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Incorporation of root-knot nematode (*Meloidogyne javanica* (Treub) Chitwood) resistance in the Zimbabwean elite Virginia tobacco (*Nicotiana tabacum* L.) germplasm: A review

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ABSTRACT

Virginia tobacco (*Nicotiana tabacum* L.) is currently the most popular field cash crop grown in Zimbabwe. The farming of Virginia tobacco in Zimbabwe started during the late 19th Century with introduced varieties which succumbed to most of the locally occurring pests and diseases. One major pest of tobacco that threatens the success of growing the crop has been the root-knot nematode [*Meloidogyne javanica* (Treub) Chitwood]. The pest attacks the root system of the crop from as early as the seedbed stage to such an extent that yield losses of above 90% can be realised if the nematode is not controlled. In Zimbabwe, the threat posed by the pest in the success of the tobacco farming industry was realised as early as the 1950s and one of the approaches used to contain it was breeding for resistance into the local tobacco germplasm among other methods such as chemical control and cultural practices like crop rotation. This paper is a review of the process that was followed in incorporating root-knot nematode resistance in flue-cured tobacco germplasm in Zimbabwe. It shows the sources/origin of the root-knot nematode (*M. javanica*) resistance currently present in the Zimbabwean Virginia tobacco (*N. tabacum*) germplasm and the methods used in transferring the resistance to elite germplasm currently used in modern breeding programmes. The current study also suggests the way forward for the future breeding research for root-knot nematode (*M. javanica*) resistance in continuity of the past 50 years of breeding effort for resistance to the pest.

Key words: Chemical control, Germplasm, Resistance, Root-knot nematode, Varieties, Virginia tobacco.

INTRODUCTION

The root-knot nematodes (*Meloidogyne* spp.), also known as eelworm, are a major pest of tobacco and other solanaceous plants in many parts of the world where the crops are grown (Onkendi *et al*., 2014). The fact that root-knot nematodes have many alternative hosts has made it very difficult to effectively deal with them in the early stages of its discovery (Gildenhuys and Dalton, 1952). Root-knot nematodes have been reported to negatively affect many crops across the world such as eggplant, tomato, potato and tobacco. *Meloidogyne* spp. is widely distributed in many parts of the world where tobacco is grown but it has been shown that the species occurring differ in each geographical area. Early studies have confirmed that there are different species of root-knot nematodes occurring in different parts of the world (Onkendi *et al*., 2014). For instance, the most prevalent root-knot nematode species in the United States of America (USA) is the *Meloidogyne incognita* (Kofoid & White) Chitwood while in Zimbabwe the prevalent species is *Melodogyne javanica* whereas in the other parts of the world, other species like *Meloidogyne arenaria* (Neal) Chitwood are more prevalent (Slana *et al*., 1976).

The early strategies aimed at controlling the pest in tobacco relied mainly on chemical control and the use of alternative hosts to the pest in crop rotation programmes (Ng'ambi *et al*., 1999). However, the cost of chemicals, land limitations which could not permit long rotations and the increasing pressure from environmentalists on the need for reduction in the use of chemicals in the environment led researchers to develop alternative approaches to deal with the root-knot nematode problem (Gildenhuys and Daulton, 1952; Selvaraj *et al*., 2014). Like in any crop production system, the use of integrated pest management including incorporating host resistance is encouraged (Thakur *et al*., 2012; Gnanavel and Natarajan, 2014). The search for tobacco germplasm resistant to *Meloidogyne* species showed that it was possible to develop root-knot nematode resistant tobacco through plant breeding efforts.

In most cases it was found that germplasm resistance was specific to a root-knot nematode species. For

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Fig 1: Root-knot nematode (*M. javanica*) resistant (A) and root-knot nematode susceptible (B) varieties planted side by side in the same unfumigated land. Root-knot nematode attack can reduce yield by 100% in extreme cases.

instance, germplasm resistant to *M. javanica* could not be resistant to *M. incognita* and *vice versa*. There were, however, instances where cross resistance was reported. Root-knot nematode resistance breeding in Zimbabwe relied mainly on the work done by researchers in the United States of America. A researcher who worked for the US Department of Agriculture by the name E. E. Clayton is credited with initiating the root-knot nematode resistance breeding work during the early 1930s (Slana *et al*., 1976). Except in a few cases, most of the germplasm used as sources or resistance came from the USA or other parts of the world through the USA after being handled by Clayton or his co-workers. There are, however, few cases where local landraces showed promise and were incorporated in the early schemes.

