SCREENING FOR DISEASE AND ROOTKNOT (*Meloidogyne javanica*) RESISTANCE ON LOCAL TOBACCO LANDRACES (*Nicotiana tabacum L.*)

BY

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Degree in Horticulture

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DECLARATION

I hereby declare that the information herein presented is my own original work and a result of my efforts and energy. All additional information from secondary sources has been credited through acknowledgements and references.

KAVHIZA NYASHA …….. …….

R125254A Signature Date

CERTIFICATION

I the undersigned confirm that Kavhiza Nyasha J., candidate for the Bachelor of Science Horticulture Honours Degree has carried out research and presented on the research project entitled:

SCREENING FOR DISEASE AND ROOTKNOT NEMATODE RESISTANCE ON LOCAL TOBACCO (*Nicotiana tabacum L.*) LANDRACES

This written document, the oral examination serve as proof that the candidate executed the study and sanction the document for submission and evaluation.

SUPERVISOR: MR MUZEMU

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ABSTRACT

Thirty local tobacco landraces collected from non-traditional tobacco belt of Zimbabwe, such as Binga, Mutoko and Masvingo were screened for resistance against Tobacco Mosaic Virus (TMV), Potato Virus Y (PVY) and rootknot nematodes (RKN). The thrust of the study was to assess the level of resistance of the landraces to these major diseases of economic importance in tobacco. A standard flue cured tobacco (FCT) variety KE1, which is susceptible to all tobacco diseases was used as a positive control. The landraces were raised in the greenhouse where standard cultural and maintenance procedures were carried out. The specimens were then artificially inoculated for TMV, PVY and RKN then assessed for resistance. Significant genotypic variation was observed amongst the landraces. There were significant differences ($P<0.001$) in TMV severity, PVY severity and rootknot galling. The study revealed that 18 of the landraces have a hypersensitive response type of resistance to TMV, whilst 3 of the 12 susceptible landraces had severity percentages as high as 100% each. In PVY significant differences were noted ($P<0.001$) and four of the landraces did not exhibit any symptoms of PVY, showing immunity, hence a severity of 0. Most of the susceptible landraces were moderate in terms of severity as shown by a grand mean of 0.5931. The highest significant severity was observed in 8 landraces including Chokotwani and Chinyoka had severities of 1, comparable to KE1 which exhibited necrotic symptoms. PVY severity therefore ranged from a lowest of 0 to a peak of 1. For (RKN) assessment there were significant differences amongst the means. Two landraces namely Bhabhane and Unknown 2 had gall scores below 2. They had scores of 1.333 and 1.667 respectively, which shows very low nematode infection. Rupadza had the highest gall score of 6.33 comparable to KE1 with a mean of 7. The landrace Bhabhane had an outstanding overall performance when put under selection pressure of the 3 diseases. Potential sources of resistance to TMV, PVY and RKN were identified in this experiment. The disease resistance genes from the landraces can therefore complement already existing resources for elite tobacco breeding in Zimbabwe.

Key words: Tobacco mosaic virus, Potato virus Y, rootknot nematodes, landraces, resistance

DEDICATION

TO MOM AND DAD

The example of hard work, tenacity, strong will and a great deal of faith that you have laid out for me, has capacitated me to complete this noble task.

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Firstly I am indebted to the Lord God Almighty who has granted me life, health, enthusiasm and opportunity, thus seeing me through the successful completion of the project. It is an immutable fact that this work could not have come to fruition were it not for the priceless and invaluable contributions of the following individuals and organisations. Mr. Muzemu who supervised this work, Mr. Chaibva for providing the technical insight and guidelines on authoring this document. My profound gratitude to Dr. Magama who gave an opportunity to carry out the study and assisted me in terms of the planning, logistics and overall execution of the project. The Plant Breeding technical team, who were always ready and willing to lend me a hand on matters concerning the project. The Tobacco Research Board (TRB), for awarding me a chance to conduct the research at Kutsaga Research Station and for availing all the necessary provisions for the successful completion of the project. The Department of Horticulture at Midlands State University, who have nurtured me and imparted useful knowledge about plant culture to me. Tatenda Bobo, a comrade on the academic front, Tatenda Chirenje a fellow agrarian, for the interesting ideas. The list can never be fully exhausted, for there are so may whose noble participation is greatly appreciated. The gratitude is better felt than expressed.

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ACCRONYMS AND ABBREVIATIONS

AREX	Agricultural Research and Extension
BTV	Bush Top Virus
CBD	Central Business District
EDB	Ethyl di Bromide
ETI	Effector Triggered Immunity
FCT	Flue Cured Tobacco
GDP	Gross Domestic Product
Kb	Kilobases
PPU	Plant Protection Unit
PVY	Potato Virus Y
SA	Salicylic Acid
TF	Transcription Factors
TMV	Tobacco Mosaic Virus
TIMB	Tobacco Industries and Marketing Board
TRB	Tobacco Research Board

CHAPTER ONE

1.0 INTRODUCTION.

The tobacco crop (*Nicotiana tabacum L.*) is of fundamental economic importance globally and the industry generates about 20 billion dollars a year (FAO, 2010). Approximately 3.9 million hectares of land are dedicated to the crop worldwide (van De Merwe: 2010). Tobacco is grown in 125 countries including Zimbabwe (Factsheet, 2008). In Zimbabwe the crop has well defined regions where it is grown traditionally. There are fast growing areas such as Doma, Guruve and Hurungwe, then there are medium growing areas such as Centenary, Harare and Concession and lastly areas such as Headlands and Rusape are deemed as slow growing regions. Production of the crop has had a positive impact and reviving effect on the rural economy of Zimbabwe, a deindustrialising country with over 80% unemployment, (Mwareya, 2014). Zimbabwe is rated as the fourth largest producer of tobacco in the world and has built a reputation of producing high quality and most sought after flue cured tobacco. Flue cured tobacco contributes about 45% of the nation's total agricultural earnings, (Mazarire *et al*, 2013).

With the same token it is imperative to appreciate that tobacco production in Zimbabwe is bedevilled by prevalence of diseases such as Tobacco Mosaic Virus (TMV); Potato Virus Y (PVY) and also root-knot nematodes. TMV reduces cured tobacco yield, quality and average price. Characteristic symptoms include an irregular pattern of dark green and light green leaf areas intermingled; stunted plant growth; leaf malformation and mosaic burn, (Beekwilder, 1999). PVY in tobacco distorts the essential quality factors, such as lowering the content of the sugars in the leaf, causing a harshness which repels consumers' tastes.

Root-knot nematodes on the other hand interrupt plant growth and development by feeding on the roots. The *Meloidogyne javanica* that affects tobacco (Nicotiana tabacum) is endoparasitic and an obligate biotrophic pathogen, hence it feeds within the root system, secreting enzymes which kill the surrounding cells (Caillaud *et al*, 2007). This disrupts both water and nutrient uptake of the plant, threatening its vigour and health. It has to be understood that tobacco is a "heavy feeder" of nutrients, meaning that when the absorption of these mineral elements is compromised, the effects are manifested by poor leaf weight due to low dry matter; harsh texture as an indication of the insufficient nutrient constitution such as

that of nitrogen which improves leaf elasticity and also inconsistent leaf colour. The recent ban of effective control chemicals of nematodes such as methyl bromide and ethyl di bromide (EDB) has exacerbated the nematode problem for most tobacco farmers since the replacement chemicals such as Metham sodium and Oxamil are not as competent.

In a bid to control tobacco diseases in Zimbabwe, dates for important operations and activities such as sowing, planting, seedling bed destruction and stalk destruction have been legislated. The dates were put in place as a strategy of curbing the spread of tobacco diseases such as TMV, PVY and root knot by depriving the pathogens and vectors of their host. This is deemed an effective way of controlling the vectors and reducing disease incidence in the crop. Another control strategy that has been employed is the crop rotation method. Crop rotation reduces pathogen populations, hence reducing amounts of inoculum and lowering probability of disease incidence the following season.

Non- adherence by farmers to the legislated dates and other important hygienic practises in tobacco culture has led to a resurgence of the diseases such as TMV; PVY and root-knot nematodes in Zimbabwe's major tobacco growing regions. The post fast track land reform programme period has seen a lot of smallholder farmers coming onto the tobacco production scene. Most of these new and inexperienced farmers lack the knowledge of the enacted dates for sowing; planting; destruction of seed-beds and of tobacco stalks in the fields. The Agricultural Research and Extension (AREX) and the Plant Protection Unit (PPU) are under resourced to disseminate information and enforce the dates. Disease incidence is therefore on the rise and there is urgent need for mitigation.

