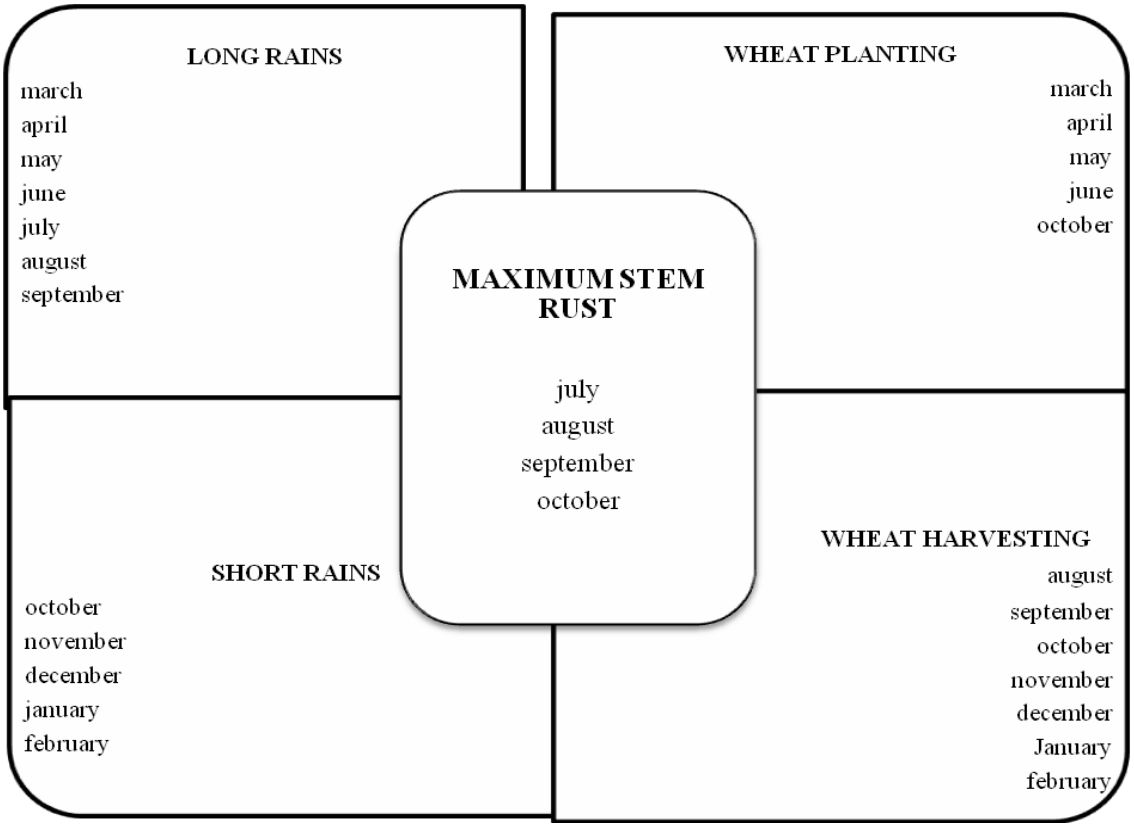

Figure 7: Periods of planting and harvesting of wheat: rainfall and maximum occurrence of airborne urediospores of stem rust in Kenya (Green *et al.*, 1968).

2.4.3 The Stem Rust Race TTKSK or 'Ug99'

In a nursery in Uganda, in 1999, susceptible type stem rust pustules (collection designated 'Ug99') were found on wheat lines known to have the stem rust resistance gene *Sr31*, a gene for which no virulence had been reported previously anywhere in the world. Similar virulence was observed in 2001 in Kenya and 2003 in Ethiopia. Race identification of earlier observations prior to 2001 could not be confirmed because of a lack of samples. Race typing (race TTKS based on *Pgt*. system of nomenclature) and DNA analysis confirmed the presence of the race in Kenya in 2005 (Wanyera *et al*., 2006). The resistance gene *Sr31* is located on the 1B/1R chromosomal translocation, a piece of rye chromosome that has been introduced into many wheat cultivars. In addition to *Sr31*, the leaf rust resistance gene *Lr26* and the stripe rust resistance gene *Yr9* are also on the 1B/1R translocation. The new race, TTKSK, commonly known as 'Ug99' blocks the vascular tissues in cereal grains including wheat, oats and barley. Unlike leaf or stripe rusts that may reduce crop yields, 'Ug99'-infected plants may suffer up to

100% yield loss (Hildebrant, 2008). It is highly damaging to wheat production and according to experiments done in affected areas it is reported to have caused yield losses of up to 71% (CIMMYT, 2007). According to FAO an estimated 80% of the wheat varieties currently being grown in East African region are considered susceptible to 'Ug99'.

By overcoming the main sources of host resistance in the varieties of wheat that are commonly grown in Africa and Asia, 'Ug99' has spread from Uganda to Kenya, Ethiopia, Sudan, Yemen and Iran (Singh *et al.*, 2006). It is now surmised that it may also be in Pakistan. It has been disseminated for long-distance by wind, with the early (unanticipated) spread to Asia purported to be due to Cyclone Gonu in early June 2007. Recently geographic information systems specialists working at CIMMYT plotted the probable trajectory of the fungus, whose spores can travel large distances on the wind (Figure 8). The wind models predicted that if the fungus crossed from Eastern Africa to the Arabian Peninsula it could easily spread to the vast wheat-growing areas of North Africa, the Middle East, Pakistan and India.

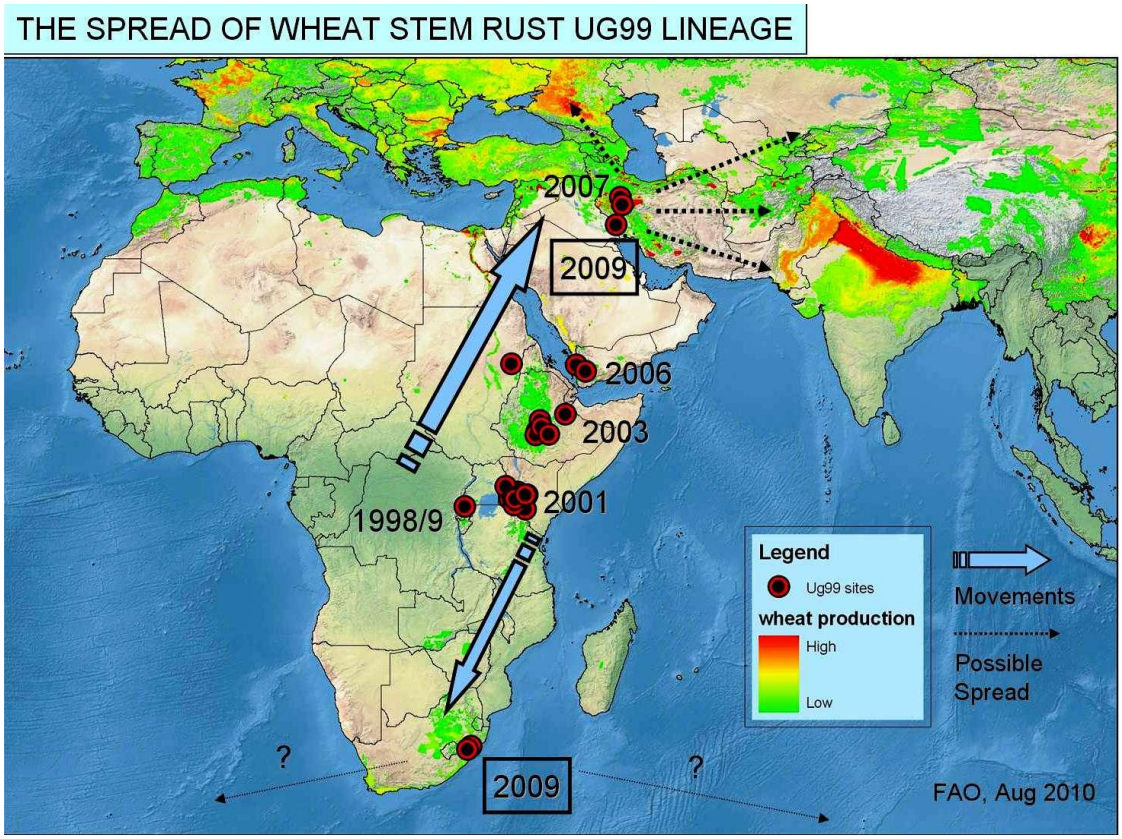

Figure 8: Potential migration routes for race 'Ug99' of the stem rust pathogen based on prevailing airflows and regional wheat production areas (Source: FAO).

2.4.4 Control of Wheat Stem Rust

2.4.4.1 Cultural control

Cultural methods such as; early planting, variety mixtures, removal of volunteers through tillage, grazing or spraying herbicides have been used either singly or in combination to ameliorate the effects of the pathogen (Roelfs, 1988). When applied independently none of these strategies have proved effective (Wanyera, *et al*., 2006). The present emphasis is focused on integrated disease management, i.e., sowing resistant cultivars, good crop husbandry and fungicide control.

2.4.4.2 Chemical Control

Fungicides remain the first line of defense against 'Ug99'. Unlike Stripe and Leaf Rust, foliar fungicide applications targeting Stem Rust ('Ug99') must be applied as soon as the disease is detected as opposed to targeting key yield determining leaves. Failure to do so will increase the risk of significant yield loss. Field trials conducted in Kenya have successfully demonstrated that 'Ug99' can be effectively controlled by the cereal-specific fungicides including Amista Xtra 280SC (azoxystrobin 200 g/L + cyproconazole 80 g/L), Folicur 250EC (tebuconazole 250 g/L), Stratego 250EC (trifloxystrobin 125 g/L + propiconazole 125 g/L), Prosaro 250EC (prothioconazole 125 g/L + tebuconazole 125 g/L) and Silvacur 375EC (tebuconazole 250 g/L + tredimenol 125 g/L). Most of these are triazole and strobirulin fungicides (Wanyera, 2009). However, the poor farmers who stand to lose most from the rust generally cannot afford fungicides, or don't have the equipment or know-how to apply it. Available fungicides are expensive, estimated at US$ 40 per crop cycle to protect one hectare in Kenya and pose risks to human health and the environment (Wanyera, 2009).

2.4.4.3 Host Plant Resistance

In the last 50 years stem rust has been effectively controlled through selection and breeding for stem rust resistance genes known as *Sr* genes. There are at least 50 *Sr* genes which confer resistance to different races of stem rust (CIMMYT, 2007). Globally, the pathogen has virulence for some of the *Sr* genes and so they cannot be deployed in wheat breeding programmes. For example; virulence has been detected to *Sr13* in Ethiopia in durum wheat areas; to *Sr24* in South Africa and India; and, to *Sr27* in Australia and South Africa. Significantly, in 1999, virulence to *Sr31* was detected in Uganda; this race has virulence to a

number of other *Sr* genes and is known as 'Ug99' (or TTKS). *Sr31* was derived from Petkus rye and has been used extensively as the main source of resistance to stem rust in breeding programmes for many wheat cultivars (CIMMYT, 2007).

Other *Sr* genes identified in wild relatives and incorporated into the wheat genome through genetic manipulation in the form of chromosome translocation and addition include *Sr25, Sr26,* and *Sr43* from *Thinopyrum elongatum, Sr37* and *Sr40* from *Triticum timopheevii, Sr32* and *Sr39* from *Aegilops speltoides,* and *Sr44* from *Thinopyrum intermedium.* These genes have been found to be effective against 'Ug99'. In addition, three novel *Sr* genes have been identified recently (temporally designated as *Sr2S, Sr5S,* and *Sr6V*) from *Aegilops speltoides* and *Haynaldia villosa*. Deployment of combinations of these *Sr* genes in wheat can provide protection against the various races of the stem rust fungus, including Ug99 (CIMMYT, 2007).

