Characterization of Plant-Parasitic Nematodes and Host Resistance in Rice Production in Tanzania

Karakterisatie van Plantenparasitaire Nematoden en Gastheerresistentie in Rijstproductie in Tanzania

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TO GOD IS THE GLORY!

Yasinta Beda Nzogela

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LIST OF ABBREVIATIONS

AIC	Akaike Information Criterion
ANOVA	Analysis Of Variance
ARCA	Africa Rice Centre
ARI	Agricultural Research Institute
ARICA	Rice Varieties for Africa
BCT	Basic Cultivation Technique
BI	Bayesian inference
RB	Rice Blast
BLB	Bacterial Leaf Blight
CARI	Competitive African Rice Initiative
CIMMYT	Centro Internacional de Mejoramiento de Maíz y Trigo
CN	Cyst Nematodes
COI	Cytochrome C oxidase subunit
DAMPs	Damage Associated Molecular Patterns
DDA	Data-Dependent MS/MS Analysis
DGO	Dorsal pharyngeal Gland Orifice
DNA	Deoxyribonucleic Acid
DPBA	Diphenylboric acid 2-aminoethyl ester
DPI	Days Post Inoculation
DTT	Dithiothreitol
EAC	East African Community
EDTA	Ethylene diaminetetraacetic acid
EPPO	European and Mediterranean Plant Protection Organization
ESI	ElectroSpray Ionization
ET	Ethylene
ETI	Effector-Triggered Immunity
ETS	Effector-Triggered Susceptibility
FAO	Food and Agriculture Organisation
GAP	Good Agricultural Practices
GC-MS	Gas chromatography-mass spectrometry
GDP	Gross Domestic Product
GTR+G	General Time reversible
HPLC	High-Performance Liquid Chromatography
HR	Hypersensitive Reaction

IAEA	International Atomic Energy Agency
IITA	International Institute of Tropical Agriculture
INRAE	Institut national de recherche pour l'agriculture, l'alimentation et Environnement
IPM	Integrated Pest Management
IRRI	International Rice Research Institute
ITS	Internal Transcribed Spacer
JA	Jasmonic Acid
KATRIN	Kilombero Agricultural Training Research Institute
KPM	Khao Pahk Maw
KULeuven	Katholieke Universiteit Leuven
LGC	Laboratory of the Government Chemist
LSD	Least Significant Difference
MAPKs	Mitogen-Activated Protein Kinases
MI	Meloidogyne Incognita
MIG	Meloidogyne incognita group
MN	Mwangaza with Nematodes
MONA	Mass Bank of North America
MS	Mass Spectrometer
NADH	Dehydrogenase subunit
NERICA	New Rice of Africa
NPK	Nitrogen, Phosphorus, Potassium
PRR	Pattern Recognition Receptors
P R	Partially resistant
PTI	Pattern-Triggered Immunity
PAL	Phenylalanine Ammonia Lyase
PAMPs	Pathogen-Associated Molecular Patterns
PCA	Principal Component Analysis
PCN	Potato Cyst Nematode
PCR	Polymerase Chain Reaction
PPN	Plant Parasitic Nematodes
PR	Pathogen Related
PVC	Polyvinyl Chloride
PVPP	Poly-VinylPolyPyrrolidone
QC	Quality Control
QTL	Quantitative Trait Loci

RCB	Randomized Complete Block
RCE	Root Crude Extracts
RF	Reproduction factor
RKN	Root-Knot Nematodes
RLK	Receptor-Like Kinases
RLN	Root Lesion Nematodes
RLP	Receptor-Like Protein
RNA	Ribonucleic acid
ROS	Reactive Oxygen Species
RT	Retention Time
RYMV	Rice Yellow Mottle Viruses
SA	Salicylic Acid
SAP	Sand Absorbent Polymers
SAR	Systemic Acquired Resistance
SAS	Statistical Analytical System
SCAR	Sequence Characterized Amplified Region
SE	Standard Error
SIR	System of Rice Intensification
SN	Supa with Nematodes
SSA	Sub-Saharan Africa
SSR	Simple Sequence Repeat
TAE	Tris-Acetate-EDTA
TARI	Tanzania Agricultural Research Institute
UK	United Kingdom
UP	Upland
UPLC	Ultra Performance Liquid Chromatography
URT	United Republic of Tanzania
USA	United States of America
USDA	United States Development Agency
VIB	Vlaams Instituut Voor Biotechnologie
WARDA	West-Africa Rice Development Association

SUMMARY

Rice is a crop that feeds the entire world, and for many years to come, it will remain the most wanted cereal globally. The role of agricultural research in development should be centred on sustainable food availability and access for people's livelihood security. Tanzanian population growth rate is very high, reaching 3% annually. Rice consumption in Tanzania has increased dramatically due to the growing population. Its increased demand creates a big quest for farmers to increase their productivity. Government agricultural policies have been centred on increased rice productivity by intensification at the given unit of land. Rice productivity is hampered by, among other factors, plant-parasitic nematodes (PPN). The most important species being root-knot nematode (RKN) (*Meloidogyne* species) and root-lesion nematodes (RLN) (*Pratylenchus species*). Management of these nematodes is challenging and demands a species -specific strategy. For developing countries like Tanzania, the most reliable way of nematode management must be relatively cheap and easy to apply. In that sense, host resistance would be farmers' best choice to manage nematode problems if the developed variety would be relatively cheap and easy to apply. However, identification of resistant cultivars for a diversity of nematode problems is a big challenge. This study aimed to characterize the nematode problems and associated rice resistance in different agroecosystems in Tanzania. The first task was to analyze the nematode problems. A diagnostic survey of rice from upland, lowland and irrigated fields was conducted, and a total of 190 soil and root samples were analyzed. PPN were extracted from soil and roots using a modified Baermann funnel technique. Nematodes were morphologically identified to the genus level, and the most prevalent genera *Pratylenchus* and *Meloidogyne* were identified to species level using both morphological and molecular methods. For *Pratylenchus*, D2D3 expansion segments of the 28S gene were amplified, and the obtained sequences were compared with those of *Pratylenchus* species in the GenBank database. The

comparison confirmed the morphological identification and revealed a population of *P. zeae*. The study of the phylogenetic relationship of the Tanzanian *Pratylenchus zeae* populations showed a high similarity (99-100%) with other *P. zeae* populations. *M. arenaria* were identified by sequencing the *Nad5* gene and by PCR using species-specific Sequence Characterized Amplified Regions (SCAR) primers. The survey revealed that RLN *P. zeae* were a major nematode parasite of rice prevailing in 100% of the samples from all rice agroecosystems. The upland rice agroecosystem is more infested than lowland and irrigated fields.

Knowledge of natural resistance and the pathogenicity of the nematodes is fundamental to explore nematode management strategies further. The use of resistant varieties is one of the most effective methods to control nematodes. Finding such varieties constituted the second part of this thesis. Several rice genotypes from East Africa and West Africa belonging to *O. sativa* and *O. glaberrima* and their interspecific hybrids (NERICA) were evaluated for resistance against *P. zeae* and RKN (*M. graminicola* and *M. javanica*) in screenhouse experiments. The evaluation was based on the number of nematodes present in the roots and their respective developmental stages except for *P. zeae* that were based solely on the number of nematodes inside the roots. It was found that cultivar Supa was resistant to *P. zeae* and showed partial resistance to *M. javanica* and *M. graminicola*. Cultivar Komboka was partially resistant to both root-knot nematodes but susceptible to *P. zeae*. Among the hybrids, NERICA 5 and the *O. glaberrima* (TOG5674, TOG5675, CG11, and CG14) were resistant to *P. zeae.* The study on the pathogenicity of *P. zeae* was conducted on Supa and SARO-5 (TXD-306) genotypes infected with different initial nematode population densities (0, 200, 500, 1000, 3000, 10000 nematodes pot−1) under simulated conditions of upland, drought and flooded conditions. The experiment was under screenhouse conditions using pots of 5L in size, and the experiment was run for six months. It was evident that *P. zeae* was able to infect the rice under all tested conditions, and the resistance of Supa to *P. zeae* was confirmed. Yield loss in terms of number and grain weight, the

number of panicles, the number of tillers, and spikelets were evaluated. *P. zeae* caused the most considerable loss under drought conditions with about 100% loss in filled grains at the highest inoculum level. The yield loss increased with increasing nematode pressure for upland and flooded conditions. The rice yield losses due to *P. zeae* were minimal under flooded conditions, and nematodes reproduced more under low initial nematode population density (200).

The resistance mechanisms were in-depth investigated in both Supa and Komboka for RKN and Supa and Mwangaza for *P. zeae*. The investigation on the possible resistance mechanisms was done along the nematodes' life cycle, from attraction, penetration, development, and reproduction. It was revealed that Supa exhibits pre-infection resistance against *P. zeae*. At the same time, both pre- and post-infection resistant mechanisms were shown by Supa and Komboka against *M. graminicola* and *M. javanica.* Post-infection mechanisms of resistance were demonstrated by juvenile emigration from the roots. The identified resistance in Supa against *P. zeae* was temperature insensitive.

In the final part of this study, root extracts, and metabolites of resistant (Supa) and susceptible (Mwangaza) rice cultivars were analyzed. Root crude extracts from Supa and Mwangaza were assayed against *P. zeae* motility. The nematostatic effect of RCE was monitored every 12 hours for 72 hours of exposure to the RCE by counting numbers of motile and non-motile nematodes under a dissecting microscope. The contents in crushed root extract from Supa were able to inhibit *P. zeae* motility. These results confirmed the pre-infection resistance. The histochemical assay revealed that nematode infection increased lignification in both Supa and Mwangaza. Lignification was localized around the vascular system and progressed to the vascular parenchyma cells at the centre. There was no lignification at the cortex cells that might be directly involved in the defense to *P. zeae*. Increased lignification in both Supa and Mwangaza after nematode infection was probably only a general defense response to protect the vascular bundle to reduce damage to the plants. Flavonoids were stained

with 0.25%, w/v, diphenylboric acid 2-aminoethyl ester (DPBA), and were found to be present more in Supa infected with nematodes than Mwangaza infected and non-infected roots indicating their possible involvement in the observed resistance.

Untargeted UPLC-MS/MS metabolomics analysis was performed on both Supa and Mwangaza to determine the global metabolite changes in compatible and incompatible rice - *P. zeae* interactions. The identified metabolites did not allow conclusively pointing out those that are responsible for early-stage Supa resistance, but the presence of metabolites such as dihydro-p-coumaroyl hexose, p-coumaroyl hexose, feruloyl hexose, cis-p-hydroxycinnamic acid, and salicylic acid are good indicators that phenylpropanoids might be involved in Supa early resistance to *P. zeae*. Furthermore, constitutive Phenylalanine Ammonia Lyase (PAL) activity in Supa was high for all sampling time points and lowered for Mwanganza. Further exploration of the metabolite candidates responsible for Supa early resistance to *P. zeae* could be done by fractionating the root extracts to find the inhibitory compounds and then analyze only the fraction that affects nematode motility.

SAMENVATTING

Rijst is een gewas dat de ganse wereld voedt en het zal ook in de toekomst een van de meest populaire granen blijven. De rol van landbouwonderzoek voor ontwikkeling moet focussen op duurzame voedselbeschikbaarheid voor voedselzekerheid. De bevolkingsgroei in Tanzania is heel hoog met 3% aangroei per jaar. Mede door deze bevolkingsgroei is er een gestegen vraag naar rijst waardoor de boeren op zoek moeten naar een hogere productiviteit. Het landbouwbeleid van de overheid spitst zich toe op een hogere rijstproductie door intensificatie. Optimale rijstproductie wordt gehinderd door meerdere factoren waaronder ook plantenparasitaire nematoden. De belangrijkste species zijn de wortelknobbelnematode (*Meloidogyne* species) en de wortellesienematoden (*Pratylenchus* species). Controle van deze nematoden is uitdagend en vraagt species-specifieke strategieën. Voor landen in ontwikkeling zoals Tanzania moet de controle goedkoop en gemakkelijk te gebruiken zijn. Op dat vlak is plantenresistentie de beste keuze voor de boeren om nematoden onder controle te houden. Het is echter niet zo eenvoudig om resistente cultivars te identificeren voor de diverse nematodenproblemen. Deze studie heeft als doel om de nematoden-problemen te identificeren in de verschillende agro-ecosystemen in Tanzania en overeenkomstige rijstresistenties te vinden. De eerste taak was om de nematoden- problemen te identificeren. Een diagnostische survey werd uitgevoerd in hoogland, laagland en geïrrigeerde rijstvelden, met een totaal aan 190 bodem- en wortelstalen. Plantenparasitaire nematoden werden geëxtraheerd uit de stalen door gebruik te maken van de aangepaste “Baermann funnel” techniek. Nematoden werden morfologisch geïdentificeerd tot op genusniveau en de meest voorkomende genera *Pratylenchus* en *Meloidogyne* werden verder geïdentificeerd tot soortniveau door een combinatie van morfologische en moleculaire methoden. Voor *Pratylenchus* werd de D2D3 regio van het 28S rRNA-gen geamplificeerd, en de bekomen sequenties werden

vergeleken met deze van *Pratylenchus* species in GenBank. De vergelijking bevestigde de morfologische identificatie en wees uit dat het species *P. zeae* was. De studie van de fylogenetische relatie met de Tanzaniaanse *Pratylenchus zeae* populaties toonde een hoge similariteit (99-100%). *Meloidogyne arenaria* werd geïdentificeerd door sequentie-analyse van het *Nad5*-gen en via PCR met species-specifieke Sequence Characterized Amplified Regions (SCAR) primers. De survey toonde aan dat de wortellesienematode *P. zeae* een belangrijke rijstparasiet is met voorkomen in 100% van de stalen van alle rijst-agroecosystemen. Het hoogland-agroecosystem is meer besmet dan laagland of geïrrigeerde velden.

Kennis van natuurlijke resistentie en de pathogeniciteit van de nematoden is essentieel om nematodebeheersstrategieën te ontwikkelen. Het gebruik van resistente variëteiten is een van de meest effectieve methoden om nematoden te controleren. Het vinden van dergelijke variëteiten vormde het tweede deel van mijn thesis waarbij meerdere rijstgenotypes uit Oost- en West-Africa behorende tot *O. sativa* en *O. glaberrima* en hun hybrieden (NERICA) geëvalueerd zijn voor resistentie tegen *P. zeae, M. graminicola* en *M. javanica* in “screenhouse” experimenten. De evaluatie was gebaseerd op aantallen nematoden aanwezig in de wortels en voor wortelknobbel-nematoden ook de stadia. De cultivar Supa bleek resistent tegen *P. zeae* en partieel resistent tegen *M. javanica* en *M. graminicola*. Cultivar Komboka was partieel resistent tegen beide wortelknobbelnematoden maar was gevoelig voor *P. zeae*. Bij de hybriden was enkel NERICA 5 resistent tegen *P. zeae*, maar minder dan *O. glaberrima* (TOG5674, TOG5675, CG11, en CG14). De studie ivm de pathogeniciteit van *P. zeae* is uitgevoerd op Supa en SARO-5 (TXD-306) geïnfecteerd met verschillende initiële nematodeninocula (0, 200, 500, 1000, 3000, 10000 nematoden per pot) onder gesimuleerde condities van hoogland, droogte en bevloeide condities. Het experiment is uitgevoerd in “screenhouse” condities met potten van 5L inhoud, en liep gedurende 6 maanden. Het was duidelijk dat *P. zeae* in staat was om rijst te infecteren onder alle geteste condities, en de resistentie van Supa tegen *P.*

zeae kon bevestigd worden. Opbrengstverliezen in termen van aantal en gewicht van zaad, en aantallen pluimen, helmen en aartjes zijn geëvalueerd. *P. zeae* veroorzaakte de aanzienlijkste verliezen onder droogtecondities met ca. 100% verlies in zaadopbrengst bij het hoogste inoculum. Bij hoogland en bevloeide condities namen de opbrengstverliezen toe met stijgende infectiedruk. De opbrengstverliezen door *P. zeae* waren minimaal bij bevloeide condities, en nematoden reproduceerden meer bij de laagste inocula (200).

De geïdentificeerde resistentie tegen *P. zeae* en wortelknobbelnematoden werd dan nader onderzocht in Supa en Komboka voor wortelknobbelnematoden en Supa vs. Mwangaza voor *P. zeae*. Het onderzoek naar de mogelijke resistentiemechanismen werd uitgevoerd door analyse van de levenscyclus, nl. attractie, penetratie, ontwikkeling en reproductie. Supa vertoonde pre-infectieresistentie tegen *P. zeae* terwijl zowel pre-als post-infectieresistentie mechanismen gevonden werden bij Supa en Komboka tegen *M. graminicola* en *M. javanica.* Post-infectiemechanismen van resistentie bestonden o.a. ook uit juvenile emigratie uit de wortels. De geïdentificeerde resistentie in Supa tegen *P. zeae* was temperatuurongevoelig.

In het finale deel van deze thesis zijn wortelextracten en metabolieten van de resistente Supa vergeleken met de gevoelige Mwangaza. Het effect van wortelextracten van Supa en Mwangaza op de beweeglijkhheid van *P. zeae* werd bestudeerd. De wortelextracten van Supa inhibeerden de beweeglijkheid van *P. zeae*. Deze resultaten bevestigen de pre-infectieresistentie. De histochemische assay toonde aan dat nematodeninfectie de lignificatie verhoogde in wortels zowel bij Supa als Mwangaza. Lignificatie was gelocalizeerd rond het vasculair systeem en zette zich voort naar het vasculair parenchyma in het centrum. Lignificatie in de cortexcellen die zou kunnen wijzen op een direct effect als afweer tegen *P. zeae* werd niet gevonden. Verhoogde lignificatie na nematodeninfectie bij zowel Supa en Mwangaza is wellicht een algemene afweerrespons om het vasculair systeem te beschermen tegen schade.

Flavonoïden werden gekleurd met 0,25% w/v diphenylboric acid 2-aminoethyl ester (DPBA), en waren meer aanwezig in Supa geïnfecteerd met nematoden dan Mwangaza met of zonder infectie wat wijst op een mogelijke betrokkenheid bij de resistentie.

UPLC-MS/MS metaboloomanalyse werd uitgevoerd om Supa en Mwangaza te vergelijken en om de veranderingen na infectie met *P. zeae* te analyseren. De geïdentifieerde metabolieten konden niet toegewezen worden aan een hogere resistentie bij Supa, maar de aanwezigheid van dihydro-p-coumaroyl hexose, p-coumaroyl hexose, feruloyl hexose, cis-p-hydroxycinnamic acid, en salicylzuur is een goede aanwijzing dat fenylpropanoïden mogelijks betrokken zijn bij de vroege resistentierespons tegen *P. zeae*. Bovendien konden we een hogere constitutieve PAL-activiteit meten in Supa in vergelijking met Mwanganza. Verder onderzoek van de metaboliet-kandidaten die mogelijks verantwoordelijk zijn voor de resistentie van tegen *P. zeae* zou kunnen gebeuren door fractionatie van de wortelextracten om de inhibitorische componenten te vinden en dan verder onderzoek te focussen op de fractie die de nematodenmobiliteit inhibeert.

CHAPTER 1: PROBLEM STATEMENT, OBJECTIVES AND OUTLINE OF THE THESIS

1.1 PLANT-PARASITIC NEMATODES, A HIDDEN ENEMY TO RICE PRODUCTIVITY

In the developing world, rice is an essential food crop, estimated to feed more than half of the world's population (Chauhan et al., 2017; Seck et al., 2012). It is the primary source of daily calories of about 1/3 of the population (Seck et al., 2012). World-wide rice demand is very high (Van Nguyen & Ferrero, 2006).

In Africa, rice consumption is growing much faster (7% per year) than any other food commodity. The driving force is increasing population, urbanization accompanied by rising income and shifting of consumers' preferences in favour of rice (Somado et al., 2008). In Sub-Saharan Africa (SSA) the average annual per capita rice consumption is estimated to be 40 kg (Van Oort et al., 2015) with the highest reported in Madagascar (Diagne et al., 2013). The increased consumption has created a significant demand for rice. The need is bigger than the production in many African countries, necessitating rice importation from Asia (Balasubramanian et al., 2007). Therefore in many SSA countries, rice has been taken as a strategic crop for food security and people's livelihood.

In Tanzania, rice is a most crucial grain, second to maize. About 60% of the population consumes rice and its derivatives (Frewer & Spatscheck, 2018; Mkonda & He, 2016). Annual per capita consumption has risen from less than 15 kg in the 1970s to more than 35-40 kg in 2015 (Kilimo Trust, 2014). With increasing income, people are moving away from consuming tubers and cassava to rice (Luzi-Kihupi et al., 2015). The country has enormous potential for land and human labour for rice production (Kilimo Trust, 2014). However, rice production has been lower than its demand, thus

driving rice to higher prices than poor and low-income households can afford. Lower rice productivity is contributed to by abiotic, biotic factors and poor seed systems. Most SSA rice farmers use uncertified local landrace seeds that are very susceptible to abiotic and biotic stresses and cause low yield. To combat the situation, the Africa Rice Centre (ARC) developed a new variety of rice called New Rice of Africa (NERICA) which originates from crossbreeding between the Asian rice (*Oryza sativa L*.) and the local African rice (*Oryza glaberrima Steud)* (Somado et al., 2008). NERICA has qualities of Asian rice (high productivity) and African rice (resistance to water stress and pathogens) and has an enormous potential when grown as upland rice (Mondal & Henry, 2018).

Biotic factors that hinder rice production in Tanzania include weeds (*Striga* spp.) (Rodenburg et al., 2016), blast (BL) (Chuwa et al., 2015; Mgonja et al., 2016), Rice Yellow Mottle Viruses (RYMV) (Alkali et al., 2017; Hubert et al., 2017a; b) and PPN.

The latter has been given less attention compared to other biotic problems because they are hidden enemies usually miss diagnosed and unnoticed by farmers (De Waele & Elsen, 2007).

Microscopic plant-parasitic nematodes (PPN) contribute largely to low rice productivity (Luc et al., 2005). Global food security is compromised by about 4100 species of described PPN (Galvan et al., 2017). The extent of rice yield losses due to these pathogens is variable and can be 50%-80% depending on cropping patterns and varieties, season and cultivation practices (Coyne et al., 1998; Khan & Ahamad, 2020; Mantelin et al., 2017; Musarrat et al., 2014; Tülek et al., 2014; Win et al., 2015). In pot experiments under different hydrology, total crop failure was recorded under drought/upland condition due to *P. zeae* (Nzogela et al., 2020a).

1.2 PROBLEM DEVELOPMENT AND JUSTIFICATION

Tanzania is the largest producer of rice in the East and Southern Africa sub-region. It has excellent natural resource potential to increase production in the next decade and take advantage of the regional market. The expanded market of rice will enhance both household and national incomes in pursuit of the poverty reduction goal (Diagne et al., 2013; Ngailo et al., 2016). To achieve the national strategy for food self-sufficiency in rice, the Tanzanian government has committed itself to transform the existing subsistence rice sub-sector to a commercially viable one through a public-private partnership. The goal is to double rice production within the long-term period of ten years, from around 890,000 t in 2008 to 1.9 million t by 2020 which has been achieved (Fig. 1.1). Through the Eastern Africa Agricultural Productivity Program, the government has aimed at promoting NERICA varieties to increase production and productivity of upland rice (Luzi-Kihupi et al., 2015).

Rice production in Tanzania is pre-dominated by small scale farmers accounting for the largest proportion. These farmers own small plots of land ranging from 1-5 acres mostly clustered together, few separated apart from each other. The majority of farmers use shared farm implements like tractors powertillers, weeder and harvester and they rely on unimproved cultivars despite their low yield.The main reasons of using unimproved cultivars is a poor rice seed system. Some farmers are not aware of the presence of improved cultivars, and others argue that improved cultivars are of low quality in terms of palatability and fetch low market price. Improved cultivars like SARO-5 can be of high yield under lowland and irrigated conditions however, breeding initiatives that resulted in the generation of this cultivar did not take into consideration nematode resistance. The reasons might be not obvious but the major one is the lack of nematologists and facilities like screening equipments and

nematode identification facilities.The nematode problems have always been underestimated by breeders, agronomists, pest management specialist/consultants and farmers at large. This indicates the low level of awareness on the nematode damage potential caused to the rice sector.

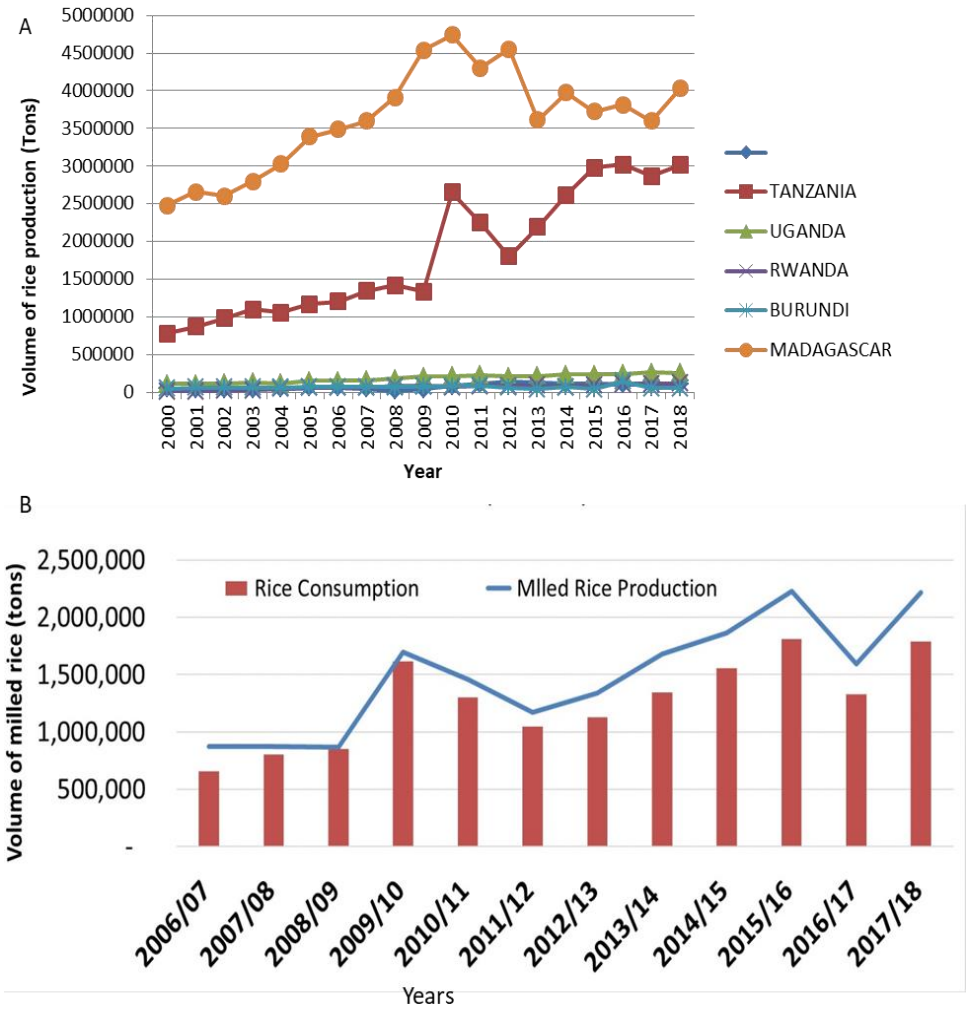

Fig. 1.1: A, Rice production in Eastern African countries, B, Milled rice production and consumption in Tanzania. Data obtained from FAOSTAT, 2020.

In Tanzania, nematode problems do exist (Table 1.1) and cause considerable damage to deferent crops, including rice (Fig. 1.2) (Talwana et al., 2015). However, the literature on the existence and extent of the problem is mainly missing. Most of the work was conducted in the early 70s. Tylor et al. (1972)

Few nematode studies have been performed in Tanzania on other crops such as tomatoes, banana, coffee and mostly concentrated on management aspect especially of RKN (Table 1.1).

Table 1.1: Studies on nematode problems in Tanzania

Type of study /Title	Nematodes dealt with / found	Source
Plant-parasitic nematodes associated with sugarcane in Kilimanjaro, Tanzania	*Helicotylenchus, Hemicycliophora, Pratylenchus, Rotylenchulus, Scutellonema,* and *Tylenchorhynchus.*	Singh et al., 2020
Root-knot nematodes associated with coffee in Tanzania	*M. decalineata*, *M. kikuyensis* and *M. africana*	Bridge, 1984
Status of *Pratylenchus coffeae* in banana-growing areas of Tanzania	*P. coffeae*	Luambano et al., 2018
Root-knot nematodes in outdoor tomatoes-Tanzania	*M. javanica*, *M. hapla* & *M. incognita*	Tily et al., 2013
Identification of root-knot nematode species occurring on tomatoes in Tanzania and resistant lines for their control	*M. javanica*, *M. hapla* & *M. incognita*	Womdim et al., 2001
Distribution and identification of nematodes in tomato farmers' fields in the selected semi-arid climates of central and northern Tanzania	*Meloidogyne, Scutellonema Helicotylenchus, Tylenchulus, Pratylenchus, Aphelenchoides Rotylenchus, Xiphinema Hemicycliophpra, Ditylenchus Criconema* and *Paratylenchus*	Misanga et al., 2018
Studies on the control of root knot nematodes on tomato in Tanzania using marigold plants, ethylene dibromide and aldicarb	Root - knot nematodes	Ijan & Mmbaga, 2008
Growing tomato in nematode infested soil and the pest implication in poorly managed post-harvested fields during the dry season in Tanzania	Root - knot nematodes	Bagarama et al., 2014
Achieving rational pesticide use in outdoor tomato production through farmers training and implementation of a technical guideline	Root - knot nematodes	Musebe et al., 2014
Molecular approach to confirm traditional identification of *Radopholus similis* sampled in Tanzania	*Radopholus similis*	Mgonja et al., 2020
Effect of natural and sesbania fallow and crop rotation on incidence of root knot nematodes and tobacco production in Tabora	*M. incognita* & *M. javanica*	Shirma et al., 2000
First report of dry rot disease of yam caused by *Scutellonema bradys* in Eat Africa	*Scutellonema bradys*	Coyne et al., 2018
Integrative taxonomy of root-knot nematodes reveals multiple independent origins of mitotic parthenogenesis	*M. africana*	Janssen et al., 2017
Ethno phytopathology and survey of tomato diseases in Morogoro Tanzania	Root - knot nematodes	Testen et al., 2015
Root-knot nematodes associated with tannia (*Xanthosoma sagittifolium*) in Tanzania	*M. arenaria* and *M. javanica*	Teri & Runkulatile, 1991

Fig. 1.2: ***P. zeae*** **infested upland rice field. Source Y.B. Nzogela**

Table 1.2; Major rice varieties grown in Tanzania and their physiological traits

Name of variety	Released year	Ecology	Growth duration (days)	Plant height (cm)	Potential yield (ton/ha)	Blast tolerance	RYMV tolerance	Drought tolerance	Palatability	Remarks
Katrin (IET 2397)	1984	Lowland	135-138	120	5.5 - 6.05	Moderate	Moderate	Medium	Low	High yielding, early to medium maturing, photoperiod insensitive, semi dwarf
TXD 85	2000	Lowland	110-120	117	5.7 - 6.0	Moderate	Moderate	Medium	Moderate	High yielding, early to medium maturing, photoperiod insensitive, semi dwarf
TXD 88	2000	Lowland	110-116	120	6.0 - 7.0	Moderate	Moderate	Medium	Moderate	High yielding, early to medium maturing, photoperiod insensitive, semi dwarf
TXD 306 (SARO-5)	2001	Lowland	120-125	118	4.5 - 5.5	Moderate	Low	Medium	High	Early to medium maturing, photoperiod insensitive, semi dwarf and scented
SUPA	Recommended	Lowland/upland	120-135	136	2.0 - 3.0	Low	Low	Medium	High	Low yielding, late maturing, strongly scented, tall, photoperiod sensitive
Shingo ya mwali	Local	Lowland	133	134.8		Low	Low	Medium	High	Popular due to its palatability
Faya Thereza	1980	Lowland	119	130		Low	Low	Medium	High	Good cooking quality
India rangi	Local	Lowland	134	130.2		Low	Low	Medium	High	Preferred in Usangu basin
Jicho la Samora	Local	Lowland	138	111.2		Low	Low	Medium		Preferred in Usangu basin
Zambia	Introduced	Lowland	81	102	3-Feb	Low	Low	Medium	High	Preferred in Kyela and Usangu basin
Kula na Bwana	Local	Lowland	134	97.5						
Rangi mbili	Local	Lowland	145	126						
Kihogo	Local	Lowland	145	118						
Name of variety	Released year	Upland/ lowland	Growth duration (days)	Plant height (cm)	Potential yield (ton/ha)	Blast tolerance	RYMV tolerance	Drought tolerance	Palatability	Remarks
Kilombero	Local	Lowland	145	139						
Afaa Mwanza	local improved	Lowland	141	126					Low	very high yielding
Subarimati	Introduced	lowland	145	113						

Increased rice production through crop intensification will agravate pest and disease problems, including nematodes, which are overlooked but need attention (De Waele & Elsen, 2007). To support the stated government initiatives on enhancing rice productivity, research on rice nematode problems is inevitable.

So far efforts have been made to promote rice production in Tanzania without considering nematodes. Plant-parasitic nematode problems in rice cultivation have not been addressed in Tanzania. Even the development of new rice cultivars has been made and cultivars released without data on nematodes for example NERICAs. Most farmers are still cultivating local varieties which have not been tested against nematode species. Rice researches have been focussed mainly on crop improvement and production constraints, including other pests than nematodes. It is a high time to address nematode problems in rice ecosystems in Tanzania as a component among the rice productivity limiting factors.

Host response and mechanisms of resistance to rice RKN *M. graminicola* has been extensively studied (Kyndt et al., 2014). But to date, rice host responses and mechanisms of resistance with other nematodes, especially *M. javanica* and *P. zeae,* are not known. It's essential, therefore, to get insight into the rice responses to these critical nematode species and explore further on sources of resistance from a wide range of rice genotypes.

Resistance screening of *O. sativa* and *O. glaberrima* and their interspecific hybrids against different species of nematodes have been conducted elsewhere but not in Tanzania (Bimpong et al., 2010; Brar et al., 1999; Cabasan et al., 2012; 2014; 2018a; Claudius-Cole et al., 2019; Das et al., 2011; De Waele et al., 2013; Diomandé, 1984; Mattos et al., 2019; Namu et al., 2019; Priya et al., 2019; Reversat & Destombes,

1998; Sharma-Poudyal et al., 2004; Win et al., 2014; Zhan et al., 2018). The results show that *O. glaberrima* is resistant to both *H. sacchari* and *Meloidogyne* species, and recently *O. glumaepatula* has proved to be strongly resistant to *M. graminicola.* As we broaden our research on nematodes, introduce new cultivars, and intensify rice cropping systems records for nematode pests will rise. Therefore, the risk might be very high as intensification is in favor of monocropping that might pose up selection for plant parasitic nematodes (Coyne et al., 2000). Continuous cropping without crop rotation increases the chances of population buildup of the plant parasitic nematodes.

So far, from screened results, no hybrids between *O. sativa* and *O. glaberrima* have shown the level of resistance as that of the parent, *O. glaberrima* (Cabasan et al., 2018a). To date, there are few discoveries of *O. sativa* genotypes that are resistant to *M. graminicola*, Zhonghua 11, KPM and LD24 (Dimkpa et al., 2016; Zhan et al., 2018). For Khao Pahk Maw a (KPM) and LD24, the resistance locus has been located at chromosome 11 (Lahari et al., 2019). Resistance mechanisms of true *O. sativa* characterized so far involve a strong HR response that is depicted at the early time of infection accompanied by accumulation of phenolic compounds (Phan et al., 2018).

Although some rice varieties are known to carry resistance genes against nematodes, this opportunity has never been used to improve great yielding varieties (Boerma & Hussey, 1992). Most of the resistant varieties are low yielding and of poor quality (Coyne et al., 2018). Therefore, screening of *O. sativa* that is directly used by farmers will be of economic i*m*pact.

1.3 SCIENTIFIC HYPOTHESIS, OBJECTIVES AND THESIS OUTLINE

The current study was undertaken to establish the base for characterization of nematode problems in Tanzanian rice cultivation for increased rice production through identifying and studying nematode resistance in *O. sativa, O. glaberrima* and their hybrids. The current study specifically aimed at

1. To carry out a diagnostic survey on plant-parasitic nematodes in selected rice fields. (Chapter 3).
2. To evaluate the popularly grown rice genotypes from *O. sativa* and *O. glaberrima* and their interspecific hybrids for their resistance to root-knot and root-lesion nematodes. (Chapter 4).
3. To compare the penetration, development and reproduction of *M. javanica* and *M. graminicola* on partially resistant *O. sativa* cultivars from East Africa. (Chapter 5).
4. To study the pathogenicity of the root-lesion nematode, *P. zeae*, on rice genotypes under different hydro-ecologies in Tanzania and characterize the host resistance to root-lesion nematode. (Chapter 6 & 7).

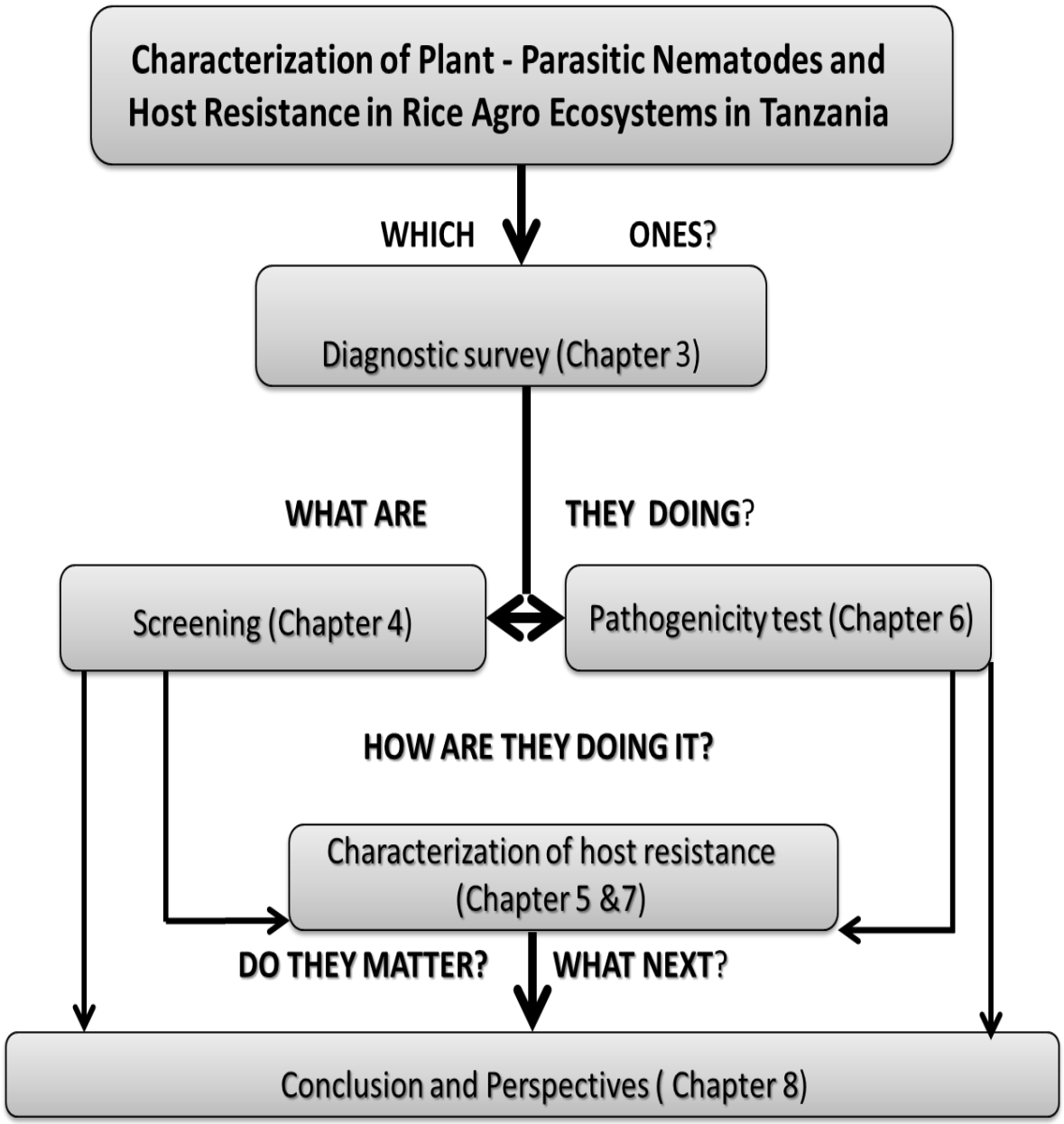

Fig. 1.3: **Schematic outline of the research topics investigated in this thesis**

CHAPTER 2: RICE IS LIFE; THE ORIGIN OF RICE CULTIVATION AND ITS ASSOCIATED NEMATODE CHALLENGES; THE AFRICAN CONTEXT

2.1 RICE AS A CROP PLANT

2.1.1 Rice morphology

Rice belongs to the grass family *Poaceae* under the genus *Oryza* (Fig.2.1). It is a self-pollinating crop with aerenchyma tissues that can diffuse oxygen from aerial parts downward to the roots (Yoshida, 1981). The plant height is, on average, 1 m with some exceptions, especially for deepwater genotypes that can go up to 5m with the rise in water level. The root system is fibrous, with functional secondary adventitious roots that are produced from the lower nodes of the culm/stem. The stem is hollow with nodes and internodes; each node generates a leaf that forms a shoot or tiller. Tillers are vegetative plant parts that bear panicles during the reproductive stage. A panicle is an inflorescence located on a terminal shoot. It is commonly determinate and droopy. Panicles bear spikelets, the floral parts of the rice plant (Yoshida, 1981). A rice flower consists of six stamens each with two anthers and a pistil with a single ovary and two stigmas. The fertilized rice ovary will form rice grain/seed (GRiSP, 2013). The rice seed contains the embryo that gives rise to embryonic leaves called plumules and a root called radicle. Seminal (primary) roots arise from the radical soon after seed germination. These roots are temporary, and the functional roots are secondary/adventitious roots arising from the lower nodes of stem/culm (Ahmed et al., 2012).

Fig. 2.1: Rice morphology; a = seed, b = stamen, c = tillers, d = adventitious roots, e = panicle, f = spikelets.

Source; https://www.goole=morphology+of+rice+plant&source=lnms&tbm=copy

2.1.2 Reproductive Biology

The growth cycle of rice usually is 3-6 months, depending on the variety and environmental conditions. The rice crop has three growth phases (Fig. 2.2) vegetative phase, reproductive phase and ripening phase (IRRI, 2002).

The vegetative phase starts from seed germination to panicle initiation, and in tropical countries like Tanzania, it takes 30-60 days (Eucord, 2012). It involves seed germination, seedling growth/elongation and tiller formation. Tillers start to emerge at

2-3 weeks after seedling emerged. Those originating from the main stem are called primary tillers which in turn form secondary and tertiary tillers (GRiSP, 2013). Varieties differ in tillering ability. The vegetative phase shows a lot of variation among different rice cultivars hence the duration is being used to differentiate between different varieties as short and long duration varieties which mature in 105-120 days and 150-160 days respectively (Vergara, 1991).

The reproductive phase starts from panicle initiation to flowering and takes about 30 days for most varieties. From panicle initiation, it takes about ten days for the panicles to be visible as a white feathery cone occurring in the main stem. Then tillers are emerging in an uneven pattern (FAO, 2019). Development of panicles gives rise to spikelets. The development of panicle in size causes the bulging of the flag leaf sheath, also called booting. At booting stage most of the non-productive tillers undergo senescence. The panicles developing further (heading) emerge from the flag leaf sheath, and the spikelets bear anthers (flowering) (Kumashiro et al., 2013). Flowering occurs a day after heading, and it takes a week for all spikelets in a panicle to flower (GRiSP, 2013).

The ripening phase starts just after flowering, and it takes about 30 days. In this stage, grains are filled with a white milky liquid. Milky liquid is an accumulation of starch and sugars. The grain increases in size and weight, the starch in the grain becomes firm, the moisture content decreases and the colour changes from green to gold (Dough stage). Panicles start changing from green to yellow, and the whole rice field looks yellowish. Finally, the individual grain gets hard and turns yellow-gold. Its moisture content becomes 20-22% and leaves at the base of the plant dry out. The crop is at its maturity stage, ready for harvest (Yoshida, 1981). The rice crop with

panicles and a high number of filled grains results into high yield. Each of the three growth stages largely determines the size of the panicle and the number of grain and degree of full grain per panicle (Ceesay, 2004).

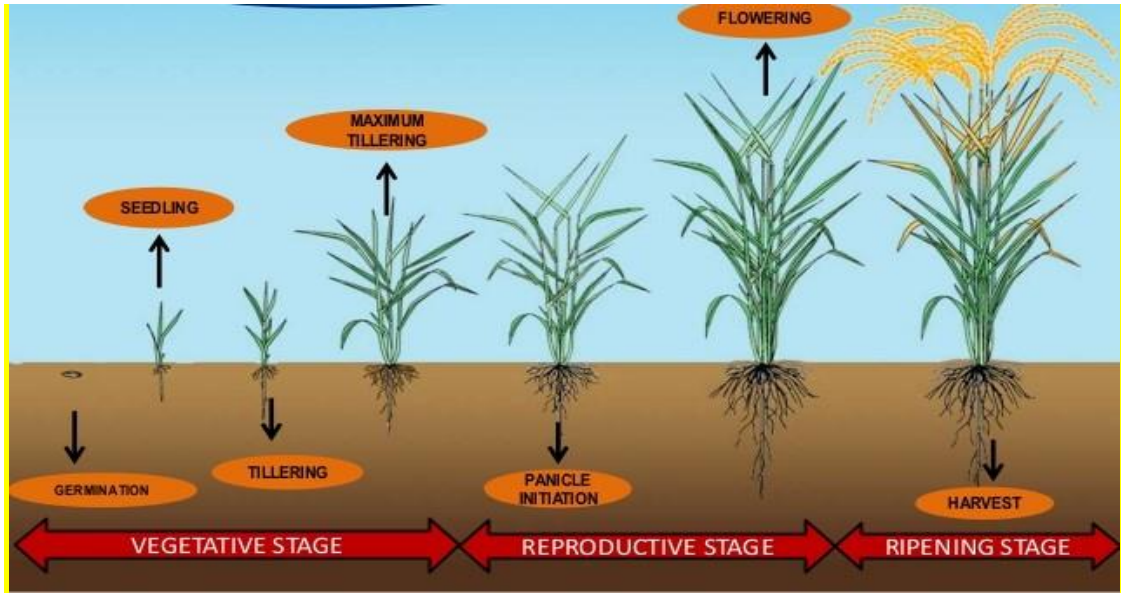

Fig. 2.2: The growth stages of the rice plant from seeding to maturity. Adapted from http://www.knowledgebank.irri.org/ericeproduction/growth_stages_of_the_rice_plant.htm).

2.2 GEOGRAPHIC ORIGIN OF CULTIVATED RICE SPECIES

The Oryza genus contains 24 species, of which 22 are wild and 2 are cultivated species. The genus *Oryza* has four species complexes sativa, Officinalis/latifolia, Ridley, and Meyeriana, among which two species complexes are well studied, Sativa, and Officinalis (Chang, 1976). The Sativa complex encompasses the two cultivated species *O. sativa* which is native to Asia and *O. glaberrima* specifically endemic to West Africa, and six weedy/wild ancestors (Red rice) (Civáň & Brown, 2017). The wild ancestors of Sativa complex include perennial rhizomatous *O. longistaminata* and *O. barthi,* endemic to Africa, *O. rufipogon,* and *O. nivara* native to Asian and Oceania, *O. melidionalis* native to Australia *and O. glumaepatula,* which is endemic to Central and South America (Solis, 2020). *Sativa* and *Officinalis* species

complex make a good source of gene-pool with AA genome and 24 chromosomes (Sié et al., 2012).

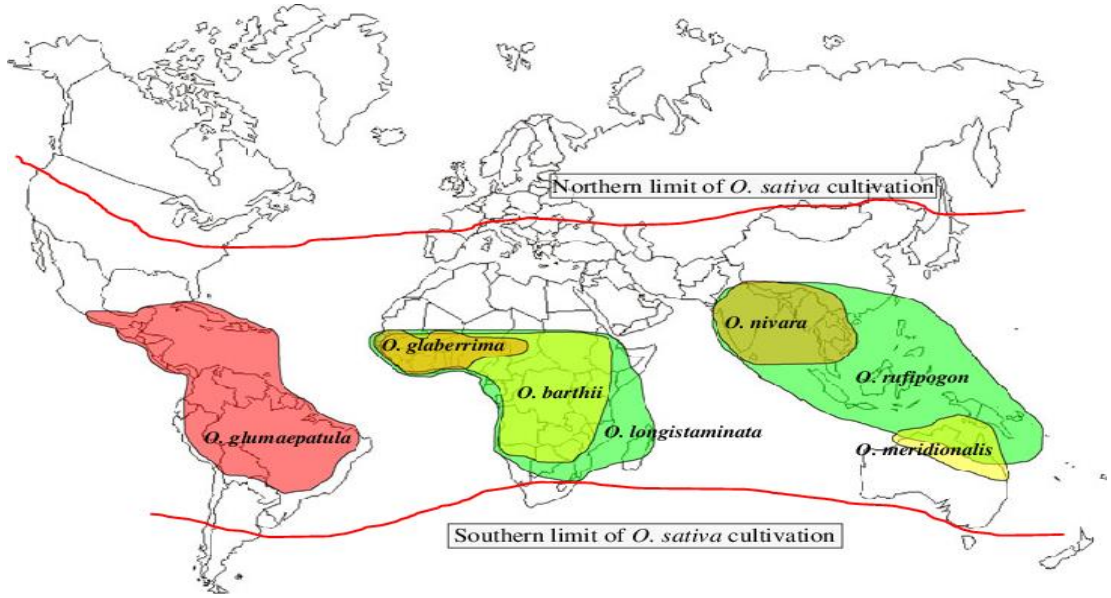

Fig. 2.3: A map showing *O. sativa* cultivation limit and its wild ancestors' distribution (Choi et al., 2019).

The centre of origin and diversity of the two cultivated species have been studied using genetic diversity, histological and archaeological shreds of evidence, and geographical distribution (Fig.2.3) (Park et al., 2019; Smith & Dilday, 2002). *Oryza sativa* (Asian rice) has three subspecies (Table 2.1A), (races) *japonica*, *indica* and *javanica* (Fuller, 2011). Recently five sub-species have been suggested within *O. sativa,* namely*, indica, aus*, *tropical japonica*, *temperate japonica*, and *aromatic* (Wang et al., 2014). *Japonica* has short and sticky grain, is cultivated at high altitude and temperate climate on dry land. The plant is short with dark green leaves. The grains are hard, rounded short, non-aromatic with low shattering characteristics (Dai et al., 2012). *Indica* has tall plants with light green leaves, long-grain, non-sticky and very aromatic, commonly cultivated in tropical Lowland and submerged environments (Chang, 1976). The third subspecies, which is not very common, is known as *javanica* or tropical *japonica.* Its characters lie between *japonica* temperate and tropical *indica.*

It has tall, broad light green leaves bearing larger and bold grains that are medium sticky due to low amylose content. All subspecies of *Oryza sativa* are believed to share the ancestor *O. rufipogon* with different perspectives on their origin of domestication in various parts of Asia (Fuller et al., 2012). It is commonly believed that the centre of the source of *O. sativa* is around the valleys of the Yangtze and Mekong rivers in China (Mogga et al., 2018). It is the most studied rice species, widely domesticated, and used as a model cereal crop (Jackson, 2016).

Table 2.1A: Characteristics of O. sativa sub-species

Characteristics	Subspecies		
	Japonica	**Indica**	**Javanica**
Height	Medium	Tall	Tall
Tillering	Low	High	Low
Lodging	Not easily	Easily	Not easily
Photoperiod	Non-sensitive	Sensitive	Non-sensitive
Grain shattering	Not easily	Easily	Not easily
Grain type	Short and round	Long to medium	Large and bold
Grain texture	Sticky	Non- sticky	Intermediate
Cool temperature	Tolerant	Sensitive	Tolerant

Source; Sweeney & McCouch (2007)

Table 2.1B: Number of rice germplasm accessions per region in AfricaRice genebank as of April 2012

Origin	*O. glaberrima*	*O. sativa*	Wild species	Total
Central Africa	83	190	101	374
East Africa	14	743	13	770
North Africa	0	53	0	53
West Africa	2,400	12,025	311	14,736
Southern Africa	0	869	53	922
Total Africa,	2,497	17,033	482	20,012
Others	0	3,153	4	3,157

Source; AfricaRice genebank review 2014

Oryza glaberrima Steud is typically known as African rice. Its origin and domestication were strictly in West Africa around the Niger River delta 3500 years ago (Veltman et al., 2019). It was selected for domestication among other African-indigenous species (Ndjiondjop et al., 2010). Its progenitor is *O. barthii* (Veltman et al.,

2019). It has two significant ecotypes, namely floating and non-floating. It is among the primary staple food in West Africa and highly appreciated for its taste and culinary qualities (Sanni et al., 2013). It is sometimes used in rituals and mostly not sold away from West African boundaries. Exceptionally it has been recorded in the Island of Zanzibar-Tanzania (Agnoun et al., 2012). In the field, the *O. glaberrima* plant can be differentiated from Asian rice by its ligule shape and panicle branching, more significant numbers of tillers with droopy leaves that make the plants very good weed competitors (Seck et al., 2012). At the maturity, the very characteristic of *O. glaberrima* is lodging and grain shatter. The plant height is ranging from 120 cm for the upland and irrigated varieties up to 1.5 meters for floating types (Sow et al., 2014). The rooting system is fibrous with rooting on lower nodes and upper nodes for non-floating and floating ecotypes, respectively (Lorieux et al., 2013). Grains are red to grey coloured. It survived abiotic and biotic stresses for a long time without human interference, and this has enabled the development of diverse resistance characters that are being exploited in various breeding programs (Seck et al., 2012). Useful traits which have been identified are resistance to pests and diseases such as weeds (Rodenburg & Johnson, 2009), nematodes (Cabasan et al., 2012; Petitot et al., 2017; Plowright et al., 1999; Soriano et al., 1999), African rice gall midge (Nwilene et al., 2002), RYMV (Thiémélé et al., 2010), *Xanthomonas oryzae* strain from Africa (Djedatin et al., 2011). *O. glaberrima* is also an excellent source of abiotic stress resistance, such as endurance to iron toxicity, salt tolerance, drought tolerance, unfavourable temperature, and excessive water (Bimpong et al., 2011; Sahrawat & Sika, 2002; Sikirou et al., 2018). However, bad characters such as lodging (Fig. 2.4), grain shattering, long seed dormancy, and low yield made it unfit for commercial purposes, and it is widely

unaccepted by farmers away from its centre of origin. Even in West Africa, the species is replaced rapidly by better yielding varieties from *O. sativa* and is only grown for a particular purpose on small lands (Mokuwa et al., 2013). To date, about 2497 accessions of *O. glaberrima* have been collected in AficaRice genebank (Ndjiondjop et al., 2014).

Fig. 2.4: ***O. glaberrima*** **grain polymorphism (on the left) and plants in lowland rice cultivation system. Adapted from Agnoun, 2009.**

2.3 NEW RICE FOR AFRICA – NERICA

O. glaberrima is a self-fertilizing crop, and the hybrids with *O. sativa* are complicated to obtain due to incompatibility barriers. This confused rice breeders who were very eager to transfer and utilize the richness in resistance to biotic and abiotic stresses from *O. glaberrima* to *O. glaberrima* x *O. sativa* hybrids. Thanks to novel techniques the eminent rice breeders, Dr Monty Jones and Dr Sie Moussa from AfricaRice were able under the rice interspecific hybridization project to develop fertile offspring through embryo rescue in 1994. The offspring were backcrossed to *O. sativa* parents to produce fertile offspring, which were baptized as New Rice for Africa - NERICA (Jones et al., 1997).

The hybrid infertility chronicle was finally broken by producing more fertile hybrids by crosses between *O. glaberrima* (CG14 and TOG5681) with *O. sativa* WAB 56-104 and IR64 for upland and lowland ecosystem respectively (Fig. 2.5) (Sie et al., 2005). The selections of best NERICAs were based on the resistance and tolerance to abiotic and biotic stress with high yield potentials (Somado et al., 2008). Through screening, NERICA has allowed researchers identifying resistance genes against major rice diseases like rice BL, RYMV and insect pests. Unfortunately, so far from the hybrids screened against different rice nematode problems, there is not one that has been reported to be utterly resistant as its *O. glaberrima* parent. Few are partially immune and tolerant to the nematodes in question (Bimpong et al., 2010). Plowright et al. (1999) found four hybrids that were less susceptible but not resistant to *M. graminicola* based on the number of females per root system. Recently a panel of hybrids was screened for resistance/tolerance to *M. graminicola,* and none of the hybrids would reveal the resistant characteristics as that of the resistant check. Most of the hybrids were either susceptible or tolerant to the nematodes based on the nematode reproduction (Cabasan et al., 2018a). In pot experiments, NERICA and other improved varieties were evaluated against *M. incognita.* All NERICAS included in the analyses were very susceptible to the nematodes based on the nematode reproduction factor (Claudius-Cole et al., 2019). Based on the negative results available so far, it is clear that further NERICA screening against nematodes is of paramount importance to find nematode-resistant genotypes.

Fig. 2.5: NERICA in the field, lowland (left) and upland (right). Adapted from Diagne (2010).

2.4 HISTORY OF RICE DOMESTICATION IN TANZANIA

In Tanzania, rice farming has not been a business but a way of life. It is only recently that the crop is being commercialized, although full mechanization for commercialization is at its infant stage and gaining support from the government (Rugumamu, 2014). The history of rice cultivation in Tanzania backdates to sea - traders from Asia through the Indian ocean by Portuguese and Arabs about 1500 years ago (Walshaw, 2010). Indica rice subspecies were introduced through traders along the coast of the Indian Ocean from Madagascar to Pemba Island in Zanzibar (Khush, 1997). The coast societies were introduced to Asian foodways that went along with Indian Ocean cultures, urbanization and Islamization, especially along the eastern African coast region in the 11^{th}-15^{th} centuries (Walshaw, 2010). The crop spread by natural means in the mainland -Tanganyika by then under Germany colonial era in 1884. The first place for rice domestication in Tanzania mainland was in Coastal regions at Rufiji river basin in Lindi and Kilwa (Fuller et al., 2012). Around the 19^{th}-century lowland rice was found cultivated around the Kilombero valley and Usangu basin in Morogoro and Mbeya regions of Tanzania mainland, respectively (Kato,

2007). In the mid-nineteen century, during the Arabic slave trade, the traders cultivated rice around their trade bases that pushed the rice cultivation to penetrate in Northern regions of Tanzania (Meertens et al., 1999). From 1930s indica rice was cultivated as a commercial crop in mainland Tanzania and had been adapted to local conditions resulting in a massive number of local cultivars (Landraces) (Mogga et al., 2018; Suvi et al., 2020).

In 1948 the first modern rice irrigation scheme was built in Kilangali Morogoro. In 1967 after Arusha declaration that changed the policy from colonialism to socialism and self- reliance few state-owned irrigation schemes were developed (Dakawa, Kapunga, Mbalali and Ruvu) that expanded the rice sector and increased the rice production (Therkildsen, 2011). However, poor management of the state-owned schemes due to financial constraints hampered the sustainability of the schemes and rice production corrupted by 1970s-1980s (Mdemu et al., 2017).

Soon after economic liberalization in Tanzania 1986, rice became very important as food and cash crop in so many societies from Lake Zone Mwanza and Shinyanga regions to Morogoro, at Kilombero river valley to Mbeya at Mbalali and Kyela (Kadigi, 2003). The most driving factor was rice demand created from demographic factors that stimulated the commercialization and expansion in rice production. The demand and Government policy attracted many private investors and spurred local small scale farmers to initiate and expand rice farming. As a result, the rice industry in Tanzania was rejuvenated, and the rice market grew across the country (Barreiro-Hurle, 2012).

Before rice commercialization in Tanzania, most of the families/ households were considering rice as a luxurious meal that was supposed to be taken on special

occasions such as marriage ceremonies, Christmas and Easter holidays for Christians and Eid – El Fitri / Maulid for Muslims (Oikeh et al., 2009). A growing rural and urban population with increased income has increased rice consumption at an annual rate of about 6% (Oikeh et al., 2009). Increased rice consumption led to the increased area under production, but production increased with limited cultivated land expansion and improved crop intensification are the main target (Katambara et al., 2013).