Small landholdings of the small-scale farmers who constitute the bulk of new tobacco growers present a challenge to the application of the crop rotation method. There is not enough land for proper crop rotation to be implemented, hence the farmers use the same fields for tobacco cultivation year in year out. This leads to the build-up of pathogen populations and increases chances of disease epidemics occurring. This is one of the probable reasons for the persistent prevalence of TMV, PVY and root knot nematodes across the tobacco production zones.

The use of resistant cultivars provides a very effective way of disease management which complements other control methods, including those cited above. This is when the genetic architecture of the plant allows it to resist specific pathogens and insects. For example a certain tobacco species may constitute genes encoding resistance to aphids which are vectors

of PVY, hence the virus will not have detrimental effects on that particular tobacco. In-built resistance has the advantage that, even if the pathogen is present and the prevailing conditions are conducive for the latter, the host can reduce the effects of the pathogenic attack, (Acquaah, 2012). The exploration of genetic sources of resistance to the problematic diseases of TMV, PVY and root knot will lead to the development of novel varieties which are resistant. This leads to a comprehensive approach towards tobacco disease control. Productivity will then be boosted and profitability of the enterprise improved.

Furthermore use of resistant tobacco varieties is cost-effective and allows for sustainable tobacco production. There is very little use of chemicals which are costly and damage the environment through residual effects. Highly resistant hosts have a supplementary effect on the less competent but environmentally friendly chemicals that are now used to control rootknot nematodes.

With the current resurgence of tobacco diseases which are of economic importance, it becomes an issue of paramount importance to explore new and various sources of disease resistance. Landraces can therefore come to the rescue. They are locally adapted cultivars which are selected by farmers based on their phenotypic characteristics. Generally landraces are perceived to survive the vagaries of both biotic and abiotic stresses because of their heterogeneity, (Anderson, 1997).

The hypothesis is that, since landraces grow successfully in the wild without any fertilizers or crop protection methods, then they must be genetically fit. The rationale of incorporating landraces in this particular study is that local tobacco landraces may house genes of agronomic importance that alleviate current challenges to tobacco production. These landraces were collected outside Zimbabwe's main tobacco growing belt. They were collected from Binga; Masvingo and Mutoko which are non-traditional tobacco growing areas. The main thrust of this approach is to capture new genes, thus widening Zimbabwe's tobacco genetic base, leading to the development of novel varieties which possess multiple disease resistance.

It has been widely recognised that landraces constitute one of the most valuable sources of genetic diversity, (Samia *et al,* 2013). In barley for example, the landraces contributed significantly as one of the founding germplasm groups of North American barley varieties, (Samia *et al,* 2013). Most powdery mildew resistance genes used in the modern cultivars of barley originated from landraces, (Czembor and Czembor, 2000). Anderson (1997), proposes

that landraces may have different mechanisms of accommodating components for various diseases and the present results indicated that tolerance could be one of them. It is only a reasonable and prudent endeavour to exploit the landraces for such benefits herein stated.

1.1 Main Objective.

- To evaluate 30 tobacco landraces for resistance to root-knot nematodes, Tobacco Mosaic Virus and Potato Virus Y and to determine their cross compatibility to the flue cured variety KE1.

1.2 Specific Objectives.

- To determine the resistance of landraces to TMV infection.
- To assess the level of resistance of the landraces to PVY infection.
- To identify the dominant PVY strain affecting susceptible landraces.
- To evaluate level of resistance of the landraces to root-knot nematodes, (*Meloidogyne javanica*) infection.

1.3 Hypotheses.

- The landraces have TMV resistance.
- At least one of the landraces has resistance to PVY.
- There is one strain of PVY which dominantly affects the landraces.
- The tobacco landraces are resistant to root-knot nematodes (RKN).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 ECONOMIC IMPORTANCE OF TOBACCO IN ZIMBABWE

Tobacco is an important cash crop, christened, "The Golden Leaf", in Zimbabwe. The annual output of the crop is on the rise, reaching a total of 216.2 million kg in the recent season of 2014-2015, (TIMB ANNUAL REPORT, 2014). The crop raked in US$685.2 million contributing positively to the mainstream economy (TIMB, 2014). Flue cured tobacco only is responsible for about 45% of the nation's total agricultural earnings, (Mazarire et al, 2013). In the year 2013 tobacco exports totalled 153 million kilogrammes (kg) generating $877, 5 million, translating to an average of $5.72 per kg, (TIMB, 2014). This massive capital inflow has a significant impact on the Gross Domestic Product (GDP) of the country and its reverberations are felt across all sectors of the economy.

There has been a dramatic increase in the number of tobacco growers from a mere 8 537 in the year 2000 to 87 166 in the 2014-2015 season, (TIMB, 2014). The post-independence era has seen a lot of new farmers coming to join the industry since tobacco offers, higher returns per unit area than most traditional crops grown in Zimbabwe. This paradigm shift from traditional cereal production by smallholder farmers to cash crop production (tobacco and cotton) is explained by the study done by (Patched, 1999 in Van de Merwe, 2012) which found that tobacco production in Zimbabwe and Malawi is 6.5 times more profitable than maize, twice as profitable as cotton and 60 times more profitable than sorghum.

The inception of contract tobacco production system in 2004 as a way of addressing the market failures to allocate productive resources to all sectors of the economy due to perceived information asymmetries has played a part in stimulating this increase in tobacco producers, (Moyo, 2012). The farmers are assured of markets and guaranteed prices since the contracting company is obliged to purchase the commodity at agreed prices and also support production through provision of inputs and technical assistance. The farmers on the other hand are compelled to produce the crop in quantities and quality standards that meet the contractor's specifications, (Nyamwanza *et al*, 2014).

2.2 CONSTRAINTS OF TOBACCO PRODUCTION IN ZIMBABWE

Inasmuch as the tobacco annual output is very high, the revenue margin is only increasing at a slothful pace. The margin of 2014-2015 only increased by 12% from that of 2013-2014, owing largely to the plummeting seasonal average prices, which fell from US$3.67 in 2014 to US$3.17 in 2015, (TIMB, 2014). One of the negative factors that have significantly contributed to this dramatic drop in prices is that of poor quality styles of tobacco. Poor quality leaf styles are only an indication of the threat posed by pests and diseases to meaningful production and also poor agronomic practises being employed in the field. Tobacco is a crop which is affected by many pests and diseases such root knot nematodes, cutworms, leaf miner, white flies, TMV, PVY, Bushy Top Virus, angular leaf spot, wildfire and many more. These pests and diseases interfere with the plant growth and development of the crop thus negatively altering the quality of the final product which is the leaf.

The huge influx of new growers who are taking up tobacco production has come with its fair share of challenges. Most of the farmers lack knowledge concerning important legislation in tobacco production. For example the farmers are not adhering to legislated dates which state that no tobacco seed should be sown before 1 June, no tobacco to be planted before 1 September, seedling beds to be destroyed by 31 December and that all tobacco stalks should be destroyed by 31 May. The issue of growers negating tobacco stalk destruction is of paramount importance as it promotes the carryover of diseases into the next season, (Reed *et al*, 2012). The Tobacco Industries and Marketing Board (TIMB) is making efforts to educate and train farmers about these dates and sustainable tobacco production, (TIMB, 2014). However this is being done at a snail pace and growers still continue to neglect the enacted dates.

Disregard to these dates and poor agronomic practises have given rise to the proliferation of diseases such as TMV, PVY, root knot and Bushy Top Virus (BTV). Of late PVY and BTV are becoming more prevalent due to late planting being practised by most farmers. Late planting promotes synchrony between the crop establishment phase and pathogen or vector breeding stage. The problems caused by these pests and diseases are then reflected in the increased cost of production in a bid to control them and also in the poor leaf quality which translates to subdued prices.

2.3 TMV AND ITS EFFECTS ON TOBACCO

Tobacco Mosaic Virus infects 199 plant species from 30 different families but most damage is done to the Solanaceous family. The virus is highly contagious for it is mainly mechanically transmitted and infection is quick and effective, (Bagley, 2001). When a plant is TMV infected it shows symptoms such as vein clearing in the upper new leaves; manifestations of mosaics in the juvenile leaves; mottling of irregularly shaped dark green patches of tissue surrounded by light green areas of tissue and wrinkling coupled with deformation of the young infected leaves. The virus translocates to all parts of the plant such as the root, corolla, seed, etc. even though visual symptoms are not present.

TMV is a member of the Tobamovirus genus and a 300nm rod shaped particle, enveloped 2130 copies of protein in a cylindrical manner. The RNA genome encodes up to four proteins that enable the virus to infect and replicate. The 180k protein by itself infects plants. The stability of TMV allows it to stay for many years in cigarettes and cigars manufactured from leaves containing the virus, (Protein Database, 2009).