Gene *Sr26,* of *Thynopirum elongatum* origin, translocated to chromosome 6AL, has been used successfully in Australia and remains effective despite its large-scale deployment in the 1980s. It is not known to be present in cultivars from other countries and the translocation used initially may confer a yield penalty (Singh *et al*., 2007).

Gene *Sr27,* of rye origin, has not been used in wheat improvement. Its deployment in triticale in Australia resulted in a rapid evolution of virulence. This gene has also become ineffective in South Africa. Strategically, this gene should be left for triticale improvement in areas where virulence is not known (Singh *et al*., 2006).

Gene *Sr36,* derived from *Triticum timopheevi,* shows very good levels of resistance to race 'Ug99' at both seedlings and adult plant stages. This gene occurs in a high frequency in US soft winter wheat. Although races with virulence for *Sr36* are common, it could be used effectively as a component for 'Ug99' resistance breeding (Macharia, 2009).
Appendix III shows some examples of the identified genes for stem rust resistance, compiled from McIntosh *et al.,* (1995).

2.5 Breeding for Multiple Resistance

Resistance to two or more diseases has been bred into individual cultivars since Orton (1909) succeeded in combining resistance to *Fusarium* wilt and root rot nematodes in cowpea and cotton. Hope and H44 wheat cultivars combined resistance to leaf rust, stem rust and covered smut (Ausemus, 1943). Multiple resistance has long been a breeding achievement in tobacco, sugar beet, corn, beans and many other crops. Some noteworthy accomplishments have been reported for cabbage (William *et al*., 1968), cucumber (Barnes, 1972; Sitterly, 1972), sugar beet (Gaskill *et al*., 1970) and tomato (Crill *et al*., 1971). Resistance in wheat to both cereal leaf beetle, *Oulema melanopus* and to stem sawfly, *Cephus cinctus* were incorporated by Wallase (1974). Multiple pest resistant alfafa lines were developed by Kindler and Schalk (1975). Multiple resistance in induced amphiploids of *Zinnia elegaans* and *Zinnia angustifolia* to three major pathogens was reported by Lewandowski and Stirmart (1983). The amphiploids posed high levels of resistance to powdery mildew, *Alternaria* blight and moderate to high levels of resistance to bacterial leaf and flower spot. Multiple resistance in rice to bacterial blight, grassy stunt disease, brown plant hopper and green leaf hopper was developed by Khush (1977a).

One of the approaches for incorporating the multiple resistance could be the screening for multiple resistance line(s) from germplasm followed by crossing and screening for more than two diseases and or insect pests in the segregation generations. Such technique is being followed in cowpea. Five thousand cowpea plants were screened for field resistance to Anthracnose, *Cercospora* leaf spot, rust and bacterial pustule. After preliminary observations on the 5,000 lines, 719 lines were selected for further evaluation in field nurseries. All 719 lines were resistant to at least one of the four diseases, 685 lines were resistant to at least two diseases, 537 lines were resistant to at least three diseases 208 lines were resistant to all four diseases and of these 28 lines were also resistant to the target spot (Williams, 1977).

Another approach could be followed by making single cross F_1 hybrids. The F_1 hybrids could be crossed with other resistant donors to make double or top crosses to combine resistance to given diseases and insects. If more donors are available for a single parasite, it is desirable to use good combiners for yield components and plant type etc. The pedigree method is suitable

for handling the segregating generations (Khush, 1977a). At the International Rice Research Institute (IRRI) in the Philippines efforts have been made to eliminate the susceptible material in the early generations. The screening starts even in the F_1 generation of a cross. Khush (1977b) elaborated the procedure citing an example of a double cross between four parents of which A, B, C and D are resistant to bacterial blight, grassy stunt disease, brown plant hopper and green leaf hopper, respectively. All of these traits are monogenic and dominant in their inheritance and are inherited independently. About 400 seeds from double cross (A/B/C/D) are obtained. The seeds are germinated and inoculated with grassy stunt virus in a green house. Approximately 50% of the seedlings are susceptible and are therefore eliminated. The remaining 200 are transplanted in the field and inoculated with bacterial blight. About 50% of these plants are susceptible and are rooted out. The remaining 100 plants are harvested separately. Two small seed samples are taken from each, and the progeny are tested for resistance to brown plant hopper and green leaf hopper. Those carrying the brown plant hopper resistance gene (50%) and those carrying the green leaf hopper resistance (50%) are identified. The F_2 populations are grown only from those carrying both the genes (25 – 30 plants), which segregate for the four resistance genes. The population is subjected to appropriate disease and insect pressures and agronomic desirable plants with multiple resistance are selected in F_3 and F_4 generation to obtain true breeding lines (Khush 1977b). IR28, IR29 and IR34 are some of the varieties of rice with multiple resistance developed at IRRI. The variety IR36 resistant to four diseases (blast, bacterial blight, tungro virus and grassy stunt) and four insect pests (brown plant hopper, green leaf hopper, stem borer and gall midge) were planted in 11 million hectares in early 1980s in Asia.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Site

The experiment was carried out in a green house and a breeding cage at Kenya Agricultural Research Institute (KARI) in Njoro from 30th March 2009 to 14th December 2010. KARI – Njoro, A National Plant Breeding Center, is situated along the Njoro – Mau Narok road in Nakuru County. The altitude is about 2185m above the sea level and it lies between 0° 20′S and 35° 56′E. The area receives an average rainfall of 939mm per annum, with a mean temperature of 14.9 °C. The site is classified as Lower Highland 2 to 3 (LH2 – LH3) agro ecological zones and has a sub humid modified tropical climate. Soil type is predominantly *mollic Andosols* (Jaetzold and Schmidt, 2007).

Njoro in general has been described as a 'hot spot' for stem rust and RWA, as a result of the favourable climatic condition (temperature and relative humidity) that encourages epidemics and promotes evolution of new stem rust physiological races and RWA biotypes (Singh *et al*., 2006; Maling'a, 2007). The climate seems to have a strong effect on the frequency of stem rust epidemics in the highlands of Kenya where most of the wheat is grown (Macharia, 2009).

3.2 Genotypes

This included three parental genotypes and their F_1, F_2 and $F_{2:3}$ populations derived from the double crosses.

3.2.1 Parental Genotypes

Three varieties of wheat were used in crosses. They include 'Kwale', 'KRWA9' and 'Cook'.
'Kwale' is a Kenyan commercial variety popular in high altitude environment with high rainfall. It is known to have good agronomic characteristics (i.e. high yielding), and susceptible to both local RWA biotypes (Maling'a, 2007; Kenduiwa, 2009) and the new stem rust race 'Ug99' (Macharia, 2009). The pedigree of this genotype is given in appendix VIII.
'**KRWA9'** is a Kenyan line of Turkish origin which has poor agronomic attributes, early maturing, high tillering ability and is resistant to the local RWA biotypes. The resistance is conferred by a single dominant gene (Pathak *et al*., 2004; Maling'a, 2007; Kenduiwa, 2009).

'Cook' is an Australian cultivar carrying stem rust resistance gene *Sr*36 derived from *Triticum timopheevi*. The gene is located on chromosome 2BS (Singh *et al*., 2006). This variety shows very good levels of resistance to stem rust race 'Ug99' at both seedling and adult plant stages (Macharia, 2009). The pedigree of this genotype is given in appendix VIII.

3.2.2 Single Crosses (SC)

SC F_1: Kwale × Cook

The F_1 seeds of 'Kwale × Cook' were provided by the breeding department of KARI-Njoro. This cross was made in 2007 in a breeding cage at KARI-Njoro and was part of the Borlaug Global Rust Initiative (BGRI) breeding programme. 'Cook' was used as a pollen donor (Macharia, 2009).

SC F_1: Kwale × KRWA9

The F_1 seeds of 'Kwale × KRWA9' were provided by the biotechnology department of KARI-Njoro. This cross was made in 2006 in a green house at KARI-Njoro during an inheritance study on lines introduced from CIMMYT. 'KRWA9' was used as pollen donor (Kenduiwa, 2009).

3.2.3 Double Crosses (DC)

DC F_1: (F_1 Kwale × Cook) × (F_1 Kwale × KRWA9)

Seeds of DC F_1 were obtained by crossing the F_1 of 'Kwale × Cook' and the F_1 of 'Kwale × KRWA9', with F_1 of 'Kwale × KRWA9' being used as a pollen donor. This was done from 30th March 2009 to 20th August 2009. Staggered planting was done at an interval of 14 days in order to synchronize flowering to enable crossing. Land was ploughed twice and harrowed once to achieve a fine tilth. Seeds were drilled at an inter-row spacing of 20 cm using DAP fertilizer at a rate of 125 kg/ha. Each crossing block had eight rows measuring 7 meters in length, making them 10.5 m^2 in area. Hand weeding was done and all insect pests and diseases were chemically controlled using Bulldock© Star (Beta-cyfluthrin 12.5g/L + chlorpyrifos 250g/L) and Amista Xtra© (azoxystrobin 200g/L + cyproconazole 80g/L), respectively.

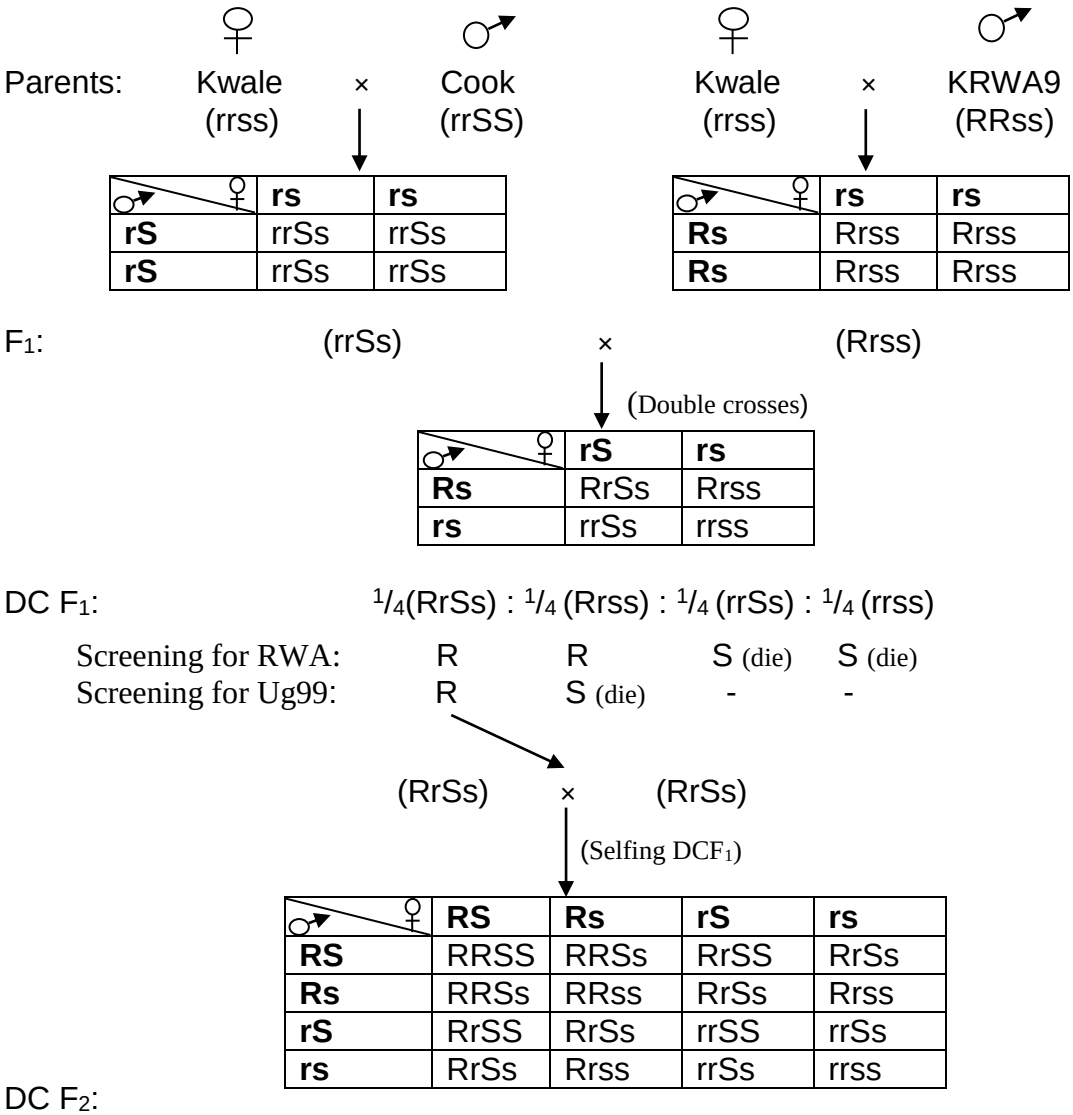

Figure 9: Scheme used for developing multiple resistant wheat germplasm through double crosses. Genotypes of parents and segregating populations are indicated. Kwale (rrss) – susceptible to both RWA and 'Ug99', Cook (rrSS) – resistant to 'Ug99' but susceptible to RWA and KRWA9 (RRss) – resistant to RWA but susceptible to 'Ug99'