Currently, rice is the second major food crop and is a source of income and ensures food security for a rural and urban growing population. It is grown in a range of environments from semi-arid regions with less than 800 mm of rainfall to humid areas experiencing more than 1500mm (Sekiya et al., 2013). Rice cultivation cuts across lowland areas to steep slopes (Sekiya et al., 2015). The rice sector employs about 24% of farming households that contributes to national Gross Domestic Product (GDP) by about 2.68% (Trevor & Lewis, 2015). Rice is grown almost in each region in Tanzania mainly by subsistence farmers in small plots of land ranging from 1- 2.5 hectares. The major producing areas are Morogoro, Mbeya, Tabora, Shinyanga and Mwanza (URT, 2019).

The rice market demand in Tanzania is vast. Recently larger private investors have joined the government effort in the transformation of the rice sector from subsistence to commercial that may grab the alarming rice demand within and outside the country (Liu & Ingabire, 2017). The government has earmarked several areas with permanent river flows, reliable year-round irrigation and suitable flat fertile land for rice investment and modern infrastructures are being implemented (Analysis, 2012). However, all these strategies will only be realized if farmers will be involved in planning and decision making.

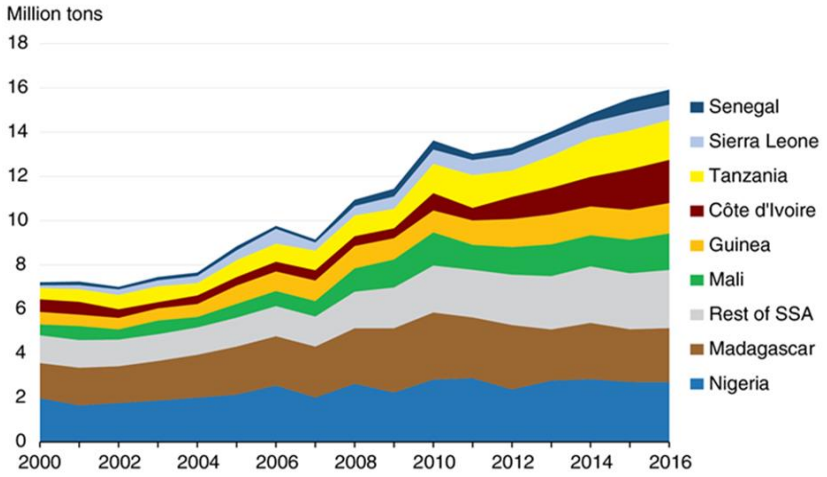

Fig. 2.6: Expansion of rice cultivated land across African countries, including Tanzania. Source: USDA, Economic research service, agricultural baseline database.

2.5 RICE AGROECOSYSTEMS IN TANZANIA

2.5.1 Geographical distribution of rice cultivation

Rice is cultivated almost throughout Tanzania. The area under cultivation is about 681,000 ha, which represents 18% of the cultivated land. The average yield ranges from 1.5 - 3 t ha−1. Production is commonly traditional and divided into three major zones.

i. Northwest/Lake Victoria zone. This covers Mwanza, Shinyanga and Tabora region. The area is under Savanna grassland covering the land so-called "Mbuga" in Swahili and receives limited annual rainfall ranging from 500mm-800mm. In this zone, rice is grown on gentle slopes under lowland rain-fed with water harvesting technique called "majaruba[1]" system (Meertens et al., 1999; Ngailo et al., 2007).

[1] Rain water harvest technique found extensively in Tanzania and is used primarily for the production of rainfed lowland rice in bunded basins. Hill slope runoff is collected from stony outcrops and grazing lands in upslope areas with cattle tracks often used as conduits. It originated in Sukumaland, around Lake Victoria. Majaruba are constructed by excavating a bunded basin by digging to a depth of 0.3 m to 0.6 m. The scooped soil is used to build a bund around the field perimeter. The bunds may have a height of between 0.3 m to 0.7 m above the ground and are used as a passage for Jaruba maintenance. Water should be uniformly distributed within the bunded area that depends largely on the general slope of the area, the bund size and leveling. Usually large bunds are found on flat land while smaller ones are found on steep slopes

ii. North zone covering Kilimanjaro and Manyara regions. Under this zone, rice is very much intensified. Most of the rice fields are under large scale irrigation schemes that enable farmers to grow rice twice to three times a year (Ikegami, 2001).

iii. South-east and South-Southwest zone. This zone covers Morogoro, Mbeya and Rukwa regions. The area receives abundant rainfall annually ranging from 800mm -2800mm (Sekiya et al., 2020). Most of the large scale rice farms are located in this zone, and it is the most rice-producing zone feeding the big cities of the country like Dar es Salaam and neighbouring countries like Burundi, Rwanda, Congo, Zambia, Mozambique and Malawi. Rice is grown in different systems from upland to lowland and irrigated. The highest price of fetching rice comes from these regions (Supa-Kyela). Rice produced from this zone has outstanding quality and aroma.

iv. Coast zone covering areas of Tanga, Pwani, Lindi, Mtwara and Zanzibar. This zone contributes a small amount to the total rice production, however, there is potential for the intensification and rice expansion (Mwaseba, 2005).

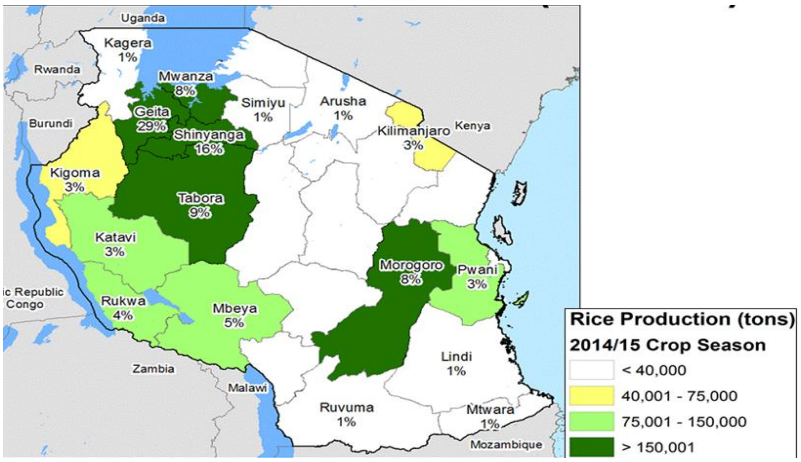

Fig. 2.7: Rice producing regions in Tanzania and the levels of production in 2014/2015. Source: Rice pedia 2.6.

2.5.2 Classification of rice cultivation systems in Tanzania

There are three major rice-cultivation systems, rain-fed upland, rain-fed lowland and irrigated lowland.

2.5.2.1 Rain-fed upland

Rain-fed upland rice constitutes 20% of the total Tanzania production, with average productivity of 1.2 t ha^{-1}. The productivity potential of rain-fed upland rice ranges between 3 and 5 t ha^{-1} (Kitilu et al., 2019). Upland rice is usually produced under aerobic conditions with/without irrigation to rainy and dry seasons from steep slopes to valley between hills, characterized by soil erosion, poor soil structure with low fertility and pH (Bucheyeki & Kadadi 2011). Upland rice fields are mainly found in Mbeya region at Kyela district and Morogoro region along with the Eastern Uluguru Mountain ranges, in Morogoro rural district and in Mvomero district (Suleiman, 2018). Traditionally rice in this agro-ecosystem is either grown alone or intercropped with other cereals, commonly maize. Upland rice competes with maize in resource allocations, especially fertilizers and labour. Often preferred varieties are Supa, Kihogo red, Kula na bwana, Mlimani and Salama.

Recently NERICA 1, 2, 4 and 7 have been introduced. The rain-fed upland rice ecosystem in Tanzania is constrained by unreliable and inadequate rainfall which leads to long periods of drought spells that increase chances of crop failure and low productivity (Ceesay, 2004). Heavy rain to flooding conditions on aerobic rice fields enhance the nutrient availability to crops and ameliorate the acidity/ alkalinity conditions of the soil (Oikeh et al., 2009). Soils from upland rice fields show a nutrient deficiency, especially in necessary critical nutrients N, P and K (De Bauw et al., 2019). The gap between the available plant nutrients that the soil can supply and the crop

requirement have to be covered by an external supply of nutrients in the form of mineral fertilizers (Mghase et al., 2010). Yet mineral fertilizer uses among upland rice farmers are very low due to unavailability at the right time and financial constraints; farmers cannot make a profit based on cost-benefit analysis (Meertens et al., 2003). Instead, farmers solve the fertility issues by subjecting the fields too long periods of bush fallow that cannot work effectively any more under alarming increases in the human population. The areas face weed pressure as a result of low labour productivity due to already limited labour availability during the cropping season (Rodenburg & Johnson, 2009). The low productivity in upland rice-based ecosystems can be addressed by the use of better yielding varieties that are drought-tolerant, weed competitive and disease-resistant, such as NERICA. The use of high yielding varieties such as NERICA would motivate and encourage the policy makers to initiate the fertilizers voucher system to rice farmers as they do for maize farmers. The fact that upland rice production is not well organized, gets low attention. Soil fertility amelioration, proper seeding and timely weeding would guarantee to achieve the production potential of 3 and 5 t ha^{-1}. Yield losses in upland rice production are in addition to the factors mentioned earlier, aggravated by pests and disease problems, including nematodes (Chidiebere-Mark et al., 2019; Rugumamu, 2014).

2.5.2.2 Lowland rain-fed

The rain-fed lowland rice ecosystem in Tanzania covers 70% of Tanzania rice total production with average productivity of 3.5 t ha^{-1}, and its production potential ranges from 4.5 to 6 t ha^{-1} (Meertens et al., 1999). According to Sekiya et al. (2020) rice fields under rain-fed lowland, the ecosystem is further classified based on the water source and farmers' water use techniques.

(i) Plain grassland (Mbuga) whereby farmers harvest water for rice cultivation by making high bunds around fields (Majaruba);

(ii) A gentle slope in a catchment basin (Catchment) whereby collected water runoff under adequate to excessive rainfall is used to flood the rice fields. Farmers rarely make bunds around their rice fields;

(iii) Flood plain whereby farmers depend on water flooding from big rivers;

(iv) Narrow valley located at inland valley bottoms with moderate slopes found along the coastal regions.

The rice crop under lowland system is customarily grown under fully flooded conditions in a regular season (Fig 2.8). However, when the weather is not favourable, especially under this era of climate change, rice may face a drought spell (Kangalawe & Liwenga, 2005). The big challenge facing the lowland rice cultivation system is water management (Bouman et al., 2007). It is the most potential ecosystem that can be intensified to meet the rising urban rice demand within and outside the country. The intensification would be achieved by the use of high yielding varieties, improved water use efficiency, good agronomic practices and external supply of inputs such as fertilizers (Senthilkumar et al., 2018). The reported challenges hindering maximum productivity of the system is iron toxicity, BL, RYMV and African rice gall midge (Mwatawala et al., 2016).

Fig. 2.8: Low land rice field at Idete village, Kilombero, Morogoro region; Source, Y. B. Nzogela.

2.5.2.3 Lowland irrigated

Since the 19th-century rice has been cultivated under irrigation in Tanzania mainly in Kilombero valley Morogoro and Usangu basin Mbeya, across the country, 461 rice irrigation schemes cover approximately 100,000 ha which was built by the government in collaboration with farmers and other agricultural developmental agencies (Sekiya et al., 2020). It includes 12% of the total area under production. Rice cultivation under this ecosystem is characterized by high levels of water management and agro-inputs (Fig.2.9). The primary source of water for irrigation is permanent rivers that flow all over the year, seasonal streams, dams or groundwater (Sekiya et al., 2017). Water can be managed by opening and closing the main water supply. The average rice productivity under this system is 3.8 t ha^{-1}, while production potential varies from 5 to 6 t ha^{-1} (Ngailo et al., 2016). Kanyeka et al. (2004) reported a yield of 4.7-8.1 t ha^{-1} from improved varieties (TXD 85 and TXD 88) and 4.5-5.9 t ha^{-1} from the local variety Supa. Lower Moshi irrigation scheme recorded a high yield of 6.6 t ha^{-1} (Ikegami, 1995). Different studies also indicate variability in rice yield from various

irrigation schemes that are very far from the productivity potential (Mdemu et al., 2017; Mwaseba et al., 2007; Nakano et al., 2015). Low productivity of irrigated rice systems is probably due to poor agronomic practices accompanied by water scarcity due to climate change and low water management.

Fig. 2.9: Lowland irrigated rice field at Dakawa irrigation scheme, Mvomero, Morogoro. Source, Y. B. Nzogela.

2.5.2.4 System of Rice Intensification (SRI)

The holistic approach, rather than the introduction of a unique advanced technology, would be required to realize the productivity of rice in farmers' fields (Mwaseba, 2005). The holistic approach led to the opening of the system of rice intensification (SRI). Unlike in conventional paddy production whereby flooding of paddy fields from transplanting to harvesting is the usual way of farming (which takes about 3,000 litres of water to produce one kilogram of rice), flooding is not required in SIR. SIR capitalizes on Good Agronomic Practices (GAPs). The GAPs include intermittent wetting and drying of rice fields, mechanical aeration of soils, early sowing with wide spacing of rice seedlings (Sekiya et al., 2017; Stoop, 2003) and improved

Basic Cultivation Technique (BCT) (Senthilkumar et al., 2018). It reduces input requirements such as inorganic fertilizers and a large number of seeds. The challenge with the SIR system is labour demand and time consumption (Bezabih et al., 2016).

2.6 RICE RESEARCH IN TANZANIA-AN UPDATE

Currently, rice research is coordinated at Tanzania Agricultural Research Institute-TARI- Ifakara, formerly called Kilombero Agricultural Training Research Institute (KATRIN). Rice research in Tanzania began in 1935 with specific objectives of screening the traditional and introduced foreign rice cultivars and improving cultivation techniques (Sekiya et al., 2020). To date, in Tanzania, the primary source of rice germplasm is by varietal introduction and selection from other countries. An excellent example of an introduced variety that is still very popular is Supa- India (Surinam V-880) (Luzi-Kihupi et al., 2015). Different varieties have been screened and released for farmers use in different rice agro-ecologies. The introduced varieties, including those from IRRI (IR-8, 54, 58, 64) were tested at various locations in the country and released for use by farmers (Singh et al., 2013a). In the early 1980s, the rice research objectives increased in addition to screening, to modern breeding (hybridization and mutation breeding). Through the Supa improvement project, improved varieties were bred at ARI-Dakawa. These varieties are TXD85, TXD88, and TXD306 (SARO-5) which are early-maturing, high-yielding, shorter varieties with pest and disease resistance and acceptable cooking and eating quality (Kanyeka et al., 2004, 2005; Msomba et al., 2004; Luzi-Kihupi et al., 2009). Next project was mutational breeding for resistance to RYMV whereby Mwangaza was developed through radiation under the International Atomic Energy Agency (IAEA-SUA project) that was effected in the early 2000s (Luzi-Kihupi et al., 2008). AfricaRice introduced lowland

and upland NERICA series, among which NERICA 1, 2, 4, 7 and WAB-450-122-BL1-DV4 for farmer uses after trials at different locations (Sekiya et al., 2015). TARI-Dakawa has released SATO1 and nine salt-tolerant cultivars. Breeding for biotic and abiotic stress has been a critical focus in rice breeding. Studies on host resistance to rice yellow mottle virus (RYMV), rice (BL), and bacterial leaf blight (BLB) have drawn much attention. However, the development of resistant varieties to the mentioned diseases is not yet realized. The big challenge being lack of knowledge on population structure, diversity of the pathogens, and host-pathogen interactions (Banwo, 2015). Rice varieties resistant/tolerant to abiotic stress such as drought and heat, cold, salt and low P and Fe have been developed in collaboration with different international partners (Kashenge-Killenga et al., 2014). Different lines are being evaluated at TARI-Ifakara for various abiotic stresses (Luzi-Kihupi et al., 2015). Apart from rice crop improvement, other research activities concerning rice farming systems, water use efficiency, rice agronomy and rice impact on socio-economic aspects have been made (Sekiya et al., 2020). Of all the researches done on rice, nowhere nematodes problems have been addressed. This might be due to lack of awareness on the existence of the problem, aggravated by the lack of nematologists who are able to identify the nematode problems.

2.7 NEMATODE PROBLEMS IN RICE CULTIVATION SYSTEMS

Plant-parasitic nematodes occur in all rice-growing environments. The rice ecosystem determines its abundance and diversity. Generally, rice plants can be attacked by diverse species of nematodes in different parts. The nematodes can be found in roots, stem, leaves and seeds; hence they are divided into two major groups based on the part of plant attacked, foliar parasites (feed on stems, leaves and

panicles) and root parasites. These nematodes exhibit diverse parasitic nature during penetration, feeding, development and reproduction on a host plant that results in damage and yield losses (Bridge et al., 2005). More than 200 species of nematodes have been described as parasites of rice (Prot & Rahman, 1994). Environmental and socio-economic factors determine their abundance and damage caused to rice production. Upland ecosystems have a higher nematode diversity than lowland and irrigated lowland (De Waele & Elsen, 2007). Therefore, changes in land use forced by human population growth and climate changes may change the existing nematode diversity pattern.

About 29 species of plant-parasitic nematodes are known to cause economic damage to rice (Bridge et al., 2005). *Meloidogyne* spp., cyst nematodes (e.g. *Heterodera sacchari*), root-lesion nematodes (*Pratylenchus* spp.), root rot nematodes (*Hirschmanniella* spp.), and foliar nematodes (*Aphelenchoides besseyi* and *Ditylenchus angustus*) are the most common and damaging nematodes of rice (Babatola & Bridge, 1979; Bridge et al., 2005; Coyne et al., 1998; 1999a; 2001; 2004; 2018; Fortuner & Merny, 1979; Gnamkoulamba et al., 2018; Pili et al., 2016; Singh et al., 2013a). Estimated rice yield reduction due to root-knot nematodes ranges from 20-98% (Soriano et al., 2000), cyst nematodes 38-100% (Coyne et al., 1999b; Audebert et al., 2000) and lesion nematodes 28% - 100% (Prasad et al., 1987; Nzogela et al., 2020a).

2.7.1 Foliar parasitic nematodes

These are nematodes that feed and reproduce on rice stems, and some species can survive in seeds. Foliar parasitic nematodes are prominent in deepwater rice (lowland and irrigated). They mainly feed ectoparasitically on newly forming

tissues of the emerging leaves, buds of the shoots and apical stems (Mendes & Godoy, 2017; Peng & Moens, 2003) although in some cases endoparasitic feeding has been observed. They enter the host plant in the presence of water at a certain level moving by the water film to the rice stem and leaves where they feed. *Aphelenchoides besseyi* causes whitening of the young leaf tips, and therefore a so-called flag leaf is a typical symptom (Ali et al., 2017). *Ditylenchus angustus* (Ufra disease) causes white patches or speckles in a splash pattern at the leaf base and leaf malformation. Attacked leaves depict twisted and distorted leaf sheath and bases (Khanam et al., 2016). Survival and dispersion of these nematodes are mainly through crop leftover in the fields, irrigation water and infected nurseries (Lambert & Bekal, 2002), and via seed in the case of *A. besseyi*. When present in the rice field, *D. angustus* and *A. besseyi* may cause a yield loss of about 70% (Plowright & Gill, 1994) and 90% respectively (Latif et al., 2013)

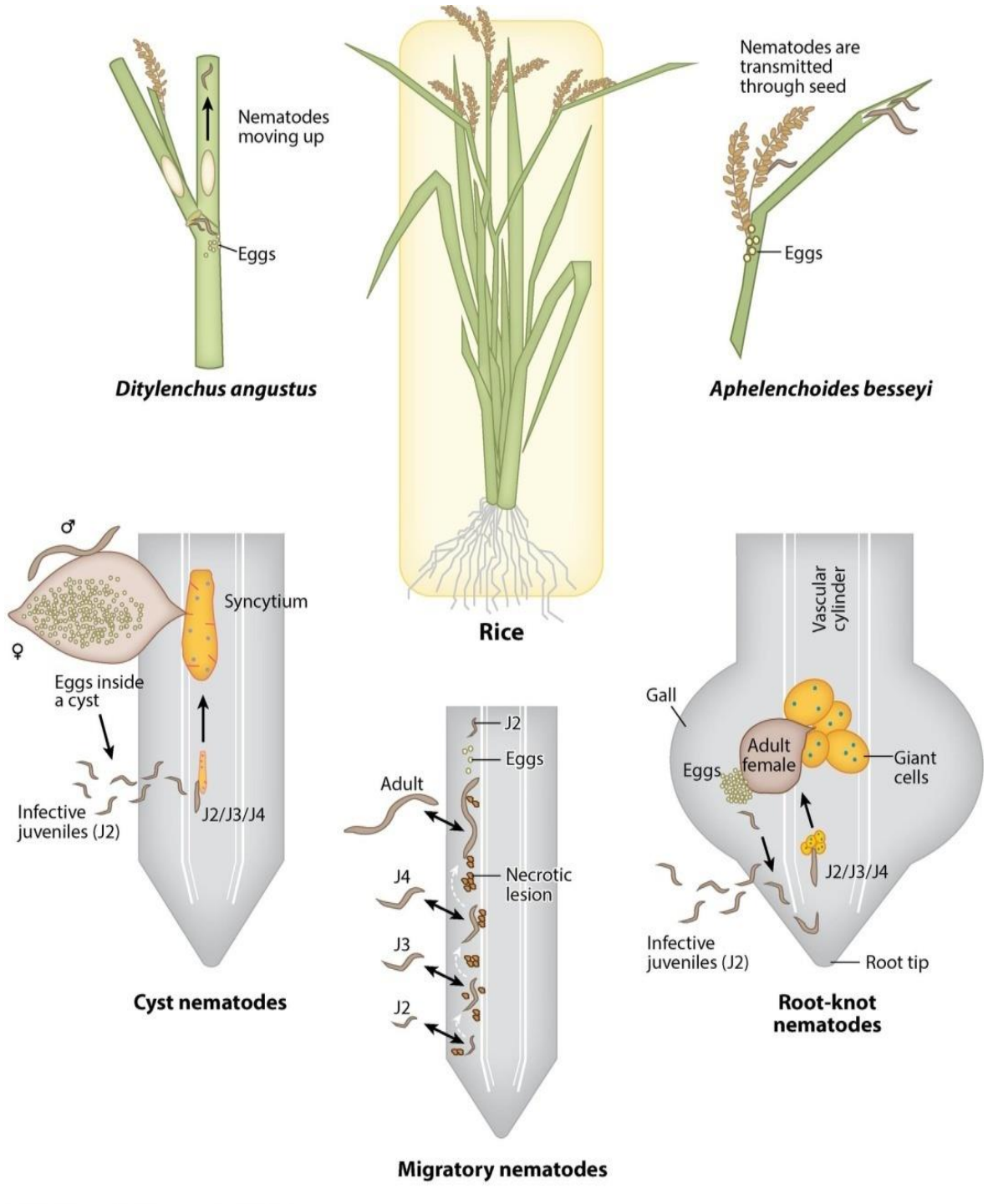

Fig. 2.10: **Different groups of rice nematodes, foliar and root endoparasites. J2,-second stage juveniles; J3,-third stage juveniles; J4,-fourth stage juveniles. The symbols ♂ and ♀ represent male and female nematodes. Source; adapted from Kyndt et al., 2014.**

2.7.2 Root parasites

2.7.2.1 Ectoparasitic nematodes

These nematodes feed on the rice root tissues by using their protruding stylet, which punctures the root and syphon the cytoplasmic content of the cell while their bodies remain outside the rice root. Their entire life cycle takes place outside the host. Nematode genera which have been found in rice fields in Africa include *Helicotylenchus, Hoplolaimus, Cliconemoides, Tylenchorhynchus, Xiphinema, Scutelonema, Cliconema, Trichodorus, Dolichodorus, Aphelenchus, Basiria, Filenchus, Hemicycliophora, Longidorus, Malenchus, Rotylenchus* and *Telotylenchus*. Most of them are found across all rice agroecosystems (Babatola, 1984; Coyne et al., 1998).

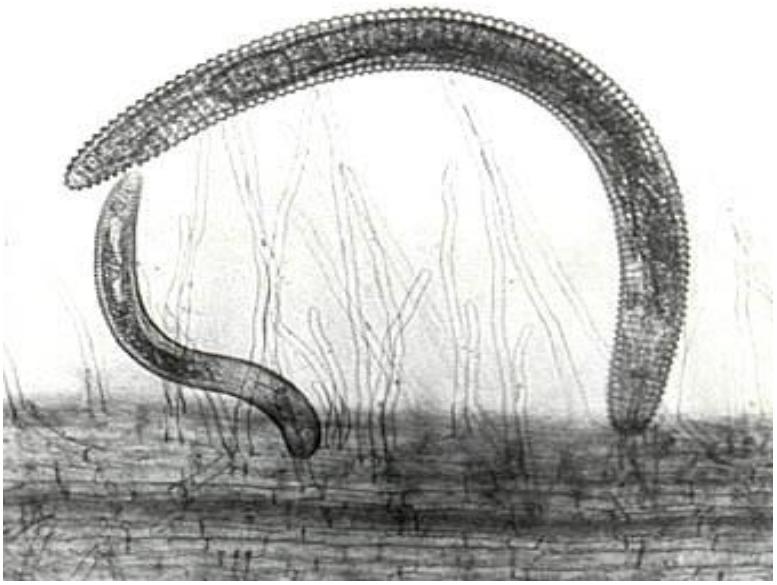

Fig. 2.11: Rice ectoparasitic nematodes, *Mesocliconema* sp: adapted from Courtesy S. W. Westcott III.

2.7.2.2 Endoparasitic nematodes

These are the most economically significant group of nematodes. They are divided into two major subgroups based on the mechanisms of feeding. The first group is **migratory endoparasitic nematodes** that are very mobile throughout their life

cycle except for eggs. They enter and exit the host root tissues at any time and stage. While inside the host, they feed and reproduce in the root tissues, although reproduction may also take place outside the host roots. They don't have a permanent place to feast on while in the root tissue, instead, they migrate while feeding and hence cause brown lesions on the roots that culminate to form cavities. Several cavities on infected roots cause root rot. Economic important genera from this group are *Pratylenchus* and *Hirschmanniella* (Back, 2009; Castillo & Vovlas, 2007; Holbein et al., 2016; Jones & Fosu-Nyarko, 2014; Jordaan et al., 1989; Lambert & Bekal, 2002; Smiley, 2010; Yu et al., 2012; Zunke, 1990).

P. zeae has been reported in African rice ecosystems in different countries mainly being the problem in upland rice (Coyne et al., 1999a; Gnamkoulamba et al., 2018; Nzogela et al., 2020a; Pili et al., 2016).

The second group are **sedentary endoparasitic nematodes** that are the most critical group that causes the most considerable economic loss to different crops, including rice (Kyndt et al., 2014)**.** As their name indicates, they have unique mechanisms of initiating a permanent relationship with host plants for their benefit that makes their entire life (except for J2 and adult males) positioning at a single suitable site, called feeding site. They feed, develop and reproduce while in their feeding sites and in so doing, they cause a lot of physiological changes on host cells. The most important genera that have been associated with rice are *Meloidogyne* (RKN) (Kaloshian & Teixeira, 2019; Mantelin et al., 2017) and *Heterodera* (CN) (Audebert et al., 2000; Coyne et al., 1999c). They can be found across rice-growing ecosystems from flooded to upland rice ecosystems at a varying magnitude of damage. Yield loss of about 80% (Khan & Ahamad, 2020; Mantelin et al., 2017) and 100% (Audebert et

al., 2000) for *M. graminicola* and *H. sacchari* respectively has been reported. Because *P. zeae* and RKN (*Meloidogyne sp.*) were the primary focus in the current study, they will be described in detail.

2.7.3 Case studies

2.7.3.1 Root Lesion Nematodes (RLN): *Pratylenchus zeae*

Root lesion nematodes (RLN) are the third most critical plant-parasitic nematodes after (RKN) and cyst nematodes (CN) (Jones et al., 2013; 2014; Kyndt et al., 2014; Singh et al., 2013b). They are polyphagous, obligate, migratory root endoparasites occurring in all agricultural regions (Jones et al., 2013). They survive by feeding and migrating within the roots of crops causing necrotic lesions which further culminate into cavities (Olowe & Corbett, 1976; Yu et al., 2012). The roots damaged by nematodes fail to absorb nutrients and water efficiently, which results in weak growth and yield reduction (Nzogela et al., 2020a; Plowright et al., 1990). The lesions in the root create entry points for and pre-dispose the roots to secondary infection by bacteria or fungi (Patel, 2001). Typical host crops of *P. zeae* are cereals, sugarcane, vegetables, legumes, banana, coffee and fruit trees (Castillo & Vovlas 2007). Unlike RKN and CN which are sedentary, RLN are migratory and less easily seen or found. They don't establish a permanent feeding relationship with their host roots; hence they are the most hidden enemies on plant roots and very difficult to deal with (Fosu-Nyarko & Jones, 2016).

P. zeae can survive in a wide range of rice agro-ecology from upland to flooded (Nzogela et al., 2020a). In Africa, it has been reported in Kenya, Malawi, Zimbabwe, Ivory Coast, Nigeria, Senegal and Tanzania (Coyne et al., 2001; Fortuner & Merny, 1979; Plowright et al., 1990). The above-ground symptoms on rice are

stunted growth, yellowing of the leaves and wilting under severe drought conditions. Below-ground symptoms include root lesions and deterioration due to root rot as a result of secondary infection (Back, 2009; Barbosa et al., 2013). Under severe infestations, roots may show some kind of knotting and branching. When present in rice fields at a population of 30 nematodes per seedling, *P. zeae* may cause a yield loss of about 30% (Prot & Savary, 1993a or b).

The life cycle of *P. zeae* (Fig. 2.10) a migratory nematode, starts from the egg by four moults leading to the adult stage. All stages of these nematodes from the second juvenile (J2) to adult are vermiform and can infect the host. The life cycle takes about 21-25 days, and the reproductive rate is highest between 28°C-30°C (Olowe & Corbett, 1976). *P. zeae* may complete several life cycles leading to several generations within a single rice-growing season (Jones & Fosu-Nyarko, 2014). The mode of reproduction is by parthenogenesis. At egg stage, *P. zeae* can survive in adverse conditions for several years under cryptobiosis or anhydrobiosis state until ideal, or comfortable environment conditions are set (Swanepoel & Loots, 1988). It can move from one field to the other through infested soils, plants and planting materials, irrigation water, mulching, crop residues and human movement from infested areas to clean fields.

2.7.3.2 Root-knot nematodes (Meloidogyne spp)

Root-knot nematodes (RKN) are the most damaging nematodes in most agricultural systems (Coyne et al., 2018; Mantelin et al., 2017; Onkendi et al., 2014). RKN species reported to be associated with rice ecosystems in the tropics *are M. graminicola, M. incognita, M. arenaria, M. javanica, M. salasi*, *M. oryzae* and *M. triticoryzae* (Chandel et al., 2002; Dutta et al., 2012; Ravindra et al., 2017). Yield losses

of about 70% have been associated with *M. graminicola* and *M. incognita*. Of all species, *M. graminicola* is the most devastating pest of rice from flooded to aerobic ecology (Bridge & Page, 1982). It is the perfect example of highly adapted root parasitism (Mantelin et al., 2017). For decades it has been found as a dominant nematode problem in Asia, recently it has been reported in Africa (Chapuis, et al., 2016) and Europe (Fanelli et al., 2017). Infested crops suffer pest injury, and up to 80% yield loss has been reported (Ravindra et al., 2017). The apomictic, mitotic parthenogenetic root-knot nematode species referred to as *Meloidogyne incognita* group (MIG) (*M. incognita, M. javanica* and *M. arenaria* have been reported on rice in different fields including Africa in Ivory Coast, Togo, Nigeria, South Africa, Egypt, Benin, and Niger (Babatola, 1980; 1984; Bridge et al., 2005; Diomandé, 1984). While *M. graminicola* is a vital pest from flooded to aerobic rice, the MIG group is poorly adapted to flooded conditions (Diomandé, 1984). They are a significant economic pest of rice under upland conditions (Kyndt et al., 2014).

Rice fields infected by RKN usually show uneven growth with patches of yellowish/chlorotic stunted crop, plants with poor tiller formation, and poorly filled or no grain formation as above-ground symptoms. Below-ground symptoms include the establishment of galls which differ in appearance depending on the species (Fig. 2.12). *M. graminicola* galls are characteristic hook-shaped formed at the root tip while those of *M. javanica* and *M. incognita* are bluntly and roundish and present on young roots not necessary at root tips (Di Vito et al., 1996; Ibrahim & Handoo, 2018; Nguyễn et al., 2014).

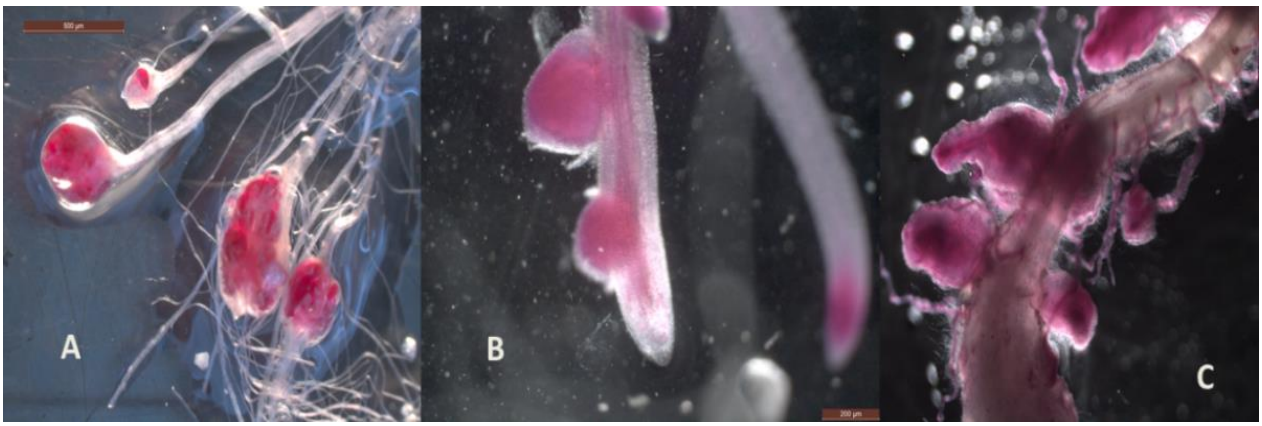

Fig. 2.12: Gall differences between A. *Meloidogyne graminicola*, B & C *Meloidogyne javanica* on susceptible rice genotype, UPLRi-5 Source, Y. B. Nzogela.

The major damage caused by RKN on rice is the effect on the development of the root system caused by multiple galls formed as response to nematode infection. The enlargement of the galls devoid the formation of fine lateral roots and the nematodes withdraw nutrients from the vascular system. These lateral roots play a role in nutrient and water absorption. Failure of that causes loss of vigour, stunting, chlorosis and weak host plant growth and development. At harvest, the infected plants bear empty spikelets which results in low yield.

The life cycle of *Meloidogyne* species (Fig. 2.10) starts from eggs which are laid in the soil or root cortex by matured females. Eggs are encapsulated in a gelatinous matrix secreted from the rectal glands of adult females that keep the eggs together and protects them from microbes/predators and adverse environmental conditions (Moens et al., 2009). First stage juveniles (J1) develop inside the egg, and the first moult occurs inside the egg to second-stage juvenile (J2). J2 hatch from the egg and find a suitable site for infection. Hatching will depend primarily on temperature and moisture although other factors like root diffusate may be involved (Čepulytė et al., 2018; Trudgill, 1997; Wesemael et al., 2006; Yang et al., 2016). J2 is the filiform infective stage which in some cases can survive free in the soil for several months in

the absence of a host. Survival mechanisms involve the deposition of lipid reserves during embryonic development, diapause and quiescence (Elling, 2013; Huang, 1994; Simons, 1973). At these two stages, eggs and J2 of RKN can be transmitted from one field to another through farming equipment, water, infected seedlings, soils or human and animal movements (Bridge et al., 2005). Once J2 has located a suitable host, it penetrates the root tip just behind the meristematic region using the stylet. Nematode stylet movement is accompanied by secretions from the subventral and dorsal oesophageal glands. The secretions contain cell wall degrading enzymes like cellulases, pectate lyase and polygalacturonase to degrade plant cell walls (Haegeman et al., 2013). Inside the rice root, J2 migrate intercellularly to the vascular cylinder where they find suitable sites to initiate a permanent feeding site called giant cells (about six modified host cells per nematode). After settlement, the feeding is commenced instantly, and they become sedentary (Bartlem et al., 2014; Kyndt et al., 2014). *M. graminicola* lays its eggs inside the gall. J2 hatch inside the root cortex. Once hatched it may exit the root to the soil, and find a suitable site for penetration on the same root or different roots, or may re-infect the maternal gall to different places by migrating from the maternal galls through aerenchymous tissues of the cortex to new sites within the same source (Bridge & Page, 1982). Upon feeding the J2 become swollen and sedentary and under favourable environment condition J2 moults to consecutive stages J3 and J4 which are non-feeding stages as they miss a functional stylet. The J4 moult to adult that may be female or male depending on the environment conditions and species. Males when present are vermiform and there is no evidence that they feed (Moens et al., 2009). The life cycle duration differs between species and for *M. graminicola* it takes 18-27 days in a suitable host and optimal temperature while

that of *M. javanica* and *M. graminicola* may take 30-40 days (Fernandez et al., 2014). The reproduction mode of *M. javanica, M. incognita* and *M. arenaria* is obligate asexual reproduction (apomixis mode of reproduction) whereby no sex mixing is involved hence they are called parthenogenetic species. Despite this parthenogenesis, these species are very successful in parasitism and show a geographical widespread (Blanc-Mathieu et al., 2017; Lunt et al. 2014). The study of genomes of these species by Blanc-Mathieu et al., (2017) shows a high level of polyploidy, hybridization and many transposable elements in their genome. The *M. graminicola* mode of reproduction is facultative meiotic parthenogenesis (outomixis) (Castagnone-Sereno et al., 2013).

2.7.4 Emerging nematode problems in rice cultivation systems

With climate change and growth of the human population, scarcity of water and land necessitates a rapid change in farming systems (Talwana et al., 2015). In Sub-Saharan Africa rice intensification is becoming inevitable. The challenges are how to intensify crop production in a sustainable way (Byerlee et al., 2014). Rice crop intensification ultimately favours cultivation without rotation that leads to changes in nematode populations (Karuri et al., 2017). In West Africa, intensification of upland rice was accompanied by a significant increase in *P. zeae* and *H. sacchari* (Coyne et al., 2001).

Global warming resulting in a change of temperature and moisture content of the soil has direct effects on plant-parasitic nematode development and survival (Singh et al., 2013b). It has been reported that with global warming, the geographical distribution range of some plant-parasitic nematodes may expand and this will enhance the spread of plant-parasitic nematodes to newer areas (Amarasena et al., 2016). For instance, lowland rain-fed rice ecosystems usually retain water at a 0.5-20

cm high throughout the rice-growing seasons. Nowadays, the ecosystem is subjected to unusual drought spells that naturally may facilitate/enhance the build-up of the plant-parasitic nematodes that were not favoured under flooded condition (Balasubramanian et al., 2007). In addition to that, shortage of water necessitates shifting from paddy system to aerobic rice system (SRI) (Bezabih et al., 2016). The aerobic rice system has been reported to favour the booming of *Meloidogyne* species in rice fields, for instance, the rice root-knot nematode *M. graminicola* in Asia. These nematodes were initially parasites of upland rice. However, due to water scarcity that subjected flooded/irrigated rice fields to aerobic conditions for a while in the rice-growing periods, promoted *M. graminicola* from minor pest to major pest (Mantelin et al., 2017). *P. zeae,* which have never been reported from the lowland/flooded rice ecosystem, have been detected in all rice agroecosystems in the current study (Nzogela et al., in prep).

Therefore, a holistic approach to address the nematode problems in different rice agroecosystems should take into account the component of drastic climate change that is making a lot of surprises in the fields.

2.7.5 Management options for rice nematodes

There are different approaches which can be deployed by farmers to manage nematodes. These involve traditional nematode management tactics like prevention of new infestation or secondary infestation by using techniques that reduce or if possible, eliminate nematode inoculum or reproduction. These methods include;

2.7.5.1 Prevention

This involves imposing regulatory and restrictive rules as phytosanitary measures to prevent any possible way of introduction of the nematodes through

different materials/ ways from outside or inside the country. It includes documented regulations, legislation or procedures to prevent any introduction or spread of nematodes/pests. The shortcoming of this method is that it requires trained taxonomists and well up-to-date equipped labs for correct, quick and proper nematode diagnosis. Sometimes decision making is very difficult as it involves somehow policy makers and politicians.

2.7.5.2 Cultural control

The efficiency of cultural control measures largely depends on the understanding of the nematode problems, hosts and environmental conditions. The cultural control measures that can reduce the nematode damage to the crop includes crop rotation, hot water treatment, dry heating, multiple cropping, weed control, soil amendments, cover crops and flooding. Setbacks are that proper nematode identification is a problem not only to farmers but also to researchers. This knowledge is highly needed for proper nematode management. (Action, 2014; Collange et al., 2011; Coyne, 2009; Coyne et al., 2018; Dawabah et al., 2019)

2.7.5.3 Chemical control

Nematicides can be used as a component of integrated nematode management or as a sole component. The only needed knowledge is the proper use; when to use, and how? The use of nematicides as a sole application should be advocated when other control measures can't be accessible/useful (Onkendi et al., 2014). When used effectively nematicides have been very efficient to control different plant parasitic nematodes. The disadvantage is their effect on the environment, public health and beneficial organisms (Hydock et al., 2013). In Tanzania a chemical called Velum have been officially registered to be used against RKN.

2.7.5.4 Biological control

This includes the use of living organisms to suppress the population or create unfavorable environment for a specific pest organism, in doing so the pest organism become less abundant and damaging than it would otherwise be. A number of organisms have been used as biological control agents against plant parasitic nematodes. These include fungi, bacteria, viruses, nematodes and other invertebrates (Luambano et al., 2019b). Most of these organisms used as a biocontrol agent against plant-parasitic nematodes are exotic organisms to the soil that might have only a temporary effect due to biological/ecological balance that will take place to balance soil organisms at equilibrium. In that sense the introduced biocontrol agent will be naturally diminished and its role will assume a minor effect to a target. Ijani & Mmbaga, 1988; Luambano et al., 2019a; Prasad et al., 1987; Ralmi et al., 2016; Roberts, 1982; Soriano & Reversat, 2003).In Tanzania a product called Mytech a Nematophagous fungus from Dudutech company have been registered for control of RKN in vegetables.

2.7.5.5 Host resistance

The use of a nematode resistant variety when available, is the most economical and effective way of dealing with nematode problems. Resistant varieties would allow farmers to produce even on heavily infested fields. Its use requires less or little knowledge on rotation and maintenance, no specialized technology required and its use is harmless to the environment. Resistant cultivars reduce the nematode population density for the next crop allowing the best use to be made of the land. The set back is that it is time consuming, searching for and development of the resistant cultivar. For high valued crops, the seeds are very expensive (Gheysen et al., 1996).

Recently, new technologies have emerged, and they are becoming central to the development of sustainable nematode management systems (Collange et al., 2011). These include genetic engineering for host resistance (Ali et al., 2017) and application of soil health biology. The soil health technique should integrate the use of eco-soil friendly farming systems like cover crops, manure and compost, that are antagonistic to nematode pests, minimum tillage to promote and sustain the beneficial microbes and predators while suppressing the plant-parasitic nematodes and other pathogens (Dixit, 2019).

Other advances in nematode management are priming, which has drawn much attention among researchers in crop protection. Priming is the tactic which involves the induced systemic resistance that prepares the plant for more effective activation of defence responses upon subsequent infection with nematodes (Mart et al., 2017). Priming can be done by chemicals, microbes or organic compounds that will enhance plant immunity against the upcoming pathogen attack (Floryszak-Wieczorek et al., 2012; Gillet et al., 2017; Ji et al., 2015; Martinez-Medina et al., 2016). The effectivity of these new technologies for field applications on a large scale remains to be realized.

Of all nematode management options, host plant resistance to nematodes is a practical, economical and environmentally friendly technique. It is compatible with other control options such as biological, cultural and chemical control. The advantage of host plant resistance over all other nematode management options is that farmers virtually do not need any skills in application. Once it is available, resource-poor farmers can use it without cash investment (Cook, 1974). Prot & Rahman (1994) verified that the inclusion of resistant cultivars in nematode control ensures increased

crop yield in case of nematode infested fields. In addition to that, identified host resistance to nematodes can be prioritized over the other mentioned management options because of its economic significance from small scale to commercial-scale farming, and a guarantee for the safety of consumers and environment (Okorley et al., 2018; Quisenberry & Scbotzko, 1994). There has been a great effort on screening for rice resistance to nematodes, though the pace is not yet wide enough to cover the important germplasm (Berliner et al., 2014; Cabasan, 2014; Cabasan et al., 2015; Devi, 2014; Fuller et al., 2008; Rana et al., 2016; Sharma-Poudyal et al., 2004; Win et al., 2014; Zhan et al., 2018). Therefore exploitation of new and elucidation of available sources of rice nematode resistance is of paramount importance.

2.7.6 Host plant resistance and tolerance to nematodes

Over time plants have evolved robust defence mechanisms against different pathogens. Resistant host plants do not allow or inhibit nematode penetration and reproduction, while susceptible host plants allow normal nematode reproduction and development (Giebel, 1982). In another scenario, a susceptible plant may be tolerant such that it endures the consequences of nematodes attack but with little damage symptoms (Boerma & Hussey, 1992). Resistance can happen at different levels as partial and high levels (Peng & Moens, 2003). Development and reproduction of nematodes in resistant host plants can be delayed/retarded or wholly blocked (Abad & Williamson, 2010). Resistant plants may interfere with the ability of the nematode on host finding and allocation, penetration of the host plant root tissues and initiation of feeding sites in case of sedentary nematodes and migration and feeding in case of migratory endoparasitic nematodes. Therefore, host plant resistance can act before and after nematode root penetration (Webster, 1975).

The characteristics of a plant involved in a plant-nematode interaction follow these terminologies: resistance/susceptibility on the one hand and tolerance/sensitivity on the other hand. These terminologies are defined as independent, relative qualities of a host plant, based on a comparison between plant varieties. A host plant may either suppress (resistance) or allow (susceptibility) nematode development and reproduction; it may suffer either slight injury (tolerance), even when heavily infected with nematodes, or many injuries (sensitivity), even when is lightly infected with nematodes (Cook, 2004).

2.7.7 Mechanisms of resistance against plant-parasitic nematodes

Plant defence against pathogens is triggered by pattern recognition receptors (PRRs) located on the cell surface (Fig 2.13) (Jones and Dangl, 2006).

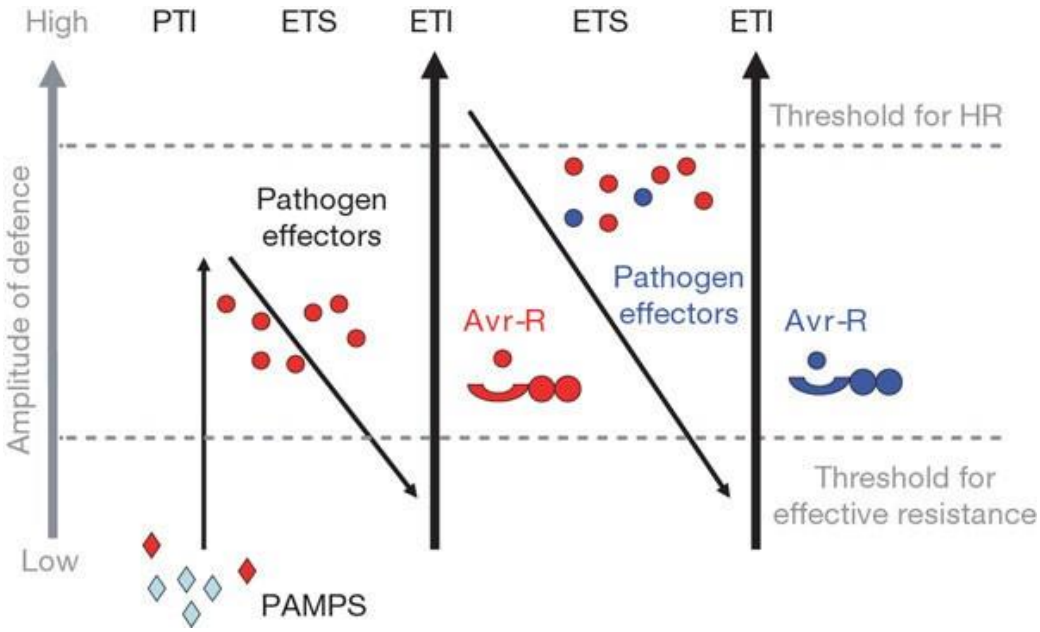

Fig. 2.13: A zigzag model to illustrate the quantitative output of the plant immune system (Adapted from Jones and Dangl, 2006).

PRRs recognise the conserved pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) from the disrupted host tissues (Henry et al., 2012; Hou et al., 2019). When PAMPs/DAMPs are detected this

leads to Pattern-Triggered Immunity (PTI). PTI is, therefore, innate or basal resistance host defence response as a result of PAMPs or DAMPs recognition by their respective pattern recognition receptor (PRRs) (Boutrot and Zipfel, 2017). PPN attacks, like other pathogens, can result in PAMPs/DAMPs that lead to PTI (Sato et al., 2019; Mendy et al., 2017). PTI may halt further nematode host colonization through the production of reactive oxygen species (ROS) and cell wall re-enforcement. The pathogens, including nematodes, may suppress PTI for their survival by producing effectors that lead to effector-triggered susceptibility (ETS) (Jones and Dangl, 2006). However, these effectors may be recognized by specific proteins from the host (R-proteins) (Przybylska & Obrępalska-Stęplowska, 2020). Once pathogens effectors are recognized this leads to effector-triggered immunity (ETI). ETI is very effective in disease/ nematode resistance and it amplifies the host defense state leading to hypersensitive cell death responses (HR) (Jones and Dangl, 2006). In nematodeshost-interaction, different R-genes have been identified and cloned. However, ETI can again be suppressed by pathogens or nematodes effectors leading to effector-triggered susceptibility that allows the pathogen or nematode establishment, development and reproduction (Jones and Dangl 2006).

There are two well-categorized resistance mechanisms of plants against plant-parasitic nematodes. The first is passive resistance which involves the preformed layers of barriers (defence) that may be physical barriers or chemical barriers. This first layer of resistance is constitutive, and most of the times, prevent the nematodes from migration to or penetration of the host roots (Giebel, 1982). It may involve cell wall lignification, production of compounds which might be toxic to the nematodes or interfere with nematode mobility and activity (Tsunoda & van Dam,

2017). For instance, α-terthienyl and derivatives of biphenyl from *Tagetes patula* L. and *Tagetes erecta* L. have been found to limit *Meloidogyne* and *Pratylenchus* infection (Sikder & Vestergård, 2020).

The second mechanism of host resistance is the active or induced resistance. In this case, the defence is activated through signals triggered by the parasitic nematodes upon infection (Erb et al., 2009; Goverse & Smant, 2014; Holbein et al., 2016; Peng et al., 2018). The outcomes of active defence will depend on the availability of the corresponding defence genes, the timing of the action and the presence of specific effectors in the nematode. The cascade of genes activated upon nematode infection has also been studied in rice (Kumari et al., 2016; Kyndt et al., 2012b). Dramatic changes in metabolite profiles, changes in hormonal pathways, induction of oxidative burst and lignification are some of the host responses upon nematode infection (Holbein et al., 2016; Kyndt et al., 2012a&b; Mendy et al., 2017; Peng et al., 2018). These responses may either strengthen the barriers against the invading nematode or weaken and destroy the invading nematodes. The degree of the outcome strength of the host against the nematodes determines the host susceptibility/resistance (De Almeida Engler et al., 2005; Motalaote et al., 1983; Williamson & Kumar, 2006). This induced defence may take place locally to contain the parasite from development and spread or systemically that calls upon different host defence weaponry away from the infection sites (Sato et al., 2019). The local defence is a speedy response of the host cells that are close to the nematodes and collapse in the form of programmed cell death or the so-called hypersensitive reaction (Kaplan & Keen, 1980).

Systemic defence takes part by sending the signals to the cells far away from the localized battlefield so that cells should keep watching out for the subsequent attack, and this is called Systemic Acquired Resistance (SAR) or induced systemic resistance (ISR) (Vallad & Goodman, 2004). The distal cells accumulate pathogenesis-related proteins with microbial activity or inhibitory proteins.

In an incompatible interaction, the hypersensitive response-cell death can inhibit nematode migration and block the formation and development of feeding sites. The HR host response may extend its effects to degradation of the feeding site neighbouring cells, which cause inadequate nutrient flow/supply for nematode development. The outcome of this response is the death of nematodes or escape of the nematodes out of the root (if not yet initiated the feeding sites) and further inhibition of nematode development and reproduction. This type of response has been observed in early *O. sativa* interaction with *M. graminicola* (Phan et al., 2018) and late *O. glaberrima* interaction with *M. graminicola* (Petitot et al., 2017).

Besides, nematode infection induces the production of secondary metabolites. These metabolites are called phytoalexins. They may act as nematode repellents, inhibitors of nematode motility, and be nemastatic or nematicidal (Atkinson, 2011; Dhakshinamoorthy et al., 2014; Rupcic et al., 2018). The constitutive presence of some secondary metabolites called phytoanticipins may also make the host root hostile for the nematodes. An excellent example of such kind of metabolites is phenylphenalenone anigorufone which was found in a Musa cultivar resistant to *Radopholus similis*. The metabolites were highly accumulated in un-infected roots and were localized inside the nematode body where they formed large lipid-anigorufone complexes that are toxic to the nematode (Hölscher et al., 2014).

Mechanisms of resistance against sedentary nematodes have been well documented. For example what is happening in the feeding sites induced by *M. graminicola* on rice documents the types and mechanisms of resistance at different time points of infection throughout the sedentary nematode life cycle (Ji et al., 2013; Kumar et al., 2014; Petitot et al., 2017; Zhou et al., 2020). However, host responses to *Pratylenchus* spp are poorly known. Linsell et al. (2014b) studied the interaction between *P. thornei* and two wheat cultivars (*Triticum aestivum*) with contrasting responses to these nematodes. They deduced that the mechanisms of resistance were post penetration, namely suppression of nematode migration in the roots, egg deposition, hatching and multiplication. Root extracts and root exudates from the resistant cultivars compared to the susceptible ones, clearly showed that the resistance mechanisms were constitutive and species-specific. The studies by Vieira et al. (2019) on the interaction between *P. penetrans* and *Medicago sativa* L. revealed the same pattern of resistance that constitutive defence plays a vital role to keep the host active against the invading nematodes and the induced protection adds on for enhanced resilience. Their study highlighted that the resistance mechanisms observed were quite different from those to other PPN on the same host and genes involved in the biosynthesis of secondary metabolites from the phenylpropanoid pathway were essential for the observed resistance.

2.7.8 Resistance genes against sedentary and migratory nematodes

Plant natural resistance to different pathogens including nematodes can be controlled by a major gene (single dominant *R*-gene) hence the state called monogenic/plant *R* –gene-mediated / vertical resistance. To the other side, when several minor genes control the resistance, it is called polygenic/horizontal resistance.

R-gene mechanisms are well elaborated in these reviews: Dangl & Jones (2001) and Jones & Dangl (2006).

Briefly, the mechanisms of an R-gene resistance are variants from hypersensitive responses (HR), going from no feeding site initiation to late necrosis and degeneration of feeding sites. So far, a well-characterized *R*-gene for nematode control that has been usefully applied in the field is the *Mi*-gene (Barbary et al., 2015). The *Mi* -gene was isolated from a wild tomato plant *Solanum peruvianum* (Williamson, 1998). It confers resistance to the most notorious tropical RKN species so-called MI-lineage, *M. incognita, M. javanica* and *M. arenaria*. The wild tomato species *Solanum peruvianum* is incompatible when crossed with the cultivated tomato *Solanum lycopersicum* syn. *Lycopersicon esculentum*. The introgression began with embryo rescue of a single interspecific cross. Luckily the hybrid was carrying the resistance gene, which was then cloned to facilitate the backcross to *S. lycopersicum* (Williamson, 1998). The backcrosses continued, and the *Mi*-gene has been widely introduced in commercial tomato varieties. To date, there are about 10 *Mi* -genes identified as *Mi-1*, *Mi-2*, *Mi-3*, *Mi-4*, *Mi-5*, *Mi-6*, *Mi-7*, *Mi-8*, *Mi-9*, and *Mi-HT* of which only five genes (*Mi-1, Mi-9*, *Mi-HT* and *Mi,3*, *Mi-5* have been mapped on chromosomes 6 and 12 respectively (El-Sappah et al., 2019).

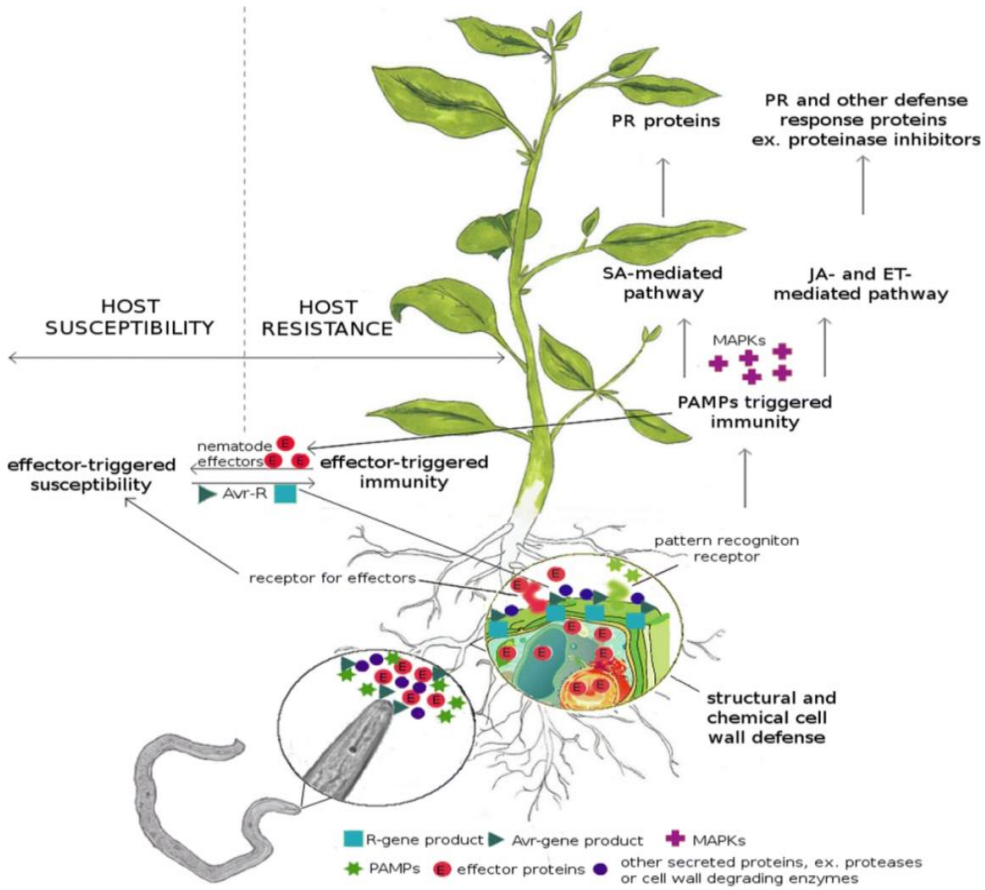

Fig. 2.14: The mechanism of natural resistance against (RKN) infection. PR proteins – pathogen-related proteins, SA – salicylic acid, JA – jasmonic acid, ET – ethylene, R gene – plant resistance gene, avrgene-gene encoding an RKN avirulence factor, MAPKs - mitogen-activated protein kinases, PAMPs - pathogen-associated molecular patterns. Adapted from Przybylska & Obrępalska Stęplowska (2020).