TMV spreads rapidly and easily thus the infection establishes very quickly. A relationship exists between the time of infection and the severity of TMV infection, (Bagley, 2001). Virus infection of younger plants causes great yield losses as compared to infection of older plants. The virus has a negative effect on the yield; grade index and value of the cured leaf. When resistant cultivars are used the reductions in yield and quality caused by the virus are lessened, whereas a field of susceptible cultivars would exhibit very high TMV incidence. In cases where TMV incidence levels are above 37% even when proper sanitation and hygiene are being practised, use of resistant cultivars proves to be a better method of control for tobacco farmers.

2.4 TMV CONTROL

Knowledge of all potential sources of inoculum is necessary if TMV is to be prevented. Primary inoculum is the term given to any overwintering source of TMV infection. Weeds belonging to the Solanaceous family such as nightshade can be alternative hosts of TMV and serve as overwintering sources of primary inoculum. Effective weed management, especially removal of the Solanaceous weeds around tobacco fields minimises potential infections of TMV virus, (Beekwilder, 1999).

Milk can be used as an antiviral agent. Applying milk a day before transplanting can help to control the mechanical spread of TMV, (Scholtof, 2008). Even though most TMV infections

emanate from primary infected plants, secondary infection is also rampant in fields affected by the virus. The most effective way of guarding against secondary spread is through proper hygienic practises, (Bagley, 2001). Complete sanitation is three dimensional and consists of worker sanitation, greenhouse sanitation and equipment sanitation.

TMV is mechanically transmitted and some operations in commercial tobacco production such as clipping which improves uniformity and stiffens the stalks may lead to massive spread of TMV. For example if one plant in the seedling bed has TMV, the virus will be spread across the whole transplant bed. The chances of the spread may be reduced by sterilising the sheers used to clip in copper oxchloride solution.

Air dried tobacco is another source of infection. When a worker smokes cigarettes; uses chewing tobacco or snuff containing air cured tobacco, they may cause infection by introducing the virus to the plant via air, (Beekwilder, 1999).

2.5 Resistance to TMV

In the year 1929 when Nolla J. was touring Colombia in South America, he noticed that there were two *N. tabacum* cultivars which did not manifest symptoms when inoculated with TMV. In an experiment carried out in Puerto Rico with the two cultivars when they were inoculated with TMV, the cultivar Ambalema showed no TMV signs, (Bagley, 2001).

Two recessive genes were reported to control TMV resistance. Further studies also reported the possibility of modifying genes involved in this form of resistance which make it more difficult to develop resistant cultivars, (Scholtof, 2008). Unfortunately after 15 years of using Ambalema as a source of resistance to TMV the results were unsatisfactory. Plants which carried Ambalema resistance would scald and wilt severely when temperatures were high. The Ambalema does not confer true resistance since the virus still continues to replicate though at a subdued rate of multiplication. Inhibition of the rate of multiplication therefore results in no TMV symptoms.

When evaluating Nicotiana species for TMV resistance in 1914, Allard observed that *N. glutinosa* did not show mottling symptoms after inoculation with TMV but developed a rot from the point of infection, (Bagley, 2001).

In 1938, Holmes managed to incorporate genetic resistance to TMV in tobacco. However some researchers are of the view that cultivars that get their resistance from *N. glutinosa* are of inferior quality. Nevertheless there are some species which also possess the resistance to

TMV for example N. repanda which also has resistance to eight other diseases. However challenges in introgressing *N. repanda* genes into *N. tabacum* proves a hindrance in the transfer of disease resistance to the latter, (Scholtof, 2008).

2.6 PVY AND ITS EFFECTS ON THE TOBACCO CROP

Potato Virus Y infects members of the Solanaceae family including tobacco, (*N tabacum L.*). The disease drastically reduces the yields and quality of the crop. In tobacco PVY causes vein clearing, mosaics and necrosis. PVY has a single stranded positive sense RNA of 9.7 kilobases (kb), with a single open reading frame, (Kim *et al,* 2014). It has been reported that there are three strains of PVY depending on the symptoms they cause in tobacco and potato plants. The PVY^{O} strain also referred to as the ordinary strain induces the mosaic patterns whilst PVY^{N} (necrotic) strain causes veinal necrosis in tobacco. PVY^{NTN}, a merger between PVY^{O} and PVY^{N} causes necrosis on potato tubers but no observable effect on tobacco. PVY^{C} induces symptoms similar to those of PVY^{O} which are mosaic patterns, (Kim *et al,* 2014). $PVY^{N:O}$ and the recombinant PVY^{NTN} isolates have a hybrid genome which constitutes segments of PVY^{O} and PVY^{N}. Consequently most isolates of $PVY^{N:O}$ induce veinal necrosis, vein clearing, stem necrosis and foliar deformation in tobacco, and these symptoms are the same that are produced by PVY^{N} and PVY^{NTN}, (Nie and Molen, 2014). In Manitoba Canada, $PVY^{N:O}$ isolates were collected and from these 3 distinct subtypes which are the severe, intermediate and mild were recognised on the basis of their pathogenicity on tobacco plants, (Nie and Molen, 2014).

Since it was identified, PVY has been perceived as a complex of various isolates. PVY^{O} and PVY^{N} strains are reported to have originated from Andean countries where they were probably adapted to infection of various Solanaceous species in their natural habitats, (Tsadeley, 2015). The strains PVY^{O} and PVY^{N} may have diverged from one common viral ancestor that followed two different evolutionary paths, probably on different hosts. In tobacco the occurrence of the necrotic symptoms caused by PVY can be associated with unbalanced nutrition, especially magnesium deficiency. PVY^{N} and PVY^{O} account for high yield losses, of up to 14-59% in PVY^{O} infected tobacco and as high as 100% for veinal necrosis caused by PVY^{N} isolates, (Tsadeley, 2015).

2.7 Transmission

PVY is spread from one plant to another by aphids; mechanical means and contact. Most PVY strains and isolates are transmissible by aphids and there are about 50 species of aphids which transmit the virus with varying efficiency, (Tsadeley, 2015). These aphids transmit the virus in a non-persistent manner, requiring less than one minute in acquisition and inoculation times. However some aphid species transmit PVY more effectively than others, though transmission efficiency will be variable among virus isolates of a certain strain and the aphid populations of a particular species. The *Myzus persicae*, aphid species is responsible for about 50% of all observed transmission.

2.8 NEMATODES AND THEIR EFFECTS ON TOBACCO

Root-knot (*Meloidogyne species*) and cyst (*Heterodera spp*) affect all crops of economic importance including tobacco and cause losses to the tune of $90 billion worldwide, (Dhandaydham *et al,* 2008). The average yield loss due to nematodes is estimated at 12% per annum, reaching a high of 20% in certain crops. The *Meloidogyne* group of nematodes has about 70 species but four of them, namely *M. incognita*; *M. javanica*; *M. arenaria* and *M. hapla* account for approximately 95% of all infections, (Dhandaydham *et al,* 2008).

Root-knot nematodes are sedentary endoparasites, hence a greater part of their active lives is spent within plant roots. They enter the roots as second stage juveniles (J2) and establish an intricate relationship with the host. The nematodes form giant cells also called nurse cells, from which they deprive the plant of water, solutes and nutrients. Once they establish their feeding sites the (J2) sedentary nematodes then start to develop in a series of moults from (J3) to (J4) where they become large female adults. When their life cycle is complete they complete insert themselves into the root tissue and lay their eggs in an external gelatinous matrix which can be viewed clearly outside the roots in the form of an egg mass, (Molinari, 2010). Hypertrophy and hyperplasia as a result of nematode action cause the formation of galls on the roots of susceptible plants.

In Nematology quantitative research is carried out to establish the relationship which exists between known pre-plant populations of plant parasitic nematodes and the effect they have on crop performance, specifically plant growth yield and quality, (Fourie *et al,* 2010). For optimum protection against nematodes to be achieved, it is very necessary to understand the relationship that exists between the quantities of the organisms at planting and the response of the host plants. Mere presence of the nematodes in the soil does not necessarily translate to

crop damage or crop yield loss because populations of these plant parasitic nematodes may be below the economic threshold levels of a particular field, (Seah *et al,* 2004).

Crop damage and yield reduction caused by nematodes depends on several factors such as prevailing nematode distribution pattern; race present; pathotype distribution; nematode multiplication rate; the specific nematode species, soil type; plant cultivar grown; previous cropping history and the environmental conditions, (Fourie *et al,* 2010).

In a study carried out by Fourie in 2010, it was reported that cultivar susceptibility to nematodes has a large effect on the damage caused to the crop. He also recommends that many nematode parameters should be considered when host resistance is being evaluated. This is so because plant resistance may represent only one or more of the many various mechanisms of resistance.