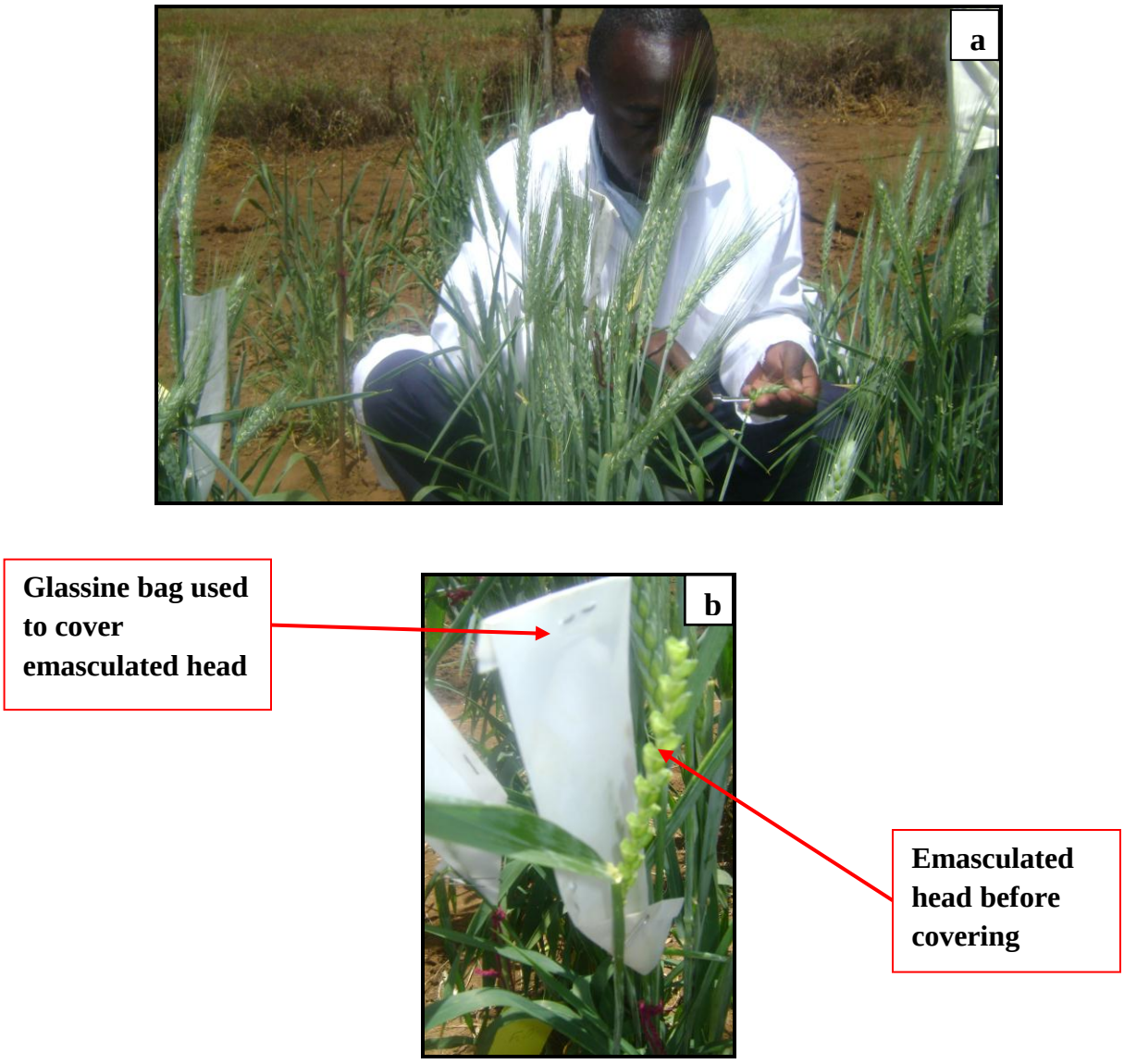

Figure 10: (a) Emasculation procedure **(b)** Emasculated head covered with a glassine bag

Making the Double Crosses

These crosses were made between 14th June and 3rd July 2009. Emasculation and pollination was effected on spikes of the intended female parents before anthers matured while still green in the florets, taking care not to damage the stigma. The emasculated ears were covered with glassine bags for 2-3 days (Figure 10b). Spikes for male parents were selected for pollination before they actually shade pollen. Pollination followed the "go-go" method (Kinyua, 1997). In this case the glassine bags on the emasculated female parents (Figure 10b) were trimmed at the top using a pair of scissors and spike selected from the male parent shaken back and forth to release the pollen grains from the protruded anthers. Optimal growth conditions including hand

weeding and watering were provided to the pollinated plants to ensure good seed development (Figure 11). Insect pests and disease were chemically controlled using Bulldock© Star (Beta-cyfluthrin 12.5g/L + chlorpyrifos 250g/L) and Amista Xtra© (azoxystrobin 200g/L + cyproconazole 80g/L), respectively. On 20th August 2009, plants were harvested using a sickle, hand threshed, cleaned, dried under the sun, properly packed in paper bags and stored in a cool dry place.

Figure 11: Crossing blocks in a breeding cage at Kenya Agricultural Research Institute-Njoro

DC F_2: (F_1 Kwale × Cook) × (F_1 Kwale × KRWA9)

The DC F_2 seeds were obtained by selfing the DC F_1 progenies that were resistant to both RWA and 'Ug99'. On 27th August 2009, one hundred and five seeds of DC F_1 were planted in ½ litre plastic pots, one seed per pot. The potting medium was a mixture of sterilized forest soil and sand at a ratio of 3:1, and 20 g of DAP fertilizer per pot. Watering was done twice a week. Eighty six out of the 105 seeds germinated. The 86 seedlings were sequentially screened against RWA and 'Ug99' to obtain a population that is resistant to both RWA and 'Ug99' which was left to self pollinate to produce DC F_2 population. (A detailed screening method against RWA and 'Ug99' is described in section "3.3 Greenhouse phenotyping of DC F_1 population").

3.3 Greenhouse phenotyping of DC F_1 population

3.3.1 Screening for resistance to RWA

On 27th August 2009, 105 seeds of DC F_1 were planted in ½ litre plastic pots, one seed per pot. The potting medium was a mixture of sterilized forest soil and sand at a ratio of 3:1, and 20 g of DAP fertilizer per pot. The potting mixture was steam autoclaved at120^{o}C to eliminate pests and disease pathogens. Watering was done twice a week. Eighty six out of the 105 seeds germinated. Seven days after seedling emergence (11th September 2009), the 86 plants of DC F_1 were screened for resistance to RWA. Russian wheat aphid culture was first reared on a susceptible variety 'Pasa' (Figure 14). Four aphids (at 4th instar stage) were placed directly on each plant using a camel hair brush at three leaf seedling growth stage i.e. stage (13) according to Zadoks (1974). The pots were gently covered with a 1 mm screen mesh to prevent aphids from escaping and other aphids/insects from getting in (Figure 12).

Figure 12: Pots covered with 1 mm screen mesh to prevent aphids from getting in and out.

A modified 1 – 9 visual scale suggested by Maling'a (2007) was used to score for RWA damage (i.e. leaf rolling, purple/white streaking and leaf chlorosis) on a single plant basis at 21 days after aphid infestation (on 1st October 2009). Plants were rated as follows:

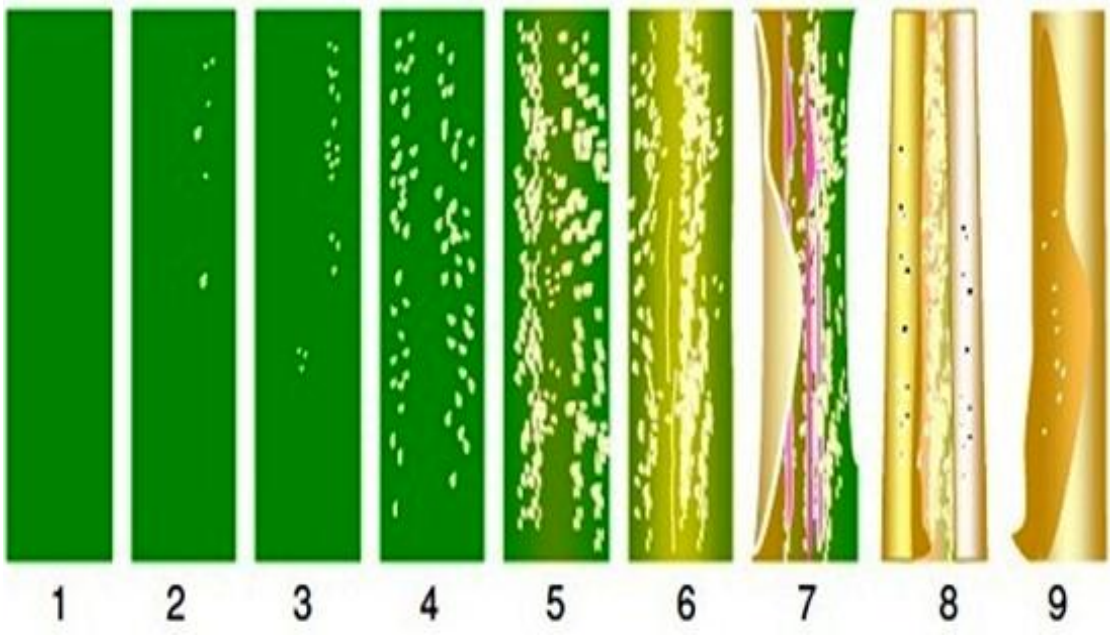

Figure 13: A plate showing the modified 1 – 9 visual scale as suggested by Maling'a (2007)

Table 5: Damage rating scale (1 – 9) used to characterize reaction types and classes to RWA on infested plants (Maling'a, 2007)

Rating	Description of symptoms	Classification of varieties
1	Small isolated chlorotic spots	Highly resistant
2	Small chlorotic spots	Highly resistant
3	Chlorotic spots in rows	Resistant
4	Chlorotic splotches	Moderately resistant
5	Mild chlorotic streaks	Moderately resistant
6	Prominent chlorotic streaks, leaves partially folded	Moderately susceptible
7	Severe streaks leaves partially rolled, flag leaf trapped	Susceptible
8	Severe streaks, leaves roll tightly	Highly susceptible
9	Plant dying	Highly susceptible

Plants showing damage scale of 1, 2, 3, 4 and 5 were grouped as resistant. After scoring for RWA damage, seedlings were sprayed with Bulldock© Star (Beta-cyfluthrin 12.5g/L + chlorpyrifos 250g/L) insecticide to kill the aphids and left for one week before being subjected to screening for stem rust race 'Ug99' resistance.