In susceptible plants, where there are no *Mi*-genes, the nematode completes its life cycle in the root by forming giant feeding cells. In the resistance case, the plant undergoes the first defence line against RKN penetration by the interaction between extracellular receptor proteins, receptor-like kinases (RLK), receptor-like protein (RLP), and nematode effectors. (c) The plant then begins the second defence line, which includes direct gene-for-gene interaction. This theory depends on direct interaction between the receptor protein of tomato and a nematode effector, which prevents the nematode from feeding. No giant cell formation is observed. (d) The other second defence line is an indirect pathway, which is referred to as the guard

hypothesis. In these cases, the virulence factor of the nematode (*Avr* genes) interacts with tomato accessory protein which is the case for *Mi*- gene.

In rice root-knot nematode (*M. graminicola*) interaction studies, natural resistance has been found in *O. sativa* genotypes. Dimkpa et al., (2016) identified two rice genotypes LD 24 (an indica from Sri Lanka) and Khao Pahk Maw (aus) that were shown clearly to be hardly infected by the devastating rice RKN. QTL-seq analysis of the bulked segregants revealed the location of the locus at chromosome 11 for both genotypes (Lahari et al., 2019).

Another promising source of exceptional natural resistance was identified in a study by Zhan et al. (2018) whereby a population of rice genotypes from *Oryza sativa* (aus, hybrid aus, indica, hybrid indica, temperate japonica, tropical japonica) were screened against *M. graminicola*. Zhonghua 11 (aus), Shenliangyou 1 (hybrid aus) and Cliangyou 4418 (hybrid indica) were highly resistant to *M. graminicola.* The resistance was confirmed in a screenhouse experiment and under field conditions. The resistant genotypes were galled less, and the penetrated J2s failed to develop into females. Further analysis of the mechanisms of resistance and genetic background of the backcross from Zhonghua 11 revealed the involvement of a dominant resistance gene (Phan et al., 2018).

However, all single resistance genes (*R*-genes) that have been identified in different herbaceous plants so far confer resistance against sedentary endoparasitic nematodes; RKN and CN (Roberts, 1995). Natural resistance to *Pratylenchus* sp is rarely found. Studies that have identified natural resistance to RLN (migratory endoparasitic) shows that QTLs commonly determine resistance/tolerance, and to date, none have been cloned (Linsel et al., 2014a). Few loci have been identified linked

to some *Pratylenchus* resistance or tolerance especially in wheat (*Triticum aestivum* L) and barley (*Hordeum vulgare L*) (Galal et al., 2014; Jayatilake et al., 2013; Linsell et al., 2014a; Williams et al., 2002). Sawazaki et al. (1987) found two dominant genes with additive effects in the maize line Col 2 (22) conferring resistance to *P. brachyrus* and *P. zeae.*

Resistance to root-lesion nematodes may involve the production of compounds that deter the nematodes from penetration and suppress the nematodes' motility and migration after infection (Linsell et al., 2014b; Sikder & Vestergård, 2020). Some of the compounds may be toxic to the nematodes such as phytoalexins and proteins that constitute a general defence of the resistant plant (Zacheo et al., 1997; Zinov'eva et al., 2004). Baldridge et al. (1998) noted high mRNA levels of genes in the phenylpropanoid pathway in a *P. penetrans* resistant alfalfa compared to a susceptible cultivar.

Therefore, the few findings on genetic and biochemical resistance to root-lesion nematodes have only shown a polygenic nature of resistance to root-lesion nematodes. These findings show how complicated it is when it comes to designing a functional nematode management programme especially for RLN taking into account that in the field the single host can be attacked by different species of plant-parasitic nematodes with varying levels of pathogenicity.

2.8 CONCLUSIONS FROM A REVIEW OF THE LITERATURE

This chapter has discussed the nematode problems in rice and the way they have been managed in different rice agroecosystems. Natural genetic host resistance is a useful means of nematode management. Identification of a natural, reliable source of nematode resistance and introgression of the identified sources of resistances into

the elite cultivars especially for RLN remains a core mission to accomplish. Rice-nematode interaction studies step out as a critical issue to understand the mechanisms of resistance. In that context, sedentary nematode-host interactions have been more detailed with than root-lesion nematodes.

So far PPN problems in rice cultivation have not been addressed in Tanzania. Rice researches have been focussed on crop improvement and production constraints, including other pests and diseases than nematodes. The symptoms of nematode infection are not unique such that they can be confused with other problems like nutrient deficiencies, drought, bacterial or fungal diseases. Generally, in low-income countries like SSA, nematode problems are underestimated. More importantly, rice in the field is often subjected to a complex of nematode problems which makes the management strategies very difficult. Minimum laboratory capacities in terms of equipment and nematology expertise are required for proper nematode problem characterization such as diagnosis, continuous nematode surveillance and formulation on nematode management strategies.

Tanzania national rice strategy is to increase rice production at a given unit of cultivated land that is rice intensification. With climate change, rice intensification will possibly lead to a rise of minor pests into significant pests, and unseen problems will be aggravated. Therefore, the current study will largely contribute to the knowledge on plant-parasitic nematode problems in rice and their interaction with the host that will build the base for proper development of plant-parasitic management strategies.

CHAPTER 3: THE OCCURRENCE OF PLANT-PARASITIC NEMATODES IN DIFFERENT RICE AGRO-ECOSYSTEMS IN MOROGORO AND MBEYA REGIONS, TANZANIA

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Manuscript in preparation

Author's contributions;

YN, AL-K, DC, WB LB and GG designed the study and methodology.

YN, excuted the study, analysed the data and wrote the manuscript.

GG edited the manuscript.

3.1 ABSTRACT

A diagnostic survey of (PPN) in rice fields was conducted in rain-fed upland/lowland and irrigated lowland agro-ecosystems in Morogoro and Mbeya regions in Tanzania. Frequency of occurrence and abundance of PPN associated with rice were determined to assess their potential threat to rice production. From 19 villages 190 rice root and soil samples were collected when the plants were at early tillering or flowering stage. PPN genera recovered were *Pratylenchus*, *Hoplolaimus*, *Meloidogyne*, *Helicotylenchus*, *Tylenchorhynchus*, *Trichodorus*, *Hemicycliophora*, *Cliconemoides* and *Xiphinema*. The most important genera found were *Pratylenchus, Hoplolaimus* and *Meloidogyne*. After species identification, it was seen that *P. zeae* predominated throughout the fields and agroecosystems with the highest population densities and frequency of occurrence (100%). *Criconemoides* was the least common, with minimal presence in upland rice ecosystem and low densities. *M. arenaria* was

detected only in soil from lowland irrigated fields. Upland fields had less diverse PPN populations but with higher densities than lowland and irrigated rice fields. *Xiphinema* was most prevalent in soils from lowland rice fields. The prevalence of *P. zeae* suggests significant parasitic potential in rice fields in Tanzania. Based on its distribution across the rice ecosystems, its potential for damage to rice production is highest in upland rice agro-ecosystem to lowland and rice agro-ecosystem. Under the lowland ecosystem, rain-fed fields are more prone to these nematodes than irrigated fields.

3.2 INTRODUCTION

Rice is the second most important food crop after maize in Tanzania and considered to be of dual purpose as a significant source of household income and food security (URT, 2019). The demand for rice in Tanzania reached 2.05 million tonnes in 2018, and it is projected to increase by 2.9% during the next five years to 2.27 million tonnes (Suleiman, 2018). In Tanzania, rice is grown all over the country, but the central producing regions are Shinyanga, Morogoro, Mbeya, Mwanza and Rukwa (Ngailo et al., 2016). The production potential varies among the agroecosystems (Kahimba et al., 2014; Katambara et al., 2013). In Tanzania, rice is grown in three major rice ecosystems, rain-fed upland, and rain-fed lowland and irrigated lowland. Each rice ecosystem is characterized by a relatively low yet different production potential depending on soil fertility and climatic conditions (Kato, 2007). For more detailed agro-ecology of rice production in Tanzania, the reader is referred to section 2.5.2. In most rice-growing areas, especially under paddy fields, rice is grown traditionally using the commonly adopted agronomic practices of rice cultivation which involves transplanting manually or seeding by broadcasting.

Despite the significant potential in terms of productive lands where rice is grown, farmers are still producing far less than the attainable yields. The yields are averaging between 1.5-3.5 t ha^{-1} while the estimated potential is between 4 and 5 t ha^{-1} (Kato, 2007). The leading causes of low yield are biotic and abiotic stress, lack of improved varieties and low soil fertility (Sekiya et al., 2017). Among the biotic stresses facing rice productivity, are pests and diseases, including nematodes (Bridge et al., 2005).

In Africa, especially West Africa, many genera of plant-parasitic nematodes are known to be associated with rice. However, *Meloidogyne* spp., *Heterodera sacchari*, *Pratylenchus* spp., and *Hirschmanniella* spp are the most frequent and damaging species (Bridge et al., 2005; Coyne et al., 1998, 1999b, 2001; Plowright et al., 1999). Recently the root-knot nematode *M. graminicola* has been reported in Madagascar (Chapuis et al., 2016). Babatola (1984) documented several genera and species of plant-parasitic nematodes in rice fields of the Southern part of Nigeria under the upland rain-fed system, and the most important species were *Pratylenchus brachyurus*, *M. incognita*, *M. javanica* and *M. arenaria*. The two latter species were co-found in upland rice fields. When 8000 eggs and larvae / L of the soil of *M. incognita* were inoculated on rice, a grain yield reduction of more than 60% occurred under controlled glasshouse environment (Babatola, 1984).

In Tanzania nematode problems have been reported on different crops including banana (Luambano et al., 2019a; Mduma et al., 2018; Mgonja et al., 2020) vegetables (Missanga & Rubanza, 2018; Nono-Womdim et al., 2002), coffee (Janssen et al., 2017) and sugar cane (Singh et al., 2020). Unfortunately, problems due to plant-parasitic nematodes may be heightened by the lack of awareness among farmers due to the microscopic nature of nematodes and the lack of typical plant symptoms. To

make the problem even more complicated, one crop within a field may be affected by multiple nematode species and their interactions with other pathogens (De Waele & Elsen, 2007).

A field survey is the first component for a nematode management strategy. You can't fight and win the enemy if you do not have full information on it. Surveys enable (i) early detection and controlling the spread of the nematode problems (ii) identifying non-infested fields that can be useful for crop production and meet the exportation requirement of international trade and (iii) lastly formulation and implementation of proper nematode management strategies. There are three categories of the survey that are commonly carried out in nematology. (i) Diagnostic /detection survey which reveals what species/genera of nematodes are associated with which host. However, it should be noted that failures to detect a certain nematode species of interest does not guarantee the absence of such a species but only means that the population of such nematode species is below detection level (Prot & Ferris, 1992). (ii) Monitoring survey which is for a check-up on the population buildup of a certain nematode species over time at a given site/field for keeping the population below the threshold through proper nematode management (Dixit, 2019). (iii) Delimiting surveys which help to establish the boundaries of an area considered to be infested by or free from a nematode pest (Singh et al., 2013a).

Smallholder farmers characterize rice farming in Tanzania although the country is endowed with extensive landscapes suitable for rice production coupled with a tremendous domestic and potential foreign market. Therefore, there is an urgent need to enhance the yield as a key to promote food self-sufficiency and security from household to national and international levels. Adding to that, strategies to combat

factors that may hinder rice productivity should be addressed. A diagnostic survey on nematode problems associated with rice was conducted to identify the species that might hamper rice productivity in Tanzania.

3.3 MATERIALS AND METHODS

3.3.1 Diagnostic survey of plant-parasitic nematodes in rice agroecosystems

3.3.1.1 Study area

Three districts in Morogoro region, namely Kilombero, Mvomero, and rural Morogoro, and one district Kyela in Mbeya region were selected for the current study (Fig. 3.1). Morogoro region samples were taken from three rice growing systems rain-fed upland, lowland and irrigated lowland, but in Mbeya region, only upland rice fields were sampled. These regions represent major rice-producing areas in Tanzania, and they differ in agro-ecological conditions as can be seen in table 3.1.

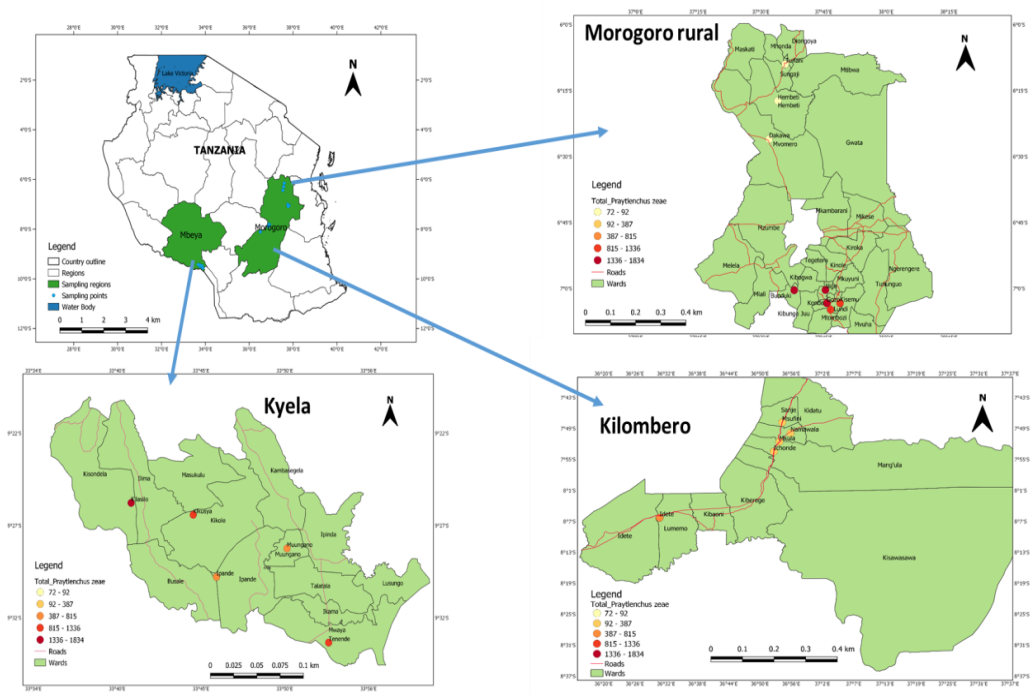


Fig. 3.1: Study area

Table 3.1: Study area geographical location

District	Longitude	Latitude	Altitude (M)	Temperature (ºC)	Rainfall (mm)
Kyela	9°25’ - 9°40’S	33°41’-33°30’E	500-1615	23ºC - 28ºC	2000-3,000
Kilombero	10°00'S - 08°40'S	35°10'- 37°22'E	290-2,600	26ºC - 32ºC	1,200-1,600
Mvomero	05° 80′ - 07°40′S	37°20 -38°05′E	300 - 400	25ºC -33ºC	800 - 2000
Morogoro rural	06°60’ - 7°29’	37°35’ -38°30’E	400 -1000	18ºC-26.9ºC	1500-1800

3.3.1.2 Sampling

Composite samples of soil and plant with its intact roots were collected from a total of 190 rice fields. Fields were selected based on access by road during the farming season (Fig. 3.1). Sampling was done when plants were at tillering or booting stage except in Matombo Morogoro region where samples were taken five weeks after seeding. Ten rice fields were selected from each village for sampling. The selected fields were at least 50 m apart. From each field, one composite sample of 10 subsamples of root and soil was collected from within the rows in a zigzag pattern 10-30 cm deep using narrow-blade shovel/ soil auger. A total of 190 soil and root samples were analyzed to provide a representative survey across the regions. The field area sampled varied from one third to one hectare. The soil and root samples were (when possible) placed in plastic bags, labelled and transported to the laboratory, analyzed for the occurrence of different root and soil rice nematodes

3.3.1.3 Nematode extraction

Nematodes were extracted using a modified Baermann funnel technique (Coyne et al., 2014) from 10 g of roots and 500 ml of soil. Roots from each sample were separated from soil, washed, dabbed dry, finely chopped and mixed thoroughly before subsampling. A subsample of 10 gram chopped roots was taken and macerated with 25 ml distilled water in a small laboratory blender, three times for 15

seconds at low speed, and the resultant mixture was incubated for 24 - 72 h. Soils were mixed thoroughly before subsampling, and a sample of 500 ml was processed using a combination of sieving and decanting followed by incubation under a modified Baermann funnel for 24 h. When applicable, (when soil is not muddy) soils were incubated for 72 h under modified Baermann funnel technique.

3.3.1.4 Nematode density and prevalence value

Nematodes were identified to the genus level and counted to determine their densities. The nematode suspensions were concentrated to 20 ml using an 18-µm sieve, homogenized, and 2 ml were sampled poured in a counting dish of 100 squares. 20 squares out of 100 located within the two principal diagonals of the counting dish were chosen, and nematodes in those squares were identified to genus level and counted under Leica compound microscope (Leica MZ13, Nussloch Germany). The counts were expressed as the number of nematodes per genus per 10 g of fresh root weight and the number of nematodes per litre of soil. Several individuals from the most dominant genera were hand-picked and fixed on slides for further identification to species level.

Prevalence of nematode genera was analyzed based on two factors: frequency and abundance per agro-ecosystem. Frequency and abundance were determined according to limits established by Fortuner & Merny (1979). The frequency (F) of a nematode genus was calculated from the number of positive samples with that genus divided by the number of total samples collected and expressed as a percentage. A nematode genus observed in at least 30% of the samples was regarded as frequent.

Abundance was determined as the summation of nematodes in 10 g of root (N/g) or soil (N/L) samples containing that genus or species, divided by the number of positive samples for that genus or species and expressed as decimal logarithm (log (x+1)).

A nematode genus/species was regarded as abundant within roots when the value was ≥ 1.3 (≥ 20 individuals/g of roots) or soil value ≥ 2.3 (≥ 200 individuals/1,000 cm^3 of soil).

3.3.1.5 Nematode morphological and molecular identification

Selected specimens from all plant-parasitic nematode genera from each agro-ecosystem were mounted on slides. The samples were processed following the glycerine-ethanol method (Ryss, 2017) and they were identified to genus level under a light microscope compound Olympus (BH-2 Japan) based on morphological features with the aid of identification key by Mekete et al. (2013). The most prevalent genera were identified to species level based on morphology, morphometric and molecular analysis. *Hoplolaimus pararobustus* identity was made solely based on the key (Handoo & Golden, 2000). No morphometrics was taken from *H. pararobustus* specimens, and no molecular studies were made for this genus. The slides with the identified genera were also sent to the Plant Protection Institute of South Africa for confirmation by taxonomic experts.

Identification of the *Pratylenchus spp* and *Meloidogyne spp*

Morphological identification of *Pratylenchus zeae*

Individuals from each sample were hand-picked from a suspension and mounted on slides for observations. From each sample, individual gravid females were hand-picked and inoculated on carrot discs for pure culture initiation. Descriptions were based on mature females. Characters commonly used to diagnose the species

are presence/absence of males, the number of head annules, shape of the head, shape of the spermatheca, length and structure of posterior uterine sac (PUS), shape of female tail and terminus, body length, stylet length, the shape of stylet knobs and structure of the lateral field. The morphological characterizations were based on both original descriptions from Castillo & Vovlas (2007) and polychromous identification keys from Ryss (2002). Morphometric measurements were done using Image J (Abràmoff et al., 2004).

Molecular identification of *Pratylenchus zeae*

P. zeae individuals were handpicked from pure cultures per carrot disc, and DNA was extracted using the proteinase K protocol as described by Subbotin et al. (2008). PCR was carried out according to Tanha Maafi et al. (2003) to amplify two regions of the nematode genome. The D2-D3 expansion segment was amplified with the forward D2A and the reverse D3B primers, and the Cytochrome C oxidase subunit 1 (COI) gene was amplified using JB3 and JB4.5 primers (Table 3.1). PCR reactions were purified using a DNA purification kit (Thermo Scientific-Germany) and sent to the commercial company LGC genomics (Germany) for sequencing.

Table 3.2: List of amplified nematode genomic regions and their corresponding primers

Nematodes sp	Target region	Primer used	Reference
Meloidogyne arenaria	NADH dehydrogenase subunit 5 (NAD5F)	NAD5F (5'-TATTTTTTGTTTGAGATATATTAG-3') NAD5R (5'-AAAAATCCCCTCGAAAAATCCACC-3')	Janssen et al. (2016)
	Species specific Sequence Characterized Amplified Region (SCAR)	MI-F (5'-GTGAGGATTCAGCTCCCCAG-3') MI-R (5'- ACGAGGAACATACTTCTCCGTCC-3')	Adams et al. (2007)
		Far(5'-TCGGCGATAGAGGTAAATGAC-3') Rar (5'-TCGGCGATAGACACTACAAACT-3')	
		Fjav (5'-GGTGCGCGATTGAACTGAGC-3') Rjav (5'-CAGGCCCTTCAGTGGAACTATAC-3')	
Pratylenchus zeae	COI gene	JB3: (5'-TTTTTTGGGCATCCTGAGGTT TAT-3') JB4:(5'– TAAAGAAAGAACATAATGAAAATG-3')	Derycke et al. (2010)

Nematodes sp	Target region	Primer used	Reference
	D2D3 rDNA expansion region of 28S	(5'-ACA AGT ACC GTG AGG GAA AGT TG -3') (5'- TCG GAA GGA ACC AGC TAC TA -3')	De Ley et al. (1999) Al-Banna et al., (2004)

For phylogenetic analysis, 21 D2-D3 sequences of *P. zeae* populations were downloaded from the GenBank, including two sequences of *Rotylenchus buxophilus* that were added as outgroup. The obtained sequences for the amplified genes were aligned using Clustal Omega (clustalo) software on the EBI server with FASTA (Pearson) as an output format (http://www.ebi.ac.uk/Tools/msa/clustalo/). The curation and automatic sequence edit were done in Gblock. The edited sequences were converted to nexus format using the Forcon http://www.es.embnet.org/embnet_common/embnet.news/vol6_1/ForCon/forcon.html. Model selection for the 28S gene was made using JModelTest2- 2.1.10 (Posada, 2008) based on Akaike Information Criterion (AIC). The nexus files were analysed using Bayesian inference (BI) in Mr Bayes v3.2.6 x 64 with The General Time reversible (GTR+G) substitution model (Ronquist et al., 2012). Markov Chain was sampled at intervals of 1000 generations. Two runs were conducted, and BI was run with 5000000 generations. The burning was 25% discarded, and tree topology was used to generate a 50% majority-rule consensus tree. Obtained phylogenetic consensus trees were visualized in Figtree software V.1.42 (http://tree.bio.ed.ac.uk/software/figtree/) with posterior probabilities.

Morphological identification of *Meloidogyne sp*

Field soil samples, in which *Meloidogyne sp*. was detected, were used to inoculate individual tomato (*Solanum lycopersicum.* cv. Carl J) plants in a sterile 500 cm^3 plastic pot and the cultures were maintained for 60 days in a screenhouse. Each village composite sample had 6 pots. For further studies, pure single species cultures

were established from a single egg mass hand-picked from each infested tomato plant and inoculated to a new tomato plant. These plants infested with a single egg mass were maintained for two months. Root-knot nematode species identifications were based on mature females taken from the established pure cultures. Twenty females per sample were hand dissected from the infected galled tomato roots and put on a glass slide one by one in a drop of water to observe the perineal pattern. The posterior end of each female was squashed. The coverslip was gently sided to exact turn on the perineal position. After having the perineal position at the right point, the pictures were taken under x20 using a camera mounted on the compound microscope Olympus (BX 50, Japan). Perineal patterns were characterized following Eisenback et al. (1981) and Jepson (1983) morphological keys. Second-stage juveniles were placed on a slide in a drop of water, immobilized by heat, covered with a glass coverslip and identification was based on both morphological description and morphometric measurements (Van Den Berg et al., 2016, 2018). All microscopic observations were done under an Olympus BX50 DIC Microscope (Olympus Optical), equipped with an Olympus C5060Wz camera. Morphometric measurements were done using Image J (Abràmoff et al., 2004).

Molecular identification of *Meloidogyne sp*

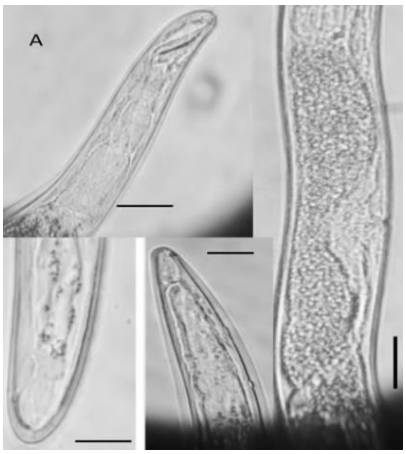
DNA was extracted using the protocol adapted from Tanha-Maafi et al. (2003). A single mature female picked from the tomato pure culture was transferred to an Eppendorf tube containing 12 µl worm lysis buffer (500 mM KCl, 100 mM Tris-Cl pH 8, 15 mM $MgCl_2$, 10 mM DTT, 4.5% Tween 20) and squashed with a pipette tip. Two microlitres proteinase K (600 µg/ml) were added, and the tubes were frozen at -80ºC for at least 10 min and then incubated at 65ºC for 1hr and 95ºC for 10min

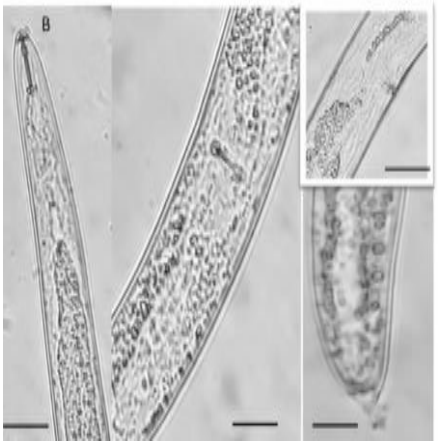
consecutively. After incubation, the tubes were centrifuged for 2 min at 14000 rpm and kept at -20ºC until use. Molecular analyses were performed on three DNA regions using the primers in Table 3.1. First, the Internal Transcribed Spacer (ITS) region was amplified using universal primers. Second, the species-specific Sequence Characterized Amplified Regions (SCAR) were amplified using MI-F/MI-R, Fjav/Rjav, Far/Rar primers for *M. incognita*, *M. javanica* and *M. arenaria* respectively (Adam et al., 2007) and lastly, confirmation was done by using the mitochondrial NADH dehydrogenase subunits 5 (*Nad5*) region (Janssen et al., 2016). Polymerase chain reaction (PCR) amplification was carried out using the standard Taq DNA polymerase mixture (Qiagen, Germany), with two µl DNA extract and 1 µl of each primer in a total volume of 30 µl. The PCR amplifications were done using a T100 Thermal Cycler (Bio-Rad), conditions were according to Adam et al. (2007) and Jansen et al. (2016) with minor modifications. Initial denaturation for 2 min at 94°C, followed by 45 cycles of 30 sec at 94°C, 30 sec at 64°C, 90 sec at 72°C, and finally an extension for 7 min at 72°C. PCR products were electrophoretically separated on a 1% agarose gel in TAE buffer at 100V for 30 minutes and stained with ethidium bromide followed by visualization on a UV transilluminator. Successful PCR reactions were purified using a DNA purification kit (Thermo Scientific) following the manufacturer's protocol and sequenced by LGC Genomics in Germany. Sequences were read and assembled in BioEdit. The sequences under study were compared with available online sequences of *Meloidogyne* and aligned in Clustal W. Species identity was confirmed based on species-specific sequences of mitochondrial NADH dehydrogenase subunits 5 (*Nad5*) region after alignment with reference sequences according to Janssen et al. (2016).

3.4 RESULTS

3.4.1 Nematode inventory

Nine plant-parasitic nematodes genera were found to be associated with rice, in upland and lowland rice-ecosystems representing the main rice-growing regions in Tanzania. These include *Pratylenchus, Hoplolaimus, Meloidogyne, Helicotylenchus, Tylenchorhynchus, Trichodorus, and Hemicycliophora, Criconemoides* and *Xiphinema* in different densities (Table 3.3A & 3.3B) and some of the nematodes genera are presented on Figure 3.2. Within the investigated rice fields, the genus *Pratylenchus* showed the highest (123/g of root and 708/1000ml soil) densities in all rice agroecosystems. The rice fields most populated with the genus *Pratylenchus* were from the upland agro-ecosystem. Here we report the presence of *P. zeae, M. arenaria* and *H. pararobustus.*





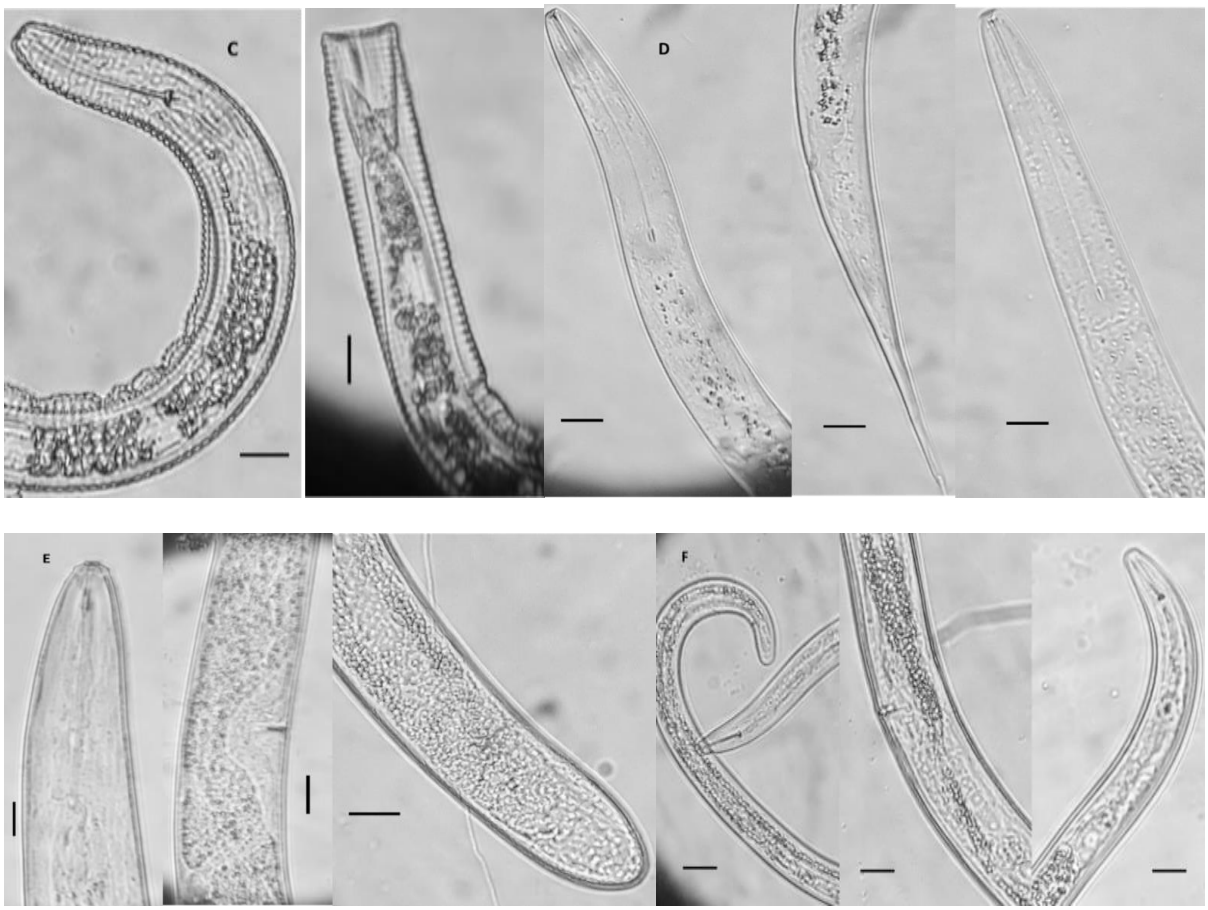


Fig. 3.2: Morphology of some of the PPN genera recovered from the rice-growing ecosystems in Tanzania. A. *Trichodorus*, B. *Helicotylenchus*, C. *Criconemoides*, D. *Hemicycliophora*, E. *Hoplolaimus,* F. *Tylenchorhynchus*. Scale bars: = 10 µm.

Table 3.3A: Population densities range and (mean) of PPN recovered from 1L of soil from rice fields in Morogoro and Mbeya regions in Tanzania

Morogoro rural	**Rice - ecosystem**	**Cropping sequence**	***P. zeae***	***M. arenaria***	***H. pararobustus***	***Helicoty-lenchus***	***Hemicycliophora***	***Xiphinema***	***Trichodorus***	***Criconemoides***	***Tylenchorhynchus***
Nige	Upland	Rice/MMaize (Mono/ inter cropping	*455-892(602)*	0	208-480(376)	10-21(14)	19-46(29)	0	0-16(3)	0-12(6)	0-33(8)
Gozo	"	Rice/Maize	344-645(528)	0	220-606(406)	0-8(3)	0	0	5-13(9)	0-11(3)	2-11(6)
Kisemu	"	Rice/Maize	437-596(521)	0	253-629(486)	0	2-11(5)	0	0	0-5(1)	1-6(2)
Nige2	"	Rice/Maize	253-501(501)	0	342-580(488)	0-11(5)	0-6(2)	0	0	0-11(3)	0-12(4)
Kibaoni	"	Rice/Maize	243-583 (451)	0	309-505(402)	0-8(2)	0-7(2)	0	2-9(5)	0-9(2)	0-11(3)
Mvomero	Irrigated - lowland										
Dakawa	"	Rice/Rice	14-56(20)	0	0	0	0	36-77(67)	23-66(55)	0	0
Hembeti	"	Rice/Rice	9-31(10)	0	0	0	0	55-99(88)	25-87(66)	0	0
Turiani	"	Rice/Rice	21-71(41)	0	0	0	0	66-101(88)	8-66(48)	0	0
Kilombero											
Kisawasawa	irrigated -lowland	Rice/Rice	26-111(58)	15-77(53)	0-4(2)	0-19(7)	0-8(2)	0-18(6)	0-16(5)	0-9(4)	0-44(6)
Msufini	"	Rice/Rice	11-49(32)	11-51(29)	0	0-6(2)	0-5(1)	0-15(4)	0-6(1)	0-12(1)	2-21(9)
Mkula	"	Rice/ Vegetable/ Legumes	5-23(13)	0-12(6)	0-6(1)	0-15(4)	0-9(2)	0-12(5)	0	0	1-6(3)
Ichonde	Rain-fed lowland	Rice/ Vegetable/ Legumes	0-31(12)	0-21(9)	0-2(1)	0	0	1-2(1)	0	0-23(4)	0-11(2)
Idete	"	Rice/ Vegetable /Legumes	3-33(11)	0-23(10)	0-11(5)	0-8(2)	0-14(4)	0-11(3)	0-11(2)	0	0-17(3)
Namawala	"	Rice/ Vegetable/ Legumes	9-33(18)	8-32(15)	0-6(2)	0	0	8-15(11)	0-16(3)	0-6(1)	0-12 (2)
Kyela											
Kilasilo	Upland	Rice/Maize	456-981(708)	0	111-301(197)	0-21(8)	0	0	0	0-19(7)	0-20(5)
Kikusya	"	Rice/Maize	106-701(272)	0	1-89(56)	0	1-6(2)	0	0	0-5(1)	0-12(3)

Morogoro rural	**Rice - ecosystem**	**Cropping sequence**	***P. zeae***	***M. arenaria***	***H. pararobustus***	***Helicoty-lenchus***	***Hemicycliophora***	***Xiphinema***	***Trichodorus***	***Criconemoides***	***Tylenchorhynchus***
Ipande	"	Rice/Maize	106-532(250)	0	45-89(72)	1-14(7)	0	0	1-3(2)	0	0-5(1)
Muungano	"	Rice/Maize	88-321(188)	0	0-84(59)	11-31(17)	1-12(6)	0	0-3(1)	0-5(1)	0-6(2)
Tenende	"	Rice/Maize	78-521(230)	0	55-89(70)	0-9(3)	2-11(7)	0	0-19(10)	0-15(3)	0-12(2)

Table 3.3B: Population densities range and (mean) of PPN recovered from 10 g of rice roots from fields in Morogoro and Mbeya regions in Tanzania

Region and district	Rice ecosystem	Cropping sequency	*Pratylenchus zeae*	*Hoplolaimus pararobustus*	*Meloidogyne arenaria*
Morogoro-rural;					
Nige	Upland	Rice/Maize	769-1761 (1232)	0-821 (383)	0
Gozo	"	Rice/Maize	687-995(839)	232-709 (471)	0
Kisemu	"	Rice/Maize	719-874 (794)	519-952 (688)	0
Nige2	"	Rice/Maize	642-1821(1018)	613-950(755)	0
Kibaoni	"	Rice/Maize	105-886 (597)	480-650 (573)	0
Mvomero;	Irrigated-lowland				
Dakawa	"	Rice/Rice	44-99(56)	1-10(5)	0
Hembeti	"	Rice/Rice	56-101(62)	0-20(7)	0
Turiani	"	Rice/Rice	13-88(44)	0-9(4)	0
Kilombero;					
Kisawasawa	Irrigated-lowland	Rice/ Vegetable	221-810 (428)	9-76 (30)	73-443 (321)
Msufini	"	Rice/ Vegetable	261-452(330)	0-9 (4)	122-412(281)
Mkula	"	Rice/ Vegetable	121-229(167)	0-7 (1)	25-89 (50)
Ichonde	Lowland rainfed	Rice/ Vegetables	101-200 (139)	0- 9(4)	11-144 (67)
Idete	"	Rice/Vegetables	42-99 (67)	0-9 (2)	51-102 (79)
Namawala	"	Rice/ Vegetables	55-101 (78)	0-16 (5)	67-99 (81)
Kyela	Upland				
Kilasilo	"	Rice /Maize	423-677 (544)	166-401 (272)	0
Kikusya	"	Rice /Maize	513-626 (561)	136-388 (257)	0
Ipande	"	Rice /Maize	529-631(572)	182-291 (243)	0
Muungano	"	Rice /Maize	534-981 (799)	125-292(222)	0
Tenende	"	Rice /Maize	401-771 (588)	99 -301(201)	0

3.4.2 Prevalence of nematodes parasitizing rice

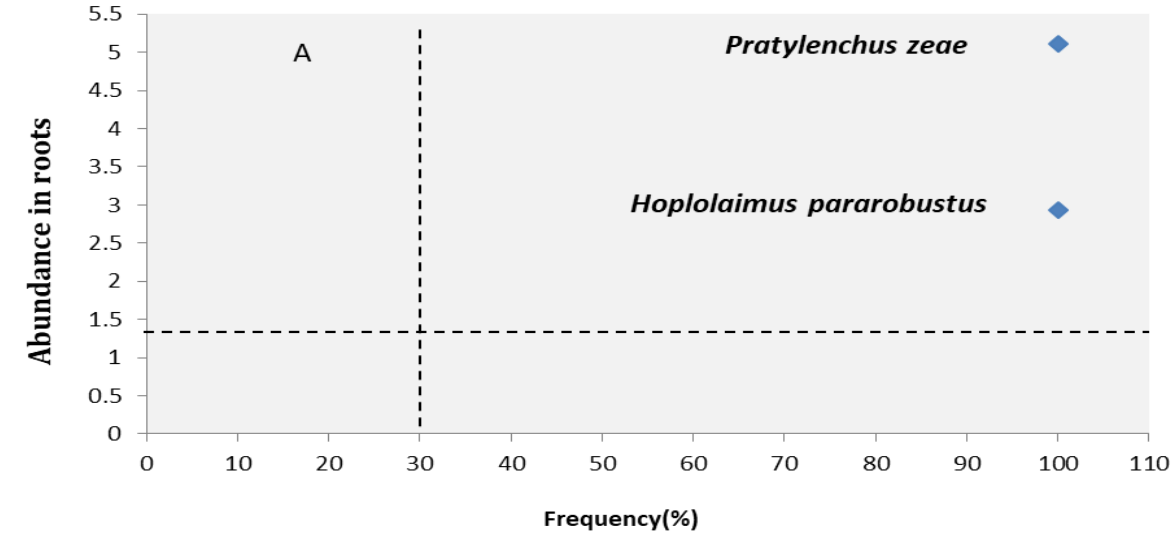
The potential for damage to rice caused by plant-parasitic nematodes was evaluated based on relative frequency and abundance of the nematode genus using the methods developed by Fortuner & Merny (1973). According to this method, groups of nematodes are distinguished by sectioning plots of abundance by relative frequency based on the threshold values (Fig. 3.3). The group at the upper right comprises the nematodes which are most prevalent in the rice fields based on abundance and relative frequency values both above the assigned thresholds.

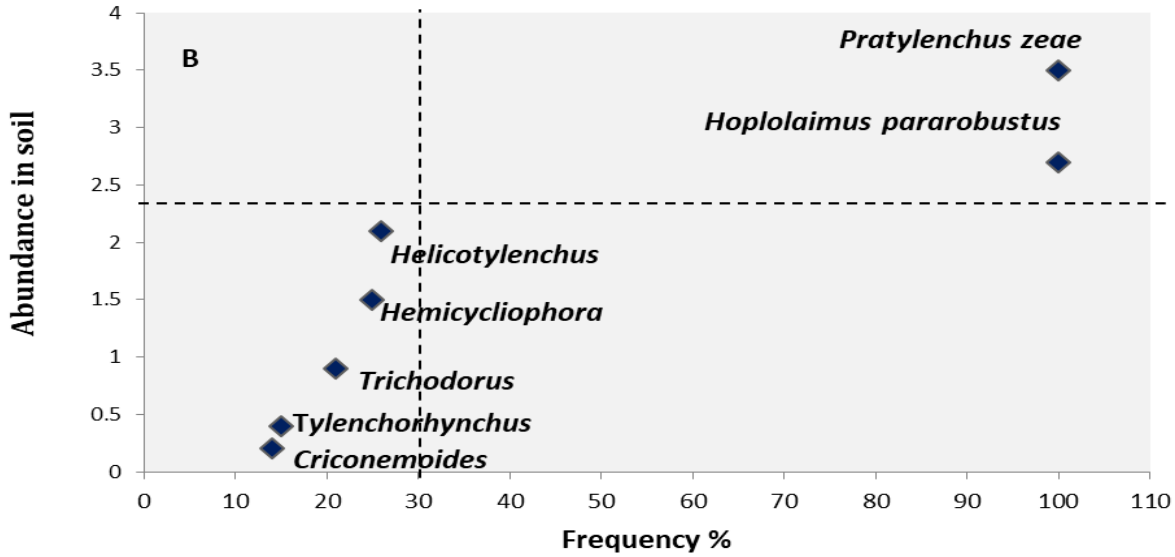
From this study, from both soil and root samples collected from lowland and upland agro-ecosystem, three genera, namely *Pratylenchus, Hoplolaimus,* and *Meloidogyne,* meet the above criterion. The first two genera were found most prevalent in both roots (Fig. 3.3A) and soils (Fig. 3.3B) from upland fields and only in roots (Fig. 3.3C) from lowland fields, and they represent migratory endoparasitic nematodes. *Meloidogyne* was mostly associated with roots (Fig. 3.3C) from lowland fields and is the only sedentary endoparasitic nematode of rice found in this study.

In all soil and root samples from upland fields, *Pratylenchus* and *Hoplolaimus* were found and were more abundant than other nematodes. In lowland root samples, *Pratylenchus, Hoplolaimus* and *Meloidogyne* prevailed at frequencies of 100%, 66% and 80% respectively. The virus vector nematodes *Xiphinema* and *Trichodorus* occurred at a frequency of 100% and 80% respectively in lowland soil samples (Fig. 3.3D). *Helicotylenchus, Hemicycliophora*, *Trichodorus, Tylenchorhynchus* and *Criconemoides* were grouped in the lower left quadrant which indicates that these nematodes were not prevalent being characterized by both low frequency and abundance in soil samples from upland rice fields (Fig. 3.3B).

3.4.3 Nematode distribution according to the rice agro-ecosystem

Low land rice-ecosystem is more diverse concerning plant-parasitic nematode as compared to upland rice-ecosystem. All genera found during this study were present in lowland soil samples (Fig 3.3B). The dominant PPN species found in higher abundances in the upland ecosystem were *P. zeae* and *H. pararobustus*. *Meloidogyne* sp. was not found in upland rice fields but only in low land rice fields. Among the major species, *P. zeae* was the most dominant one across all rice agro-ecosystems (Fig. 3.4). It has the highest relative frequencies and abundances in all root samples from all agroecosystems (Fig. 3.3) while *Criconemoides* was the least recovered from all rice-agro ecosystems with minimal occurrence in upland rice ecosystem. *M. arenaria* and *Xiphinema* were only found in lowland agro-ecosystem (Fig. 3.3 A & B). Their high frequencies (85% and 100%) coupled with low abundances (1.5 and 1.7) rank them the second most important rice parasitic nematodes in lowland ecosystem. The rest of the nematode genera were more abundant and frequent in lowland ecosystem than an upland ecosystem.


A
Pratylenchus zeae
Hoplolaimus pararobustus
Abundance in roots
Frequency(%)
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
10
20
30
40
50
60
70
80
90
100
110


B
Pratylenchus zeae
Hoplolaimus pararobustus
Helicotylenchus
Hemicycliophora
Trichodorus
Tylenchorhynchus
Criconemoides
Abundance in soil
Frequency %
4
3.5
3
2.5
2
1.5
1
0.5
0
0
10
20
30
40
50
60
70
80
90
100
110

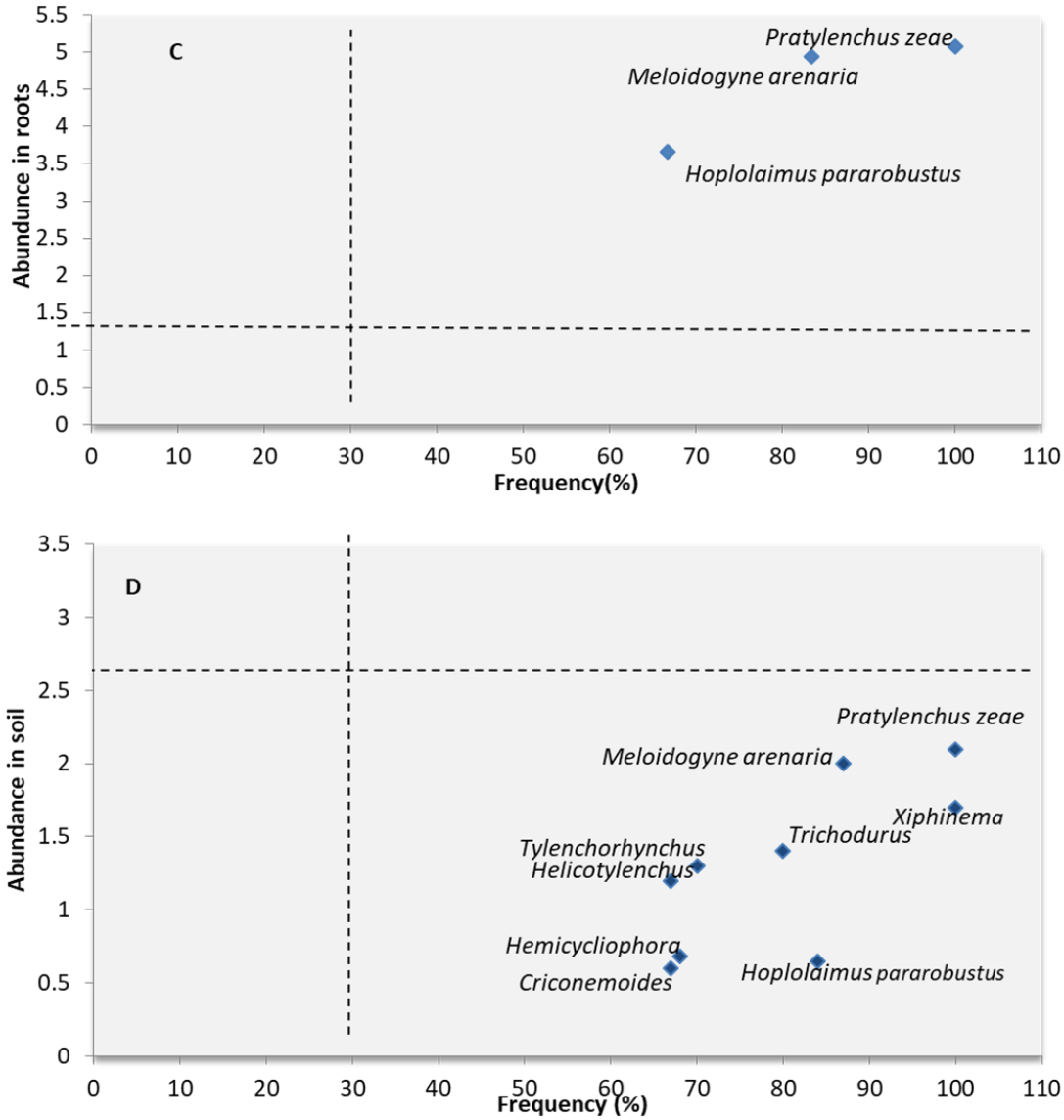


Fig. 3.3: **Frequency and abundance of PPN genera associated with roots and soil from the upland rice ecosystem (A & B) and lowland rice ecosystem (C & D). Dotted vertical lines represent nematode frequency limit (30%), and the dotted horizontal lines represent the abundance threshold =1.3 for roots samples and 2.3 for soil samples according to Fortuner and Merny (1973). A nematode genus is regarded as frequent in the soil or the roots when it is observed in at least 30% of the samples. A nematode genus is considered to be abundant if abundance value in roots ≥ 1.3 (≥ 20 individuals/g of roots) and if a value in soil ≥ 2.3 (≥ 200 individuals/1,000 cm3 of soil).**

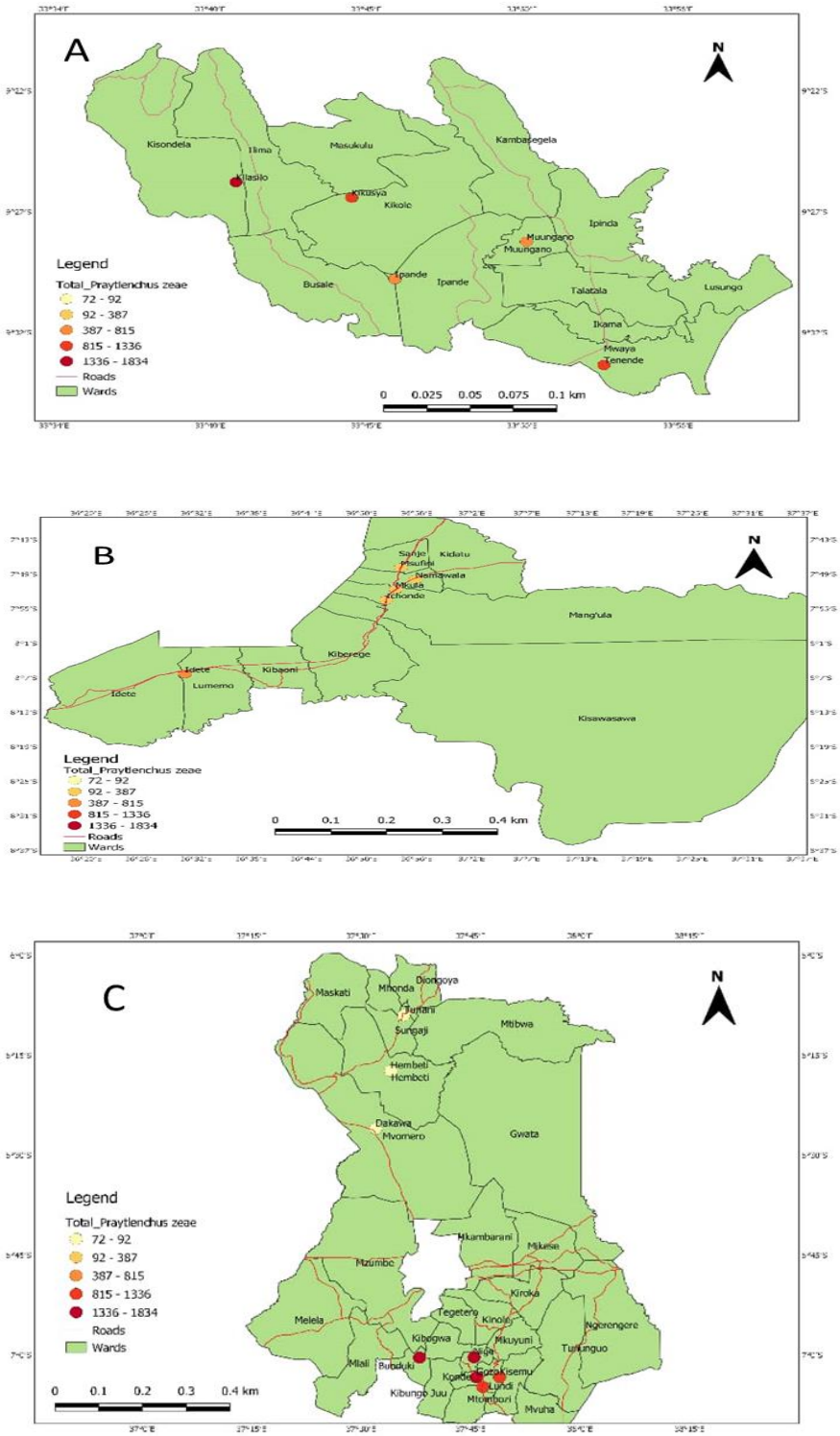


Fig. 3.4: **Maps showing *P. zeae* infestation levels across four districts, A. Kyela, B; Kilombero, C; Morogoro rural, and Mvomero. Different colour on legend shows a range of nematode densities and red shows the hot spot.**

3.4.4 Morphological and molecular characterization of the major nematode genera recovered from the rice-growing ecosystem

***Pratylenchus zeae,* Graham 1951-morphological and morphometric characterization**

***P. zeae* descriptions**

Body slim, after fixation straight with faint annuli. Lateral field with four incisures extending along tail beyond phasmids occupies 19% of corresponding body diameter. Lip region with three annulations, hemispherical and flat at the middle. Labial framework heavily sclerotized extending into the body about one annule. Stylet very strong with a round basal knob. Dorsal pharyngeal gland orifice close to stylet knob opening posterior to the stylet base. Pharyngeal gland lobe ventrally overlapping intestine. Vulva posterior, spermatheca round, small, without sperms. Phasmids slightly posterior to middle of the tail. Tail terminus pointed with no variation within the population.

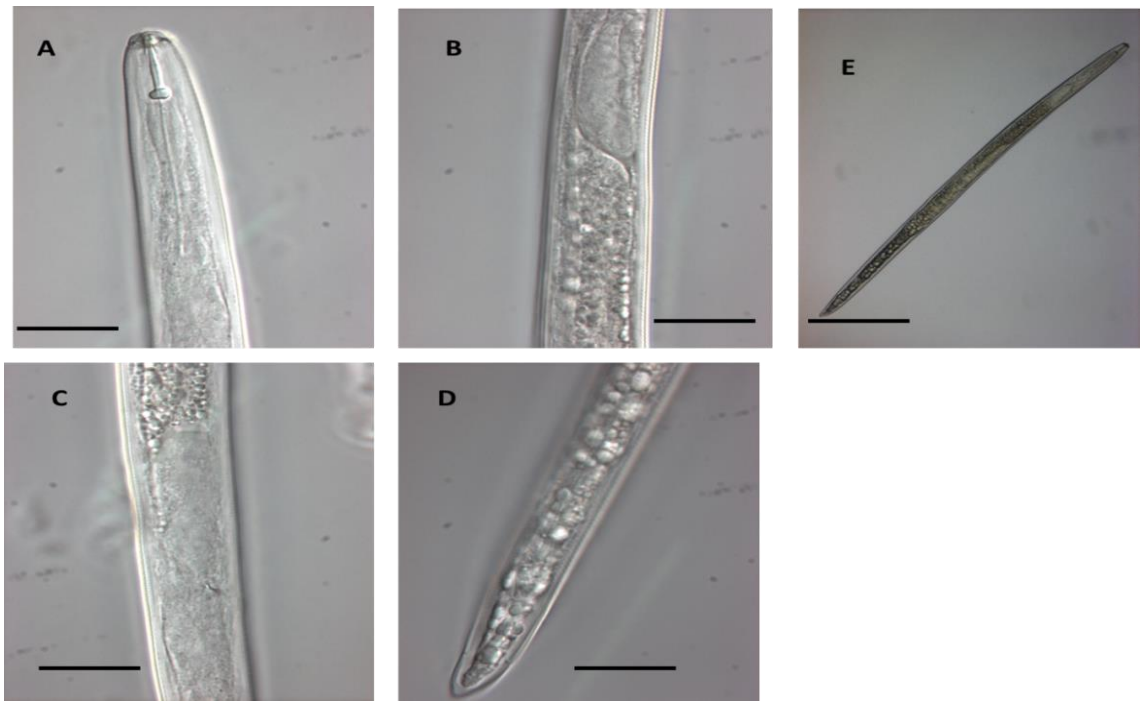


Fig. 3.5: ***P. zeae*, light microscopy photograph of a female. A; Anterior region, B; Pharyngeal overlap, C; Vulva region, D; Tail terminus, E; Entire female. (Scale bars: A-D = 10 µm; E = 150 µm.**

Molecular characterization of *P. zeae*

Four sequences (363 bp) for the COI gene and D2-D3 expansion segment of 28S ribosomal gene (704 bp) were obtained based on individual nematodes from 4 sampled districts. The variations of the COI and D2D3 sequences obtained for this population of *P. zeae* were 0.0-0.1% (0-1 bp) and 0-0.4% (0-4 bp) respectively. For phylogenetic analysis, homologous sequences of similar size to the studied population from the same region were obtained from Genbank through Blastn search. The search revealed 99% similarity with the deposited sequences of *P. zeae* into GenBank accession number KU198934.1 (Troccoli et al., 2016) for COI and KT033000.1 for the D2D3 expansion segment of 28S RNA gene (Pili et al., 2016). After Bayesian inference (BI) analysis with closely related sequences, the population under study shared a maximally supported clade with other *P. zeae* populations (Fig. 3.6). From morphometric features, combined with morphological and molecular data, it was noted that the specimens had features congruent with *P. zeae* (Troccoli et al., 2016).

Table 3.4: Measurements of females of *P. zeae* from rice fields in Tanzania. All measurements are in µm (except for ratio) and in the form: mean ± SD (range)

Character in µm	Graham T. W. (1951)	Roman, J. & Hirschmann H. (1969)	Pili et al. (2016)	Tatiana et al. (2016) Costa-Rica	Present study
n	-	50	10	20	20
L	360-580	540.10 ± 5.4 (463.1-657.2)	437±39.01 (383 - 502)	504 ± 37.9 (434-556)	387 ±52.3 (352-500)
a	25-30	27.190 ±36 (20.5-32.8)	20.72±1.01 (19.78-22.88)	25.8±1.8 (23.1–29.8)	17.2±7.02 (17.9-20.9)
b	5.4-8.0	6.53 ±0.07 (5.5-7.9)	6.43±0.75 (5.25 to 7.25)	-	5.513±1.09 (4.19-6.84)
b’	-	-	-	4.1±0.5 (3.2–4.7)	3.91±0.65 (3.06-4.79)
c	17-21	15.220 ±0.19 (13.0-17.7)	16.13±1.48 (13.41 -18.2)	17.4±4.0 (12.5–23.9)	13.44±1.1 (12.2-14.91)
c’	-	-		2.4±0.4 (1.5–3.1)	2.64±0.35 (2.38-2.89)
V%	68-76	70.94 ± 0.16 (69.0-75.0)	71.84±1.05 (69.75 -73.21)	71.1±1.8 (67.3–74.4)	69.76±2.8 (67-75)
Stylet length	15-17	15.50±0.08 (13.6-16.6)	14.76±0.44 (13.8 -15.3)	15.0±0.6 (14.0–16.0)	15.1±0.46(14.6-17)
DGO	-	2.38±0.04 (1.8-3.0)	-	-	2.73 (1.6- 3.72)
S-E pore	-	88.14 ± 0.92(74.8-104.4)	-	81.4±6.7 (65.0–95.3)	59.48±7.23 (48.4-68)
Lip height	-	2.45 ± 0.02(2.4-3)	-	-	2.87±0.32 (2.5-3.12)
Lip width	-	7.810 ± 0.32 (7.2-8.4)	-	-	8.34±0.42 (7.9-8.64)
Max. body diam.	-	19.80 ± 0.34(16.2-24.0)	21.1±5.56 (17-25)	33.2± 3 (28.9-38.2)	19.36±3.2 (13-23.6)
Tail length	-	35.57 ± 0.44 (24.0-40.2)	27.1±1.66 (24 - 29)	27.3±5.8 (21.0–33.0)	28.9±3.24 (25-34.5)

Abbreviations are defined as in Siddiqi (2000). n =Number of specimens on which measurements are based; L =Overall body length; a =body length / greatest body diameter; b = body length / distance from anterior to esophago-intestinal valve; c= body length / tail length; V% =distance of vulva from anterior.