The exploitation of natural genetic resistance of host plants to nematodes is the most economically feasible strategy of controlling nematodes in the less economically developed countries in the subtropical and tropical regions of the world, (Molinari, 2010). In the high input modern production systems of the more economically developed countries plant resistance is increasingly becoming important as most chemicals used to control nematodes are being restricted or banned. Managing nematodes has been a cumbersome task for more than 5 decades and the most effective way of doing so has been the use of toxic fumigants. However the development of a nematicide takes about 10 years and multi millions to come up with a competent product, (Seah *et al*, 2004).

2.9 Nematode Resistance

Genetic resistance to nematodes can be dominant; recessive or additive in expression. It may be conferred by either single major genes or by a combination of 2 or more genes or quantitative trait loci. Several of the characterised genes confer resistance to the most economically important and widely distributed nematode groups such as *Meloidogyne species (spp)*; *Globodera spp* and the *Heterodera spp*. The Mi-1.2 tomato gene has been found to be the one which encodes for the resistance of the 3 most diffused species of root-knot nematodes, namely *Meloidogyne incognita*, *M. javanica* and *M. arenaria.*

It is also important to note that the Mi-1.2 gene also confers resistance to particular isolates of the potato aphid, *Macrosiphum euphorbiae* and also 2 biotypes of the white fly (Bemisia tabaci), another nuisance pest in tobacco culture. It is the only resistance gene (R gene) that is known to confer resistance against several disease groups of pests. The tomato plant to root-

knot interactions serve as a model on which to base the assessment of the defence response of resistant plants to nematodes. The tomato resistance is exhibited as hypersensitive reaction which induces rapid localised cell death, occurring just 12 hours after inoculation of the roots with second stage (J2) nematodes as they attempt to form feeding sites, (Molinari, 2010).

However in crops of high value, application of resistance as management strategy has its practical limitations. Resistance unlike chemical control is target specific, thus it may be effective in controlling a particular species, biotype or isolates whilst most nematicides are polyspecific and offer more comprehensive nematode protection, (Dhandaydham *et al,* 2008).

2.10 LANDRACES AS POTENTIAL SOURCES OF RESISTANCE

With the current resurgence of tobacco diseases which are of economic importance, it becomes an issue of paramount importance to explore new and various sources of disease resistance. Landraces can therefore come to the rescue. They are locally adapted cultivars which are selected by farmers based on their phenotypic characteristics. Generally landraces are perceived to survive the vagaries of both biotic and abiotic stresses because of their heterogeneity, (Anderson, 1997).

The assumption is that, since landraces grow successfully in the wild without any fertilizers or crop protection methods, then they must be genetically fit. The rationale of incorporating landraces in this particular study is that local tobacco landraces may house genes of agronomic importance that alleviate current challenges to tobacco production. These landraces were collected outside Zimbabwe's main tobacco growing belt. They were collected from Binga; Masvingo and Mutoko which are non-traditional tobacco growing areas. The main thrust of this approach is to capture new genes, thus widening Zimbabwe's tobacco genetic base, leading to the development of novel varieties which possess multiple disease resistance.

It has been widely recognised that landraces constitute one of the most valuable sources of genetic diversity, (Samia *et al,* 2013). In barley for example, the landraces contributed significantly as one of the founding germplasm groups of North American barley varieties, (Samia *et al,* 2013). Most powdery mildew resistance genes used in the modern cultivars of barley originated from landraces, (Czembor and Czembor, 2000). Anderson (1997), proposes that landraces may have different mechanisms of accommodating components for various diseases and the present results indicated that tolerance could be one of them. It is only a reasonable and prudent endeavour to exploit the landraces for such benefits herein stated.

In a study done by Czembor (2000), where 19 were investigated+ Moroccan barley (*Hordeum vulgare*) landraces for resistance to powdery mildew, 15 of them showed resistance. What is more interesting is that the distribution of reaction type readings indicated that most of the tested landraces had more than gene for resistance. Samia *et al* (2013), when conducting trials and assessing the productivity of the barley landraces against that of commercial varieties, found that there are drastic changes in genotype order between productivity levels revealing that landraces are superior under mid to low production conditions. The findings are very relevant as they give the suggestion that some of the landraces possess traits that may not have been captured in the current elite varieties.

In cassava (*Manihot esculenta*), locally adapted and traditionally grown cultivars have been reported to possess a number of important traits including considerable tolerance to indigenous pests and diseases, (Adebola, 2008).

CHAPTER 3

3.0 Material and Methods

3.1 Research Site.

The experiment was carried out in a greenhouse at Kutsaga Research Station, in the 2014-2015 growing season. Kutsaga is located 15km East from Harare's central business district (CBD). The predominat soil groups in this region are regosols; siallitic; fersiallitic; paraferralitic and the orthoferralitic, (Mugandani et al 2012). According to Mugandani et al, (2012), these soils are highly suitable for agricultural use.

Table 3.1: Experimental site description

Trial Site	GIS Position	Soil Type	Altitude (masl)	Average rainfall (mm)	Natural Region
Kutsaga Research Station	17'55''S3108''E	Light sands pH5.2	1479	600-800	2

3.2 Description of the plant material.

The experiment consisted of 30 tobacco landraces collected outside Zimbabwe's main tobacco growing belt. Some of them are early maturing whilst others are of a slow growth habit. The standard commercial variety KE1 was used as positive control. The variety KE1 was chosen based on its susceptibility to all tobacco diseases including PVY, TMV and rootknot nematodes.

3.3 Treatments.

Table 3.2: Landrace codes and names used in the study

Landrace code	Landrace name	Landrace code	Landrace name
1	Nalokotwani	17	Chinyoka
2	Kalyatamvuvi	18	Fodya
3	Unknown	19	Chikwarimba
4	Nalubotu	20	Rupadza
5	Nalubotu	21	Chikwarimba
6	Chingambe	22	Bhabhane
7	Cholokotwani	23	Unknown
8	Nalukotwani	24	Chambwa
9	Nalukotwani	25	Unknown
10	Chokotwani	26	W36
11	Chikwarimba	27	W170
12	Chikwarimba	28	W171
13	Chikwarimba	29	W172
14	Fodya	30	W173
15	Gwini	31	KE 1
16	Unknown	32	K M10 (root knot nematode susceptible control)

3.4 Experimental Design

In this experiment a Random Complete Block Design (RCBD) with three replications was used. There were 32 treatments in each block. Each tobacco landrace served as an individual treatment. Shade was the blocking factor in this experiment.

3.5 Seedling Raising

Leader pots of diameter 10cm were filled with sterilized soil mixed with basal fertilizer compound C at 2g (30kg/Ha) per pot per week. The seeds of each treatment were sown in the 10cm leader pots and then covered with vermiculite. Each treatment was sown in 2 pots, thus the total number of pots sown was 64.Four weeks after transplanting the seeds were individually transplanted into 10cm diameter, leader pots. Ten pots of individual seedlings were planted. Inoculation of the seedlings with the viruses (PVY and TMV) and rootknot nematodes was done 3 weeks after transplanting when the plants had attained at least 5 leaves.

3.6 Fertilizer Management

To make sure that the plants remained healthy, buoyant and lush green till the time for evaluation, a fertilizer regime was put in place. The plants received 2 grams (30kg/Ha) of ammonium nitrate (A/N) per plant every fortnight. This practise also makes for clear manifestation of the signs and symptoms in the disease trials, thus making them easy to identify.

3.7 Water Management.

The plants were watered every day to meet the crop water requirements. However in cases where there was enough antecedent moisture, application of water would be skipped to guard against waterlogging.

3.8 Statistical analysis.

One way Analysis of Variance, (ANOVA) was performed using software, GenStat 17th Edition. The means were separated using Duncan's Multiple Range Test (DMRT).

3.9 Tobacco Mosaic Virus (TMV).

The tobacco landraces shall be tested for their resistance against TMV. KE1 and will be used as the standard control.

3.10 Inoculum preparation.

Leaves from affected plants previously inoculated with TMV, were collected. The leaves were grinded with a pestle and mortar, adding some distilled water to make a smooth solution. Sap was separated from the fibrous material through the use of sieves. The sap was collected and put into flasks ready for use.

3.10.1 Inoculation.

The plants were inoculated upon reaching 3 weeks from the date of transplanting. Celite an abrasive (powder) was put in the petri dishes and mixed with the TMV inoculum. A cotton swab would then be dipped (immersed) into the inoculum and gently rubbed against plant leaves.

3.10.2 Assessment.

Three weeks after inoculation the visual checks were carried out on the plants to see if there was presence or absence of TMV. Those plants identified to be giving mottling (mosaic) symptoms were recoded as the susceptible landraces. The ones exhibiting a local lesion of hypersensitive reaction (HR) or lacking signs and symptoms of TMV were deemed resistant landraces.