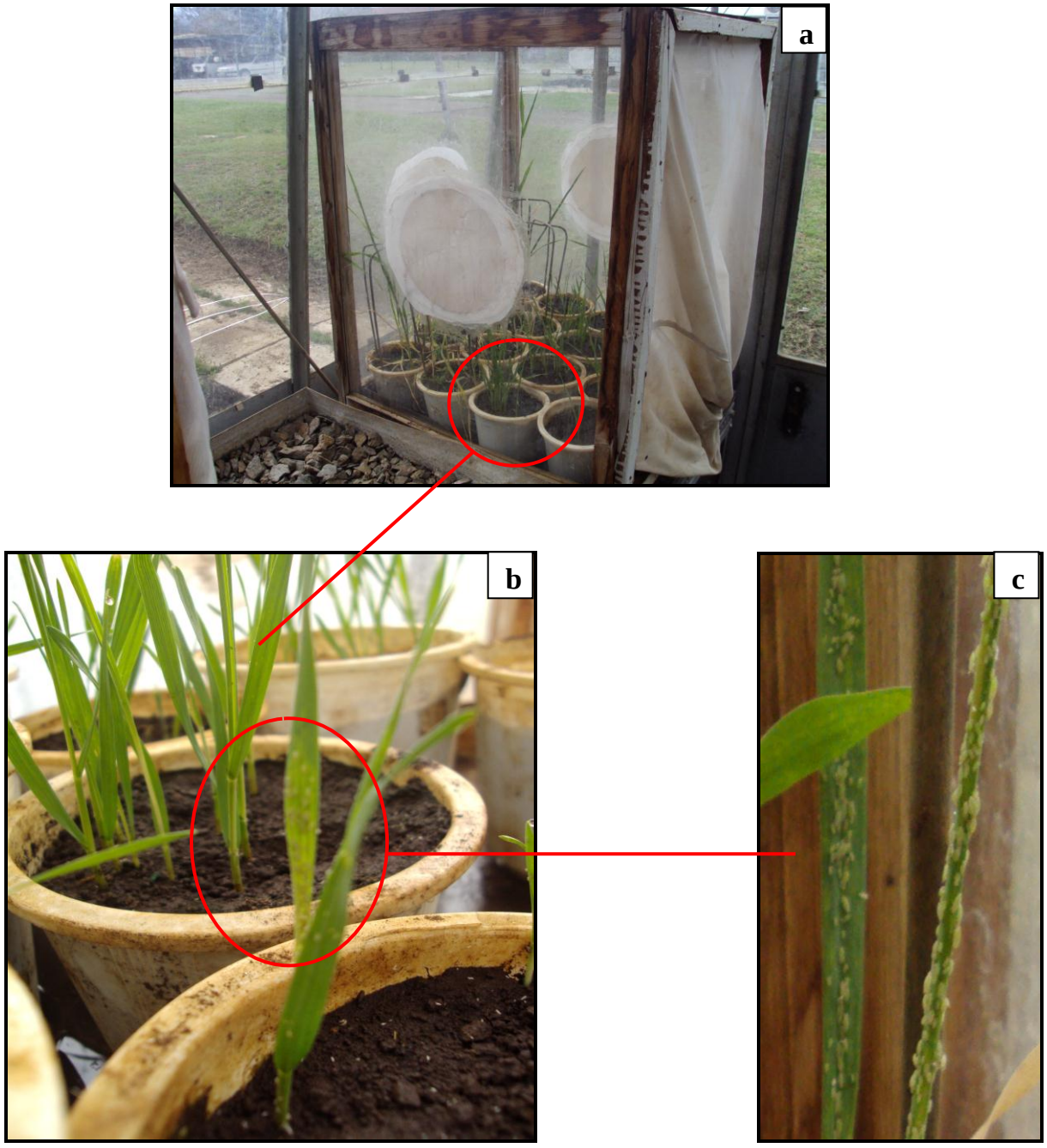

Figure 14: (a) A cage used for raring Russian wheat aphids **(b)** Russian wheat aphids conditioned on a susceptible variety Pasa **(c)** Close-up of a colony of Russian wheat aphids after multiplication.

3.3.2 Screening for resistance to stem rust race 'Ug99'

On 8th October 2009, the DC F_1 plants were screened for resistance to stem rust race 'Ug99'. The inoculum was first isolated by biased sampling from experimental plots of wheat cultivar 'Kwale' in KARI-Njoro by picking single large isolated pustules on stems, cutting them off with a sterile scalpel and placing them into glassine bags. The sampled pustules were removed using a spatula and the spores suspended in a drop of distilled water and light mineral oil Tween®-20 solution in a glass tube (Figure 15a).

Figure 15: (a) Glass tube containing a solution of stem rust inoculum. **(b)** Increasing the inoculum using susceptible variety 'Morocco'. **(c)** A vacuum pump used to collect spores. (d) Collected spores on a petri dish.

The mixture was stirred vigorously using a spatula, while diluting with distilled water. The suspension was sprayed using a hand operated sprayer on to seven-day-old seedlings of susceptible wheat cultivar 'Morocco' to increase the inoculum (Figure 15b). The inoculated seedlings were air-dried before placing in a dark dew chamber. Seedlings were incubated in the dew chamber for 16 hours at 18 ^{o}C, and then placed on a greenhouse bench at 22–30 ^{o}C under ambient photo period. The urediniospores that developed after fourteen-days were collected using a vacuum-pump (Figure 15c and 15d), dried in the laboratory under anhydrous gel and then stored in refrigerator. The stored spores were later used for race identification which was based on 20 differential set for North America (Jin *et al*., 2008) (Appendices IV and V). After race 'Ug99' confirmation, the collected urediniospores were used for 'Ug99' screening in the greenhouse.

The inoculum/spores (isolates of 'Ug99') were suspended in distilled water, at a concentration of 1mg urediospores/100ml of distilled water (Welty *et al.*, 1992), in which 1 – 2 drops of the surfactant Tween®-20 were added followed by agitation to produce the desired inoculation suspension. This mixture was applied on the plants through an atomizer with a fine nozzle with the trays placed on a revolving table to ensure uniform coverage. Inoculated plants were then left overnight in the growth chamber at 16 – 20 ^{o}C in the dark. To optimize spore germination, germ tube growth and appressorium formation, high humidity conditions were created within the chambers by pouring water in the troughs. The polythene masking its walls was also sprayed with a fine mist of distilled water. Twenty hours post inoculation, the plants were removed from the growth chambers and allowed to slowly dry in the well light cubicles at a temperature of 15 – 28 ^{o}C where they were watered for 14 days. On the 15th day (23rd October 2009), scoring for the plant reaction was done on the basis of the 0 – 4 scale (Figure 16) proposed by Stakman *et al.,* (1962).

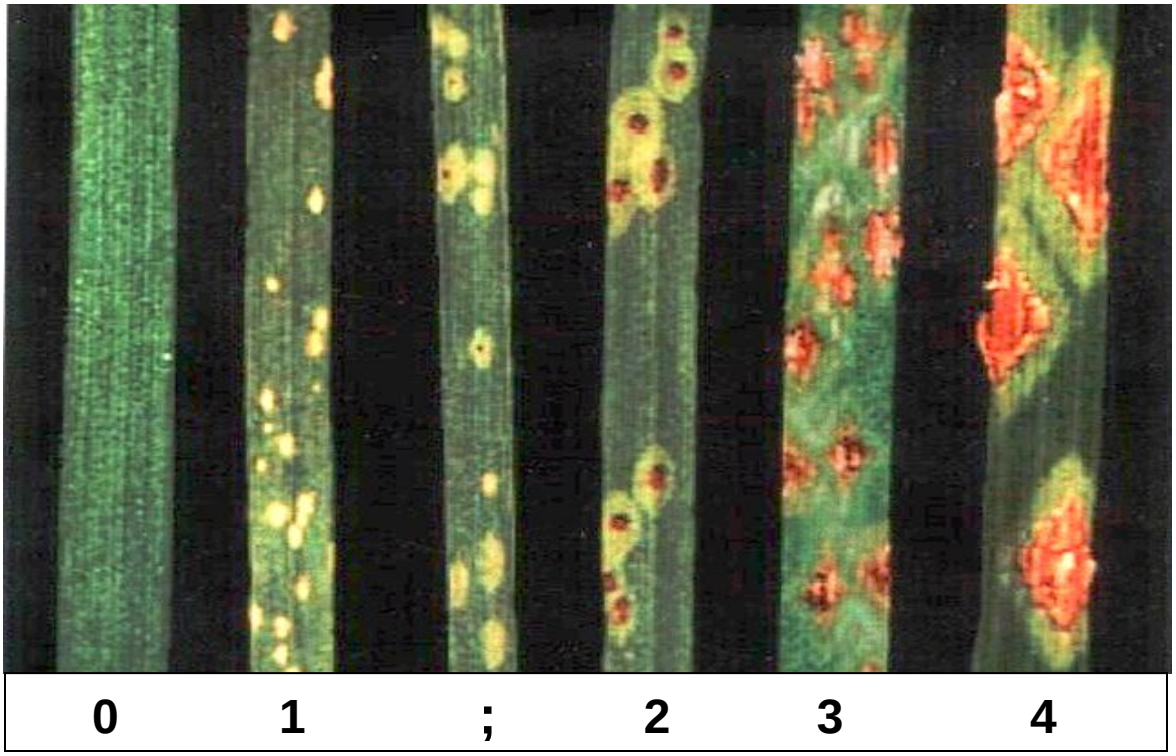

Figure 16: A plate showing the scale proposed by Stakman *et al*., (1962)

Table 6: A scale used for rating plant reaction 14 days post-inoculation (Stakman *et al.*, 1962)

Infection Type	Host response	Disease symptoms
0	Immune	No visible uredinia
0;	Highly resistant	Very few hypersensitive chlorotic and necrotic flecks
;	Resistant	Chlorotic and necrotic flecks
1	Resistant	Small uredinia with necrosis
2	Resistant	Small to medium sized uredinia with characteristic chlorotic, necrotic border
3	Moderately resistant / susceptible	Medium sized uredinia with chlorosis
4	Susceptible	Large pustules without chlorosis

Plants showing damage scale of 0, **;** , 1 and 2 were grouped as resistant while plants showing damage scale of 3 and 4 were grouped as susceptible.

On 5th January 2010, DC F_1 plants were harvested using a sickle, hand threshed, cleaned, dried under the sun, properly packed in paper bags and stored in a cool dry place.

3.4 Greenhouse phenotyping of DC F_2 population

3.4.1 Screening for resistance to RWA

Seedlings of DC F_2 were planted on 14th February 2010 in ½ litre plastic pots, one seed per pot. The potting medium was a mixture of sterilized forest soil and sand at a ratio of 3:1, and 20 g of DAP fertilizer per pot. The potting mixture was steam autoclaved at 120^{o}C to eliminate pests and disease pathogens. Watering was done twice a week. On 28th February 2010 (7 days after seedling emergence) 104 seedlings of DC F_2 were infested with RWA to facilitate the screening for RWA resistance. Four aphids (at 4th instar stage) were placed directly on each plant using a camel hair brush at three leaf-seedling growth stage i.e. stage (13) according to Zadoks (1974). The pots were gently covered with a 1 mm screen mesh to prevent aphids from escaping and other aphids/insects from getting in. On 21st March 2010 (21 days after aphid infestation), A modified 1 – 9 visual scale suggested by Maling'a (2007) was used to score for RWA damage (i.e. leaf rolling, purple/white streaking and leaf chlorosis) as described in part 3.3.1 of this thesis. Plants showing damage scale of 1, 2, 3, 4 and 5 were grouped as resistant while those that scored 6, 7, 8 and 9 were grouped as susceptible. After scoring for RWA damage, seedlings were sprayed with Bulldock© Star (Beta-cyfluthrin 12.5g/L + chlorpyrifos 250g/L) insecticide to kill the aphids and left for one week before being subjected to screening for stem rust race 'Ug99' resistance.