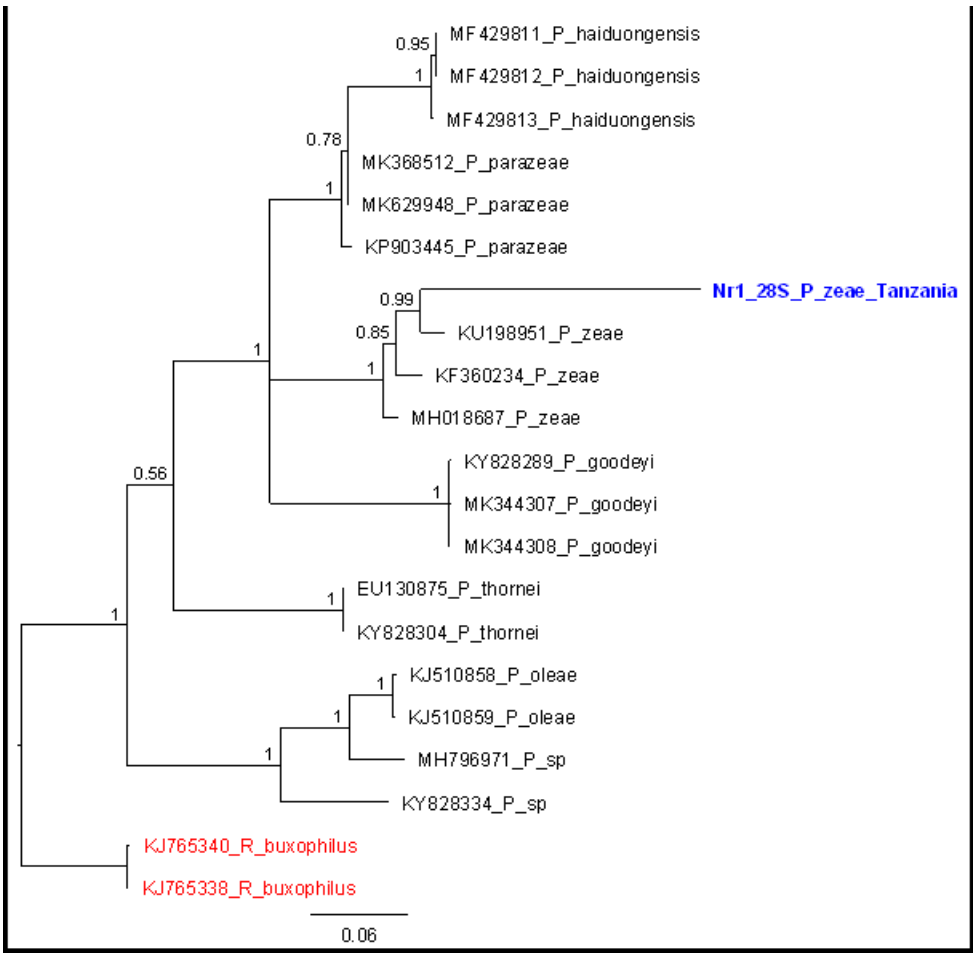


Fig. 3.6: **Phylogenetic relationship based on the 28S sequences of *P. zeae* isolates and other Pratylenchus spp: a Bayesian inference majority rule of consensus tree reconstructed using the GTR+G model. The tree was rooted using *Rotylenchus buxophilus*.**

***Meloidogyne arenaria* (Chitwood 1949)-Morphological characterization**

Second-stage juveniles (J2) description

J2 are short (424.5-586.0) (515.0 µm) and slender (19.9-18.6) (19.2 µm). The stylet is very long (9.72-15.7) (11.9 µm) and slightly sclerotized. The oesophageal gland overlaps the intestine ventrally. Tail slightly long 58.9 (62-55.4 µm), with the hyaline poorly defined bearing a finely rounded to pointed tip with clear terminus. Rectum undilated (Fig. 3.7). Means and standard error of the means of morphometric were compared with the previously done work to delineate the species. However, the morphometrics was outside the previously described reports (Table 3.4).

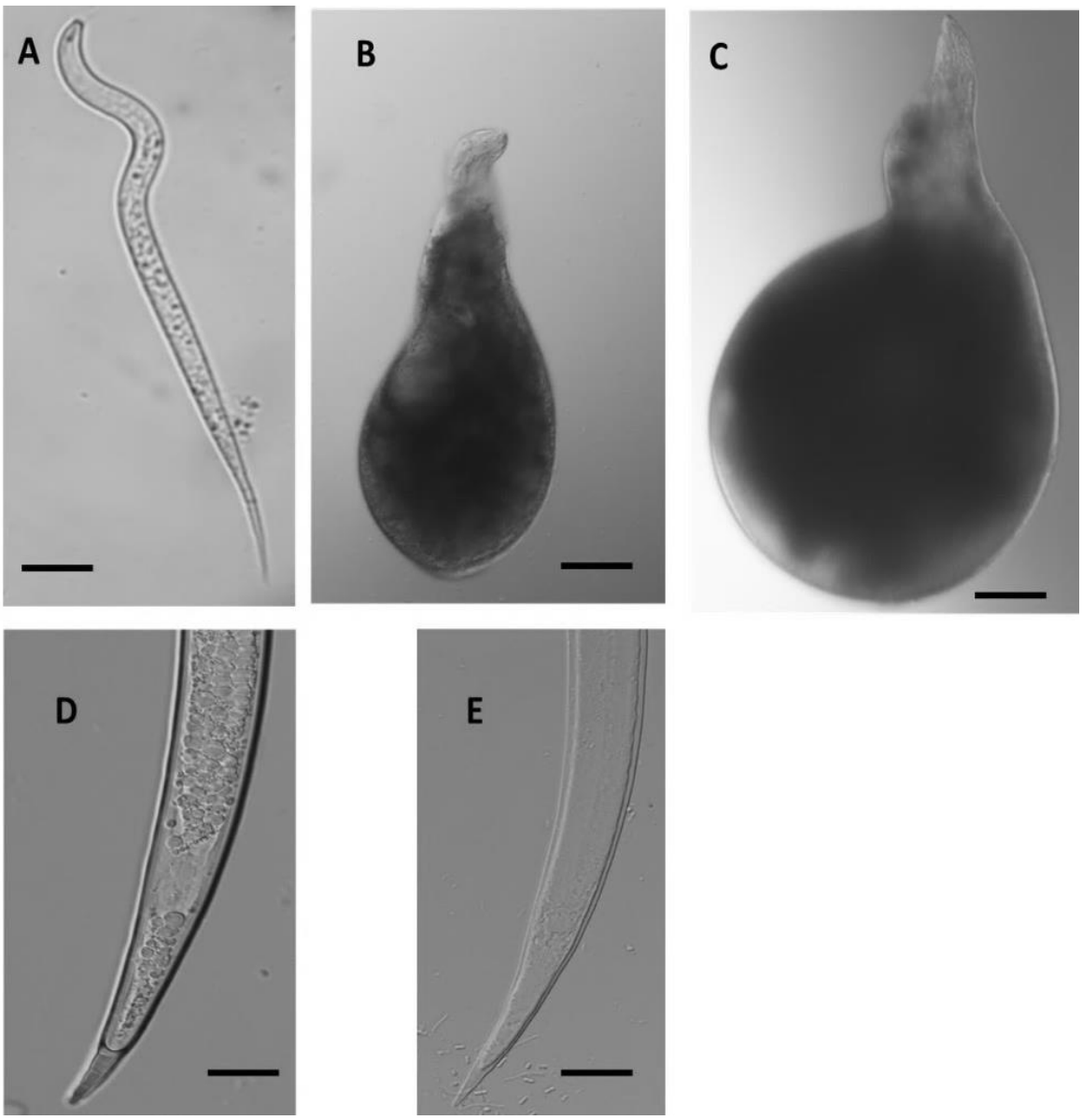


Fig. 3.7: **Light microscope photograph of *M. arenaria* from Tanzania. A; Entire second stage mobile infective juvenile (J2), B; immobile young female, C; Mature immobile female, D, E; Tail terminus of J2. (Scale bars: A-C, = 100 µm; D- E = 20 µm)**

Table 3.5: Morphometrics of the second-stage juvenile (J2) of *M. arenaria* on rice in Tanzania and other populations. All measurements are in µm (except for the ratio) and in the form of mean ± SD (range)—abbreviations as in Table 3.3.

Character (µm)	Present study	*M. arenaria* Chitwood 1949	*M. arenaria* Cliff & Hirschmann 1985c	*M. arenaria* Garcia & Sanchez-Puerta (2012)	*Osman et al., 1985*
n	20	-	-	20	50
L	450.0 ± 26.5 (430.0 - 480.0)	398-605 (504)	398-605 (504)	419.6-493.69 (458.5)	413-556 (494)
a	29.4 ± 2.3 (26.9 - 31.4)	-	-	13.88-16.95 (15.35)	24.9-36.7 (31.2)
b	7.26±0.34 (8.17-8.97)	-	-	5.3-5.4 (5.3)	
b'	7.4 ± 0.3 (7.0 - 7.5)	-	-	4.6-7.8 (4.8)	
c	8.3 ± 0.5 (8.0 - 8.6)	-	-	8.9-9.2 (8.0)	6.9-8.4 (7.5)
c'	6.6 ± 0.4 (6.3 - 6.8)	-	-	6.8-7.1 (6.2)	
DGO	4.4 ± 0.2 (4.1 - 4.5)	3.3 (3 – 3.5)	4 (3-5)	4.5-4.9 (4.50)	2.5-5 (4.0)
Stylet length	12.5 ± 0.8 (11.0 - 13.0)	12.5 (11.4 – 13.3)	11 (10-12)	11.7-13.6 (12.1)	10.9-14.4 (12.8)
Pharynx length	61.8 ± 2.1 (60.0 - 65.0)	-	-	58-66 (59.9)	
Excretory pore	88.7 ± 1.2 (88.0 - 90.0)	-	-	86.9-92 (88.2)	71-102 (90)
Max. body diameter	15.4 ± 1.2 (14.0 - 17.0)	-	-	13.9-16.9 (14.9)	13.8-15.99 (14.8)
Diameter at anus	8.8 ± 0.6 (8.0 - 9.0)	-	-	9.0-9.9 (9.2)	10.0-13.1 (11.5)
Tail length	54.2 ± 2.2 (50.0 - 60.0)	40.9 – 57.1 7.39 (48.1)	44-69 (56)	49.56-59.4 (49.9)	53.1-75.0 (66.1)
Hyalin	13.6 ± 3.3 (10 – 14)	12.8 – 13.8 0.36 (13.1)	6-13 (9)	11.8-13.9 (13.80)	8.1-14.4 (10.8)

Abbreviations are defined as in Siddiqi (2000). n = Number of specimens on which measurements are based; L = Overall body length; a = body length / greatest body diameter; b = body length / distance from anterior to esophago-intestinal valve; c = body length / tail length; V% = distance of vulva from anterior; DGO = Dorsal Gland Orifice; S-E pore = the ventral opening of the secretory-excretory system.

Twenty female perineal patterns were observed, and moderate morphological variations within the sample were noted (Fig.3.8). The perineal shapes commonly observed were rounded to oval. The dorsal arc from low in most cases to high (Fig. 3.8 A). Smooth to wavy lines at post-anal region (Fig.3.8 B & D) broken or continuous intermittently forming shoulders (Fig.3.8 C). Morphology of the female perineal pattern has been used as a distinguishing character between root-knot nematodes; however, for *M. arenaria* it is a very variable and not reliable character for species identification. In the current study, perineal pattern matched to some extent

with those of the original descriptions of *M. arenaria.* Majority of dorsal arches were low flattened and rounded to high and squarish with shoulders (Fig 3.8 C) typical of *M. arenaria* perineal pattern. Still, variations were observed among the different individuals. All vulva lips were smooth without invaginations. The shoulders characteristics of *M. arenaria* were visible in most of the samples.

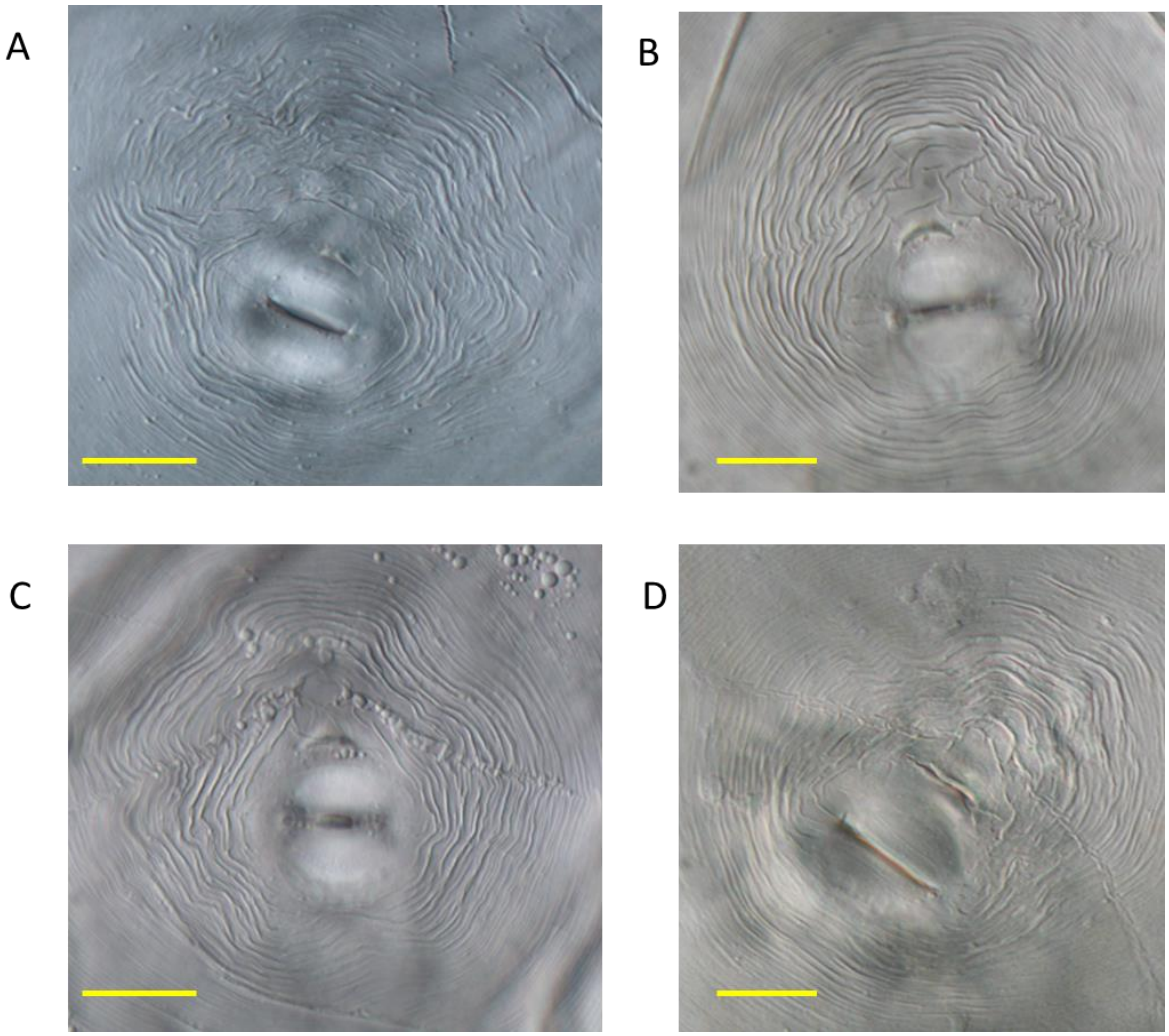


Fig. 3.8: A representative perineal pattern of a mature female of *M. arenaria* showing variations within the same population. Scale bar = 20µm. A; Perineal pattern with broken lines, B & D; Perineal pattern with high dorsal arc C; Perineal pattern showing shoulders.

***Meloidogyne arenaria* (Chitwood 1949)-Molecular characterization**

Sequences of *Meloidogyne* species from ITS region gave results to 100% for three root-knot nematodes species, *M. incognita*, *M. arenaria* and *M. javanica.* Using SCAR primers, no bands were obtained for *M. javanica* and *M. incognita* specific - SCAR primers respectively. However, bands of 420 base pair were observed using Far TCGGCGATAGAGGTAAATGAC and Rar TCGGCGATAGACACTACAAACT-specific SCAR primers for *M. arenaria* (Fig. 3.9). The mitochondrial NADH dehydrogenase subunits 5 (*Nad5*) sequence was shown to be identical to the *M. arenaria* reference sequence from Janssen et al. (2016) which confirmed the species identity.

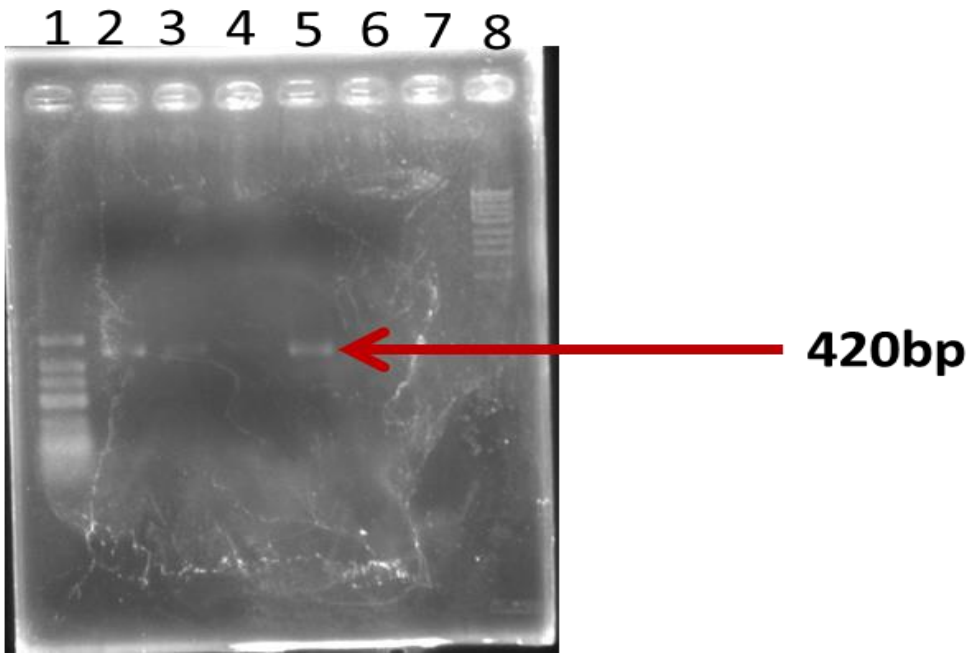


Fig. 3.9: Agarose gel electrophoresis of the polymerase chain reaction (PCR) products in lanes 2, 3, 4 & 5 showing the fragment of 420 base pair amplified with the species-specific primers pair Far/Rar for *M. arenaria*. Lane 1 & 8 is 100bp & 1kb-ladder.

3.5 DISCUSSION

The results from the present diagnostic survey have given a good indication of plant-parasitic nematode diversity on rice in rice agroecosystems in Tanzania. The

nine identified plant-parasitic nematodes genera have been previously reported from different rice fields elsewhere (Bridge et al., 2005; Coyne et al., 1998; 1999a; 2001; Gilces et al., 2016; Gnamkoulamba et al., 2018; McDonald et al., 2003; Namu et al., 2018; Pascual et al., 2014; Shahabi et al., 2016; Udo et al., 2011). Different nematode genera occurred in the same field, which results in a complex interaction and aggravates the effect on the host (Mokrini et al., 2016). Among the plant-parasitic nematode genera documented in the current study, *Pratylenchus*, *Meloidogyne,* and *Xiphinema* are among the top ten crucial plant-parasitic nematodes (Jones et al., 2013). The cultivar and rice agro-ecosystem determine this plant-parasitic nematode prevalence and abundance in rice fields (Coyne et al., 2001; Pascual et al., 2014; Win et al., 2011; 2015). Moreover, the increasing intensification of rice production in different rice ecosystems, production systems that involved intercropping and crop sequences of maize to rice and cultural practices employed could have contributed to the distribution of these nematode genera. In this case for example in rice-maize cropping systems, nematode densities seemed to be higher than in rice-vegetable/legume cropping systems, especially for the root-lesion nematode *P. zeae* because maize and rice are both cereals and a favorite host of *P. zeae*. Further in lowland and irrigated rice ecosystems farmers usually grow the high yielding improved cultivars that are commonly susceptible to *P. zeae* eg. SARO-5 and Komboka. This may lead to population build up. *P. zeae* for instance becomes more abundant when rice is rotated with maize than with any other crop species. For further nematode management strategies cropping sequences that take into account the presence and densities of *P. zeae* should be considered. *P. zeae* has been reported to prevail and cause yield losses in upland rice in West Africa (Coyne et al., 1998, 2004), India

(Prasad et al., 1987), The Philippines (Pascual et al., 2014) and Brazil. In the upland rice fields in The Philippines, *P. zeae* was found in 79% of the samples (Pascual et al., 2014). Due to their polyphagous nature, these nematodes may be a threat to rice production in Tanzania. The changes in farming systems from traditional to more intensive rice farming with projected elevated temperatures coupled with intermittent wetting and drying of the rice fields due to scarcity of water may probably exacerbate the nematode problem even in areas with low nematode densities (Coyne et al., 2018; Elling, 2013; Onkendi et al., 2014). In upland rice fields in Tanzania, farmers commonly grow cereals in continuous cycles like maize, rice and sorghum, and sometimes rice is intercropped with legumes like beans, peanuts or green gram. All these crops are a host of *P. zeae* that could explain the high incidences and densities found on upland rice agro-ecology.

Meloidogyne species, especially the common three, so-called MI-lineage, *M. incognita*, *M. javanica* and *M. areanaria* are complicated species to tell apart (Pagan et al., 2015). *M. arenaria* is believed to be an evolving species that has characteristics falling between *M. javanica* and *M. incognita* (Janssen et al., 2016). The identity of the Meloidogyne sp. was analysed using different regions of the genome. Specific SCAR primers were able to separate the three species (Adam et al., 2007) and the samples were identified to contain only *M. arenaria* which was then confirmed by sequencing the mitochondrial *Nad5* gene (Janssen et al., 2016). A combination of morphological features, morphometrics, and molecular information led us to conclude that the RKN samples from the rice fields contained *M. arenaria*. In Tanzania, *M. arenaria* has been reported parasitizing other crops, especially vegetables (Pagan et al., 2015). *M. arenaria* is a very polyphagous nematode that can infect almost every

plant (García et al., 2012). The isolation of *M. arenaria* in lowland soil samples might be due to the cropping system. During the off-season, farmers prefer to use the same pieces of land from rice to grow vegetables such as tomatoes, eggplants, okra, sweet potatoes which are excellent hosts of RKN. However, J2 was recovered from rice root samples taken from these fields, and it was also multiplied on rice for the culture which justifies that *M. arenaria* also is a rice parasite (García et al., 2012).

3.6 CONCLUSION

From the current study, it was indicated that *P. zeae* was the most prevalent plant-parasitic nematode associated with rice in all Tanzanian rice agro-ecologies. To our knowledge, this is the first study to report the nematode problems in rice fields in Tanzania. The information on the densities and prevalence of these nematodes in Tanzanian rice fields is very useful in designing and implementing nematode management strategies. The study forms a base for further research on nematode problems such as pathogenicity and searching for natural sources of resistance against these nematodes, especially from local rice cultivars.

CHAPTER 4: THE RESPONSE OF *ORYZA SATIVA*, *ORYZA GLABERRIMA* AND NERICA TO THE ROOT-LESION NEMATODE *PRATYLENCHUS ZEAE* (GRAHAM) AND THE ROOT-KNOT NEMATODE *MELOIDOGYNE GRAMINICOLA* (GOLDEN AND BIRCHFIELD)

Yasinta Beda Nzogela, Ashura Luzi Kihupi, Godelieve Gheysen

Manuscript in preparation

Author's contributions;

YN, AL-K, and GG designed the study and methodology

YN excuted the study analysed the data and wrote the manuscript.

GG edited the manuscript

4.1 ABSTRACT

Different rice genotypes belonging to *Oryza sativa*, *Oryza glaberrima* and their interspecific hybrids were screened for resistance against a population of *P. zeae* isolated from rice fields in Tanzania. The hybrids (NERICAs) were further evaluated against the RKN *M. graminicola*. Both experiments were conducted under screenhouse conditions. The evaluation was based on the number of nematodes present in the roots at 30 days post-inoculation (dpi) for *P. zeae,* and *M. graminicola* number of nematodes, developmental stages and galling severity was assessed at 18 dpi. All *O. glaberrima* genotypes under study were resistant to *P. zeae*. Among the *O. sativa* genotypes tested, Supa showed to be resistant to *P. zeae*. NERICA were either partially resistant or susceptible to *P. zeae* except for NERICA5 which was resistant. Our study showed a large variation in susceptibility to *M. graminicola* infection among

the NERICA genotypes examined. Resistance comparable to the resistant reference genotype TOG5674 was not found among NERICAs evaluated, but NERICA2 was less susceptible to *M. graminicola in* terms of galling severity and nematode reproduction and development.

4.2 INTRODUCTION

Through AfricaRice, NERICA varieties which are interspecific progeny between Asian rice, *Oryza sativa* and African rice, *Oryza glaberrima* have been introduced to Tanzania in different rice-producing areas (Luzi-Kihupi et al., 2015). NERICA varieties are resistant to multiple abiotic and biotic factors. They have a high yielding capacity in low input soils where smallholder farmers lack the means to irrigate or apply chemical fertilizers or pesticides (Futakuchi et al., 2013).

The most important nematode species are *Meloidogyne* and RLN *Pratylenchus* (De Waele & Elsen, 2007; Onkendi et al., 2014). *M. graminicola* is widely distributed primarily in Asia and is considered as the most economically important pest of Asian rice *O. sativa* (De Waele & Elsen, 2007; Mantelin et al., 2017). Yield loss up to 50% might occur due to severe infestation of *M. graminicola* in upland, rain-fed and direct-seeded field conditions (Cabasan et al., 2018b; Kyndt et al., 2014). However, other RKN like *M. javanica* (Treub) Chitw have also been reported to cause a severe loss in rice production (Coyne et al., 1998; Namu et al., 2018; Negretti et al., 2017). *P. zeae* is prominent in rice fields, mainly upland rice in West Africa and Brazil (Bellé et al., 2017; Coyne et al., 1998) but they have also been reported in Kenya (Pili et al., 2016) and Tanzania (current study).

The use of resistant cultivars is a low cost and sustainable option for the control of nematodes in the long term, which does not impose unwanted changes in

traditional agronomic practices (Peng & Moens, 2003). Genetic host resistance can be found from landraces, wild ancestors or lines from breeding programs. Whether to be directly recommended to be used by farmers will depend on the good agronomic characteristics (Zwart et al., 2019).

Sources of nematode resistance can be inherited from the donor in either a polygenic manner or as a single dominant resistance gene conferring resistance due to recognition of a specific avirulence protein in the nematode (Cook, 2004). Resistance sources against *M. graminicola* have been found in *Oryza longistaminata* and *O. glaberrima* (Brar et al., 1999; Cabasan et al., 2015; Soriano et al., 1999). Diomande (1984) and Plowright et al. (1999) found resistance to *M. incognita* in *O. glaberrima.* Unlikely *O. glaberrima, O. sativa* is generally very susceptible to these root-knot nematodes. Few reports on *O. sativa* resistance to *M. graminicola* have been recently re-evaluated for their resistance status against these nematodes, and none of them was genuinely resistant but partially resistant or susceptible to *M. graminicola* (Cabasan et al., 2018a). The study by Dimkpa et al. (2016), reported the resistance of one *O. sativa indica* accession from Sri Lanka LD 24, and the Khao Pahk Maw accession of *aus* subpopulation from Thailand to *M. graminicola*. These genotypes were hardly having one gall and one to five developed females, respectively. Recently also a Chinese *O. sativa* cultivar was found to be resistant to *M. graminicola* (Zhan et al., 2018) and the resistance is controlled by one dominant gene (Phan et al., 2018). Rice resistance to root-lesion nematodes (*P. zeae*) has been less reported. Only Pili et al., (2016) reported the variety Supa being resistant to root-lesion nematodes and little effort has been devoted to study rice root-lesion nematode interaction. What is Supa? Supa is *O. sativa* indica type most preferred variety in the Eastern and Southern

Africa region due to its aroma. It was introduced in Tanzania from Surinam in South America during the variety testing series in Kilosa and other areas through 1968 to the early 1970s when the Rice improvement program of 1965 was redesigned at Ilonga Agricultural research (Revised to TARI-Ilonga). Therefore, the current study is conducted to explore some popular rice varieties, NERICA and their parental lines for rice resistance sources against *M. graminicola* and *P. zeae*.

4.3 MATERIALS AND METHODS

4.3.1 Nematode inoculum

The *P. zeae* population was isolated from infected rice roots under upland conditions in Matombo Morogoro Tanzania, and a pure culture was established and maintained on carrot discs (Kagoda et al., 2010). Nematode inoculum was extracted from carrot discs. Emerging nematodes mixed stages were washed off the carrot that was incubated at 28ºC for two months. The nematode suspension was homogenized and quantified. The *M. graminicola* culture was provided by Prof. Dirk De Waele (University of Leuven, Belgium) and was initially isolated from rice in the Philippines. *M. graminicola was* maintained on *O. sativa* cv. Nipponbare in potting soil or on the grass *Echinochloa crus-galli*. Three-month-old infected plants were used to extract the J2 of *M. graminicola* using a modified Baermann method (Coyne et al., 2014).

4.3.2 Rice genotypes

The rice genotypes consisted of 9 *O. sativa* genotypes which are locally preferred varieties by farmers in Tanzania, NERICA series from 1-10 except NERICA 6 and four *O. glaberrima* genotypes reported as resistant to *M. graminicola* in reports and publications (Table 4.1). The resistant reference was *O. glaberrrima* TOG5675

and the susceptible *O. sativa*- UPLRi-5 (Cabasan et al., 2012; Soriano et al., 1999). NERICAs tested against *P. zeae* were further evaluated against *M. graminicola*. NERICA5 was not assessed because of poor germination of the seeds.

4.3.3 Experimental design

Two sets of experiments were carried out. The first experiment was to evaluate the response of *O. glaberrima*, *O. sativa* and 9 NERICAs against *P. zeae* (Fig.4.1)

Fig. 4.1: Screening experimental set up in rice culture room at Ghent University

The second experiment screened NERICA varieties against *M. graminicola.* Rice seeds were germinated on Petri dishes lined with moist tissue paper and sealed with tape. The Petri dishes containing seeds were kept in the environmental chamber under dark condition for four days. Pre-germinated rice seedlings were transplanted in polyvinylchloride SAP tubes of 18 cm height and 5 cm diameter filled by Ssand and absorbent polymer mixture (Reversat et al., 1999). Water and nutrients were delivered

in form of Hoagland's nutrient solution 3 times a week. Fourteen - day-old rice seedlings in SAP medium were inoculated with ± 300 and ± 200 freshly harvested nematodes of either *P. zeae* or *M. graminicola* respectively per plant as previously described (Nahar et al., 2011). The experiments were conducted in a rice culture room at Ghent University under controlled environmental conditions (26/24ºC day/night temperature, 70% relative humidity, 12/12 h light/dark cycle and light intensity was 150µmol/m² s (Fig.4.1). Nematodes were counted in roots stained with acid fuchsin under microscope.

Table 4.1: List of rice genotypes screened against nematodes

Genotype	Accession number/Source	Ecotype	species
TOG5674	AfricaRice	Low land	*O. glaberrima*
TOG5675	AfricaRice	Low land	*O. glaberrima*
CG11	AfricaRice	Low land	*O. glaberrima*
CG14	AfricaRice	Upland	*O. glaberrima*
WAB450	AfricaRice	Upland	*O. glaberrima*
UPLRi5	IRRI	Upland	*O. sativa*
Komboka	ARI-Katrin-IR05N 221	Lowland	*O. sativa*
Supa	Local variety	Lowland/upland	*O. sativa*
Zambia	Local variety	Lowland	*O. sativa*
Saro	ARI-Dakawa	Lowland	*O. sativa*
TXD88	ARI-Katrin - Ifakara	Lowland	*O. sativa*
Tai	ARI-Dakawa - IR03A 262	Lowland	*O. sativa*
Lunyuki	ARI-Katrin-IR05N 221	Lowland	*O. sativa*
Mwangaza	Mutant from Supa	Lowland/upland	*O. sativa*
08fan6	ARI- Dakawa - China	Lowland	*O. sativa*
NERICA1	AfricaRice - WAB 450-IBP-38-HB	Upland	Interspecific-hybrid
NERICA2	AfricaRice -WAB 450-1-1-P31-1-HB	Upland	Interspecific-hybrid
NERICA3	AfricaRice - WAB 450-IBP-28-HB	Upland	Interspecific-hybrid
NERICA4	AfricaRice - WAB 450-IBP-91-HB	Upland	Interspecific-hybrid
NERICA5	AfricaRice - WAB 450-11-1-1-P24-HB	Upland	Interspecific-hybrid
NERICA7	AfricaRice - WAB 450-IBP-20-HB	Upland	Interspecific-hybrid
NERICA8	AfricaRice - WAB 450-1-BL1-136-HB	Upland	Interspecific-hybrid
NERICA9	AfricaRice - WAB 450-BL1-136-HB	Upland	Interspecific-hybrid
NERICA1	AfricaRice - WAB 450-11-1-1-P41-HB	Upland	Interspecific-hybrid

The experimental layout consisted of a randomized complete block (RCB) design with eight replications and the experiment was performed twice. Evaluations were done at 18 and 30 dpi, for *M. graminicola* and *P. zeae,* respectively. At harvest, root and shoot length was recorded. Infected roots were washed off SAP, cleaned and damped dry with tissue paper, and fresh root weight was measured.

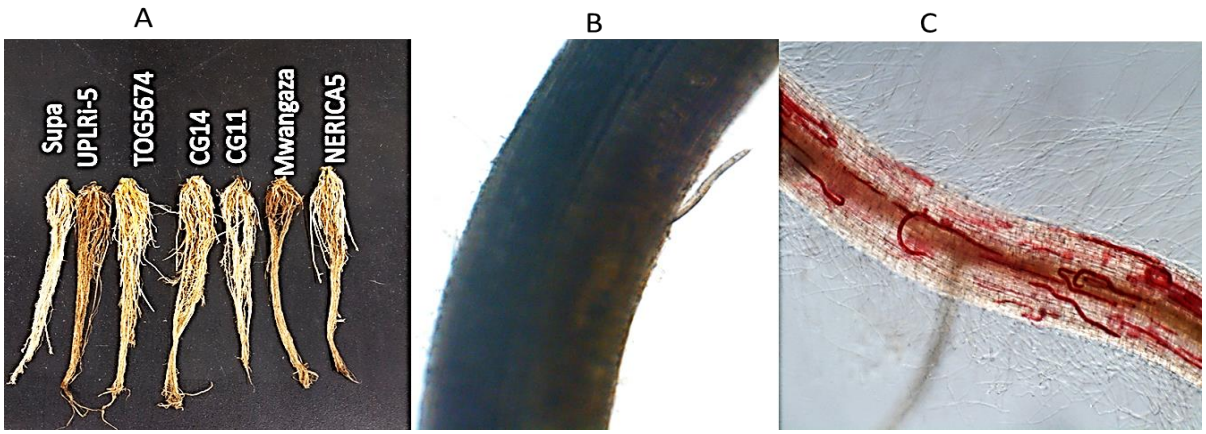


Fig. 4.2: **Rice roots infected with *P. zeae*. A & B; rice roots before staining with acid fuchsin, C; Acid fuchsin-stained root as seen under dissecting microscope.**

To visualize the galls and nematodes, infected roots were stained in boiling acid fuchsin 0.013% and 0.8% acetic acid for 3 minutes. Boiled roots were washed under running tap water, distained in a solution of acidified glycerol to remove excess stains. Genotype evaluation was done by counting the number of galls and nematodes inside the root under a dissecting microscope (S8APO-Leica- Switzerland), for *P. zeae* reproduction factors were calculated as Pf/Pi (Pf=final nematode population in the root; Pi=number of nematodes inoculated to the plant). Host response designation of each genotype as resistant, partially resistant or susceptible was assigned based on methodology by Dochez (2004). For *M. graminicola* infection levels of the plants were evaluated by counting number of galls, developmental stages as females, J3, and J4

compared to the resistant and susceptible reference genotypes. The experiment was performed twice with similar results, only results from one experiment are shown.

4.3.4 Statistical analysis

Data from each experiment were analyzed separately. Nematode data were subjected to log(x+1) transformation before analysis to meet the assumption of analysis of variance (ANOVA). Data were analyzed using XLSTART software. Tukey's ($P<0.05$) was applied for comparisons of factor level means for fresh root weight, the severity of root galling and reproduction factor. To rate the host response of the genotypes the average final nematode population (Pf) of each genotype under evaluation was compared with that of TOG5675 (resistant) and UPLRi-5 (susceptible) reference genotypes using Dunnett's test ($P<0.05$). Host response status was assigned as per table 4.2.

Table 4.2: Identification of the host response of *Oryza sativa*, *O. glaberrima* and their hybrid genotypes to *P. zeae*, infection based on a comparison with the reaction of a susceptible (UPLRi-5) and a resistant (TOG5674) reference rice genotype

Comparison with UPLRi-5	Comparison with TOG5674	Host response of the genotype
Significantly different	Not significantly different	Resistant
Not significantly different	Significantly different	Susceptible
Significantly different	Significantly different	Partially resistant
Not significantly different	Not significantly different	Inconclusive

4.4 RESULTS

4.4.1 Host response of rice genotypes to *P. zeae*

The results from the *P. zeae* infection experiment on the rice genotypes 30 days after inoculation are presented in table 4.3. Lunyuki had the highest root weight, and O8fan6 had the lowest root weight. The hybrids NERICA2 and NERICA1 had the highest and lowest root weight, respectively. Nematode reproduction factor ranged from 0.1 for CG14 to 1.9 for UPLRi-5. Among the *O. sativa* genotypes under

evaluation, Supa had the lowest reproduction factor (0.2) followed by Lunyuki (0.8). NERICA5 had the smallest reproduction factor (0.2) among other NERICAs followed by NERICA2 (0.8) while NERICA1 had the highest nematode reproduction factor of 2.6. The response of *O. glaberrima* to *P. zeae* was fascinating. All *O. glaberrima* did not allow *P. zeae* reproduction hence they were rated resistant to *P. zeae.* Among all *O. sativa* evaluated only Supa was resistant to the nematodes. The NERICA group showed a wide range of responses from highly susceptible to resistant. They were rated either partially resistant or susceptible to *P. zeae* except for NERICA5 that was resistant.

4.4.2 NERICA responses to *Meloidogyne graminicola*

The response of NERICA series against *M. graminicola* was evaluated based on root galling, the total number of nematodes and number of developed females. All these parameters were compared with the susceptible genotype UPLRi-5 and the resistant genotype TOG5674 as controls. TOG5674 had the lowest number of galls, the number of nematodes per plant, and number of developed females per plant. At the same time, All NERICA were found to be susceptible to *M. graminicola* by having a significantly ($p<0.05$) higher number of galls (Fig. 4.3), number of nematodes per plant (Fig. 4.4) and number of developed females per plant (Fig. 4.5) than the resistant TOG5674. Among the NERICA genotypes, NERICA1 was very susceptible and NERICA2, 3 and 10 were the least susceptible to *M. graminicola.*

Table 4.3: Reproduction of *P. zeae*, host response of *Oryza sativa*, *Oryza glaberrima* and interspecific hybrid NERICA and resistant -TOG5674 and susceptible - UPLRi5 reference genotypes 30 days after inoculation with ± 300 nematodes. Data are means ± standard deviation (N=8). Means in the same column followed by the same letter are not significantly different P<0.05) according Tukey's multiple comparison analysis. Comparisons between the number of nematodes in roots of the rice genotypes under study with the susceptible reference UPLRi-5 and the resistant reference TOG5674 were made using Dunnet test. *: indicates significantly and ns: not significantly different (P<0.05). Host response designation based on phenotype, R: Resistant; PR: Partially resistant; S: Susceptible to *P. zeae* infection.

Rice genotypes	Fresh root weight	Number of nematodes/g of root	Number of nematodes/ plant	RF= (Pf/Pi)	UPLRi5^{S}	TOG5674^{R}	Host response based on Dunnet test	Host response based on the RF (Pf/Pi)
TOG5674	0.73±0.1abc	75±20a	55±9a	0.2	*	_	**R**	**R**
TOG5675	0.79±0.7abcde	103±18a	70±12a	0.2	*	ns	**R**	**R**
CG14	0.91±0.2bcdef	101±15a	41±13a	0.1	*	ns	**R**	**R**
CG11	0.64±0.1ab	483±130cdefg	64±11a	0.2	*	ns	**R**	**R**
WAB450	0.96±0.2bcdefg		97±9a	0.3	*	ns	**R**	**R**
UPLRi5	1.18±0.2fghi		569±105i	1.9		*	**S**	**S**
Komboka	1.13±0.2efghi	528±130defg	547±44i	1.9	ns	*	**S**	**S**
Supa	1.34±0.3i	45±13a	60±10a	0.2	*	ns	**R**	**R**
Zambia	1.02±0.1cdefgh	360±67bcde	363±38c	1.2	*	*	**PR**	**S**
Saro	0.64±0.1ab	617±143fgh	385±43cd	1.3	*	*	**PR**	**S**
TXD88	0.90±0.1bcdef	530±91efg	474±48efgh	1.6	*	*	**PR**	**S**
Tai	1.11±0.4efghi	360±69bcde	394±51cde	1.3	*	*	**PR**	**S**
Lunyuki	2.14±0.4j	115±28a	237±34b	0.8	*	*	**PR**	**R**
Mwangaza	0.87±0.1bcdef	474±63cdefg	406±41cde	1.4	*	*	**PR**	**S**
08fan6	0.50±0.1a	1122±290i	544±86hi	1.8	ns	*	**S**	**S**
NERICA1	1.06±0.8defghi	734±72h	776±36j	2.6	*	*	**S**	**S**
NERICA2	1.33±0.3hi	190±41ab	245±26b	0.8	*	*	**PR**	**R**
NERICA3	1.04±0.2cdefgh	351±55bcd	360±24c	1.2	*	*	**PR**	**S**
NERICA4	0.99±0.3cdefg	447±49cdef	439±20cdef	1.5	*	*	**PR**	**S**
NERICA5	1.06±0.8cdefghi	48±8a	51±8a	0.2	*	ns	**R**	**R**
NERICA7	1.11±0.3efghi	328±42bc	360±27c	1.2	*	*	**PR**	**S**
NERICA8	1.04±0.2cdefgh	506±72cdefg	522±65fghi	1.7	ns	*	**S**	**S**
NERICA9	0.75±0.3abcd	641±149gh	459±32defg	1.5	*	*	**PR**	**S**
NERICA10	1.25±0.4ghi	431±63cde	534±69ghi	1.8	ns	*	**S**	**S**

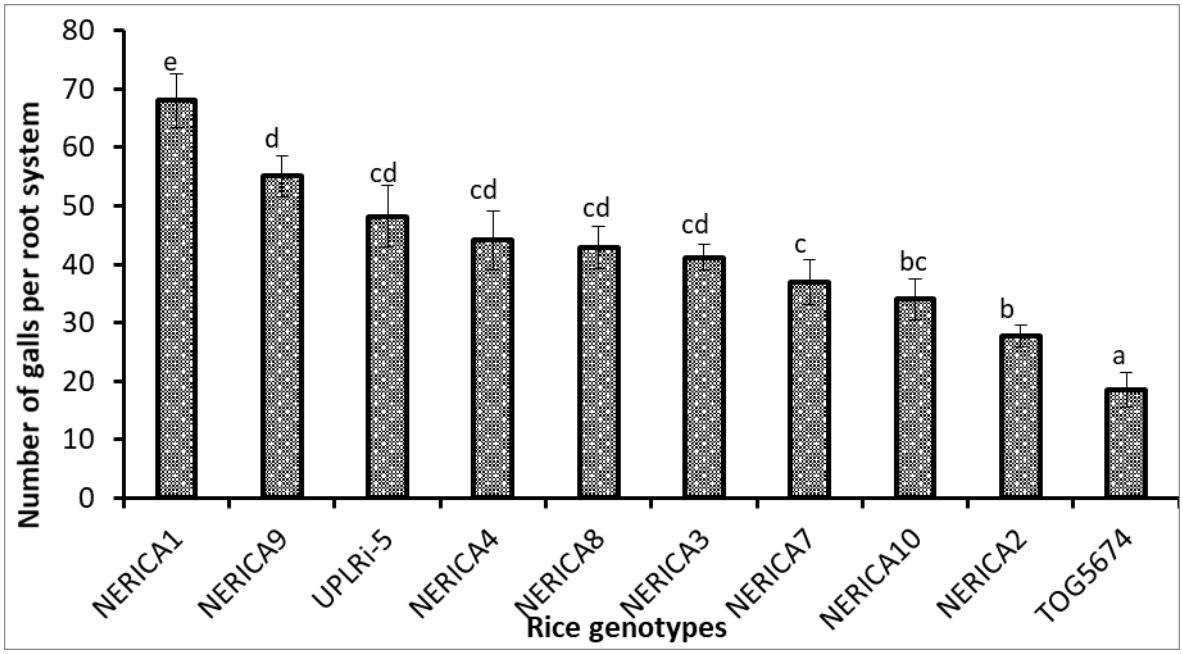


Fig. 4.3: **Root galling of NERICA series 18 days after inoculation with *M. graminicola*. Roots were stained with acid fuchsin and number of galls counted under a dissecting microscope. Each bar shows the average number of galls. Different letters indicate significant ($p < 0.05$) differences among the means according to Tukey's multiple comparison analysis. Data represent mean and standard deviation of 8 plants per treatment. The experiment was repeated once with similar results. The results shown are from one experiment.**

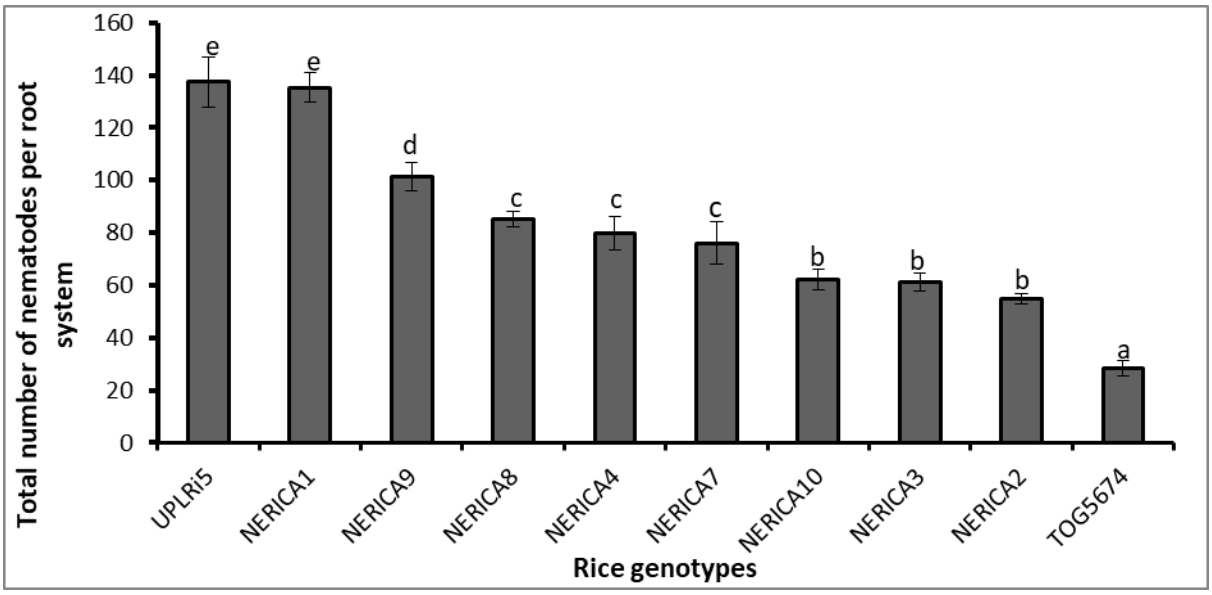


Fig. 4.4: **Total numbers of nematodes in the roots of NERICA series 18 days after inoculation with *M. graminicola*. Roots were stained with acid fuchsin and numbers of nematodes inside the roots were counted under a dissecting microscope. Each bar shows the average number of nematodes. Different letters indicate significant ($p < 0.05$) differences among the means according to Tukey's multiple comparison analysis. Data represent mean and standard deviation of 8 plants per treatment. The experiment was repeated once with similar results. The results shown are from one experiment.**

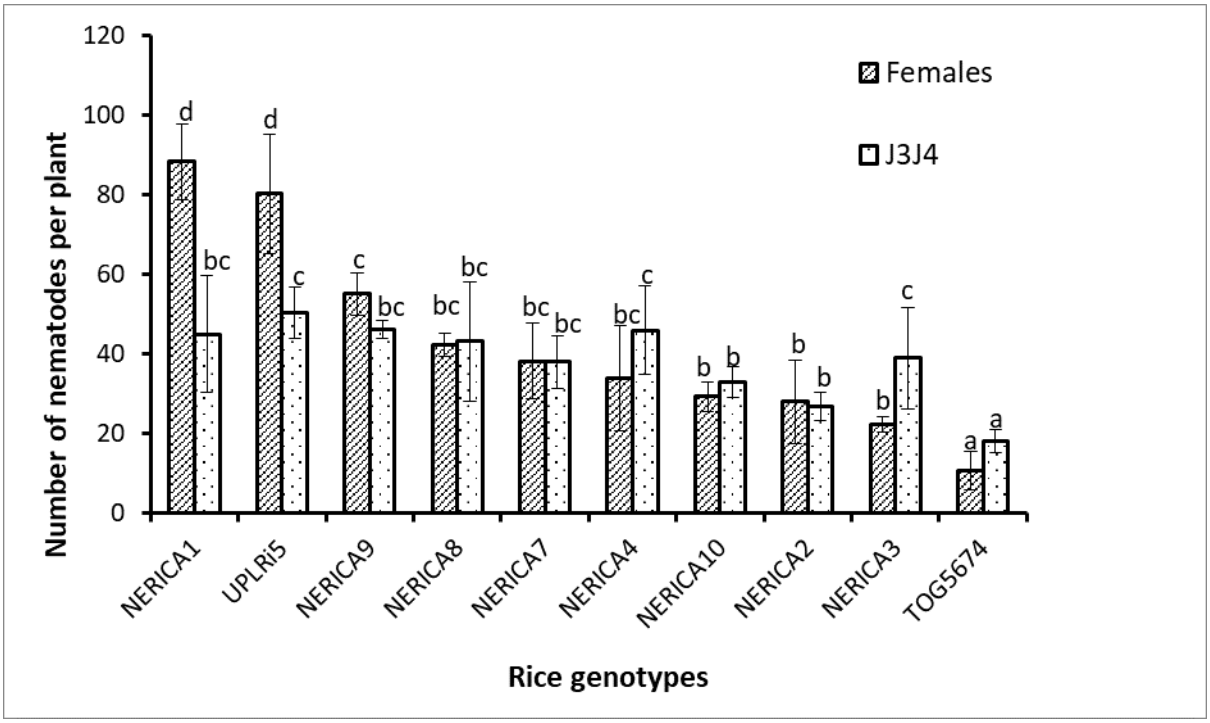


Fig. 4.5: ***M. graminicola*** **development into J3, J4 and females in the roots of NERICA 18 days after inoculation. Roots were stained with acid fuchsin and numbers of J3, J4 and females inside the roots were counted under a dissecting microscope. Each bar shows the average number of developmental stages. Different letters indicate significant ($p < 0.05$) differences among the means according to Tukey's multiple comparison analysis. Data represent mean and standard deviation of 8 plants per treatment. The experiment was repeated once with similar results. The results shown are from one experiment.**

4.5 DISCUSSION

Plant resistance to parasitic nematodes is defined as the ability of the plant to inhibit or lessen nematode penetration and reproduction (Trudgill, 1991). Among the methods used to measure the resistance of the plant is to compare nematode reproduction and development in test genotypes with well-known resistant and susceptible genotypes as reference (Table 4.2) (Boerma & Hussey, 1992). The response of rice genotypes to plant-parasitic nematodes can be evaluated based on the reproductive factor (RF), galling and level of development from J2 to mature females that lay eggs (for RKN) (Cook, 2004; Peng & Moens, 2003). The reproduction factor is obtained by taking a ratio of final to initial nematode populations, Pf/Pi. A resistant genotype should score a nematode reproduction factor less than 1. In the

current study, the mentioned parameters were used to screen rice genotypes against *P. zeae* and *M. graminicola.*

Among the tested genotypes, all *O. glaberrima,* and *O. sativa* – Supa cultivar, and NERICA5 were resistant to *P. zeae*. That can be directly utilized by farmers in the *P. zeae* infested fields where *P. zeae* causes significant yield losses (Coyne et al., 1998, 2001; 2018; Plowright et al., 1990). In Tanzania, rice farmers' varietal selection and market criteria for pricing are primarily based on aroma /palatability (Sekiya et al., 2020). It was interesting to find out that Supa, which is a preferred variety by farmers in lowland and upland rice agroecosystem is resistant to *P. zeae*. Farmers adopted the variety probably because of its extra-long and strongly scented (aromatic) kernels (pers. comm. Luzi-Kihupi 2019). After over fifty years of its cultivation in the country, Supa is taken as a local variety. From a technical point of view, according to Dr Luzi-Kihupi, a rice breeder, after 15-20 years of cultivation, a variety deteriorates very fast probably because of crossing and mutations. For this reason, irrespective of its origin, Supa is considered a traditional variety.

Galling responses of NERICA to *M. graminicola* were widely different, indicating that there is diversification in the genetic background among the NERICA series. Still, all of them were more susceptible than the *O. glaberrima* genotypes.

Host response of plants to nematode infections may differ with genotypes and nematode species. Still, in the current study, NERICA 1 has been identified to be very susceptible to both *P. zeae* and *M. graminicola,* two nematodes with a different lifestyle. Remarkably most of NERICA tested genotypes were moderate to highly susceptible to both nematodes, thus indicating a high risk of introducing these NERICA genotypes in areas infested with *P. zeae* and *M. graminicola.* However, there is a need

for a collaborative effort to screen all NERICAs for reaction to plant-parasitic nematodes, especially RKN, CN and RLN before releasing them for farmers use.

On the other hand, Supa may be recommended to be used by farmers in nematode infested fields in combination with other nematode control measures like crop rotation, biological control and good agronomic practices to maximize yields. Pili et al. (2016) found in his field survey meagre numbers of *P. zeae* infecting rice roots in Kwale county Kenya. Further analysis showed that the cultivar of rice grown by farmers in those fields was Supa which is resistant to the nematodes. Therefore Pili *et al.* (2016) and the current study confirm that Supa resistance to *P. zeae* is depicted in both greenhouse and field conditions. Characterization of the mechanisms of Supa responses to *P. zeae* is hereby suggested.

4.6 CONCLUSION

From the current study, we have found Supa variety to be resistant to *P. zeae*, a positive promising output for rice farmers whose fields are infested with these nematodes. The genotype will be a focal point in future *P. zeae* – host interaction studies.

CHAPTER 5: COMPARISON OF THE PENETRATION, DEVELOPMENT AND REPRODUCTION OF *MELOIDOGYNE JAVANICA* AND *MELOIDOGYNE GRAMINICOLA* ON PARTIALLY RESISTANT *ORYZA SATIVA* CULTIVARS FROM EAST AFRICA

This chapter is adapted from Yasinta Beda Nzogela, Ashura Luzi-Kihupi, Dirk De Waele, and Godelieve Gheysen

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Author's contributions;

YN, AL-K, and GG designed the study and methodology.

YN, excuted the study, analysed the data and wrote the manuscript.

GG & DW edited the manuscript.

5.1 ABSTRACT

The response of the rice (*O. sativa*) genotypes Komboka and Supa to rice root-knot nematodes *Meloidogyne javanica* and *Meloidogyne graminicola* was evaluated in plant room conditions*. O. glaberrima* TOG5674 and CG14 were used as resistant control for *M. graminicola* and *M. javanica* respectively while UPLRi-5 (*O. sativa*) was included as susceptible check for both nematode species. None of the two genotypes under investigation was completely resistant to the root-knot nematodes. However, both genotypes were found to be partially resistant to these nematodes. *M. graminicola* was more aggressive on these rice genotypes than *M. javanica*. For both nematode species, significantly less galling was found in Komboka and Supa than in the susceptible UPLRi-5.

Further analysis showed that Komboka and Supa were significantly less favourable than UPLRi-5 for juvenile penetration, development into adult females and reproduction. Differential emigration of second-stage juvenile (J2) from the roots of Supa and Komboka contributed to the observed partial resistance to *M. javanica* and *M. graminicola*. Nematodes that successfully penetrated and developed in Supa and Komboka showed aberrant phenotypes. Supa and Komboka are here reported to be the new source of resistance to *M. javanica* and adding to the existing *M. graminicola* sources of resistance. They may be directly recommended to be used by farmers in *M. javanica,* and *M. graminicola* infested fields.

5.2 INTRODUCTION

Rice is the staple and commercial crop for more than one-third of the world population (Mantelin et al., 2017). In Tanzania rice (*O. sativa*) is the second most important cereal crop after maize (*Zea mays*), and its production is essential for food security. It is produced across all regions of the country under a wide range of hydrological ecologies. This includes rain-fed upland, rain-fed lowland and highly controlled irrigation schemes (Luzi-Kihupi et al., 2015; Ngailo et al., 2016; Sekiya et al., 2017). RKN and RLN (Pratylenchidae) are the most economically damaging widespread plant-parasitic nematodes in non-flooded lowland and upland rice ecosystems (Nicol et al., 2011; Bridge et al., 2018). The dominant species of RKN are *M. graminicola, M. javanica* and *M. incognita*. Among the three primary RKN species *M. graminicola* is in Asia currently the major cause of yield failure in tropical aerobic rice (De Waele and Elsen, 2007; Kreye et al., 2009; Win *et al.,* 2016; Mantelin et al., 2017). It is well adapted to flooded conditions, and yield losses of up to 87% have been reported (Soriano et al., 2000). When *M. graminicola* is well managed a yield increment of about 12-80% has been reported (Soriano & Reversat, 2003; Padgham

et al., 2004). *M. graminicola* has been known to dominate the rice ecosystems from flooded to aerobic/upland ecosystems, mainly in Asia. Still, of recently, *M. graminicola* has been found in rice fields in Africa - Madagascar (Chapuis et al., 2016) and Europe - Italy (Fanelli et al., 2017).

M. javanica is prominent in *Solanaceae* though it has been found occasionally to infect rice. Under high population initial density of 8000 eggs and juveniles of *M. javanica* per dm^3 soil, grain yield of rice decreased by 40% (Babatola 1984). Damage by these nematodes is more severe under upland conditions (Fademi 1984). In Africa, *M. javanica* has been found associated with rice especially in upland rice fields for example in Egypt, Comoro Island, Nigeria, Ivory Coast, Ghana, Benin, Nigeria, Ivory Coast and Madagascar (Coyne et al., 1999a; 2018). The life cycle of the two RKN species differs*. M. graminicola* lay eggs inside the gall (root cortex) and the second stage juvenile hatches inside the gall. Juveniles can remain in the maternal gall or migrate intercellularly through the aerenchymatous tissues of the cortex to new feeding sites within the same root or may exit the root and find a suitable place for infection. This behaviour appears to be an adaptation by *M. graminicola* to flooded conditions enabling it to continue multiplying within the host tissues even when roots are deeply covered by water. Hence, this makes *M. graminicola* very successful and able to establish in all rice growing ecosystems (Bridge and Page, 1982). Unlike *M. graminicola, M. javanica* lay their eggs to the outer surface of the root. Eggs are enclosed in a gelatinous egg-matrix. Adult females have their egg masses attached to their posterior end protruding out of the root cortex, which can be easily detached from the root (Zijlstra et al., 2000).