3.10.3 Potato Virus Y.

In this part of the experiment the 30 indigenous tobacco landraces were tested for their resistance against PVY. Variety KE1 was used as the positive control.

3.10.4 Inoculum preparation.

Fresh necrotic leaves of plants affected with PVY were collected from the late December crop infested with the disease. The leaves exhibited both mosaic and necrotic symptoms of PVY. Upon collection the leaves were crushed using a blender and adding some distilled water to produce a smooth inoculum. The desired amount of inoculum for sufficient inoculation of all plants was approximately 500 millilitres.

3.10.5 Crushing of leaves.

The leaves were first stripped off the midrib, leaving the lamina only. This was done to ensure that the midrib does not chock the blender during the crushing process. About 500mls of inoculum was produced per crushing session. All fibrous material were strained from the inoculum using sieves. The solution was then put in flasks ready for use.

3.10.6 Inoculation.

In this experiment mechanical inoculation was done to the plants. The inoculation process took place 3 weeks after transplanting when the seedlings had attained at least 5 leaves per plant. A buffer called celite which has an abrasive effect on the leaves was put into petri dishes and mixed with the inoculum. A cotton swab was then dipped (immersed) in the inoculum/buffer solution and gently rubbed against the leaves of the plants.

3.10.7 Assessment.

Four weeks after inoculation the plants were observed for symptoms of infection with PVY, (Doroszewska *et al,* 2010). The symptoms were closely examined and classified into 2 main strains of PVY namely the necrotic strain and the mosaic producing strain. The degree of severity and extent of strain on the individual plant was also considered. This is so because some plants may show symptoms for both strains, albeit there is one that is always dominant and it will be noted. A key denoting the type of strain and severity was employed, where B1 and B2 represented the mosaic and necrotic strains respectively. Severity was rated from 1 to 3, with 1 being slight infection; 2 as mild infection and 3 showing severe infection. For example plants that showed mosaics only and which had a small portion of the plant tissue affected would be coded B11.

3.11.0 Root-knot nematode inoculation and assessment.

3.11.1 Preparation of nematode inoculum.

Root knot nematode cultures were collected from the Kutsaga Land 3 that is infested with nematodes. Roots with conspicuous galls were selected, washed gently with tap water and identification of *Meloidogyne javanica* was carried out. A single egg mass from the female was used to raise a pure culture of *M javanica*.

Hatched second stage juveniles (J2s) were collected from the watch glass and diluted with fresh water, (Barre *et al,* 2013). The nematode suspension was then inoculated into the rhizosphere of 15 days old tomato seedlings raised in sterilized soil and transplanted into 20 centimeter diameter pots with approximately 2.5 kilogrammes of soil. An inoculum density of two J2s per gram of soil was used so as to raise a pure culture. Regular additions of moisture to the pots were done by letting the water pass through the 325micrometer (µm) mesh sieve to avoid contamination through water. The egg mass cultures multiplied in a period of about 60 days, (Barre *et al,* 2013).

3.11.2 Collecting root-knot nematode juveniles for inoculum.

The tomato plants infected with root-knot nematodes were removed from the pots and gently rinsed in a bucketful of water or under a running tap. The roots were cut into 1-2 centimetres sections. The roots were placed in a large beaker containing 0.5% sodium hypochlorite (NAOCL) and shaken for 2-3 minutes. The suspension was then poured through a 250 µm sieve nested over a 25 µm sieve to collect the eggs. Eggs and juveniles captured on the 25 µm sieve were be rinsed under running water then the sieve was washed in a beaker using a bottle and distilled water. Roots from the 250 µm sieve were placed into a beaker and the whole process above would be repeated to release any remaining eggs or juveniles. The egg number was determined. The eggs were put into a test tube for ready use as inoculum.

3.11.3 Nematode inoculation.

In this experiment the standard amount of inoculum per plant was 5 000 eggs. It was requisitely ensured that the soil is moist enough and not saturated before inoculation. Two pencil holes were made around each plant. The inoculum was stirred or agitated by a magnetic stirring plate at low speed to ensure even distribution of inoculum. The nematode inoculum was applied evenly to the plant using a pipette. The holes were covered with soil. The pots were then left to settle for 24 hours before watering. Maintenance of the plants for 6-8 weeks was done to allow for nematode stress reaction.

3.11.4 Root-knot nematode assessment.

Eight weeks after inoculation the plants were removed from the pots, had their roots washed free of soil and root-knot indices determined**.** In this study the Nasbaum and Daulton scale (1960) was used to determine the root-knot nematode indices. The scale has 8 infection classes with index values ranging from 0-100.

CHAPTER FOUR

RESULTS

4.1 TMV SCREENING

Table 4.1 Responses of the landraces to TMV infection

TREATMENTS	TMV SEVERITY
Bhabhane	0.00[a]
Chambwa	0.00[a]
Chingambe	0.00[a]
Chokotwani	0.00[a]
Cholokotwani	0.00[a]
Kalyatamvuvi	0.00[a]
Nalokotwani	0.00[a]
Nalubotu 1	0.00[a]
Nalubotu 2	0.00[a]
Nalukotwani 1	0.00[a]
Nalukotwani 2	0.00[a]
Unknown 1	0.00[a]
Unknown 4	0.00[a]
W170	0.00[a]
W171	0.00[a]
W172	0.00[a]
W173	0.00[a]
W36	0.00[a]
Chikwarimba 2	70.00[b]
Chikwarimba 5	73.33[bc]
Chikwarimba 4	80.00[cd]
Chikwarimba 3	83.33[de]
Fodya 1	83.33[def]
Unknown 2	86.67[defg]
Chikwarimba 1	90.00[defgh]
Gwini	93.33[egh]
Rupadza	93.33[efgh]
Unknown 3	93.33[efgh]
Chinyoka	100.00[h]
Fodya 2	100.00[h]
KE1	100.00[h]
p-value	(P<.001)
S.E.D	4.544
C.V	15%

Multiple comparison test showed significant differences of TMV incidence and severity amongst the landraces. The least significant disease score of zero was recorded in 18 landraces including Bhabhane; Cholokotwani; Nalukotwani and Kalyatamvuvi which tested negative to TMV. The landrace Chikwarimba 2 with severity percentage of 70% was significantly different, ($P<0.001$) to all other treatments but comparable, ($P<0.001$) to Chikwarimba with mean percentage of 73.33%. Analysis of variance showed no significant differences ($P>0.001$) amongst Chikwarimba 4; Chikwarimba 3; Fodya 1; Unknown 2;

Chikwarimba 1, Gwini; Rupadza and Unknown 3. The percentage of severity ranged from 80% to 93.33%. The highest TMV severity percentage was recorded in the landraces, Chinyoka and Fodya 2 with a 100% severity. These two landraces were statistically comparable, (P>0.001) to the positive control KE1.

4.2 PVY SEVERITY

Significant statistical differences (p<0.001) were recorded on PVY severity of the inoculated tobacco landraces as shown in figure 4.2. Four of the treatments, Cholokotwani; Nalukotwani 1; Nalukotwani 2 and Bhabhane did not show any signs of infection. They were immune to the virus thus their means were not separable (P>0.001). However these treatments were significantly different from all the other treatments. Statistically comparable means (P>0.001) were observed for Nalubotu 2; W171; Nalubotu 1; W173 and W36 but landrace W36 with a mean of 0.2933 was also comparable with Nalukotwani with a mean of 0.3300. Nalukotwani and Kalyatamvuvi were statistically similar. The landraces Unknown 4 and Unknown 1 were significantly different from the rest of the treatments but comparable to Chingambe (P>0.001).

The disease severity of Chambwa was statistically similar to both Chingambe and Chikwarimba 3. However the severity of Chikwarimba 3 was significantly higher than that of Chingambe, (P<0.001). Fodya 1 was comparable (P>0.001), with both Gwini and Chikwarimba 2 but Chikwarimba 2 had a significantly higher, (P<0.001) severity of 0.7900 than Gwini (0.7167) and Fodya 1 was intermediate (0.7300). Another statistical group constituted of Unknown 2, Rupadza and Chikwarimba 1 with means of 0.8367; 0.8900 and 0.8967 respectively. However Unknown 2 was also statistically similar to Chikwarimba 2 with a mean severity of 0.7900. The highest significant severity was recorded for the nine following treatments, Chikwarimba 1; Chikwarimba 4; Chikwarimba 5; Chinyoka; Chokotwani,; Fodya 2; Unknown 3; W170 and W172, which were statistically comparable, (P>0.001) to the positive control KE1. A severity of 1 (100%) was recorded for each of them. Figure 4.1 shows the trend of severity that was observed after assessment.