3.4.2 Screening for resistance to stem rust race 'Ug99'

On 29th March 2010, the DC F_2 plants were inoculated with 'Ug99' isolates to facilitate the screening for resistance to stem rust race 'Ug99'. The isolates/spores of 'Ug99' were suspended in distilled water at a concentration of 1mg urediospores/100ml of distilled water (Welty *et al.,* 1992), in which 1 – 2 drops of mineral oil Tween®-20 were added followed by agitation to produce the desired inoculation suspension. The suspension was applied on the plants in the evening using a hand sprayer with a fine nozzle. Inoculated plants were then left overnight in the growth chamber at 16 – 20 ^{o}C in the dark. To optimize spore germination, germ tube growth and appressorium formation, high humidity conditions were created within

the chambers by pouring water in the troughs. The polythene masking its walls was also sprayed with a fine mist of distilled water. Twenty hours after inoculation, the plants were removed from the growth chambers and allowed to slowly dry in the well light cubicles at a temperature of 15 – 28 ^{o}C where they were watered for 14 days. On 13th April 2010 (15 days after inoculation), scoring for the plant reaction was done on the basis of the 0 – 4 scale proposed by Stakman *et al.*, (1962) (Table 6/Figure 16) as described in part 3.3.2 of this thesis. Plants showing damage scale of 0, **;** , 1 and 2 were grouped as resistant while plants showing damage scale of 3 and 4 were grouped as susceptible.

On 15th July 2010, DC F_2 plants were harvested individually using a sickle. The individual heads from each plant were hand threshed, cleaned, dried under the sun and properly packed separately in medical enveloped and stored in a cool dry place.

3.5 Screening of DC $F_{2:3}$ families

On 25th July 2010, Seeds from each of the DC F_2 plants that were resistant to both RWA and 'Ug99' and were harvested individually and packed separately in medical envelopes were planted in rows, each row containing seeds from each medical envelope (i.e. head to row), to make DC $F_{2:3}$ families. They were planted in special wooden boxes and placed on a tray in a greenhouse as shown in Figure 17 below:

Figure 17: Seedlings of DC $F_{2:3}$ families planted in special wooden boxes placed on a tray in a greenhouse at KARI-Njoro

The planting medium was a mixture of sterilized forest soil and sand at a ratio of 3:1. The planting medium was steam autoclaved at 120^{o}C to eliminate pests and disease pathogens. The planting mixture was then amended with the equivalent of 125 kg DAP /ha. Seeds were drilled at inter-row spacing of 15 cm. Watering was done twice a week. In this case 48 rows were planted and were used to facilitate the selection of only plants that were homozygous dominant to the two resistance genes and leave the plants that were still segregating.

3.5.1 Screening for resistance to RWA

On 11th August (seven days after seedling emergence), the 48 rows of the DC $F_{2:3}$ families were subjected to screening for RWA resistance. RWA infestation was done using camel brush by placing 3 aphids per plant at seedling growth stage (13) according to Zadoks (1974). The seedlings were then covered with a 1 mm screen mesh to prevent aphids from escaping and other aphids/insects from getting in (Figure 18). On 22nd August 2010 (21 days after infestation), scoring was done using a 1 - 9 damage scale as describe by Maling'a, (2007) (Table 5/Figure 13). The plants were then sprayed with an insecticide, Bulldock© Star (Beta-cyfluthrin 12.5g/L + chlorpyrifos 250g/L) to kill the aphids and left for one week before being subjected to 'Ug99' resistance screening.

Figure 18: DC $F_{2:3}$ families in special wooden boxes and covered with 1mm screen mesh after RWA infestation

3.5.1 Screening for resistance to stem rust race 'Ug99'

On 29th August 2010, the selected resistant rows against RWA i.e. non-segregating were subjected to screening for stem rust ('Ug99') resistance. 'Ug99' inoculum was suspended in distilled water at a concentration of 1mg urediospores/100ml of distilled water (Welty *et al.,* 1992), in which 1-2 drops of the surfactant Tween®-20 were added followed by agitating to produce desired inoculum. The inoculum was applied on the plants in the evening using a hand sprayer with a fine nozzle. Inoculated plants were then left overnight in the growth chamber at 16 – 20 ^{o}C in the dark. To optimize spore germination, germ tube growth and appressorium formation, high humidity conditions were created within the chambers by pouring water in the troughs. The polythene masking its walls was also sprayed with a fine mist of distilled water. Twenty hours after inoculation, the plants were removed from the growth chambers and allowed to slowly dry in the well light cubicles at a temperature of 15 – 28 ^{o}C where they were watered for 14 days. On 14th September 2010 (15 days after inoculation), scoring for the plant reaction was done on the basis of the 0 – 4 scale proposed by Stakman *et al.,* (1962) (Table 6 /Figure 16) as described in part 3.3.2 of this thesis. Plants showing damage scale of 0, **;** , 1 and 2 were grouped as resistant while plants showing damage scale of 3 and 4 were grouped as susceptible.

On 14th December 2010 the DC $F_{2:3}$ were harvested harvested using a sickle, hand threshed, cleaned, dried under the sun, properly packed in paper bags and stored in a cool dry place.

3.6 Data Analysis

Data collected on Parents, $F_{1,}$ DC F_1, DC F_2 and DC $F_{2:3}$ was subjected to *chi*-square "goodness of fit" using GenSTAT 12th edition (version 12.1) to determine the mode of inheritance of RWA and stem rust race 'Ug99' (*Sr36*) resistance genes.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Range of damage score of parents, DC F_1 and DC F_2 populations to RWA

The seedling reactions of parents, F_1 and double crosses (DC F_1 and DC F_2) derived from Kwale/Cook//Kwale/KRWA9 are given in Table 7. Seedlings of the resistant parent 'KRWA9' generally showed a resistant reaction (Figure 19c). All seedlings of 'KRWA9' were highly to moderately resistant (classes 2, 3, 4 and 5; scale of 1–9) with a mean score of 3.3 (Table 7). This is in agreement with previous studies by Maling'a (2007) and Kenduiwa (2009) which showed that the breeding line 'KRWA9' was resistant to the local RWA biotypes. Seedlings had open leaves with vigorous growing leaves. Although leaf folding was seen in some plants, no leaf rolling or purple/white streaking was observed on any leaves (Figure 19c). Leaf rolling and streaking are major symptoms of RWA infestation (Walters, 1984; Tolmay, 2006). 'KRWA9' is a true breeding line and establishes a basis of identifiable constant behavior and any changes can be due to the interaction with other genes.

Seedlings of susceptible parents, 'Kwale' and 'Cook' showed a susceptible reaction, with damage scores of 6, 7, 8 and 9 (Table 7). This is because these two varieties have no RWA resistance genes (Maling'a, 2007 and Macharia, 2009). Most seedlings of 'Kwale' and 'Cook' had severe chlorotic streaking, leaf folding, leaf rolling and died from RWA after 14 days of infestation (Figure 19 a and b). Four seedlings of the susceptible parent 'Kwale' were resistant 21 days after aphid infestation and were excluded from the study as they were thought to be escapes. However, after 21 days, 70% of the plants had a score of 7 and above indicating resistant reaction.

'Kwale' and 'Cook' are true breeding cultivars which are high yielding with very good agronomic attributes and most farmers in Kenya and Australia are growing them commercially but are highly susceptible to RWA. In Kenya, 'Kwale' is grown by more than 80% of the wheat farmers in Narok County. This County alone accounts for about 60% of the local wheat production (KARI, 2008).

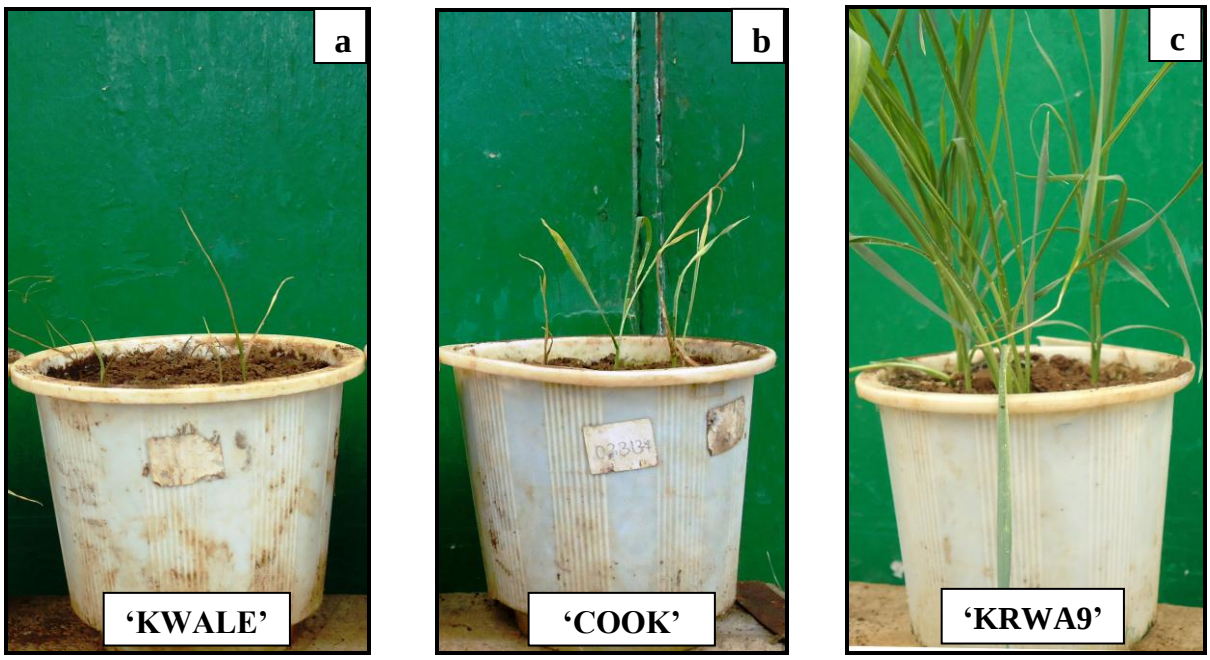

Figure 19: (a) Susceptible variety 'Kwale' and **(b)** 'Cook' dying from RWA 21 days after infestation **(c)** Resistant line 'KRWA9' 21 days after RWA infestation.

The F_1 population derived from 'Kwale' × 'KRWA9' showed a resistant reaction with damage scores of 2, 3, 4 and 5, recording a mean score of 3.5 (Table 7). This is because all the F_1 progenies were heterozygous for the resistance gene. The union of gametes carrying different alleles, one dominant from the resistant line and a recessive from the susceptible line produces a heterozygous individual (Hartl and Clark, 2007). On the other hand, the F_1 population derived from 'Kwale' × 'Cook' showed a susceptible reaction with damage scores of 6, 7, 8 and 9, recording a mean score of 7.3 (Table 7). This is because none of the two parents has RWA resistance genes. The double recessive homozygote *(rrss)* is expected to be susceptible. This confirms previous studies by Maling'a (2007) and Macharia (2009) which showed that both 'Kwale' and 'Cook' were susceptible to RWA.

