Trichoderma as a functional fungal group in the rhizosphere of maize and wheat under conservation and conventional agricultural practices

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Declaration

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December 2021

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Summary

The contribution of agriculture in South Africa to the economy, is one of those major drivers of employment in South Africa. The two most produced commercial crops in SA are maize and wheat. In chapter one, the importance of these crops and the effect of farming practices such as conventional and conservation agriculture were briefly compared and discussed. Furthermore, the importance of microorganisms in agriculture as well as their role in various biological processes that occurs in soil are briefly discussed. Particular attention was given to the role of *Trichoderma* spp. as they interact and form relationships with other soil organisms. This was followed by a brief discussion on the taxonomic history of *Trichoderma* spp. and the application of *Trichoderma* spp. in the industrial and agricultural sectors.

Chapter two is the first of three research chapters and discussed the isolation and identification of *Trichoderma* species from wheat soil in the Western Cape. All isolates in this chapter were collected from agricultural soil only. The identification and classification of species was primarily based on macro features and amplification of internal transcribed spacer (ITS) regions. Thereafter, the final identification was done by combining two markers (ITS and Elongation factor 1 alpha (TEF1)). Ninety-one (91) strains of *Trichoderma* spp. which resolved into seven species that were identified as *T. virens, T. saturnisporum, Trichoderma* sp., *T. gamsii, T. koningiopsis, T. velutinum,* and *T. spirale*. It was also reported that *T. gamsii* was the predominant species. In addition, crop rotation practices resulted in a higher number of strains and species when it is compared with the monoculture practices.

Chapter three is similar to Chapter 2 and focuses on the identification of *Trichoderma* species, on maize from different geographical areas in KwaZulu-Natal and the Free State. Soil samples were collected from sites with crop rotation as well as monoculture practices. From isolations, 337 strains were recovered from maize soil representing 11 *Trichoderma* species. Seven species have been isolated previously in South Africa. However, five species namely, *T. velutinum, T. rifaii, T. paratroviride, T. neokoningii* and *T. peberdyi* are being reported for the first time in South Africa. Distribution of the species significantly differed between crop rotation and monoculture practices, with crop rotation sites resulting in a higher number of species than monoculture practices. Furthermore, *T. gamsii* and *T. hamatum* were the most abundant species isolated.

In chapter four the potential functions of different strains were investigated. The results suggest that each certain function in *Trichoderma* spp. could be strain specific. This chapter determined the abilities of *Trichoderma* strains to solubilize phosphate and produce indole acetic acid. These two metabolic factors (solubilization of phosphate and production of indole acetic acid) were evaluated because it is known that they could be used for primary identification of species that might have the capacity to improve plant growth. Findings indicated that the majority of strains were able to solubilize phosphate and pH reduction play a vital role in this case. *T. koningiopsis* NNC066 solubilized the maximum amount of phosphate whereas *Trichoderma* sp. K4 solubilized the least amount of phosphate. Moreover, no strains were able to produce indole acetic acid (IAA) in the absence of tryptophan (L-TRP). Although, the amendments of the media with L-TRP, enabled all strains to produce the IAA where maximum amount obtained at 41.90 µg/ml by *T. gamsii* NNC019, while the least amount was at 0.30 µg/ml by *Trichoderma* sp. K1.

Opsomming

Die bydrae van die landbou in Suid -Afrika tot die ekonomie, is een van die belangrikste dryfvere vir werk in Suid -Afrika. Die twee mees geproduseerde kommersiële gewasse in SA, is mielies en koring. In hoofstuk een is die belangrikheid van hierdie gewasse en die effek van boerderypraktyke soos konvensionele en bewaringslandbou kortliks vergelyk en bespreek. Verder word die belangrikheid van mikroörganismes in die landbou sowel as hul rol in verskillende biologiese prosesse wat in die grond voorkom, kortliks bespreek. Spesifieke aandag is gegee aan die rol van *Trichoderma* spp. as hulle interaksie het en verhoudings met ander grondorganismes vorm. Dit is gevolg deur 'n kort bespreking oor die taksonomiese geskiedenis van *Trichoderma* spp. en die toepassing van *Trichoderma* spp. in die industriële en landbousektor.

Hoofstuk twee is die eerste van drie navorsingshoofstukke en bespreek die isolasie en identifisering van *Trichoderma* -spesies uit koringgrond in die Wes -Kaap. Alle isolate in hierdie hoofstuk is slegs uit landbougrond versamel. Die identifisering en klassifikasie van spesies was hoofsaaklik gebaseer op makrokenmerke en versterking van interne getranskribeerde afstandhouers (ITS) streke. Daarna is die finale identifikasie gedoen deur twee merkers (ITS en Elongation factor 1 alpha (TEF1)) te kombineer. Een-en-negentig (91) stamme van *Trichoderma* spp. wat opgeneem het in sewe spesies wat geïdentifiseer is as *T. virens, T. saturnisporum, Trichoderma sp., T. gamsii, T. koningiopsis, T. velutinum* en *T. spirale*. Daar is ook berig dat *T. gamsii* die oorheersende spesie is. Boonop het wisselboupraktyke 'n groter aantal stamme en spesies tot gevolg gehad as dit vergelyk word met die monokultuurpraktyke.

Hoofstuk drie is soortgelyk aan hoofstuk 2 en fokus op die identifisering van *Trichoderma*-spesies, op mielies uit verskillende geografiese gebiede in KwaZulu-Natal en die Vrystaat. Grondmonsters is versamel vanaf terreine met wisselbou sowel as monokultuurpraktyke. Uit isolasies is 337 stamme gevind uit mieliegrond wat 11 *Trichoderma* -spesies verteenwoordig. Sewe spesies is voorheen in Suid -Afrika geïsoleer. Vyf spesies, naamlik *T. velutinum, T. rifaii, T. paratroviride, T. neokoningii* en *T. peberdyi*, word egter vir die eerste keer in Suid -Afrika aangemeld. Die verspreiding van die spesies het aansienlik verskil tussen wisselbou- en monokultuurpraktyke, met wisselbouplekke wat 'n groter aantal spesies as monokultuurpraktyke tot gevolg gehad het. Verder was *T. gamsii* en *T. hamatum* die mees voorkomende spesies wat geïsoleer is.

In hoofstuk vier is die moontlike funksies van verskillende stamme ondersoek. Die resultate dui daarop dat elke sekere funksie in *Trichoderma* spp. kan stamspesifiek wees. Hierdie hoofstuk bepaal die vermoëns van *Trichoderma* -stamme om fosfaat op te los en indool -asynsuur te produseer. Hierdie twee metaboliese faktore (oplosbaarheid van fosfaat en produksie van indool -asynsuur) is geëvalueer omdat dit bekend is dat dit gebruik kan word vir primêre identifisering van spesies wat die vermoë het om plantgroei te verbeter. Bevindings het aangedui dat die meerderheid stamme fosfaat kon oplos en pH -vermindering speel in hierdie geval 'n belangrike rol. *T. koningiopsis* NNC066 het die maksimum hoeveelheid fosfaat opgelos terwyl Trichoderma sp. K4 het die minste hoeveelheid fosfaat opgelos. Boonop kon geen stamme indool-asynsuur (IAA) produseer in die afwesigheid van tryptofaan (L-TRP). Alhoewel die wysigings van die media met L-TRP alle stamme in staat gestel het om die IAA te produseer waar die maksimum hoeveelheid op 41,90 µg/ml verkry is deur *T. gamsii* NNC019, terwyl die minste hoeveelheid op 0,30 µg/ml was deur *Trichoderma* sp. K1.

Dedication

This work is dedicated to my lovely family; Eunice Thabisile Ndaba (Mother), Gawu Phillemon Ndaba (Father), Mbali Nombulelo (Sister), Mthobisi Kwanele (Late brother), Mthokozi Jabulani (Brother), Mncedisi Siphelele (Brother), and Njabulo Mfanafuthi (Brother).

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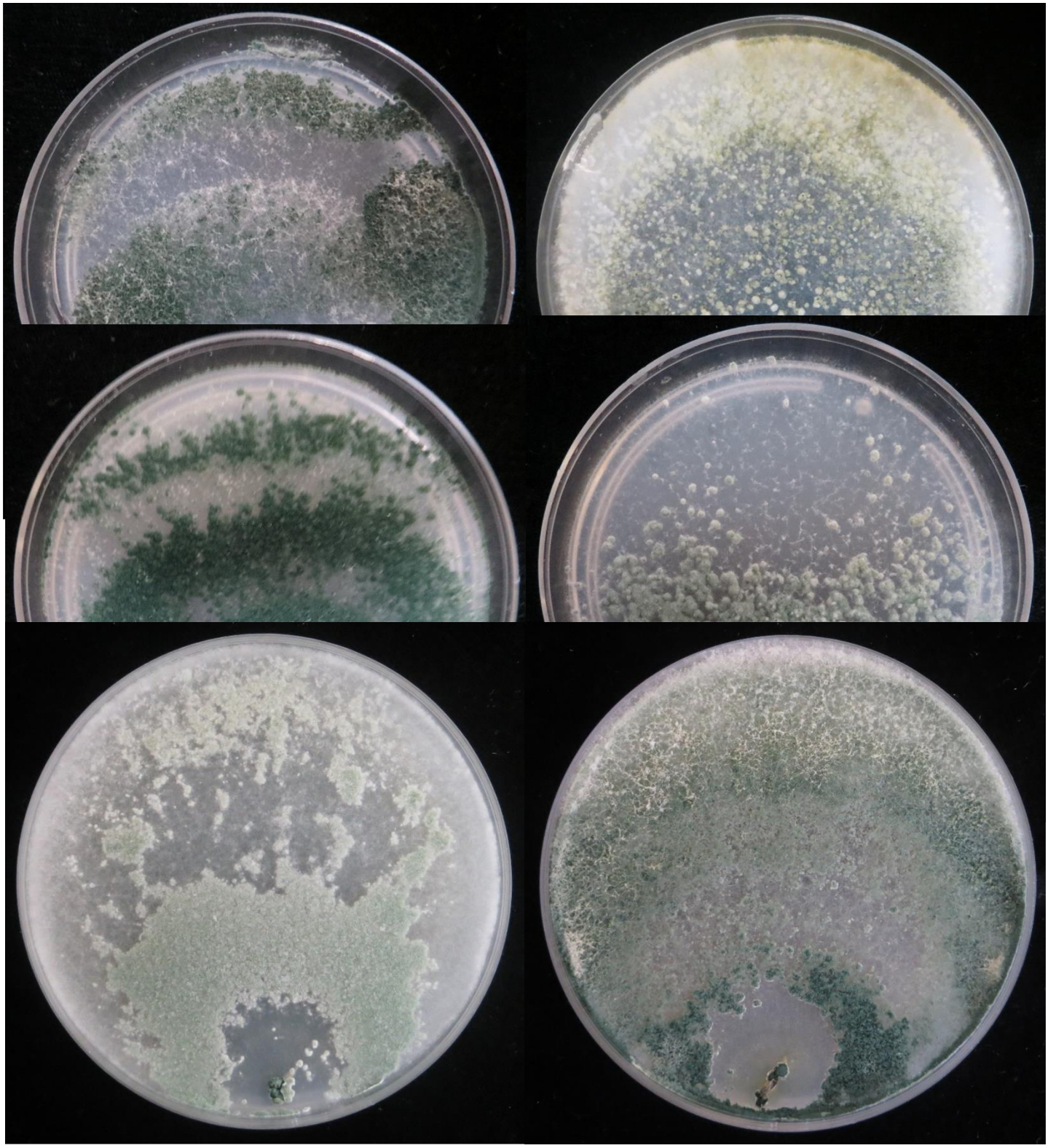
Chapter 1: Literature Review



Introduction

The South African agricultural sector is important for economic growth as it contributes between 2.5 and 3% towards the national gross domestic products (GDP) (Poonyth *et al*., 2001; Musvoto *et al*., 2015). Despite the relatively low contribution towards the national GDP, agriculture remains the highest contributor to local economies (Musvoto *et al*., 2015) and could play an important role in the reduction of poverty as it employs a large fraction of the workforce (Van Rooyen *et al*., 1987; Van Zyl *et al*., 1988; Van Rooyen and Sigwele, 1998; Poonyth *et al*., 2001). In addition, agriculture is the main contributor to food security for an increasing population (Godfray *et al*., 2010).

Food security faces various challenges including climate change, an increase in the human population, and high poverty rates (Godfray *et al*., 2010; FAO, 2016). These challenges place pressure on the agricultural sector to produce higher yields of commercial crops. The production of these crops will be enhanced using conventional farming techniques which might result in more problems. For instance, the incorporation of agrochemicals may have a detrimental effect on soil health in the long run (Matson *et al*., 1997; MacDonald and McBride, 2009). However, one of the solutions to these challenges in South Africa is the application of green economy methods (Musvoto *et al*., 2015; Loiseau *et al*., 2016; Consolo *et al*., 2020).

The green economy is defined as a practice that aims to implement approaches and techniques that reduce environmental damage (Pearce *et al*., 1991; Musvoto *et al*., 2015; Loiseau *et al*., 2016). Advantages of the green economy include the reduction of carbon emissions and waste, increased energy and water production, limited habitat destruction and limited loss of ecological structure (Pearce *et al*., 1991; Musvoto *et al*., 2015; D'Amato *et al*., 2017). One such example would be the use of biological entities such as microbes to produce valuable products (enzymes, fertilizers, antimicrobial compounds, nanoparticles, etc.) (Kusari *et al*., 2008; Singh *et al*., 2016; Leylaie and Zafari, 2018).

Conservation (regenerative) agriculture is one of the green economy approaches that should be considered as an alternative to conventional farming practices. This type of practice focuses on restoration of soil since it only allows minimum or no soil disturbance (Erenstein *et al*., 2012; Fiorini *et al*., 2020). Also, the incorporation of agrochemicals is low or not applied at all in this practice. The agrochemicals are usually substituted with biological fertilizers that are produced from various microorganisms (Mukherjee *et al*., 2014; Olanrewaiu *et al*., 2017; Kribel *et al*., 2020). *Trichoderma* is among those genera that are predominantly utilized in agriculture for different functions (Mukherjee *et al*., 2014; Bischof *et al*., 2016; Mahato *et al*., 2018).

Trichoderma species are found in various environments including soil, plants, and water (Gams and Bissett, 1998; Druzhinina and Kubicek, 2005; Brotman *et al*., 2013). *Trichoderma* species have the ability to produce various enzymes and other bioactive compounds that contribute in fighting against soil-borne pathogens (Shalaby *et al*., 2013; Abo-Elyousr *et al*., 2014; Elshahawy *et al*., 2017), and have been used as biocontrol agents against various pathogenic microorganisms (Samuels, 1996; Waghunde *et al*., 2016; Mutawila *et al*., 2016). In addition, recent studies on this genus evaluated their capacity to improve plant growth. *Trichoderma* spp. were found to exert positive traits for plant growth such as the production of plant hormones and solubilizing minerals (Gravel *et al*., 2007; Saber *et al*., 2017; Herrera-Jaminez *et al*., 2018; Bader *et al*., 2020; Mendes *et al*., 2020). Therefore, the isolation and identification of this genus could help in improvement of commercial crops.

Commercial crops play an important role in supporting the economy of the country (FAO, 2016; Dube *et al*., 2019). Major global crops including maize, wheat and rice serve as staple for many people. Among these crops, maize and wheat are the most produced crops in South Africa compared to other African countries (Wallington *et al*., 2012; Ekwomadu *et al*., 2018; Mupangwa *et al*., 2019). Therefore, focusing on these crops could contribute to food security for the South African population by 2050 (Godfray *et al*., 2010; FAO, 2016; Dube *et al*., 2019).

Important commercial crops

Maize

Maize (*Zea mays*) belongs to the family *Poaceae* which originated from Mexico 7000 years ago (Ranum *et al*., 2014; BFAD, 2015), and was distributed by colonizers and traders all over the world. Its success can be contributed to its ability to survive in various environmental conditions and, therefore, proved to be a successful crop on most continents including Africa (Abassian, 2006). Maize is the basic staple crop that is cultivated in Sub-Saharan Africa (Byerlee and Heisey, 1997), with South Africa the leading region (Muller *et al*., 2016; Musokwa *et al*., 2019).

South Africa produces approximately 8 million tons of maize per year on 3.1 million ha land (FAOSTAT, 2019). White maize is mostly consumed by people as food while yellow maize is widely consumed as animal fodder (Greyling and Pardey, 2018). Furthermore, yellow maize is extensively grown in the northern hemisphere countries including China, whereas white maize is mostly found or cultivated in countries such as Mexico and Southern Africa (Abassian, 2006; BFAP, 2015). Maize is grown for a

variety of reasons around the world, with animal feed being the most common, followed by industrial uses and human consumption (Nuss and Tanumihardjo, 2010; Wallington *et al*., 2012; Ekwomadu *et al*., 2018; Mupangwa *et al*., 2019).

Wheat

Wheat (*Triticum aestivum* L) is the second largest grain crop in South Africa (Dube *et al*., 2019; Naledzani *et al*., 2019) as it is consumed as food and serves as livestock feed (Shewry, 2009). It is the second most widely used staple food in the world after maize (Hussain and Shah, 2002; Dugassa *et al*., 2019). In South Africa, wheat is produced at higher rates compared to other Sub-Saharan African countries (Demeke and Di Marcantonio, 2013; Dugassa *et al*., 2019).

Growing of wheat was first recorded in South-eastern parts of Turkey about 10 000 years ago (Shewry, 2009). In South Africa, wheat was first grown approximately 368 years ago, although, the commercial cultivation of this crop was only established from 1910 onwards (Du Plessis, 1933; Nhemachena and Kirsten, 2017). The commercialization of wheat in South Africa resulted in improving food security because it can also be used for the production of important food products such as alcoholic beverages, cereals, bread, etc. (Stander, 2012; Nhemachena and Kirsten, 2017; Naledzani *et al*., 2019).

Soil health and the production of maize and wheat

Maize and wheat are the most consumed crops in South Africa as food (Demeke and Di Marcantonio, 2013; FAO, 2016; Muller *et al*., 2016; Musokwa *et al*., 2019; Dugassa *et al*., 2019). In addition to these crops being consumed as food and used to produce feeds, their residues are also used to generate environmentally friendly energy (bio-energy) (Urosevic and Gvozdenac-Urosevic, 2012; FAO, 2016; Batidzirai *et al*., 2016). However, their inconsistency in production could limit them in other beneficial uses such as bioenergy (Varvel *et al*., 2008; Scarlat *et al*., 2011).

Lower production of maize and wheat crops could be compounded by environmental factors such as the decline in soil fertility and the occurrence of diseases (MacDonald and McBride, 2009; Ncube *et al*., 2011; Kucukakyuz *et al*., 2016; FAO, 2016; Lu *et al*., 2017). The decline in soil fertility can be attributed to intensification of farming practices such as the overuse of the agrochemicals and disturbance of soil (Matson *et al*., 1997; Bouwman *et al*., 2013). This may result in eliminating essential nutrients and disturbing microbial diversity in soil (Matson *et al*., 1997; Singh *et al*., 2016; Mahanty *et al*., 2017;

Srivastava *et al*., 2019; Mattoo *et al*., 2021; Zhai *et al*., 2021). Furthermore, the use of agrochemicals might lead to soil and water pollution (Savci, 2012; Singh *et al*., 2016). This could be resolved, or at least be minimized by employing conservation agricultural practices, such as crop rotation, the use of cover crops, minimum or no tilling, and application of bio-fertilizers, bio-pesticides, and bio-fungicides. In this scenario the application of *Trichoderma* spp. could be used to enhance plant growth and also protect against pathogenic microorganisms (Rudresh *et al*., 2005; Saravanakumar *et al*., 2013; Chagas *et al*., 2016; Sharma *et al*., 2019; Khoshmanzar *et al*., 2020).

Overview of conventional and conservation agricultural practices

Conventional agriculture is known as a practice that involves the disturbance of soil, focuses more on the use of agrochemicals (Morrison-Whittle *et al*., 2017; Tal, 2018) and has been used as the main farming technique over the last couple of decades due to high and intensified production requirements in response to a rapid growing population (Godfray *et al*., 2010; Seufert *et al*., 2012; FAO, 2016). The introduction of agrochemicals in the 20th century in order to the improve plant growth (fertilizers) and control diseases (pesticides) (Johnston and Mellor, 1961; Schultz, 1964; Johnson *et al*., 2003; McArthur and McCord, 2017; Carvalho *et al*., 2017) contributed to the massive increase in yield (Fig. 1.1). However, the overuse of agrochemicals resulted in higher levels of environmental toxicity which in turn had deleterious effects on the surrounding ecosystems (Matson *et al*., 1997; Tilman *et al*., 2002; Aktar *et al*., 2009; Savci, 2012; Bouwman *et al*., 2013; Amaral and Abelho, 2016; Sheahan *et al*., 2017; Mahanty *et al*., 2017; Sellare *et al*., 2020).