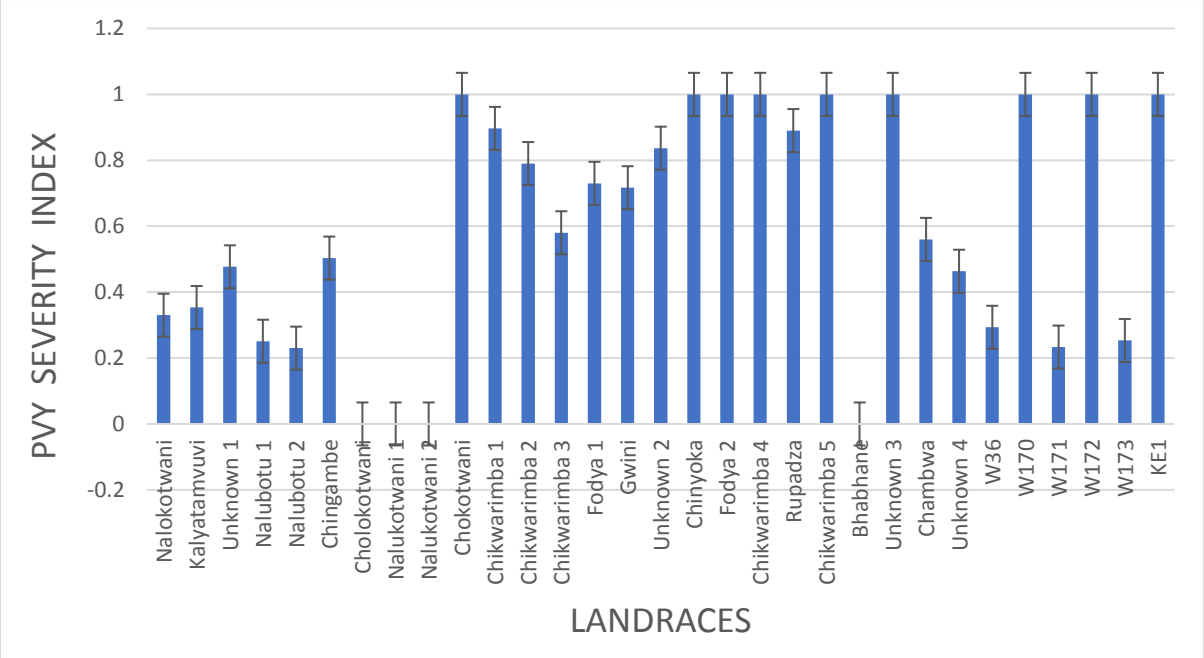

Figure4.1 PVY severity on the tobacco landraces.

4.3 Evaluation of landraces for root-knot resistance

Analysis of variance showed significant differences (P<0.001), amongst the landraces on their responses to rootknot nematode infection. Bhabhane had the lowest significant score of 1.333, only comparable (P>0.001), to two other landraces, Unknown 2 and Fodya 1 with means of 1.667 and 2 respectively. A significant difference (P<0.001) was noted between Bhabhane with the lowest score and Rupadza with the highest gall score of 6.333. The differences amongst the rest of the treatments were not of significant statistical importance (P>0.001). Fodya 2; Chikwarimba 1; Chikwarimba 4 and Rupadza with means of 4.667; 5.333; 5.667 and 6.333 respectively had high gall ratings comparable to the positive control KE1 with a mean score of 7.000. Figure 4.3 shows the different responses of the tobacco landraces to rootknot nematode attack, when they were rated against the Dalton and Nasbaum scale (1960).

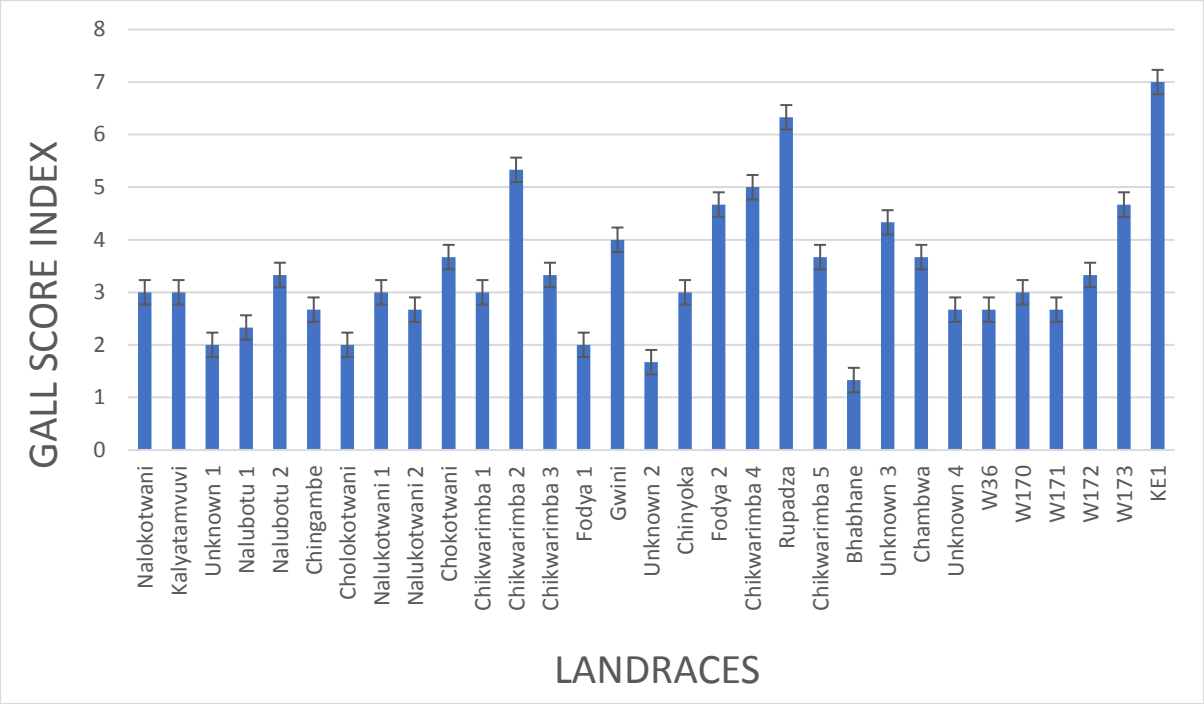

Figure 4.*2*: Gall scores obtained after roots of inoculated plant were rated.

CHAPTER FIVE

5.0 DISCUSSION

The evaluation of new potential sources of disease resistance has become an issue of paramount importance in tobacco disease management. There were varied performances amongst the local landraces when they were subjected to pest and disease pressure. Nevertheless as expected some of landraces exhibited some considerable resistance to TMV; PVY and rootknot nematodes.

5.1 TMV SCREENING

Following inoculation and assessment for TMV 18 of the local tobacco landraces proved to be resistant to TMV. They all exhibited the hypersensitive response type of resistance. This type of resistance was first discovered in *Nicotiana glutinosa* and is encoded by a gene called the N gene (Bagley, 2001). The landraces had localised necrotic lessions at the virus points of entry. The reaction prevents systemic spread of the virus by causing cell death at the region of infection. *Nicotiana rustica* is another species which shows the hypersensitive type of reaction. None of the resistant landraces exhibited asymptomatic resistance, thus all them possess the N gene or its allele for resistance to TMV.

Another point to note is that 10 of the landraces which were resistant to TMV such as Nalokotwani, Chokotwani, Kalyatamvuvi, Bhabhane, Nalubotu 1, Cholokotwani. have been collected from the same geographical area of Binga, thus their genotypes maybe similar. The fact that landraces are adapted to the environments which they are found is in synchrony with the fact that the resistant landraces from Binga all possess the same type of resistance to TMV.

The other eight resistant landraces such as Unknown 1; Unknown 4; W170; W173; W171; W172 and W36 were collected from Mutoko and Masvingo. There is a high probability that the genotypes of these landraces maybe similar to those from Binga, the only variation being the phenotypic expression and the name by which they are known. The morphology and physiology of the landraces from Masvingo and Mutoko is the same with that of the Binga landraces. They are all adapted to similar environmental conditions and also exhibit the same type resistance to TMV. The fact that all of the landraces are indigenous explains the same type of resistance that was observed.

Most of the resistant landraces are of the *N. rustica* type. In cases where the landraces exhibited phenotypic differences though they are all of the *N. rustica* origin and have the same type of resistance (hypersensitive reaction), the phenomenon is due to intraspecific variation in terms of morphology. Nifong (2008) postulates that this species shows elevated levels of phenotypic owing to reorganisation and speciation. In a study that Nifong (2008) carried out using N. rustica accessions, all of them were found to have a hypersensitive response kind of resistance. This may explain the phenomenon whereby all the resistant landraces are of the *N. rustica* species.