The DCF_1 and DCF_2 populations exhibited both susceptible and resistant reactions with damage scores of 2–9 (Table 7) as some seedlings were susceptible and others were resistant. This reaction follows the law of segregation which states that hereditary characters are determined by genes which occur in pairs and in the formation of gametes the factors are segregated so that only one is transmitted by a particular gamete. When male and female

gametes fuse to form a zygote the diploid status is restored. By combining the two kinds of male and female gametes in all possible ways the relative proportions of susceptible and resistant types are obtained (Hartl and Clark, 2007). Some of the seedlings are homozygous dominant, some homozygous recessive and others heterozygous. The homozygous dominant and heterozygous progenies for RWA resistance gene will show a resistant reaction while the homozygous recessive progenies will show a susceptible reaction to RWA. The mid-parent value (mean score) of DCF_1 was 5.5 which was lower than the mid-parent value of 'KRWA9', 3.5 (Table 7) because only 25% of the genetic ratio is retained in the double cross. Nevertheless, the F_1 was still resistant, with DCF_2 mid-parent value of 5.0. This segregation exhibited in the DCF_1 and DCF_2 populations where there are identifiable resistant plants. The fact that they have 'Kwale' characteristics in them shows that the RWA resistance genes can easily be introgressed to a local adapted cultivar.

4.2 Inheritance of RWA resistance gene

Seedlings of parent 'Kwale' (P_1) were generally susceptible to RWA. Out of 180 plants evaluated for RWA, a total of 176 plants were susceptible (Table 7). This is in agreement with previous studies which showed that 'Kwale' has no RWA resistance genes (Maling'a, 2007; Kenduiwa, 2009). However, the four seedlings of the parent 'Kwale' were resistant probably because they were escapes and were excluded from the study. Similarly, seedlings of the parent 'Cook' (P_2) were all susceptible to RWA (Table 7). The results meant that 'Cook', an Australian commercial variety, has no resistance gene to RWA. According to CSIRO, the Australia's national science agency, RWA has never been detected in Australia. The 3rd parent 'KRWA9' (P_3) generally showed a resistant reaction to RWA (Table 9). This is because it is known to have a single dominant gene that confers resistance to RWA (Pathak *et al.,* 2004; Maling'a 2007; Kenduiwa, 2009). All the F_1 progenies of 'Kwale × Cook' were susceptible to RWA (Table 7). This is because neither 'Kwale' nor 'Cook' has genes that confer resistance to RWA. On the other hand, all the F_1 progenies of 'Kwale × KRWA9' showed resistant reaction to RWA (Table 7). This is because they were all heterozygous for the resistance gene in 'KRWA9'.

The double cross F_2 (DCF_2) progenies of 'Kwale/Cook//Kwale/KRWA9' showed a segregation ratio of 3:1 (3 resistant: 1 susceptible) as *chi*-square value (χ^2 =2.38, Crit.=3.841) showed a

good fit to the expected ratios (Table 7). This means that the gene conferring resistance to RWA is a major gene present in 'KRWA9'. The resistance to RWA in bread wheat is mostly controlled by single dominant genes (Saidi and Quick, 1996; Pathak *et al*., 2004, Maling'a, 2007) with the possible exceptions of recessive gene in PI 294994 wheat (Elsidaig and Zwer, 1993) and a single recessive gene *dn3* in an accession of *Aegilops tauschii* line SQ24 (Nkongolo *et al.*, 1991a).

Table 7: Seedling reaction of parents, single crosses and double crosses to RWA and the *Chi*-square (χ^2) values

Test Entry	Pop.	No. of plants tested	Number of Plants with damage score									Mean score	Ratios Observed R:S	Ratios Expected R:S	χ^2	P- value
			1	2	3	4	5	6	7	8	9					
Parents																
Kwale	P_1	180	0	0	0	1	3	70	51	41	18	7.2	4:176	-	-	-
Cook	P_2	155	0	0	0	0	0	42	71	20	22	7.1	0:155	-	-	-
KRWA9	P_3	125	0	33	45	18	29	0	0	0	0	3.3	125:0	-	-	-
Single Crosses																
Kwale × Cook	F_1	178	0	0	0	0	0	55	38	57	28	7.3	0:178	0:1	0.00	1.00 ns
Kwale × KRWA9	F_1	140	0	21	42	57	20	0	0	0	0	3.5	140:0	1:0	0.00	1.00 ns
Double Crosses																
(Kwale/Cook) × (Kwale/KRWA9)	DC F_1	86	0	1	11	18	14	15	6	17	4	5.5	44:42	1:1	0.05	0.829 ns
	DC F_2	104	1	6	21	24	19	20	6	5	2	5.0	71:33	3:1	2.38	0.128 ns

Scale: 1-3 = Resistant, 4-5 = Moderately resistant, 6 = Moderately susceptible, 7-8 = Susceptible, 9= Very susceptible.
R = Resistant, S = Susceptible, P = probability, ns = No significant difference.
†Significance limit of χ^2 ($P < 0.05$, df = 1, Crit. V=3.841).

4.3 'Ug99' infection type of parents, F_1, DC F_1 and DC F_2 populations

Seedlings of the resistant parent 'Cook' generally showed immune and resistant reactions (Figure 20c), with infection types of 0, ; and 1. This is because 'Cook' is known to carry the stem rust resistance gene, *Sr36*, which confers resistance to stem rust race 'Ug99' (Singh *et al*., 2006; Macharia, 2009). 'Ug99' susceptible parents, 'Kwale' and 'KRWA9', produced susceptible reactions (Figure 20a and b) of infection types 3 and 4 (Table 8) because the two have no resistance genes to stem rust race 'Ug99'. Most seedlings had medium to large sized pustules without necrotic spots (Figure 20a and b). A large pustule without a necrotic spot is a characteristic of high susceptibility because the plant does not exhibit hypersensitive reaction. Hypersensitive reaction is characterized by a chlorotic or necrotic spot (fleck) where a few host cells die near the point of infection. No sporulation occurs (Roelfs, 1988). This meant that the plants did not kill the cells around the fungal pathogen allowing it to feed, grow and spread on other parts of the plant. 'Kwale' is a true breeding cultivar and most farmers in Kenya are growing it commercially but it is susceptible to stem rust race 'Ug99' posing a great challenge to the Kenyan wheat farmers.

The F_1 population derived from 'Kwale' × 'Cook' showed a resistant reaction, with infection types of 0, ; and 1 (Table 8). This means that all the progenies were heterozygous for the resistance gene *Sr36*. The union of gametes carrying different alleles, one dominant from the resistant line and a recessive from the susceptible line produces a heterozygous individual. The F_1 population derived from 'Kwale' × 'KRWA9' produced a susceptible reaction with infection types of 3 and 4 (Table 8). This is because none of the two parents i.e. 'Kwale' and 'KRWA9' that were used to make this cross has 'Ug99' resistance genes.

All DCF_1 and DCF_2 populations reacted to stem rust with infection types of 0, ; , 1, 2, 3 and 4 (Table 8). This follows the law of segregation which states that hereditary characters are normally determined by genes which occur in pairs and in the formation of gametes the factors segregate so that only one is transmitted by a particular gamete. When male and female gametes fuse to form a zygote the double chromosome number is restored. By combining the two kinds of male and female gametes in all possible ways the relative proportions of susceptible and resistant types are obtained (Hartl and Clark, 2007).

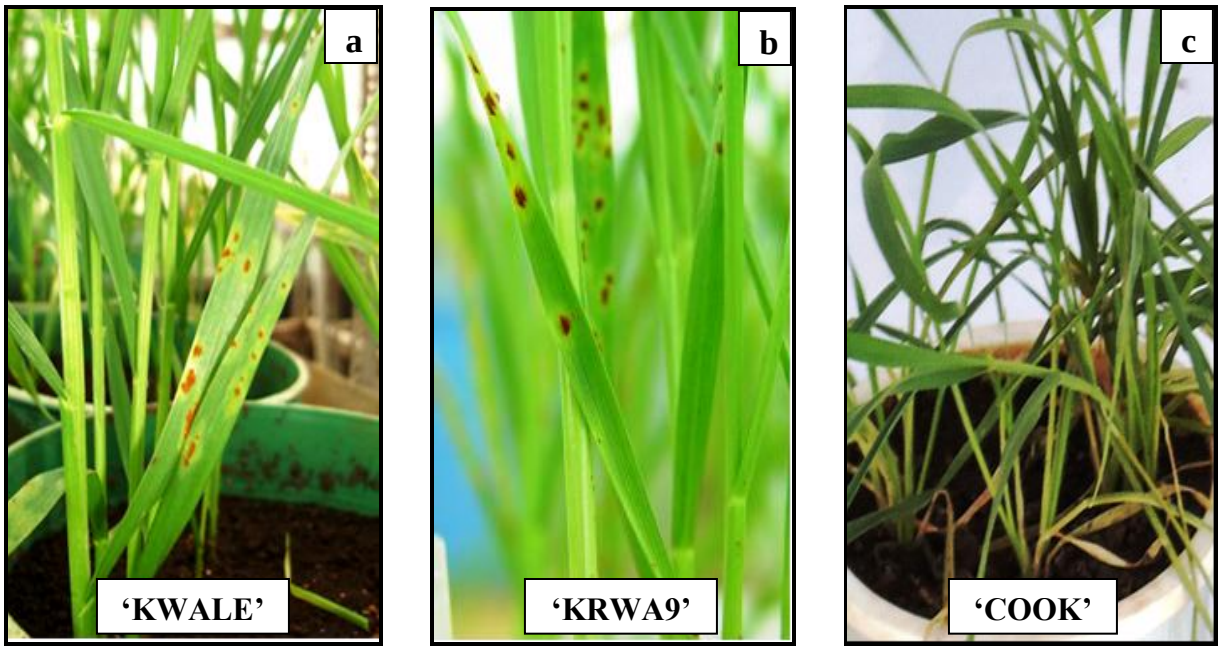

Figure 20: (a) Seedlings of susceptible parents 'Kwale' and **(b)** 'KRWA9' with medium to large sized pustules without necrotic spots 14 days post inoculation **(c)** Seedlings of parent 'Cook' exhibiting immunity to 'Ug99' 14 days post inoculation.

Some of the seedlings were homozygous dominant, some homozygous recessive and others heterozygous. The homozygous dominant and heterozygous progenies for 'Ug99' resistance gene *Sr36* will show a resistant reaction while homozygous recessive progenies will show a susceptible reaction to stem rust race 'Ug99'.

4.4 Inheritance of 'Ug99' resistance gene *Sr36*

Seedlings of parent 'Kwale' (P_1) were generally susceptible to stem rust race'Ug99' with only 7 out of 180 seedlings showing an immune reaction (Table 8). This is because 'Kwale' has no resistance gene to 'Ug99' (Macharia, 2009). The 7 seedlings showed immune reaction probably because of disease escape and they were excluded from the study. The seedlings of parent 'KRWA9' (P_3) were also susceptible to 'Ug99' (Table 8) meaning that the line 'KRWA9' has no resistance to stem rust race 'Ug99'. Seedlings of parent 'Cook' (P_2) showed resistance reaction to 'Ug99' (Table 9). This was expected because previous studies showed that the variety 'Cook' carries stem rust resistance gene *Sr36* which confers resistance to 'Ug99' (Singh *et al*., 2006; Macharia, 2009).

All the F_1 progenies of 'Kwale' × 'Cook' showed resistant reaction to 'Ug99' (Table 8). This is because the entire population was heterozygous to resistance gene *Sr36* indicating complete dominance of resistance over susceptibility. All the F_1 progenies of 'Kwale' × 'KRWA' were susceptible to 'Ug99' (Table 9), evidence that none of them has a resistance gene to 'Ug99'. The DCF_2 population segregated in the ratio of 3:1 (3 resistant: 1 susceptible). *Chi*-square values (χ^2 =1.95, Crit.=3.841) showed consistence with the expected ratio (Table 8). These results indicated the presence of a single dominant gene (*Sr36*) that provided resistance to race TTKSK ('Ug99').

The *Sr36* gene was derived from *Triticum timopheevi*. The gene occurs in high frequency in US soft winter wheat (Singh *et al*., 2006). Although races with virulence for *Sr36* have been detected, it is immune to TTKSK and could still be used effectively as a component for TTKSK ('Ug99') resistance breeding together with other *Sr* genes.