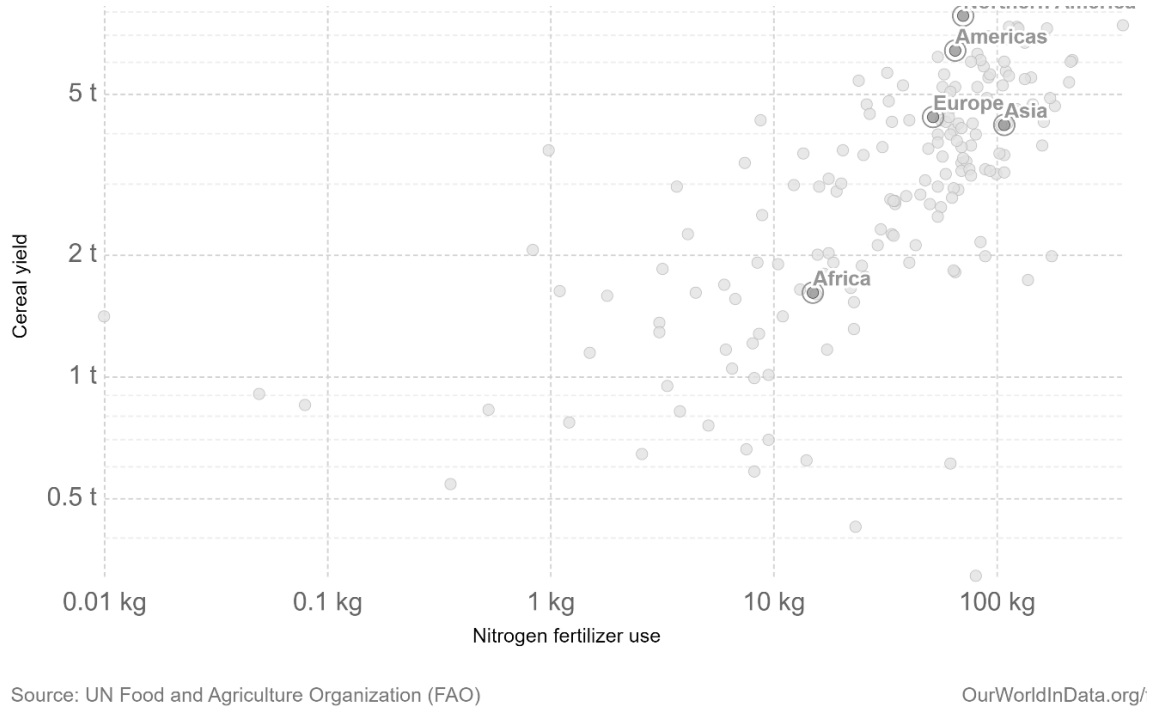


Figure 1. 1. Cereal production rate versus fertilizer use in 2017, cereal yield are measured in tonnes per hectare. Fertilizer use is measured in kilograms of nitrogenous fertilizer applied per hectare of cropland

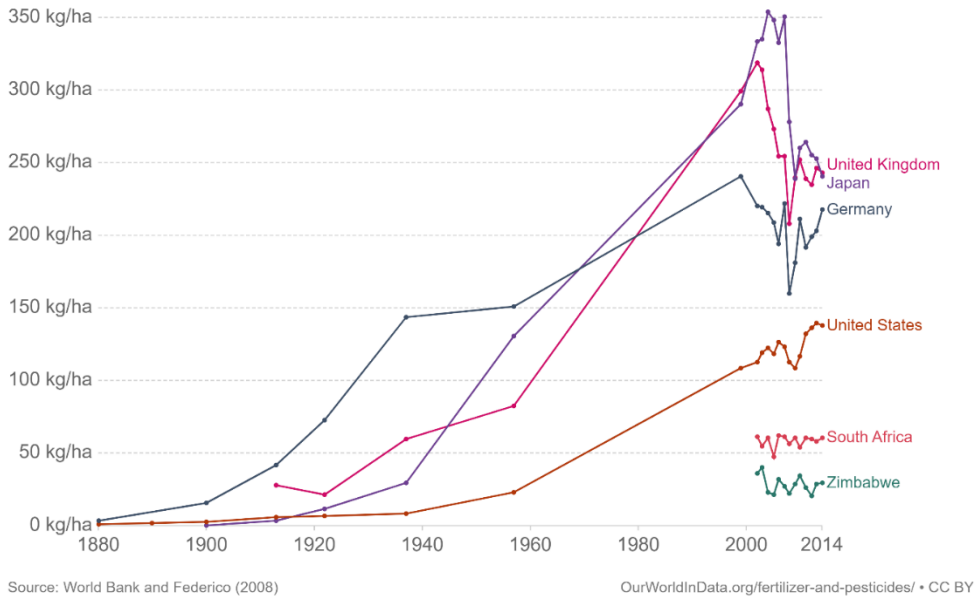


Figure 1. 2. Average fertilizer rates for selected countries over the long-run, measured in kilograms of nutrient per hectare of arable land

Conventional agricultural practice currently receives criticism due to its adverse effect on the environment, especially soil health (Matson *et al*., 1997; Savci, 2012; Mahanty *et al*., 2017). While it is common knowledge that the application of agrochemicals increase yield and reduce pests, the effect on the general microbial community is unknown, but considered to be detrimental. Wickings *et al*. (2016) found that chemical fertilizers have an adverse effect on microbial diversity. However, another study revealed that the application of agrochemicals (glyphosate) increased the number of fungi and actinomycetes while the number of bacteria was reduced (Araújo *et al*., 2003). This is not always good as agrochemicals in conventional farming eliminate certain groups of microorganisms, because the chemicals select for microorganisms by changing their environment (Araújo *et al*., 2003; Kalia and Gosal, 2011; Wickings *et al*., 2016; Lupatini *et al*., 2017). The disadvantages of conventional farming, notably a decrease in microbial diversity, and increasing microbial resistance against pesticides, created the opportunity to adopt alternative ways to improve crop production and disease protection (Araújo *et al*., 2003; Wickings *et al*., 2016).

Conservation agriculture is regarded as a sustainable farming practice since it focuses on producing higher crop yields while avoiding or minimizing adverse environmental effects (Williams, 2002; Erenstein *et al*., 2012; Fiorini *et al*., 2020). In this practice, the use of organic or bio-fertilizers is combined with minimal or no soil tillage. Minimal or no till has been shown to increase microbial diversity and soil organic matter, improve soil health, and maintain optimum moisture content (Gomiero *et al*., 2011; Wang *et al*., 2016; Fiorini *et al*., 2020). Conservation agriculture also involves the use of other crops and livestock to manage soil health and diseases in a production system.

The use of crop and animal management on a single farm is known as integrated crop/livestock farming (Thornton and Herrero, 2001; Hilimire, 2011). There are some advantages that are associated with this method including improved soil quality, enhance yields, controlled pests, and weeds, and improved land-use efficiency (Clark, 2004; Russelle *et al*., 2007; Hilimire, 2011). The combination of various crops or animals will depend on your desired function or product. For example, poultry can be used to control weeds or pests (Clark and Gage, 1996; Tanaka *et al*., 2008) and ruminants can transform forage to manure for fertilizer (Weller and Bowling, 2007). There are also some challenges associated with livestock as they account for around 18% of greenhouse gas emissions (Reynolds *et al*., 2015). However, as compared to extensive agricultural practices, this approach is meant to keep agriculture sustainable, less environmentally detrimental, and produces greater or equivalent yields (Wang *et al*., 2016; Fiorini *et al*., 2020).

The role of microorganisms in agriculture

Microorganisms are crucial components of soil as they contribute to plant health (Khan *et al*., 2009; Lundberg *et al*., 2012; Bulgarelli *et al*., 2013). The use of selected microorganisms in the agricultural sector is increasing (mostly in developed countries) due to their positive attributes such as being eco-friendly and cost-effective compared to agrochemicals (Vaxevanidou *et al*., 2015; Singh *et al*., 2016). In an ideal world, expensive agrochemicals should be replaced by the use of biological fertilizers. Agrochemicals have far reaching damaging effects on the ecosystem, especially on soil composition which eventually impacts soil health as well as in extreme conditions leads to eutrophication (Savci, 2012; Bouwman *et al*., 2013; Mahanty *et al*., 2017).

The beneficial effects of microorganisms include their ability to fix atmospheric nitrogen, decompose organic waste, stimulate plant growth hormones and control soil pathogenic microorganisms (Sabry *et al*., 1997; Reinhold-Hurek and Hurek, 1998; Harman *et al*., 2004; Yadav *et al*., 2009; Kapri and Tewari, 2010; Verma *et al*., 2010; Singh *et al*., 2011; Singh *et al*., 2016; Mahato *et al*., 2018; Kucuk *et al*., 2019). These properties have an important role in regulating the plant growth. Furthermore, some microorganisms confer resistance toward biotic and abiotic factors, and this has been observed from various studies as they reported that the growth of different plants was improved under stress conditions when microorganisms were employed (Shukla *et al*., 2012; Zhao *et al*., 2015; Zhang *et al*., 2016; Habib *et al*., 2016).

The potential of microorganisms to improve plant growth and distribute or solubilize nutrients in agriculture has been widely documented (Kapri and Tewari, 2010; Zhang *et al*., 2016; Mahato *et al*., 2018). The most commonly utilized microorganisms in agriculture are bacteria, fungi, and yeast, although the common applications are more based on the use of fungi and bacteria (Mahanty *et al*., 2017). Bacteria and fungi stimulate the growth of the plant through different mechanisms which will not be discussed in this review (Kapri and Tewari, 2010; Zhang *et al*., 2016; Mahato *et al*., 2018; Setyaningrum *et al*., 2019). Genera that are commonly used in agriculture include *Bacillus* (Navon, 2000; Borriss, 2011), *Rhizobia* (Dardanelli *et al*., 2010; Vargas *et al*., 2017), *Penicillium* (Kucey, 1998; Pradhan and Sukla, 2006), *Aspergillus* (Mwajita *et al*., 2013), and *Trichoderma* (Harman, 2000, Harman *et al*., 2004; Yadav *et al*., 2009).

Trichoderma is one of various microorganisms that have been used in the development of biological fertilizers and biological control agents (Mukherjee *et al*., 2014; Kucuk *et al*., 2019), due to its beneficial functions. However, little research has been done in Africa and Asia on the prevalence of this group in natural or agricultural settings (Yadav *et al*., 2009; Druzhinina *et al*., 2010; Shukla *et al*., 2012; Du Plessis *et al*., 2018; Setyaningrum *et al*., 2019). *Trichoderma* has a cosmopolitan distribution and can be isolated from a number of sources such as soil, decaying trees, and plant tissues (Brotman *et al*., 2013; Jiang *et al*., 2016; Du Plessis *et al*., 2018). A number of studies isolated this genus from rhizospheric soils and plant tissues (endophytes) (Belayneh *et al*., 2010; Contreras-Cornejo *et al*., 2016; Frisvad *et al*., 2018).

Rhizosphere soil has a diverse microbial community and most of the processes occurs in rhizosphere space which enables it to be used for isolation of *Trichoderma* (Kalam *et al*., 2017; Benitez *et al*., 2017). Endophytic *Trichoderma* on the other hand, are also preferable to be isolated due to their useful properties (Strobel, 2003; Kusari *et al*., 2008; Chaverri *et al*., 2015). Rhizospheric and endophytic microorganisms have the ability to colonize the plants roots without causing any diseases (Vázquez *et al*., 2000; Zhang *et al*., 2013), but rather benefit the plant. Furthermore, endophytic microorganisms were found to exhibit various beneficial properties such as the production of antimicrobial compounds and helping host plants develop resistance against biotic and abiotic factors (Kusari *et al*., 2008; Mastouri *et al*., 2012; Singh and Dubey, 2020). Therefore, the exploration of these habitats is essential in order to identify microorganisms that can render valuable properties.

Endophytes

Endophytes are defined as microorganisms that are capable of colonizing the internal parts of plants without causing disease (Schulz and Boyle, 2006; Wallace and May, 2018; Collinge *et al*., 2019). Bacon and White (2000, 2016) also defined endophytes as non-pathogenic fungi or bacteria that occupy the inner parts of plant tissues such as stem, roots, flower, and seeds. Although endophytic organisms were recognized 100 years ago, these organisms were not well studied and little interest was paid to them, until the mid-twentieth century when researchers started to isolate them from the internal part of the plant tissues (Johnson and Whitney, 1992; Pereira *et al*., 1993; Strobel, 2003; 2018). It is a known fact that most endophytes may play a vital role in the agricultural, medical, and biotechnological industries (Strobel, 2003; Kusari *et al*., 2008).

Endophytes have been reported to produce important biological compounds (Kusari *et al*., 2008; Ronsberg *et al*., 2013; Leylaie and Zafari, 2018; Lai *et al*., 2020; Singh and Dubey, 2020). These biological compounds are able to exhibit different functions such as antimicrobial activity and inducing resistance against biotic and abiotic stresses (Kusari *et al*., 2008; Suryanarayanan, 2013; Singh and Dubey, 2020; Khalil *et al*., 2021). For instance, the production of hypercirin by endophytic fungi has antibiotic and antiviral activity (Kusari *et al*. 2008). However, Ronsberg *et al*. (2013) and Khalil *et al*. (2021) reported that some of the compounds produced by endophytes had no effect against tested pathogens. Therefore, it is important to further assess the compounds from endophytes before developing these as biopesticides.

Rhizosphere

The rhizosphere is a part of soil that is near the roots of plant (Curl and Truelove, 2012). The rhizosphere acts as a habitat for a large number of living organisms, including microorganisms, and insects (McNear Jr, 2013; Benitez *et al*., 2017). Many of the soil processes take place in this part of the plant (Bakker *et al*., 2013; McNear Jr, 2013). Despite a large diversity of microorganisms in the rhizosphere, the ones that are of interest to the Agri-industry are those that exhibit beneficial characteristics (Mukherjee *et al*., 2012; Collinge *et al*., 2019).

Beneficial microorganisms are essential for plant health (Mukherjee *et al*., 2012; Van Dam and Bouwmeester, 2016; Mahato *et al*., 2018; He *et al*., 2019). These microorganisms employ different mechanisms to achieve this such as mycoparasitism, competition, and antibiosis (Contreras-Cornejo *et al*., 2016; Habib *et al*., 2016; Venturi and Keel, 2016; Xiang *et al*., 2017; Benitez *et al*., 2017; Yan *et al*., 2017; He *et al*., 2019). These mechanisms are mainly based on competition for nutrients and the production of antimicrobial compounds and are the main mechanisms employed by *Trichoderma* to control pathogens (Vinale *et al*., 2008; Verma *et al*., 2007; John *et al*., 2010; Qualhato *et al*., 2013).

Trichoderma interaction with pathogenic microorganisms

Competition for nutrients and space, antibiosis, and mycoparasitism are the most typical methods of interaction between pathogenic microbes and *Trichoderma* spp. (Whipps and Davies, 2000; Whipps, 2001; Harman *et al*., 2004; Qualhato *et al*., 2013; Latz *et al*., 2018). Competition for nutrients and space can be easily observed on agar plates (confrontational assays) where *Trichoderma* spp. and pathogenic microorganisms compete nutrients such as carbon and nitrogen (Whipps, 2001; Contreras- Cornejo *et al*. 2016; Jiang *et al*., 2016). A number of *Trichoderma* spp. has the capacity to outgrow plant pathogens that are usually associated with causing diseases to commercial crops (John *et al*., 2010; Jiang *et al*., 2016).

Antibiosis is the secretion of antimicrobial compounds by microorganisms to inhibit or destroy other microorganisms in the region of their growth area (Verma *et al*. 2007). During antibiosis, antimicrobial proteins and various other metabolites are produced to hinder the growth of competing species (Whipps, 2001; Latz *et al*., 2018). For instance, metabolites produced by *Trichoderma* species were able to reduce the gray mold caused by *Botrytis cinerea* on tomato (Vinale *et al*., 2008). In addition, Katoch *et al*. (2019) found that metabolites (*Tribacopin* AV) produced by endophytic *Trichoderma* species have an antifungal activity but not antibacterial activity.

Mycoparasitism is defined as the killing of one fungus by another (Ridout *et al*., 1988; Whipps and Davies, 2000; Mukherjee *et al*., 2012; Qualhato *et al*., 2013). *Trichoderma* spp. are known for their ability to parasitize other fungi, although their mycoparasitism is not easy to demonstrate in *situ* (Verma *et al*., 2007). The mycoparasitism of *Trichoderma* spp. is known to be initiated or induced by the production of hydrolytic enzymes (Ridout *et al*., 1988; Antal *et al*., 2000; Harman *et al*., 2004; Qualhato *et al*., 2013; Latz *et al*., 2018). All of these mechanisms are of importance when it comes to evaluating biological control abilities of any strain.

Trichoderma overview

The genus *Trichoderma* (*Hypocrea*) is commonly found in soil and on decaying trees and are known to produce important industrial enzymes (Zachow *et al*., 2009; Belayneh Mulaw *et al*., 2010; Blaszczyk *et al*., 2011; Jaklitsch and Voglmayr, 2015). In addition, *Trichoderma* spp. are used as biological control agents and biological fertilizers (Gams and Bissett, 1998; Druzhinina and Kubicek, 2005; Brotman *et al*., 2013; Sandle, 2014; Jaklitsch and Voglmayr, 2015; Contreras-Cornejo *et al*., 2016). Some *Trichoderma* spp. are opportunistic pathogens in mammals, including humans with compromised immune systems (Druzhinina *et al*., 2006; Sandle, 2014; Recio *et al*., 2019). However, the use of *Trichoderma* spp. could be beneficial to improve the growth of crops (Saravanakumar *et al*., 2013; Chagas *et al*., 2016; Contreras-Cornejo *et al*., 2016; Khoshmanzar *et al*., 2020).

The morphological characteristics of *Trichoderma* spp. are studied on various culture media namely, potato dextrose agar (PDA), cornmeal dextrose agar (CMD), and malt extract agar (MEA). Colony characteristics that are normally seen on these culture media are greenish, bluish, black, or greyish colonies. Occasionally white to yellow mycelium-like structures are observed. Under the microscope, strains are usually seen as small, green, or white conidia, in the presence of phialides (lageniform to ampuliform) on profusely or slightly branched conidiophores (Hassan *et al*., 2014; Hassan *et al*., 2019). However, some *Trichoderma* species are so similar that morphological identification alone is not enough to differentiate between them (Samuels *et al*., 2010; Jaklitsch *et al*., 2013; Du Plessis *et al*., 2018).

Various traditional methods have been used to identify and distinguish between different *Trichoderma* species. Most of these methods were based on macroscopic and microscopic characters as well as the secondary metabolites they produce (Degenkolb *et al*., 2008; Frisvad *et al*., 2008). However, none of these methods can differentiate between closely related species as they are morphologically similar. In addition, the production of secondary metabolites by *Trichoderma* are strain specific and not necessarily species specific (Horinouchi, 2007; Frisvad *et al*., 2008; Samuels and Ismaiel, 2009; Samuels *et al*., 2010).

Trichoderma spp. are known to produce various types of secondary metabolites, such as terpenoids, pyrones, indolic derived compounds, siderophores, and enzymes (Mukherjee *et al*., 2013; Contreras-Cornejo *et al*., 2016; Frisvad *et al*., 2018). These secondary metabolites can be seen as facilitators of chemical communication in soil communities (Contreras-Cornejo *et al*., 2016). Furthermore, some secondary metabolites play an important role in initiating the mechanisms that lead to the improvement of plant growth, tolerance to biotic and abiotic stresses and fighting against pathogenic fungi (Waghunde *et al*., 2016; Kashyap *et al*., 2017; Sharma *et al*., 2019).

Trichoderma spp. have been found to be resistant to various types of toxins and xenobiotic compounds (Harman *et al*., 2004; Oros *et al*., 2011; Cocaign *et al*., 2013). These can be any harmful compounds including chemical fungicides, and pesticide residues. *Trichoderma* species tend to vary in terms of tolerance to these compounds (Oros *et al*., 2011). This was observed when alkanols were tested against *Trichoderma* spp. which showed the tolerance efficiency to be different from species to species (Oros *et al*., 2011). In addition, *Trichoderma* species (*T. virens* and *T. reesei*) shown resistance towards highly toxic pesticide residue such as 3, 4-dicloroaniline (Cocaign *et al*., 2013).

Trichoderma is also known for colonizing the inner parts of plant tissues (Harman, 2006; Bae *et al*., 2011). Colonization of plant tissues by *Trichoderma* can alter the metabolic processes of plants and also regulate the metabolism (Contreras-Cornejo *et al*., 2016). This can result in the production or secretion of hormones or compounds that are important in triggering the growth of plants, and absorption of nutrients and minerals (Harman *et al*., 2004). *Trichoderma* spp. that has the ability to inhabit the internal parts of plant tissues are considered to be endophytes.

Understanding the diversity of *Trichoderma* is important because of its variety of uses and application (Herman *et al*., 2004; Cummings *et al*., 2016). However, our understanding of *Trichoderma* diversity in Africa is still inadequate (Druzhinina *et al*., 2006; Du Plessis *et al*., 2018). A recent study revealed that the majority of the known *Trichoderma* spp. were only recently detected in South Africa (Du Plessis *et al*., 2018). Exploring this genus in previously unexplored regions may thus aid in the identification of native and some novel species.

Taxonomic history of *Trichoderma* spp.

The name *Trichoderma* is derived from a Latin and Greek word *Tricho* meaning 'hair', and *derma* meaning 'skin' in Latin. In 1794, Persoon described four species, *T. viride*, *T. aureum*, *T. roseum*, and *T. nigrescens* (Bisby, 1939; Samuels, 1996; Druzhinina *et al*., 2006; Jaklitsch, 2009; Zeng and Zhuang, 2019). Three of the species (i.e. *T. aureum*, *T. roseum*, and *T. nigrescens*) were later found to be unrelated to *Trichoderma* which resulted in their exclusion from the genus (Bissett, 1984, 1991; Mukherjee *et al*., 2013). Rafai (1969) identified nine aggregate species and suggested that each aggregate species might consist of different sub-species if new methods for identification become available. Later, an in-depth revision of aggregate species was done, and aggregates species were grouped into four sections representing 27 species (Bissett, 1984, 1991).

The introduction of molecular methods in the 19th century enabled researchers to identify and describe *Trichoderma* species more precisely. Kindermann *et al*. (1998) and Dodd *et al*. (2000) introduced the use of molecular methods to describe *Trichoderma* species. This method was initially based on the internal transcribed spacer regions (ITS sequences) (Kindermann *et al*., 1998; Dodd *et al*., 2000), although later the ITS regions was shown to provide low resolution for the identification of *Trichoderma* species (Kuhls *et al*., 1997; Jaklitsch *et al*., 2006; Hatvani *et al*., 2007; Jaklitsch, 2009).

The identification of *Trichoderma* species was improved using protein coding genes which were employed to increase the robustness of the phylogenetic analysis (Lieckfeldt *et al*., 1998). These include the translation elongation factor 1-alpha (*tef1*) (Hermosa *et al*., 2004; Lu *et al*., 2004; Overton *et al*., 2006), endochitinase (*chi18-5 or ech42*), RNA polymerase II (*rpb2*) (Jaklitsch and Voglmayr, 2015), actin (*act*), and calmodulin (*cal1*) (Lieckfeldt *et al*., 1998) gene regions. Moreover, these gene regions were found to be more reliable compared to the ITS gene region (Overton *et al*., 2006; Samuels, 2006; Jaklitsch and Voglmayr, 2015). Among them, *tef1* was considered to provide accurate identification, and provides the highest resolution in the different clades (Samuels, 2006; Jaklitsch *et al*., 2006; Jaklitsch and Voglmayr, 2015).

There are a number of databases that are commonly used for accurate taxonomically identification of *Trichoderma* spp. The National Center for Biotechnology Information (NCBI) database is normally used to compare sequences via blastn search. However, the other databases such as International Commission on *Trichoderma* Taxonomy (ICTT) (https://trichoderma.info/trichoderma-taxonomy-2020/), Multilocus Identification System for *Trichoderma* (MIST) (http://mmit.china-cctc.org/), and Trichokey (https://www.trichokey.com/index.php) should be prioritized as they give more reliable results (Duo *et al*., 2020; Cai and Druzhinina, 2021). Therefore, using these databases in conjunction with NCBI database could potentially yield accurate results if used properly.

To date 375 species have been recognized in *Trichoderma* (Zeng and Zhuang, 2019; Cai and Druzhinina, 2021). The limited diversity studies of *Trichoderma* in other countries bring hope that more species could be identified (Druzhinina *et al*., 2006; Du Plessis *et al*., 2018). Thus, the accurate identification of this genus is important due to its applications and ecological importance.