Inasmuch as the N gene provides resistance against TMV, previous studies have shown that it is associated with poor economic quality; reduced yield and quality of the leaf of FCT. Therefore if the landraces have the N gene type of resistance, the incorporation of this gene into FCT may confer resistance to TMV but compromising the yield potential of the varieties to which it has been introgressed. On the other hand it has to be appreciated that the gene responsible for localised lesion reaction exhibited by the local landraces may be another gene which is allelic to the N gene (Beekwilder, 1999).

The variation of the landraces in their responses to TMV which caused significant differences in terms of incidence and severity is attributable to the genetic differences between the various species of tobacco from which the landraces belong. The landraces are of different species therefore variation is smaller within the same species (intraspecific variation) and greater between species. Interspecific variation is the most prominent cause of the differences whereby other landraces exhibit resistance to TMV whilst 12 other landraces are susceptible.

It has to be noted that some of the landraces which were found to be susceptible to TMV for instance Gwini; Chikwarimba 5; Fodya 1 and Fodya 2 were highly phenotypically similar to Flue Cured Tobacco (FCT). Their morphological characteristics in terms of leaf shape, flower colour; leaf nodes, etc. were the same as those of FCT. This phenotypic similarity also suggests the genetic proximities between the susceptible landraces and FCT. Most FCT cultivars are highly susceptible to TMV, for example the positive control in the experiment KE1 with an incidence and severity of 100%, hence the gene of resistance is absent in FCT as in the susceptible landraces similar to commercial *N. tabacum.*

5.2 PVY SEVERITY

The significant differences on PVY severity of the local tobacco landraces may be due to the fact that, the inoculum did not constitute pure strains of the virus. Rather the inoculum was

prepared from random leaves of PVY infected late plants, thus there is high likelihood that the inoculum had several different strains contained in it. The occurrence of both mosaic and necrotic symptoms on the infected landraces proves this point. However differences in genetic constitution of the landraces had significant role to play in the manifestation of these symptoms for instance treatments such as Chikwarimba 5; Chinyoka; W171 and the control KE1 were the only ones that exhibited necrosis.

In *Nicotiana spp.,* the manifestation of necrotic symptoms may also be also be associated with nutrient deficiency especially magnesium deficiency, (Tsedaley, 2015). This may also further explain the occurrence of necrosis in the three landraces and the control. The magnesium concentration may have been so low as to promote exhibition of necrotic symptoms. The results of the study however show that most of the landraces are susceptible to PVY^{O}, since a significant proportion of them displayed mottling and mosaic symptoms, caused by this particular strain on the tobacco crop.

For those four which did not show any symptoms and exhibited signs of immunity, the type of resistance might be that of a single recessive gene known as the *va* gene, which was a result of a deletion of the *Va* locus that determines susceptibility to PVY. There are three allelic forms of the *va* gene, namely va^0, va^1 and va^2, with va^0 being the most effective allele, (Czubacka and Doroszewska, 2014). These are the alleles responsible for the different mechanisms of protection, one for restriction of the intercellular movement of a virus and the other to reduce the accumulation of the virus in a single cell.

Furthermore it has been noted that in cultivars where a very high concentration of cytokinines (CK) is present, the resistance to PVY is more pronounced and the symptoms are repressed, (Synkova *et al,* 2006). In a study by Synkova *et al* (2006), there was a correlation between symptom development and virus protein content found in the infected plants. In plants where no symptoms were observed, very low concentrations of viral proteins had accumulated in the plants. The increase in the cytokinines is due to the increase in inactive (*N. glucosides*) or storage forms of CK. The rationale for this is that for virus replication to take place, the levels of active CK must first be reduced. Hence if the CK are in excessive supply in the post inoculation phase, the virus's rate of replication is highly inhibited, thus no symptoms of the virus are exhibited on the plants.

5.3 EVALUATION FOR ROOTKNOT NEMATODES RESISTANCE

The landrace Bhabhane had a very interesting result scoring slightly above 1 on the Dalton and Nasbaum scale which means that there was trace infection and there were about 5 galls per plant root. The fact that Bhabhane also displayed no symptoms when assessed for PVY, may confirm that it possesses the *Mi 1.2* gene which confers resistance to rootknot nematodes (*M. javanica*), potato aphid (*Macrosiphum euphorbiae*) and also two biotypes of whitefly (*Bemisia tabaci*), (Molinari, 2010). Studies by (Fellers *et al,* 2002) revealed that N. tabacum houses gene which provides resistance to rootknot nematodes which is also tightly linked or pleiotropic with a veinal necrosis systemic hypersensitive response to infection by PVY.

Mi mediated resistance is expressed as hypersensitive reaction causing rapid and localised cell death, which can be observed 12 hours after inoculation of the roots with J2 juveniles as they will be attempting to establish feeding sites. It seems that *Mi* resistance is mediated by a salicylic acid (SA) dependent defence pathway (Atamian *et al,* 2011). When the roots are attacked by the nematodes, overproduction of (SA) is stimulated leading to an impaired mitochondrial phosphorylation efficiency, hence the necrosis of those cells involved in hypersensitive reaction.

Most of the tobacco landraces inoculated with rootknot nematodes (*M. javanica*) exhibited moderate degree of galling because 23 of the 30 landraces had a rating of 4 out of 8 and an index value of 25. This shows that a huge proportion of the tobacco landraces has moderate resistance to *M. javanica*. It may also be due to the nematode races being les virulent. In Nematology the virulent populations are characterised by their ability to reproduce significantly on the resistant host plants, hence suppressing or preventing reproduction of avirulent populations of the same species. The performance of the nematodes on the resistant landraces Bhabhane, Unknown 2 and Fodya 1 which had gall scores below 2, shows that this particular population of nematodes is less virulent.

The attack of the nematodes varies with environmental conditions. In agronomic systems, the nematodes are heterogeneous for virulence factors, such that if the population lacks virulent individuals selection does not take place. On the other hand in greenhouses and labs selection can be a protracted adaptation which involves genome rearrangements of the progeny of the avirulent individuals under selection.

Not only do plants make use of physical barriers and chemical defences but they also utilise inducible immune responses which are regulated by complex signalling networks essentially

at the transcriptional level. Transcription factors therefore play a vital role in the regulation of temporal and spatial expression patterns of genes that are involved in plant defence responses.

There are two categories of immune receptors which trigger defence associated transcriptional reprogramming in plants. First there is PAMP, (pathogen associated molecular patterns) triggered immunity, (PTI) whereby recognition of PAMP chemical signatures that seem to be widely conserved among certain pathogen clads are mediated by pattern recognition receptors (PRRs). Generally plants respond to the PAMP perception with a basal defence, (weakened immune reaction), which limits the growth and spread of the pathogen. The second category of plant immune receptors is known as disease resistance (R) proteins, which are able to reorganise pathogen effectors thus activating effector triggered immunity (ETI). This a strong immune response that results in incompatible plant-pathogen or pest interaction. ETI is triggered by a pair of complementary host resistant genes avirulence conferring effector genes, (Atamian *et al*, 2011).

Several studies have revealed that PTI based defence and ETI utilise related signalling pathways involving the defence hormones, salicylic acid (SA) and jasmonic acid (JA). Transcription factors (TFs) control plant immune responses by way of regulating JA signalling pathway for example in tomato (*Solanum lycopersicum*), the TFs SlWRKY72a and SlWRKY72b were shown to be involved in *Mi 1* mediated resistance as well as basal defences against potato aphid and rootknot nematodes, (Atamian *et al*, 2011). In another species of tobacco, N. attenuata, the TFs NaWRKY3 and NaWRKY6 were shown to be required for resistance against larvae of the tobacco hornworm (*Menduca sexta*). Silencing of the WRKY TFs through stable transformation resulted in an impaired JA accumulation confirming that the TFs control plant immune responses by regulating the JA signalling pathway.

The chief aim of coming up with nematode resistant varieties is to protect the yield potential of the crop by measurable restriction of nematode pest production. Therefore the 3 landraces Bhabhane; Unknown 2 and Fodya 1 have useful potential as sources of resistance to rootknot nematodes and may be integrated in subsequent nematode assessment trials. Introgression of the resistance genes into FCT has been made easier by the advancement of techniques such as embryo culture; protoplast fusion, somatic hybridisation which circumvent incompatibilities that rise as a result of interspecific hybridisation. This also reduces the time taken for the

introgression of the resistance genes into a variety of interest, unlike using the classical breeding route. This increases the feasibility of coming up with novel varieties that possess resistance to rootknot nematodes.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSION

Eighteen of the landraces had a hypersensitive response type of resistance to TMV. Four of the landraces namely Bhabhane, Cholokotwani; Nalukotwani 1 and Nalukotwani 2 were immune to PVY. The dominant strain of PVY affecting the landraces was the mosaic inducing strain PVY^{O}. Three landraces had a gall score below 2 on the Dalton and Nasbaum scale (1960). The landrace Bhabhane had an outstanding performance, for it proved resistant to all the diseases tested for in the trial. It therefore exhibited multiple disease resistance.