The process of coming up with root-knot nematode resistance in Zimbabwean Virginia tobacco took a long period that spanned over 50 years. This is the journey that resulted in the current possibility by Zimbabwean tobacco growers to plant a crop on unfumigated lands and still get a decent crop yield.

Constitution of the initial root-knot nematode (*M. Javanica*) breeding populations in the 1940s

Crosses which formed the foundation of breeding for root-knot nematode (*M. javanica*) resistance in flue-cured tobacco in Zimbabwe were made during the early 1940's. Selections for root-knot nematode resistance from the early crosses started during the same time but most of the evaluation of the selected plants that resulted in meaningful gains was done by Gildenhuys and Daulton during the 1951-52 season (Gildenhuys and Daulton, 1952). Of note during this season's line evaluations was the inclusion of a promising primitive local line, W37, obtained from the Ngorima Area near Chimanimani (Gildenhuys and Daulton, 1952). These early results of studies involving *N. tabacum* selections showed that there was no resistance at all among the cultivated species of tobacco in Zimbabwe. It was also discovered that root-knot nematode resistance was controlled by several different genes in the tobacco germplasm under study (Gildenhuys and Daulton, 1952). Only some wild species of *Nicotiana* like *Nicotiana longiflora* Cavanilles selection W83 showed low infection indices to *M. javanica*. Linkage of some root-knot nematode resistance major genes to undesirable growth traits like small leaf size and the dark green colour was also reported (Gilgenhuys and Daulton, 1952).

Variety studies on resistance to root-knot nematodes during the 1950s reported two types of root-knot nematode resistance. The first functioned through resistance to invasion by the root-knot nematodes and was found in *Nicotiana repanda* Willdenow ex Lehmann while the second resistance functioned by unsuitability of a variety to acting as a host as in *N. longiflora* (Gildenhuys and Daulton, 1952). In the second type of resistance, root galling occurs but there is non-completion of the life cycle by the pathogen (Raeber and Schwappenhauser, 1964). Further work on breeding for root-knot nematode resistance in Zimbabwe became focussed on introgressing these two forms of resistance into elite cultivated flue cured tobacco (*N. tabacum*).

Inter-specific crosses involving *N. repanda* and *N. longiflora* were therefore made and selections with high root-knot nematode (*M. javanica*) resistance were identified (Raeber and Schweppenhauser, 1964). Even though initial studies had ruled out *N. tabacum* as a potential source of resistance to root-knot nematodes, local line and variety introduction screening for resistance to the pest continued through the 1950s until in another test involving the *N. tabacum* type breeding lines, a line coded R392 showed very high resistance to the nematode in the seedbed and the field (Raeber and Schweppenhauser, 1966). The other promising lines from the *N. tabacum* breeding program were R392, R643, R670, R723, R727 and R247 (Raeber and Schweppenhauser, 1966; Raeber and Schweppenhauser, 1968b).

Since *N. longiflora* and *N. repanda* had proved to be the most promising potential sources of root-knot nematode resistance, breeding programmes in these two species continued (Raeber and Schweppenhauser, 1966). This led to the isolation of *N. longiflora* lines with a very high degree of *M. javanica* resistance. In this population, resistance was higher in selfed generations than the back cross generations leading to suggestions that the resistance was monofactorial. This higher level of resistance appeared to be controlled by modifier genes as well. *N. repanda* derived lines with resistance or immunity to *M. javanica* were also identified from a greenhouse program (Raeber and Schweppenhauser, 1968a).

The *N. tabacum* derived root-knot nematode resistant lines RK1 and RK2 with narrow leaves were first tested for agronomic performance during the 1970-71 farming season with other selections from the same programme. Most of the lines evaluated showed that they needed further improvement (Raeber and Smeeton, 1971).