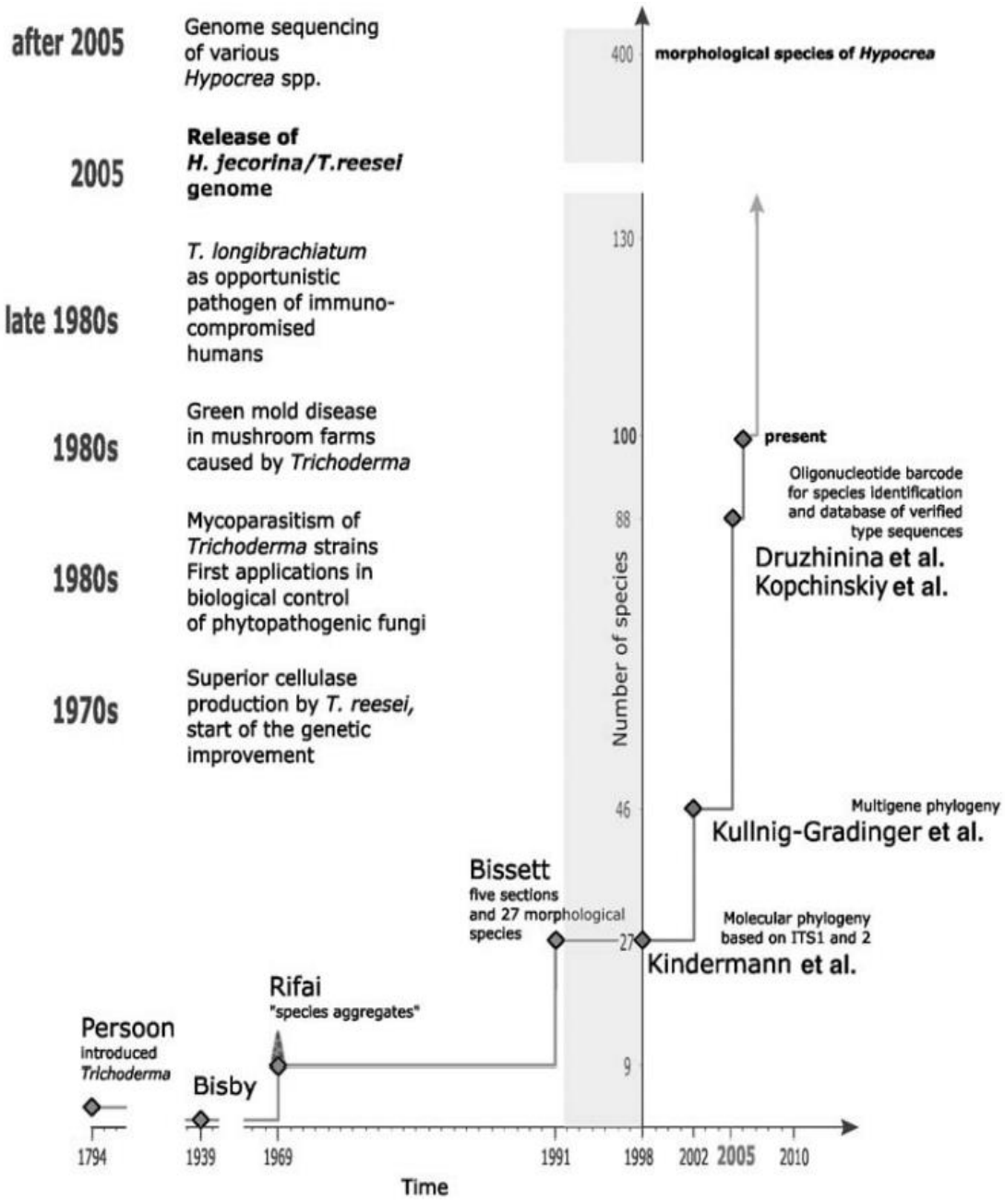


Figure 1. 3. Diagrammatic representation of taxonomic history of *Trichoderma* species (Re-printed from Druzhinina *et al*., 2006)

Characteristics of *Trichoderma* spp. colonies:

Macroscopic characterization

Morphological characterization of *Trichoderma* species has been based on various types of growth media such as Oats Agar (OA), Cornmeal Dextrose Agar (CMD), Synthetic Nutrient poor Agar (SNA), Potato Dextrose Agar (PDA), and Malt Extract Agar (MEA) (Gams and Bissett, 1998; Jaklitsch, 2009; Hassan *et al*., 2019). SNA and CMD usually produce morphological characters that are commonly found in natural environments (Samuels, 2004). The conidium production is more abundant on CMD than on SNA. Colony morphology should be studied early during the development, in order to visualize conidiophores and phialides. The ideal temperature for *Trichoderma* spp. growth is 25℃. Several morphological traits can be observed to identify *Trichoderma* species according to their colony morphology (Jaklitsh, 2009).

Colony growth rate: This should be prioritized when investigating *Trichoderma* strains based on their colony morphology (Kim *et al*., 2012). This is because growth rates of colonies may differ due to various conditions ranging from media and temperature to incubation time. The colony radius is expected to be measured daily for seven days (Jaklitsch, 2009). The majority of *Trichoderma* strains have a rapid growth rate and can fill an entire 90 mm Petri dish in less than a week under optimum conditions.

Conidium formation: Conidia of *Trichoderma* species are different from each other in terms of texture. The texture of conidia depends on type of media used. Moreover, structures or textures that may be obtained from colonies are as follows (Jaklitsch, 2009):

- Fertile pustule formation : distinct dense opaque conidiation structures.
- Tufts: are also called ‘fluffy tufts’ appear as loose cotton like structures.
- Granules or shrubs: grow on the agar like sand texture (Fig. 1.4 H and I).
- Effuse conidia formation: conidia form on the surface layer of mycelium that develops out of the substrate and no pustules are formed.
- Pustule and effuse conidia formation: they simultaneously produce conidia effuse and form pustules.

Colony odors: a coconut-like odor on CMD and PDA can be observed in some species such as *T. atroviride, T. asperellum, T. gamsii, T. viride, T. afroharzianum, T. camerunense,* however, this can be

impractical for the identification of species since not all strains exhibit this trait (Jaklitsch, 2009; Chaverri *et al*., 2015).

Conidium color: *Trichoderma* species normally are characterized by greyish green to dark green conidia on CMD and SNA, while other species form yellow conidia on media such as PDA and MEA (Samuels, 1996; Du Plessis *et al*., 2018).

Reverse coloration: The majority of *Trichoderma* spp. usually does not form any color on the reverse of CMD or SNA, while PDA can reveal the brown or yellow reverse pigmentation (Fig. 1.4. F-G).

Zonation: Some *Trichoderma* spp. can be stimulated by light to sporulate (Betina and Farkas, 1998). *Trichoderma* colonies are sporulating more in the light conditions than those in the dark (Du Plessis, 2018). Thus, colonies appearance and texture can be affected (Kim *et al*., 2012).

Exudates: this forms at the end of hyphae tips whereby exudates are produced in different colors ranging from hyaline, green, or brownish yellow.

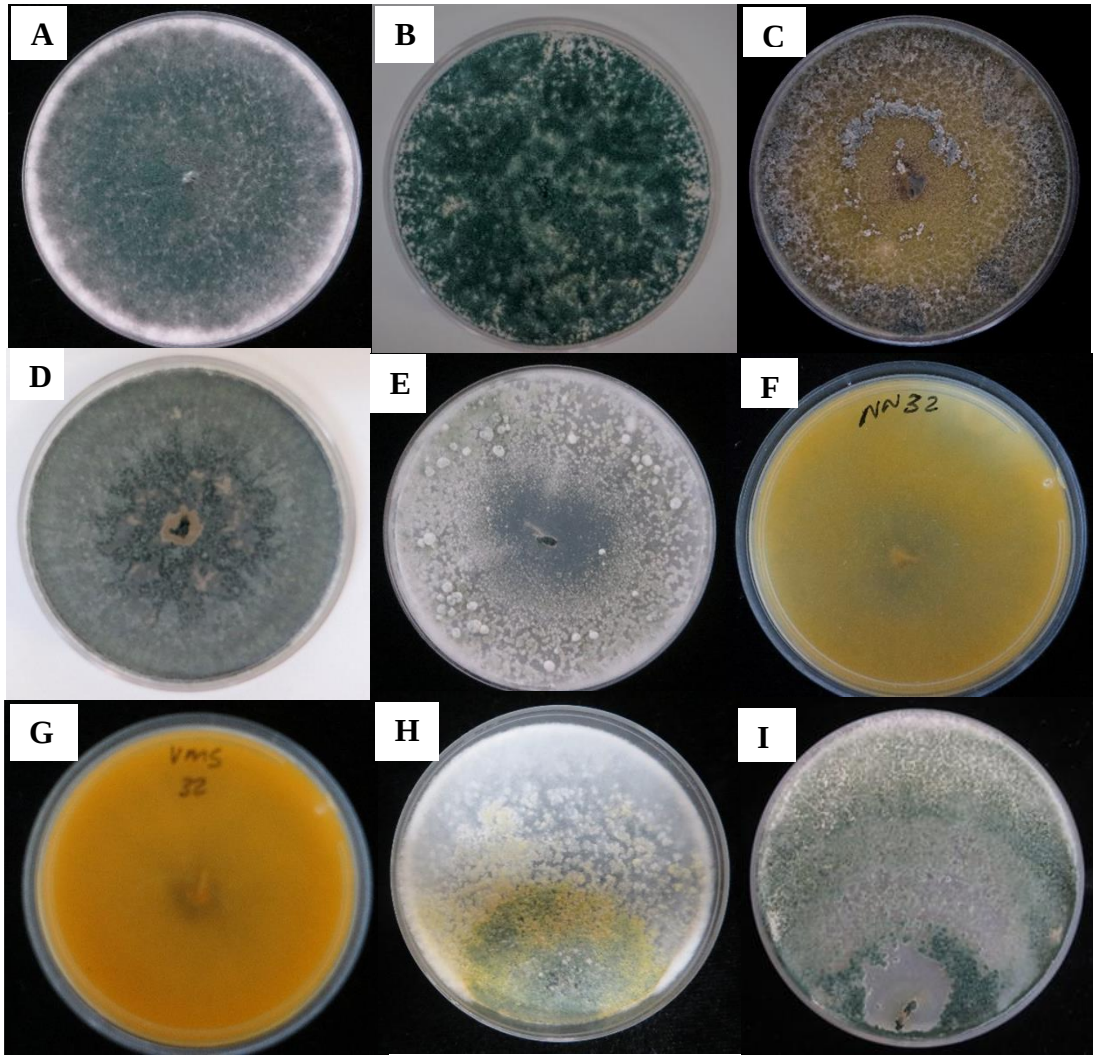


Figure 1. 4. Illustrations of macroscopic features of *Trichoderma* spp. **A**. *T. neokoningii* (PDA), **B**. *T. asperellum* (PDA), **C**. *T. peberdyi* (PDA), **D**. *T. saturnisporum* (PDA), **E**. *T. koningiopsis* (PDA), **F**. *T. afroharzianum* (Reverse view on PDA), **G**. *T. spirale* (Reverse view on PDA), **H**. *T. gamsii* (CMD), **I**. *T. rifaii* (CMD)

Microscopic characterization

Microscopic characterization of *Trichoderma* strains is normally performed using colonies grown on media such as PDA, CMD or SNA, mounted using a 3% KOH solution or lactic acid (Jaklitsch, 2009; Samuels *et al*., 2012). Slides prepared using 3% KOH may results in brown conidia, on the other hand with lactic acid conidia will be blue, green, or hyaline. Slides should not be prepared from old or very mature cultures, since it will be difficult to view complete structures. Relevant microscopic structures of *Trichoderma* spp. are as follows:

Type of phialide: *Trichoderma* spp. usually form lageniform, flask-shaped, lanceolate, or subulate phialides which are curved or slender. Phialides can arise in whorls or occur solitary.

Regular or irregular conidiophores. Regular conidiophores can be tree or pyramid-like with many branches at the base and less branching on the top while an irregular conidiophore is recognized by showing unpaired branching from the stipe.

Conidium shape: Conidia (Fig. 1.5 J-L) can be ellipsoidal, subglubose, globose, oblong, or oval-shaped conidia.

Chlamydospores: Chlamydospores can be absent or present in *Trichoderma* spp. Noticeable traits of chlamydospores are globose to pyriform, smooth, or rough, thick-walled. These structures are normally seen in older cultures (from 10 days upwards) especially when using CMD medium.

Conidiophore hyphal elongations: Commonly observed in *Pachybasium* clade, and hyphal elongations form at the terminal of conidiophores. Hyphal elongations can be sterile or fertile (Bissett, 1991).

Conidiophore type: *Trichoderma* spp. develop various types of conidiophores which can be used to classify species:

- *Acremonium*-like: Basic conidiophores, characterized by one or few phialides which originate directly from stalk-like support.
- *Verticillium*-like: Unbranched or scarcely branched conidiophores and whorls of phialides originate on the same level.
- *Trichoderma*-like: Conidiophores are elastic and branched which may be irregular at right angles. Phialides are lageniform, bent, sometimes repetitive.
- *Pachybasium*-like: classified by having more thick branches and flask-like phialides. Conidiophore commonly ending in simple or branched, sterile, or fertile elongations.

- *Gliocladium*-like: Unbranched conidiophores, with penicillin-like structure having more or less parallel phialides at the apex.

Intercalary phialides: It is described by phialides that resembles the formation of nodes-like (septum-like), as seen in *Longibrachiatum* clade.

Conidium ornamentation: *Trichoderma* conidia can display various ornamentations and appear as warted, smooth, partially roughened, or roughened. Ornamentations can be easily observed from mature conidia (Fig. 1.5 A).

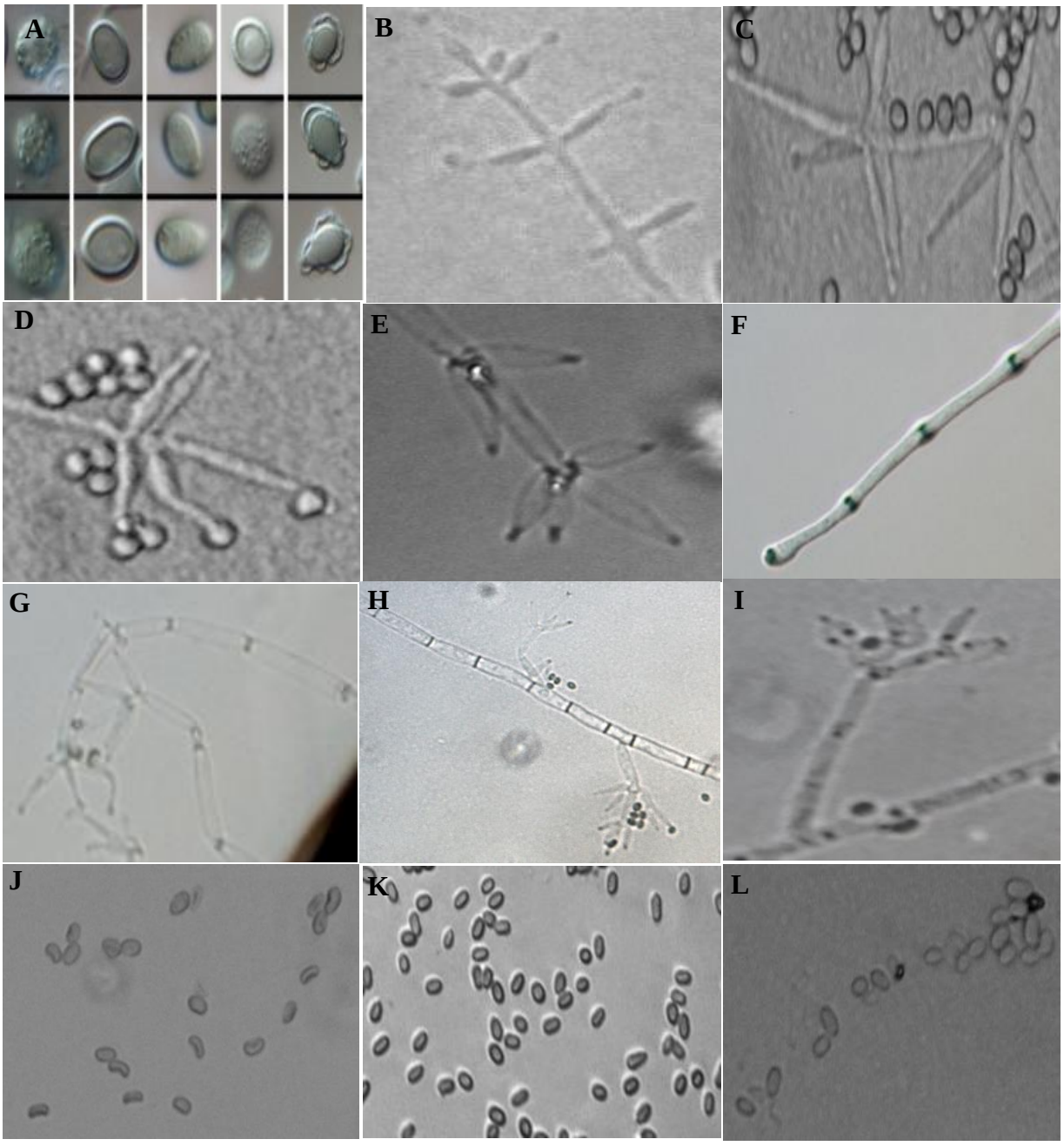


Figure 1. 5. Illustrations of microscopic features of *Trichoderma* spp.: **A.** Conidium ornamentations (Re-printed from Du Plessis, 2015), **B-E.** Phialides, **F-I.** Conidiophores**, J-L.** Conidia

Application of *Trichoderma* spp.

The application of *Trichoderma* spp. is growing as this genus have various interesting industrial and agricultural applications (Mukherjee *et al*., 2014; Bischof *et al*., 2016; Mahato *et al*., 2018).

Industrial application

Trichoderma species have been used for various industrial purposes such as the production of enzymes, antimicrobial compounds, and bioremediation agents (Wen *et al*., 2005; Saravanakumar and Kathiresan, 2014; Borin *et al*., 2015; Yao *et al*., 2015; Carvalho *et al*., 2017; El Aty *et al*., 2018). This genus is known for producing important enzymes such as cellulases and other hydrolytic enzymes (Lorito *et al*., 1993; Wen *et al*., 2005; Carvalho *et al*., 2017). Enzymes have various roles that they play, for instance one of the xylanases produced by *T. stromaticum* have the ability to improve softness and volume of wheat and grain breads (Carvalho *et al*., 2017).

Trichoderma species and other genera were investigated for their ability to produce xylanases on different substrates such as wheat bran, rice straw, peach palm and potato peels (Mander *et al*., 2014; Mostafa *et al*., 2014; Carvalho *et al*., 2017; El Aty *et al*., 2018). It was shown that almost all *Trichoderma* spp. produce higher xylanases on the above-mentioned substrates when compared to other genera, although this is also strain specific. This was proven when other genera were investigated for their ability to produce xylanase, and they produced it at an optimum level when wheat bran was used as a substrate (Mander *et al*., 2014; Mostafa *et al*., 2014). Thus, the enzymes production will depend on the type of substrate used for them to be produced at an optimum level.

Trichoderma also produce enzymes that are important in dye removal (Saravanakumar and Kathresan, 2014). Dyes from industries have an adverse impact in the environment especially when discharged into the water sources. Various methods are being used to remove dyes or to decrease their toxicity before discharging them into the environment (Garg *et al*., 2004; Anjaneyulu *et al*., 2005; Akar *et al*., 2013; Chew and Ting, 2016). Among these approaches the use of *Trichoderma* is a promising method since it has been reported to degrade malachite green dye by producing laccase (Saravanakumar and Kathresan, 2014).

Agricultural application

Fungicides are widely used in agriculture to protect crops from pathogenic fungi (Mutawila *et al*., 2016). Fungicides are inorganic in nature and their continuous and long-term use in agriculture has had led to the production of toxic residues which ultimately had an adverse effect on the surrounding environments (Matson *et al*., 1997; Elshahawy *et al*., 2017). Adverse effects include the development of resistance against fungicides, and health issues in mammals (Avenot and Michailides, 2007; Miles *et al*., 2014; Lucas *et al*., 2015). Therefore, biological control agents could be an alternative approach in plant protection (Mukherjee *et al*., 2014; Elshahawy *et al*., 2017).

Trichoderma spp. have been extensively utilized as biological control agents since 1920 (Samuels, 1996; Waghunde *et al*., 2016; Mutawila *et al*., 2016; Morales-Rodriguez *et al*., 2018) against a variety of plant pathogens (Shalaby *et al*., 2013; Abo-Elyousr *et al*., 2014; Elshahawy *et al*., 2017). For example, *Trichoderma* strains were able to suppress *Sclerotium cepivorum* which causes onion white rot disease (Shalaby *et al*., 2013; Elshahawy *et al*., 2017), as well as *Alternaria porri* responsible for onion purple blotch disease (Abo-Elyousr *et al*., 2014). Moreover, some studies have shown the potential of *Trichoderma* strains to control other pathogens namely, *Sclerotium delphinni* (Mukherjee *et al*., 2014), *Fusarium* head blight (Sarrocco *et al*., 2011; Matarese *et al*., 2012; Sarrocco *et al*., 2013), *Rhizoctonia solani*, and *Sclerotium oryzae* (Swain *et al*., 2018).

Various microorganisms such as bacteria, fungi, and actinomycetes play an important role in enhancing plant growth (Shoresh and Herman, 2008; Kapri and Tewari, 2010; Doni *et al*., 2014). Microorganisms could improve plant growth by solubilizing phosphate, and producing phytohormones (e.g., indole acetic acid) (Sabry *et al*., 1997; Harman, 2000; Yedidia *et al*., 2001; Kapri and Tewari, 2010; Saravanakumar *et al*., 2013; Zhao and Zhang, 2015; Li *et al*., 2015). *Trichoderma* is one of the genera that is known to improve plant growth through solubilizing phosphate and the production of phytohormones (Kapri and Tewari, 2010; Saravanakumar *et al*., 2013; Saber *et al*., 2017; Bononi *et al*., 2020).

Phosphorus is important for plant growth (Richardson, 2000; Maguire *et al*., 2005; Saravanakumar *et al*., 2013). Phosphorus in the soil can be in the fixed form of either $Ca_3(PO_4)_2$ and $FePO_4$ or $AlPO_4$ in alkaline and acidic soil, respectively (Grant *et al*., 2001; Kapri and Tewari, 2010). Plants may not get the required amount of phosphorus for their growth even though it is abundant in the soil, as its insoluble nature makes it inaccessible to plants (Grant *et al*., 2001; Kudoyarova *et al*., 2017; Alori *et* al., 2017). However, microorganisms can make it available for plant uptake through various mechanisms (Jones and Oburger, 2011; Saravanakumar *et al*., 2013; Bader *et al*., 2020). There are two mechanisms predominantly known

for making phosphate available for uptake by plants such as the (1) dissolution of phosphate containing minerals through a combination of soil acidification and the production of organic acids, or (2) an enzymatic reaction by phosphatases (Cunningham and Kuiack, 1992; Jones and Oburger, 2011). The first mechanism occurs mostly in phosphate limiting environments since it helps in solubilizing and mobilizing insoluble mineral-bound phosphates, while the second mechanism is normally used in controlling phosphate in natural environments (Jones and Oburger, 2011). Fungi are considered to be better at solubilizing insoluble phosphate than bacteria (Kucey, 1983; Turan *et al*., 2006; Rajankar *et al*., 2007; Gupta *et al*., 2007; Sembiring, 2017). However, some studies have shown that bacteria were able to solubilize phosphate more effectively compared to fungi (Alam *et al*., 2002; Mwajita *et al*., 2013; Hussein and Joo, 2015; Zhang *et al*., 2020). These different findings could be due to different methods used to screen phosphate, or the strains that were used in an experiment.

Trichoderma strains were evaluated in many studies for their ability to enhance various plant growth through solubilizing phosphate (Rajankar *et al*., 2007; Saravanakumar *et al*., 2013; Chagas *et al*., 2016; Khoshmanzar *et al*., 2020; Mendes *et al*., 2020; Kribel *et al*., 2020). For instance, soybean plants were enhanced by *Trichoderma* spp. that have the ability to solubilize phosphate, showing an increase in plant height and roots in treated plants compared to untreated ones (Bononi *et al*., 2020). The solubilization of phosphate by *Trichoderma* has been associated with the production of organic acids and enzymes, as well as a reduction in pH (Saravanakumar *et al*., 2013; Zuniga-Silgado *et al*., 2020; Tandon *et al*., 2020; Bononi *et al*., 2020), although, some studies did not observe any production of organic acids and reduction in pH when solubilization of phosphate were assessed (Altomare *et al*., 1999; Rudresh *et al*., 2005; Chagas *et al*., 2016). In addition to *Trichoderma* spp. being phosphate solubilizers, this genus also can produce indole acetic acid (IAA) which is regarded as another metabolic factor that helps in the plant growth (Gravel *et al*., 2007; Hussein and Joo, 2015; Herrera-Jaminez *et al*., 2018; Bader *et al*., 2020; Mendes *et al*., 2020).