Options to control these nematodes are limited and unsustainable. These include flooding (Coyne et al., 1999c; 2018; Das et al., 2011) which is limited by water scarcity and availability (Kreye et al., 2009), nematicides which are not practical due to the low-cost benefit ratio and hazardousness to the environment and crop rotation which is hampered by the presence of the broad host range of these nematodes (Win et al., 2014; 2016). In this context, searching for resistant or tolerant rice genotypes against these nematodes would offer the best alternative for the management of these nematodes in different rice ecosystem.

Natural plant resistance is the cost-effective, most promising, practical, reliable and safe alternative nematode control strategy, but the availability of resistance sources limits it. Most commonly grown *O. sativa* cultivars are very susceptible to these nematodes. Resistance to rice RKN has been identified mostly in African rice *O. glaberrima* Steud and *O. longistaminata* (Diomandé, 1984; Brar et al., 1999; Plowright et al., 1999; Cabasan et al., 2012). However, few studies have identified resistant *O. sativa* rice genotypes to these nematodes (Yik et al., 1979; Sharma-Poudyal et al., 2004; Prasad et al., 2006; Kumar et al., 2014; Dimkpa et al., 2016; Phan et al., 2018; Zhan et al., 2018). Breeding for resistance or tolerance to biotic and abiotic factors including PPN has been a big dream to the rice breeders in Africa. Development of New Rice for Africa (NERICA), progenies of interspecific crosses between the more productive Asian rice (*O. sativa*) and the more rugged African species (O*. glaberrima*) opened the door searching for resistance to PPN (Jones et al., 1997a). The main objective of the breeding work that led to the NERICAs was to combine in one cultivar the high yielding attribute of *O. sativa* rice species with the resistance attribute of the indigenous *O. glaberrima* to various biotic and abiotic stresses of the African environment (Jones and Fosu-Nyarko, 1994; 1997a; 1997b).

This triggered the further exploration of resistance genes for biotic and abiotic stress, including nematodes. Several screening studies have been carried out searching for nematode resistance in interspecific progenies (Plowright et al., 1999; Lorieux et al., 2003; Bimpong et al., 2010; Claudius-Cole et al., 2018). However, the identified nematode resistance in *O. glaberrima* has not been successfully introgressed in interspecific progenies. There are no hybrids found yet that express resistance compared to that of *O. glaberrima* parents. Plowright et al. (1999) found the interspecific progenies were less susceptible to *M. graminicola* than their *O. sativa* parents indicating inheritance of the resistance to *M. graminicola* was quantitative. Afolami and Orisajo (2003) tested 14 NERICA lines released by West African Rice Development Authority (WARDA) against *M. incognita* and found all progenies were susceptible to the nematodes. This shows that there is still a big room for screening interspecific progenies for resistance against PPN.

Host resistance is measured by the degree of nematode reproduction and may occur at different stages of the nematode's life cycle (Trudgill, 1991). Reproduction of the RKN in their host is an output of successful invasion initiation, establishment and maintenance of their feeding sites, which determine the number of nematodes that can cause damage to the host (Ehwaeti et al., 1999). So far identified mechanisms of resistance of rice to RKN has been of three types; First, reduced penetration of the infective second-stage juvenile (J2) in resistant genotypes. This is influenced by root exudates, the aggressiveness of the nematode species and physical barriers such as lignin and callose depositions. In this case, J2 may fail to penetrate the root or enter in lower numbers (Proite et al., 2008). Secondly, equal J2 penetration between resistant and susceptible rice genotypes but delayed development and hence low reproduction in resistant genotype (Cabasan et al., 2012).

Third, an early hypersensitive response (HR) like a reaction that is preventing giant cell formation and further development of the nematodes and ultimately leading to nematode death (Goverse and Smant 2014; Kyndt et al., 2014; Petitot et al., 2017). Fourth, are late resistance responses which involve degradation of the giant cells and failure in nematode development (Di Vito et al., 1996; Kyndt et al., 2014).

Rice-nematode interaction and characterization of the different mechanisms of resistance have been studied much more for *M. graminicola* than *M. javanica* (Petitot et al., 2017; Zhan et al., 2018). The pathogen-host interaction between *M. javanica* and rice is not very well studied. Di Vito et al. (1996) identified the mechanisms of resistance of rice cultivar Hakurt Monton against *M. javanica* one month after nematode inoculation that was associated with necrotic tissue and undersized or no giant cell formation and failed nematode development. There is no more information concerning rice *M. javanica* interaction. Therefore, there is a vast knowledge gap on the rice host responses to *M. javanica* and hence mechanisms of resistance. Searching for *M. javanica* and *M. graminicola* sources of resistance in rice germplasm and thus characterization of their mechanisms of resistance is of paramount importance.

The current study, therefore aimed at 1. Screening for resistance to *M. javanica* from rice (*O. sativa*) germplasm that is commonly grown in Tanzania including *O. glaberrima* which have been reported to be resistant to *M. graminicola*. 2. Characterizing the mechanisms of the identified resistance to *M. javanica* and *M. graminicola* in Supa and Komboka by comparing the two nematodes in terms of nematode penetration development and reproduction. For that three phases were considered in the life cycle; penetration, rate of development from J2 to J3 and J4 and from J4 to adult egg-laying female. Reproduction was measured by egg mass counting

and emigration from the root was analysed as a possible mechanism of resistance in rice against these RKN. Four experiments were carried out to accomplish the stated objectives. The first experiment aimed to screen selected rice genotypes from those widely grown by farmers in Tanzania for possible root-knot nematode resistance. The tested genotypes included 10 *O. sativa*, 4 *O. glaberrima* which are reported to be resistant to *M. graminicola* (Cabasan et al., 2012) and 2 NERICA varieties, which are interspecific hybrids of *O. sativa* and *O. glaberrima* released in Tanzania (Table 5.1.). These were screened against a *M. javanica* population from Tanzania. The second experiment was to test the responses of Supa and Komboka, the rice genotypes that were identified to be partially resistant to *M. javanica* in the first experiment against *M. graminicola*. Then the resistance mechanism of Supa and Komboka was analysed in experiment three through observation of the developmental stages. Experiment four was executed to test for the emigration of J2 from the root. *O. glaberrima* TOG5674 and CG14 were used as resistant control for *M. graminicola* (Cabasan et al., 2012) and *M. javanica* respectively while UPLRi-5 (*O. sativa*) was included as susceptible check for both nematode species (Soriano *et al.,* 1999). Komboka is a Tanzanian local adapted cultivar with a mild aroma, good grain quality developed by IRRI in 2013. The cultivar has a high tolerance to most diseases and thrives well in drought stress rain-fed lowland ecosystems (Malemba et al., 2017).

5.3 MATERIALS AND METHODS

5.3.1 Plant materials

All *O. sativa* rice and NERICA seeds (see table 5.1.) were provided by AfricaRice centre in Morogoro Tanzania while TOG5674, TOG 5675, CG14, and UPLRi-5 were given by the AfricaRice headquarter in Benin.

5.3.2 Source of nematode inoculum and culture maintenance

The *M. javanica* population used in Tanzania (screening experiment) was a pure culture from Kibaha Sugarcane Research Institute primarily isolated from infected tomato plants from Mlali Morogoro Tanzania. The culture was established from a single egg-mass characterized and identified, multiplied and maintained on the Cal J tomato variety grown in sterile soil in the glasshouse at 28°C - 30°C, 75% relative humidity, 12 h: 12 h, light: dark cycle. The *M. javanica* inoculum used at Ghent University (resistance analysis experiment) was a pure culture from INRA-France, multiplied on tomato cultivar Moneymaker grown in sterile soil in pots kept at 28°C-29°C, 70% relative humidity, 12h: 12h, light: dark cycle. When the cultures were two months old, egg-masses were handpicked from the infected tomato plant roots placed on the falcon sieves of 200µm aperture embedded in the six-well plate containing distilled water and subsequently incubated for 72 hours at 26-27°C. Freshly hatched juveniles were collected. The collected nematode suspension was then thoroughly homogenized, and a subsample of 5 ml was poured on a counting dish. With the aid of a microscope and a counter, the nematodes were counted three times in 5 ml aliquots to calculate the nematode density used for inoculation. The *M. graminicola* culture was from the University of Leuven, Belgium and was initially isolated from rice in the Philippines. It was maintained on *O. sativa* cv. Nipponbare in potting soil or on the grass *Echinochloa crusgalli*. Three-month-old infected plants were used to extract the J2 of *M. graminicola* using a modified Baermann method (EPPO, 2013).

5.3.3 Screening for resistance to *M. javanica* of 16 rice genotypes

This was the first experiment conducted in the screenhouse at the Sokoine University of Agriculture in Morogoro - Tanzania, where the temperature was 30 ± 3°C (Fig. 5.1). Seeds of the rice genotypes were pre-germinated in Petri dish containing

moist tissue paper at room temperature for five days in the dark. One 5-days-old seedling was transplanted into (40 X 10) cm - polyvinyl chloride (PVC) tubes filled with sterilized sand and coconut fibre in the ratio of 3:1. The medium was saturated at planting (100% of the soil volume filled with water) and kept at field capacity (50% of the soil volume filled with water) during inoculation. Two-weeks-old plants were inoculated with ±150 *M. javanica* J2 by pipetting an aqueous nematode suspension around the base of each seedling at approximately 5cm deep. One day after nematode inoculation, the plants were watered at field capacity simulating upland conditions. 10 ml of Hoagland's nutrient solution was added three times per week, and the plants were maintained in the screenhouse for 45 days. The experimental layout consisted of a randomized complete block (RCB) design with 12 replications. At harvest, plants were removed from the tubes, and the roots were gently washed and cleaned thoroughly for galling root assessment and nematode extraction. Galls on the roots were visually rated on a 0-5 scale (Cabasan et al., 2018a). Fresh root weights were recorded, and the roots were cut into 1 cm sections. J2 were extracted using a modified Baermann filter technique (Itsede & Akpheokhai, 2013; Cai et al., 2019) by incubating the roots for 14 days. The nematode suspensions were collected for two days interval and counted using a dissection microscope. After each collection, the autoclaved tap water was replaced for further extraction of the J2 until there was no further juvenile emergence. Final J2 (Pf) population was calculated, and classification of the host response of the rice genotypes as resistant, partially resistant, susceptible was based on the standard methodology (Dochez et al., 2009). The Rf of nematodes was calculated based on the final number of nematodes in the root (P_f) divided by the number of nematodes initially inoculated to the plant (P_i).

Fig. 5.1: Eperimental set up for screening for resistance to *M. javanica* of 16 rice genotypes

The second experiment was carried out to assess the reactions of Supa and Komboka against *M. graminicola*. Rice seeds were pre-germinated in Petri dish having moist tissue paper at 30°C for five days in the dark. The seedlings were transplanted singly in specially made polyvinylchloride (PVC) tube containing Sand and Absorbent Polymers (SAP) (Reversat et al., 1999; Nahar *et al.,* 2011). Fourteen – days -old rice seedlings were inoculated with ±300 freshly harvested J2 per plant as previously described (Nahar et al., 2011). Rice seedlings were grown in a plant room at 27-28 ˚C, 12 hr/ 12 hr light regime and relative humidity of about 70-75%. The plants were fertilized with 10ml of Hoagland's nutrient solution per plant three times a week. The response of each rice genotype was evaluated 21 days post-inoculation (dpi) using acid fuchsin staining (Nahar et al., 2011). Eight plants were analysed per genotype in terms of the number of galls and number of nematodes [Females and juveniles] per plant. The experiment was performed twice.

5.3.5 Penetration, development and reproduction of *M. graminicola* and *M. javanica* on Supa and Komboka cultivars

In experiment 3, a time-course study was conducted to compare the penetration and subsequent nematode development of two RKN species on two partial resistant rice cultivars at six-time points during the life cycle. Rice genotypes used in this experiment were Supa and Komboka identified to be partially resistant to *M. graminicola* and *M. javanica* in the first and second experiments. Rice seeds were pre-germinated, transplanted and inoculated as described in the second experiment. Six sampling points were made at 1, 3, 7, 14, 21, 28 and 1, 3, 7, 14, 21, 30 dpi for *M. graminicola* and *M. javanica*, respectively. The experimental design was a factorial experiment with two RKN x 6 days dpi x 8 replications and was repeated once in time. On each sampling point, eight plants per genotype and nematode species were analysed for nematode penetration, development and reproduction (Cabasan et al., 2012). Root systems were washed gently in tap water to clear the root adhered SAP, blotted dry using tissue paper, and weighed. Roots were stained with 1% boiling acid fuchsin for 3 minutes, followed by distaining of the root in acidified glycerol (Bridge & Page, 1982).

Under a dissecting microscope, infection levels and subsequent development were analysed at 1, 3 and 7 days post-inoculation by counting the total number of galls per root system. Galls were excised, transferred to a Petri dish with a drop of glycerol and dissected. Different nematode developmental stages inside the galls were counted as vermiform non-swollen and sausage-shaped J2, globose juvenile with a spiked tail as J3 (Fig. 5.2). At 14, 21, and 28/30 dpi, development and reproduction were analysed by counting the number of galls, a total number of nematodes, J2, J3, globose juveniles with development of the reproductive system as J4, fully developed roundish females, egg-laying females, and egg-masses

(Cabasan et al., 2012). Percentages of each developmental stages of the observed nematodes within the galls per plants were calculated to get more insight on the rate of development and reproduction at 14, 21, 28/30 dpi as

$$\text{Individual Developmental Stage (\%)} = \frac{\textit{Number of individual developmental stage}}{\textit{Total number of nematodes at that sampling time}} \times 100$$

The proportion of individual developmental stages of partially resistant genotypes was compared to that of the susceptible check.

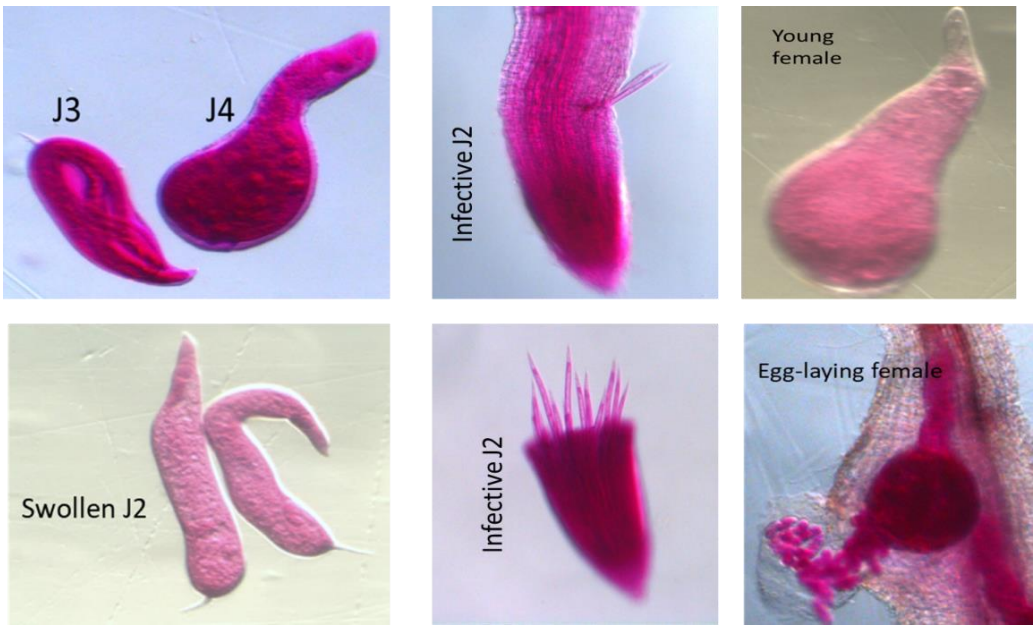

Fig. 5.2. RKN developmental stages

5.3.6 Emigration experiment

In the fourth experiment, the exit of J2 from partially resistant Supa and Komboka was tested. Seeds were germinated, transplanted and inoculated with *M. javanica* and *M. graminicola* as described in 5.3.5. At 3 dpi the roots of the infected plants were removed from the SAP-tubes, their roots cleared of all adhering SAP and immediately immersed in a 50 ml falcon tube containing 30 ml of Hoagland nutrient solution. The tubes were wrapped with aluminium foil to create a dark environment and were left for seven days at room temperature. The nutrient solution was changed three times at 3rd 5th and 7th day and at each change nematodes migrated out of the roots was enumerated under dissecting microscope. At the end of the experiment, the total number of nematodes emigrated from the root system was calculated.

5.3.7 Statistical analysis

Data from each experiment were analysed separately for each nematode species, and time point using Statistical analytical System (SAS). The data were subjected to “proc univariate normal plot” and “proc glm” procedures to test for normality and homogeneity of the variance respectively before analysis of variance. The data set that did not fulfil the assumptions of normality and homogeneity of variance were subjected to log(X+1) or arcsine transformation. The data were then subjected to a one-way analysis of variance (ANOVA). Means of fresh root weight, root galling severity and reproduction factor were compared and separated using Tukey multiple comparison analysis for the screening experiment. Comparisons between the number of nematodes in roots of the rice genotypes under study with the susceptible reference UPLRi-5 and the resistant reference TOG5674 were made using Dunnet test and host responses were designed as resistant, partially resistant or susceptible. Fisher’s least significance difference (LSD) at ($P \leq 0.05$) was used to

separate means for the other experiments where the treatments were not more than 4.

5.4 RESULTS

5.4.1 Screening for resistance to *M. javanica* of 16 rice genotypes

Root galling severity, reproduction and host response of *O. sativa, O. glaberrima* and their interspecific hybrid (NERICA) rice genotypes 45 days after inoculation with *M. javanica* are presented in Table 5.1**.** Supa and Zambia had the highest (4.76) and lowest (0.87) fresh root weight, respectively. Supa and Komboka had galling root indices of 1.6 and 2.5 that were significantly ($P<0.05$) lower than that of UPLRi-5 but significantly ($P<0.05$) higher compared with TOG5675. The highest number of J2 per root system (869) was recovered from TXD 88. Supa had a low number of J2 per root system among the *O. sativa*, that was five times less compared with UPLRi-5 (159 vs 797) but still two times more compared with TOG5675^{R} (159 vs 84). CG11 had the lowest number of J2 (18) per root system.

Nematode reproduction factor (Rf) in the *O. sativa* genotypes examined ranged from 1.6 (Supa) to 8.6 (TXD 88) while the Rf of UPLRi-5 was 7.95 and that of TOG5675 was 0.8. Based on the Rf and gall rating of 10 *O. sativa* genotypes examined, Supa and Komboka were classified as partially resistant, while all others were susceptible to *M. javanica.* None of the NERICA but all four *O. glaberrima* genotypes included in this experiment were resistant to *M. javanica.*

Table 5.1. Reproduction of *M. javanica*, host response and severity of root galling of *O. sativa*, *O. glaberrima* and interspecific hybrid NERICA and resistant - TOG5674 and susceptible - UPLRi5 reference genotypes grown under upland condition in polyethylene tubes of 40 x10 cm, 45 days after inoculation with ± 150 second-stage juveniles. Data were log-transformed before analysis to meet the conditions for ANOVA. Data are means ± standard deviation (N=8). Means in the same column followed by the same letter are not significantly different P<0.05) according Tukey's multiple comparison analysis. Comparisons between the number of second-stage juveniles in roots of the rice genotypes under study with the susceptible reference UPLRi-5 and the resistant reference TOG5674 were made using Dunnet test. *: indicates significantly and ns: not significantly different (P<0.05). Host response designation based on phenotype, R: Resistant; PR: Partially resistant; S: Susceptible to *M. javanica* infection.

Rice genotypes	Fresh root weight (g)	No. of J2 /g of root	No. of J2 / root system	Root galling index	Rf	UPLRi5	TOG 5674	Host Response
TOG 5674	2.14±0.25	28±21.56	53.17±7.63	0.34±0.06a	0.40±0.07a	*	-	R
TOG 5675	1.55±0.36	56.06± 13.31	84±12.74	0.58±0.13a	0.64±0.127a	*	ns	R
CG11	1.89±0.47	10.50±2.47	18.99±2.84	0.16±0.00a	0.14±0.028a	*	ns	R
CG14	2.02±0.47	44.35±10.51	85.92±12.01	0.64±0.11a	0.65±0.12a	*	ns	R
Zambia	0.87±0.19	665.08±170.09	578.83±12.17	3.33±0.24c	3.93±0.12cd	ns	*	S
Mwangaza	1.56±0.312	525.82±131.76	784.50±54.35	4.01±0.15de	5.59±0.54d	ns	*	S
SARO-5	2.32±0.34	367.19±52.05	837.83±51.27	4.16±0.21e	5.92±0.51d	ns	*	S
NERICA4	1.8±0.18	462.40±57.66	822.83±9.81	4.1±0.07e	5.53±0.09d	ns	*	S
O8fan6	1.63±0.24	455.41±66.82	729.50±24.25	3.28±0.26de	5.00±0.24d	ns	*	S
UPLRi-5	1.77±0.09	450.95±29.19	797.17±32.29	3.96±0.31de	5.53±0.32d		*	S
NERICA1	1.43±0.14	613.31±90.38	822.58±56.80	4.12±0.58de	5.85±0.56d	ns	*	S
TAI	1.56±0.32	451.39±118.96	669.17±40.23	3.76±0.12cd	4.66±0.40cd	ns	*	S
Supa	4.76±0.62	39.70.09±8.23	159.58±32.43	1.60±0.32b	1.26±0.32bc	*	*	PR
TXD88	4.05±0.41	216.29±25.76	869.08±82.28	4.41±0.53de	6.33±0.82de	ns	*	S
LH1	1.19±0.58	529.94±175.92	582±65.56	3.17±0.18cd	4.20±0.65cd	ns	*	S
Komboka	2.56±0.53	106.79±31.49	259.58±28.05	2.51±0.29b	1.85±0.28bc	*	*	PR

5.4.2 Host response of Supa and Komboka to *M. graminicola*

After finding that Supa and Komboka were partially resistant to *M. javanica,* their response against *M. graminicola* was analysed. The number of galls and number of nematodes inside the roots was counted in acid fuchsin stained roots 21 days after nematode inoculation.

Supa and Komboka showed low susceptibility to *M. graminicola* but still significantly (P<0.05) different from the resistant TOG5674, hence they are partially resistant to these nematodes as well (Fig. 5.3). The susceptible genotype UPLRi-5 had 47 galls per root system (Fig. 5.3A) with the highest number of females (130) in its roots at 21 days post-inoculation (Fig. 5.3B). Komboka and Supa number of galls and female were low (20 and 23 respectively) compared to a susceptible reference, females (40 and 44 respectively) per root system but significantly higher than TOG5674 (13 galls, 19 females).

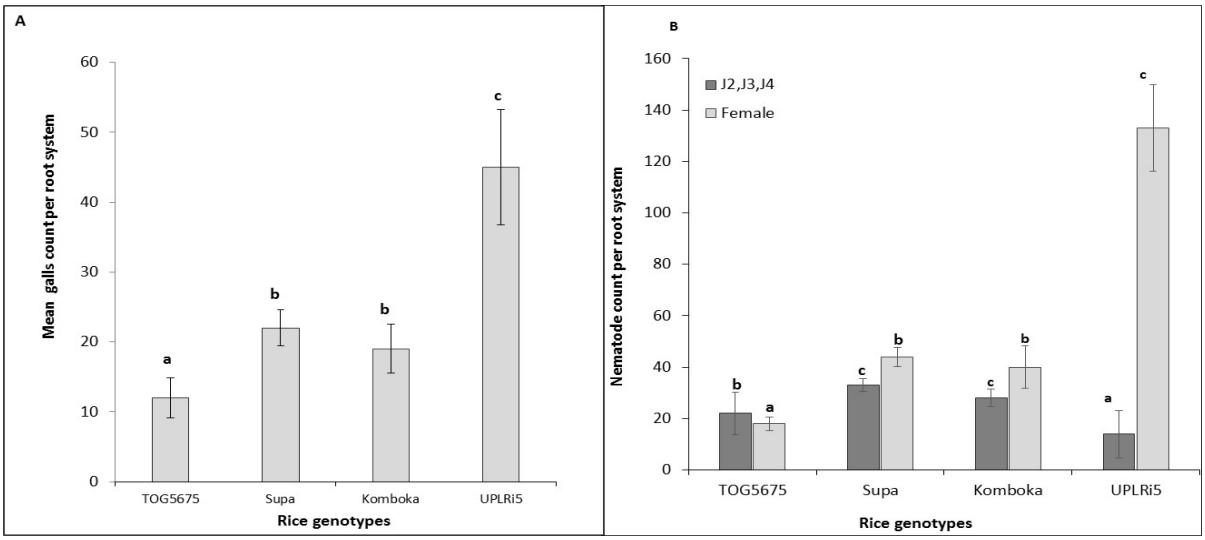

Fig.5.3: Susceptibility of Supa and Komboka compared to TOG5675 and UPLRi-5 as resistant and susceptible references to root-knot nematode *M. graminicola* 21 days post-infection. Eight plants were analysed per genotype, and the response was evaluated based on (A) Average number of galls and (B) the average number of nematodes (females and juveniles) inside the roots infected with ± 300 J2. Each bar with standard error (±SE) represents the average number of galls or nematodes (females and juveniles). Different letters on error bars indicate significantly different infections (P<0.05) according to Fishers Least Significant Difference (LSD). The experiment was performed twice with similar output.

5.4.3 Penetration, development and reproduction of *M. graminicola* and *M. javanica* on Supa and Komboka cultivars

In a more detailed study, Supa, Komboka and susceptible and resistant references were infected with *M. javanica,* and *M. graminicola* and roots were analysed for galling and nematode development stages at several time points after inoculation. The results confirmed Supa and Komboka to be partially resistant to these nematodes.

5.4.3.1 Number of galls

M. javanica gall development was visible at 3 dpi in all tested genotypes (Fig. 5.4A). The highest number of galls was found in UPLRi-5 at 30 dpi, while the resistant CG14 had a low number of galls throughout the sampling time points. In Supa and Komboka, the number of galls at 3 dpi and 7 dpi were significantly ($p<0.001$) lower than for UPLRi-5. At 14 dpi, Supa gall numbers were comparable to those of the resistant CG14, and at 21 dpi, Komboka had several galls similar to CG14.

Root swellings due to *M. graminicola* infection were observed from 3 dpi in all rice genotypes, including the resistant check. A large variation in galling was found among the rice genotypes under study as a response to *M. graminicola* infection (Fig. 5.4B). Initially, Supa had the highest (32) number of galls, however, from 7 to 14 dpi, the number of galls in Supa and Komboka started to decline. From 14 to 28 dpi, the number of galls in Supa and Komboka were significantly ($p<0.001$) lower than the susceptible UPLRi-5^S, but higher than the resistant TOG5674.

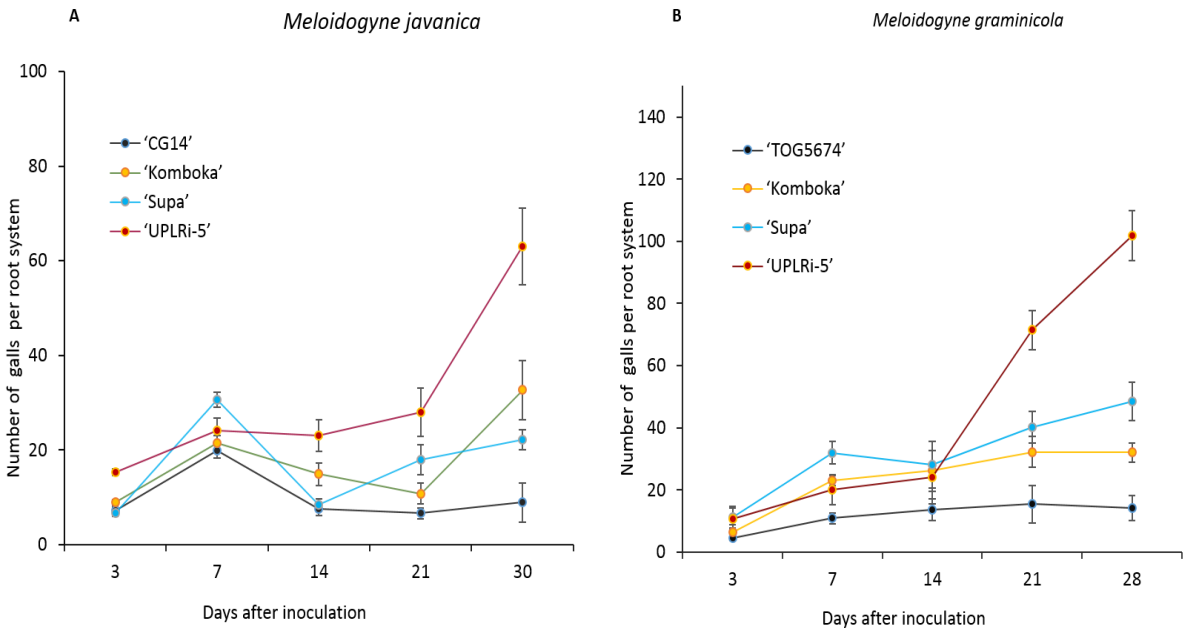

Fig.5.4: **Galling responses of rice plants to (A) *M. javanica* (B) *M. graminicola* in the roots of partially resistant (Supa & Komboka), as compared with resistant (TOG5674/CG11) and susceptible (UPLRi-5) rice genotypes at 3, 7, 14, 21, 28/30 days post-inoculation with approximately 300 juveniles per plant. Data points are least-square means from 8 replicated plants (two experiments X eight plants per days post-inoculation). Bars indicate the ± standard error of the mean.**

5.4.3.2 Abnormal gall phenotypes on Supa and Komboka

Interesting nematode-rice interaction phenotypes were observed during this study (Figure 5.5 & 5.6). The galls on the partially resistant Supa and Komboka were generally numerous but occupied by lower numbers of nematodes as compared to the susceptible reference (Fig. 5.5 A, B and C). *M. graminicola* galls depicted the typical hook-like structure as in the susceptible rice, but *M. javanica* galls often developed at the initiation site of lateral roots, leading to a lateral swelling. The nematodes penetrated the primordial cells, which led to the unique gall structure that made the nematode looks like a hook laterally from the primary roots (Fig. 5.5 F). Reduced gall size forced the nematodes to protrude from the galls (Figure 5.6, B, D, and E) as compared to the control plants (Fig 5.6C and 5.6F). These phenotypes were observed from 14 dpi onward.

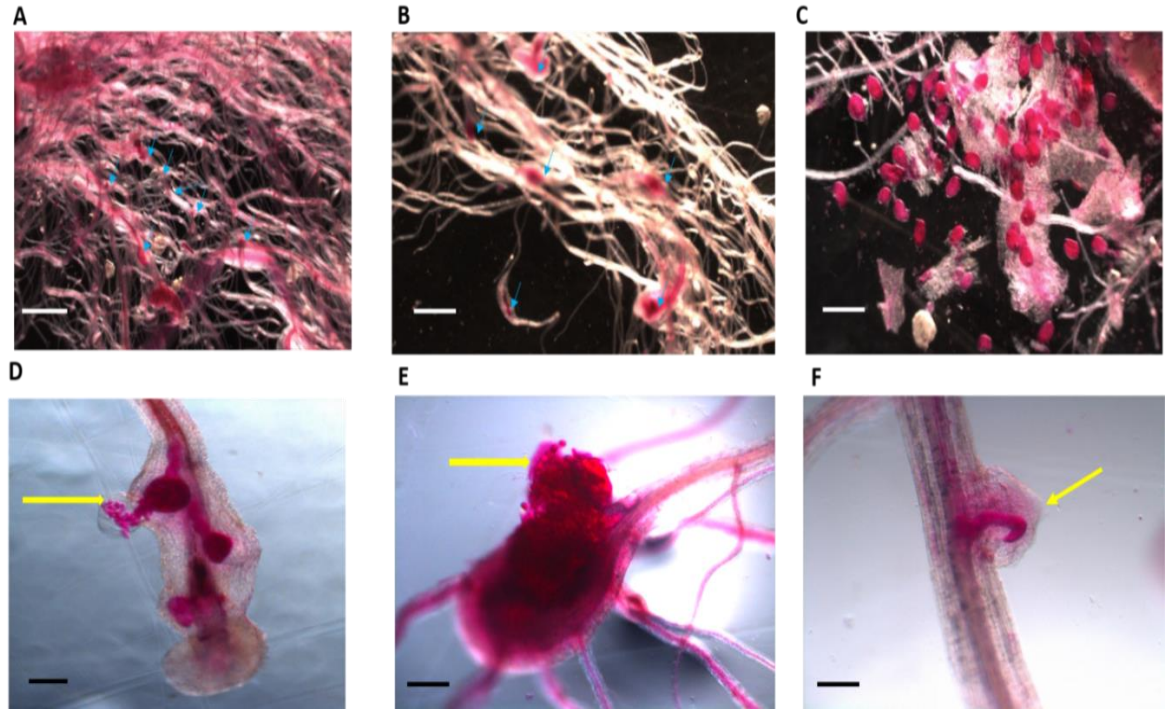

Fig. 5.5: **Fuchsin-stained galls of *M. javanica* and *M. graminicola* partially resistant Supa and Komboka. Small and numerous galls (blue arrows) on (A) Supa and (B) Komboka roots 14 dpi occupied mostly with a single nematode as compared to (C), susceptible UPLRi-5 which had several nematodes in one gall. (D) The egg-laying female of *M. javanica* on Komboka (yellow arrow) with reduced size of egg-masses as compared to that of susceptible UPLRi-5 (E) at 21 dpi. (F) A special gall shape which was observed commonly for *M. javanica* on Supa and Komboka. The galls were frequently formed at the lateral root initiation site only on partially resistant genotypes. Scale bar for a, b, and c = 100mm; d, e and f = 500µm.**

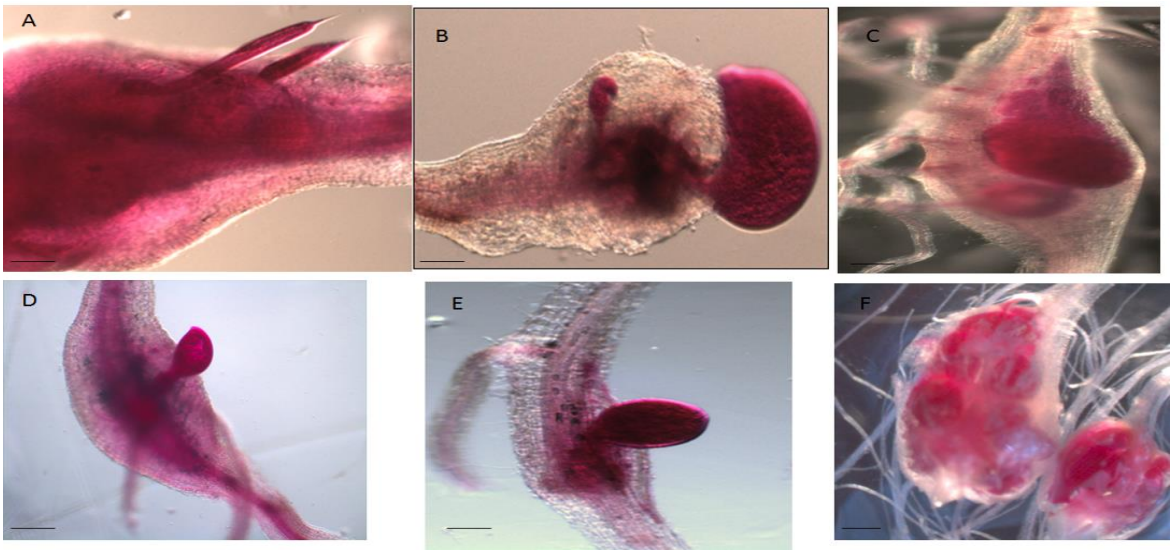

Fig. 5.6: **Distinct phenotypes of the Supa-*M. graminicola* and *M. javanica* interaction that had developed in partially resistant genotypes revealing distinct resistance responses (post-infection) which alter the normal phenotype of the nematodes and the galls at 14 dpi. A and B show J3, J4 of *M. graminicola* unsuccessfully struggling to establish a comfortable feeding site suitable for their development, which resulted in their protrusion out of the root cortex. In contrast, in (C) the root of the susceptible reference UPLRi-5 nematode is well encapsulated in the gall. The same phenotypes were observed with *M. javanica* -Supa interaction, as shown in D, and E, compared to (F) the susceptible UPLRi-5. Scale bar = 500µm.**

5.4.3.3 Penetration and post-infection development of *M. graminicola* and *M. javanica* juveniles in Komboka and Supa roots

M. javanica had been able to penetrate the roots of all rice genotypes understudy at 1 dpi (Fig. 5.7A). However, significantly ($p<0.001$) less J2 were present in CG14, Supa and Komboka roots than in UPLRi-5 at both time points. *M. javanica* J2 was able to develop into J3 at 7 dpi in all tested rice genotypes, but UPLRi-5 roots had the highest J3 number that was significantly ($p<0.001$) different from CG14, Supa and Komboka. Similar to *M. javanica,* J2 of *M. graminicola* had started to invade the roots of all rice genotypes at 1 dpi (Fig. 5.7B). The number of nematodes was very low inside the roots of TOG5674. The number of J2 that had penetrated the roots of Supa and Komboka was similar but significantly ($p<0.001$) lower than UPLRi-5 and significantly higher than TOG5674 at both 1 and 3 dpi. At 7 dpi, the development of both nematodes J2 to J3 was evident in all rice genotypes (Fig. 5.8A & B) with Supa and Komboka having a comparable (25 and 30) J3 number that was significantly ($p<0.001$) lower than in UPLRi-5 (77).

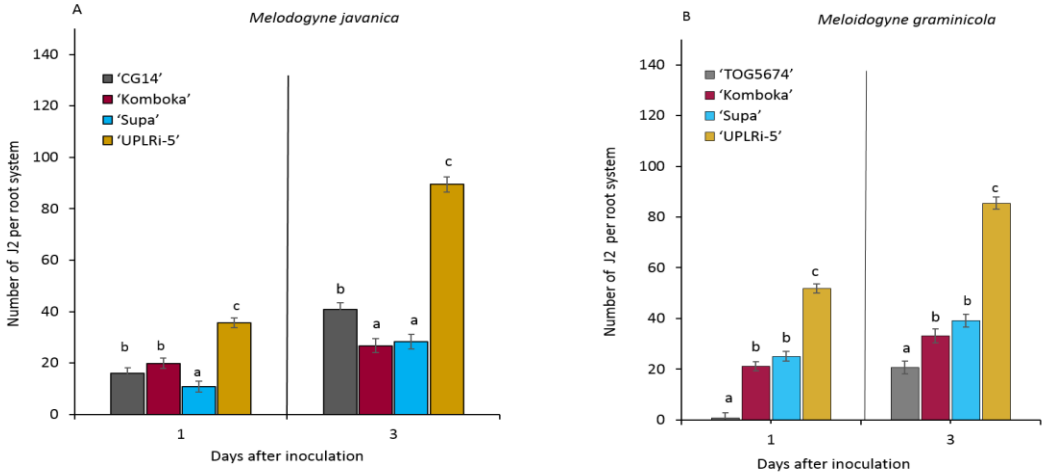

Fig. 5.7: **Number of J2 of (A) *M. javanica* (B) *M. graminicola* that invaded the roots of partially resistant Komboka and Supa rice genotypes compared to resistant TOG5674/CG14 and susceptible UPLRi5 rice genotypes at 1 and 3 days post-inoculation with ± 300 J2. Eight plants were analysed per genotype, and the response was evaluated based on the average number of juveniles inside the roots. Each bar with standard error (±SE) represents the mean number of juveniles. Means followed by the same letter in the same dpi are not significantly different at $p<0.05$ according to Fishers Least Significant Difference (LSD). The experiment was performed twice with similar output.**

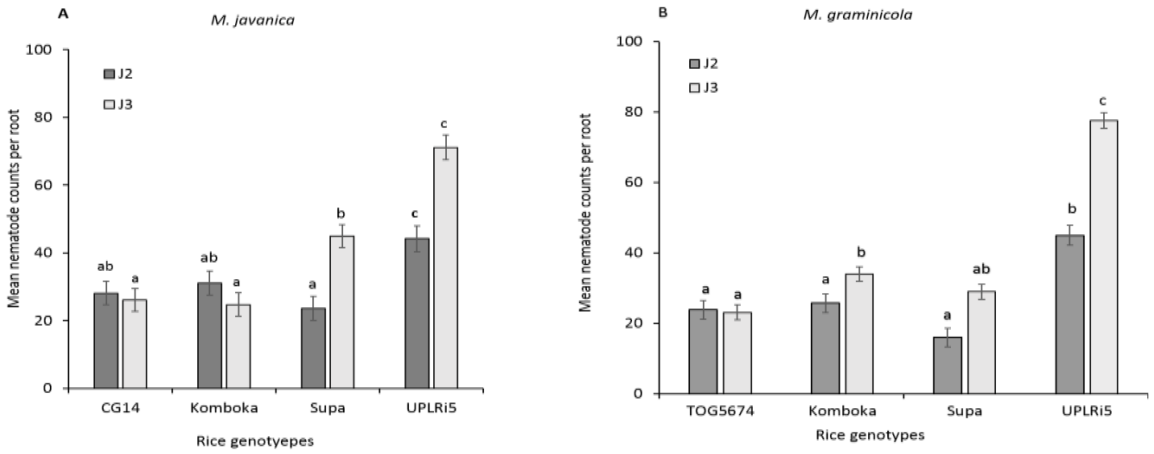

Fig. 5.8: **Invasion and subsequent development of second-stage juvenile (J2) into third stage juvenile (J3) of (A) *M. javanica,* (B) *M. graminicola* in the root of partially resistant Komboka and Supa rice genotypes compared to resistant TOG5674/CG14 and susceptible UPLRi5 rice genotypes at seven days post-inoculation with ± 300 J2. Eight plants were analysed per genotype. The response was evaluated based on the average number of juveniles inside the roots. Each bar with standard error (±SE) represents the mean number of juveniles. Means followed by the same letter in the same developmental stage are not significantly different at p<0.05 according to Fishers Least Significant Difference (LSD). The experiment was performed twice with similar output.**

5.4.3.4 Adult development and reproduction of *M. graminicola* and *M. javanica* on Komboka and Supa

For *M. javanica,* more females had developed at 14 dpi in UPLRi-5 roots than in other genotypes (Table 5.2). Only 26% and 28% of the nematodes present in the roots developed into females and egg-laying females, respectively in Komboka. Supa showed the same trend as Komboka except that; it had fewer nematodes than Komboka. In both partially resistant genotypes specifically, the female development rate was significantly ($p<0.001$) lower than that of UPLRi-5. More nematodes were still lagging in their J2, J3 & J4 developmental stages. At 21 dpi J2 from the second generation developed in all genotypes except for the resistant check. At 30 dpi, significantly ($p<0.001$) less J3 from the second generation was observed in Supa and Komboka than in UPLRi-5 indicating the longer duration of J2 stages in these rice genotypes. The proportion of females and egg-laying females in UPLRi-5 continued to decline to shift the nematode populations from the first to the second generation.

Table 5.2: **The number of *M. javanica* and rate of development (proportion of nematodes in percentage column-wise) on Supa and Komboka rice genotypes and their respective resistant and susceptible references. Nematode development at 14, 21 and 30 days after inoculation (rows) is compared to the susceptible rice genotype UPLRi-5 (–). Means followed by * are significantly and ns not significantly different (P >0.05) to susceptible reference rice genotype according to Fishers Least Significant Difference (LSD). (-) No nematodes of the developmental stage indicated were observed for the rice genotype on that date. The experiment was performed twice with similar output.**

Days post-inoculation	Rice genotypes	Total number of nematodes	Percentage change in each developmental stage						
			J2	J3	J4	Females	Egg-laying females	Second generation	
								J2	J3
	Komboka	52	10ns	19*	17*	26*	28*	-	-
	Supa	32	25*	26*	11ns	16*	22*	-	-
	UPLRi5^{S}	40	14	7	2	66	11	-	-
14	CG14^{R}	31	30*	32*	16*	11*	11ns		
	Komboka	26	-	14*	16*	30ns	27*	13*	
	Supa	46	-	10ns	2ns	29ns	24*	35*	
	UPLRi5^{S}	62	-	5	1	36	46	12*	
21	CG14^{R}	13	-	4ns	38*	40ns	18*	0*	
	Komboka	88	-	-	-	28*	46*	18ns	8*
	Supa	85	-	-	-	26*	37*	22ns	15*
	UPLRi5^{S}	295	-	-	-	2	10	23	65
28	CG14^{R}	31	-	-	-	24*	50*	25ns	1*

^{S} Susceptible reference genotype; ^{R} Resistant reference genotype

At 14 dpi *M. graminicola* juveniles had developed into females and even egg-laying females in all rice genotypes although at variable rates (Table 5.3). At this time point, most of *M. graminicola* J2 have normally moulted into J3; however, in Supa, Komboka and TOG5674 a significant number of them were delayed in their development. At 21 dpi the proportion of egg-laying females reached the highest peak (64%), and that of females started to decline in the susceptible genotype. A substantial proportion of nematodes in the roots of TOG5674, Supa and Komboka, were delayed in J3 and J4 stages of their development. At 28 dpi more J2 had developed into second-generation J3 and eggs continue to hatch, and many more J2 were present in the UPLRi-5 roots. However, only 10%, 1%, and no J3 from the second generation were observed in Komboka, Supa and TOG5674 respectively.

Table 5.3: **The number of *M. graminicola* and rate of development (proportion of nematodes in percentage e column-wise) on Supa and Komboka rice genotypes and their respective resistant and susceptible references. Nematode development at 14, 21 and 28 days after inoculation (rows) are compared to the susceptible rice genotype UPLRi-5 (–). Means followed by * are significantly and ns not significantly different (P >0.05) to susceptible reference genotype according to Fishers Least Significant Difference (LSD). (-) No nematodes of the developmental stage indicated were observed for the rice genotype on that date. The experiment was performed twice with similar output.**

Days post-inoculation	Rice genotypes	Total number of nematodes	Percentage change in each developmental stage						
			J2	J3	J4	Females	Egg-laying females	Second generation	
								J2	J3
14	Komboka	61	26*	22*	38*	13*	1*	-	-
	Supa	73	14*	17 ns	27 ns	32	10*	-	-
	UPLRi-5^{S}	109	0	11	26	42	21*	-	-
	TOG5674^{R}	35	15*	46*	16*	23*	0*	-	-
21	Komboka	55	-	17*	18*	29*	36*	0*	-
	Supa	91	-	10*	16*	24*	47*	3*	-
	UPLRi-5^{S}	150	-	0	1	17	64	18	-
	TOG5674^{R}	33	-	30*	10*	12 ns	40*	8*	-
28	Komboka	84	-	-	17*	27*	52*	4*	0*
	Supa	90	-	-	7 ns	24*	53*	15*	1*
	UPLRi-5^{S}	151	-	-	0	7*	40	43*	10
	TOG5674^{R}	76	-	-	40*	18*	42 ns	0*	0*

^{S} Susceptible reference genotype; ^{R} Resistant reference genotype

Nematode fecundity was measured in terms of egg masses produced per gram of root for both nematode species. The number of egg masses per gram of root was significantly ($p<0.001$) lower in Supa and Komboka than in the susceptible genotype for both *M. graminicola* and *M. javanica* at 14, 21, and 28/30 days post-inoculation (Fig. 5.9).

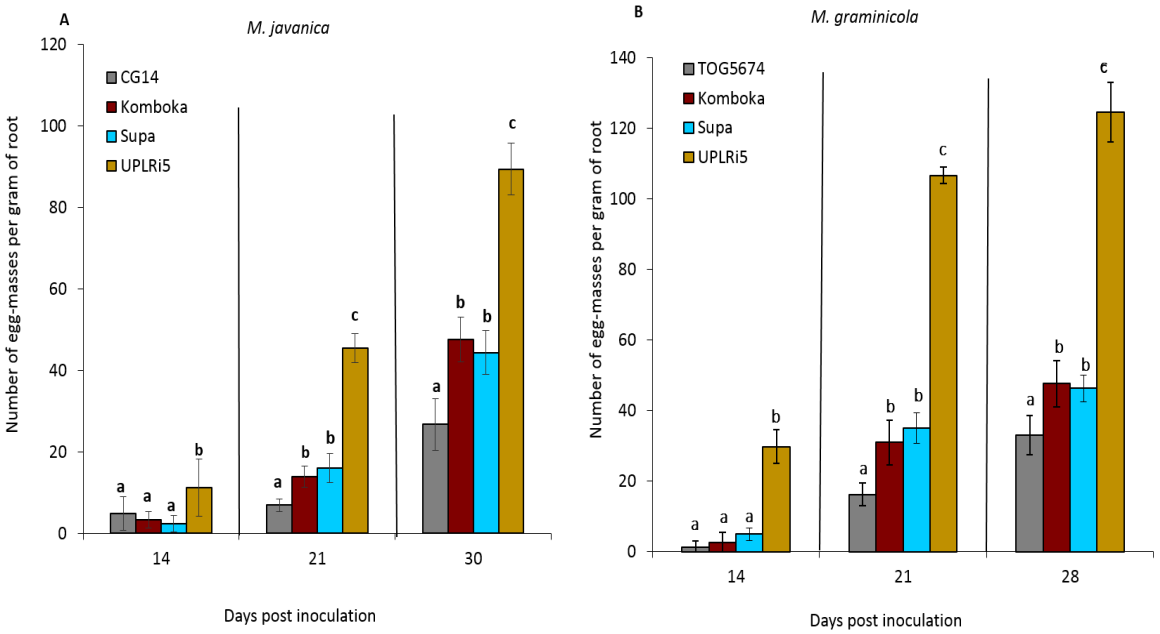

Fig. 5.9: **The number of *M. javanica* and *M. graminicola* egg masses per root weight (g) of partially resistant Komboka and Supa compared to resistant TOG5674/CG14 and susceptible UPLRi5 rice genotypes at 14, 21, 30 days post-inoculation with ± 300 J2. Eight plants were analysed per genotype. The response was evaluated based on the average number of egg masses on/inside the roots. Each bar with standard error (±SE) represents the mean number of egg-masses. Means followed by the same letter at the same sampling point across genotypes are not significantly different at p<0.05 according to Fishers Least Significant Difference (LSD). The experiment was performed twice with similar output.**

5.4.3.5 Second stage juvenile (J2) emigration from the roots of partially resistant Komboka and Supa.

Fig. 5.10 shows average counts of J2 of *M. javanica* and *M. graminicola* emigrated out of the partially resistant genotypes as compared to resistant and susceptible references for all sample dates. More *M. javanica* and *M. graminicola* J2 emigrated from the roots of Komboka and Supa compared to the resistant and susceptible references.

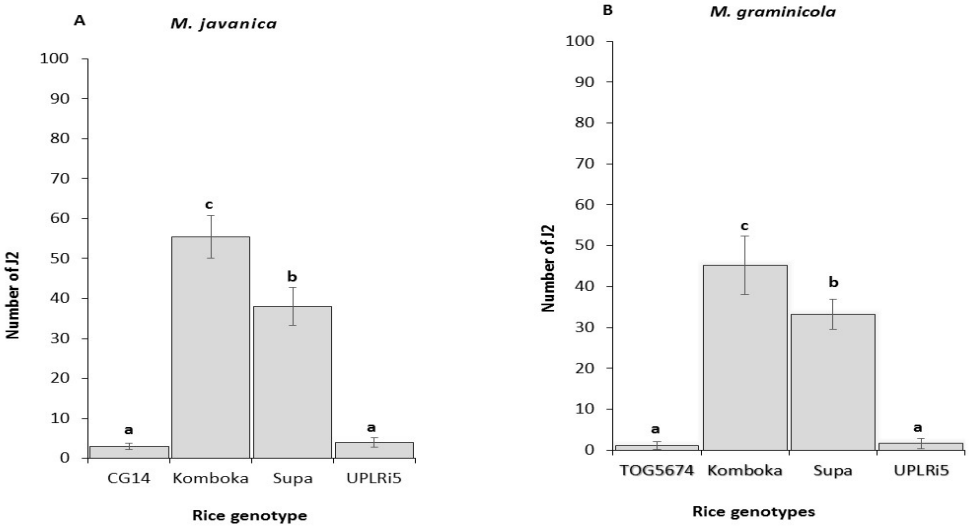

Fig 5.10: The number of (A) *M. javanica* and (B) *M. graminicola* second-stage juveniles (J2) emigrating out of the root of partially resistant Supa and Komboka compared to resistant CG14/TOG5674 and susceptible UPLRi5 rice genotypes from 3-7 post-inoculation with ±300 J2. The rice plants were kept in Hoaglands' nutrient solution for seven days. The suspension was collected three times (at 3, 5, and 7) days post-inoculation and a total number of nematodes counted per genotypes by summing of all nematodes collected for the 3-time points. Means followed by the same letter are not significantly different at p<0.05 according to Fishers Least Significant Difference (LSD). The experiment was performed twice with similar output.

5.5 DISCUSSION

To our knowledge, there is very little information documenting the *Meloidogyne javanica* rice interaction (Diomandé, 1984). *M. javanica* is commonly found associated with rice in rain-fed upland systems (Coyne et al., 1999a; Di Vito et al., 1996; Negretti et al., 2017) and its importance is less than that of *M. graminicola* in flooded conditions. *M. graminicola* egg-masses are layed inside the gall. Contrary to *M. graminicola*, *M. javanica* egg-masses protrude from the root tissue in a gelatinous matrix attached to the posterior end of the female as in other hosts for instance tomato which might be the reason for its lower success in flooded rice ecosystems. However, *M. javanica* may cause economic damage, especially to upland rice (Onkendi et al., 2014) necessitating the search for resistant genotypes.

In screening for resistance, it's imperative to use known resistant and susceptible references for comparison (Peng & Moens, 2003). For this study, it wasn't

possible to find the information with regards to *M. javanica* resistant and susceptible reference rice genotypes. The only research done by Diomandé (1984) depicted that CG11 was shown to be resistant to *M. javanica* under field conditions. However, we did not have access to sufficiently viable seed of CG11. Based on the available information, CG14 was chosen as a resistant reference. In addition to that, CG14 is one of the parents of the NERICAs, and its seed viability is excellent. However, from the screening experiment in the current study, it was evident that the resistance status of CG14 to *M. javanica* is not as strong as that of CG11.

It's therefore recommended to use CG11 as an accurate resistant check during *M. javanica* - rice responses studies if viable seeds are available. UPLRi-5 was used as a susceptible check because it is an upland cultivar and frequently used as a reference for root-knot nematode host responses studies (Das et al., 2011; De Waele et al., 2013; Kumar et al., 2014). In this study, we evaluated the responses of 10 *O. sativa,* 4 *O. glaberrima* and 2 NERICA with *M. javanica. We* showed that these nematodes could infect, develop and reproduce on *O. sativa* however; their reproduction on *O. glaberrima* is hampered. Genotypes from *O. sativa* that were resistant to *M. javanica* similar to the reference genotype CG14 and other *O. glaberrima* genotypes under evaluation could not be found in a pool of screened genotypes. Nevertheless, in terms of susceptibility among the *O. sativa* genotypes under study, Supa and Komboka were found to be less susceptible to *M. javanica* compared to the susceptible reference genotype UPLRi-5 and these genotypes are worth to be more investigated.

Resistance mechanisms may act either before infection or after infection. A pre-infection mechanism is mostly characterized by the pre-existing barriers hindering nematode penetration. Reduced nematode penetration has been commonly observed

as a pre-infection mechanism against nematodes, for example in *O. sativa* (Dimkpa et al., 2016; Kumar et al., 2014) and *O. glaberrima* (Cabasan et al., 2015) against *M. graminicola.* Reduced number of J2 in resistant genotypes has been associated with the emigration of nematodes from the root and mostly within 1- 4dpi range. Bendezu & Starr (2003) found that the emigration of J2 from the root soon after penetration contributed to the resistance of the peanut cultivar COAN to *M. arenaria*.

Delayed nematode development is one among other characteristics of post-infection resistance mechanisms. In the current study, J2 in Supa and Komboka roots were arrested in their further development. This was observed from 7dpi after which *M. javanica* maturity was delayed in Supa and Komboka. Delayed maturity affected the fecundity of the developed females; hence lower numbers of egg-masses were produced per gram of roots. These observations confirmed the lower reproduction factor observed during the screening study, whereby Supa and Komboka had 1.06 and 1.73 reproduction factor, respectively.

Sources of resistance to *M. graminicola* have been found in *O. glaberrima* (Cabasan et al., 2015b;2018a; Soriano et al., 1999) and some few *O. sativa* genotypes (Dimkpa et al., 2016; Phan et al., 2018). Therefore we found of significance to test the identified partially resistant genotypes to *M. javanica* against *M. graminicola,* a known pest of economic importance in rice production in Asia (Wang et al., 2017). The number of galls, J3, J4 and females were less in Supa and Komboka than in UPLRi-5 but significantly higher than in TOG5674 indicating that these genotypes are partially resistant to *M. graminicola* as well. TOG5674 and UPLRi-5 genotypes were found to be useful as resistant and susceptible check respectively for *M. graminicola.* These findings are in congruence with other studies (Cabasan et al., 2015; Das et al., 2011; De Waele et al., 2013).

The galling index has been used as a good indicator of resistance in different root-knot nematode host interactions including rice (Anwar & McKenry, 2002; Mota et al., 2013; Win et al., 2016). However, Supa and Komboka had gall numbers close to the susceptible genotype at the early infection stage (at 7dpi for *M. javanica* and 3, 7 and 14dpi for *M. graminicola)*. Contrary to the susceptible reference these galls were mostly occupied by single nematodes. The number of galls started to decline from 14 dpi to 30 dpi suggesting gall dissolution probably as a result of exit or death of nematodes and recovery of the root tissues from the infection (Kaplan & Keen, 1980; Pegard et al., 2005; Petitot et al., 2017; Roman & Triantaphyllou, 1969). These observations indicate that the mechanism of resistance in Supa and Komboka to *M. javanica* and *M. graminicola* is already pronounced at the early stage of infection. In some roots dissolved galls lead to the protrusion of the female nematode bodies outside of the root tissues. Somewhere between J3 and females, the life cycle was delayed indicating that the mechanism of resistance is not only less penetration but also delayed development. The aforementioned kind of resistance mechanisms has also been observed in *O. glaberrima* TOG5681, whereby degeneration of giant cells and presence of some males were observed from 15 dpi onward for *M. graminicola* (Petitot et al., 2017). For *M. incognita* in resistant cotton (Anwar & Mckenry, 2000) and cowpea (Das et al., 2008) nematodes were able to infect and form galls in the root on both resistant genotype and the susceptible genotype, however, from 14 dpi onward deteriorations occurred for both galls and nematodes in the resistant plants. Therefore, it should be noted that root galls may or may not be a good indicator of host resistance in root-knot nematode host interactions that is most important to be observed especially at early time points of infection. Its interpretation should be made with care (Nyczepir et al., 1999).

We have shown here that part of the Supa and Komboka resistance is contributed to by J2 emigration from the root. A study in *Medicago* hypothesizes that the reasons for the emigration of J2 might be the lack of essential nutrients needed for feeding site initiation and development (Dhandaydham et al., 2008). The specific stimulus for the J2 exit from the roots of Supa and Komboka genotypes remains unknown and warrants further study.

The current study has identified Supa and Komboka rice genotypes to be partially resistant to *M. javanica* and *M. graminicola.* The resistance of Supa and Komboka is associated with reduced penetration of J2, the emigration of J2 from the root, delayed development of J2 to adult and reduced or delayed fecundity.

The study adds more on available knowledge on resistance mechanisms of rice to root-knot nematodes. This information is beneficial for breeders and management of root-knot nematode problems in rice, especially in poor resource farmers that can not afford expensive control measures like the use of chemicals (Coyne et al., 2018). In the fields, damage of these nematodes to the rice is a function of initial nematode density at the onset of seedlings (McLeod et al., 2001). Nematode population increases in soil depend on the number of generations per growing season, which is an outcome of nematode development and reproduction factor (Pegard et al., 2005). The identified Supa and Komboka mechanisms of resistance, which are reduced penetration and emigration of nematodes and delayed nematode development, may contribute to reduced population build-up hence lower nematode population during the seeding. Therefore, Supa and Komboka may be directly recommended to be used by farmers in *M. javanica* and *M. graminicola* contaminated fields.

CHAPTER 6: PATHOGENICITY OF THE ROOT-LESION NEMATODE, *PRATYLENCHUS ZEAE*, ON RICE GENOTYPES UNDER DIFFERENT HYDRO-ECOLOGIES IN TANZANIA

This chapter is adapted from: Yasinta Beda NZOGELA, Sofie LANDSCHOOT, Ashura-Luzi KIHUPI, Danny L. COYNE, and Godelieve GHEYSEN.