6.2 RECOMMENDATIONS

The landrace Bhabhane and other landraces which proved considerable resistance to any of the diseases for example Cholokotwani and Nalukotwani 1 against PVY; Unknown 2, Cholokotwani and Fodya 1 against rootknot nematodes and Kalyatamvuvi and W36 against TMV, to be crossed with FCT and the F1 generation tested for disease resistance.

The study to be carried out in the field as an agronomic trial. The pathogens, for example nematodes tend to be more virulent under field conditions. This is mainly due to the heterogeneous populations found in the field. Growing the landraces which exhibited resistance to the diseases would allow an opportunity to evaluate their aggregate performance in the field. Moreover tobacco is a field crop, hence it is necessary to evaluate the behaviour of the landraces under field conditions.

Molecular genetic characterisation has to be performed on the landraces in future. Phenotypic evaluation and characterisation has limitations regarding knowledge of the specific genes responsible for resistance and the mechanisms and the pathways they influence. Molecular characterisation will reveal information about these genes and present an opportunity for their maximum exploitation.

REFERENCES

Acquaah, G., (2012), Principles of Plant Genetics and Plant Breeding, Second Edition, Wiley-Blackwell, U.S.A.

Adebola K., 2008, Genetic Erosion: Landraces to the Rescue; University of Nigeria, Nigeria.

Anderson J. 1997, Landraces A Source of Genetic Diversity: *Cucumis sativa*, University of Kentucky, U.S.A.

Atamian, H.S., Eulgem, T., Kaloshian, I., (2011), *SlWRKY70* required for *Mi* mediated resistance to aphids and nematodes, Springer-Verlag, Berlin.

Bagley, C., (2001), Controlling Tobacco Mosaic Virus in Tobacco through Resistance, Virginia Polytechnic and State University.

Barre, R.S., Rajalingam, G., Bethi N., Palaniappan, G., (2013), Search for Resistance to infecting Rootknot nematode (*Meloidogyne incognita*, Race 2), infecting Sunflower (*Helianthius anuus*), Journal of Microbiology and Biotechnology Research, vol. 4, 59-62

Beekwilder, K.M., (1999), The Inheritance of Resitance to Tobacco Mosaic Virus in Tobacco Introductions, Virginia Polytechnic Institute and State University, U.S.A.

Czembor, J.H. and Czembor H.J., (2000), Powdery Mildew Resistance in Barley Landraces from Morocco, Journal of Phytopathology, vol. **148,** 277-288, Berlin.

Czubacka, A., Doroszewska, T., (2014), Effectiveness of combining *va* mediated resistance with a lettuce mosaic coat protein gene and a potato virus Y polymerase RNA gene and a Potato Virus Y gene in protection of tobacco hybrids from Potato Virus Y, Journal of Food, Agriculture and Environment, vol.13, 36-42, Poland.

Dhandaydham B., Prevish T. and Singh J., 2008, The Effects of Population Density on the Reproductive Factor of *Meloidogyne javanica* inoculated on the peanut crop, Journal of Microbiology and Biotechnology Research, vol. 7, 35-52.

Doroszewska, T. and Kruvisazky P., 2010, Charaterization of necrotic strains of Potato Virus Y strains in tobacco, Journal of Food, Agriculture and Environment, vol.21, 43-67, Poland.

FAO, 2010, STATISTICAL SURVEY ON TOBACCO PRODUCTION, U.S.A.

FACTSHEET, 2008, Trends in Tobacco Production, Turkey.

Fellers, J.P., Trembley, D., Handest, M.F. and Lommel, S.A., (2002), The Potato Virus Y M(S)N(R) Nlb-replicase is the elicitor of a veinal necrosis hypersensitive response in rootknot nematodes, Molecular Plant Pathology, U.S.A.

Fourie, H., McDonald, A.C. and Waele, D.D., (2010), Relationships between initial population densities of *Meloidogyne incognita* race 2 and nematode population development in terms of variable soybean resistance, Journal of Nematology, vol. 42, 55-61, South Africa.

Mazarire, M., Gasura, E. and Garwe, D., (2013), Potential of marker assisted selection for Tobacco mosaic tobamovirus resistance in tobacco breeding, African Journal of Biotechnology, vol. 12, 3783-3789, Zimbabwe.

Molinari, S. (2010), Natural genetic and plant resistance, as a control strategy to plant-parasitic nematodes alternative to pesticides, Springer-Verlag, Italy.

Moyo, M., (2012), Effectiveness of a contract farming arrangement: A case study of tobacco farmers in Mazowe district in Zimbabwe, Stellenbosch University, South Africa.

Mugandani, R., Wuta, M., Makarau, A. and Chipindu, B., (2012), RE-CLASSIFICATION OF AGRO-ECOLOGICAL REGIONS OF ZIMBABWE WITH CONFORMITY CLIMATE VARIABILITY AND CHANGE, African Crop Science Journal, vol. 20, 361-369, Zimbabwe.

Mwareya R, 2014, Tobacco production trends in Zimbabwe, Stellenbosch University, South Africa

Nasbaum and Dalton, 1960, Gall classification scale, North Carolina University, U.S.A.

Nie, X. and Molen, T. A., (2015), Host recovery and reduced virus level in the upper leaves after Potato Virus Y infection occurs in tobacco and tomato but not in potato plants, *Viruses*, vol. 7, 680-698, Canada.

Nifong, J.M., (2008), Assessment of the Diversity and Resistance Properties within a collection of *Nicotiana rustica*, North Carolina State University, U.S.A.

Nyamwanza, T., Mapetere, D., Mavhiki, S., Nyamwanza, L., and Tumai, K., (2014), COTTON INDUSTRY'S STRATEGIC RESPONSES TO SIDE MARKETING OF

COTTON BY CONTRACT FARMERS IN ZIMBABWE, European Journal of Business and Economics and Accountancy, Vol. 2, Progressive Academic Publishing U.K., Zimbabwe.

PROTEIN DATA BANK, (2009), MOLECULE OF THE MONTH: TOBACCO MOSAIC VIRUS, www.pdb.org, accessed (November 10 2015).

Reed, D., Peek, D.R., Johnson C.S., Semtner, P.J., Wilkinson, C.A., (2012), BURLEY TOBACCO PRODUCTION GUIDE, Virginia State University, U.S.A.

Scholthof, K.B.G., (2008), Tobacco Mosaic Virus: The Beginning of Plant Virology, Texas University, U.S.A.

Seah, S.J., Yaghoobi, J., Rossi, M. and Williamson V., (2004), The nematode resistance gene, *Mi 1*, is associated with an inverted chromosomal segment in susceptible compared to resistant tomato, University of California, U.S.A.

Synkova, H., Semoradova, S., Schnablova, R., Muller, K., Posilova, J., Ryslava, H., Malbeck, J. and Cerovska, N., (2006), Effects of Biotic stress caused by Potato Virus Y on Photosynthesis in *ipt* transgenic and control tobacco (*Nicotiana tabacum L.*), Science Direct, Czech Republic.

Tobacco Industries and Marketing Board, 2014, Annual Statistical Report, TIMB, Zimbabwe.

Tsedaley, B., (2015), A Review Paper on Potato Virus Y (PVY) Biology, Economic Importance and its Management, Journal of Biology, Agriculture and Healthcare, vol. 5, 2015, Ethiopia.

Van de Merwe, F., (2012), Tobacco farming- the growers' perspective, Farmer's Weekly, 52-54, South Africa.

Appendices

Appendix 1 Analysis of Variance for TMV Incidence

Variate: TMV_INCIDENCE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	8.60	4.30	0.14	
REP.*Units* stratum					
TREATMENTS	30	179690.32	5989.68	193.42	<.001
Residual	60	1858.06	30.97		
Total	92	181556.99			

Appendix 2 Analysis of Variance for PVY Severity

Variate: DISEASE_SEVERITY

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.000286	0.000143	0.09	
REP.*Units* stratum					
TREATMENTS	30	11.880929	0.396031	240.07	<.001
Residual	60	0.098981	0.001650		
Total	92	11.980196			

Appendix 3 Analysis of Varaiance for Rootknot Nematodes Gall Scoring

Variate: GALL_SCORES

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.03563	0.01781	0.37	
REP.*Units* stratum					
TREATMENTS	30	9.53506	0.31784	6.63	<.001
Residual	60	2.87782	0.04796		
Total	92	12.44851			