Table 8: Seedling reaction of parents, single crosses and double crosses to 'Ug99' and the *Chi*-square (χ^2) values

Test Entry	Pop.	No. of plants tested	Number of Plants with Infection Type						Ratios		χ^2	P- value
			0	;	1	2	3	4	Observed R:S	Expected R:S		
Parents												
Kwale	P_1	180	5	2	0	0	99	74	7:173	-	-	-
Cook	P_2	155	94	59	2	0	0	0	155:0	-	-	-
KRWA9	P_3	125	0	0	0	0	56	69	0:125	-	-	-
Single Crosses (SC)												
Kwale × Cook	F_1	178	95	80	3	0	0	0	178:0	1:0	0.00	1.00 ns
Kwale × KRWA9	F_1	140	0	0	0	0	72	68	0:140	0:1	0.00	1.00 ns
Double Crosses (DC)												
(Kwale/Cook)	DC F_1	44	11	6	3	0	16	8	20:24	1:1	0.36	0.546 ns
× (Kwale/KRWA9)	DC F_2	71	23	21	4	0	14	9	48:23	3:1	1.95	0.162 ns

Scale: 0=Immune, **;**=Highly resistant, 1-2=Resistant, 3=Moderately susceptible, 4=Very susceptible.
R = Resistant, S = Susceptible, P = probability, ns = No significant difference.
†Significance limit of χ^2 ($P < 0.05$, df = 1, Crit. V=3.841).

4.5 Pyramiding of RWA and 'Ug99' resistance genes

The term 'gene pyramiding' is used to refer to a way of determining and introducing multiple genes which impart resistance to an independent insect pest/pathogen, or impart resistance to a single pest through independent host pathways (Nevo *et. al.*, 2002).

The data collected during RWA seedling reaction of DC F_1 and DC F_2 progenies (Table 7) showed a segregation ratio of 3:1 drawing a conclusion that the RWA resistance gene in line 'KRWA9' is a single dominant gene whose inheritance can be predicted. Therefore, the gene was easily introgressed into 'Kwale' a Kenyan commercial wheat variety which has good agronomic attributes but is susceptible to RWA. On the other hand data collected during 'Ug99' seedling reaction of DC F_1 and DC F_2 progenies (Table 8) also showed a segregation ratio of 3:1 which was an expression of a single dominant gene *Sr36* which was also easily introgressed into 'Kwale'. The expression of the two genes at the same time meant that they were successfully pyramided.

When the data of RWA and 'Ug99' damage recorded on DC F_2 in Table 7 and 8 is combined and tested against several two gene models, the data fits the ratio 9:3:3:1 (Table 9).

Table 9: *Chi*-square values (χ^2) Goodness of fit of DC F_2 population to two gene models

Model	Total no. of plants	Ratios		χ^2	P-value
		Observed	Expected		
9:3:3:1	104	48:23:25:8	9:3:3:1	4.03	0.259 ns
6:3:3:4	104	48:23:25:8	6:3:3:4	21.09	< 0.001*
3:6:3:4	104	48:23:25:8	3:6:3:4	55.75	< 0.001*
12:3:1	104	48:23:8	12:3:1	12.5	0.002**
9:3:4	104	48:23:8	9:3:4	14.97	<0.001**
9:6:1	104	48:23:8	9:6:1	8.79	0.012**
15:1	104	48:56	15:1	173.17	<0.001***
9:7	104	48:56	9:7	4.26	0.039***

*Significance limit of χ^2 ($P < 0.05$, df = 3, Crit. V=7.815). ns = No significant difference.
**Significance limit of χ^2 ($P < 0.05$, df = 2, Crit. V=5.991).
***Significance limit of χ^2 ($P < 0.05$, df = 1, Crit. V=3.841).

This means that the DC F_2 population shows a segregation ratio of 9:3:3:1 (9 resistant to both RWA and 'Ug99': 3 resistant to RWA but susceptible to 'Ug99': 3 resistant to 'Ug99' but susceptible to RWA : 1 susceptible to both RWA and 'Ug99"). This is obtained because both the RWA and 'Ug99' resistance genes are single dominant genes located on different chromosomes i.e. they are not linked. *Sr36* is located on chromosome 2BS (Jin *et al.*, 2008) while it is highly suspected that the resistance gene in 'KRWA9' might be *Dn1* (Masinde, 2012) which is located on chromosome 7D, similar to all the other known *Dn* genes which are all located on the D genome (Du Toit, 1987; Saidi and Quick, 1996; Pathak *et al.*, 2004). The fact that the two genes are not linked means that they were inherited independently hence the ratio 9:3:3:1. This is explained by the Mendelian law of independent assortment, which states that alleles of different genes assort independently of one another during gamete formation (Hartl and Clark, 2007). While Mendel's experiment with mixing one trait always resulted in a 3:1 ratio between dominant and recessive phenotypes, mixing two traits (dihybrid cross) showed ratios of 9:3:3:1. Independent assortment occurs during meiosis I in eukaryotic organisms, specifically metaphase I of meiosis to produce gametes with a mixture of the organism maternal and paternal chromosomes (Hartl and Clark, 2007). In this case the chromosomes that end up in a newly formed gamete are randomly sorted from all possible combinations of parent chromosomes. Because gametes end up with a random mix instead of a pre-defined "set" from either parent, gametes are therefore considered assorted independently. As such, the gametes can end up with any combination of 'Kwale', 'Cook', and 'KRWA9' chromosomes.

In this study, the homozygous DC $F_{2:3}$ population clearly demonstrated that it is possible to get one population that is resistant to both RWA and stem rust. This population can be advanced to early generations and selections made within preliminary and advanced yield trials for future variety release. With the constant mutations leading to evolution of new stem rust races and RWA biotypes, the strategy can be incorperated into the national wheat breeding programme to develop wheat varieties that have multiple resistance to insect pests and diseases. Gene pyramiding has been proposed and applied to enhance resistance to disease and insect pests by selecting for two or more than two genes at a time. For instance in rice, pyramids have been developed against bacterial blight and blast (Khush, 1977a).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In this study, germplasm resistant to both RWA and stem rust race TTKSK ('Ug99') was developed by pyramiding the RWA resistance gene in 'KRWA9' and 'Ug99' resistance gene *Sr36*. The genes proved to be dominant with single crosses exhibiting ratios of 3:1. The genes were simply inherited and easy to transfer into 'Kwale', a Kenyan commercial variety. The inheritance patterns of these genes have been elucidated. The fact that the two genes were not linked, they were inherited independently. This study has clearly demonstrated that it is possible to get one population that is resistant to both RWA and stem rust. This population can be advanced to early generations and selections made within preliminary and advanced yield trials for future variety release.

5.2 Recommendations

The following recommendations can be made:

1. Though races with virulence for *Sr36* have been reported, the gene is immune to the race TTKSK ('Ug99') and could still be used effectively as a component for 'Ug99' resistance breeding together with other *Sr* genes.

2. This material can be advanced to early generations by backcrosses or topcrosses and selections made within preliminary and advanced yield trials for future variety release.

3. Continuous screening should be done on this material in order to detect any new virulence due to continuous mutations that leads to evolution of new stem rust races and RWA biotypes.

4. This germplasm need to be screened in different agro ecological zones of the country because of variation in disease and insect pressure before being used in resistance breeding.

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APPENDICES

Appendix I: A list of bread wheat varieties released in Kenya from 1920 to 2012.

VARIETY	YEAR	VARIETY	YEAR	VARIETY	YEAR
EQUATOR	1920	**KENYA EAGLE**	1959	**GOBLET**	1973
KENYA-324	1920	**KENYA CURLEW**	1959	**KENYA NYATI**	1973
KENYA B-256-G	1920	**KENYA HAWK**	1959	**KENYA KIBOKO**	1973
KENYA GOVERNOR	1925	**KENYA QUAIL**	1959	**KENYA MBWEHA.**	1974
KENYA STANDARD	1930	**AFRICA MAYO**	1960	**KENYA KURO**	1974
KENYA-112	1936	**KENTANA YAQUI**	1960	**KENYA BATA**	1974
KENYA-58	1937	**KENYA JAY**	1962	**KENYA NUNGU**	1975
KENYA-131	1939	**KENYA GRANGE**	1962	**KENYA NYOKA**	1975
KENYA-122	1939	**KENYA PAGE**	1963	**K.TEMBO**	1975
REGENT	1939	**SALMAYO**	1963	**K.PAKA**	1975
KENYA- 117-A	1939	**TAMA**	1963	**KENYA KIFARU**	1976
KENYA-291	1946	**GABRINO.**	1963	**K.KULUNGU**	?
KENYA-294-B-2 A-3	1947	**LENANA.**	1963	**K.FAHARI**	1977
KENYA-261	1949	**FRONTHATCH.**	1963	**K.NYANGUMI**	1979
KENYA-318-AJ-4 A-1	1949	**MENCO.**	1963	**K.NGIRI**	1979
KENYA-318	1949	**CATCHER.**	1963	**KENYA ZABADI**	1979
KENYA PLOUGHMAN	1950	**BAILEY**	1964	**KENYA-4792-K-1B-4A**	1979
KENYA SETTLER	1950	**MORRIS**	1964	**KENYA PAA**	1980
KENYA-350	1951	**GEM.**	1964	**K.KONGONI**	1981
KENYA-337	1951	**FANFARE**	1964	**K.POPO**	1982
KENYA-184-P	1951	**FURY-KEN.**	1964	**KENYA NYUMBU**	1982
KENYA-360-H	1951	**KENYA HUNTER**	1964	**KENYA TUMBILI**	1984
KENYA-321	1954	**KENYA PLUME**	1965	**KWALE**	1987
KENYA FARMER.	1954	**KENYA KUDU**	1966	**K.CHIRIKU**	1989
KENYA-356-A	1955	**KENYA LEOPARD.**	1966	**PASA**	1989
KENYA-354	1955	**KENYA CHEETAH**	?	**NGAMIA**	1993
KENYA-261-E	1955	**KENYA CIVET.**	1966	**DUMA**	1993
KENYA-356-B	1956	**ROMANY**	1966	**MBEGA**	1993
KENYA-362	1956	**BEACON-KEN.**	1966	**MBUNI**	1993
KENYA-358-AA	1956	**BOUNTY.**	1967	**K.HEROE**	1999
KENYA-358	1956	**TOKEN-KEN.**	1967	**CHOZI**	1999
KENYA-363	1957	**BONNY.**	1967	**K.YOMBI**	1999
KENYA-358-R	1957	**MENTOR.**	1967	**NJORO BW II**	2006
KENYA-358-P	1957	**TROPHY.**	1968	**K.IBIS**	2008
KENYA-358-AC	1957	**KENYA SUNGURA**	1969	**KENYA ROBIN**	2010
KENYA-362-B-1 E-4	1958	**KENYA KANGA**	1971	**EAGLE 10**	2012
KENYA-339	1958	**KENYA BONGO**	1971	**KINGBIRD**	2012
KENYA-291-J-1-I-1	1958	**BREWSTER.**	1971	**SUNBIRD**	2012
KENYA-6297-2	1958	**K.SWARA**	1972		
KENYA-362-B-1 A	1958	**K.MAMBA**	1972		

Appendix II: The known RWA genes, sources and their chromosomal locations.