Indole acetic acid (IAA) is an auxin that contributes to root hair development which then results in the efficient use of nutrients (Gravel *et al*., 2007; Saber *et al*., 2017; Herrera-Jaminez *et al*., 2018). Previous studies have shown that *Trichoderma* species have the potential of producing indole acetic acid (Gravel *et al*., 2007; Hussein and Joo, 2015; Herrera-Jaminez *et al*., 2018; Bader *et al*., 2020; Mendes *et al*., 2020). The production of indole acetic acid resulted in the growth improvement of different plants (Kotasthane *et al*., 2015; Chagas *et al*., 2016; Khoshmanzar *et al*., 2020). However, some studies indicated that the production of IAA by *Trichoderma* did not have a positive correlation with the growth improvement (Hoyo-Carvajal *et al*., 2009; Kotasthane *et al*., 2015; Nieto-Jacobo *et al*., 2017). This could

be due to the fact that *Trichoderma* properties are strain specific (Hoyo-Carvajal *et al*., 2009; Kotasthane *et al*., 2015). Also, the methods of evaluating strains that can produce IAA could be another contributing factor since quantitative and qualitative assays might not give the same results. Despite the fact that in some cases IAA production by *Trichoderma* strains has an adverse relationship with plant growth, it is however considered a required assay to determine if a given *Trichoderma* strain has the ability to increase plant development.

Trichoderma spp. can tolerate stressful conditions by producing a variety of compounds (defense related enzymes) (Chandra *et al*., 2004; Mastouri *et al*., 2012; Shukla *et al*., 2012; Zhang *et al*., 2014). Among these stressful conditions, abiotic and biotic factors are sometimes the limiting factors in agriculture as they may lower production yield of crops. *Trichoderma* could potentially play an essential role in helping plants to confer resistance to biotic and abiotic stresses (Bjorkman *et al*., 1998; Mastouri *et al*., 2010; Shukla *et al*., 2012). Various studies have been reported that *Trichoderma* species proved to be beneficial to plant growth even when stress conditions were experienced (Mastouri *et al*., 2010; Shukla *et al*., 2012; Zhang *et al*., 2014). This was due to the increased levels of stress related proteins produced by *Trichoderma*. For example, the seeds treated with *Trichoderma* strains resulted in the improved growth of tomato roots and shoots compared to untreated seeds (Mastouri *et al*., 2012).

Trichoderma species have been used to improve growth of different crops including maize and wheat (Mastouri *et al*., 2012; Saravanakumar *et al*., 2013; Zhang *et al*., 2014; Mahato *et al*., 2018; Nepali *et al*., 2020). Various studies have indicated the capabilities of different strains of *Trichoderma* to increase the maize and wheat growth parameters such as roots, stem, and yield (Saravanakumar *et al*., 2017; Mahato *et al*., 2018; Nepali *et al*., 2020). These findings are an indication that *Trichoderma* spp. can be successfully used as a biofertilizer to minimize the use of synthetic fertilizers. Therefore, *Trichoderma* spp. could be used for multifunctional purposes in agricultural fields.

Conclusion

Maize and wheat in South African economy have huge impact because most people and animals depend on these crops as a primary source of food. The cultivation of these crops in South Africa is commonly based in conventional practices rather than conservation practices. This is because intensive agricultural practices (conventional) have been known to produce higher yields which is required for an increasing population. However, in this century the conventional method for cultivating crops needs to be adapted to mitigate their negative impact on the environment, and particularly on soil health. Conservation agricultural practices could potentially deliver in this goal, ensuring optimum yield is obtained while minimizing negative impacts in the surrounding ecosystems. One element of conservation agriculture is the use of microorganisms as biofertilizer or biological control agents. The genus *Trichoderma* has been widely explored for its protection against pathogens and potential to improve plant growth. *Trichoderma* improves plant growth via the solubilization of phosphate and the production of phytohormones (Indole acetic acid). It has been noted that majority of *Trichoderma* strains that solubilize phosphate and produce IAA have been reported to be the good candidates for development of bio-stimulants. The diversity of *Trichoderma* species have been widely documented, however in South Africa there is still a gap in terms of isolating and identifying this genus. This was highlighted by a recent study that was done in South Africa (Du Plessis *et al*., 2018). The identification of this genus in agricultural soils could potentially yield native strains that could enhance plant growth via production of phytohormones and solubilization of phosphate.

Research questions

- Does agricultural soil from Western Cape, KwaZulu-Natal and Free State provinces in South Africa differ in terms of *Trichoderma* species distribution?
- Does *Trichoderma* strains from wheat and maize rhizosphere soil have the potential to solubilize phosphate and produce indole acetic acid?

Research Aims

- To isolate and identify naturally occurring *Trichoderma* spp. in the rhizosphere of maize and wheat under conventional and conservation agricultural practices
- To evaluate the ability of *Trichoderma* species to exhibit plant growth properties such as, production of auxins (indole acetic acid) and nutrients acquisition (solubilization of phosphate)

Research Objectives

- To isolate and identify *Trichoderma* spp. from rhizosphere soil of maize and wheat.
- To investigate the impact of farming practices on the presence of different *Trichoderma* spp.
- To identify *Trichoderma* strains that can solubilize phosphate and produce indole acetic acid.

Significance of the research

Limited research has been done on *Trichoderma* species isolated in South Africa, thus this research can strengthen the existing knowledge of this genus in SA. Furthermore, this research also hopes to identify *Trichoderma* species that can solubilize phosphate and produce indole acetic acid that could be researched further to understand their effectiveness in natural soils. These results could increase the pool of species that used in the development of bio-fertilizers, as literature indicates that these strains have the potential to improve crop development. In addition, these indigenous or local *Trichoderma* strains could have a better chance of being commercialized since they have been accustomed to the South African environment.

Literature cited

Abassian, A., 2006. Maize: International market profile. Background paper for the competitive agriculture in sub-Saharan African, economic and social department, trade and markets, division, Food and Agriculture Organisation of the United Nations. Washington, p. 25.

Abd El Aty, A.A., Saleh, S.A., Eid, B.M., Ibrahim, N.A. and Mostafa, F.A., 2018. Thermodynamics characterization and potential textile applications of *Trichoderma longibrachiatum* KT693225 xylanase. *Biocatalysis and Agricultural Biotechnology*, *14*, pp.129-137.

Abo-Elyousr, K.A., Abdel-Hafez, S.I. and Abdel-Rahim, I.R., 2014. Isolation of *Trichoderma* and evaluation of their antagonistic potential against *Alternaria porri*. *Journal of Phytopathology*, *162*(9), pp.567-574.

Akar, T., Ozkara, E., Celik, S., Turkyilmaz, S. and Akar, S.T., 2013. Chemical modification of a plant origin biomass using cationic surfactant ABDAC and the biosorptive decolorization of RR45 containing solutions. *Colloids and Surfaces B: Biointerfaces*, *101*, pp.307-314.

Aktar, W., Sengupta, D. and Chowdhury, A., 2009. Impact of pesticides use in agriculture: their benefits and hazards. *Interdisciplinary toxicology*, *2*(1), pp.1-12.

Alam, S., Khalil, S., Ayub, N. and Rashid, M., 2002. In vitro solubilization of inorganic phosphate by phosphate solubilizing microorganisms (PSM) from maize rhizosphere. *International Journal of Agriculture and Biology*, *4*(4), pp.454-458.

Alori, E.T., Glick, B.R. & Babalola, O.O. 2017. Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Frontiers in Microbiology*, 8, pp.1–8.

Altomare, C., Norvell, W.A., Björkman, T. and Harman, G.E., 1999. Solubilization of phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22. *Applied and Environmental Microbiology*, *65*(7), pp.2926-2933.

Amaral, F. and Abelho, M., 2016. Effects of agricultural practices on soil and microbial biomass carbon, nitrogen and phosphorus content: a preliminary case study. *Web Ecology*, 16(1), pp. 3-5.

Andersson, B.E., Lundstedt, S., Tornberg, K., Schnürer, Y., Öberg, L.G. and Mattiasson, B., 2003. Incomplete degradation of polycyclic aromatic hydrocarbons in soil inoculated with wood-rotting fungi and their effect on the indigenous soil bacteria. *Environmental Toxicology and Chemistry: An International Journal*, *22*(6), pp.1238-1243.

Anjaneyulu, Y., Chary, N.S. and Raj, D.S.S., 2005. Decolourization of industrial effluents–available methods and emerging technologies–a review. *Reviews in Environmental Science and Bio/Technology*, *4*(4), pp.245-273.

Antal, Z., Manczinger, L., Szakacs, G., Tengerdy, R.P. and Ferenczy, L., 2000. Colony growth, in vitro antagonism and secretion of extracellular enzymes in cold-tolerant strains of *Trichoderma* species. *Mycological Research*, *104*(5), pp.545-549.

Araújo, A.D., Monteiro, R.T.R. and Abarkeli, R.B., 2003. Effect of glyphosate on the microbial activity of two Brazilian soils. *Chemosphere*, *52*(5), pp.799-804.

Avenot, H.F. and Michailides, T.J., 2007. Resistance to boscalid fungicide in *Alternaria alternata* isolates from pistachio in California. *Plant Disease*, *91*(10), pp.1345-1350.

Bacon, C.W., and White, J. (Eds.)., 2000. Microbial Endophytes (1st ed.). CRC Press, Boca Raton. https://doi.org/10.1201/9781482277302

Bacon, C.W. and White, J.F., 2016. Functions, mechanisms and regulation of endophytic and epiphytic microbial communities of plants. *Symbiosis*, 68(1-3), pp. 87-98.

Bader, A.N., Salerno, G.L., Covacevich, F. and Consolo, V.F., 2020. Native *Trichoderma harzianum* strains from Argentina produce indole-3 acetic acid and phosphorus solubilization, promote growth and control wilt disease on tomato (*Solanum lycopersicum L.*). *Journal of King Saud University-Science*, *32*(1), pp.867-873.

Bae, H., Roberts, D.P., Lim, H.S., Strem, M.D., Park, S.C., Ryu, C.M., Melnick, R.L. and Bailey, B.A., 2011. Endophytic *Trichoderma* isolates from tropical environments delay disease onset and induce resistance against *Phytophthora capsici* in hot pepper using multiple mechanisms. *Molecular Plant-Microbe Interactions*, *24*(3), pp.336-351.

Bakker, P.A., Berendsen, R.L., Doornbos, R.F., Wintermans, P.C. and Pieterse, C.M., 2013. The rhizosphere revisited: root microbiomics. *Frontiers in Plant Science*, *4*, p.165.

Baroncelli, R., Zapparata, A., Piaggeschi, G., Sarrocco, S. and Vannacci, G., 2016. Draft whole-genome sequence of *Trichoderma gamsii* T6085, a promising biocontrol agent of *Fusarium* head blight on wheat. *Genome Announcements.*, *4*(1), pp.e01747-15.

Batidzirai, B., Valk, M., Wicke, B., Junginger, M., Daioglou, V., Euler, W. and Faaij, A.P.C., 2016. Current and future technical, economic and environmental feasibility of maize and wheat residues supply for biomass energy application: Illustrated for South Africa. *Biomass and Bioenergy*, *92*, pp.106-129.

Belayneh Mulaw, T., Kubicek, C.P. and Druzhinina, I.S., 2010. The rhizosphere of *Coffea arabica* in its native highland forests of Ethiopia provides a niche for a distinguished diversity of *Trichoderma*. *Diversity*, *2*(4), pp.527-549.

Benitez, M.S., Osborne, S.L. and Lehman, R.M., 2017. Previous crop and rotation history effects on maize seedling health and associated rhizosphere microbiome. *Scientific Reports*, 7(1), p. 15709.

Betina, V. and Farkas, V., 1998. Sporulation and light-induced development in *Trichoderma*. *Trichoderma and Gliocladium: Basic Biology, Taxonomy and Genetics*, *2*, pp.131-151.

BFAP, 2015. Adding value to the South African maize industry. https://ageconsearch.umn.edu/record/279763/files/ADDING VALUE IN THE SOUTH AFRICAN_ MAIZE VALUE CHAIN 13 April 2017.pdf

Bisby, G.R., 1939. *Trichoderma viride* Pers. ex Fries, and notes on *Hypocrea*. *Transactions of the British Mycological Society*, *23*(2), pp.149-168.

Bischof, R.H., Ramoni, J. and Seiboth, B., 2016. Cellulases and beyond: the first 70 years of the enzyme producer *Trichoderma reesei*. *Microbial Cell Factories*, *15*(1), p.106.

Bissett, J., 1984. A revision of the genus Trichoderma. I. Section *Longibrachiatum* sect. nov. *Canadian Journal of Botany*, *62*(5), pp.924-931.

Bissett, J., 1991. A revision of the genus *Trichoderma*. III. Section *Pachybasium*. *Canadian Journal of Botany*, *69*(11), pp.2373-2417.

Bissett, J., 1991. A revision of the genus *Trichoderma*. IV. Additional notes on section *Longibrachiatum*. *Canadian Journal of Botany*, *69*(11), pp.2418-2420.

Bissett, J., 1992. *Trichoderma atroviride*. *Canadian Journal of Botany*, *70*(3), pp.639-641.

Bissett, J., Gams, W., Jaklitsch, W. and Samuels, G.J., 2015. Accepted *Trichoderma* names in the year 2015. *IMA fungus*, *6*(2), pp.263-295.

Björkman, T., 2004. Effect of *Trichoderma* colonization on auxin-mediated regulation of root elongation. *Plant Growth Regulation*, *43*(1), pp.89-92.

Björkman, T., Blanchard, L.M. and Harman, G.E., 1998. Growth enhancement of shrunken-2 (sh2) sweet corn by *Trichoderma harzianum* 1295-22: effect of environmental stress. *Journal of the American Society for Horticultural Science*, 123(1), pp. 35-40.

Błaszczyk, L., Popiel, D., Chełkowski, J., Koczyk, G., Samuels, G.J., Sobieralski, K. and Siwulski, M., 2011. Species diversity of *Trichoderma* in Poland. *Journal of applied genetics*, *52*(2), pp.233-243.

Bononi, L., Chiaramonte, J.B., Pansa, C.C., Moitinho, M.A. and Melo, I.S., 2020. Phosphorus-solubilizing *Trichoderma* spp. from Amazon soils improve soybean plant growth. *Scientific Reports*, *10*(1), pp.1-13.

Boonchan, S., Britz, M.L. and Stanley, G.A., 2000. Degradation and mineralization of high-molecular-weight polycyclic aromatic hydrocarbons by defined fungal-bacterial cocultures. *Appl. Environ. Microbiol.*, *66*(3), pp.1007-1019.

Borin, G.P., Sanchez, C.C., de Souza, A.P., de Santana, E.S., de Souza, A.T., Leme, A.F.P., Squina, F.M., Buckeridge, M., Goldman, G.H. and de Castro Oliveira, J.V., 2015. Comparative secretome analysis of *Trichoderma reesei* and *Aspergillus niger* during growth on sugarcane biomass. *PLoS One*, *10*(6), p.e0129275.

Borriss, R., 2011. Use of plant-associated Bacillus strains as biofertilizers and biocontrol agents in agriculture. In *Bacteria in Agrobiology: Plant growth responses* (pp. 41-76). Springer, Berlin, Heidelberg.

Bouwman, L., Goldewijk, K.K., Van Der Hoek, K.W., Beusen, A.H., Van Vuuren, D.P., Willems, J., Rufino, M.C. and Stehfest, E., 2013. Exploring global changes in nitrogen and phosphorus cycles in agriculture induced by livestock production over the 1900–2050 period. *Proceedings of the National Academy of Sciences*, *110*(52), pp.20882-20887.

Branković, G., Pajić, V., Zivanović, T., Dodig, D., Kandić, V., Knežević, D. and Đurić, N., 2018. Genetic parameters of *Triticum aestivum* and *Triticum durum* for technological quality properties in Serbia. *Zemdirbyste-Agriculture*, *105*(1), pp.39-48.

Brotman, Y., Landau, U., Cuadros-Inostroza, Á., Takayuki, T., Fernie, A.R., Chet, I., Viterbo, A. and Willmitzer, L., 2013. *Trichoderma*-plant root colonization: escaping early plant defense responses and activation of the antioxidant machinery for saline stress tolerance. *PLoS Pathogens*, *9*(3), p.e1003221.

Bulgarelli, D., Schlaeppi, K., Spaepen, S., Van Themaat, E.V.L. and Schulze-Lefert, P., 2013. Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology*, *64*, pp.807-838.

Byerlee, D. and Heisey, P.W., 1997. Evolution of the African maize economy. *Africa's Emerging Maize Revolution*, pp .9-22.

Cai, F. and Druzhinina, I.S., 2021. In honor of John Bissett: authoritative guidelines on molecular identification of *Trichoderma*. *Fungal Diversity*, *107*(1), pp.1-69.

Card, S., Johnson, L., Teasdale, S. and Caradus, J., 2016. Deciphering endophyte behaviour: the link between endophyte biology and efficacious biological control agents. *FEMS Microbiology Ecology*, 92(8).

Carvalho, F.P., 2017. Pesticides, environment, and food safety. *Food and Energy Security*, *6*(2), pp.48-60.

Cerniglia, C.E., 1984. Microbial metabolism of polycyclic aromatic hydrocarbons. In *Advances in Applied Microbiology* (Vol. 30, pp. 31-71). Academic Press.

Chagas, L.F.B., De Castro, H.G., Colonia, B.S.O., De Carvalho Filho, M.R., Miller, L.D.O. & Chagas, A.F.J. 2016. Efficiency of *Trichoderma* spp. as a growth promoter of cowpea (*Vigna unguiculata*) and analysis of phosphate solubilization and indole acetic acid synthesis. Revista brasileira de botânica. 39(2), pp.437–445.

Chandra, A., Pathak, P.S., Bhatt, R.K. and Dubey, A., 2004. Variation in drought tolerance of different Stylosanthes accessions. *Biologia Plantarum*, *48*(3), pp.457-460.

Chaverri, P., Branco-Rocha, F., Jaklitsch, W., Gazis, R., Degenkolb, T. and Samuels, G.J., 2015. Systematics of the *Trichoderma harzianum* species complex and the re-identification of commercial biocontrol strains. *Mycologia*, *107*(3), pp.558-590.

Chew, S.Y. and Ting, A.S.Y., 2016. Common filamentous Trichoderma asperellum for effective removal of triphenylmethane dyes. *Desalination and Water Treatment*, *57*(29), pp.13534-13539.

Clark, E.A., 2004. Benefits of re-integrating livestock and forages in crop production systems. *Journal of Crop Improvement*, *12*(1-2), pp.405-436.

Clark, M.S. and Gage, S.H., 1996. Effects of free-range chickens and geese on insect pests and weeds in an agroecosystem. *American Journal of Alternative Agriculture*, *11*(1), pp.39-47.

Cocaign, A., Bui, L.C., Silar, P., Tong, L.C.H., Busi, F., Lamouri, A., Mougin, C., Rodrigues-Lima, F., Dupret, J.M. and Dairou, J., 2013. Biotransformation of Trichoderma spp. and their tolerance to aromatic amines, a major class of pollutants. *Applied and environmental microbiology*, *79*(15), pp.4719-4726.

Collinge, D.B., Jørgensen, H.J.L., Latz, M., Manzotti, A., Ntana, F., Rojas Tayo, E.C. and Jensen, B., 2019. Searching for novel fungal biological control agents for plant disease control among endophytes. *Endophytes for a Growing World*, p. 25.

Consolo, V.F., Torres-Nicolini, A. and Alvarez, V.A., 2020. Mycosinthetized Ag, CuO and ZnO nanoparticles from a promising *Trichoderma harzianum* strain and their antifungal potential against important phytopathogens. *Scientific Reports*, *10*(1), pp.1-9.

Contreras-Cornejo, H.A., Macías-Rodríguez, L., del-Val, E. and Larsen, J., 2016. Ecological functions of *Trichoderma* spp. and their secondary metabolites in the rhizosphere: interactions with plants. *FEMS Microbiology Ecology*, *92*(4), p.fiw036.

Cook, R.J. and Baker, K.F., 1983. The nature and practice of biological control of plant pathogens. *American Phytopathological Society*. St. Paul.

Cummings, N.J., Ambrose, A., Braithwaite, M., Bissett, J., Roslan, H.A., Abdullah, J., Stewart, A., Agbayani, F.V., Steyaert, J. and Hill, R.A., 2016. Diversity of root-endophytic *Trichoderma* from Malaysian Borneo. *Mycological Progress*, 15(5), p. 50.

Cunningham, J.E. and Kuiack, C., 1992. Production of citric and oxalic acids and solubilization of calcium phosphate by *Penicillium bilaii*. *Applied and Environmental Microbiology*, *58*(5), pp.1451-1458.

Curl, E.A. and Truelove, B., 2012. *The Rhizosphere* (Vol. 15). Springer Science & Business Media.

D'Amato, D., Droste, N., Allen, B., Kettunen, M., Lähtinen, K., Korhonen, J., Leskinen, P., Matthies, B.D. and Toppinen, A., 2017. Green, circular, bio economy: A comparative analysis of sustainability avenues. *Journal of Cleaner Production*, *168*, pp.716-734.

Dardanelli, M.S., Carletti, S.M., Paulucci, N.S., Medeot, D.B., Caceres, E.R., Vita, F.A., Bueno, M., Fumero, M.V. and Garcia, M.B., 2010. Benefits of plant growth-promoting *rhizobacteria* and *rhizobia* in agriculture. In *Plant growth and health promoting bacteria* (pp. 1-20). Springer, Berlin, Heidelberg.

Degenkolb, T., Von Doehren, H., Fog Nielsen, K., Samuels, G.J. and Brückner, H., 2008. Recent advances and future prospects in peptaibiotics, hydrophobin, and mycotoxin research, and their importance for chemotaxonomy of *Trichoderma* and *Hypocrea*. *Chemistry & Biodiversity*, *5*(5), pp.671-680.

Demeke, M. and Di Marcantonio, F., 2013. Understanding the performance of food production in sub-Saharan Africa and its implications for food security. *Journal of Development and Agricultural Economics*, *5*(11), pp.425-443.

Dodd, S.L., Lieckfeldt, E. and Samuels, G.J., 2003. *Hypocrea atroviridis* sp. nov., the teleomorph of *Trichoderma atroviride*. *Mycologia*, *95*(1), pp.27-40.

Doni, F., Isahak, A., Zain, C.R.C.M. and Yusoff, W.M.W., 2014. Physiological and growth response of rice plants (Oryza sativa L.) to *Trichoderma* spp. inoculants. *Applied, microbiology and biotechnology express*, *4*(1), p.45.

Dou, K., Lu, Z., Wu, Q., Ni, M., Yu, C., Wang, M., Li, Y., Wang, X., Xie, H., Chen, J. and Zhang, C., 2020. MIST: a multilocus identification system for *Trichoderma*. *Applied and Environmental Microbiology*, *86*(18).

Druzhinina, I. and Kubicek, C.P., 2005. Species concepts and biodiversity in Trichoderma and Hypocrea: from aggregate species to species clusters?. *Journal of Zhejiang University. Science. B*, *6*(2), p.100.

Druzhinina, I.S. and Kubicek, C.P., 2017. Genetic engineering of *Trichoderma reesei* cellulases and their production. *Microbial Biotechnology*, *10*(6), pp.1485-1499.

Druzhinina, I.S., Komoń-Zelazowska, M., Kredics, L., Hatvani, L., Antal, Z., Belayneh, T. and Kubicek, C.P., 2008. Alternative reproductive strategies of *Hypocrea orientalis* and genetically close but clonal *Trichoderma longibrachiatum*, both capable of causing invasive mycoses of humans. *Microbiology*, *154*(11), pp.3447-3459.

Druzhinina, I.S., Kopchinskiy, A.G. and Kubicek, C.P., 2006. The first 100 *Trichoderma* species characterized by molecular data. *Mycoscience*, *47*(2), p.55.

Druzhinina, I.S., Kopchinskiy, A.G., Komoń, M., Bissett, J., Szakacs, G. and Kubicek, C.P., 2005. An oligonucleotide barcode for species identification in *Trichoderma* and *Hypocrea*. *Fungal Genetics and Biology*, *42*(10), pp.813-828.