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Author's contributions;

YN, AL-K, and GG designed the study and methodology

YN excuted the experiments and wrote the manuscript

SL and YN analysed the data

GG & AL-K edited the manuscript

6.1 ABSTRACT

The root-lesion nematode, *P. zeae*, is commonly found in upland rice fields. The impact of the nematode on rice production was measured in a screenhouse experiment. Two farmer-adapted *Oryza sativa* cultivars, Supa ('SurinamV-880') and 'SARO-5' ('TXD 306') were used under flooded, upland and drought water regimes imposed at seven days post-inoculation of mixed stage nematodes (200, 500, 1000, 3000 and 10000 plant^{-1}). Growth and yield parameters were recorded, and the experiment was terminated after five months. Supa was shown to be resistant to *P. zeae,* while 'SARO-5' was susceptible. *P. zeae* reduced the growth and yield of both cultivars, though more for 'SARO-5' than for Supa. Yield decreased with increasing final nematode densities. *P. zeae* reproduction was highest at 200 and 500 inoculum levels and under upland water conditions. The yield of 'SARO-5' was higher than that

of Supa under flooded conditions and with no or 200 and 500 nematode inoculum levels, but with high *P. zeae* inoculum, Supa yield was better than 'SARO-5'.

6.2 INTRODUCTION

Rice (*Oryza sativa*) is the second most important cereal crop after maize (*Zea mays*) in Tanzania. Rice production is undertaken across all regions of the country under a wide range of hydrological ecologies including rain-fed upland, rain-fed lowland and highly controlled irrigation schemes (Luzi-Kihupi et al., 2015; Ngailo et al., 2016; Sekiya et al., 2017). In Tanzania, rice is used as both a source of food, as and income that constitutes a major crop for food security (Mwaseba et al., 2007). However, production potential varies markedly, mainly due to ecological conditions (Kahimba et al., 2014; Katambara et al., 2013). In Tanzania, there are three main rice-growing systems, rain-fed upland, rain-fed lowland and irrigated lowland (Mwatawala et al., 2016; Van Oort & Zwart, 2018). Rain-fed upland rice constitutes 20% of the total Tanzania production, with an average productivity of 1.2 t ha^{-1}. The productivity potential of rain-fed upland rice ranges between 3 and 5 t ha^{-1} (Kanyeka et al., 1994; Kitilu et al., 2019). Upland rice is usually produced under aerobic conditions with/without irrigation characterised by rainy and dry seasons (Bucheyeki et al., 2011; Mwaseba et al., 2007). The rain-fed upland rice ecosystem in Tanzania is constrained by unreliable and inadequate rainfall, low soil fertility, soil erosion, weed competition, insect pests and diseases (Bucheyeki et al., 2011). The rain-fed lowland rice ecosystem in Tanzania covers 68% of Tanzania rice total production with average productivity of 3.5 t $ha^{-1,}$ and its production potentials range from 4.5 to 6 t ha^{-1} (Meertens et al., 1999, 2003). Fields under rain-fed lowland rice ecosystem are located in valley bottoms, river floodplains (referred to as *Mbuga* in Kiswahili) and swampy areas (Meertens, 1999). Lowland irrigated rice cultivation is characterised by high

levels of water management, often in irrigated schemes constructed for this purpose. It covers 12% of the total area under production with an average productivity of 3.8 t ha^{-1}, while production potential varies from 5 to 6 t ha^{-1} (Ngailo et al., 2007; 2016).

Weed infestation, insect pests and diseases are among the biotic constraints affecting rice in Tanzania (Banwo, 2015). Although only a little information is available with regards to distribution and damage of PPN, a preliminary assessment on their distribution and incidence in selected rice fields was recently provided. Results indicated that the lesion nematode, *P. zeae*, is predominant in all rice agroecosystems but is highly abundant and frequently occurs in upland rice fields (Nzogela, pers. comm.). In West Africa *P. zeae* was found to be prevalent in upland rice fields (Coyne, et al., 1998;1999) second only in economic importance to cyst nematodes. However, there have been fewer studies on *P. zeae* than on the RKN. *Pratylenchus* is migratory and challenging to work with (Jones & Fosu-Nyarko, 2014; Vieira et al., 2017; Yu et al., 2012). Most studies documenting the pathogenicity of these nematodes on rice have been conducted in Asia under upland conditions (Plowright et al., 1990) for example in India (Prot & Savary, 1993). In West Africa, Coyne et al. (2001) demonstrated that increasing densities of *P. zeae* were associated with the increasing number of consecutive rice cropping. Rice yield losses due to *P. zeae* were reported in the Philippines (Aung & Prot, 1990; Prot & Savary, 1993). *P. zeae* is commonly associated with several alternative host crops of importance, such as maize (*Zea mays*), sorghum (*Sorghum bicolour*), sugarcane (Blair & Stirling, 2007), coffee, tobacco, cotton, finger millet, soybean, tomato, sweet potato, wheat, peanut, barley and cowpea (Fortuner & Merny, 1979). *P. zeae* is associated with economic losses in various crops (Castillo et al., 1998; Castillo & Vovlas, 2007). Several studies have demonstrated maize yield losses due to *P. zeae* parasitism, including in Kenya (Arim

et al., 2006) Egypt (Youssef, 2013) and Uganda (Kagoda et al., 2010; Talwana et al., 2015). In pot studies, the yield of sorghum was reduced with an initial inoculum density (P_i) of 500 *P. zeae* in a 20 cm diameter pot (Cuarezma & Trevathan, 1985) and 600 *P. zeae* plant^{-1} (Castillo et al., 1998).

The level of available water and its management has apparent implications for rice production. Similarly, the rice production system and available water/moisture are known to strongly influence nematode infection (Cabasan et al., 2018b). For instance, host location and penetration (Bridge & Page, 1982), migration (Tandingan et al., 1996) and development of nematodes (Win et al., 2015) have been known to be affected at different levels of soil moisture content.

The current work aimed to determine the pathogenicity of *P. zeae* and its impact on rice production under different water regimes. The commonly cultivated *O. sativa* landrace Supa and improved 'SARO-5' ('TXD 306') were used for this study.

6.3 MATERIALS AND METHODS

6.3.1 Experimental setup and nematode inoculation

6.3.1.1 Soil and seedling establishment

Pot experiments were conducted at Sokoine University of Agriculture, Tanzania, in the screenhouse at ambient temperatures of 26-31°C. Pots (5 l) filled with 3 kg of water-saturated sterilised sandy clay soil, pH 6.0, were used. Pre-germinated 5-day-old rice seedlings of Supa and 'SARO-5' were transplanted in the pots and watered regularly as required to maintain the soil-water at field capacity. The seedlings were thinned to one per pot at 14 days after transplanting.

6.3.1.2 Rice genotypes

Seeds of Supa and 'SARO-5' were obtained from AfricaRice at the Sokoine

University of Agriculture, Morogoro, Tanzania. Supa is widely grown in Tanzania under a range of hydrological conditions. It thrives well, is aromatic, has long grains, a pleasant taste and commands a high price at market. Physiological maturity of Supa is reached at 110-120 days, while 50% flowering may take 60-100 days. Spikelet fertility is about 90%, and it yields up to 2.5-3.0 t ha^{-1}. 'SARO-5' is an early maturing (90-100 days) improved cultivar, which takes 50-75 days to 50% flowering. It has a high yielding potential of between 4.3-6.5 t ha^{-1} and is explicitly grown in irrigated and lowland rice ecosystems.

6.3.1.3 Nematode inoculum

P. zeae was initially isolated from upland rice in Tanzania and maintained as a single species monoxenic culture on carrot discs (Kagoda et al., 2010). Nematodes were rinsed off when most began appearing on the surface of the carrot discs. The nematode suspensions (mixed life stages) were homogenized, quantified using a dissecting microscope and standardised to meet each inoculum level. Mixed life stages of *P. zeae* in 3 ml of sterile water were inoculated on individual plants at the following five density levels (P_i), 0, 200, 500, 1000, 3000 and 10000, by pipetting the suspension into a 4 cm deep depression made around the base of the seedling. Non-inoculated pots (control) received the same volume of sterile water.

6.3.1.4 Water regime and fertiliser application

One week after nematode inoculation three water regimes, flooded (F), upland (U) and drought (D) conditions, were simulated for each of the treatments. Flooded pots were maintained permanently flooded until maturity with the water level 5 cm above the soil surface, and then allowed to dry to field capacity one week before harvesting. Upland pots were watered as required to maintain the soil moist at all times

until rice maturity. Water was added to drought pots when the level of water tension, measured with a tensiometer, in the soil fell below -50 Kpa, at which point most plant leaves were beginning to roll in response to drought stress.

Plants received fertiliser, as NPK at a rate of 90:60:60 kg ha^{-1}, 7, 40 and 75 days after transplantation. The experiment was arranged in a split-split plot with water regime as the main plot, cultivar as sub-plot and inoculum level as sub-sub plot using six replications per treatment. The experiment was terminated at 20 weeks after transplanting when all plants were at the maturity stage, although 'SARO 5' matured faster than Supa.

6.3.2 Data collection

6.3.2.1 Plant growth parameters

Rice plants were measured weekly from week one after nematode inoculation to week 11, and then just before the onset of the flowering stage, at the flowering stage and harvest. Numbers of tillers were counted per plant at 30 days after nematode inoculation, flowering stage and harvest. Days to 50% flowering were recorded when 50% of panicles of plants for each treatment had anthers exerted. At harvest, the number of panicles per plant, the number of spikelets, grains, filled grains and unfilled grains per panicle were recorded, as well as filled grain weight. Shoot fresh weight and shoot dry weight, following oven drying at 75°C for 48 h were recorded. Root length and weight were measured at harvest. Percent spikelet fertility, determined by pressing the spikelet between the forefinger and thumb touching along the spikelet to determine whether it was filled or not, was calculated using:

$$\frac{\text{Number of fertile spikelets per plant (fully filled and partly filled > 50\%)}}{\text{Total number of spikelets per plant}} \times 100$$

Fully and partially (> 50%) filled spikelets were all recorded as fertile. Plant grain yield was recorded after adjusting to 14% moisture content. Percentage yield loss per plant was calculated using:

$$\frac{\text{(Yield of non infected – yield of infected)}}{\text{Yield of non infected}} \times 100$$

6.3.2.2 Nematode densities

At harvest, all roots were carefully removed from each pot, gently rinsed under running tap water to remove any adhering soil and then dabbed dry on tissue paper. Roots were placed on a labelled extraction tray, chopped finely and a 10 g root sub-sample was blended in a kitchen blender for 15 s. The soil from each pot was thoroughly mixed in a basin, and nematodes were extracted from a 300 ml sub-sample. The extraction tray method was used to recover nematodes from roots and soil (Coyne et al., 2014). The nematode suspensions from each tray were collected in a labelled disposable plastic cup daily over four days, reduced to 20 ml using a 20 µm sieve and the nematode density established from a 2 ml aliquot on a dissecting microscope; the total for each tray over four days was calculated. Fresh tap water was added to each tray containing soil and root samples immediately after collection. Final nematode population (P_f) and reproduction factor (RF) was calculated using combined root and soil data.

6.3.3 Statistical analysis

The R software package (R Core Team, 2017) version 3.4.2 was used for all statistical analysis of data. As the assumptions normality (Shapiro test) and homoscedasticity (Levene test) for an ANOVA were in most cases not met, all data were analysed using the non-parametric Kruskal-Wallis (Kruskal) test. With a Kruskal-Wallis test, it is not possible to test for interactions between factors; therefore, for this analysis, the data were separated according to water regime and cultivar, and the effect of the nematode P_i on a particular dependent variable tested. Where significant differences ($P < 0.05$) were observed, a pairwise comparison of the groups using a Dunn test was performed.

The data are presented as box plots, which provides a graphical view of the median (horizontal line) and quartiles (Q1 - Q3, box). An outlier is defined as a data point that is located outside the whiskers of the box plot, outside 1.5× the interquartile range above the upper and lower quartiles. Pearson correlation coefficient was calculated on the yield component and nematode data to identify any association with yield components and between fixed variables. Polynomial regression analysis was carried out to explore the respective relationships amongst the yield loss against, P_i levels. at different water regime for each cultivar. Principal Component Analysis (PCA) was executed to visualise yield factor patterns, groups/clusters, and trend of yield components as influenced by P_i to assess any association of P_i with water regime.

6.4 RESULTS

6.4.1 Effect of *P. zeae* on rice plant growth

Plants infected with *P. zeae* had stunted growth, especially for ‘SARO-5’ under drought regime (Fig. 6.1). Yellowing of the lower leaves was commonly observed, mostly for ‘SARO-5’ from 1000-10000 P_i treatment. Most ‘SARO-5’ plants

exposed to higher P_i under drought regime dried and died at around 50 days post-inoculation. Root systems of non-inoculated plants were healthy and intact. Roots of Supa inoculated plants under all water regimes were heavily darkened, and new roots were regenerating. Most of 'SARO-5' inoculated plant roots were heavily damaged, necrotised and beginning to rot, especially at higher (3000 and 10000) P_i inoculation levels. Observable symptoms of *P. zeae* infection included lesions, discolouration of the root system and the loss of fibrous roots. The symptoms were more prominent for 'SARO 5' than for Supa.

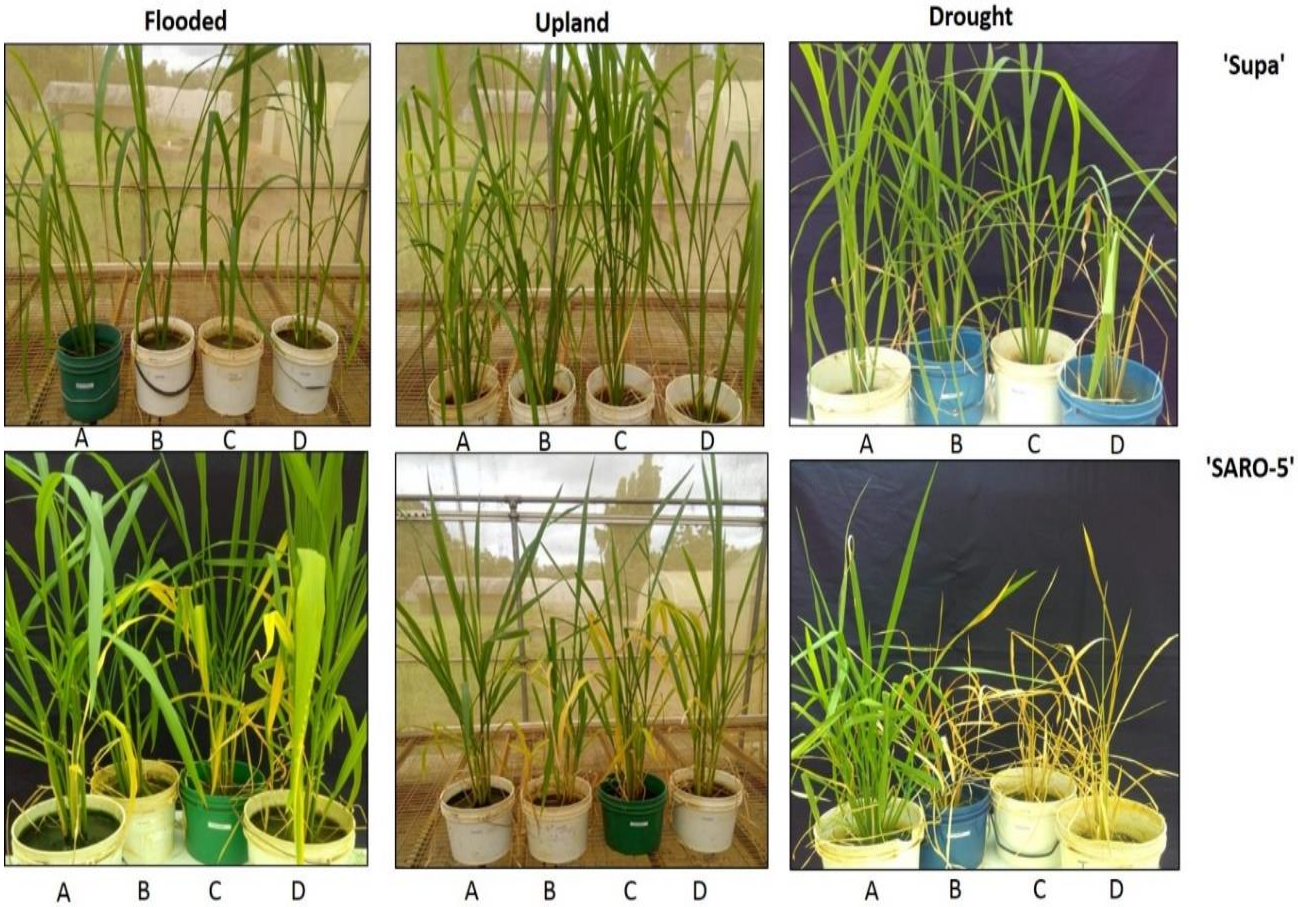
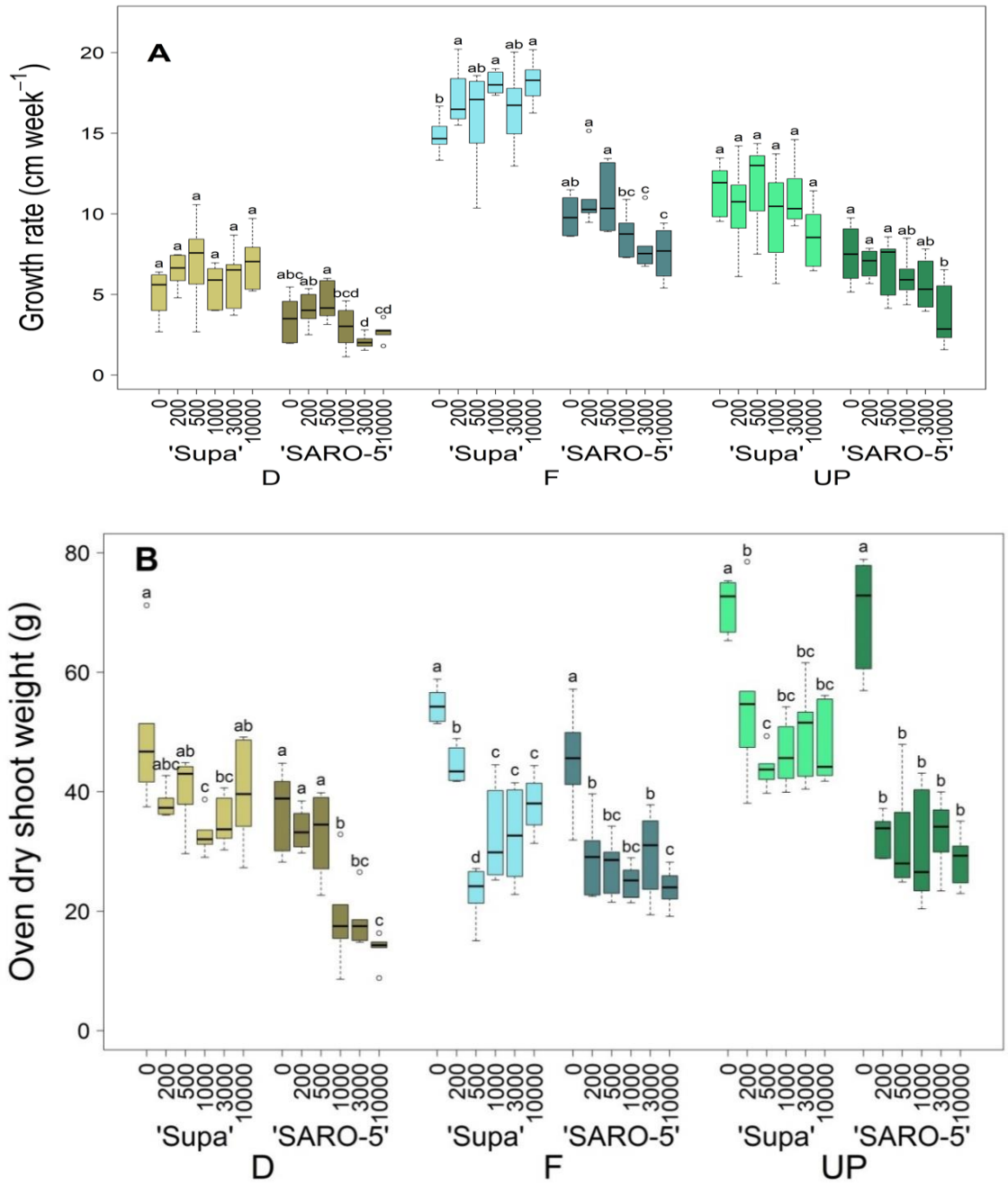


Fig. 6.1: Above-ground symptoms due to *P. zeae* infection on Supa and SARO-5 rice cultivars grown under flooded upland and drought water regime. Infected plants with Pi level of A: 0, B: 1000, C: 3000, D: 10000 nematodes per pot. Obvious symptoms are the yellowing of the lower leaves of SARO-5 under flooded water regime and stunted growth and drying of rice plants under drought 50 days after nematode inoculation.

Growth rates of the plants at one week after nematode inoculation differed between treatments (Fig. 6.2.). Generally, the growth rate of Supa differed significantly ($P < 0.05$) at P_i levels of 200, 1000 and 10000 only under the flooded regime, while that of 'SARO-5' differed significantly ($P < 0.05$) at P_i levels 3000 and 10000 at both drought and flooded regimes and only at 10000 P_i level under upland water regime.



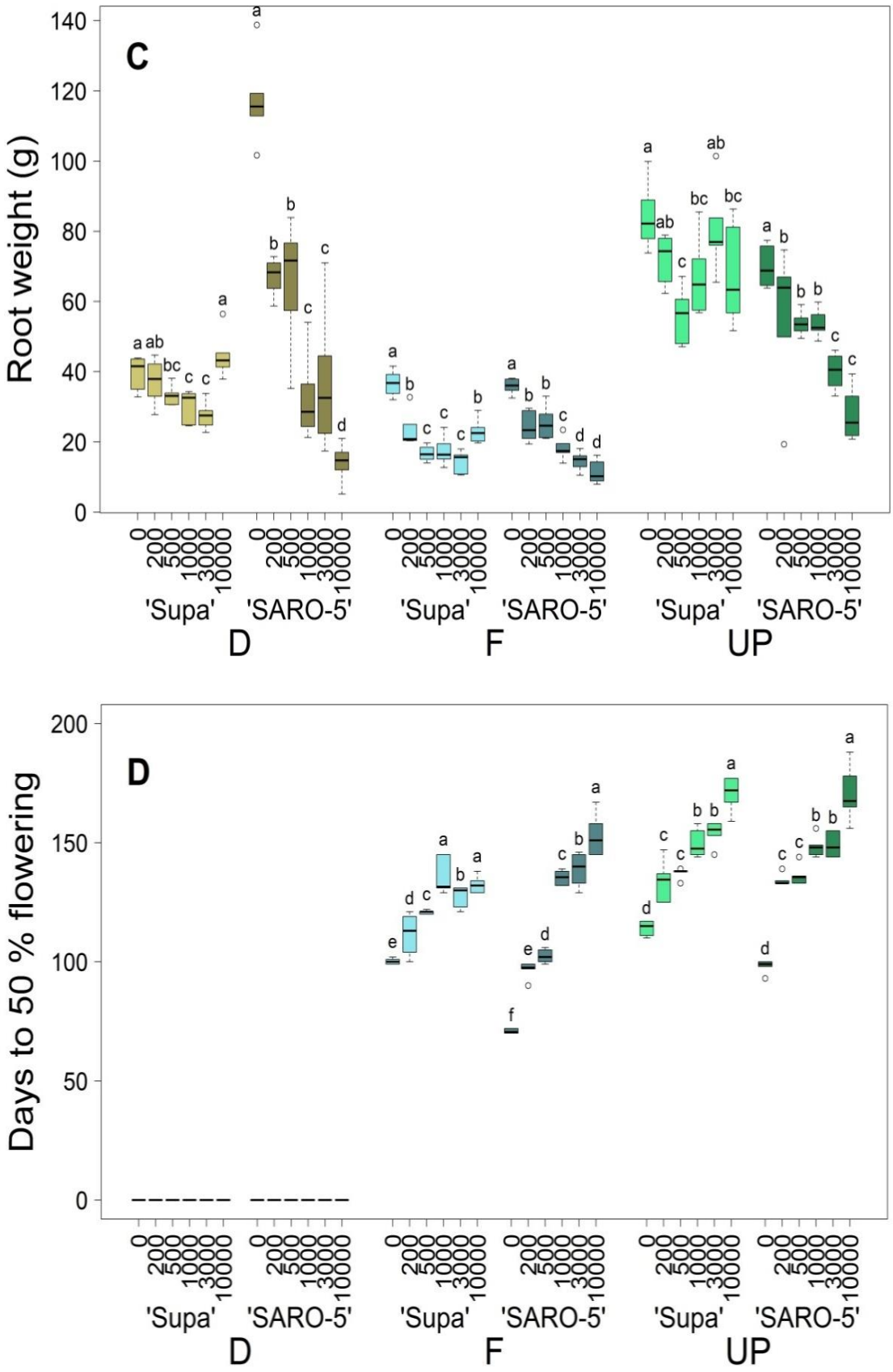


Fig. 6.2: **Effect of initial *P. zeae* density on (A) growth rate, (B) shoot biomass, (C) root weight and (D) days to 50% flowering of Supa and SARO-5 rice cultivars grown under flooded (F), upland (UP) and drought (D) water regime at harvest. Different letters between Pi levels for each cultivar indicate significant differences (p-value < 0.05) according to Dunn test.**

P. zeae inoculation decreased oven-dry shoot weight of both cultivars under all water regimes except at P_i of 200 and 500 nematodes under drought regime for both cultivars and 10000 nematodes for Supa under drought regime (Fig. 6.2B).

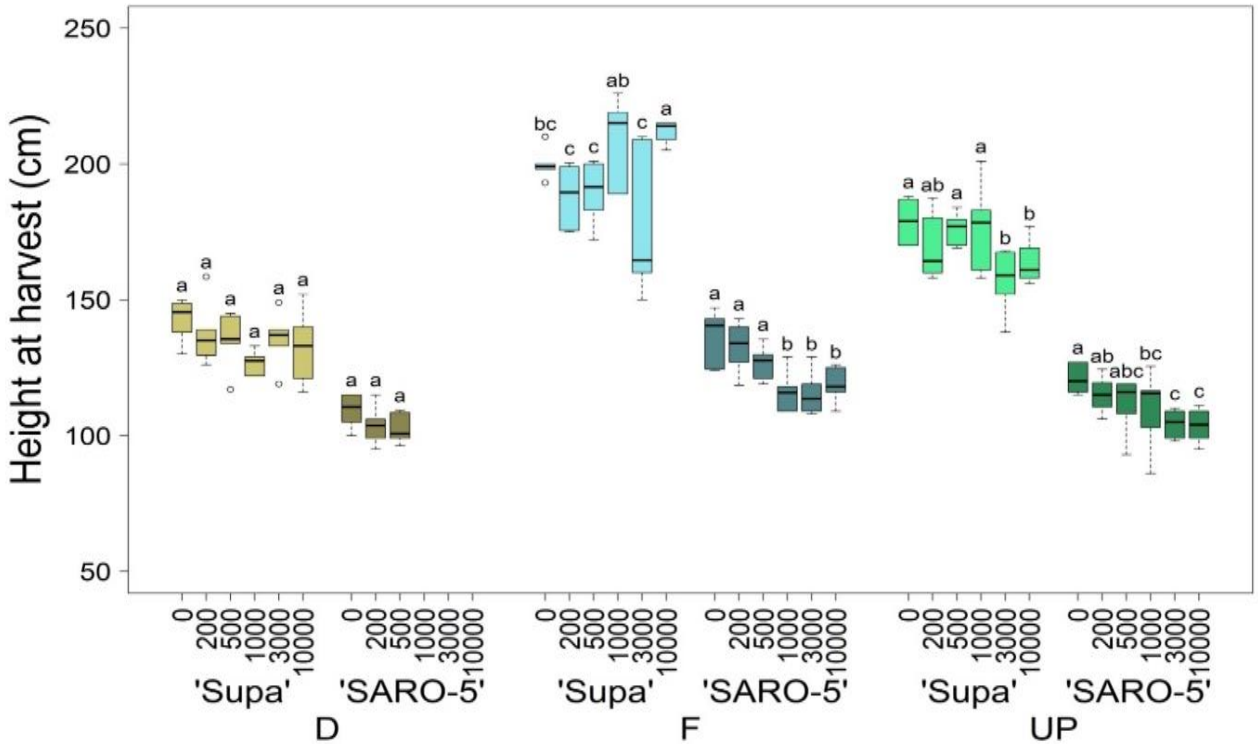


Fig. 6.3: Effect of initial *P. zeae* density on plant height at harvest of Supa and SARO-5 rice cultivars grown under flooded (F), upland (UP) and drought (D) water regime. Different letters between Pi levels for each cultivar indicate significant differences (p-value < 0.05) according to Dunn test.

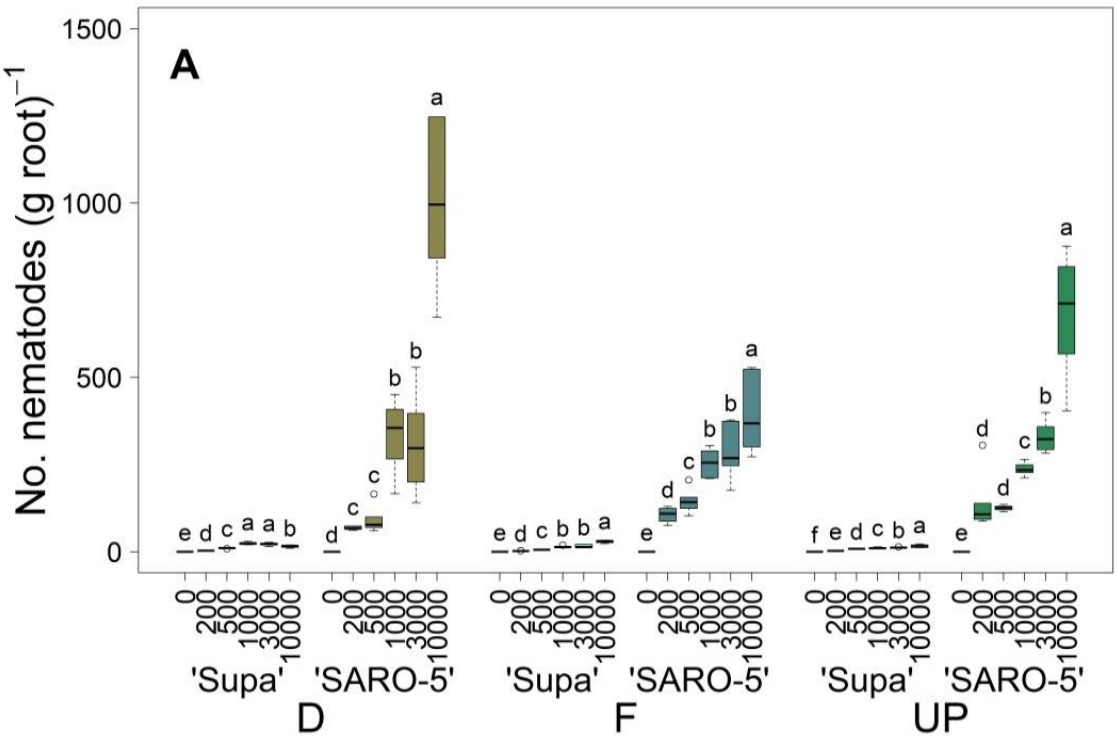
Root weight of Supa was significantly decreased ($P < 0.05$) at P_i of 500, 1000, 3000 under drought regime, 200-10000 P_i under the flooded regime, and 500, 1000 and 10000 P_i under upland water regime. 'SARO-5' root weight was markedly reduced upon nematode infection at all P_i levels under all water regimes (Fig. 6.2C)

Time to 50% flowering was significantly ($P < 0.05$) prolonged for inoculated plants under all water regimes and at all P_i levels (Fig. 6.2D) for both cultivars. Under drought regime, all plants, including control, failed to flower, even at 160 days after transplanting. Height of the plant at harvest was significantly ($P < 0.05$) reduced at flooded and upland water regimes at 1000, 3000 and 10000 P_i levels, but only at 3000

and 10000 P_i level for Supa (Fig. 6.3).

6.4.2 Nematode assessment

Final nematode populations (P_f) per pot progressively increased ($P < 0.05$) with increasing P_i across water regimes for both cultivars (Fig. 6.4A). However, a decreasing trend of final nematode population build-up was observed from drought to upland to flooded water regime. Supa showed resistance to *P. zeae.* It sharply restricted the buildup of nematode populations, and its RF was consistently less than one at all P_i levels (Fig. 6.4A, B), which led to lower P_f in Supa compared with 'SARO-5', especially at higher inoculum levels. Nematode reproduction in 'SARO-5' was inversely proportional to increasing P_i under all water regimes (Fig. 6.4B). The highest and lowest RFs were observed at a P_i of 200 and 10000, respectively, under all water regimes.



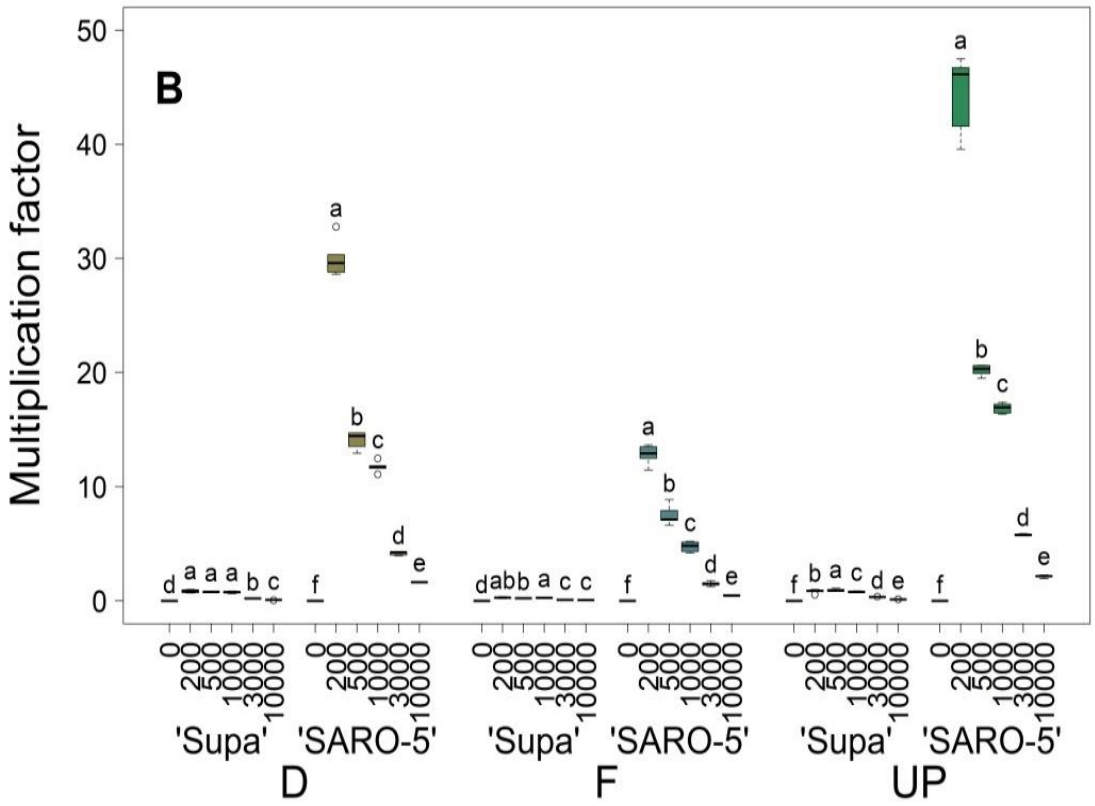
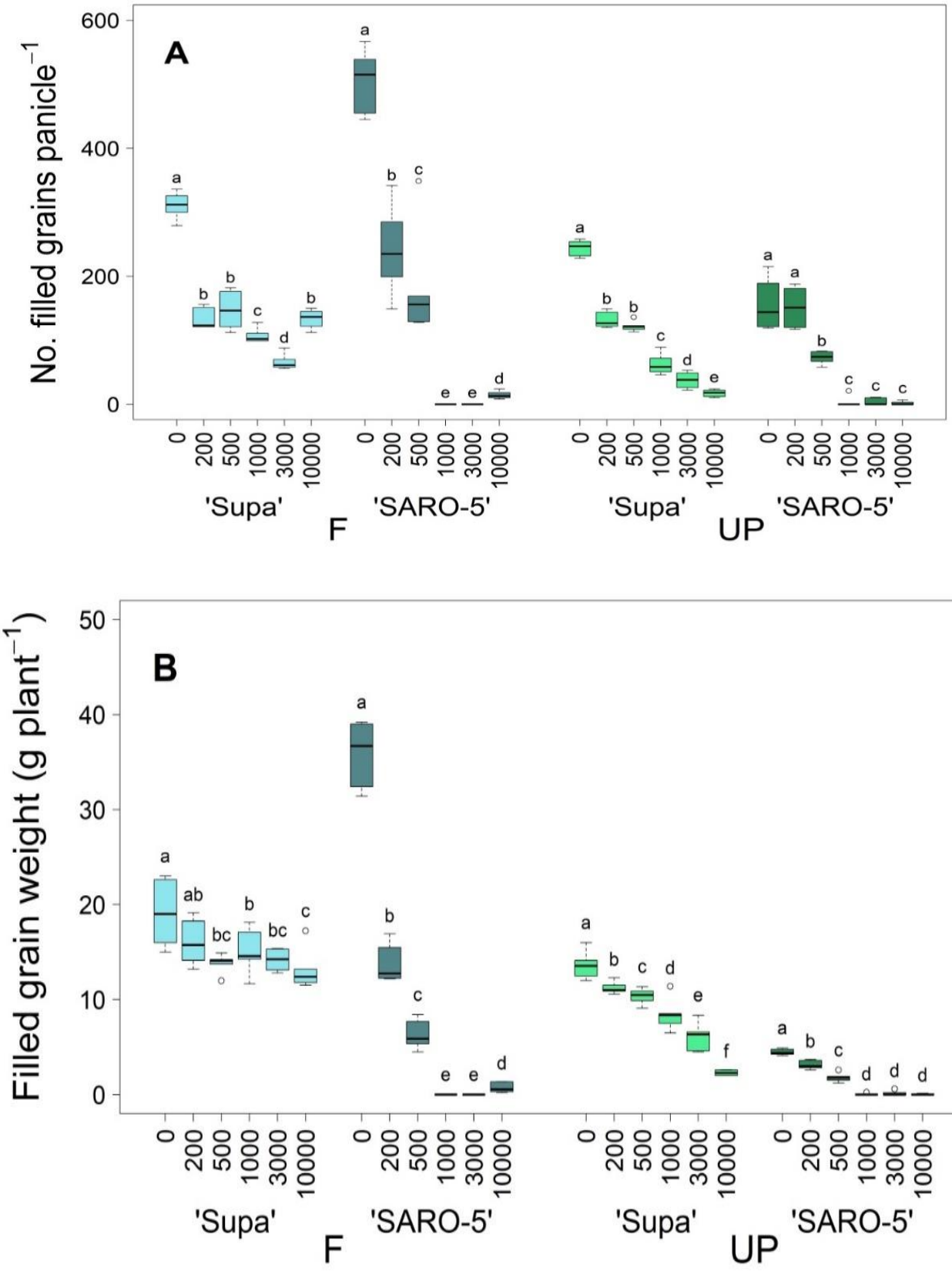


Fig. 6.4: ***P. zeae*** **reproduction at different initial nematode densities on Supa and SARO-5 rice cultivar grown under flooded, upland and drought water regimes with (A) number of nematodes per gram of fresh root weight (B) multiplication rate. Different letters between Pi levels under each cultivar indicate significant differences (p-value < 0.05) according to Dunn test.**

6.4.3 Effect of *P. zeae* on rice yield

There was no grain obtained from plants under drought regime as none of the plants was able even to flower, while some did not survive. The number of filled grains per panicle (Fig. 6.5A) and filled grain weight per plant (Fig. 6.5B) were significantly ($P < 0.05$) reduced following *P. zeae* inoculation as compared to non-inoculated plants across all P_i levels and water regimes except at 200 P_i for 'SARO - 5' and Supa under upland and flooded water regimes, respectively. It was further reflected in spikelet fertility and yield losses (Fig. 6.5C, D). Generally, the yield components varied between P_i levels, for both cultivars under all water regimes. 'SARO-5 was more vulnerable to nematode pressure under all water regimes than Supa at all P_i levels. Under both water regimes, almost no yield was recorded for

'SARO-5' at P_i levels of 1000, 3000 and 10000.



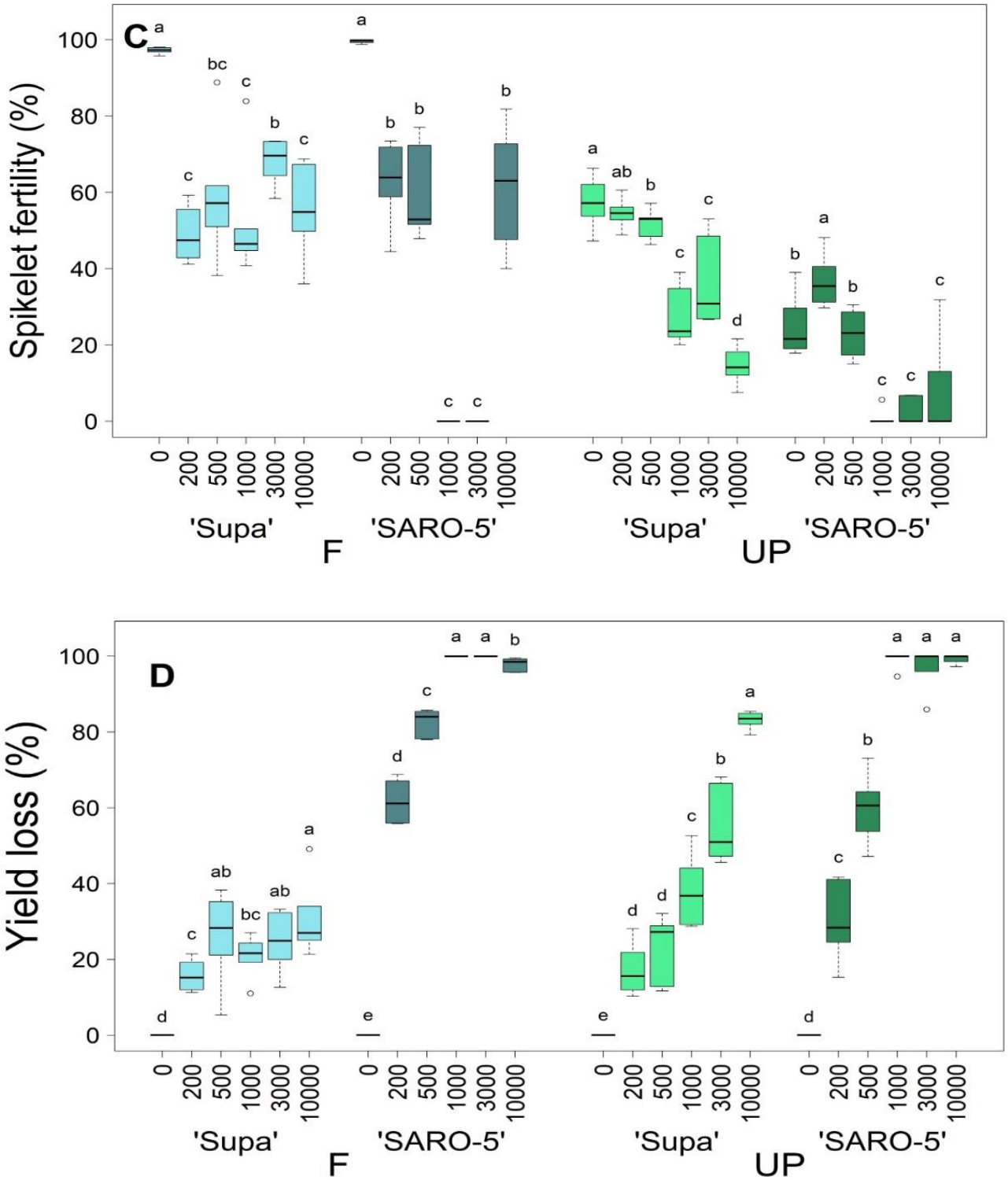


Fig. 6.5: **Effect of initial *P. zeae* density on rice yield components; (A) number of full grains per panicle (B) % of fertile spikelet (C) weight of whole grain per plant in grams (D) % yield loss per plant. Different letters between Pi levels for each cultivar indicate significant differences (p-value < 0.05) according to Dunn test.**

6.4.4 The relationship between rice yield components, yield loss, initial nematode densities and water regime

6.4.4.1 Pearson correlation between variables

In Figure 6.6 the correlation between rice yield parameters and *P. zeae* P_i and P_f are displayed. All yield parameters are significantly negatively correlated to P_i levels and P_f ($P < 0.001$) except the number of unfilled grains per panicle. There was a significantly strong positive association between rice yield loss and P_f (r = 0.88) and P_i levels, (r = 0.70). Yield loss was significantly ($P < 0.001$) and strongly negatively associated with filled grain weight per plant (actual yield) (r = -0.81). P_i levels were significantly ($P < 0.001$) positively correlated with yield loss and significantly ($P < 0.001$) negatively correlated (r = 0.80) with the number of grains per panicle. Grain filling was strongly negatively correlated with P_i, P_f and yield loss and strongly positively correlated with grain weight and spikelet fertility.

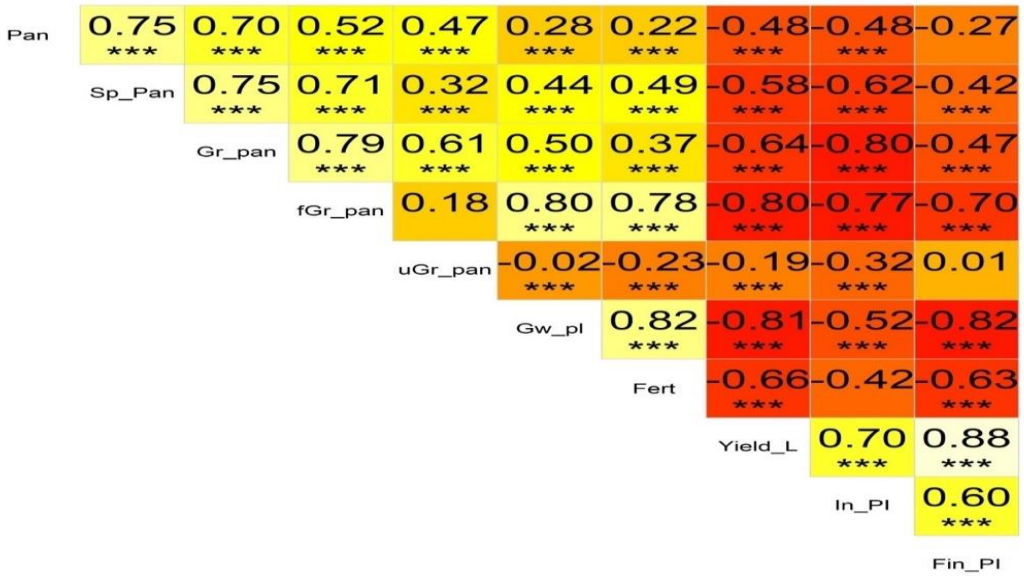


Fig. 6.6: **A correlogram shows Pearson correlations between the yield parameters and initial and final *P. zeae* densities; the number of panicles per plant (Pan), number of spikelet per panicle (Sp_pan), number of grains per panicle (Gr_pan), number of filled grains per panicle (fGr_pan), number of unfilled grains per panicle (uGr_pan), grain weight per plant (Gw_pl), spikelet fertility (Fert), initial nematode density (In_Pi), final nematode population density (Fin_Pl) and yield loss (Yield_L). Colours yellow to red indicate increment in correlation significance negatively or positively. Correlations marked with *** are significant at α = 0.001.**

6.4.4.2 Polynomial regression analysis

For both cultivars, Supa and SARO-5, Pi was significantly ($P < 0.05$) associated with yield loss under both upland and flooded conditions (Fig. 6.7A & B, & Fig. 6.8A & B. For Supa, R2 = 0.94 for upland; R2 = 0.59 for flooded water regime).

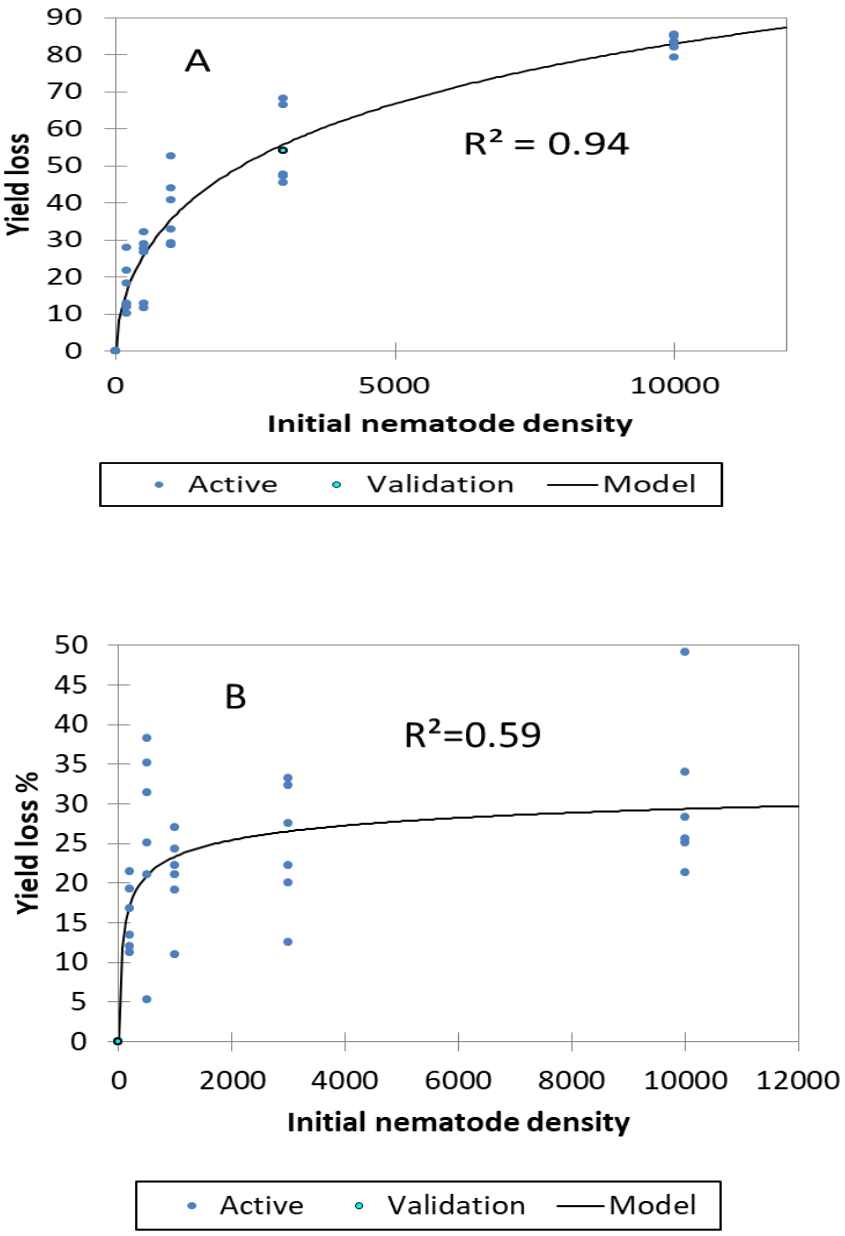


Fig. 6.7: Relationship of Supa yield loss as affected by different initial nematode densities under A, upland and B, lowland water regime using a third degree polynomial function.

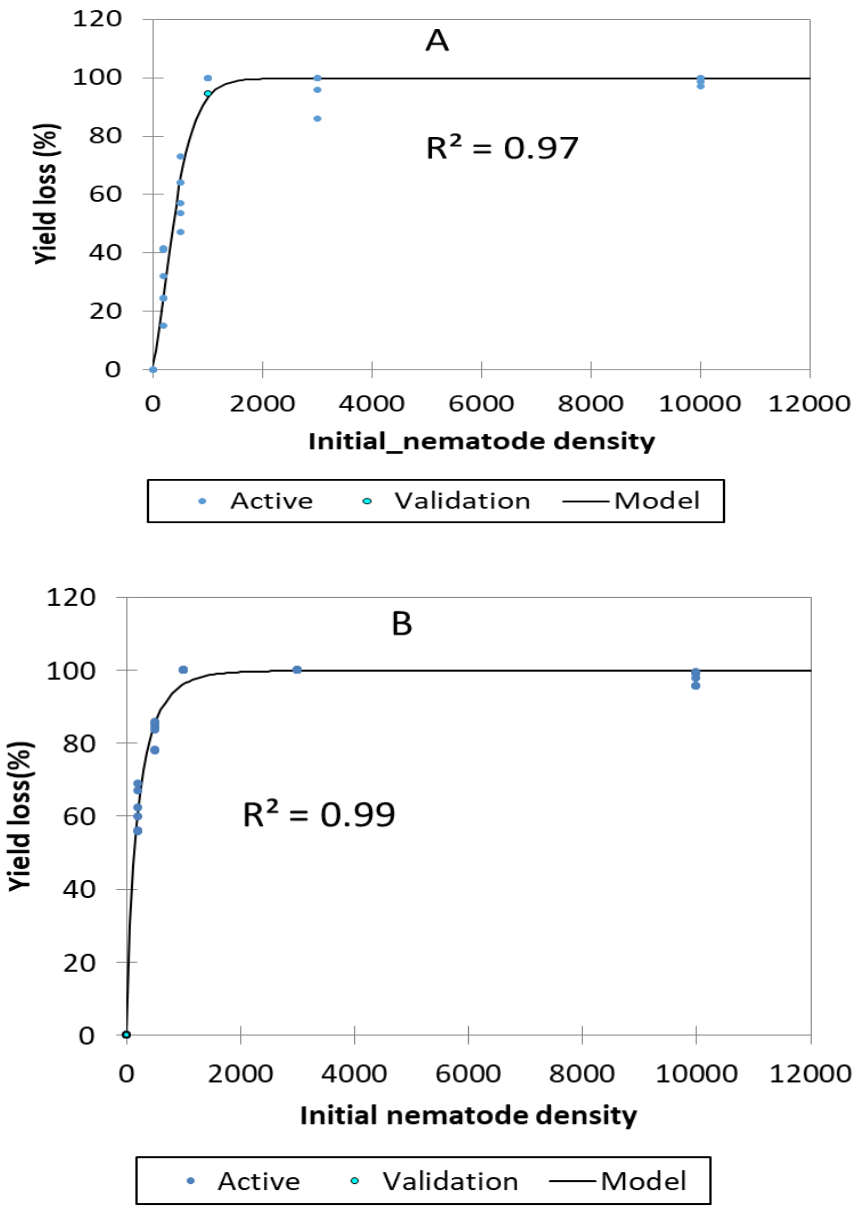


Fig. 6.8: **Relationship of SARO-5 yield loss as affected by different initial nematode densities under A, upland and B, lowland water regime using a third degree polynomial function.**

6.4.4.2 Principle component analysis

The principal component analysis was performed to reveal the pattern of variation of different yield attributes, including yield loss as affected by P_i levels (Fig. 6.9A) and water regimes (Fig. 6.9B). Two components, PCA1 and PCA2, were shown to contribute most to the variation of the yield components and yield loss as affected by P_i and water regime (flooded and upland). The vector directions represent the direction of greatest increase of that yield component, and the vector length provides

a measure of the importance for that yield component. In contrast, the vector angle indicates a measure of the correlation between the components.

PCA1 has an eigenvalue of 2.1820 (variances of the principal components) and explains about 57% of the total variation in the yield component, as affected by P_i. PCA2 has an eigenvalue of 1.37 and explains an additional 22% of the total variation in the dataset. Therefore, 79% of the total variation of the yield component affected by P_i is explained. From the bi-plot, yield loss is mainly positioned under PCA1, in the direction of increasing P_i, indicating that as P_i level increases there is variation in the yield component that leads to an increase in yield loss. Yield loss due to *P. zeae* is the cumulative effect of P_i on all the individual yield components; hence it's positioning at the far extreme to PCA1 and close to PCA2.

The number of filled grains per panicle contributes much to the yield; thus, its vector is placed in the opposite direction to the yield loss vector, with a large angle between them indicating strong negative relationships. Spikelet fertility, grain weight and filled grain are closely associated, indicating strong positive relationships. The number of grains per panicle is linked to the number of panicles per plant. Grain filling has a higher loading on PCA1, with a reciprocal relationship with yield loss as this variable is an important yield component strongly affected by increasing P_i. The bi-plot (Fig. 6.9B) shows the separation between the flooded and upland conditions in determining their effect on rice yield components. The upland water regime is more important than the flooded regime concerning the observed variation of the yield components. However, the importance of spikelet fertility filled grain per panicle and grain weight per plant is evident for the increase in rice yield parameters from the upland to flooded water regime.

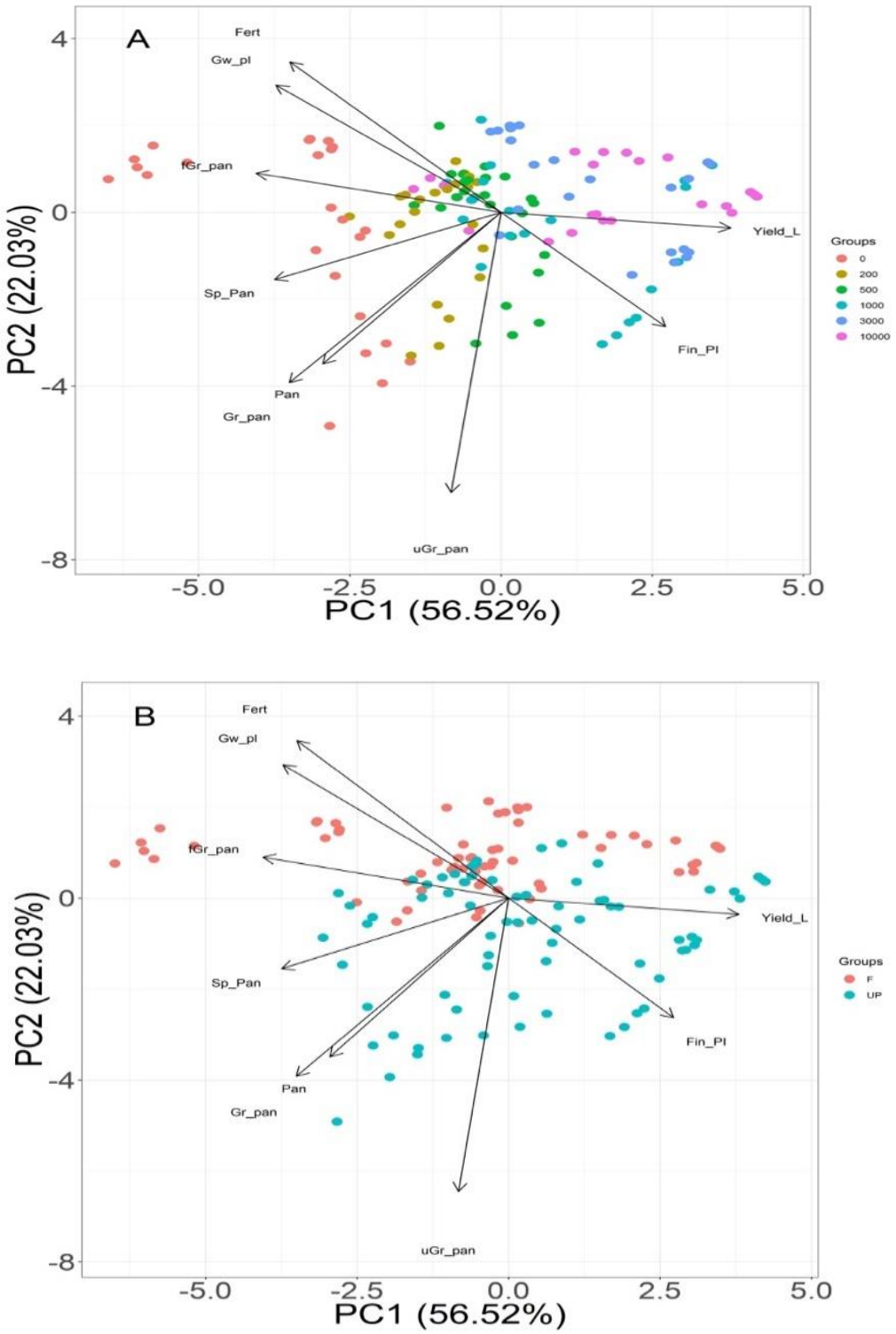


Fig. 6.9: **Biplots (PCA analysis) show the geometric distance and direction of the yield components and yield loss vectors. Fert = Spikelet fertility; Gw_pl = Grain weight per plant; fGr_pan = filled grain per panicle; Sp_pan = spikelet per panicle; Pan = panicles per plant; Gr_pan = grain per panicle; uGr_pan = unfilled grain per panicle; Yield L = yield loss per plant. In (A) the data points (scores) are coloured according to nematode Pi levels, in (B) according to the water regime. F = flooded water regime; UP = upland water regime. Black arrows overlaying the score plot correspond to eigenvectors for different yield components and yield loss.**

6.5 DISCUSSION

To our knowledge, this is the first report of *P. zeae* and rice interaction study under different water conditions in a controlled environment. There is little knowledge with regards to the effect of water regime on infection, survival and reproduction of some *Pratylenchus* spp. (da Silva & Munkvold, 2010). We have shown that in flooded conditions, the reproduction and, hence, final *P. zeae* densities were lower than in upland water regimes. Grain yield was better in flooded than upland conditions, indicating that the effect of *P. zeae* on rice is dependent on water regime and that flooded conditions suffer less nematode damage than upland conditions. *P. zeae* is a well-adapted pest to upland conditions (Bridge et al., 2018; Coyne et al., 2018; 1998; Pili et al., 2016; Plowright et al., 1990).