Source of Variation	Wheat Type	Chromosomal location	Origin of accession	Resistance gene	Mode of resistance
PI 137739	Hard White Spring	7D (Schroeder-Teeter *et al*., 1994)	Iran (Du Toit, 1987)	*Dn1*	Antibiosis and antixenosis (Du Toit 1987, 1989a)
PI 262660	Hard White Winter	7DL (Ma *et al*., 1998)	Bulgaria (Du Toit, 1987)	*Dn2*	Antibiosis and antixenosis (Du Toit 1987, 1989a)
Triticum tauschii	-	-	SQ24 (Nkongolo *et al*., 1991a)	*dn3*	Unknown
PI 372129	Hard Red Winter	1DS	Former Soviet Union (Nkongolo *et al*. 1991b; Saidi and Quick, 1996).	*Dn4*	Tolerance (Saidi and Quick, 1996)
PI 294994	Hard Red Winter	7DL (Du Toit, 1987; Marais and Du Toit, 1993)	Bulgaria (Marais and Du Toit, 1993)	*Dn*5	Tolerance, antibiosis and antixenosis (Du Toit 1987, 1989b Smith *et al*., 1992)
PI 243781	Winter wheat	7DS	Iran (Saidi and Quick, 1996)	*Dn*6	Tolerance and Antibiosis (Miller *et al*., 2003
Rye accession	-	Transferred to 1RS in wheat (Liu *et al*., 2001)	-	*Dn*7	Antixenosis (Kogan and Ortman, 1978)
PI 294994	Hard Red Winter	7D (Liu *et al*., 2001)	Bulgaria (Marais and Du Toit, 1993)	*Dn*8	Unknown
PI 294994	Hard Red Winter	1D (Liu *et al*., 2001)	Bulgaria (Marais and Du Toit, 1993; Liu *et al*., 2001)	*Dn*9	Unknown
PI 220127	Winter wheat	7D (Liu *et al*., 2001)	Afghanistan (Harvey and Martin, 1990)	*Dnx*	Unknown
PI 220350	Chinese wheat Lin-Yuan207	1DL (Liu., 2001)	China(Liu, 2001)	*Dny*	Unknown

Appendix III: Some examples of the identified genes for stem rust resistance, compiled from McIntosh (1995).

Gene	Common sources	Typical seedling IT	Chromosome Location
Sr 1=Sr9d			
Sr 2	Hope, H44-24, Hopps	4	3BS
Sr 3, Sr4	Marquillo-not available in separate lines	_	_
Sr 5	Kanred, Reliance, Thatcher, Chris, Manitou, Hochzucht	00;	6D
Sr 6	Kenyan lines(e.g. Kenya 58), Red Egyptian, Africa 43, Eureka, McMurachy, Kentana 52, Chris, Manitou, Selkirk, Gamut	0;	2D
Sr 7a	Egypt Na 101, many Kenyan lines, Kentana 52	13	4BL
Sr 7b	Marquis, Hope, Spica, Renown, Selkirk, Chris, Manitou, Khapstein	2	4BL
Sr 8a	Red Egpytian, Mentana, Frontana, Rio negro	2	6A
Sr 8b	Barleta Benvenuto, Klein Titan, Klein Cometa	X	6A
Sr 9a	Red Egyptian	1+ 2-	2BL
Sr	Many Kenyan lines(e.g., Kenya 117a, Kenya Farmer), Frontana, Gamenya, Festival, Gamut	2	2BL
Sr 9c	Reserved for *Sr Tt1*(*Sr 36*) which was later found not to be an *Sr9* allele		
Sr9d	Hope, H-44-24, Lancer, Scout, Lawrence, Renown, Redman	0;2	2BL
Sr9e	Vernstein, Vernal emmer	0;1	2BL
Sr9f	Chinese Spring	0;2	2BL
Sr 9g	Thatcher, Kubanka, Acme	22+	2BL
Sr10	Egyptian Na95, Kenyan lines	0;X	-
Sr 11	Lee, Gabo, Kenya Farmer, Charter, Sonora 64, Tobari 66, Yalta, Mendos	12-	6BL
Sr 12	Thatcher, Windebri, Egret, Chris, Manitou	X	3BS
Sr 13	Khapstein, Mdden	2-2	6Aβ
Sr 14	Khapstein	12	1BL
Sr 15	ASII, Axminster, Festival, Norka, Thew, Normandie	$0;1^{+}$	7AL
Sr 16	Thatcher, Reliance	2	7AL
Sr 17	Hope, Renown, Redman, Lawrence, Spica, Warigo, Aotea	0;X	7BL
Sr 18	Marquis, Reliance, many wheat lines	0;2	1D
Sr19	Marquis	1^{-}	2B
Sr 20	Marquis, Reliance	2	2B
Sr 21	Einkorn(*Triticum monococcum*), tetraploid and hexaploid derivatives	12^{-}	2A

Sr22	Einkorn(*Triticum monococcum*),tetraploid and hexaploid derivatives	12^{-}	7AL
Sr 23	Exchange, Etoile de Choisy, Selkirk, Warden	23	4A
Sr 24	*Agropyron elongatum*, Agent, Blueboy II, Cloud, Fox, Sage	12^{-}	3DL
Sr25	*Agropyron elongatum*,Agatha, Sears 7D/Ag translocations	12^{-}	7DL
Sr 26	*Agropyron elongatum*, Knott's 6A/Ag translocations, Eagle, Kite	12^{-}	6Aβ
Sr 27	*Secale cereale*, Wheat-rye translocation WRT 238-5	0;	3A
Sr 28	Line AD, Kota	0;	2BL
Sr 29	Etoile de Choicy	23^{-}	6Dβ
Sr 30	Webster, Festiguay	2^{-}	5DL
Sr 31	*Secale cereale*, 1B/1R translocations, Aurora, Kavkaz, Lovrin,Neuzucht, Veery, Weique	0;1	1BL/ 1RS
Sr 32	*Triticum speltoides* derivatives	2^{-}	2A,2B,2D
Sr33	RL 5405 (Tetra Canthatch/ *Aegilops squarrosa*)	$1^{-}2$	1DL
Sr34	*Triticum comosum*, compare, various translocation lines	$1^{-}2$	2A,2D
Sr35	Triticum monococcum derivatives, Arthur	0;	3AL
Sr36	*Triticum timopheevii*, CI 12632, CI 12633, Cook, Idaed 59, Timgalen, Timvera, formerly *SrTt1*	$0;1^{+}$	2BS
Sr 37	Steinwedel/ *Triticum timopheevii* derivative	0;	4Aβ

Appendix IV: A key for defining the *Puccinia graminis tritici* - code races of *Puccinia graminis* f. sp. *tritici*

	Subset[a]	Infection type produced on host lines with *Sr*			
	1	5	21	9e	7b
	2	11	6	8a	9g
	3	36	9b	30	17/13
Pgt – code	4	9a	9d	10	Tmp
	5	24	31	38	McN
B		Low	Low	Low	Low
C		Low	Low	Low	High
D		Low	Low	High	Low
F		Low	Low	High	High
G		Low	High	Low	Low
H		Low	High	Low	High
J		Low	High	High	Low
K		Low	High	High	High
L		High	Low	Low	Low
M		High	Low	Low	High
N		High	Low	High	Low
P		High	Low	High	High
Q		High	High	Low	Low
R		High	High	Low	High
S		High	High	High	Low
T		High	High	High	High

[a]Pgt. - code consists of the designation for subset 1 followed by that for subset 2, 3,4 and 5.

Appendix V: Identification of race Ug99 (TTKSK) of wheat stem rust

USDA-Differential line	***Sr* gene**	**Infection Type (IT)[a]**	**Virulence/avirulence**
ISr5-Ra	5	4	H
CnS-T-mono	21	3	H
Vernstine	9e	4	H
ISr7b-Ra	7b	4	H
ISr11-Ra	11	4	H
ISr6-Ra	6	4	H
ISr8a-Ra	8a	4	H
CnSr9g	9g	4	H
W2691SrTt-1	36	0;	L
W2691Sr9b	9b	4	H
BtSr30Wst	30	3	H
Combination VII	17	4	H
ISr9a-Ra	9a	4	H
ISr9d-Ra	9d	3	H
W2691Sr10	10	3	H
Triumph 64	Tmp	2	L

[a]ITs at the seedling stage following the descriptions of Stakman *et al*. (1962), where IT - 0, ;, 1, 2, or combinations thereof were considered low (L), infection types, and 3 & 4 were considered high (H), infection types.

Appendix VI: Growth stages of small grains

CODE	Description	CODE	Description
0	**GERMINATION**	37	Flag leaf just visible
00	Dry seed	38	Flag leaf ligule just visible
01	Start of imbibitions	**4**	**BOOTING**
03	Imbibition complete	41	Flag leaf sheath extending
05	Radicle emerged from seed	43	Boots just visible swollen
07	Coleoptile emerged from seed	45	Boots swollen
09	Leaf just at coleoptiles tip	47	Flag leaf sheath opening
1	**SEEDLING GROWTH**	49	First awns visible
10	1st leaf through coleoptiles	**5**	**EAR EMERGENCE**
11	1st leaf unfolded	51	1st spikelet of ear emerged
12	2 leaves unfolded	53	One-fourth of ear emerged
13	3 leaves unfolded	55	One-half of ear emerged
14	4 leaves unfolded	57	Three-fourths of ear emerged
15	5 leaves unfolded	59	Emergence of ear completed
16	6 leaves unfolded	**6**	**FLOWERING**
17	7 leaves unfolded	61	Beginning of flowering
18	8 leaves unfolded	65	Flowering half-way complete
19	9 leaves unfolded	69	Flowering complete
2	**TILLERING**	**7**	**MILK DEVELOPMENT**
20	Main shoot only	71	Seed water ripe
21	Main shoot and 1 tiller	73	Early milk
22	Main shoot and 2 tillers	75	Medium milk (An increase in the solids of the liquid of the endosperm is notable when crushing the seed between fingers)
23	Main shoot and 3 tillers	77	Late milk
24	Main shoot and 4 tillers	**8**	**DOUGH DEVELOPMENT**
25	Main shoot and 5 tillers	83	Early dough
26	Main shoot and 6 tillers	85	Soft dough (Fingernail

			impression not held)
27	Main shoot and 7 tillers	87	Hard dough (Fingernail impression not held; head losing chlorophyll)
28	Main shoot and 8 tillers	**9**	**RIPENING**
29	Main shoot and 9 tillers	91	Seed hard (difficult to divide by thumbnail)
3	**STEM ELONGATION**	92	Seed hard (can no longer be dented by thumbnail)
30	Pseudostem erection	93	Seed loosening in daytime
31	1st node detectable	94	Over-ripe; straw dead/ collapsing
32	2nd node detectable	95	Seed dormant
33	3rd node detectable	96	Visible seed giving 50% germination
34	4th node detectable	97	Seed not dormant
35	5th node detectable	98	Secondary dormancy induced
36	6th node detectable	99	Secondary dormancy lost

(Adopted from Zadok *et al*., 1974)

Appendix VII: Rainfall and temperature data recorded during the experiment period (2009 - 2010).

Year	Month	Rainfall (mm)	Maximum (ºC)	Minimum (ºC)
2009	January	21.7	25.0	9.0
	February	5.7	27.0	8.0
	March	24.8	28.0	9.0
	April	62.7	26.0	9.0
	May	173.8	24.0	9.0
	June	13.6	24.0	8.0
	July	42.2	23.0	7.0
	August	56.3	25.0	9.0
	September	45.1	25.0	8.0
	October	74.8	22.0	10.0
	November	62.2	23.0	9.0
	December	76.7	23.0	10.0
2010	January	42.9	23.3	9.0
	February	157	25.0	10.5
	March	184.1	23.0	10.0
	April	140.4	23.0	10.0
	May	180.8	22.0	11.0
	June	51.8	22.0	9.0
	July	166.1	21.0	9.0
	August	240	21.0	9.0
	September	172.2	23.0	8.0
	October	109.9	22.0	10.0
	November			
	December			

Appendix VIII: Pedigree of the three parental material used in this study

Parent	Origin	Pedigree	Reaction to RWA	Reaction to 'Ug99'
Kwale	Kenya	KAVKAZ/TANORIL F-71/3/MAYA 4//BLUEBIRD/INIA	S	S
Cook	Australia	SCOUT PURE LINE SELECTION	S	R
KRWA9	CIMMYT	UNKNOWN	R	S