Druzhinina, I.S., Kubicek, C.P., Komoń-Zelazowska, M., Mulaw, T.B. and Bissett, J., 2010. The Trichoderma harzianum demon: complex speciation history resulting in coexistence of hypothetical biological species, recent agamospecies and numerous relict lineages. *BMC Evolutionary Biology*, *10*(1), pp.1-14.

Druzhinina, I.S., Seidl-Seiboth, V., Herrera-Estrella, A., Horwitz, B.A., Kenerley, C.M., Monte, E., Mukherjee, P.K., Zeilinger, S., Grigoriev, I.V. and Kubicek, C.P., 2011. *Trichoderma*: the genomics of opportunistic success. *Nature Reviews Microbiology*, *9*(10), p.749.

Du Plessis AJ. The history of small-grains culture in South Africa. In: Annals of the University of Stellenbosch. Vol 8. Stellenbosch: University of Stellenbosch; 1933. pp. 1652-1752.

Du Plessis, I.L., 2015. The diversity of *Trichoderma* spp. in South Africa, MSc Thesis, Stellenbosch University, South Africa.

Du Plessis, I.L., Druzhinina, I.S., Atanasova, L., Yarden, O. and Jacobs, K., 2018. The diversity of *Trichoderma* species from soil in South Africa, with five new additions. *Mycologia*, *110*(3), pp.559-583.

Dube, E., Kilian, W., Mwadzingeni, L., Sosibo, N.Z., Barnard, A. and Tsilo, T.J., 2019. Genetic progress of spring wheat grain yield in various production regions of South Africa. *South African Journal of Plant and Soil*, 36(1), pp. 33-39.

Dugassa, A., Belete, K. and Shimbir, T., 2019. Response of Wheat (*Triticum aestivum* L.) to Different Rates of Nitrogen and Phosphorus at Fiche-Salale, Highlands of Ethiopia. *International Journal of Plant Breeding*, *6*(1), pp.474-480.

Ekwomadu, T.I., Gopane, R.E. and Mwanza, M., 2018. Occurrence of filamentous fungi in maize destined for human consumption in South Africa. *Food Science & Nutrition*, *6*(4), pp. 884-890.

El Aty, A.A.A., Saleh, S.A., Eid, B.M., Ibrahim, N.A. and Mostafa, F.A., 2018. Thermodynamics characterization and potential textile applications of *Trichoderma longibrachiatum* KT693225 xylanase. *Biocatalysis and Agricultural Biotechnology*, 14, pp. 129-137.

Elad, Y., Barak, R., Chet, I. and Henis, Y., 1983. Ultrastructural studies of the interaction between *Trichoderma* spp. and plant pathogenic fungi. *Journal of Phytopathology*, *107*(2), pp.168-175.

Elshahawy, I.E., Saied, N., Abd-El-Kareem, F. and Morsy, A., 2017. Biocontrol of onion white rot by application of *Trichoderma* species formulated on wheat bran powder. *Archives of Phytopathology and Plant Protection*, *50*(3-4), pp.150-166.

Erenstein, O., Sayre, K., Wall, P., Hellin, J. and Dixon, J., 2012. Conservation agriculture in maize-and wheat-based systems in the (sub) tropics: lessons from adaptation initiatives in South Asia, Mexico, and Southern Africa. *Journal of sustainable agriculture*, *36*(2), pp.180-206.

FAOSTAT, 2019 Accessed from FAO.org/faostat/en/#data/QC/visualize (Available on the 25 March 2021).

Filizola, P.R.B., Luna, M.A.C., de Souza, A.F., Coelho, I.L., Laranjeira, D. and Campos-Takaki, G.M., 2019. Biodiversity and phylogeny of novel *Trichoderma* isolates from mangrove sediments and potential of biocontrol against *Fusarium* strains. *Microbial Cell Factories*, *18*(1), p.89.

Fiorini, A., Boselli, R., Maris, S.C., Santelli, S., Ardenti, F., Capra, F. and Tabaglio, V., 2020. May conservation tillage enhance soil C and N accumulation without decreasing yield in intensive irrigated croplands? Results from an eight-year maize monoculture. *Agriculture, Ecosystems & Environment*, *296*, p.106926.

Food and Agriculture Organization (FAO) (2016) *The state of food and agriculture, 2016, The Eugenics review*. Available online: http://www.fao.org/3/a-i6030e.pdf (accessed on 03 November 2020).

Frisvad, J.C., Andersen, B. and Thrane, U., 2008. The use of secondary metabolite profiling in chemotaxonomy of filamentous fungi. *Mycological Research*, *112*(2), pp.231-240.

Frisvad, J.C., Møller, L.L., Larsen, T.O., Kumar, R. and Arnau, J., 2018. Safety of the fungal workhorses of industrial biotechnology: update on the mycotoxin and secondary metabolite potential of *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei*. *Applied Microbiology and Biotechnology*, *102*(22), pp.9481-9515.

Gams, W., and Bissett, J., 1998. Morphology and identification of *Trichoderma*. CP Kubicek, GE Harman (Eds.), *Trichoderma* & *Gliocladium*, Vol.1, Basic Biology, Taxonomy, and Genetics, Taylor & Francis Ltd., London, pp. 3-34.

Garg, V.K., Amita, M., Kumar, R. and Gupta, R., 2004. Basic dye (methylene blue) removal from simulated wastewater by adsorption using Indian Rosewood sawdust: a timber industry waste. *Dyes and pigments*, *63*(3), pp.243-250.

Giunta, F., Pruneddu, G., Zuddas, M. and Motzo, R., 2019. Bread and durum wheat: Intra-and inter-specific variation in grain yield and protein concentration of modern Italian cultivars. *European Journal of Agronomy*, *105*, pp.119-128.

Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M. and Toulmin, C., 2010. Food security: the challenge of feeding 9 billion people. *Science*, *327*(5967), pp.812-818.

Gomiero, T., Pimentel, D. and Paoletti, M.G., 2011. Environmental impact of different agricultural management practices: conventional vs. organic agriculture. *Critical Reviews in Plant Sciences*, 30(1-2), pp. 95-124.

Grant, C.A., Flaten, D.N., Tomasiewicz, D.J. & Sheppard, S.C. 2001. The importance of early season phosphorus nutrition. *Canadian Journal of Plant Science*. 81(2),pp.211–224.

Gravel, V., Antoun, H. and Tweddell, R.J., 2007. Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with Pseudomonas putida or Trichoderma atroviride: possible role of indole acetic acid (IAA). *Soil Biology and Biochemistry*, *39*(8), pp.1968-1977.

Greyling, J.C. and Pardey, P.G., 2018. Measuring Maize in South Africa: The Shifting Structure of Production During the Twentieth Century, 1904–2015. *Agrekon*, pp.1-21.

Grover, M., Ali, S.Z., Sandhya, V., Rasul, A. and Venkateswarlu, B., 2011. Role of microorganisms in adaptation of agriculture crops to abiotic stresses. *World Journal of Microbiology and Biotechnology*, 27(5), pp.1231-1240.

Gupta, N., Sabat, J., Parida, R. and Kerkatta, D., 2007. Solubilization of tricalcium phosphate and rock phosphate by microbes isolated from chromite, iron and manganese mines. *Acta Botanica Croatica*, *66*(2), pp.197-204.

Gupta, R., SINGAL, R., SHANKAR, A., KUHAD, R.C. and SAXENA, R.K., 1994. A modified plate assay for screening phosphate solubilizing microorganisms. *The Journal of General and Applied Microbiology*, *40*(3), pp.255-260.

Habib, Sheikh Hasna, Kausar, Hossain & Saud, Halimi Mohd. 2016. Plant Growth-Promoting Rhizobacteria Enhance Salinity Stress Tolerance in Okra through ROS-Scavenging Enzymes. *BioMed research international*. 2016, pp.1–10.

Hallmann, J., Quadt-Hallmann, A., Mahaffee, W.F. and Kloepper, J.W., 1997. Bacterial endophytes in agricultural crops. *Canadian Journal of Microbiology*, *43*(10), pp.895-914.

Harman, G.E., 2000. Myths and dogmas of biocontrol changes in perceptions derived from research on *Trichoderma harzinum* T-22. *Plant disease*, *84*(4), pp.377-393.

Harman, G.E., 2006. Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology*, *96*(2), pp.190-194.

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I. and Lorito, M., 2004. *Trichoderma* species—opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology*, *2*(1), p.43.

Hassan, M.M., Farid, M.A. and Gaber, A., 2019. Rapid identification of *Trichoderma koningiopsis* and *Trichoderma longibrachiatum* using sequence-characterized amplified region markers. *Egyptian Journal of Biological Pest Control*, *29*(1), p.13.

Hassan, M.M., Gaber, A. and El-Hallous, E.I., 2014. Molecular and morphological characterization of *Trichoderma harzianum* from different Egyptian soils. *Wulfenia J*, *21*, pp.80-96.

Hatvani, L., Antal, Z., Manczinger, L., Szekeres, A., Druzhinina, I.S., Kubicek, C.P., Nagy, A., Nagy, E., Vágvölgyi, C. and Kredics, L., 2007. Green mold diseases of *Agaricus* and *Pleurotus* spp. are caused by related but phylogenetically different *Trichoderma* species. *Phytopathology*, *97*(4), pp.532-537.

He, Y., Wu, Z., Wang, W., Liu, X. and Ye, B.C., 2019. Bacterial community and phosphorus species changes in pepper rhizosphere soils after *Pseudomonas putida* Rs-198 inoculation. *Rhizosphere*, *11*, p.100164.

Hermosa, M.R., Keck, E., Chamorro, I., Rubio, B., Sanz, L., Vizcaíno, J.A., Grondona, I. and Monte, E., 2004. Molecular characterization of biocontrol agents. *Bulletin-OILB-SROP*, *27*(8), pp.165-168.

Herrera-Jiménez, E., Alarcón, A., Larsen, J., Ferrera-Cerrato, R., Cruz-Izquierdo, S. and Ferrera-Rodríguez, M.R., 2018. Comparative effects of two indole-producing *Trichoderma* strains and two exogenous phytohormones on the growth of *Zea mays* L., with or without tryptophan. *Journal of Soil Science and Plant Nutrition*, 18(1), pp. 188-201.

Hilimire, K., 2011. Integrated crop/livestock agriculture in the United States: A review. *Journal of Sustainable Agriculture*, *35*(4), pp.376-393.

Horinouchi, S., 2007. Mining and polishing of the treasure trove in the bacterial genus Streptomyces. *Bioscience, biotechnology, and biochemistry*, *71*(2), pp.283-299.

Hoyos-Carvajal, L., Orduz, S. and Bissett, J., 2009. Growth stimulation in bean (Phaseolus vulgaris L.) by Trichoderma. *Biological control*, *51*(3), pp.409-416.

Hussein, K.A. and Joo, J.H., 2015. Isolation and characterization of rhizomicrobial isolates for phosphate solubilization and indole acetic acid production. *Journal of the Korean Society for Applied Biological Chemistry*, *58*(6), pp.847-855.

Jaklitsch, W.M. and Voglmayr, H., 2015. Biodiversity of *Trichoderma* (*Hypocreaceae*) in Southern Europe and Macaronesia. *Studies in Mycology*, *80*, pp.1-87.

Jaklitsch, W.M., 2009. European species of *Hypocrea* Part I. The green-spored species. *Studies in Mycology*, *63*, pp.1-91.

Jaklitsch, W.M., Samuels, G.J., Dodd, S.L., Lu, B.S. and Druzhinina, I.S., 2006. *Hypocrea rufa/Trichoderma viride*: a reassessment, and description of five closely related species with and without warted conidia. *Studies in Mycology*, *56*, pp.135-177.

Jaklitsch, W.M., Samuels, G.J., Ismaiel, A. and Voglmayr, H., 2013. Disentangling the *Trichoderma viridescens* complex. *Persoonia: Molecular Phylogeny and Evolution of Fungi*, *31*, p.112.

Jiang, H., Zhang, L., Zhang, J.Z., Ojaghian, M.R. and Hyde, K.D., 2016. Antagonistic interaction between *Trichoderma asperellum* and *Phytophthora capsici* in vitro. *Journal of Zhejiang University-Science B*, *17*(4), pp.271-281.

John, R.P., Tyagi, R.D., Prévost, D., Brar, S.K., Pouleur, S. and Surampalli, R.Y., 2010. Mycoparasitic Trichoderma viride as a biocontrol agent against *Fusarium oxysporum* f. sp. adzuki and *Pythium arrhenomanes* and as a growth promoter of soybean. *Crop Protection*, *29*(12), pp.1452-1459.

Johnson, J.A. and Whitney, N.J., 1992. Isolation of fungal endophytes from black spruce (*Picea mariana*) dormant buds and needles from New Brunswick, Canada. *Canadian Journal of Botany*, *70*(9), pp.1754-1757.

Johnson, M., Hazell, P. and Gulati, A., 2003. The role of intermediate factor markets in Asia's green revolution: lessons for Africa? *American Journal of Agricultural Economics*, *85*(5), pp.1211-1216.

Johnston, B.F. and Mellor, J.W., 1961. The role of agriculture in economic development. *The American Economic Review*, *51*(4), pp.566-593.

Jones, D.L. and Oburger, E., 2011. Solubilization of phosphorus by soil microorganisms. In *Phosphorus in action* (pp. 169-198). Springer, Berlin, Heidelberg.

Kalam, S., Das, S.N., Basu, A. and Podile, A.R., 2017. Population densities of indigenous Acidobacteria change in the presence of plant growth promoting rhizobacteria (PGPR) in rhizosphere. *Journal of Basic Microbiology*, 57(5), pp.376-385.

Kalia, A. and Gosal, S.K., 2011. Effect of pesticide application on soil microorganisms. *Archives of Agronomy and Soil Science*, *57*(6), pp.569-596.

Kapri, A. and Tewari, L., 2010. Phosphate solubilization potential and phosphatase activity of rhizospheric *Trichoderma* spp. *Brazilian Journal of Microbiology*, 41(3), pp.787-795.

Kashyap, P.L., Rai, P., Srivastava, A.K. and Kumar, S., 2017. *Trichoderma* for climate resilient agriculture. *World Journal of Microbiology and Biotechnology*, *33*(8), p.155.

Katoch, M., Singh, D., Kapoor, K.K. and Vishwakarma, R.A., 2019. Trichoderma lixii (IIIM-B4), an endophyte of Bacopa monnieri L. producing peptaibols. *BMC microbiology*, *19*(1), pp.1-10.

Khalil, A.M.A., Abdelaziz, A.M., Khaleil, M.M. and Hashem, A.H., 2021. Fungal endophytes from leaves of *Avicennia marina* growing in semi-arid environment as a promising source for bioactive compounds. *Letters in Applied Microbiology*, *72*(3), pp.263-274.

Khan, M.S., Zaidi, A. and Wani, P.A., 2009. Role of phosphate solubilizing microorganisms in sustainable agriculture-a review. *In Sustainable Agriculture* (pp. 551-570). Springer, Dordrecht.

Khoshmanzar, E., Aliasgharzad, N., Neyshabouri, M.R., Khoshru, B., Arzanlou, M. & Asgari Lajayer, B. 2020. Effects of *Trichoderma* isolates on tomato growth and inducing its tolerance to water-deficit stress. *International journal of environmental science and technology* (Tehran). 17(2), pp.869–878.

Kim, C.S., Shirouzu, T., Nakagiri, A., Sotome, K., Nagasawa, E. and Maekawa, N., 2012. *Trichoderma mienum* sp. nov., isolated from mushroom farms in Japan. *Antonie van Leeuwenhoek*, *102*(4), pp.629-641.

Kindermann, J., El-Ayouti, Y., Samuels, G.J. and Kubicek, C.P., 1998. Phylogeny of the genus *Trichoderma* based on sequence analysis of the internal transcribed spacer region 1 of the rDNA cluster.

Kotasthane, A., Kotasthane, A., Agrawal, T., Agrawal, T., Kushwah, R., Kushwah, R., Rahatkar, O.V. & Rahatkar, O.V. 2015. In-vitro antagonism of *Trichoderma* spp. against *Sclerotium rolfsii* and *Rhizoctonia solani* and their response towards growth of cucumber, bottle gourd and bitter gourd. European journal of plant pathology. 141(3), pp.523–543.

Kribel, S., Qostal, S., Ouazzani Touhami, A., Selmaoui, K., Chliyeh, M., Benkirane, R. and Achbani, E.H., 2020. Effects of *Trichoderma* on growth and yield of wheat and barley and its survival ability on roots and amended rock phosphate growing substrates. *Current Research in Environmental & Applied Mycology (Journal of Fungal Biology)*, *10*(1), pp.400-416.

Kubicek, C.P., 2013. Systems biological approaches towards understanding cellulase production by *Trichoderma reesei*. *Journal of Biotechnology*, *163*(2), pp.133-142.

Kubicek, C.P., Herrera-Estrella, A., Seidl-Seiboth, V., Martinez, D.A., Druzhinina, I.S., Thon, M., Zeilinger, S., Casas-Flores, S., Horwitz, B.A., Mukherjee, P.K. and Mukherjee, M., 2011. Comparative genome sequence analysis underscores mycoparasitism as the ancestral life style of *Trichoderma*. *Genome Biology*, *12*(4), p.R40.

Kucey, R.M.N., 1983. Phosphate-solubilizing bacteria and fungi in various cultivated and virgin Alberta soils. *Canadian Journal of Soil Science*, *63*(4), pp.671-678.

Kucey, R.M.N., 1988. Effect of *Penicillium bilaji* on the solubility and uptake of P and micronutrients from soil by wheat. *Canadian Journal of Soil Science*, *68*(2), pp.261-270.

Küçük, Ç., Cevheri, C. and Mutlu, A., 2019. Stimulation of barley (*Hordeum vulgare* L.) growth with local *Trichoderma* sp. isolates. *Applied ecology and environmental research*, 17(2), pp.4607-4614.

Küçükakyüz, K., Çatav, Ş.S. and Ensarioğlu, K., 2016. Effects of biochar application to soils on seedling growth of wheat. *Agriculture and Food*.

Kudoyarova, G.R., Vysotskaya, L.B., Arkhipova, T.N., Kuzmina, L.Y., Galimsyanova, N.F., Sidorova, L.V., Gabbasova, I.M., Melentiev, A.I. and Veselov, S.Y., 2017. Effect of auxin producing and phosphate solubilizing bacteria on mobility of soil phosphorus, growth rate, and P acquisition by wheat plants. *Acta physiologiae plantarum*, *39*(11), pp.1-8.

Kuhls, K., Lieckfeldt, E., Samuels, G.J., Meyer, W., Kubicek, C.P. and Börner, T., 1997. Revision of *Trichoderma* sect. *Longibrachiatum* including related teleomorphs based on analysis of ribosomal DNA internal transcribed spacer sequences. *Mycologia*, *89*(3), pp.442-460.

Kumar, B.L. and Gopal, D.S., 2015. Effective role of indigenous microorganisms for sustainable environment. *3 Biotech*, 5(6), pp.867-876.

Kusari, S., Lamshöft, M., Zühlke, S. and Spiteller, M., 2008. An endophytic fungus from *Hypericum perforatum* that produces hypericin. *Journal of Natural Products*, *71*(2), pp.159-162.

Lai, Daowan, Ziling Mao, Zhiyao Zhou, Siji Zhao, Mengyao Xue, Jungui Dai, Ligang Zhou, and Dianpeng Li. "New chlamydosporol derivatives from the endophytic fungus *Pleosporales* sp. Sigrf05 and their cytotoxic and antimicrobial activities." *Scientific reports* 10, no. 1 (2020), pp.1-9.

Latz, M.A., Jensen, B., Collinge, D.B. and Jørgensen, H.J., 2018. Endophytic fungi as biocontrol agents: elucidating mechanisms in disease suppression. *Plant Ecology & Diversity*, pp. 1-13.

Leylaie, S. and Zafari, D., 2018. Antiproliferative and antimicrobial activities of secondary metabolites and phylogenetic study of endophytic Trichoderma species from Vinca plants. *Frontiers in microbiology*, *9*, p.1484.

Li, C., Lin, F., Li, Y., Wei, W., Wang, H., Qin, L., Zhou, Z., Li, B., Wu, F. and Chen, Z., 2016. A β-glucosidase hyper-production *Trichoderma reesei* mutant reveals a potential role of cel3D in cellulase production. *Microbial Cell Factories*, *15*(1), p.151.

Li, R.X., Cai, F., Pang, G., Shen, Q.R., Li, R. and Chen, W., 2015. Solubilisation of phosphate and micronutrients by *Trichoderma harzianum* and its relationship with the promotion of tomato plant growth. *PLoS One*, *10*(6), p.e0130081.

Lieckfeldt, E., Samuels, G.J., Börner, T. and Gams, W., 1998. *Trichoderma koningii*: neotypification and *Hypocrea* teleomorph. *Canadian Journal of Botany*, *76*(9), pp.1507-1522.

Loiseau, E., Saikku, L., Antikainen, R., Droste, N., Hansjürgens, B., Pitkänen, K., Leskinen, P., Kuikman, P. and Thomsen, M., 2016. Green economy and related concepts: An overview. *Journal of Cleaner Production*, *139*, pp.361-371.

Lu, B., Druzhinina, I.S., Fallah, P., Chaverri, P., Gradinger, C., Kubicek, C.P. and Samuels, G.J., 2004. *Hypocrea/Trichoderma* species with *pachybasium*-like conidiophores: teleomorphs for *T. minutisporum* and *T. polysporum* and their newly discovered relatives. *Mycologia*, *96*(2), pp.310-342.

Lu, J., Hu, J., Zhao, G., Mei, F. and Zhang, C., 2017. An in-field automatic wheat disease diagnosis system. *Computers and Electronics in Agriculture*, *142*, pp.369-379.

Lucas, J.A., Hawkins, N.J. and Fraaije, B.A., 2015. The evolution of fungicide resistance. *Advances in Applied Microbiology*, *90*, pp.29-92.

Lugtenberg, B.J., Caradus, J.R. and Johnson, L.J., 2016. Fungal endophytes for sustainable crop production. *FEMS Microbiology Ecology*, *92*(12).

Lundberg, D.S., Lebeis, S.L., Paredes, S.H., Yourstone, S., Gehring, J., Malfatti, S., Tremblay, J., Engelbrektson, A., Kunin, V., Del Rio, T.G. and Edgar, R.C., 2012. Defining the core *Arabidopsis thaliana* root microbiome. *Nature*, *488*(7409), pp.86-90.

Lupatini, M., Korthals, G.W., de Hollander, M., Janssens, T.K. and Kuramae, E.E., 2017. Soil microbiome is more heterogeneous in organic than in conventional farming system. *Frontiers in Microbiology*, 7, p. 2064.

MacDonald, J.M. and McBride, W.D., 2009. The transformation of US livestock agriculture scale, efficiency, and risks. *Economic Information Bulletin* No. (EIB-43) EconomicResearch Service: Washington, DC, USA.

Maguire, R.O., Chardon, W.J. and Simard, R.R., 2005. Assessing potential environmental impacts of soil phosphorus by soil testing. *Phosphorus: agriculture and the environment*, *46*, pp.145-180.

Mahanty, T., Bhattacharjee, S., Goswami, M., Bhattacharyya, P., Das, B., Ghosh, A. and Tribedi, P., 2017. Biofertilizers: a potential approach for sustainable agriculture development. *Environmental Science and Pollution Research*, 24(4), pp.3315-3335.

Mahato, S., Bhuju, S. and Shrestha, J., 2018. Effect of *Trichoderma viride* as biofertilizer on growth and yield of wheat. *Malays. Journal of Sustainable Agriculture*, 2(2), pp. 1-5.