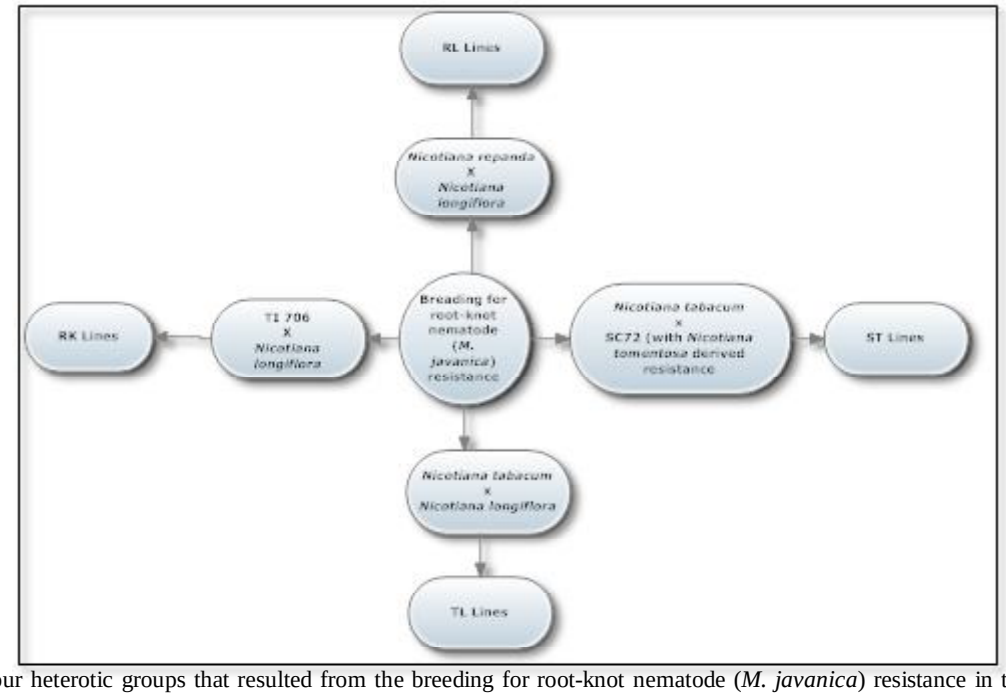


Fig 2: The four heterotic groups that resulted from the breeding for root-knot nematode (*M. javanica*) resistance in the Zimbabwean flue-cured tobacco (*N. tabacum*).

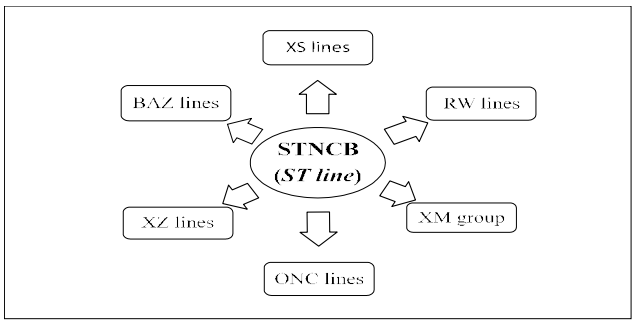


Fig 3: Breeding lines to which the ST root-knot nematode (*Meloidogyne javanica*) resistance was transferred through the line STNCB.

From the *N. tabacum* root-knot nematode resistance breeding programme, selection 4-1-1 showed promise while selection 185-1-1 and 188-1-1 were the most promising from the *N. repanda* breeding programme (Raeber and Smeeton, 1973a).

Creating well defined heterotic groups for resistance to root-knot nematodes (*M. Javanica*)

After the discovery that resistance to root-knot nematode (*M. javanica*) could be obtained from the three species, N. *tabacum*, *N. repanda*, and *N. longiflora*, selections were done from crosses involving the three species. Some root-knot nematode resistance also came from *Nicotiana tomentosa* Goodspeed through the American variety SC72. Lines developed from the crosses involving *N. repanda* and *N. longiflora* were coded RL lines and from this cross the lines RL-1-2-1 and RL-2-1-1 showed very high levels of root-knot nematode resistance (Raeber and Smeeton, 1973b). RL2-1-1 was also confirmed to be the line with the best root-knot nematode resistance during the year 1984 (Raeber and Smeeton, 1980b; Raeber and Smeeton, 1984b). The only potential limitation to these lines is that the L gene from *N. longiflora* is associated with Angular leaf spot susceptibility.