Indeed, the current study has shown that there are highly significant differences in growth parameters and yield of inoculated plants compared to the non-inoculated ones growing in upland conditions. Although effects of *P. zeae* under flooded water regime were less pronounced than in the upland water regime for both cultivars, at the highest P_i levels (10000), irrespective of the type of water regime, 'SARO-5' had the highest yield losses. Under the upland water regime, even at low (200) P_i level 'SARO-5' yielded less than Supa. This might indicate that upland field conditions, where rice is direct-seeded, and seedlings are attacked by *P. zeae* at a very early growth stage, result in severe yield losses. The yield loss under flooded water regime was unexpected. The observed yield loss under flooded water regime in the current study could be therefore explained by the fact that the nematodes were inoculated three weeks after transplanting. Watering regime was introduced one week after nematode inoculation that allowed a better invasion of the plant by *P. zeae* before flooding (Babatola & Bridge, 1979).

Nevertheless, we observed that even after penetration of the roots by nematodes, flooding reduced the crop damage by *P. zeae*. Therefore, it is evident that flooded conditions harm nematode reproduction and not just on host finding and penetration. Under natural environment, at lowland rain-fed rice production system, where rice crops face a fluctuating moisture regime from flooding to intermittent drought, the yield loss can be exacerbated by the presence of *P. zeae* (Plowright et al., 1990). Furthermore, with climate change, where water availability is becoming a serious problem, most of the fields that used to be flooded are experiencing prolonged periods of drought, which is a favourable environment for multiplication of these nematodes (Colagiero & Ciancio, 2011).

P. zeae could not reproduce well in Supa (RF about one or even lower) indicating resistance to these nematodes. Pili et al. (2016) reported similar results during a screening experiment with a *P. zeae* population from Kenya. Therefore, Supa is resistant to at least two different *P. zeae* populations. By contrast, *P. zeae* had a high RF on 'SARO-5', which resulted in high P_f and consequently, 100% yield loss demonstrating that this cultivar is very susceptible to *P. zeae*. It has been frequently noted that RF of *P. zeae* decreases with increasing P_i level, probably due to increasing competition for survival as determined by the capacity of the roots (Nicol & Ortiz-Monasterio, 2004). The higher the nematode population pressure, the more the damage to the roots leading to a reduced root capacity to support the increasing nematode densities (Nicol & Ortiz-Monasterio, 2004; Sahoo & Sahu, 1993).

The growth rate of Supa was not affected by different P_i levels, while that of 'SARO-5' was affected significantly at P_i levels of 3000 and 10000. The reduced growth of 'SARO-5' under medium and high P_i levels indicates the role played by *P. zeae* on damage to rice roots and would imply that 'SARO-5' is not as tolerant as

Supa. Nevertheless, other growth parameters and yield are also affected by nematode infection in Supa, as discussed below. Under field conditions in Côte d'Ivoire at different hydrology environments, *P. zeae* reduced the growth of rice in the early season (Coyne et al., 2001).

In the current study, 200 nematodes pot^{-1} inoculated three weeks after transplanting had a significant detrimental effect on filled grain weight and hence rice yield, indicating the aggressiveness of these nematodes to susceptible rice. We have evidence that the lowest initial nematode densities used in the current study (200 pot^{-1} or 66.6 $(kg\ soil)^{-1}$) exist in rice fields in Tanzania where rice is grown continuously as a mono-crop or intercropped with maize (Nzogela *et al*., unpubl.). The current results are similar to those of Diomandé (1984) and Plowright et al. (1990; 1999) who observed growth retardation of 'UPLRi-5' grown under field conditions, while the growth rate of 'IR63' was significantly reduced at a P_i of 630-3000 $(100\ cm^3\ soil)^{-1}$ under glasshouse conditions. Sahoo & Sahu (1993) observed damage caused by *P. zeae* on rice grown in pots containing 2 kg of soil at a P_i level of 1000 $seedling^{-1}$. However, 200 nematodes pot^{-1} was the lowest initial *P. zeae* density used in the current study, and it can be hypothesised that even lower levels may cause yield loss (Prot & Savary, 1993).

Nematode damage on rice plants leads to poor grain filling and hence poor grain yield. From the polynomial analysis, both rice cultivar yield losses were significantly ($\alpha = 0.001$) related to P_i levels; similar results were observed by Prot & Savary (1993). These results clearly show that initial nematode density is very important in determining the impact of the nematodes on the crop. Damage of the seedlings by nematodes at early stage has a huge impact on the final yield. However, it is clear that the relation is very steep at the lower inocula and then levels off at the

higher Pi. The better fitting model could be used to predict the damage with an initial nematode density in the field, however, many more data including in field trials would be needed for this.

In the current study, PCA analysis depicted how to yield components of rice, which are highly related to each other, are affected by different P_i and P_f under different water regimes. Two main distinct groups of P_i levels were determined, low P_i levels (0, 200 and 500 nematodes) and high P_i levels (1000, 3000 and 10000 nematodes). This information may be valuable for *P. zeae* management in rice production areas. It is clearly shown that under low *P. zeae* populations (0 - 500 nematodes (5 kg of soil)$^{-1}$) the damage is moderate, which may not cause economic loss. This information, if supplemented with other field studies on *P. zeae* yield loss, may be useful for farmers in making decisions as to when the management of *P. zeae* is essential.

Both rice Supa and 'SARO' plants inoculated at P_i level 3000 and 10000, grown under upland and drought conditions experienced delayed flowering and maturity. This indicates, among others, the effects of nematode infection on plant physiological processes (Babatola & Bridge, 1979). RLN cause heavy root damage reducing the absorption of essential nutrients for plant growth and development. For example, during the study on mineral deficiency, a negative relationship was shown between *P. zeae* density and Fe and Zn contents in rice straw under field conditions (Coyne et al., 2004). Besides, RLN - rice interaction induces defence against the nematodes, which probably leads to lower rice growth and reproduction. Transcriptome analysis by Kyndt et al. (2014) revealed changes in primary and secondary metabolism in rice shoots upon root-lesion nematode infection. Gene expression involved in chlorophyll biosynthesis was reduced in infected plants. These would be among other reasons for delayed flowering under nematode infestation and

hence low yield. The situation was more difficult under drought conditions, whereby plants suffered greatly from the dual stress.

P. zeae are migratory nematodes, and all life stages may enter and exit the host root. The reasons for exiting and re-entering roots are unclear. When they exit the host roots under flooded conditions, they may be less able to re-penetrate. It is hypothesised that under flooded conditions nematodes utilise their energy reserves in maintaining osmotic balance and by undirected movement, perhaps because the dilution of root exudates impairs host-finding (Cabasan et al., 2018b; Win et al., 2011, 2015).

Based on the current study it can be concluded that *P. zeae* causes economic damage to rice grown in a screenhouse environment and yield losses to depend on P_i, the rice genotype, and the water regime in which the crop is raised. Supa is a preferred genotype to be grown under all rice ecosystems when there is detectable *P. zeae* in the soil. However, when *P. zeae* is not a problem, 'SARO-5' is a better yielding genotype in irrigated and flooded rice ecosystems in Tanzania. Further investigations on rice yield loss caused by *P. zeae* under field conditions in different water regimes will add more information on the damage by these nematodes. Supa was confirmed as resistant against *P. zeae* in the current study; however, the mechanism of resistance is unknown. Therefore, investigations to clarify the genetics and mechanism of Supa resistance to *P. zeae* should be a priority for future research. The present study highlights a key issue of how hydrology may change the pest status of *P. zeae*. This information is useful, especially currently when climate change impacts are inevitable, and rice will be grown more frequently with less water.

CHAPTER 7: CHARACTERIZATION OF RESISTANCE OF RICE (*ORYZA SATIVA*) CULTIVAR SUPA TO ROOT-LESION NEMATODES *PRATYLENCHUS ZEAE*

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Manuscript in preparation

Author's contributions;

YN, GG, and LB designed the study and methodology

YN excuted the experiments, analysed the data and wrote the manuscript. Part of the experiment (Instrumentation for metabolite analysis) was done by Geert Goeminne from Vlaams Instituut voor Biotechnologie (VIB)

WD & YN analysed the metabolite data

GG & LB edited the manuscript

7.1 ABSTRACT

Understanding the mechanisms of rice resistant is the primary step that can provide rice breeders and molecular biologist with the necessary principles to develop nematode-resistant rice plants. Attraction, invasion, post inflectional development and reproduction of *P. zeae* in the resistant rice genotype Supa and susceptible rice genotype Mwangaza were compared to identify the stages at which resistance occurs. There was a significant difference in nematode attraction and penetration in rice roots when both resistant and susceptible rice genotypes were grown in the same or in different pots at 1, 2, 5 days after nematodes inoculation (dpi). Root extracts from Supa inhibited nematode motility. Further characterization of Supa and Mwangaza revealed the involvement of lignification and accumulation of flavonoids in Supa more than Mwangaza. Phenyl-Alanine-Lyse (PAL) activity was very high in Supa at the early time

point of infection. Metabolome analysis revealed more accumulation of secondary metabolites in uninoculated root tissue than the inoculated ones. Supa depicts pre-infection resistance mechanisms against *P. zeae*, and the identified resistance is temperature insensitive.

7.2 INTRODUCTION

The root-lesion nematodes (RLN) *Pratylenchus spp* have been reported to attack numerous crops including cereals, with *P. zeae* being dominant in rice and maize (Coyne et al., 1998; Fouad, 2016; Gilces et al., 2016; Matute & Anders, 2012; 2010; Nzogela et al., 2020a; Pili et al., 2016; Walter & Karssen, 2015). The rice yield loss due to *P. zeae* is in most cases underestimated; however, Nzogela et al. (2020a) have shown in a screenhouse experiment that the damage is substantial and that total crop failure may occur under drought stress. Likewise, in rice fields, *P. zeae* has been associated with reduced yield, especially in upland rice ecosystems (Aung & Prot, 1990; Coyne et al., 2001; Plowright et al., 1990; Prot & Savary, 1993). *P. zeae* reproduce by parthenogenesis (Olowe & Corbett, 1976) and its life cycle takes 21-25 days in rice under optimal conditions (personal observation). First stage juveniles (*J1*) develop and moult to infective second-stage juveniles (J2) inside eggs. J2 emerge from the egg and undergo a further three moults in the soil or host roots, resulting sequentially in *J3*, *J4* and adults. Stages from *J2* to adult are all motile and infective: they can enter at any site of the root, feed, exit, and re-enter (Jones & Fosu-Nyarko, 2014).

The infection of *P. zeae* on rice involves four stages: root recognition, penetration, feeding and migration, and reproduction. Unlike sedentary (root-knot and cyst) nematodes that induce permanent feeding sites like giant cells and syncytia respectively, *P. zeae* is mobile throughout its life cycle except for eggs, thus causing

massive destruction of the infected roots and pre-disposing the roots to secondary infections by other soil pathogens (Castillo & Vovlas, 2007). Once *P. zeae* is inside the host roots, it keeps on feeding and migrating intracellularly by puncturing cell walls and feeding on the content of cortical root cells (Sahoo & Sahu 1993). Cortical damage and reduced root proliferation minimize the plant's ability to absorb water and nutrients from the soil, leading to a reduced yield (Nzogela et al., 2020a). The observable symptoms on rice are plant stunting, wilting, premature yellowing of the leaves and poor root development with stubby characteristics (Fosu-Nyarko et al., 2016).

Generally, there has been limited success in controlling PPN. The limitations of chemical pesticides as well as the use of biological agents and cultural control strategies have brought increasing attention to efficient and cost-effective alternative control measures. The identification and implementation of host resistance is the best and most cost-effective way of managing *P. zeae* in rice production (Brar et al., 1999; Das et al., 2011; Dochez et al., 2009; Faske, 2013; Gheysen et al., 1996; Plowright et al., 1999; Soriano et al., 1999; Win et al., 2016). Natural resistance is the ability of plants to hinder nematode reproduction and development (Dababat et al., 2018; Moens & Perry, 2009; Peng & Moens, 2003; Trudgill, 1991). Resistance can be constitutive or induced. Constitutive defences are based on preformed barriers, which includes physical barriers like rigid cell walls and chemical barriers like phytoanticipins (Balmer et al., 2013). Physical barriers restrict nematode penetration in the host roots (Galeng-Lawilao et al., 2019; Sheedy & Thompson, 2009) bestowing pre-infection resistance.

In induced resistance, plants are capable of recognizing and reacting to pathogens and pests by triggering a cascade of defence responses (Jones & Dangl, 2006). Among the mechanisms involved are the induction of hormonal pathways and the production of secondary metabolites (Gheysen & Mitchum, 2019; De Vleesschauwer et al., 2013; Kyndt et al., 2014; Petitot et al., 2017).

In nematode-host interactions, secondary metabolites play a crucial role. These metabolites are either constitutively present (phytoanticipins) (Balmer et al., 2013; Chaudhary & Atamian, 2017), such as sclareol, which inhibits invasion of root-knot nematodes in *Arabidopsis* and tomato roots (Fujimoto et al., 2015), or are induced by infection (phytoalexins). Phytoalexins have been found to play a role in *Medicago sativa* resistance against root-lesion nematodes *P. penetrans* (Ahmed et al., 2017; Chinnasri et al., 2006). Constitutive secondary metabolites may act at the early stage of host-pathogen interaction by either attracting or repelling or preventing the pathogens from locating the host (Kihika et al., 2017).

The phenylpropanoid pathway is a key secondary metabolic pathway in host defence to nematodes (Dhakshinamoorthy et al., 2014; Fujimoto et al., 2015; Hölscher et al., 2014; Ji et al., 2015; Khanam et al., 2018; Kumari et al., 2016). Phenylpropanoid metabolism produces compounds that function as structural barriers (lignin), protectants (antioxidants, phytoalexins), toxins (coumarins) and signalling molecules (salicylic acid) in plant defence against a spectrum of invaders including nematodes (Vogt, 2010). Phenylalanine ammonia-lyase (PAL) is the first enzyme in the phenylpropanoid pathway. It converts L-Phenylalanine into *trans*-cinnamic acid and is considered as a key enzyme in the production of phenylpropanoids and flavonoids (Ahmed et al., 2017; Vogt, 2010; Zhang & Liu, 2015). PAL activity has also been related to lignification upon nematode infection (Khanam et al., 2018).

Flavonoids are well-known plant secondary metabolites produced specifically for plant protection against herbivore pests including nematodes (Chin et al., 2018; Ogo et al., 2016; Treutter, 2006; Wang et al., 2018; Weston & Mathesius, 2013). In several host-pathogen interactions, pathogenicity is an outcome of active suppression of the flavonoid pathway (Lee et al., 2014; Park et al., 2016). Ji et al. (2013) demonstrated that expression of chalcone synthase, a key enzyme in flavonoid biosynthesis, was suppressed in *M. graminicola* feeding sites at seven days post-infection (dpi). The same gene was induced at early stages of infection of white clover (*Trifolium repens*) by *M. javanica* and flavonoids were detected in the feeding site at 48 hours after nematode infection. However, at 4-5 dpi chalcone synthase gene expression was suppressed (Hutangura et al., 1999). Various researches support the argument that flavonoids are either constitutively produced by plants or induced upon nematode infection. They are directly involved in host defence mechanisms (Bacetty, 2008; Bogner et al., 2017; Čepulytė et al., 2018; Dhakshinamoorthy et al., 2014; Holbein et al., 2016; Hölscher et al., 2014; Huang et al., 2015; Ji et al., 2013; Kyndt et al., 2012b; Ohri & Pannu, 2018; Wuyts, 2006). For instance, the suppression of flavonoid biosynthesis in Arabidopsis rendered the plants susceptible to *H. schachtii* (Sun et al., 2013).

Lignin protects the host plant from nematodes through different mechanisms. These include forming a mechanical barrier for nematode penetration and further migration in the host roots. It reduces the susceptibility of the plant cell wall to cell wall modifying enzymes from the nematodes and imposes impermeability barriers to flow of nutrients and toxins. These mechanisms may lead to a hostile environment that will eventually deter the nematodes from feeding and development (Cartwright et al.,

1981; Dhakshinamoorthy et al., 2014; Galeng-Lawilao et al., 2019; Huang et al., 2016; Khanam et al., 2018; Veronico et al., 2018).

Most of the studies on *Pratylenchus* – host interaction have been done on wheat, barley and maize mainly on the genetics of host resistance (Yu et al., 2012), for instance in wheat and barley against *P. thornei* and *P. neglectus* respectively (Thompson et al., 2012; Yu et al., 2012) and in maize to *P. zeae* (Kagoda, 2010; Kagoda et al., 2011; Sawazaki et al., 1987). However, in other host –nematode interactions, such as RKN, the resistance mechanisms have been functionally characterized (Pegard et al., 2005; Cabasan, 2014; Dhakshinamoorthy et al., 2014; Linsell et al., 2014b; Khanam et al., 2018; Galeng-Lawilao et al., 2019; Nzogela et al., 2020b).

There is a knowledge gap concerning biochemical and biological resistance mechanisms of cereals to *Pratylenchus* species. What is known so far is on wheat interaction with *P. thornei* (Linsell et al., 2014b). This knowledge is crucial because it can shed light on the nature, timing and action of resistance genes and can be used to identify different resistance genes which could be pyramided for more durable resistance. Furthermore, identification of resistance mechanisms at a particular stage in the nematode's life cycle or within a specific tissue of root may increase the efficiency of screening for resistance in a breeding programme, thus reducing the associated cost and time, especially for RLN.

Recently, rice (*O. sativa*) cultivar Supa has been identified to be resistant to *P. zeae* (Chapter 4) and partially resistant to root-knot nematodes *M. javanica* and *M. graminicola* (Chapter 5) (Nzogela et al., 2020a; 2020b; Pili et al., 2016). Characterization of the resistance of Supa to RKN revealed pre- and post-infection resistance mechanisms that included reduced penetration, abnormal gall and

nematode development, reduced reproduction of the nematodes and emigration of the *J2* from the root 2-7 days post-inoculation (Nzogela et al., 2020b). For nematode management through environmentally friendly approaches, it is important to understand the plant-nematode interaction at different levels in order to design effective strategies for nematode resistance in crop. Therefore, it is vital to assess Supa resistance mechanisms against *P. zeae* at different angles, from plant invasion, penetration by nematodes and development and associated factors from the lifestyle, in this case, root-lesion nematodes. Therefore, the current study hypothesized that Supa resistance to *P. zeae* occurs at pre-penetration by decreasing nematode motility hindering penetration and migration. In contrast, post-root penetration resistance involves delayed or suppressed nematode development and reproduction.

The temperature has an impact on hatching, development and reproduction of plant-parasitic nematodes and may affect resistance (Pudasaini et al., 2008; Rashid et al., 2017). Cabasan et al. (2016) reported that a temperature regime of 34°C/31°C compromised the resistance of African rice *O. glaberrima* TOG5675 and TOG5674 to *M. graminicola*. Therefore, it is vital to assess Supa resistance against *P. zeae* under varying temperatures.

Several experiments were carried out to investigate the stated hypotheses, where and how the observed Supa resistance affects *P. zeae*.

7.3 MATERIALS AND METHODS

7.3.1 Nematode culture

P. zeae was isolated from the upland rice fields at Matombo Morogoro, Tanzania. The pure culture was established and maintained on carrot discs according to the procedure by Kagoda et al. (2010). Cultures were stored at 28°C and sub-cultured every two months. To collect the nematodes for inoculation, cultures in which

nematodes had already moved out of the carrot discs were selected, and nematodes washed with sterile tap water. Collected nematodes were concentrated using a 20 µm sieve (Retsch, Germany) and were allowed to settle down. The nematode suspension was then reduced to 5 ml in a 10 ml falcon tube. Six milligrams of streptomycin sulphate (Sigma-Aldrich, Inc. Bornem, Belgium) was dissolved in 10 ml of sterile tap water and filtered through a 0.2 µm filter using a 10 ml sterile syringe. From the prepared streptomycin solution, 5 ml was pipetted into the nematode suspension and incubated for one hour at room temperature to surface sterilize the nematodes. Nematodes were then washed four times for one hour with sterile tap water. Finally, the nematode suspension was centrifuged, and the re-suspension volume was adjusted to the required concentration for inoculation.

7.3.2 Rice genotypes, seed germination and nematode inoculation

AfricaRice, Morogoro, Tanzania provided *O. sativa* Supa and Mwangaza seeds. Mwangaza is a mutant from Supa developed through Gamma irradiation mutagenesis at the International Atomic Energy Agency Seibersdorf Laboratories Vienna (Luzi-Kihupi et al., 2008) and is very susceptible *to P. zeae* (Chapter 4). Supa has been identified to be resistant to *P. zeae* (Pili et al., 2016; Nzogela et al., 2020a). AfricaRice headquarters provided the *O. glaberrima* TOG5674 and UPLRi-5 seeds, Cotonou, Benin. TOG5674 was found to be resistant to *P. zeae* (Chapter 4), while UPLRi-5 is susceptible (Plowright et al., 1990). Seeds were germinated on Petri dishes lined with moist sterile tissue paper kept at 30°C in the dark for five days. The seedlings were transplanted singly in polyvinyl chloride (PVC) tubes (diameter: 2.5 cm; height: 15 cm) lined with a plastic sheet containing Sand and Absorbent Polymer (SAP) (Reversat et al., 1999). Rice seedlings were grown in a plant room with 27-28 ˚C, 12hr /12hr light regime and 70-75% relative humidity of about. The plants were fertilized

with 10 ml of Iron-modified Hoagland's nutrient solution per plant three times a week. Twenty-one – days -old rice seedlings were inoculated with 300 mixed stage-nematodes of *P. zeae*. Non-inoculated rice plants were used as control.

7.3.3 Penetration Assay

The rate of *P. zeae* penetration was assessed at 1, 2, and 3 dpi in resistant and susceptible cultivars with eight replicates. Rice seedlings were germinated and transplanted, as described in section 7.3.2. Three hundred mixed life stages of *P. zeae* were inoculated to 21 days-old seedlings. For penetration assessment, roots were harvested, cleaned and stained in boiling acid fuchsin for 3 minutes followed by rinsing under running tap water to remove excess stain. The stained roots were placed in a beaker containing a clearing solution of 1:1:1 lactic acid, glycerol and distilled water for 4 hours, after which roots were rinsed under running tap water. Nematodes that penetrated the roots were then counted under a dissecting microscope (S8APO-Leica-Switzerland). The experiment was repeated once.

7.3.4 Preferential attraction of *Pratylenchus* to resistant or susceptible cultivars

In this experiment, a single seedling of Supa in combination with one seedling of each of the genotypes; Mwangaza, UPLRi5, or TOG5674 (germinated and grown as described in section 7.3.2) were transferred with their roots intact in SAP and grown in identical plastic pots of 6 cm diameter and 12 cm height. Four combinations with eight replicates were executed. Before seedling transfer, in each pot, a layer of dry SAP was spread to cover the bottom part of the pot. This was done to provide a layer of medium for nematode movement. Ten grams of moist sand was put at the centre of the two transplanted seedlings, and 300 nematodes in 500 µl sterile tap water were inoculated at the centre of the two seedlings (a combination of Supa and

Mwangaza, UPLRi-5 or TOG5674). The distance from the seedlings to the point of nematode inoculation was about 2 cm. The whole pot was then filled with moist SAP. Plants were maintained in the same conditions, as explained in section 7.3.2. Roots were harvested at 5 dpi, washed to remove adhering SAP and processed as described in section 7.3.3. Nematodes that were attracted to and subsequently penetrated the roots were counted under a dissecting microscope (S8APO-Leica- Switzerland). The attraction was assessed by determining the per cent of nematodes that penetrated either Supa or other genotypes in each combination.

7.3.5 Assessment of the impact of root extracts on nematode motility

To test for anti-nematode properties of the Root Crude Extracts (RCE) from the resistant and susceptible rice cultivars an in-vitro bioassay of nematode motility was conducted. The methodology was adapted from Linsell et al. (2014b) with modifications. Rice seeds were dehusked and surface sterilized with 70% ethanol, followed by a solution containing 4% sodium hypochlorite and a few drops of Tween-20. Seeds were kept in sodium-hypochlorite on a shaker for 45 minutes. The seeds were then rinsed five times with sterile distilled water under sterile condition. Sterile rice seeds were germinated, grown and inoculated as described in 7.3.3. At 2 and 5 dpi, roots were harvested, washed to remove adhered SAP, weighed and immediately crushed in liquid nitrogen. The root powder was suspended in cold, sterile tap water on ice and the suspensions were centrifuged at speed 14 000 rpm at 4 °C. The supernatants were collected and adjusted to a concentration of 4 mg/ml of fresh weight root tissue in water. The adjustment was made to minimize the differences between big and small roots. RCE assays were conducted using Costar® 96-well cell culture containing 100 µl of RCE.

The wells were inoculated with 50 surface-sterilized mixed life stages of *P. zeae* in 10 µl. The wells were covered by a thin plastic sealing film to avoid evaporation. Nematodes in sterile tap water were included as a control. Six biological and eight technical replicates per genotype were analyzed (2 genotypes replicated six times and RCE from each genotype replicated eight times). The nematostatic effect of RCE was monitored every 12 hours for 72 hours of exposure to the RCE by counting the number of motile and non-motile nematodes under a dissecting microscope (S8APO-Leica, Switzerland). Nematodes were defined as immotile if they were straight and they did not move after probing with a fishing needle. After 72 hours, nematodes were transferred in distilled tap water left for 12 hours, and the number of immotile nematodes was counted again.

7.3.6 Effect of temperature on Supa resistance against *P. zeae*

The result of temperature on Supa resistance to *P. zeae* was assessed at 1, 2, 5, 10, 15, and 25 dpi with eight replicates, in comparison with a susceptible control. Rice seedlings were germinated and transplanted, as described in section 7.3.2. *P. zeae* was inoculated with 500 mixed life stages to 21 days-old seedlings. The inoculated plants were grown at 28°C, 22ºC, and 32ºC with 16h light/8h dark, 80–85% relative humidity. Roots were harvested, cleaned, and stained in boiling acid fuchsin, and nematodes development was assessed by counting the number of nematodes inside the roots under a dissecting microscope (S8APO-Leica- Switzerland as described in 7.3.3.

7.3.7 Phenylalanine ammonia-lyase (PAL) activity assay

PAL activity in rice roots was assayed, according to Camacho-Cristóbal et al. (2002) with minor modifications. Root samples stored at -80˚C were ground in liquid

nitrogen and 100 mg root powder was homogenized in 500 µl extraction buffer (50 mM sodium phosphate buffer pH7 containing 2% (w/v) poly-vinylpolypyrrolidone (PVPP), 2 mM EDTA, 18 mM-mercaptoethanol and 0.1% (v/v) Triton X-100) and centrifuged at 10 000 rpm for 10 min at 4˚C. The chemicals were purchased from Sigma-Aldrich, Inc. Bornem, Belgium. The supernatant was used as an enzyme source. To start the enzymatic reaction, 20 µl of the supernatant was mixed with 500 µl 50 mM sodium phosphate buffer (pH 7), followed by addition of 50 µl 20 mM L-Phenylalanine as a substrate. Parallel control samples without L-Phenylalanine addition were included. The reaction mixture was transferred to glass cuvettes, and PAL activity was determined by measuring the amount of *trans*-cinnamic acid using the spectrophotometer (BioRad Smart Spec Plus) at 290nm, before and after incubation at 40°C in a water bath for 45 minutes. The reaction was stopped by incubation on ice for 5 minutes. The *trans*-cinnamic acid produced per minute was calculated using the following formula:

$$PAL\ activity\ \left(\frac{U}{g}\right) = \frac{\left(OD_{sample} - OD_{control}\right) \times \frac{V_{total}}{V_{sample}} \times \frac{W}{V_{assay} \times 0.1}}{T}$$

W: the weight of the sample (100 mg)

V_{total}: the total volume of the enzymatic reaction (570 µl)

V_{sample}: the volume of the sample (20 µl)

V_{assay}: the volume of assay buffer (500 µl)

T: reaction time (45 minutes).

Six replications were used per treatment. One unit (U) of PAL activity was defined as the amount of the enzyme that produced one nmol cinnamic acid per hour. The control samples included all ingredients, except that enzyme extract was substituted for buffer.

7.3.8 Histochemical staining of lignin and flavonoids in rice root sections

Fresh roots from infected and non-infected plants at 2 and 5 dpi (18 and 21 days post transplanting) were washed to remove any adhering SAP. From eight roots selected for histochemical analysis, four roots per treatment were freshly cut into cross-sections of 100 to 150 µm thick using a sharp razor-blade under the dissecting microscope (S8APO-Leica- Switzerland). Within 5 minutes, the sections from infected and non-infected root samples were treated with two drops of 2% phloroglucinol (Sigma–Aldrich, Germany) in absolute ethanol for 2 minutes, followed by two drops of concentrated HCl for another 2 minutes. Stained root sections were mounted in 50% glycerol on glass slides and examined by light microscopy (Nikon digital microscope equipped with a Nikon Color View III camera for image capture) in bright field mode. Digital images were taken, and lignification was identified by dark red coloration.

For the detection of flavonoids, about ± 1.5 cm pieces (four pieces randomly cut from the root tips) of the other four roots from 2 and 5dpi and non-infected rice samples were fixed in a 50 mM sodium phosphate buffer (pH 7.2) containing 4% paraformaldehyde and 1% glutaraldehyde and subsequently dehydrated in a graded series of ethanol (Sigma–Aldrich, Germany) (30%, 50%, 75%, 80%, 100%) each for 2 hours. Dehydrated samples were vacuum-infiltrated with Technovit 7100 (Heraeus Kulzer, Wehrheim, Germany) hardener solution under high pressure. The infiltrated root samples were finally embedded in plastic cubes of 1 cm^2, filled with Technovit 7100 histo-embedding medium. A Leica RM2265 motorized rotary microtome (Leica Microsystems, Nussloch, Germany) was used to produce ten µm thick cross-sections. For each rice genotype at 2 and 5 dpi, three root samples of at least four different seedlings were sectioned and randomized on 20 microscopic slides. For each sampling point, at least ten slides were randomly chosen for staining. Flavonoids were

stained with 0.25%, w/v, diphenylboric acid 2-aminoethyl ester (DPBA) (Sigma-Aldrich, Inc., Bornem, Belgium) containing 0.02% (v/v) Triton X-100 (Sigma-Aldrich, Inc., Bornem and mounted on 50% glycerol (Sigma-Aldrich, Inc., Bornem). Samples were visualized with a Nikon A1R confocal laser scanning microscope (Nikon Instruments-Europe Amsterdam, The Netherlands) mounted on a Nikon Ti-E inverted epifluorescence body equipped with a 10X/0.45 Plan Apo immersion objective (Nikon) and Galvano scanner. A 404-nm laser diode was used for the excitation of dye, and emission from 429 nm to 748 nm was detected with a 32 channel spectral detector with 10 nm spectral resolution.

7.3.9 Metabolomics profiling

Metabolic analysis of Supa and Mwangaza was carried out on infected and non-infected 25 days old plants. Samples were harvested at ten days post-inoculation with 300 mixed life stages of *P. zeae*. Rice seedlings were sterilized as described in 7.3.5, germinated, and transplanted as described in section 7.3.2. On harvest, roots were separated from shoots, snap-frozen by liquid $N_{2,}$ and homogenized using mortar and pestle. Sixty milligrams of homogenized samples were transferred to 1.5 ml Eppendorf tubes and 1 mL of 90% HPLC grade MeOH (Sigma-Aldrich, Inc., Bornem-Belgium) and 10% Milli-Q water were added to the samples. Samples were shaken 15 minutes at 70°C to mix well. This was followed by centrifugation at 4°C and 14,000 rpm for 30 min. The supernatant was transferred to a new Eppendorf tube without any pellet debris. The supernatant was freeze-dried in a vacuum evaporator (speedVac evaporator-Thermofisher) until completely dry, and the dry weight of the samples was determined for normalization purposes. A hundred microliters of cyclohexane (Sigma-Aldrich, Inc., Bornem) followed by 100 µl distilled water was added to the dried liquid phase and vortexed until dissolved. The suspension was centrifuged at 4°C and

16,000 rpm for 10 minutes, and two layers were formed. 80 µl of the suspension from the bottom layer was pipetted using a very narrow extended length pipette tips (Thermo-Fischer Scientific, Germany) to 96 well plates.

UPLC analysis. All samples were analyzed on a Waters Acquit Ultra Performance Liquid Chromatography (UPLC) system coupled with a Waters Vion IMS QTOF (Ion Mobility Spectrometry Quadrupole Time of flight) mass spectrometer (MS) equipped with an electrospray ionization (ESI) source operating in negative or positive ionization mode. A linear gradient was run from 95% aqueous formic acid (0.1%, buffer A) to 50% acetonitrile (0.1% formic acid, buffer B) in 30 min, followed by a concave gradient (curve 3) in 10 min to 100% buffer B by using a flow rate of 350 µl/min and a column temperature of 40 °C. Full MS spectra (m/z 50 – m/z 1,500) were recorded at a scan rate of 10 Hz. The following ESI parameters were used: capillary voltage 2.5 kV, desolvation temperature 550 °C, source temperature 120 °C, desolvation gas 800 L/h, and cone gas 50 L/h. Lock correction was applied. In addition to full MS analysis, a pooled sample was subjected to data-dependent MS/MS analysis (DDA, exclusion duration = 10 s) using the same separation conditions as above. DDA was performed between m/z 100 and m/z 1,200 at a scan rate of 10 Hz and MS -> MS/MS transition collision energy of 6 eV. The collision energy was ramped from 24 to 35 eV and from 30 to 70 eV for the low and high mass precursor ions, respectively. Quality Control (QC) samples were included for retention time (RT) and mass accuracy stability monitoring during sample analysis. All biological samples were analyzed at random, and for every sample, a unique chromatogram was generated.

Analysis of metabolome data. All features were normalized to sample dry mass, filtered based on a class frequency threshold of 100% (i.e., a feature was only retained if it could be detected in all samples belonging to at least one class) and

filtered based on relative abundance (features with an overall abundance below 0.005% of the most abundant peak were removed). The resulting feature list was uploaded to the MetaboAnalyst server (v. 4.0) (Chong et al., 2018) and pre-processed by log-transformation and mean-centring. The class separation was assessed visually based on PCA plots, and significant features were identified based on ANOVA, followed by Tukey's HSD post hoc test.

Features were putatively annotated by matching MS/MS spectra against the in-house VIB PhytoComp database and the public Mass Bank of North America (MONA) database for negative mode MS data and MONA alone for the positive mode data. MS/MS spectra were also visually compared to those in the database. For matches against the PhytoComp database, retention time was used as an additional information source. Mummichog pathway analysis was performed through the Mummichog algorithm (v. 1.0) (Li et al., 2013) as implemented on the Metaboanalyst server (v. 4.0) (Chong et al., 2018).

7.3.10 Statistics

Data from each experiment, except the histochemical staining and metabolome data, were analyzed separately using Statistical Analytical System (SAS-Michigan, USA). The data were subjected to "proc univariate normal plot" and "proc glm" procedures to test for normality and homogeneity of variance, respectively. The data sets that did not fulfil the assumptions of normality, homogeneity of variance, and % data were subjected to log(X+1) or arcsine transformation. The data were then subjected to Analysis of Variance (ANOVA) to determine differential responses among genotypes to nematode infection. Means were compared and separated using Fisher's least significant difference (LSD) at the 0.05 significance level ($P \leq 0.05$). When a significant effect was observed, the individual mean comparisons were made between

the resistant rice genotypes and the susceptible / control Mwangaza/UPLRi-5 using Dunnett's test.

7.4 RESULTS

7.4.1 The resistance of Supa to *P. zeae* function at the pre-penetration stage

P. zeae could successfully penetrate all rice genotypes from day one (Fig. 7.1), but the number of *P. zeae* that we're able to penetrate the roots was highest in Mwangaza and lowest in Supa. *P. zeae* continue to enter into the roots of the susceptible genotypes more than of the resistant genotype at 2 and 5 dpi. Groups of nematodes were found penetrating Mwangaza roots at the same entry point and moving in groups within the roots (Fig. 7.2D, E, & F). The most preferred penetration zones were the elongation zone, at the junction of lateral branches, and the seminal main root axes. Very rarely, the root hairs were found to be penetrated. Once penetrated the roots, matured females immediately started depositing eggs. Eggs were found in the root at one dpi.

Attraction to and subsequent root penetration by *P. zeae* in Supa was assessed in the presence of either a resistant (TOG5674) or a susceptible (Mwangaza or UPLRi5) genotype (Fig. 7.3) in three combinations. Significantly ($p < 0.05$) fewer *P. zeae* were attracted to and penetrated the roots of Supa compared to the other genotypes when different genotypes were available for penetration.

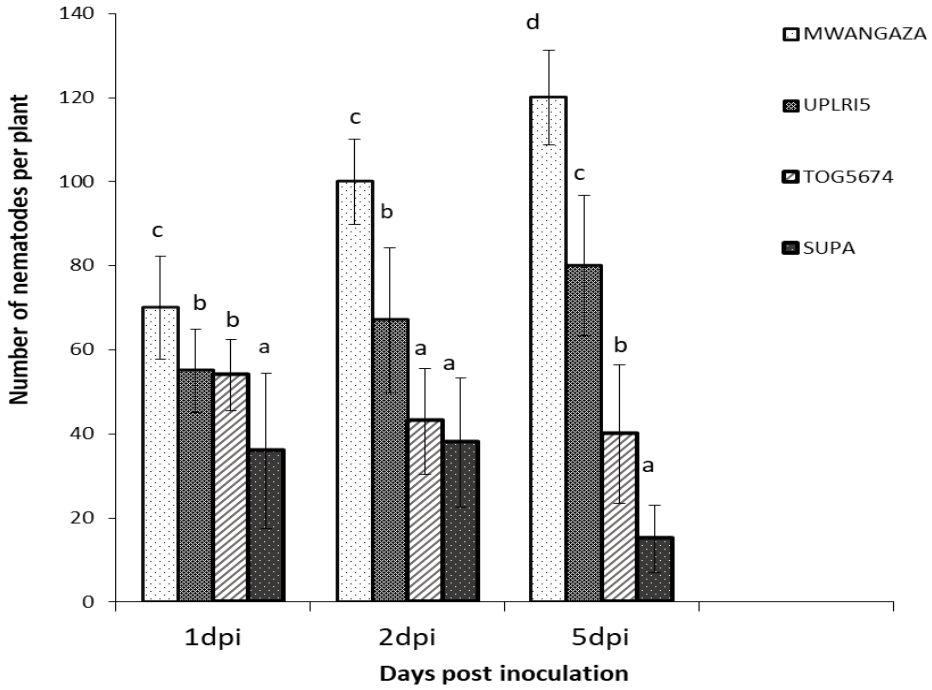


Fig.7.1: ***P. zeae*** **penetration on different rice genotypes grown in Sand Absorbent Polymer (SAP). About 300 nematodes were inoculated 14 days after transplantation, and plants were uprooted at 1, 2 and 5 days post-inoculation (dpi). Each bar shows the average number of nematodes that had penetrated the roots at different time points. Different letters at a given time point indicate significant (p< 0.05) differences among the means according to Fisher's Least Significant Difference (LSD) test. Data represent mean and standard error of 8 plants per treatment. The experiment was repeated once with similar results.**

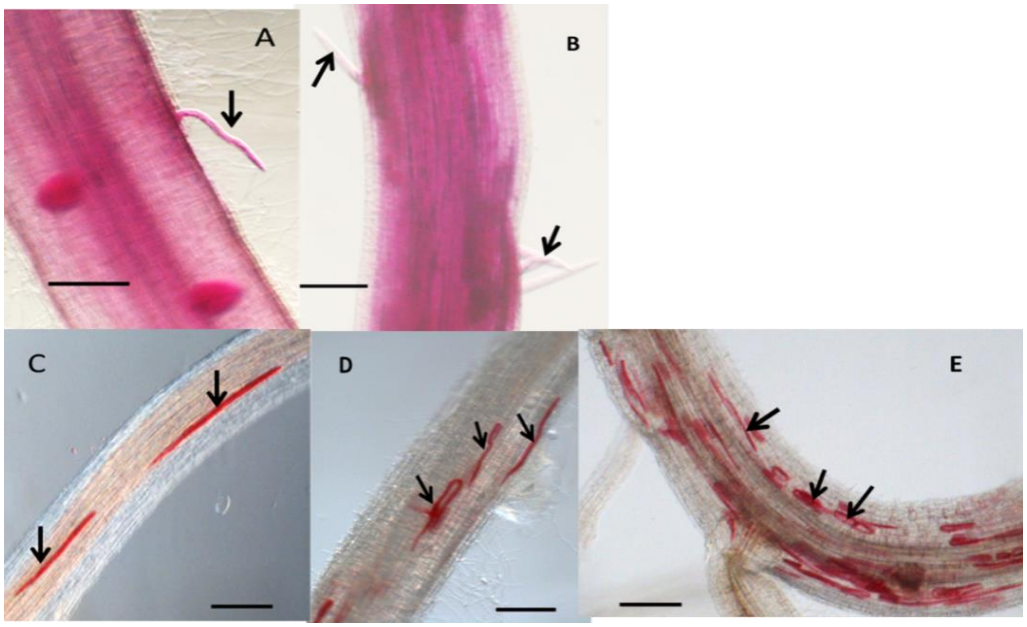


Fig. 7.2: **Root penetration of** ***P. zeae*** **in the roots of Supa and Mwangaza. Nematodes (stained with acid fuchsin) are indicated with black arrows. A and B,** ***P. zeae*** **penetrating the roots of Supa and Mwangaza respectively, C and D, few nematodes in Supa roots and E, many nematodes in the root of Mwangaza. Scale bar = 1 mm.**

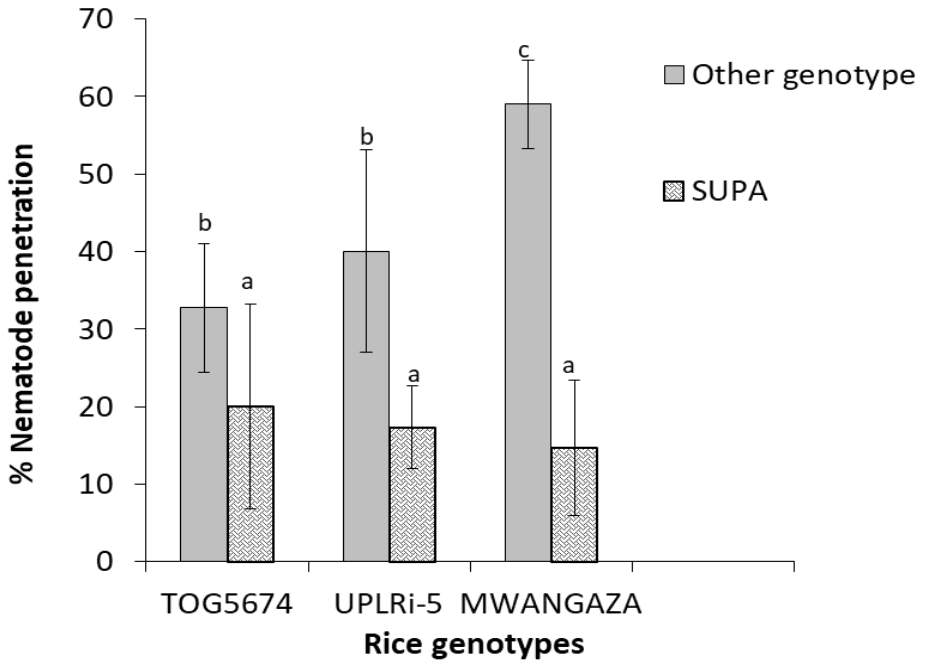


Fig. 7.3: Penetration of *P. zeae* (measured as penetration in %) in three combinations of resistant (Supa) and susceptible rice genotypes assessed at five days after inoculation with 300 nematodes. Each bar shows the average % number of nematodes that had penetrated the roots. Different letters indicate significant (P < 0.05) differences among the means, according to Fisher's Least Significant Difference (LSD) test. Data represent mean and standard error of 8 plants per treatment. The experiment was repeated once with similar results.

7.4.2 Supa root extract reduces nematode motility

The effect of root crude extracts (RCE) from Supa and Mwangaza on *P. zeae* motility was assessed by visually inspecting motility of the nematodes 2dpi and 5dpi. After 72 hours of exposure, there was a significant higher immobilization (p = 0.05) of *P. zeae* in RCE from Supa compared to water (Fig. 7.4.). The effect of RCE from Supa on *P. zeae* motility appeared irreversible. No nematodes recovered after exposure in water for another 24 hours (data not shown).

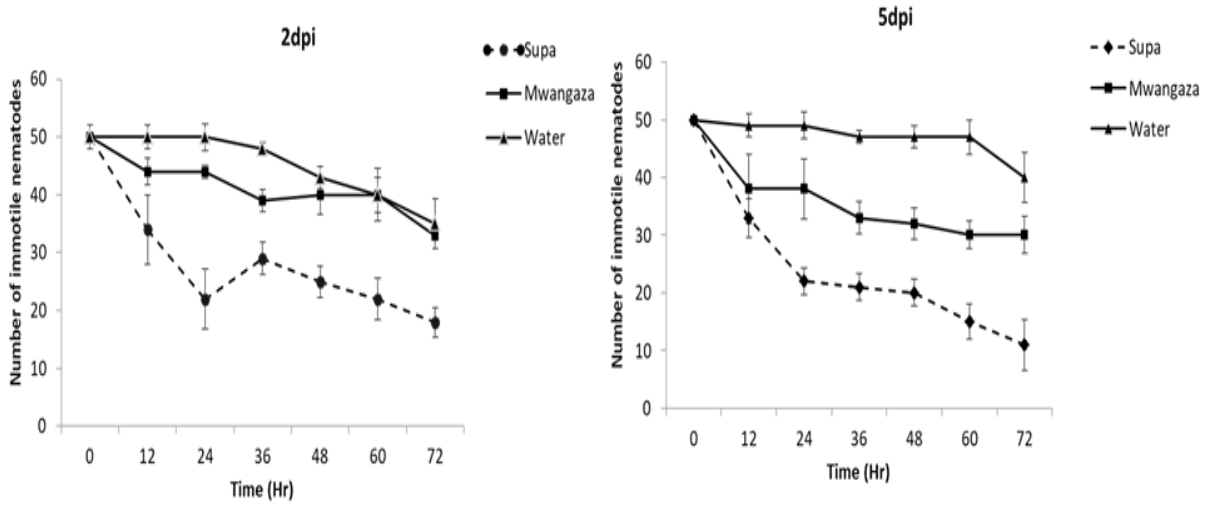


Fig. 7.4: **Nematode motility over 72 hours incubation in nematode infected root crude extracts from Supa and Mwangaza at A. 2dpi and B. 5 dpi. The number of nematodes that are mobile in the suspensions is plotted over time. Water was included as a control to the RCE. Data points indicated by symbols represent means and standard errors of 8 plants per treatment. The results are of the replicated experiment.**

7.4.3 Supa-resistance to *P. zeae* functional at high temperature (32°C)

The effect of temperature on *P. zeae* infection in the resistant Supa and the susceptible Mwangaza was assessed after inoculating the rice plants with ± 500 nematodes (Fig. 7.5.). In the Mwangaza genotype, *P. zeae* reproduced lowest at 22°C and highest at 28°C with 28°C being optimum for *P. zeae* reproduction. In Supa, the nematodes penetrated the roots least at 22°C and highest at 28°C and 32°C. Either the number of nematodes in the roots increased at each sampling point in Mwangaza, while for Supa, the number started to decrease at 5, 10, 15, and 25 dpi. Observations show that the number of nematodes in Supa were 10-fold less than that in Mwangaza. The resistance in Supa is not broken at higher temperatures, as the small increase in nematode numbers at 28°C is only visible at the very early stages.

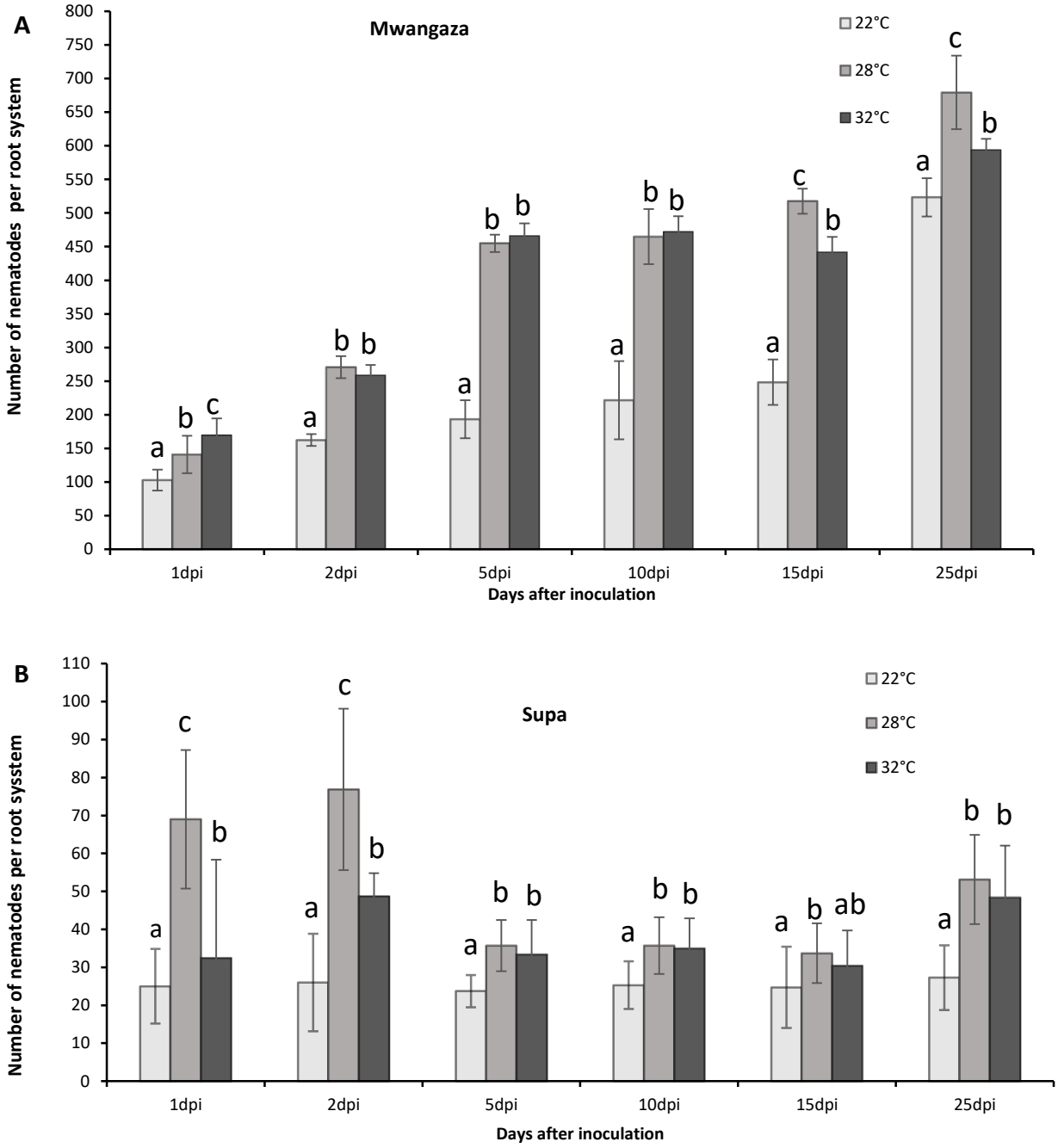


Fig. 7.5: **Effect of temperature on *P. zeae* reproduction A, on Mwangaza, B, on Supa. The mean number of nematodes for three temperature levels for each of the six sampling points (dpi). Bar height indicates the mean and error bars +/- standard error. Means sharing the same letter at the same sampling point do not differ significantly at the 95% confidence level based on the LSD mean comparison method.**

7.4.4 Phenylalanine Ammonia-Lyase (PAL) activity, lignin and flavonoids are higher in Supa

The phenylpropanoid pathway has been implicated in plant defence to nematodes (Wuyts et al., 2006a). PAL activity was analyzed in roots of Supa and

Mwangaza at 1, 2 and 5 dpi (Fig. 7.6A). The experiment was repeated, but sampling was done at 10dpi (Fig. 7.6B). PAL activity was significantly ($p = 0.05$) higher in Supa than in Mwangaza at five dpi After inoculation with *P. zeae,* PAL activity in Supa initially decreased slightly at 1 and 2 dpi but increased 4-fold ($p = 0.05$) compared to Mwangaza infected with nematodes and by at least 2-fold compared to non-inoculated plants at five dpi. Constitutive PAL activity in Supa was high for all sampling time points and vice versa for Mwanganza. The trend was the same even at 10dpi (Fig. 7.6B).

Lignification of root cell walls was detected by histochemical staining (red colouration) of the root sections using the Weisner reagent (Fig. 7.7). The intensity of lignin accumulation differed among Supa and Mwangaza and varied across sampling times. Non-inoculated plant root sections for both Supa and Mwangaza showed low lignification, present only around the metaxylem (Fig. 7.7A-D). At one dpi, lignin accumulation was detected in both genotypes around the root stele and surrounding the xylem and phloem, but the intensity was much higher in Supa (Fig. 7.7E-I) than Mwangaza. At five dpi lignin accumulated more in Mwangaza roots than in Supa. The lignifications extended further from the secondary cell wall of central vascular sclerenchyma cells to the endodermis. At this sampling time, cortical cells of Mwangaza were disrupted due to nematode movement. Large grey-brown lesions were noted on Supa roots (G).