Mander, P., Choi, Y.H., Pradeep, G.C., Choi, Y.S., Hong, J.H., Cho, S.S. and Yoo, J.C., 2014. Biochemical characterization of xylanase produced from Streptomyces sp. CS624 using an agro residue substrate. *Process Biochemistry*, *49*(3), pp.451-456.

Marti, J. and Slafer, G.A., 2014. Bread and durum wheat yields under a wide range of environmental conditions. *Field Crops Research*, *156*, pp.258-271.

Martinez, D., Berka, R.M., Henrissat, B., Saloheimo, M., Arvas, M., Baker, S.E., Chapman, J., Chertkov, O., Coutinho, P.M., Cullen, D. and Danchin, E.G., 2008. Genome sequencing and analysis of the biomass-degrading fungus *Trichoderma reesei* (syn. *Hypocrea jecorina*). *Nature Biotechnology*, *26*(5), p.553.

Mastouri, F., Björkman, T. and Harman, G.E., 2010. Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. *Phytopathology*, 100(11), pp. 1213-1221.

Mastouri, F., Björkman, T. and Harman, G.E., 2012. *Trichoderma harzianum* enhances antioxidant defense of tomato seedlings and resistance to water deficit. *Molecular Plant-Microbe Interactions*, *25*(9), pp.1264-1271.

Matarese, F., Sarrocco, S., Gruber, S., Seidl-Seiboth, V. and Vannacci, G., 2012. Biocontrol of Fusarium head blight: interactions between Trichoderma and mycotoxigenic Fusarium. *Microbiology*, *158*(1), pp.98-106.

Matson, P.A., Parton, W.J., Power, A.G. and Swift, M.J., 1997. Agricultural intensification and ecosystem properties. *Science*, *277*(5325), pp.504-509.

Mattoo, R., Umashankar, N. and Raveendra, H.R., 2021. Contrasting rhizosphere microbial communities between fertilized and bio-inoculated millet. *Rhizosphere*, *17*, p.100273.

McArthur, J.W. and McCord, G.C., 2017. Fertilizing growth: Agricultural inputs and their effects in economic development. *Journal of development economics*, *127*, pp.133-152.

McNear Jr, D.H., 2013. The rhizosphere-roots, soil and everything in between. *Nature Education Knowledge*, *4*(3), p.1.

Mendes, J.B.S., da Costa Neto, V.P., de Sousa, C.D.A., de Carvalho Filho, M.R., Rodrigues, A.C. and Bonifacio, A., 2020. *Trichoderma* and *bradyrhizobia* act synergistically and enhance the growth rate, biomass and photosynthetic pigments of cowpea (*Vigna unguiculata*) grown in controlled conditions. *Symbiosis*, *80*(2), pp.133-143.

Menendez, A.B. and Godeas, A., 1998. Biological control of *Sclerotinia sclerotiorum* attacking soybean plants. Degradation of the cell walls of this pathogen by *Trichoderma harzianum* (BAFC 742). *Mycopathologia*, *142*(3), pp.153-160.

Miles, T.D., Miles, L.A., Fairchild, K.L. and Wharton, P.S., 2014. Screening and characterization of resistance to succinate dehydrogenase inhibitors in *Alternaria solani*. *Plant Pathology*, *63*(1), pp.155-164.

Monaco, C., Perello, A., Alippi, H.E. and Pasquare, A.O., 1991. *Trichoderma* spp.: a biocontrol agent of *Fusarium* spp. and *Sclerotium rolfsii* by seed treatment. *Advances in Horticultural Science*, pp.92-95.

Morales-Rodríguez, C., Bastianelli, G., Aleandri, M., Chilosi, G. and Vannini, A., 2018. Application of *Trichoderma spp*. complex and biofumigation to control damping-off of *Pinus radiata* D. Don caused by *Fusarium circinatum* Nirenberg and O'Donnell. *Forests*, *9*(7), p.421.

Morrison-Whittle, P., Lee, S.A. and Goddard, M.R., 2017. Fungal communities are differentially affected by conventional and biodynamic agricultural management approaches in vineyard ecosystems. *Agriculture, Ecosystems & Environment*, 246, pp. 306-313.

Mostafa, F.A., El Aty, A.A.A. and Wehaidy, H.R., 2014. Improved Xylanase production by mixing low cost wastes and novel co-culture of three marine-derived fungi in solid state fermentation. *Int J Curr Microbiol App Sci*, *3*, pp.336-349.

Mukherjee, A.K., Kumar, A.S., Kranthi, S. and Mukherjee, P.K., 2014. Biocontrol potential of three novel *Trichoderma* strains: isolation, evaluation and formulation. *3 Biotech*, *4*(3), pp.275-281.

Mukherjee, M., Mukherjee, P.K., Horwitz, B.A., Zachow, C., Berg, G. and Zeilinger, S., 2012. *Trichoderma*–plant–pathogen interactions: advances in genetics of biological control. *Indian Journal of Microbiology*, 52(4), pp. 522-529.

Mukherjee, P.K., Horwitz, B.A., Herrera-Estrella, A., Schmoll, M. and Kenerley, C.M., 2013. *Trichoderma* research in the genome era. *Annual Review of Phytopathology*, *51*, pp.105-129.

Muller, M.F., Barnes, I., Kunene, N.T., Crampton, B.G., Bluhm, B.H., Phillips, S.M., Olivier, N.A. and Berger, D.K., 2016. *Cercospora zeina* from maize in South Africa exhibits high genetic diversity and lack of regional population differentiation. *Phytopathology*, *106*(10), pp. 1194-1205.

Mupangwa, W., Thierfelder, C., Cheesman, S., Nyagumbo, I., Muoni, T., Mhlanga, B., Mwila, M., Sida, T.S. and Ngwira, A., 2019. Effects of maize residue and mineral nitrogen applications on maize yield in conservation-agriculture-based cropping systems of Southern Africa. *Renewable Agriculture and Food Systems*, pp. 1-14.

Musokwa, M., Mafongoya, P. and Lorentz, S., 2019. Evaluation of agroforestry systems for maize (*Zea mays*) productivity in South Africa. *South African Journal of Plant and Soil*, 36(1), pp. 65-67.

Musvoto, C., Nortje, K., De Wet, B., Mahumani, B.K. and Nahman, A., 2015. Imperatives for an agricultural green economy in South Africa. *South African Journal of Science*, *111*(1-2), pp.01-08.

Mutawila, C., Halleen, F. and Mostert, L., 2016. Optimisation of time of application of *Trichoderma* biocontrol agents for protection of grapevine pruning wounds. *Australian Journal of Grape and Wine Research*, *22*(2), pp.279-287.

Mwajita, M.R., Murage, H., Tani, A. and Kahangi, E.M., 2013. Evaluation of rhizosphere, rhizoplane and phyllosphere bacteria and fungi isolated from rice in Kenya for plant growth promoters. *SpringerPlus*, *2*(1), p.606.

Naledzani, Z., Chaminuka, P., Nhundu, K., Machethe, C.L. and Liebenberg, F., 2019. Economic value of quality restrictions on the wheat industry in South Africa. *Agrekon*, pp.1-11.

Navon, A., 2000. *Bacillus thuringiensis* application in agriculture. In *Entomopathogenic bacteria: from laboratory to field application* (pp. 355-369). Springer, Dordrecht.

Nazifa, T.H., Ahmad, M.A., Hadibarata, T. and Aris, A., 2019. Bioremediation of Diesel Oil Spill by Filamentous Fungus *Trichoderma reesei* H002 in Aquatic Environment. *International Journal of Integrated Engineering*, *10*(9).

Ncube, E., Flett, B.C., Waalwijk, C. and Viljoen, A., 2011. *Fusarium* spp. and levels of fumonisins in maize produced by subsistence farmers in South Africa. *South African Journal of Science*, *107*(1-2), pp. 1-7.

Nepali, B., Subedi, S., Bhattarai, S., Marahatta, S., Bhandari, D. and Shrestha, J., 2020. Bio-fertilizer activity of *Trichoderma viride* and *Pseudomonas fluorescens* as growth and yield promoter for maize. *Agraarteadus*, *31*(2).

Nhemachena, C.R. and Kirsten, J., 2017. A historical assessment of sources and uses of wheat varietal innovations in South Africa. *South African Journal of Science*, *113*(3-4), pp.1-8.

Nieto-Jacobo, M.F., Steyaert, J.M., Salazar-Badillo, F.B., Nguyen, D.V., Rostás, M., Braithwaite, M., De Souza, J.T., Jimenez-Bremont, J.F., Ohkura, M., Stewart, A. and Mendoza-Mendoza, A., 2017. Environmental growth conditions of Trichoderma spp. affects indole acetic acid derivatives, volatile organic compounds, and plant growth promotion. *Frontiers in plant science*, *8*, p.102.

Ninh, H.T., Grandy, A.S., Wickings, K., Snapp, S.S., Kirk, W. and Hao, J., 2015. Organic amendment effects on potato productivity and quality are related to soil microbial activity. *Plant and Soil*, 386(1-2), pp. 223-236.

Nuss, E.T. and Tanumihardjo, S.A., 2010. Maize: a paramount staple crop in the context of global nutrition. *Comprehensive Reviews in Food Science and Food Safety*, *9*(4), pp.417-436.

Olanrewaju, O.S., Glick, B.R. & Babalola, O.O. 2017. Mechanisms of action of plant growth promoting bacteria. World journal of microbiology & biotechnology. 33(11), pp.1–16.

Oros, G., Naár, Z. and Cserháti, T., 2011. Growth response of Trichoderma species to organic solvents. *Molecular informatics*, *30*(2-3), pp.276-285.

Overton, B.E., Stewart, E.L. and Geiser, D.M., 2006. Taxonomy and phylogenetic relationships of nine species of *Hypocrea* with anamorphs assignable to *Trichoderma* section *Hypocreanum*. *Studies in Mycology*, *56*, pp.39-65.

Pascale, A., Vinale, F., Manganiello, G., Nigro, M., Lanzuise, S., Ruocco, M., Marra, R., Lombardi, N., Woo, S.L. and Lorito, M., 2017. *Trichoderma* and its secondary metabolites improve yield and quality of grapes. *Crop Protection*, 92, pp. 176-181.

Pearce, D.W., Barbier, E.B., Markandya, A., Turner, R.K., Barrett, S. and Swanson, T., 1991. *Blueprint 2: greening the world economy* (Vol. 2). Earthscan.

Pereira, J.O., Azevedo, J.L. and Petrini, O., 1993. Endophytic fungi of Stylosanthes: a first report. *Mycologia*, *85*(3), pp.362-364.

Ponisio, L.C., M'Gonigle, L.K., Mace, K.C., Palomino, J., de Valpine, P. and Kremen, C., 2015. Diversification practices reduce organic to conventional yield gap. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1799), p. 20141396.

Poonyth, D., Hassan, R., Kirsten, J.F. and Calcaterra, M., 2001. Is agricultural sector growth a precondition for economic growth? The case of South Africa. *Agrekon*, *40*(2), pp.269-279.

Pradhan, N. and Sukla, L.B., 2006. Solubilization of inorganic phosphates by fungi isolated from agriculture soil. *African Journal of Biotechnology*, *5*(10).

Qualhato, T.F., Lopes, F.A.C., Steindorff, A.S., Brandao, R.S., Jesuino, R.S.A. and Ulhoa, C.J., 2013. Mycoparasitism studies of Trichoderma species against three phytopathogenic fungi: evaluation of antagonism and hydrolytic enzyme production. *Biotechnology letters*, *35*(9), pp.1461-1468.

Rajankar, P.M., Tambekar, P.R.D. and WATE, S., 2007. Study of phosphate solubilization efficiencies of fungi and bacteria isolated from saline belt of Puma river basin. *Research Journal of Agriculture and Biological Sciences*, *3*(6), pp.701-703.

Ranimol, G., Venugopal, T., Gopalakrishnan, S. and Sunkar, S., 2018. Production of laccase from *Trichoderma harzianum* and its application in dye decolourisation. *Biocatalysis and Agricultural Biotechnology*, 16, pp. 400-404.

Ranum, P., Peña-Rosas, J.P. and Garcia-Casal, M.N., 2014. Global maize production, utilization, and consumption. *Annals of the New York Academy of Sciences*, *1312*(1), pp. 105-112.

Recio, R., Meléndez-Carmona, M.Á., Martín-Higuera, M.C., Pérez, V., López, E., López-Medrano, F. and Pérez-Ayala, A., 2019. *Trichoderma longibrachiatum*: an unusual pathogen of fungal pericarditis. *Clinical Microbiology and Infection*. 25(5), pp.586-587

Reinhold-Hurek, B. and Hurek, T., 1998. Life in grasses: diazotrophic endophytes. *Trends in Microbiology*, *6*(4), pp.139-144.

Reynolds, L.P., Wulster-Radcliffe, M.C., Aaron, D.K. and Davis, T.A., 2015. Importance of animals in agricultural sustainability and food security. *The Journal of Nutrition*, *145*(7), pp.1377-1379.

Richardson, A.E. 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. *Australian Journal of Plant Physiology*. 28(9), pp.897–906.

Ridout, C.J., Coley-Smith, J.R. and Lynch, J.M., 1988. Fractionation of extracellular enzymes from a mycoparasitic strain of *Trichoderma harzianum*. *Enzyme and Microbial Technology*, *10*(3), pp.180-187.

Rifai, M.A., 1969. A revision of the genus *Trichoderma*. *Mycological papers*, *116*, pp.1-56.

Rönsberg, D., Debbab, A., Mándi, A., Wray, V., Dai, H., Kurtán, T., Proksch, P. and Aly, A.H., 2013. Secondary metabolites from the endophytic fungus Pestalotiopsis virgatula isolated from the mangrove plant Sonneratia caseolaris. *Tetrahedron Letters*, *54*(25), pp.3256-3259.

Rudresh, D.L., Shivaprakash, M.K. and Prasad, R.D., 2005. Tricalcium phosphate solubilizing abilities of *Trichoderma* spp. in relation to P uptake and growth and yield parameters of chickpea (*Cicer arietinum* L.). *Canadian Journal of Microbiology*, *51*(3), pp.217-222.

Russelle, M.P., Entz, M.H. and Franzluebbers, A.J., 2007. Reconsidering integrated crop–livestock systems in North America. *Agronomy Journal*, *99*(2), pp.325-334.

Saber, W.I., Ghoneem, K.M., Rashad, Y.M. and Al-Askar, A.A., 2017. *Trichoderma Harzianum* WKY1: an indole acetic acid producer for growth improvement and anthracnose disease control in sorghum. *Biocontrol Science and Technology*, *27*(5), pp.654-676.

Sabry, S.R., Saleh, S.A., Batchelor, C.A., Jones, J., Jotham, J., Webster, G., Kothari, S.L., Davey, M.R. and Cocking, E.C., 1997. Endophytic establishment of *Azorhizobium caulinodans* in wheat. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *264*(1380), pp.341-346.

Samuels, G.D., Lu, S., Petrini, B., Schroers, O. and Druzhinina, H., 2006. The *Trichoderma Koningii* Morphological Species. *Studies in Mycology*, *56*, pp.67-133.

Samuels, G.J., 1996. *Trichoderma*: a review of biology and systematics of the genus. *Mycological Research*, *100*(8), pp.923-935.

Samuels, G.J., 2006. *Trichoderma*: systematics, the sexual state, and ecology. *Phytopathology*, *96*(2), pp.195-206.

Samuels, G.J., Ismaiel, A., Bon, M.C., De Respinis, S. and Petrini, O., 2010. Trichoderma asperellum sensu lato consists of two cryptic species. *Mycologia*, *102*(4), pp.944-966.

Samuels, G.J., Ismaiel, A., Mulaw, T.B., Szakacs, G., Druzhinina, I.S., Kubicek, C.P. and Jaklitsch, W.M., 2012. The *Longibrachiatum* clade of *Trichoderma*: a revision with new species. *Fungal Diversity*, *55*(1), pp.77-108.

Sandle, T., 2014. *Trichoderma*. *Encyclopedia of Food Microbiology* (Second Edition), pp. 644-646.

Saravanakumar, K. and Kathiresan, K., 2014. Bioremoval of the synthetic dye malachite green by marine *Trichoderma* sp. *SpringerPlus*, *3*(1), pp.1-12.

Saravanakumar, K., Arasu, V.S. and Kathiresan, K., 2013. Effect of *Trichoderma* on soil phosphate solubilization and growth improvement of *Avicennia marina*. *Aquatic Botany*, *104*, pp.101-105.

Saravanakumar, K., Li, Y., Yu, C., Wang, Q.Q., Wang, M., Sun, J., Gao, J.X. and Chen, J., 2017. Effect of *Trichoderma harzianum* on maize rhizosphere microbiome and biocontrol of *Fusarium* Stalk rot. *Scientific Reports*, *7*(1), pp.1-13.

Sarrocco, S., Matarese, F., Somma, S., Rossi, F., Moretti, A. and Vannacci, G., 2011. Biocontrol of Fusarium Head Blight (FHB) in a multitrophic perspective. *IOCB/WPRS Bull*, *71*, pp.123-127.

Sarrocco, S., Moncini, L., Pachetti, G., Moretti, A., Ritieni, A. and Vannacci, G., 2013. Biological control of Fusarium head blight under field conditions. *IOCB-WPRS Bull*, *86*, pp.95-100.

Savci, S., 2012. Investigation of effect of chemical fertilizers on environment. *Apcbee Procedia*, *1*, pp.287-292.

Scarlat, N., Blujdea, V. and Dallemand, J.F., 2011. Assessment of the availability of agricultural and forest residues for bioenergy production in Romania. *Biomass and Bioenergy*, *35*(5), pp.1995-2005.

Schultz, T.W. 1964. Transforming traditional agriculture /. New Haven, Conn. :: Yale University Press.

Schulz, B. and Boyle, C., 2006. What are endophytes? In *Microbial root endophytes* (pp. 1-13). Springer, Berlin, Heidelberg.

Schuster, A. and Schmoll, M., 2010. Biology and biotechnology of Trichoderma. *Applied Microbiology and Biotechnology*, *87*(3), pp.787-799.

Sellare, J., Meemken, E.M. and Qaim, M., 2020. Fairtrade, Agrochemical Input Use, and Effects on Human Health and the Environment. *Ecological Economics*, *176*, p.106718.

Sembiring, M., 2017. Bacterial and fungi phosphate solubilization effect to increase nutrient uptake and potatoes (*Solanum tuberosum L.*) production on Andisol Sinabung area. *Journal of Agronomy*, *16*(3), pp.131-137.

Setyaningrum, T., Indradewa, D., Priyatmojo, A. and Sulistyaningsih, E., 2019, March. *Trichoderma asperellum* inoculation on shallots productivity in coastal sand lands. In IOP Conference Series: *Earth and Environmental Science* (Vol. 250, No. 1, p. 012094). IOP Publishing.

Seufert, V., Ramankutty, N. and Foley, J.A., 2012. Comparing the yields of organic and conventional agriculture. *Nature*, *485*(7397), pp.229-232.

Shah, S., Nasreen, S. and Sheikh, P.A., 2012. Cultural and morphological characterization of *Trichoderma* spp. associated with green mold disease of *Pleurotus* spp. in Kashmirm. *Res. J. Microbiol*, *7*(2), pp.139-144.

Shalaby, M.E., Ghoniem, K.E. and El-Diehi, M.A., 2013. Biological and fungicidal antagonism of *Sclerotium cepivorum* for controlling onion white rot disease. *Annals of Microbiology*, *63*(4), pp.1579-1589.

Sharma, S., Kour, D., Rana, K.L., Dhiman, A., Thakur, S., Thakur, P., Thakur, S., Thakur, N., Sudheer, S., Yadav, N. and Yadav, A.N., 2019. *Trichoderma*: Biodiversity, Ecological Significances, and Industrial Applications. In *Recent Advancement in White Biotechnology Through Fungi* (pp. 85-120). Springer, Cham.

Sheahan, M., Barrett, C.B. and Goldvale, C., 2017. Human health and pesticide use in sub-Saharan Africa. *Agricultural Economics*, *48*(S1), pp.27-41.

Shewry, P.R., 2009. Wheat. *Journal of Experimental Botany*, *60*(6), pp.1537-1553.

Shoresh, M. and Harman, G.E., 2008. The molecular basis of shoot responses of maize seedlings to *Trichoderma harzianum* T22 inoculation of the root: a proteomic approach. *Plant physiology*, *147*(4), pp.2147-2163.

Shukla, N., Awasthi, R.P., Rawat, L. and Kumar, J., 2012. Biochemical and physiological responses of rice (*Oryza sativa* L.) as influenced by *Trichoderma harzianum* under drought stress. *Plant Physiology and Biochemistry*, 54, pp. 78-88.

Silva, Í.S., dos Santos, E.D.C., de Menezes, C.R., de Faria, A.F., Franciscon, E., Grossman, M. and Durrant, L.R., 2009. Bioremediation of a polyaromatic hydrocarbon contaminated soil by native soil microbiota and bioaugmentation with isolated microbial consortia. *Bioresource Technology*, *100*(20), pp.4669-4675.

Singh, J.S., Pandey, V.C. and Singh, D.P., 2011. Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. *Agriculture, Ecosystems & Environment*, 140(3-4), pp.339-353.

Singh, M., Dotaniya, M.L., Mishra, A., Dotaniya, C.K., Regar, K.L. and Lata, M., 2016. Role of biofertilizers in conservation agriculture. *In Conservation Agriculture* (pp. 113-134). Springer, Singapore.

Singh, P., Kim, Y.J., Zhang, D. and Yang, D.C., 2016. Biological synthesis of nanoparticles from plants and microorganisms. *Trends in Biotechnology*, *34*(7), pp.588-599.

Singh, R. and Dubey, A.K., 2020. Isolation and characterization of a new endophytic actinobacterium *Streptomyces californicus* strain ADR1 as a promising source of anti-bacterial, anti-biofilm and antioxidant metabolites. *Microorganisms*, *8*(6), p.929.

Srivastava, A.K., Mboh, C.M., Faye, B., Gaiser, T., Kuhn, A., Ermias, E. and Ewert, F., 2019. Options for Sustainable Intensification of Maize Production in Ethiopia. *Sustainability*, *11*(6), p. 1707.

Srivastava, P.K., Shenoy, B.D., Gupta, M., Vaish, A., Mannan, S., Singh, N., Tewari, S.K. and Tripathi, R.D., 2012. Stimulatory effects of arsenic-tolerant soil fungi on plant growth promotion and soil properties. *Microbes and Environments*, p.ME11316.

Stander CJ. The economics of cultivar improvement research in the South African wheat industry [MSc thesis]. Pretoria: University of Pretoria; 2010.

Strobel, G., 2018. The emergence of endophytic microbes and their biological promise. *Journal of Fungi*, 4(2), p. 57.

Strobel, G.A., 2003. Endophytes as sources of bioactive products. *Microbes and infection*, *5*(6), pp.535-544.

Subedi, S., 2015. A review on important maize diseases and their management in Nepal. *Journal of Maize Research and Development*, *1*(1), pp. 28-52.

Suryanarayanan, T.S., 2013. Endophyte research: going beyond isolation and metabolite documentation. *Fungal Ecology*, 6(6), pp. 561-568.

Swain, H., Adak, T., Mukherjee, A.K., Mukherjee, P.K., Bhattacharyya, P., Behera, S., Bagchi, T.B., Patro, R., Khandual, A., Bag, M.K. and Dangar, T.K., 2018. Novel *Trichoderma* strains isolated from tree barks as potential biocontrol agents and biofertilizers for direct seeded rice. *Microbiological Research*, *214*, pp.83-90.