There are lines with root-knot nematode resistance that also came from the cross involving *N. tabacum* and a line called SC72 (Fig 2). SC72 is an American variety with high levels of *M. incognita* resistance from *N. tomentosa* which was found to impart some resistance to *M. javanica*. The lines from this cross were coded ST lines and the best lines from this cross were ST1-2-8 and ST3-2-6 (Raeber and Smeeton, 1980a). The ST lines originate from the cross SC72 × RKT15-1-1. The line RKT15-1-1 was locally bred from the SC72 and has shown better resistance to *M. javanica* than SC72 (Raeber and Smeeton, 1984a). The root-knot nematode resistant ST lines were improved on their White Mould resistance and quality by crossing them to NC89 and TB22. TB22 was chosen because it possesses dominant resistance to White Mould while NC89 is renowned for its good quality and possession of an S gene for resistance to root-knot nematodes. This scheme gave rise to the popular root-knot nematode resistant lines commonly known as STNCA and STNCB in the flue-cured tobacco breeding industry in Zimbabwe (Fig 3). To be specific the best lines from these crosses were STNCA-1, STNCA-3, STNCA -5, STNCA-14, STNCB-2, and STNCB-5 (Raeber and Smeeton, 1980b). *N. tabacum* was also crossed to *N. longiflora* and the lines derived were coded TL lines. The best line from this inter-specific hybrid's segregants is TL-13-2 (Raeber and Smeeton, 1984b).

A cross involving a line from the USA called TI706 and *N. longiflora* was also constituted and segregating lines selected. TI706 is a line that was discovered by Clayton in the US to possess resistance to *M. incognita* during his pioneering work on breeding for resistance to root-knot nematode resistance in tobacco. From the segregants a line called RKA-4-1-1 was rated the best for root-knot nematode resistance and stored as V641 in the TRB accession register (Raeber and Smeeton, 1973b). Resistance to root-knot nematodes was combined with Alternaria leaf spot resistance through a cross of 22S2-48-9 to lines coded ARK lines.

Exploiting incorporated root-knot nematode resistance in elite flue-cured tobacco variety development

After the development of the major heterotic groups for resistance to root-knot nematodes, subsequent work involved the use of the developed lines in the improvement of elite Virginia tobacco lines. This work to incorporate root-knot nematode resistance gained through earlier on breeding efforts started in the early 1990s through the coordination of Anne Jack. The period saw the reconstitution of populations that gave different cured leaf styles but possessing root-knot nematode resistance (Du Toit *et al*., 2000). Only the ST type of root-knot nematode resistance was, however, utilised (Fig 3) in the development of the most commonly used populations in Zimbabwe namely, XS, XM, XZ, BAZ, RW and the ONC population (Du Toit *et al*., 1999). The other three heterotic groups have not been exploited yet.

CONCLUSION AND RECOMMENDATIONS

Breeding for root-knot nematode (*M. javanica*) resistance in flue-cured tobacco (*N. tabacum*) in Zimbabwe spanned a period of 50 years before resistant varieties became available to growers. The early grown varieties were all susceptible to root-knot nematodes. The present root-knot nematode resistance in the Zimbabwean tobacco germplasm was introgressed from N. *repanda, N. longiflora* and *N. tomentosa* through recurrent breeding. There is evidence that some selections from *N. tabacum* also contributed to the present root-knot nematode resistance but to a lesser extent. All the root-knot nematode resistance breeding work that spanned over 50 years resulted in the development of four groups of lines with varying but high root-knot nematode resistance coded TL, RL, ST and the ARK lines. Of these four groups, only the ST group (especially STNCB) has been exploited in the development of the flue-cured tobacco varieties and the other groups have been shelved. A lot of inter-crosses among the lines possessing the ST root-knot nematode resistance have been done resulting in potential loss of useful variation for future exploitation. Variation comes from the use of distantly related line crossing. It is, therefore, recommended that future breeding programmes in Zimbabwe use the other three groups of root-knot nematode r`esistance in order to reap the benefits of wide variability and the possibility of coming up with superior lines and varieties. It looks like the present elite germplasm has reached a plateau in terms of root-knot nematode resistance potential.

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