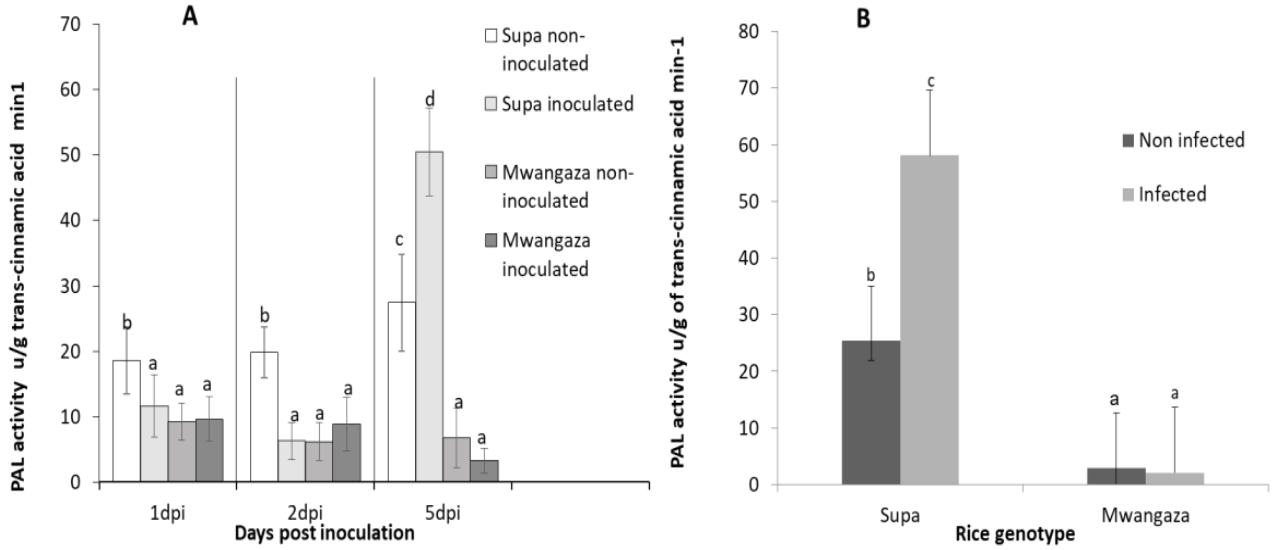


Fig. 7.6: **Phenylalanine ammonia-lyase activity in Supa and Mwangaza rice roots at A. 1, 2 and 5 and B. 10 days post-inoculation with *P. zeae*. Averages of 8 plants per treatment, cultivar and time point. Averages across the sampling point followed by the same letter are not significantly different ($p < 0.05$) according to Fisher's Least Significant Difference (LSD) test. Data represent means and standard errors of 8 plants per treatment.**

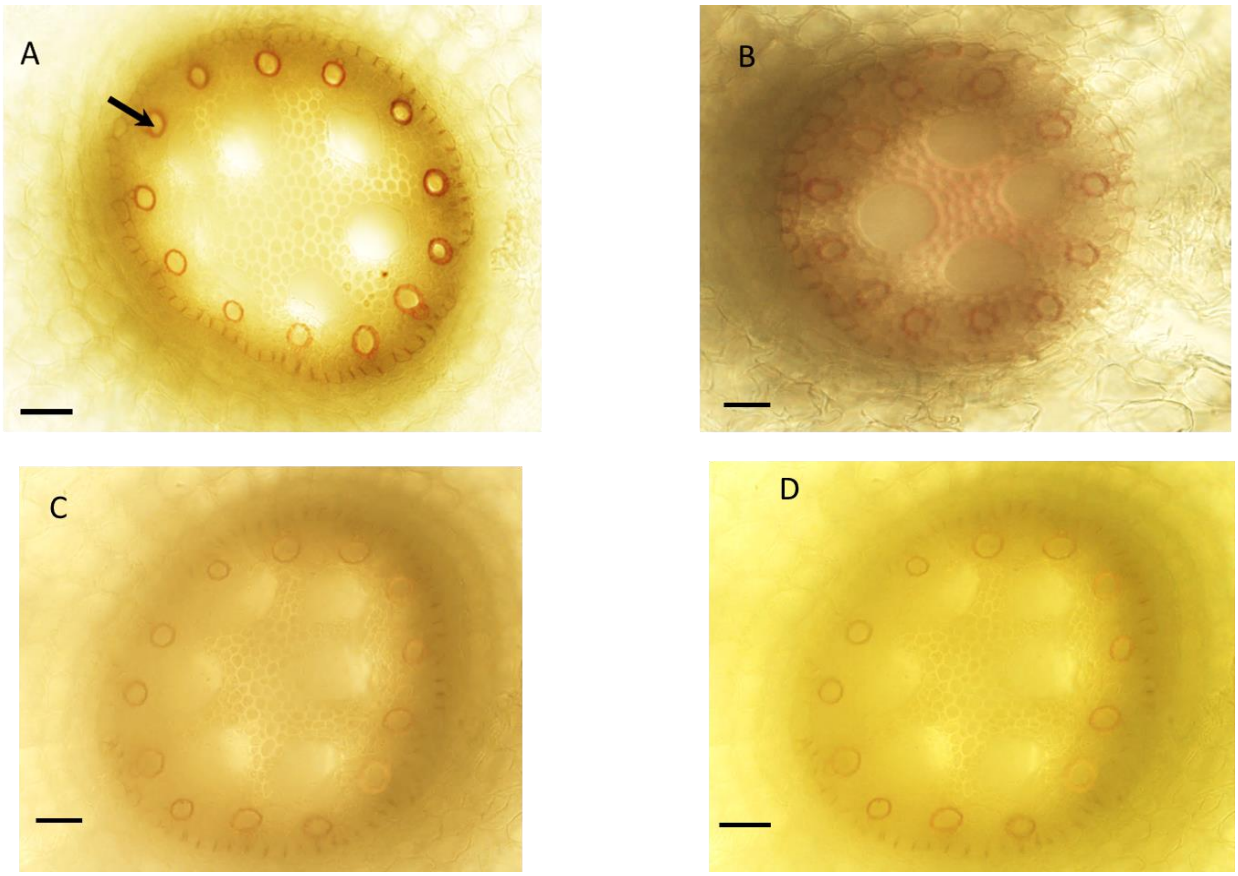


Fig. 7.7: **Fresh uninfected root cross-sections of Supa A and B and Mwangaza C and D. Lignification of Supa metaxylem stained in red indicated by black arrows at 18 and 21 days after germination. No lignification in Mwangaza roots (C and D) Scale bars =200µm. All images were captured using a bright field microscope.**

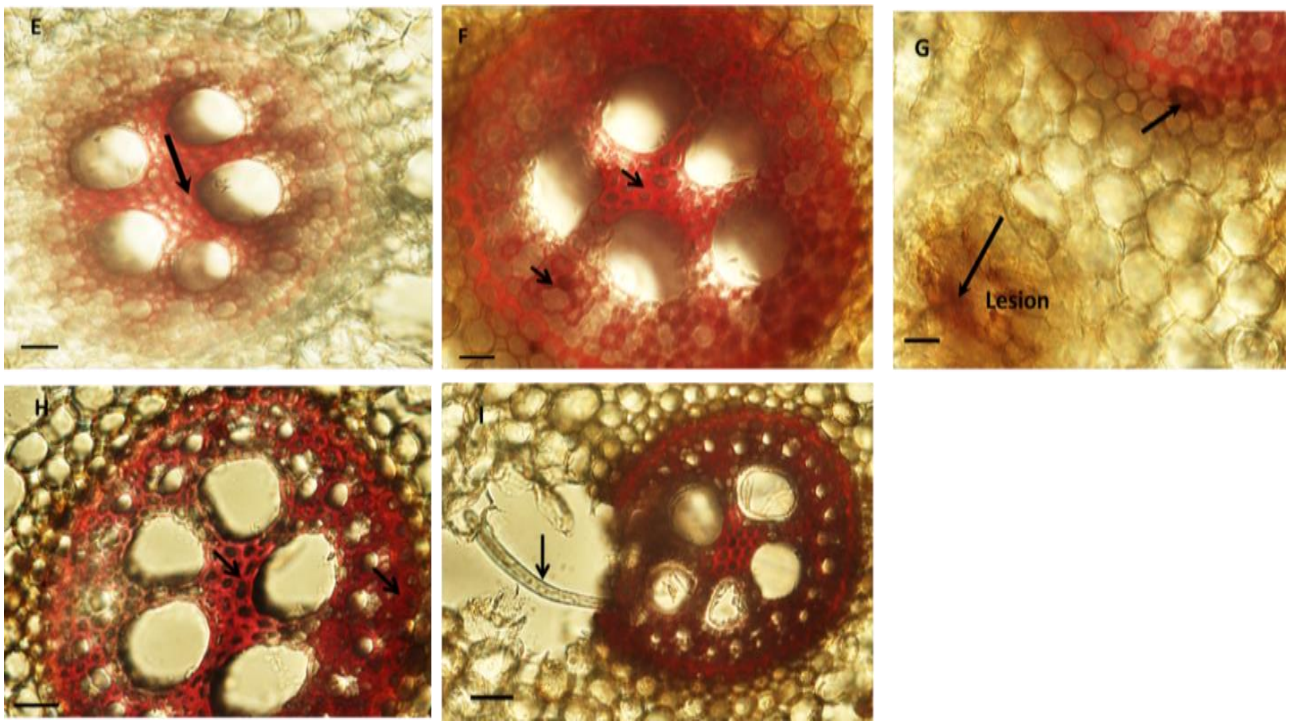


Fig. 7.8: ***P. zeae*** **infected rice roots showing entire sclerenchyma cell walls and vascular bundle lignification. E, F and G show Supa roots, H and I shows Mwangaza roots at 2 and 5 dpi respectively. G shows a root lesion on Supa root and in I a nematode (black arrow) penetrating the vascular bundle of Mwangaza root at 5dpi. Scale bar= 100µm**

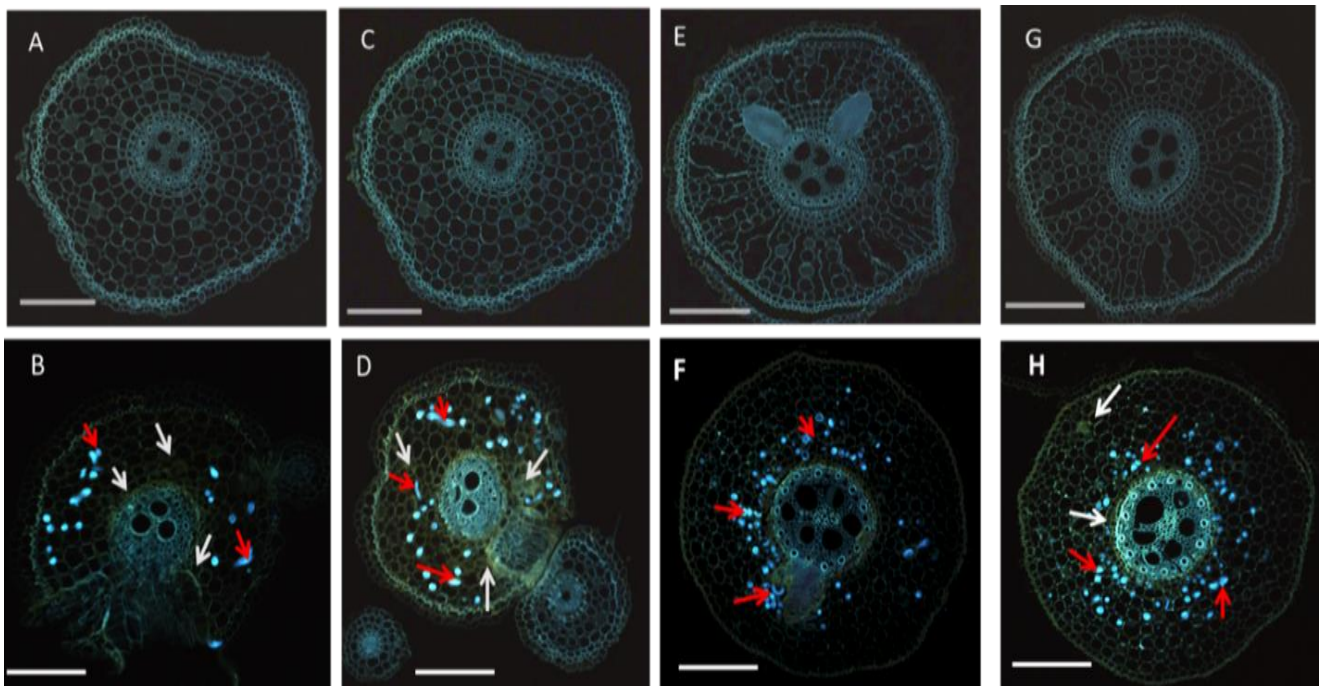


Fig. 7.9: **Histochemical localization of flavonoids in cross-sections of uninfected; A and C Supa, E and G Mwangaza; and *P. zeae*-infected rice roots of Supa (B and D) and Mwangaza (F and H) at 2 (B and F) and five dpi (D and H). White arrows show localization of fluorescence of flavonoid compounds and red arrows indicate nematodes. Scale bar = 400µm.**

Infected and non-infected rice root sections from Supa and Mwangaza plants were stained with Diphenylboric acid 2-aminoethyl ester (DPBA) to detect flavonoids (Fig. 7.8). Supa infected root sections showed fluorescent compounds ranging from bright yellow-green and pale yellow around the cells in the cortical and aerenchyma cells, indicating the presence of flavonoids. These fluorescent compounds were never observed in the vascular cells. No fluorescence was detected in non-infested root sections from Supa, and no fluorescence was observed in neither infested nor non infested root sections of Mwangaza for both sampling points.

7.4.5 A global metabolome analysis shows apparent differences between the susceptible and resistant cultivar while differences between infected and non-infected plants are subtle

Untargeted UPLC-MS/MS metabolomics was performed on both Supa and Mwangaza to determine the global metabolite changes in compatible and incompatible rice - *P. zeae* interactions, (Fig. 7.9). After filtering, unique features were retained in the negative and positive ionization mode (Table 7.1). At a p-value threshold of $p < 0.05$ (FDR-adjusted), features in negative and positive ionization mode that showed significant differential abundance in at least one treatment (Table 7.2).

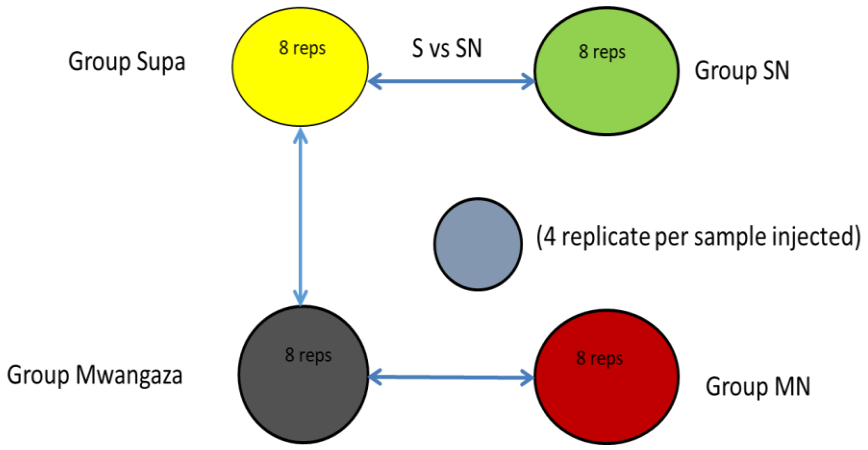


Fig. 7.10: Scheme of metabolome analysis experimental design indicating four groups and different comparisons of Supa (S) and Supa infected with nematodes (SN), Mwangaza (M) and Mwangaza infected with nematodes (MN). Each group contains eight biological replicates.

Volcano plots were created to quickly visualize the differences between control and test group for each comparison (Fig. 7.10). To highlight the number of compounds that were significantly different, all compound ions that were coloured red had a p-value < 0.01 and a fold change that is larger than 20. These results clearly show that the quantity of a large number of compounds is different between Supa and Mwanganza. Still, much fewer differences are seen when Supa or Mwangaza are compared to their 'Nematode-treated" group (Supa-Nematodes and Mwangaza-Nematodes respectively). For the samples infected with nematodes, it is clear that in both cultivars compounds are accumulating compared to their control group without nematodes, only a few compounds (with a fold change > 20) have decreased in intensity.

Table 7. 1: Summary of compound ion detection in the ESI- (negative ionization) and ESI+ (positive ionization) sample set

	ESI-	ESI+
Number of compound ions detected	4122	9641
Number of compounds after filtering	3606	8485
Number of compounds with MSe spectra	3439	8326
Number of compounds with DDA MS/MS spectra	639	1410
Number of compounds annotated using biological databases	67	12
Number of annotated compounds derived from the phenylpropanoid pathway	35	2

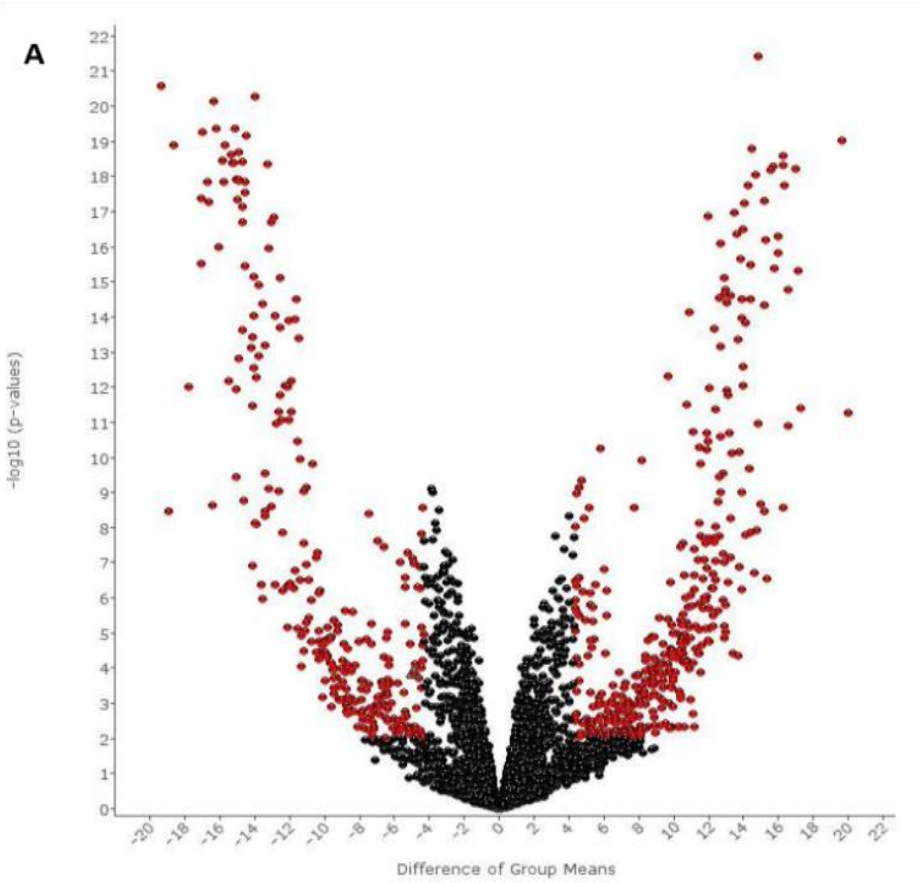
Table 7.2A: A Summary of univariate analysis of all compounds in positive ionization mode

Groups comparison	Number of significant different compound ions ($p \leq 0.01$)	% of all compound ions	Number of compound ions up	Number of compound ions down	Number of annotated compounds
M vs S	2208	26.02	1067	1141	12
M vs MN	562	6.63	154	408	12
S vs SN	370	4.36	69	301	12

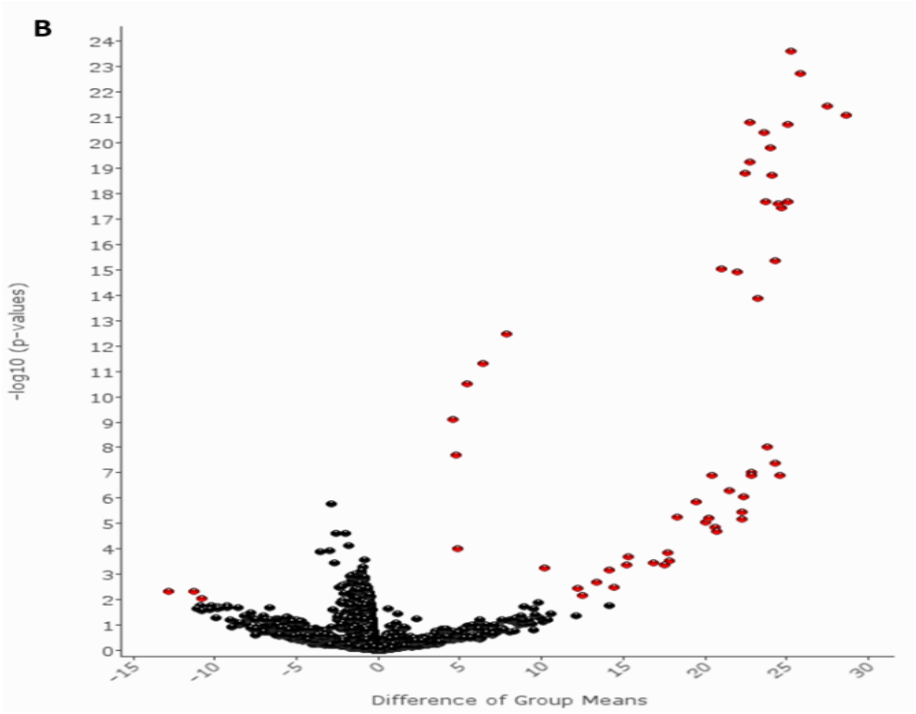
Table 7.2B. Summary of univariate analysis of all compounds in negative ionization mode

Groups comparison	Number of significant compound ions ($p \leq 0.01$)	% of all compound ions	Number of compound ions up	Number of compound ions down	Number of annotated compounds
M vs S	1239	34.35	674	565	66
M vs MN	120	3.33	75	45	66
S vs SN	194	5.37	51	143	66

S vs M


A
-log10 (p-values)
Difference of Group Means

S vs SN


B
-log10 (p-values)
Difference of Group Means

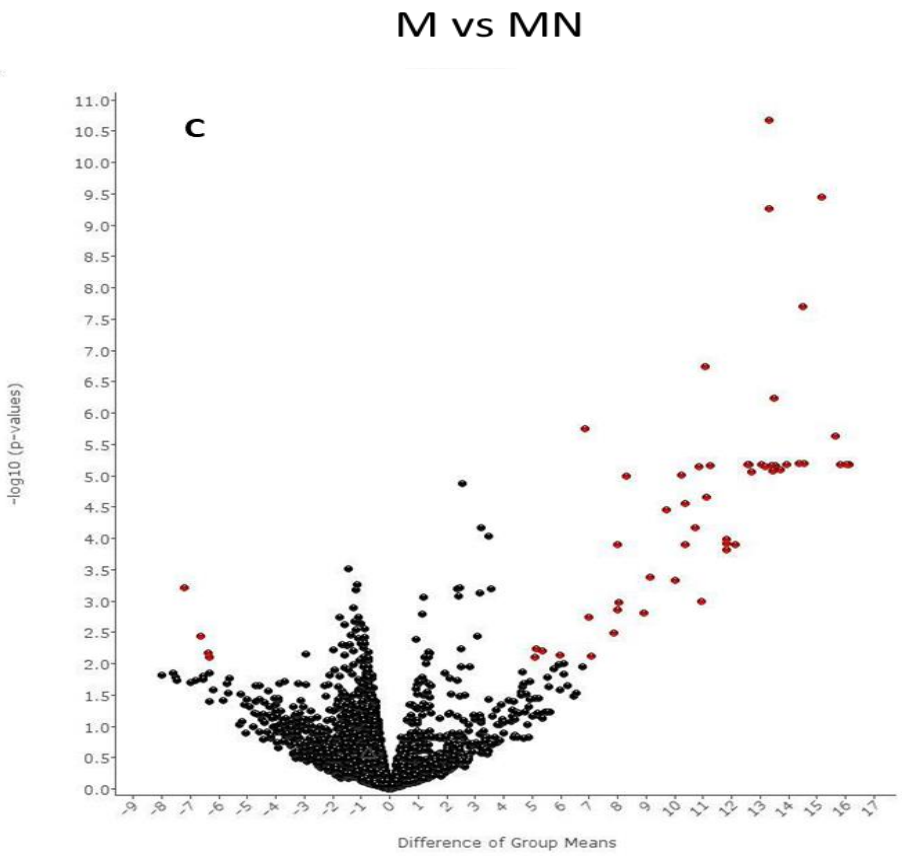


Fig. 7.11: Volcano plots of all detected features. Each dot on the plot is one feature, and the "outliers" on this graph represent the most highly differentially abundant features. All compound ions that are coloured red have a p-value < 0.01 and a fold change larger than 20. A; Supa vs. Mwangaza, B; Supa vs Supa with nematode C; Mwangaza vs Mwangaza with nematodes.

A PCA plot of the first two principal components shows an explicit confirmation of class separation between Supa and Mwangaza in both positive and negative ionization mode. Figure 7.11 shows the separation between Supa and Mwanganza through principal component 1 (PC1) both for the negative as the positive ionization mode. There is also a clear distinction between plants with and without *P. zeae* infection in negative ionization mode, but not in positive ionization mode.

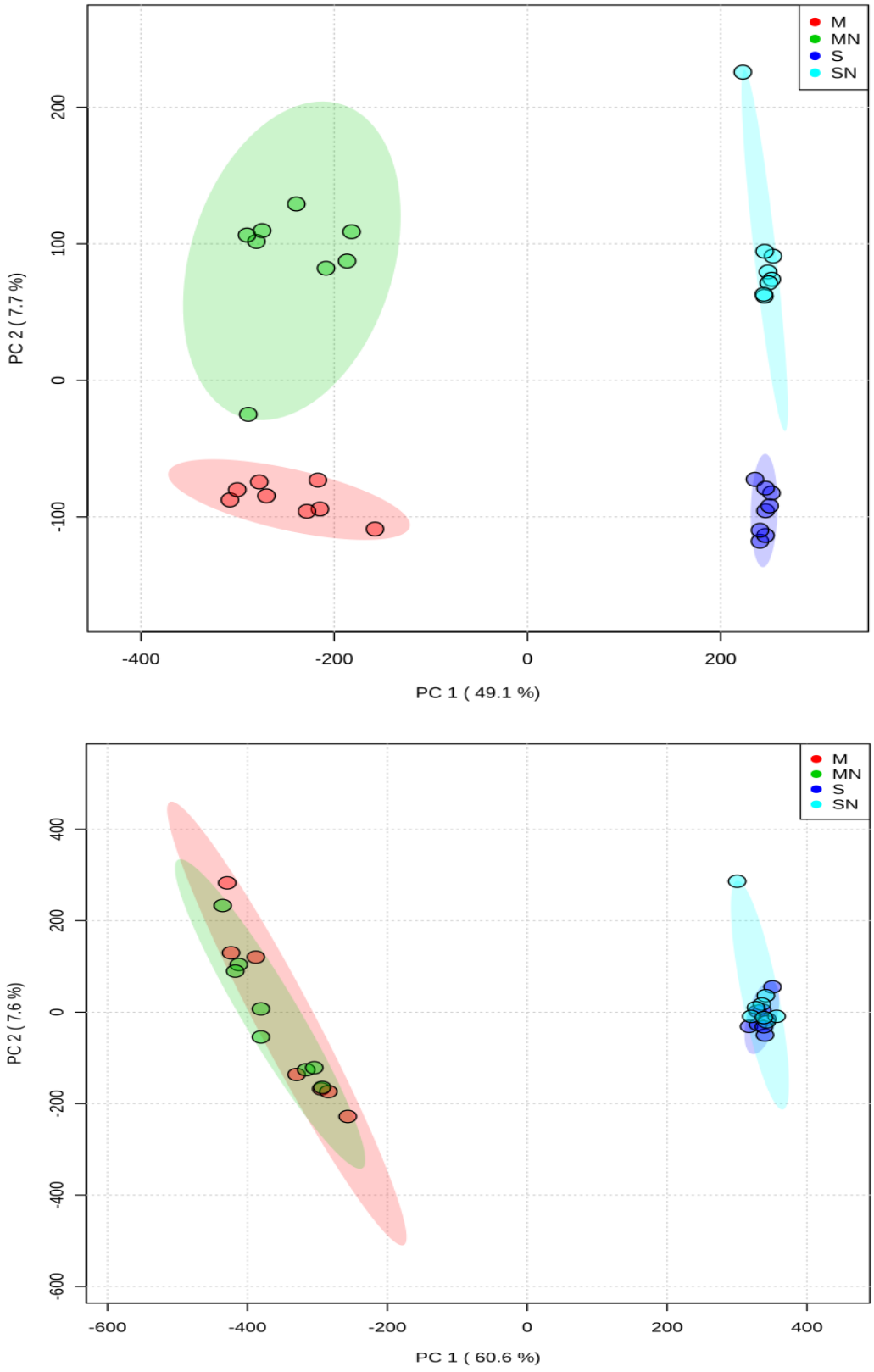


Fig. 7.12: **PCA plot of untargeted root metabolome profiling of the Supa and Mwangaza rice genotypes (S, M) with and without infection by *P. zeae* (SN, MN) via negative (A) and positive (B) ionization mode in UPLC-MS/MS. The different colours represent different treatments; M= Mwangaza; MN= Mwangaza infected with nematodes; S= Supa; SN= Supa infected with nematodes. The shaded ellipses represent 95% confidence ellipses.**

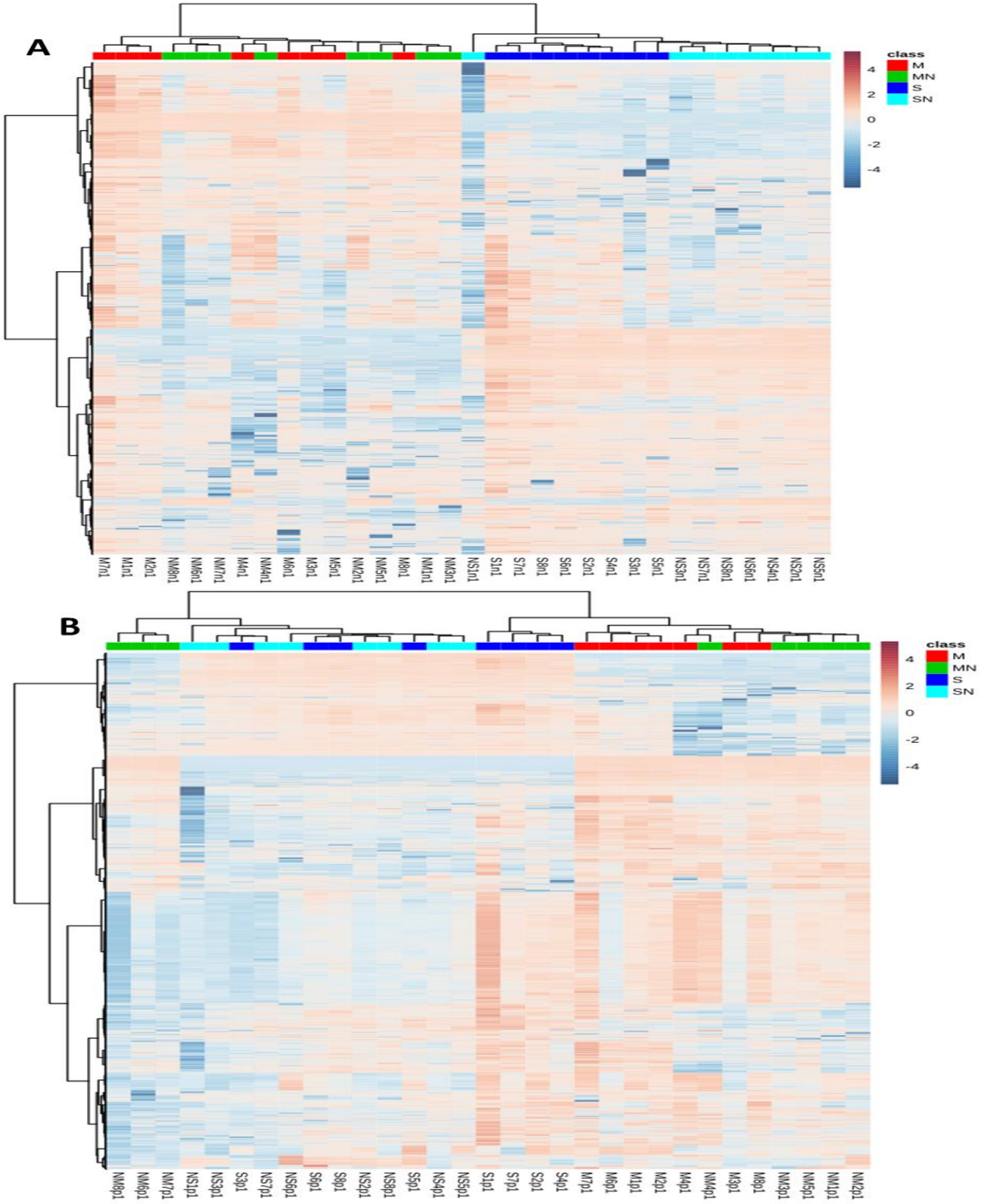


Fig. 7.13: **Hierarchical clustering analysis of negative (A) and positive (B) modes UPLC-MS/MS data for the four rice genotype/infection combinations (M: Mwangaza, no nematode; S: Supa, no nematode; MN: Mwangaza with *P. zeae*; SN: Supa with *P. zeae*). The dendrograms show the relationship between the different treatments, with each cell representing a feature coloured according to its relative abundance. Features with similar abundance patterns are clustered together.**

To further observe the patterns of filtered metabolites abundances, heat map analyses were performed, and hierarchical clustering was done to visualize the abundance patterns (Fig. 7.12). This confirmed the separation between Supa and Mwangaza, especially in negative ionization mode and less evident in positive ionization mode. The separation between nematode-infected and non-infected samples within each genotype was less obvious, with only the Supa genotype in negative ionization mode showing a separation of metabolite abundances between infected and non-infected plants.

7.4.6 Analysis of known metabolites in Supa and Mwangaza cultivars

Matching against biological databases enabled the putative annotation of 66 metabolites in negative mode and 12 metabolites in positive mode. Thirty-five out of 66 negative and 2 out of 12 positive mode metabolites were derived from the phenylpropanoid pathway, including various conjugates of quinic acid, vanillic acid and ferulic acid as well as the plant hormone salicylic acid. Most of the metabolites showed a lower baseline abundance in Supa compared to Mwangaza, and almost similar abundance in *P. zeae* Supa and Mwangaza infected plants.

To evaluate the effect of nematodes infection on Supa and Mwangaza metabolites, the significantly different features ($p < 0.05$) that are different between Mwangaza and Mwangaza + *P. zeae*, between Supa and Supa + *P. zeae* or both were made for positive and negative ionization modes (Fig.7.13). The metabolites which were common in both cultivars were all up-in abundances after nematode infection in both Supa and Mwangaza.

Fig. 7.14: Venn diagram showing the number of significantly different features in A. negative mode and B. Positive mode using FDR adjusted p-values (p < 0.05) that are different between Mwangaza and Mwangaza + *P. zeae*, between Supa and Supa + *P. zeae.*

7.4.7 Modified metabolic pathways in incompatible versus compatible interaction

To get further insight into the nature of the interaction between *P. zeae* and rice at the metabolome level, the Mummichog pathway analysis algorithm was used to identify possibly enriched pathways. A total of 33, 27, and 34 metabolic pathways were identified by the algorithm in the pairwise comparisons between Supa and Mwangaza, Supa without and with nematodes and Mwangaza without and with nematodes respectively. Among the identified pathways, two pathways were significantly (p=0.05) altered: “Phenylpropanoid biosynthesis” and “Phenylalanine metabolism”. The phenylpropanoid biosynthesis pathway activity appears to be unchanged in Supa after nematode infection, whereas it changes in Mwangaza. There is a significant (p= 0.05) difference between Mwangaza and Supa in phenylpropanoid pathway activity.

7.5 DISCUSSION

The current study investigated the resistance mechanisms of rice cultivar Supa to *P. zeae* thorough inspection of the early steps in the infection process and a

combination of histochemical and metabolomic analysis. The results shed more light on the pre-infection mechanism of resistance depicted by Supa (Nzogela et al., 2020a). When given a choice, nematodes migrated to and preferentially penetrated the susceptible genotypes Mwangaza and UPLRi5 and even the resistant TOG5764 compared to Supa. The presence of nematodes in Supa roots decreased from 1 to 5 days post-inoculation in contrast to what happened in the susceptible genotypes (Fig. 7.1 & 7.2). This observation indicates that probably some host compounds interact negatively with the nematodes soon after root penetration. This made the root environment not conducive for nematode survival; thus; nematodes may exit the host roots. Hölscher et al. (2014) found the resistance mechanisms in Banana against *Radopholus similis* involved secondary metabolites called phenylphenalenones. These compounds from banana roots were negatively affecting the nematodes after penetration. The compounds acted as nematostatic and nematicidal and their effects were concentration-dependent.

The current study shows that barriers at the early stage of the interaction play a vital role in the identified resistance mechanisms of Supa to *P. zeae*. This finding is congruent with studies in the *Radopholus similis*-banana interaction (Dhakshinamoorthy et al., 2014; Valette et al., 1998; Wuyts et al., 2007), but is in contrary to studies where suppression of invasion was not found to be a key resistance mechanism against *Pratylenchus* species and other migratory nematode species (Farsi et al., 1995; Khanam et al., 2018; Linsell et al., 2014b).

The temperature may affect both host resistance and nematode development and reproduction (Acosta & Malek, 1979; Amarasena et al., 2016; Cabasan et al., 2016; Mizukubo & Adachi, 1997; Namu et al., 2018; Thompson et al., 2015). Moreover, the effect of some secondary metabolites may also be temperature

sensitive (López-Pérez et al., 2005; Tock & Usakabe, 2017). Analysis of nematode reproduction in the compatible and incompatible interactions under different temperatures showed that *P. zeae* reproduced significantly more in Mwangaza than Supa under all tested temperatures and that the temperature had no effect on Supa resistance against *P. zeae*. The stability of the identified *P. zeae* resistance in Supa over the tested temperature indicates that this is a useful resistance source to be used in environments with variable temperatures.

When nematodes are exposed to a toxic environment, they become inactive and immobile. Root crude extracts (RCE) from non-infected Supa immobilized higher numbers of *P. zeae* compared to the water control and Mwangaza RCE. This motility suppression suggests that Supa RCE has metabolites which are toxic to nematodes. Similar effects on other *Pratylenchus* species have been reported with RCE from resistant wheat genotypes (Linsell et al., 2014b). Seenivasan (2018) also reported *R. similis* motility suppression with RCE from resistant banana. Phenolic acids and flavonoids in plant root exudates have been characterized as allelopathic substances that can inhibit nematode motility; this motility inhibition probably contributed to lower nematode infection of Supa roots (Gao et al., 2018).

The phenylpropanoid pathway is a key pathway that contributes intermediaries in the biosynthesis of secondary metabolites such as phenolic esters, flavonoids and coumarins as well as the cell wall polymer lignin. Phenylalanine ammonia-lyase (PAL) is a key enzyme in the Phenylpropanoid biosynthesis pathway. PAL activity was analysed to elucidate whether this enzyme is involved in Supa defence against *P. zeae*. Constitutive PAL activity in Supa was high throughout all sampling time points. Nematode-induced PAL activity in Supa was higher at five dpi than Mwangaza, suggesting that PAL activity plays a role in Supa's resistance to *P.*

zeae. PAL activity increases host response to abiotic stress, wounding and pathogen infection (Alamgir et al., 2016; Edens et al., 1995; Fortunato et al., 2014; Hung & Rohde, 1973; Starr et al., 2014; Wuyts et al., 2006b).

In potato resistant to *G. rostochiensis,* PAL activity was higher than in the susceptible cultivar before nematode infection (Giebel, 1982). Khanam et al. (2018) demonstrated that PAL activity contributed to cultivar Manikpukha resistance against the migratory stem rice nematode *D. angustus* and that inhibition of PAL activity by chemical compounds compromised the resistance. PAL activity was induced in a resistant banana cultivar after *R. similis* infection. The activity was six-fold higher at seven days after nematode infection than in the susceptible genotype (Wuyts et al., 2006b). PAL activity in Mwangaza was induced by nematode infection at 1 and 2 dpi and decreased at five dpi, implying that at the initial stage of *P. zeae* infection PAL activity is activated, and then suppressed by the nematodes. Baldridge et al. (1998) working with *P. penetrans* resistant alfalfa found high mRNA levels of genes in the phenylpropanoid pathway as compared to a susceptible cultivar.

Lignin accumulation in cell walls is a potential physical barrier to pathogen infection, including nematodes (Veronico et al., 2018). Nematode migration and feeding are limited as lignified cell walls hinder the access of secreted nematode enzymes to cell wall polysaccharides (Dhakshinamoorthy et al., 2014). Nematode infected Supa and Mwanganza showed high lignification of the cell walls around the vascular bundles. This could result from the general defence mechanism to protect the vascular bundle from nematode infection or damage (Wuyts et al., 2007). Although Mwangaza has lignified cell walls around the vascular tissues, *P. zeae* was able to overcome that barrier and disrupted the cell walls of the vascular cells (Fig 7.8 D). Mwangaza had a higher number of nematodes penetrating the roots at all-time points

under investigation, which suggests that although lignification occurred in infected Mwangaza roots, it did not inhibit *P. zeae* penetration and migration toward the vascular tissue. The current results could be further supported by additional quantitative lignin analysis and temporal analysis of PAL activity in compatible and incompatible rice interaction with *P. zeae* to elucidate their association further.

Untargeted metabolome profiling was conducted to gain more insight on resistance mechanisms of Supa against *P. zeae.* A vast difference in metabolite profile was found between Supa and Mwangaza (Table 7.1 & 7.2). This might explain their difference in *P. zeae* susceptibility. Nematode infection-induced relatively minor changes in the metabolome profile of both Supa and Mwangaza. Metabolites that accumulated due to nematode infection were generally common between Supa and Mwangaza (Fig. 7.13). This suggesting that constitutive resistance mechanisms might play a more important role in Supa resistance against *P. zeae* than induced responses. About half of the annotated metabolites either belong to the phenylpropanoid pathway or to pathways that use phenylpropanoid products as intermediaries, indicating that this pathway might play a key role in the identified resistance. Our experiments show that Supa possesses early time point resistance mechanisms such as impaired nematode penetration and migration. This is probably caused by nematode immobilization, and higher PAL enzymatic activity might lead to the production of compounds that are unfavourable for nematode feeding and migration within the roots of resistant genotype.

The metabolomics experiment performed in this study did not allow conclusive identification of the metabolite(s) responsible for early-stage Supa resistance. However, the presence of metabolites such as dihydro-p-coumaroyl hexose, p-coumaroyl hexose, feruloyl hexose, and cis-p-hydroxycinnamic acid and

salicylic acid are good indicators that phenylpropanoids might be involved in Supa early resistance to *P. zeae*. Wuyts et al. (2006c), reported the inhibitory effect of salicylic acid and other phenylpropanoid-derived products to *R. similis* and *M. incognita*. Constitutive accumulation of salicylic acid in Supa may contribute to the observed early resistance mechanism.

Flavonoid localization showed high accumulation in infected Supa roots as early as two days post-infection. Since flavonoids are biosynthesized using the phenylpropanoid metabolite 4-hydroxycinnamoyl-CoA, this is additional evidence that the phenylpropanoid pathway might be involved in Supa resistance against *P. zeae*. Metabolome pathway analysis shows that there are significant differences in phenylalanine metabolism and phenylpropanoid biosynthesis pathways between Supa and Mwangaza. Therefore, based on annotated metabolites, we suggest that resistance of Supa RLN *P. zeae* depends at least in part on the phenylpropanoid pathway. Identifying which intermediaries and derivatives of the path are most important to the observed resistance of Supa to *P. zeae* requires further research.

7.6 CONCLUSION

We report here that Supa resistance mechanisms against *P. zeae* is early and fast depicted as local responses to avoid root invasion through both immobilizing the nematodes and strengthening the barriers against the nematode invasion. Soon after infection, the nematodes face difficulties in migration, feeding and hence reproduction. The resistance mechanisms appear more constitutive than induced, which is confirmed by the metabolome analysis. The current study provides a strong platform for designing further studies on rice-RLN interaction. The studies include genetic analysis for the identification of the genes involved in resistance and further metabolome mining to identify metabolites involved in early Supa resistance against

RLN. These metabolites could be used as biomarkers in marker-assisted plant breeding or crop protection for the development of new crop protection agents. Histochemical staining of lignin and flavonoids shows the involvement of the phenylpropanoid pathway in the identified Supa resistance mechanisms against *P. zeae*. It is therefore suggested that the analytical quantification and identification of phenylpropanoids in Supa roots could be made to establish the role of phenylpropanoids in resistance mechanisms of rice against the RLN *P. zeae*.

CHAPTER 8: GENERAL CONCLUSION AND FUTURE OUTLOOK

8.1 REFLECTION ON THE PREVALENCE OF *PRATYLENCHUS ZEAE* IN LOWLAND AND IRRIGATED RICE FIELDS IN TANZANIA

Rice is one of the most important crops in Tanzania that contributes to people's food and livelihood security (Mwaseba et al., 2007). The rice demand in the country is very high due to the rising population, which requires more food; however, there is a decline in per capita food availability. This renders to rice intensification for increased production (Katambara et al., 2013; Nasrin et al., 2015).

Rice production intensification systems may elevate pests and diseases by changing the existing pest and disease status, and minor/unseen problems might be a threat to the rice crop. Rice productivity is very low in Tanzania due to lack of quality inputs, improved techniques, and cultivars, inadequate diagnostic capacity for pests and diseases (Coyne et al., 2018; World Bank, 2015). Climate changes have brought a significant impact on pests and diseases, crop production systems, and water availability. For rice production to increase, a holistic approach is needed to address dealing with multiple rice production limiting factors adequately, including plant-parasitic nematodes (Sekiya et al., 2020). Knowing the problem is the necessary foundation for problem management.

It was not known if there are nematode problems in rice ecosystems in Tanzania. The first exercise done by the current study was, therefore, to identify the nematode problems existing in rice agroecosystems in Tanzania and single out the most abundant and prevalent species for further characterization (Chapter 3). The work has covered upland, lowland, and irrigated rice ecosystems. The current study

deployed both morphological and molecular techniques to characterize the nematode problems from rice fields. The nematode problems we have identified from rice fields in Tanzania are commonly found also in rice fields in other African countries. *Pratylenchus zeae* is a major lesion nematode in rice fields in West Africa, Kenya, Zimbabwe, South Africa, and now Tanzania.

However, while in the countries mentioned above, *P. zeae* have been reported on upland rice fields only, in the current study, these nematodes were also isolated from upland lowland and irrigated rice agro-ecosystem. Climate change renders to changes in farming systems and crop agronomy at large and minor nematodes might become major constraints in various conditions. In Tanzania, most of the rice fields under lowland conditions that were customarily flooded from transplanting to harvesting are now subjected to intermittent wetting and drying due to water shortage during the rice-growing season. This condition makes somehow lowland rice fields resembling upland conditions (Sekiya et al., 2017) that may favour the production of other crops more than rice like vegetables during off season.These crops and favourable moist in soils in lowland rice fields may favovous the development and establishment of *P. zeae*.

The little use of water in lowland rice fields for water-saving is part of the System of Rice Intensification (SRI). SRI is a technique of rice cultivation under low-water conditions resembling those of aerobic conditions and usually has the benefit of higher yield with lower input (Sekiya et al., 2017, 2020). Its disadvantage is that it might favour the establishment and development of nematodes that were not a major problem under flooded conditions. These nematodes might be *P. zeae* (which could be the case in this study especaill under lowland conditions), *M. graminicola*, *M. javanica*, *M. arenaria* and *M. incognita.* The mentioned nematodes *are* well known to

infect rice under upland conditions (Coyne et al., 1999a; Dutta, 2012; Somasekhar & Prasad, 2012; Castillo & Vovlas, 2007; Pascual et al., 2014; Pili et al., 2016; Prot & Savary, 1993).

In most fields from the upland rice ecosystem, *P. zeae* densities were much higher than in lowland and irrigated ecosystem, indicating that although *P. zeae* were found under lowland and irrigated rice, their favourable conditions still are the upland rice ecosystem. This might be due to intercropping practices done with upland rice fields that include maize which is a very favourable host of *P. zeae*. It was further revealed in the pathogenicity test (Chapter 6) that *P. zeae* reproduced highly under upland with adequate (field capacity) moisture content. Under flooded condition with a susceptible rice cultivar, the *P. zeae* final density did not outweigh that from simulated upland conditions. *P. zeae* might be adapting to flooded conditions so they can survive under anaerobic environments. However, the information on how *P. zeae* can infect rice roots under flooded conditions is limited. In our study, *P. zeae* were inoculated on rice roots under field capacity moisture content, and the three rice ecosystems were simulated one week after nematode infection. The same applies to the rice field under lowland conditions. In addition to the survey done in rice fields, samples were taken from selected rice seedling nurseries; however, we did not find any plant-parasitic nematodes in those samples. Usually, rice seedlings are transplanted in fields when the soil is wet (muddy) under both lowland and irrigated conditions. Wet/muddy condition is not ideal for nematode root infection due to the excessive moisture content, but after transplanting the fields are allowed to drain water for one week. This is done for proper root recovery from transplantation shock and establishment. Then the fields are flooded (irrigated). For rain-fed lowland, seedling transplantation commonly coincides with fewer rains at the beginning of the rainy season, with less

flooding of the fields. Importantly, it should be noted that the rice variety Supa is the preferred rice variety in lowland rice fields, and we have shown that Supa is resistant against *P. zeae* (Chapter 4 & 6). This could justfy why low densities of *P. zeae* were recovered from lowland and irrigated rice fields. However, the noted *P. zeae* densities would have been aggravated by the cropping sequences that favoured the *P. zeae* reproduction. Further study on the population dynamics of these nematodes is recommended. On the other hand, most of the upland rice fields were growing NERICA 1. This could explain the high *P. zeae* population densities found in upland rice fields.

RKN, more specifically *M. arenaria,* were only found in lowland rice fields. This may be explained by the fact that most farmers use the fields after the main rice harvest to grow vegetables such as tomatoes, cucumber beans and onions. Among these vegetables, some are a perfect host for RKN. It can be elucidated that the nature of isolation of *M. arenaria* was mainly due to rotational crops (vegetables) grown after the main crop (rice). This should be taken care off because *M. arenaria* can infect rice and that could be a good source of spread to other rice fields and to the next crop as well. But the fact that these nematodes were detected only in lowland fields does not gurantee their absence in other rice fields for instance upland fields. It should be noted that their detection might have been missed during the survey. There is about a 5% probability of missing the species during survey.

8.2 'SUPA' A SUPER RICE GENOTYPE FROM EAST AFRICA BEATS FULLY *PRATYLENCHUS ZEAE* AND PARTLY ROOT-KNOT NEMATODES

The use of natural resistance once found is a feasible and environmentally friendly way of plant-parasitic nematode management (Saucet et al., 2016). In chapter

4, Supa was identified to be resistant to *P. zeae*. These results were in agreement with those of Pili et al. (2016), who found a low number of nematodes associated with rice in upland fields in Kwale county, Kenya. The study pointed out that farmers in Kwale County were growing Supa in their fields. Supa, as a farmer's preferred variety, can thus be directly used by farmers in *P. zeae* infested fields. Supa is well adapted to all rice agro-ecosystems so it can also be used in upland conditions where fields are infested with *P. zeae* and be promoted as a cultivar to beat the climate change. The use of Supa cultivar should be integrated well in crop rotation with other non-cereal crops. For upland fields oil crops which fetch a high price in the market like sesame, sunflower groundnuts, and sweetpotatoes are suggested. For lowland fields vegetables such as onion, green gram and cabbages are highly recommended. These recommendations should also take into account the other side of Supa. It is susceptible to rice BL and (RYMV) (Hubert et al., 2017b; Mgonja et al., 2016), and yield is relatively low compared to improved cultivars like SARO-5. Therefore, besides the direct use by farmers in *P. zeae* infested fields, Supa can be used for future rice breeding to combine *P. zeae* resistance with other beneficial characteristics.

Supa is resistant to *P. zeae,* but its mutant Mwangaza is very susceptible to *P. zeae*. The current study used Supa and its mutant Mwangaza to study the mechanism of Supa resistance against *P. zeae,* specifically on where, when and how resistance affects *P. zeae* (Chapter 6). This was done by characterization of the nematodes' attraction, penetration, and reproduction. The results have shown that *P. zeae*, when given a choice, was attracted to and penetrated in Mwangaza roots more than in Supa roots, implying that the resistance already acts at the early stage of the rice-nematode interaction. Crushed root suspensions from Supa were able to inhibit nematode motility more than those from Mwangaza, confirming that Supa roots

contained inhibitory compounds. These results are congruent with what has been found in the *P. thornei* -wheat interaction (Linsell et al., 2014b).

In-situ-localization of lignin in both compatible and incompatible *P. zeae*-rice interactions found that lignin accumulated in the cell walls lining the endodermis and more on the vascular bundle in both Supa and Mwangaza after nematode infection. This implies the protection of the vascular bundle from invading nematodes, and it has been referred to as a general defence response against vascular bundle destruction also in banana-R similis interaction (Wuyts et al., 2007). However, the accumulation of lignin increased in Supa as the time after infection elapses. This went in hands with the activity of Phenylalanine Ammonia-Lyase (PAL) a well know enzyme responsible for the first steps of lignin synthesis that were more active in Supa with and without nematodes infection than Mwangaza proving the early resistance strategy of Supa against *P. zeae.* The incompatible interaction between Supa and *P. zeae* was characterized by the accumulation of flavonoids that was congruent with Seenivasan, (2018). All these results together opened up new insights for further resistance associated-biochemical characterization in Supa.

Nowadays, metabolomics studies have gained considerable attention due to the useful information that can be extracted for studying various biological systems (Zinov'eva et al., 2004). To this end, untargeted metabolite analysis was performed on both infected and uninfected Supa (resistant) and Mwangaza (susceptible) roots using UPLC-MS/MS. The results provided evidence for constitutive resistance to *P. zeae* infection. Supa and Mwangaza are very different at the metabolite level, but neither Supa nor Mwangaza showed a substantial metabolite change after *P. zeae* infection. However, the observed differential metabolites in Supa and Mwangaza after nematode infection might have a very significant effect on resistance. Indeed, what matters is not

the number of altered metabolites but the impact of these metabolites on nematodes (Yu & Zhang, 2017). For example, in the *Radopholus similis*-Banana interaction, only one metabolite was found to play a key role as an anti-nematode compound (Hölscher et al., 2014).

Therefore, the characterization and identification of every metabolite generated from the experiment become crucial. The identification of some resistance-related metabolites such as p-coumaric, cinnamic, and ferulic acids being abundant in Supa are of interest. It was not possible to find out which specific metabolites are essential in the *P. zeae*-rice interaction; however, a good foundation was laid for further characterization using targeted metabolites analysis techniques.

In the field, plants are usually challenged by multiple PPN (Mokrini et al., 2019). To find resistance to RKN in rice is important because most of the *O. sativa* rice genotypes are susceptible to these nematodes (Plowright et al., 1999). For that reason, the current study evaluated the rice genotypes that had been studied against *P. zeae* also to the important RKN *M. graminicola* and *M. javanica*. Their reproduction and development during a single life cycle were assessed (chapter 4 and 5). The rice genotypes Supa and Komboka were partially resistant to both nematodes. Further characterization of the mechanisms of resistance revealed post-infection mechanisms contributing to reduced nematode development and reproduction. Similar results were found elsewhere (Cabasan et al., 2012; Galeng-Lawilao et al., 2019a; Kumar et al., 2014). However, also post penetration emigration from the root contributed to the observed resistance in Supa and Komboka. This has been observed in other RKN resistance studies on peanut cultivar COAN, woody plants, cotton, forage grasses and Myrobalan plums (Bendezu & Starr, 2003; Esmenjaud, 2016; Saucet et al., 2016; Silva et al., 2013; Voisin et al., 1999).

The post-infection resistance includes gall dissolution resulting in aberrant gall and nematode phenotypes. Galls dissolution is a host response indicating the failure of nematodes to maintain the formed feeding sites. This might have been contributed to by flavonoids, which were localized in *P. zeae* infected roots (Chapter 7). Flavonoids are said to interfere with feeding site development (Chin et al., 2018); however, to ascertain this, histochemical localization of flavonoids in Supa and Komboka after RKN infection is necessary. The resistant rice plants might be producing some metabolites that might be directly toxic to the nematodes leading to death of nematodes as has been seen in banana by Hölscher et al. (2014). The two mentioned reasons might be the cause of gall dissolution, but the exact cause of gall dissolution is still an open question. Petitot et al. (2017) found no relationship between gall disappearance and phenolic accumulation in the *M. graminicola*-*O. glaberrima* interactions.

So far, the current study has identified the cultivar Supa to be resistant to *P. zeae* and partially resistant to *M. graminicola* and *M. javanica*. However, it is not known if the resistance to different nematodes in Supa is due to one major gene or several minor genes and if the same minor/ major gene confers resistance to nematodes of a different lifestyle. There could be several genes for resistance in the genome. Therefore a further study, which would include genotyping of Supa to generate SNPs revealing candidate loci for identification of the resistance, will be important to clarify the current findings.

In *O. glaberrima,* it is thought that resistance against RKN is controlled by multiple minor genes (Petitot et al., 2017). In the case of *O. sativa* resistance against *M. graminicola*, studies on cultivar Zhonghua 11, accessions LD 24 and Khao Pahk Maw (KPM) hypothesise that may be R-gene is involved. However, it has not yet been proven to be one gene, but it is one locus, and in the case of QTLs it is multiple genes on multiple loci (Dimkpa et al., 2016; Lahari et al., 2019; Phan et al., 2019).

Recently an analysis of these two accessions was done against *M. javanica* and *P. zeae*, and KPM confers resistance to both nematodes. At the same time, LD24 was resistant to *M. javanica* but susceptible to *P. zeae* (Lahari et al., 2020) which shows that resistance of one genotype can be very diverse, ranging from specific to broad, and single to many genes conferring resistance. In that case, it is not known if the resistances to RKN and *P. zeae* are due to the same locus or even the same gene. Sometimes a single resistance gene can give resistance to quite diverse pathogens. A good example is the *Solanum peruvianum Mi-1* gene conferring resistance to various root-knot nematodes species and also aphids and whiteflies. On the other hand, the *Gpa* and *Gro-4* genes from potato render resistance to only one species and some of their pathotypes of potato cyst nematode (PCN) (Paal et al., 2004).

Host resistance can be changed by many factors, including temperature (Cabasan et al., 2016). This study investigated if the identified Supa resistance succumbs to higher temperatures, and we found that Supa resistance is still active at a higher temperature (32ºC). The stability of the determined *P. zeae* resistance in Supa over the tested temperatures indicates that this is a useful resistance source to be used in environments with variable temperatures.

8.3 NEW RICE FOR AFRICA –NERICA AGAINST RICE NEMATODES; A WAY FORWARD

The available information was that most NERICAs showed variable resistance to nematodes (cyst, root-knot nematodes, and root lesion nematodes) (Bimpong et al., 2010; Cabasan et al., 2018a; Plowright et al., 1999). The current study identified NERICA5 to be resistant to *P. zeae* however; the challenge was that seed germination was very poor. Most of the interspecific upland NERICAs under this study were susceptible to *P. zeae*, *M. graminicola,* and *M. javanica.* Unfortunately, NERICA that inherited the best traits of the two species *O. glaberrima* (nematode resistance) and *O. sativa* (high yield) is yet to be found. The resistance of *O. glaberrima* against RKN seems to be controlled by multiple minor genes. This kind of resistance is challenging to transfer to interspecific hybrids, which could be the reason why most of the NERICAs are susceptible to the nematodes. NERICAs were released in Tanzania for the rain-fed upland in 2009, and farmers' adoptions were very positive along the coast and Morogoro region (Mghase et al., 2010). The most attractive farmer's adoption criteria were high yielding potential under a limited amount of rainfall and soil nutrients. This attracted farmers under the rain-fed lowland rice ecosystem to adopt NERICA too. NERICAs performance is outstanding compared to local upland cultivars under nutrient and water stress conditions. In Tanzania, a large percentage of rice fields will continue to depend on rainfall.

Therefore, NERICAs promotion to upland and lowland ecosystems as a way forward to mitigate the impact of climate change, especially water shortage, should be encouraged despite being susceptible to nematodes. However, in some *P. zeae* infested fields where nematode populations are very high, farmers should be advised to use Supa instead. For the future, more NERICAs are yet to be tested against

different PPN and to explore resistance to nematodes from NERICAs and NERICAs next generations “Advanced Rice Varieties for Africa” (ARICA). In addition, studies on other stresses like Striga, iron toxicity, phosphorus, RYMV, Xanthomonas, and BL diseases (Diagne et al., 2010) should be conducted on those NERICAs.

8.4 FUTURE OUTLOOK

The findings of this study have opened up our understanding of nematode problems on rice in Tanzania and laid a foundation for further research. The study describes the first effort to combine different approaches for the analysis of the Supa resistance against the RLN *P. zeae*. These techniques are biological, histochemical, and metabolomics techniques. Some thematic areas that can be further elucidated are:

1. Population dynamics of *P. zeae* in rice fields. This should take into account the edaphic and environmental factors to delineate how these factors modulate the spatial and temporal population patterns of the nematodes. This information is vital in decision making on when and how to manage these nematodes.
2. Screening of rice for RLN resistance is time-consuming and cumbersome work involving counting actual nematodes inside the roots (done in the current study), especially in laboratories with fewer nematology facilities such as extraction equipment for easy counting of the nematodes. A simple and less time-consuming evaluation technique should be developed.
3. In-depth study of phenylpropanoids in *P. zeae* rice compatible and incompatible interaction by using chemical inhibitors for example α-aminooxyacetic acid (AOA), 2-aminoindan-2-phosphonic acid (AIP) and α-aminooxy-b-phenyl propionic acid (AOPP) to alter the phenylpropanoid

pathway. Quantitative chemical analysis (lignin, ROS, and total phenolics) to support the generated histological data will widen the understanding of the role of these compounds in the *P. zeae* rice interaction.

4. Metabolomics is the ultimate level of post-genomic analysis that reflects both transcriptional and post-transcriptional regulation. This thesis generated beneficial metabolite information derived from rice-*P.zeae* interaction. Not all the generated data were characterized and identified due to the lack of a rice metabolomics database. The identified metabolites in the current study are those that have apparent matches in biological databases. Therefore, for the metabolite experiment to be comprehensive, the following can further be done

- Phytochemical profiling of Supa and Mwangaza genotypes for the detection of marker compounds by integrating different analytical techniques and using a powerful complementary chemical analytical technique like Gas chromatography-mass spectrometry (GC-MS) to identify and quantify a significant percentage of metabolites.
- Fractionating the root extracts to find the inhibitory compounds and then analyze only the fraction that has effects on nematodes.
- In-vitro testing of the identified compounds for their nematicidal or nematostatic activity.

The current study has gathered information on nematode problems in rice production ecosystems and how the issues identified can be managed using a very efficient, convenient and less cost management strategy - host resistance. Well designed and structured nematode problem investigations, starting with nematode problem identification from the rice fields, characterization of the problem and way out on how the identified nematode problem can be managed is shown in this thesis. The

identification of the *P. zeae* resistant - Supa cultivar is of convenient application for rice farmers. More importantly, the pathogenicity study with rice yield loss attributes due to *P. zeae* is beneficial information for nematode management decision making. The in-depth research of rice-interaction with RKN such as *M. javanica* along the nematode life cycle adds value to this thesis.

8.5 RECOMMENDATIONS

1. Awareness creation for farmers, rice researchers and extensionists on *P. zeae* problems in rice fields.
2. Soil fertility issues should be addressed adequately for decreasing the effects of these nematodes on rice yield.
3. To identify the best strategies that can be used for utilization of identified sources of resistance to *P. zeae* from Supa at present and into the future.
4. Studies on rice nematodes in Tanzania, and the interaction with their host, has just begun. For the output of this research to be fully utilized and realized, further nematode research is required by developing a functional nematology lab at the Sokoine University of Agriculture. The information on the prevalence of nematodes problems in rice fields should be incorporated in the National Rice Research Programs to implement the appropriate measures for nematode management and breeding for resistance. And to spearhead the nematology field in Tanzania a nematology curriculum should be developed at university level.

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ANNEXES

ANNEX 1: CURRICULUM VITAE

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Education.

- 2014-2020 PhD in Applied Biological Science: Plant Faculty of Bioscience Engineering, Ghent University, Belgium.
- 2009-2011 Masters of Science in Nematology, Ghent University, Belgium (Great distinction)
- 2003- 2006 Bachelor of Science (B.Sc.), Agronomy, (Second class (Upper Division), The Sokoine University of Agriculture, Morogoro, Tanzania.

Professional experience.

- 2007 – Present Senior Agricultural officer, Sokoine University of Agriculture.
- 2006 – 2007 Agronomist, Kagera Sugar Company L.td and BIOSUSTAIN Tanzania.

Publications with peer reviewing.

- **Nzogela, Y, B.,** Landschoot, S., Kihupi, A., Coyne, D. L. & Gheysen, G. (2020). Pathogenicity of the root-lesion nematode, *Pratylenchus zeae*, on rice genotypes under different hydro-ecologies in Tanzania. *Nematology* 22, 221–233
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