Tal, A., 2018. Making conventional agriculture environmentally friendly: moving beyond the glorification of organic agriculture and the demonization of conventional agriculture. *Sustainability*, 10(4), p. 1078

Tanaka, D.L., Karn, J.F. and Scholljegerdes, E.J., 2008. Integrated crop/livestock systems research: Practical research considerations. *Renewable Agriculture and Food Systems*, *23*(1), pp.80-86.

Tandon, A., Fatima, T., Shukla, D., Tripathi, P., Srivastava, S. and Singh, P.C., 2020. Phosphate solubilization by *Trichoderma koningiopsis* (NBRI-PR5) under abiotic stress conditions. *Journal of King Saud University-Science*, *32*(1), pp.791-798.

Thornton, P.K. and Herrero, M., 2001. Integrated crop–livestock simulation models for scenario analysis and impact assessment. *Agricultural systems*, *70*(2-3), pp.581-602.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R. and Polasky, S., 2002. Agricultural sustainability and intensive production practices. *Nature*, *418*(6898), pp.671-677.

Trethowan, R.M., Reynolds, M., Sayre, K. and Ortiz-Monasterio, I., 2005. Adapting wheat cultivars to resource conserving farming practices and human nutritional needs. *Annals of Applied Biology*, *146*(4), pp.405-413.

Turan, M., Ataoğlu, N. and Şahın, F., 2006. Evaluation of the capacity of phosphate solubilizing bacteria and fungi on different forms of phosphorus in liquid culture. *Journal of Sustainable Agriculture*, *28*(3), pp.99-108.

Urošević, D.M. and Gvozdenac-Urošević, B.D., 2012. Comprehensive analysis of a straw-fired power plant in Vojvodina. *Thermal science*, *16*(suppl. 1), pp.97-106.

Van Dam, N.M. and Bouwmeester, H.J., 2016. Metabolomics in the rhizosphere: tapping into belowground chemical communication. *Trends in Plant Science*, 21(3), pp. 256-265.

Van Rooyen, C.J., Fenyes, T.I. and Van Zyl, J., 1987. A comparison of the contribution and relative performance of agriculture in Southern Africa. *Development Southern Africa*, *4*(2), pp.183-198.

Van Rooyen, J. and Sigwele, H., 1998. Towards regional food security in southern Africa: a (new) policy framework for the agricultural sector. *Food Policy*, *23*(6), pp.491-504.

Van Zyl, J., Nel, H.J.G. and Groenewald, J.A., 1988. Agriculture's contribution to the South African economy. *Agrekon*, *27*(2), pp.1-9.

Vargas, L.K., Volpiano, C.G., Lisboa, B.B., Giongo, A., Beneduzi, A. and Passaglia, L.M.P., 2017. Potential of rhizobia as plant growth-promoting rhizobacteria. In *Microbes for legume improvement* (pp. 153-174). Springer, Cham.

Vargas, W.A., Mukherjee, P.K., Laughlin, D., Wiest, A., Moran-Diez, M.E. and Kenerley, C.M., 2014. Role of gliotoxin in the symbiotic and pathogenic interactions of *Trichoderma virens*. *Microbiology*, *160*(10), pp.2319-2330.

Varvel, G.E., Vogel, K.P., Mitchell, R.B., Follett, R.F. and Kimble, J.M., 2008. Comparison of corn and switchgrass on marginal soils for bioenergy. *Biomass and bioenergy*, *32*(1), pp.18-21.

Vaxevanidou, K., Christou, C., Kremmydas, G.F., Georgakopoulos, D.G. and Papassiopi, N., 2015. Role of indigenous arsenate and iron (III) Respiring microorganisms in controlling the mobilization of arsenic in a Contaminated soil Sample. *Bulletin of Environmental Contamination and Toxicology*, 94(3), pp.282-288.

Vázquez, M.M., César, S., Azcón, R. and Barea, J.M., 2000. Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum*, *Pseudomonas*, *Trichoderma*) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. *Applied Soil Ecology*, *15*(3), pp.261-272.

Venturi, V. and Keel, C., 2016. Signaling in the rhizosphere. *Trends in Plant Science*, 21(3), pp. 187-198.

Verma, J.P., Yadav, J., Tiwari, K.N. and Lavakush, S.V., 2010. Impact of plant growth promoting rhizobacteria on crop production. *Int J Agric Res*, *5*(11), pp.954-983.

Verma, M., Brar, S.K., Tyagi, R.D., Surampalli, R.Y. and Valero, J.R., 2007. Antagonistic fungi, *Trichoderma* spp.: panoply of biological control. *Biochemical Engineering Journal*, *37*(1), pp.1-20.

Vinale, F., Sivasithamparam, K., Ghisalberti, E.L., Marra, R., Barbetti, M.J., Li, H., Woo, S.L. and Lorito, M., 2008. A novel role for Trichoderma secondary metabolites in the interactions with plants. *Physiological and molecular plant pathology*, *72*(1-3), pp.80-86.

Waghunde, R.R., Shelake, R.M. and Sabalpara, A.N., 2016. *Trichoderma*: A significant fungus for agriculture and environment. *African Journal of Agricultural Research*, *11*(22), pp.1952-1965.

Wallace, J.G. and May, G., 2018. Endophytes: The Other Maize Genome. *In The Maize Genome* (pp. 213-246). Springer, Cham.

Wallington, T.J., Anderson, J.E., Mueller, S.A., Kolinski Morris, E., Winkler, S.L., Ginder, J.M. and Nielsen, O.J., 2012. Corn ethanol production, food exports, and indirect land use change. *Environmental Science & Technology*, *46*(11), pp. 6379-6384.

Walters, W.A., Jin, Z., Youngblut, N., Wallace, J.G., Sutter, J., Zhang, W., González-Peña, A., Peiffer, J., Koren, O., Shi, Q. and Knight, R., 2018. Large-scale replicated field study of maize rhizosphere identifies heritable microbes. *Proceedings of the National Academy of Sciences*, 115(28), pp. 7368-7373.

Wang, Z., Liu, L., Chen, Q., Wen, X. and Liao, Y., 2016. Conservation tillage increases soil bacterial diversity in the dryland of northern China. *Agronomy for sustainable development*, *36*(2), p.28.

Weller, R.F. and Bowling, P.J., 2007. The importance of nutrient balance, cropping strategy and quality of dairy cow diets in sustainable organic systems. *Journal of the Science of Food and Agriculture*, *87*(15), pp.2768-2773.

Wen, Z., Liao, W. and Chen, S., 2005. Production of cellulase by *Trichoderma reesei* from dairy manure. *Bioresource Technology*, *96*(4), pp.491-499.

Whipps, J.M. and Davies, K.G., 2000. Success in biological control of plant pathogens and nematodes by microorganisms. In *Biological control: measures of success* (pp. 231-269). Springer, Dordrecht.

Whipps, J.M., 2001. Microbial interactions and biocontrol in the rhizosphere. *Journal of experimental Botany*, *52*(suppl_1), pp.487-511.

Wickings, K., Grandy, A.S. and Kravchenko, A.N., 2016. Going with the flow: Landscape position drives differences in microbial biomass and activity in conventional, low input, and organic agricultural systems in the Midwestern US. *Agriculture, Ecosystems & Environment*, 218, pp. 1-10.

Williams, C.M., 2002. Nutritional quality of organic food: shades of grey or shades of green? *Proceedings of the Nutrition Society*, 61(1), pp. 19-24.

Williams, J., Clarkson, J.M., Mills, P.R. and Cooper, R.M., 2003. A selective medium for quantitative reisolation of *Trichoderma harzianum* from *Agaricus bisporus* compost. *Appl. Environ. Microbiol.*, *69*(7), pp.4190-4191.

Wu, H., Shabala, L., Zhou, M. and Shabala, S., 2014. Durum and bread wheat differ in their ability to retain potassium in leaf mesophyll: implications for salinity stress tolerance. *Plant and Cell Physiology*, *55*(10), pp.1749-1762.

Xiang, Ni, Lawrence, Kathy S, Kloepper, Joseph W, Donald, Patricia A & McInroy, John A. 2017. Biological control of Heterodera glycines by spore-forming plant growth-promoting rhizobacteria (PGPR) on soybean. *PloS one*. 12(7), p.e0181201.

Yadav, A.N., 2019. Endophytic Fungi for Plant Growth Promotion and Adaptation under Abiotic Stress Conditions. *Acta Scientific Agriculture*, 3, pp. 91-93.

Yadav, R.L., Shukla, S.K., Suman, A. and Singh, P.N., 2009. *Trichoderma* inoculation and trash management effects on soil microbial biomass, soil respiration, nutrient uptake and yield of ratoon sugarcane under subtropical conditions. *Biology and Fertility of soils*, *45*(5), pp.461-468.

Yan, Y., Kuramae, E.E., de Hollander, M., Klinkhamer, P.G. and van Veen, J.A., 2017. Functional traits dominate the diversity-related selection of bacterial communities in the rhizosphere. *The ISME journal*, 11(1), p. 56.

Yao, L., Teng, Y., Luo, Y., Christie, P., Ma, W., Liu, F., Wu, Y., Luo, Y. and Li, Z., 2015. Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by *Trichoderma reesei* FS10-C and effect of bioaugmentation on an aged PAH-contaminated soil. *Bioremediation Journal*, *19*(1), pp.9-17.

Yedidia, I., Srivastva, A.K., Kapulnik, Y. and Chet, I., 2001. Effect of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. *Plant and soil*, *235*(2), pp.235-242.

Zachow, C., Berg, C., Müller, H., Meincke, R., Komon-Zelazowska, M., Druzhinina, I.S., Kubicek, C.P. and Berg, G., 2009. Fungal diversity in the rhizosphere of endemic plant species of Tenerife (Canary Islands): relationship to vegetation zones and environmental factors. *The ISME journal*, *3*(1), pp.79-92.

Zeng, Z.Q. and Zhuang, W.Y., 2019. Two New Species and a New Chinese Record of *Hypocreaceae* as Evidenced by Morphological and Molecular Data. *Mycobiology*, *47*(3), pp.280-291.

Zhai, Y., Chen, L., Liu, G., Song, L., Arenas-Lago, D., Kong, L., Peijnenburg, W. and Vijver, M.G., 2021. Compositional and functional responses of bacterial community to titanium dioxide nanoparticles varied with soil heterogeneity and exposure duration. *Science of the Total Environment*, *773*, p.144895.

Zhang, F., Yuan, J., Yang, X., Cui, Y., Chen, L., Ran, W. and Shen, Q., 2013. Putative *Trichoderma harzianum* mutant promotes cucumber growth by enhanced production of indole acetic acid and plant colonization. *Plant and Soil*, *368*(1), pp.433-444.

Zhang, J., Feng, L., Ouyang, Y., Hu, R., Xu, H. and Wang, J., 2020. Phosphate-solubilizing bacteria and fungi in relation to phosphorus availability under different land uses for some latosols from Guangdong, China. *Catena*, *195*, p.104686.

Zhang, S., Gan, Y. and Xu, B., 2014. Efficacy of *Trichoderma longibrachiatum* in the control of *Heterodera avenae*. *BioControl*, *59*(3), pp.319-331.

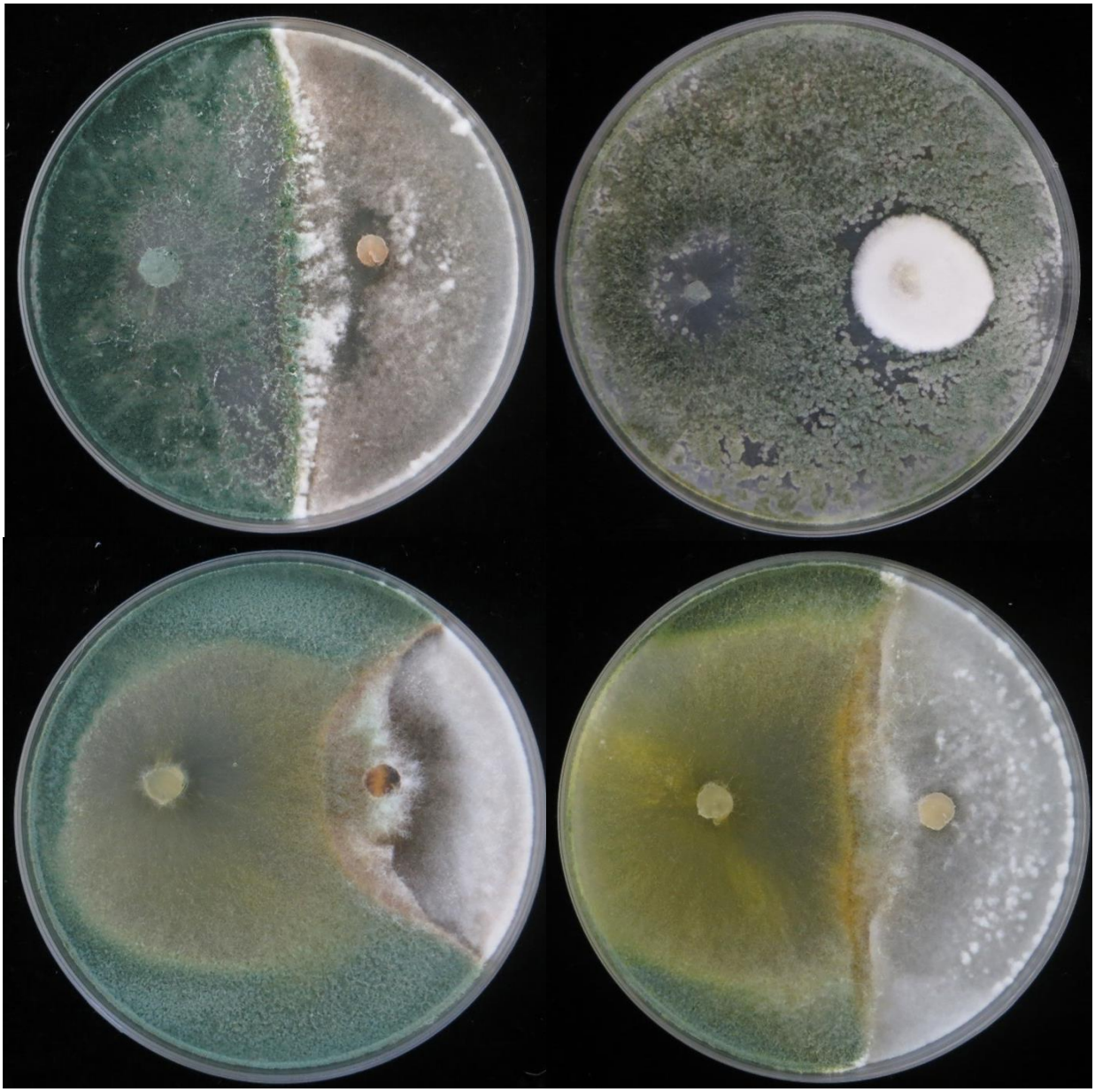
Zhang, S., Gan, Y. and Xu, B., 2016. Application of plant-growth-promoting fungi *Trichoderma longibrachiatum* T6 enhances tolerance of wheat to salt stress through improvement of antioxidative defense system and gene expression. *Frontiers in plant science*, *7*, p.1405.

Zhao, L. and Zhang, Y.Q., 2015. Effects of phosphate solubilization and phytohormone production of *Trichoderma asperellum* Q1 on promoting cucumber growth under salt stress. *Journal of Integrative Agriculture*, *14*(8), pp.1588-1597.

Zhou, L., Tang, K. and Guo, S., 2018. The plant growth-promoting fungus (PGPF) *Alternaria* sp. A13 markedly enhances Salvia miltiorrhiza root growth and active ingredient accumulation under greenhouse and field conditions. *International Journal of Molecular Sciences*, 19(1), p.270.

Zúñiga-Silgado, D., Rivera-Leyva, J.C., Coleman, J.J., Sánchez-Reyez, A., Valencia-Díaz, S., Serrano, M., de-Bashan, L.E. and Folch-Mallol, J.L., 2020. Soil type affects organic acid production and phosphorus solubilization efficiency mediated by several native fungal strains from Mexico. *Microorganisms*, *8*(9), p.1337.

Chapter 2: *Trichoderma* spp. isolated from rhizosphere soil of wheat in Western Cape, South Africa



Abstract

Wheat is the second most consumed staple crop in South Africa. However, from 2000 - 2019, South Africa's wheat output figures showed a decline. The decline in the production of wheat is mostly caused by factors such as a decline in soil fertility, climate change, and plant diseases. Synthetic fertilizers are used in the intensive agricultural practice to overcome these issues. However, it has been reported that this practice is not sustainable. Therefore, the implementation of other methods, such as conservation practices which are better for the environment, is required. Conservation practices allow the use of bio-fertilizers developed from different microorganisms to be used as an alternative to agrochemicals, and these include *Trichoderma*. *Trichoderma* have already been used for plant protection and development in a number of cropping systems. In this study, 91 strains of *Trichoderma* spp. were isolated from the wheat rhizospheres, under different management practices (crop rotations and monoculture) and identified using molecular and taxonomy methods. Seven *Trichoderma* species were identified namely *T. gamsii, T. koningiopsis, T. spirale, T. saturnisporum*, *T. velutinum*, *T. virens*, and *Trichoderma* sp NNC105. *T. gamsii* was found to be the most dominant species in all agricultural practices. *T. velutinum* was reported for the first time in South Africa. Other *Trichoderma* species isolated were previously reported from South Africa. Overall, it was noted that fields under crop rotation resulted in a higher number of species compared to fields under monoculture. The isolation and identification of *Trichoderma* species in South Africa is needed since we have limited knowledge in the diversity and distribution of this genus in this region. Furthermore, regional *Trichoderma* strains could open other paths to further develop *Trichoderma* based products for use as bio-fertilizers and biocontrol agents.

Introduction

Trichoderma spp. (*Hypocreaceae*) was described by Persoon 227 years ago (Samuels, 1996; Zeng and Zhuang, 2019). This group of fungi are known to produce industrial important enzymes, control plant diseases and improve plant growth (Harman *et al.*, 2004; Kubicek *et al*., 2008; Sadfi-Zouaoui *et al.*, 2009). Bissett *et al.* (2015) recognized 254 species of *Trichoderma*, and this has now increased to 375 species currently known (Du Plessis *et al*., 2018; Zeng and Zhuang, 2019; Cai and Druzhinina, 2021). *Trichoderma* spp. are commonly found in all types of habitats such as natural soils, decaying wood (Hosseyni-Moghaddam and Soltani, 2014), plant material, agricultural habitats, living plants (Cummings *et al.*, 2016), the human body, water-related environments, air and settled dust (Samuels, 1996; Jaklitsch *et al.*, 2006; Mouton *et al*., 2012; Kredics *et al*., 2014).

Trichoderma strains have the ability to colonize the inner parts of plant tissues and play a crucial role in plant development (Kredics *et al*., 2014; Cummings *et al*., 2016). These strains produce various compounds inside plant tissues that can induce systemic resistance, which ultimately aid in enabling plants to fight against pathogenic microorganisms. For example, *T. virens* has been reported to produce peroxidases and synthesize tepernoids which contribute to the induction of host resistance (Baek *et al*., 1999; Howell *et al*., 2000). In addition, some strains compete and parasitize other pathogenic or non-pathogenic microorganisms (Kubicek *et al*., 2008; Cummings *et al.*, 2016). *Trichoderma* spp. can, therefore, have a beneficial effect on plants while controlling diseases caused by microorganisms.

Secondary metabolites produced by *Trichoderma* spp. can also have a positive effect on plants (Silva *et al.*, 2019). These secondary metabolites play a significant role in controlling soil-borne diseases and also promote plant growth (Hosseyni-Moghaddam and Soltani, 2014). In addition, these secondary metabolites can also be used in other areas such as medicine and the industrial sector (Mukherjee *et al.*, 2013; Frisvad *et al.*, 2018).

To date, 28 *Trichoderma* species have been reported from South Africa (Du Plessis, 2018). Bisby was the first person to isolate *Trichoderma* strains in South Africa (Bisby, 1939). Other research followed, although most of them focused on evaluating the potential biocontrol of *Trichoderma* species (Askew and Laing, 1994 a, b; Kotze *et al*., 2011; Mutawila *et al*., 2011). In another study, *Trichoderma* species were isolated during surveys of fungi occurring on diseased *Acacia mearnsii* in South Africa (Roux and Wingfield, 1997). Their findings showed that *Trichoderma* were excluded from those fungi causing disease since they formed no lesions. Mouton *et al.* (2012) also reported the presence of *Trichoderma* sp. in a study conducted to evaluate culturable fungi from marine environments. A number of large

monographic studies that focused on diversity of *Trichoderma* species, included strains from South Africa (Jaklitsch *et al*., 2006; Druzhinina *et al*., 2008; Kubicek *et al*., 2008; De Respinis *et al*., 2010; Druzhinina *et al*., 2010). To date, only one survey was conducted to study the diversity of *Trichoderma* in South Africa (Du Plessis *et al*., 2018).

Trichoderma spp. are extensively used for their beneficial characteristics. Some studies suggested that locally sourced strains may be more effective than imported strains (Phua *et al*., 2011; Roese *et al*., 2017). In South Africa, *Trichoderma* diversity has not been extensively investigated (Du Plessis *et al*., 2018) for use in commercial products. The most abundant species found in South Africa include *T. harzianum*, *T. viride*, *T. orientalis* and *T. saturnisporum* (Du Plessis *et al*., 2018). Du Plessis *et al*. (2018) isolated 161 strains of *Trichoderma* from natural soils and found some species to be novel, and in some cases endemic to this region.

The aim of this study was, therefore, to isolate and identify *Trichoderma* species from rhizosphere of wheat plants in Western Cape, South Africa. Rhizosphere was selected because it composed of various types of organisms which varies from microorganisms to insects (McNear Jr, 2013; Benitez *et al*., 2017). In addition, it is recognized that this environment allows for a variety of processes to occur (Kalam *et al*., 2017; Benitez *et al*., 2017), with a strong selective pressure from the plant.

Materials and Methods

Collection of soil samples and isolation

Figure 2. 1. Map indicating the sampling sites (scale bar = 50 km)

Soil was collected from three wheat farms (Langgewens, Hopefield, and Tygerhoek) in April (Pre-Plant), June (Germination), July (Top dressing), and October 2019 (at Harvest) from the Western Cape province, South Africa (Fig. 2.1. & Table 2.1). Samples were taken from the same spot or location throughout, and 3 replicates samples were taken from each camp. Rhizosphere soil was sampled by removing plants with their roots. The samples were then placed in sterile polyethene bags and kept at 4°C before processing in the lab. For each sample about 10 g of soil was mixed with 100 ml saline solution in sterile conical flasks. The soil suspension was left on a shaker at 26°C, 121 rpm for 1 hr. The dilutions were prepared using saline solution from 1×10^{-1} to 1×10^{-3}, and from each dilution 0.1 ml mixture was spread onto PDA medium (Neogen, UK) supplemented with antibiotics namely 50 ppm dichloran, 50 ppm chloramphenicol and 100 ppm streptomycin (Applichem, SA). The plates were incubated at 26°C for 7

days. The plates were observed under a stereo microscope (NIKON SMZ800, Japan) and all colonies that resembled *Trichoderma* were sub-cultured onto malt extract agar (MEA) (Biolab, Merck, Modderfontein) (Crous *et al*., 2009; Jacklitsch, 2009; Du Plessis *et al*., 2018).

Distribution of *Trichoderma* species under different agricultural practices

Sites for isolation represent two agricultural practices namely crop rotation and monoculture (Table 2.1). The *Trichoderma* species distribution was analysed by calculating percentages of strains isolated from each practice using Microsoft Excel 2016.

Table 2. 1. Agricultural practices used for the isolation of *Trichoderma* spp. on different sites

Farm names	Practice	Treatment	GPS coordinate
Langgewens	Crop rotation	Wheat after canola	S33°16.996'E018°42.414
		Wheat after medic	S33°17.017'E018°42.434
	Monoculture	Wheat after wheat	S33°16.906'E018°42.484
Tygerhoek	Crop rotation	Wheat after canola	S34°09.913'E019°54.582
		Wheat after medic	S34°09.863'E019°54.559
	Monoculture	Wheat after wheat	S34°09.900'E019°54.585
Hopefield	Crop rotation	Wheat after canola	S33°02.108'E018°26.195
		Wheat after medic	S33°01.924'E018°26.222

DNA extraction, PCR and Sequencing

Genomic DNA was extracted from *Trichoderma* cultures grown on MEA using bacterial/fungal DNA Mini-Prep kit (Zymo research, USA) according to manufacturer's instructions. Polymerase Chain Reactions (PCRs) were conducted as described by White *et al*. (1990) using the following primers; ITS1 and ITS4 (White *et al*., 1990) to amplify the ITS1- 5.8s- ITS2 rDNA region and EF1F and EF2R (Jacobs *et al*., 2004; Du Plessis *et al*., 2018) to amplify the partial elongation factor 1α gene, respectively. PCR reactions were set up in 10 µl volumes, which consisted of the following, 5 µl Kapa *Taq* Ready mix (KM 1000, KAPA Biosystem), 0.2 µl of each primer (0.2mM), 0.5ng of gDNA template, and 4.1 µl milliQ H_2O. The thermal cycle for ITS were set up with an initial denaturing step of 94 ℃ for 5 minutes followed by 40 cycles consisting of 30 seconds denaturing at 94 ℃, 30 seconds annealing at 56 ℃ and

45 seconds extending at 72 ℃ and a final extension step of 7 minutes at 72 ℃ was used. The EF1α thermal cycle were set up with an initial denaturing step at 96 ℃ for 5 minutes followed by 40 cycles consisting of 30 second denaturing at 94 ℃, 30 seconds annealing at 51 ℃, and 90 seconds extending step at 72 ℃, with a final extension step at 72 ℃ for 5 minutes. Sequencing reactions were set up in 10 µl volume with the following: 1 µl DNA (amplified DNA), 1.25 µl Buffer, 1 µl BigDye, and 1 µl forward primer (0.2mM) with 5.75 µl H_2O. Thermal cycle conditions were set up with an initial denaturing at 96 ℃ for 1 minute followed by 25 cycles of denaturing at 96 ℃ for 10 seconds, annealing at 50 ℃ for 10 seconds and extension step at 60 ℃ for 4 minutes. Sequence reaction products were analysed using an ABI Prism 310 genetic analyser at Central Analytical Facilities (CAF, Stellenbosch University).

Phylogenetic analyses

Sequences were opened and trimmed using Chromas 2.6.6 version (Technelysium, DNA Sequencing Software, Australia) (Available from: http://technelysium.com.au/wp/) and compared to the National Center for Biotechnology Information (NCBI) database using a blast-n search option. Species isolated from agricultural soils were compared to the ex- type strains based on previous studies (Jacklitsch, 2009; Bissett *et al*., 2015; Du Plessis *et al*., 2018; Inglis *et al*., 2020). Sequences were aligned using MAFFT from Geneious Prime 2021.0.3 (Kearse *et al*., 2012; Katoh and Standley, 2013). Thereafter, the EF1α and ITS1 datasets were concatenated. Mega-X (Kumar *et al*., 2018) was used to construct maximum likelihood phylogenetic trees, where branched strengths were assessed by bootstrap using 1000 replicates.

Morphological characterization

Trichoderma species were grown on PDA, SNA, and CMA (Fluka Analytical, Sigma-aldrich, USA) with 2% D (+) glucose monohydrate (KIMIX, Chemicals and Lab Suppliers) for seven days at 26 ℃. Harris (2000) modified tape method was used to prepare the microscope slides. All the microscope slides were prepared from 7 day old cultures. Conidiophores, conidia, and phialide structures were viewed using a compound microscope (Nikon Eclipse E800, Japan) with differential interference contrast capabilities and a CFI plain Apochromat VC 100X lens.

Results

Isolation of *Trichoderma* species

A total number of 91 strains were isolated from wheat rhizosphere soil in Western Cape Province in South Africa (Table 2.3). These strains resulted in 7 *Trichoderma* species and were identified as *Trichoderma velutinum*, *Trichoderma* sp NNC105, *Trichoderma virens*, *Trichoderma spirale*, *Trichoderma gamsii*, *Trichoderma koningiopsis*, and *Trichoderma saturnisporum*, respectively (Table 2.2 and Table 2.3). These species resolved in four (4) clades, namely the *Harzianum* clade, *Virens* clade, *Viride* clade, and *Longibrachiatum* clade (Table 2.2). All the identifications of species were performed using morphological features and phylogenetic analysis.

No *Trichoderma* species were isolated at Hopefield farm during any of the sampling times. Tygerhoek and Langgewens sampling resulted in 30 and 61 strains, respectively. The five species isolated at Tygerhoek were *T. virens, T. spirale, T. saturnisporum, Trichoderma* sp NNC105, and *T. gamsii*. At Langgewens we isolated four *Trichoderma* species namely *T. gamsii, T. saturnisporum, T. koningiopsis*, and *T. velutinum*. Moreover, wheat after wheat, wheat after canola, and wheat after medic isolated 4, 4, and 6 *Trichoderma* spp. respectively (Fig. 2.2). *T. gamsii* and *T. saturnisporum* were widely distributed across all sites (Fig. 2.2).

Distribution of *Trichoderma* spp. at different agricultural practices

Monoculture resulted in 23% of the total number of strains (Table 2.3 and Fig. 2.3). On the other hand, crop rotation resulted in 77% of the total number of strains (Table 2.3 and Fig. 2.4). Only four *Trichoderma* species, *T. spirale, T. saturnisporum, T. velutinum,* and *T. gamsii* (Table 2.3 & Fig. 2.2), were isolated from fields where monoculture is practiced. Crop rotation resulted in seven *Trichoderma* species. All species that were isolated from monoculture sites were also reported under crop rotation with the additional species identified as, *T. virens, Trichoderma* sp. NNC105, and *T. koningiopsis* (Table 2.3 & Fig. 2.2).

T. gamsii was found to be the most dominant species across all sites, since it resulted in a higher number of strains both from monoculture (9) and crop rotations (25) (Table 2.3). The fewest number of strains in the monoculture system was *T. spirale* and *T. saturnisporum*, whereas in the crop rotation system it was *Trichoderma* sp NNC105 with only one strain (Table 2.3 and Fig. 2.4). Overall, it appears that different farming practices select for different species consortia.

Table 2. 2. Sections and species obtained in this study

Sections	Clades	Species names	First report in SA
Pachybasium	*T. harzianum*	*T. velutinum*	YES
		Trichoderma sp.	YES
Trichoderma	*T. virens*	*T. virens*	NO
		T. spirale	NO
	T. viride	*T. gamsii*	NO
		T. koningiopsis	NO
Longibrachiatum	*T. longibrachiatum*	*T. saturnisporum*	NO

Table 2. 3. Number of *Trichoderma* strains from different practices

***Trichoderma* spp.**	**Number of strains per practice**	
	Monoculture	Crop rotation
T. virens	0	13
T. spirale	2	2
T. saturnisporum	2	11
T. velutinum	8	6
T. gamsii	9	25
T. koningiopsis	0	12
Trichoderma sp.	0	1
Total	**21**	**70**

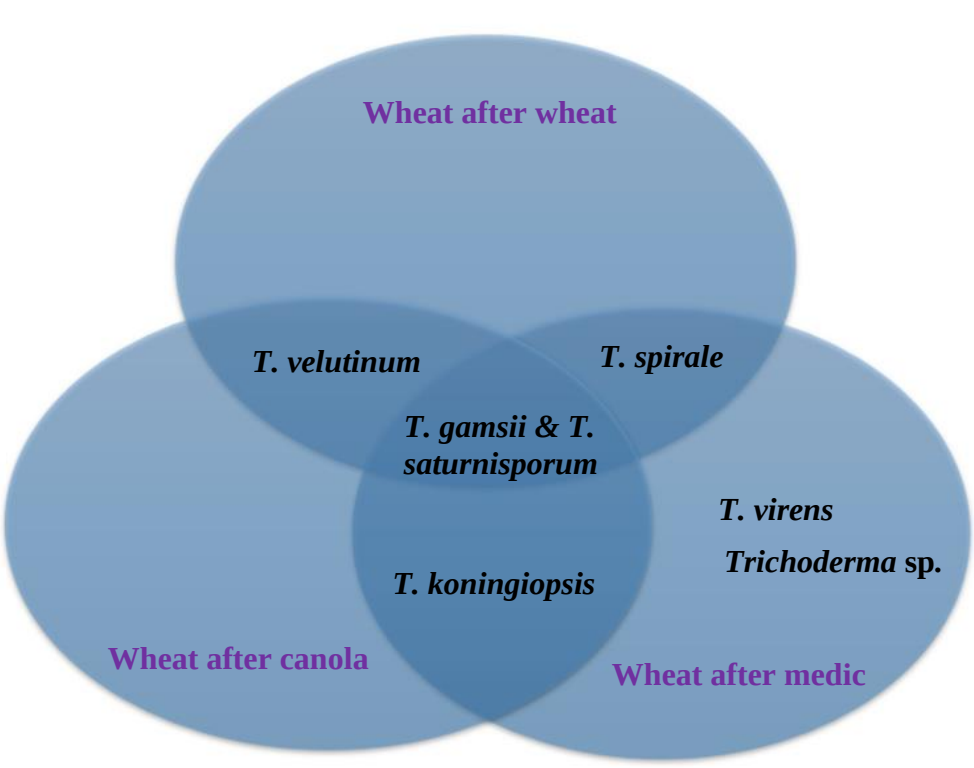


Figure 2. 2. Representation of *Trichoderma* spp. at different treatments used in agricultural practices (crop rotation and monoculture)

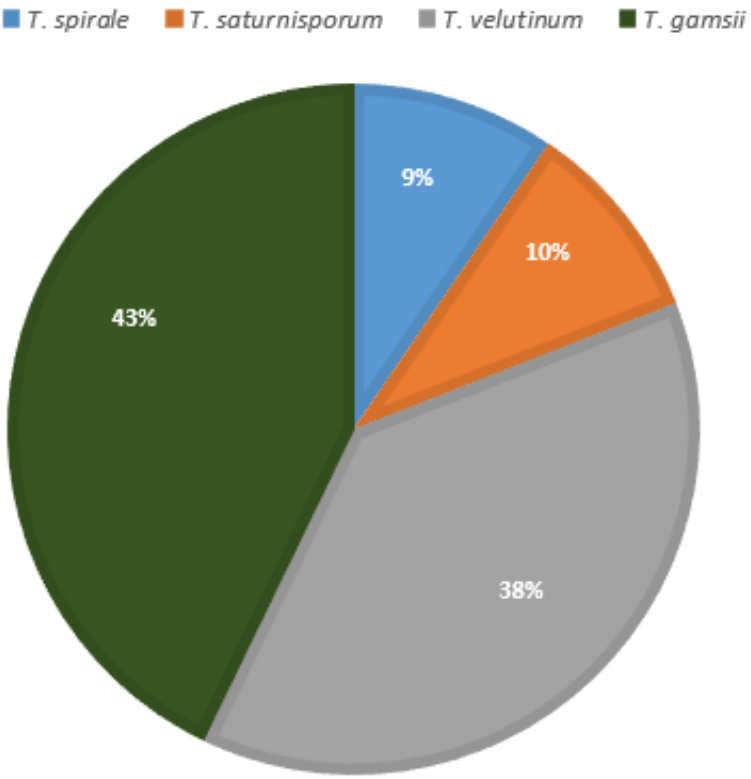


Figure 2. 3. Distribution of *Trichoderma* species under monoculture

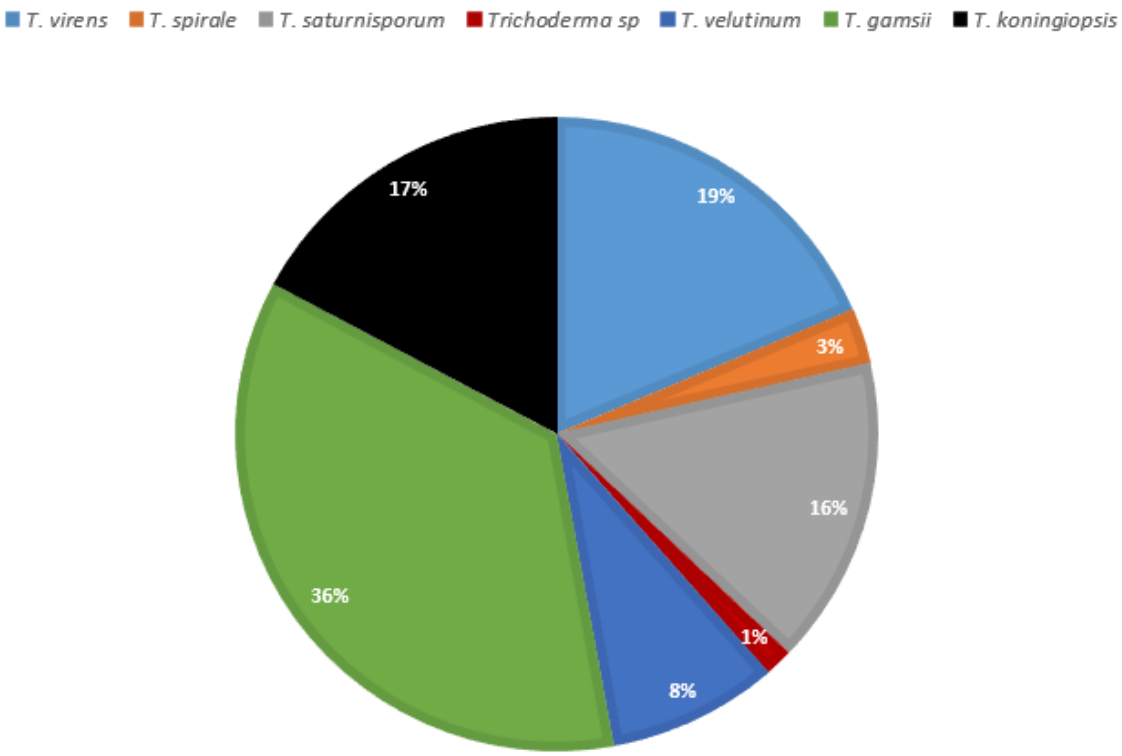


Figure 2. 4. Distribution of *Trichoderma* species under crop rotation

Phylogenetic analyses

The ITS1 and TEF1 markers were combined for construction of the maximum likelihood phylogenetic trees. The initial identification was based on morphological features and clade classification was based on TEF1 gene sequences. The resulting sequences had approximately 540 bp and 700 bp for ITS1 and TEF1, respectively. All clades were supported by the significant bootstrap values greater than 75 based on the phylogenetic analyses using the two genes.

Trichoderma longibrachiatum clade in the current study only consists of *T. saturnisporum* (Fig. 2.5). All strains from this clade grouped with ex-type and other reference *T. saturnisporum* strains that were previously identified. *T. citrinoviride* and *T. pseudokoningii* are phylogenetically closely related to *T. saturnisporum* but were not isolated in this study.

Trichoderma harzianum clade consists of two species identified as *T. velutinum* and *Trichoderma* sp. (Fig. 2.7). This is the first report of *T. velutinum* from South Africa (Fig. 2.9). *T. velutinum* clustered with other strains that were recognized in previous studies. In addition, only one strain (NNC105) grouped separately within *T. harzianum* complex species in this study. This strain could potential be a novel species in this complex since it clustered separately from others within this clade (Fig. 2.7).

The *Trichoderma virens* clade composed of *T. spirale* and *T. virens* (Fig. 2.6). *T. spirale* strains that were identified previously grouped with all of the strains that were obtained in this study. *T. spirale* obtained from this study formed a sub-clade and are phylogenetically closely related to *T. hunanense*. *T. virens* strains isolated in the current study grouped with other reference strains of *T. virens* and are phylogenetically closely related to their sister clade *T. crassum*.

Trichoderma viride clade in the current study resulted in two species and were assigned to *T. gamsii* and *T. koningiopsis* (Fig. 2.8). *T. gamsii* strains from this study clustered together with ex-type and reference strains of *T. gamsii* that were previously identified. *T. koningiopsis* strains from this study also grouped with ex-type strain and other *T. koningiopsis* reference strains that were isolated from previous studies (Samuels *et al*., 2006; Du Plessis *et al*., 2018). *T. koningiopsis* are phylogenetically closely relate to *T. ovalisporum* and *T. viride* (Fig. 2.8).

Table 2. 4. Strains and accession numbers for ITS1 and TEF1 sequences used in phylogenetic tree of *T. longibratium* clade, *T. virens* clade, *T. viride* clade, and *T. harzianum* clade

Taxon	Strain no.	NCBI GeneBank accession numbers ITS1	TEF1
T. saturnisporum	ATCC 28023	X93977	JN388897
T. saturnisporum	CBS 886.72	X93974	AY937414
T. saturnisporum	PPRI 20675	KX267811	KX267790
T. saturnisporum	IMI146852		AY865642
T. saturnisporum	DAOM:220787		KJ713203
T. saturnisporum	**NNC060**	**MZ677275**	**MZ869089**
T. saturnisporum	**NNC056**	**MZ677277**	**MZ826874**
T. saturnisporum	**NNC086**	**MZ677276**	**MZ869079**
T. saturnisporum	**NNC061**	**MZ677278**	**MZ826881**
T. saturnisporum	**NNC030**	**MZ677282**	**MZ826873**
T. saturnisporum	**NNC002**	**MZ604904**	**MZ707793**
T. saturnisporum	**NNC033**	**MZ677279**	**MZ826879**
T. saturnisporum	**NNC029**	**MZ677283**	**MZ826872**
T. saturnisporum	**NNC032**	**MZ677280**	**MZ826878**
T. saturnisporum	**NNC031**	**MZ677281**	**MZ826877**
T. saturnisporum	**NNC001**	**MZ604903**	**MZ707792**
T. saturnisporum	**NNC003**	**MZ604905**	**MZ707794**
T. saturnisporum	**NNC107**	**MZ677274**	**MZ869098**
T. longibrachiatum	PPRC S58	EU401568	EU401618
T. longibrachiatum	CBS 816.68	EU401556	EU401591
T. andenensis	DAOM 220821		EU280042
T. andenensis	GJS 90-140		AY956321
T. citronoviride	DAOM 139758	EU330960	EU338334
T. citronoviride	GJS 90-111		JN175591
T. pseudokoningii	GJS 81-300	DQ083025	AY937429
T. pseudokoningii	DAOM 167678	EU280097	EU280037
T. rossicum	DAOM 230011	HQ342419	AY937441
T. rossicum	DAOM 233977	EU280089	EU280062
T. flagellatum	PPRC-ET7		FJ763149
T. flagellatum	PPRC-ET58		FJ763184
T. ghanense	DAOM 220800	EU280100	EU280043
T. ghanense	GJS 95-137	NR 120299	AY937423
T. parareesei	TUB F-430		GQ354351
T. parareesei	TUB F-1066		GQ354353

Table 2.4. (continued)

Taxon	Strain no.	NCBI Gene Bank accession numbes	
		ITS1	TEF1
T. pinnatum	GJS 02-120		JN175572
T. pinnatum	GJS 04-100		JN175571
T. orientale	G.J.S 88-81	EU401550	EU401581
T. orientale	G.J.S 91-157	EU401558	EU401593
T. koningii	CBS 457.96	MH862585	AF456909
T. koningii	CBS 979.70	DQ323410	DQ288994
T. koningii	GJS 00-168	DQ323427	DQ307571
T. koningii	GJS 89-122	AY380902	AY376045
T. koningii	GJS 96-120	DQ109536	DQ109548
T. koningii	GJS 96-119	DQ323424	DQ289003
T. koningii	PPRI20670	KX267804	KX267783
T. gamsii	TRS 580	KP009332	KP008925
T. gamsii	GJS 05-111	DQ841730	DQ841722
T. gamsii	GJS 06-09		KT028598
T. gamsii	GJS 92-60	DQ315448	DQ307529
T. gamsii	PPRI 20680	KX267817	KX267796
T. gamsii	GJS 04-09	DQ315459	DQ307541
T. gamsii	**NNC040**	**MZ695257**	**MZ869068**
T. gamsii	**NNC053**	**MZ695260**	**MZ869085**
T. gamsii	**NNC050**	**MZ695251**	**MZ869083**
T. gamsii	**NNC049**	**MZ695248**	**MZ869086**
T. gamsii	**NNC036**	**MZ695262**	**MZ869076**
T. gamsii	**NNC038**	**MZ695259**	**MZ869078**
T. gamsii	**NNC052**	**MZ695261**	**MZ826884**
T. gamsii	**NNC055**	**MZ695256**	**MZ826882**
T. gamsii	**NNC039**	**MZ695254**	**MZ869082**
T. gamsii	**NNC037**	**MZ695258**	**MZ826887**
T. gamsii	**NNC047**	**MZ695250**	**MZ869088**
T. gamsii	**NNC045**	**MZ695249**	
T. gamsii	**NNC051**	**MZ695252**	**MZ869084**
T. gamsii	**NNC046**	**MZ695255**	
T. gamsii	**NNC043**	**MZ695247**	
T. gamsii	**NNC048**	**MZ695253**	**MZ869074**
T. gamsii	**NNC034**	**MZ695246**	**MZ826876**
T. gamsii	**NNC115**	**MZ695245**	**MZ869103**
T. gamsii	**NNC063**		**MZ869071**
T. gamsii	**NNC062**	**MZ695241**	**MZ869090**
T. gamsii	**NNC019**	**MZ695233**	**MZ816943**

Table 2.4. (continued)

Taxon	Strain no.	NCBI Gene Bank accession numbers ITS1	TEF1
T. gamsii	**NNC057**	**MZ695239**	**MZ869077**
T. gamsii	**NNC021**	**MZ695235**	**MZ826864**
T. gamsii	**NNC110**	**MZ695244**	**MZ869099**
T. gamsii	**NNC104**	**MZ695242**	**MZ869096**
T. gamsii	**NNC020**	**MZ695234**	**MZ826863**
T. gamsii	**NNC106**	**MZ695238**	
T. gamsii	**NNC023**	**MZ695237**	**MZ826866**
T. gamsii	**NNC022**	**MZ695236**	**MZ826865**
T. gamsii	**NNC058**	**MZ695240**	
T. gamsii	**NNC103**	**MZ695243**	
T. koningiopsis	**NNC112**	**MZ677316**	**MZ869101**
T. koningiopsis	**NNC113**	**MZ677315**	**MZ869102**
T. koningiopsis	**NNC080**	**MZ677319**	**MZ869093**
T. koningiopsis	**NNC068**	**MZ677326**	**MZ826880**
T. koningiopsis	**NNC067**	**MZ677320**	**MZ869087**
T. koningiopsis	**NNC076**	**MZ677317**	**MZ869091**
T. koningiopsis	**NNC079**	**MZ677318**	**MZ869092**
T. koningiopsis	**NNC066**	**MZ677322**	**MZ869072**
T. koningiopsis	**NNC069**	**MZ677321**	**MZ869080**
T. koningiopsis	**NNC099**	**MZ677323**	**MZ826875**
T. koningiopsis	**NNC070**	**MZ677324**	**MZ826886**
T. koningiopsis	**NNC081**	**MZ677325**	**MZ826885**
T. koningiopsis	GJS 91-6	DQ313135	DQ307539
T. koningiopsis	GJS 95-175	AF456923	AF456910
T. koningiopsis	PAN12-49	MK322716	MK16070
T. koningiopsis	GJS 93-20	DQ313140	DQ284966
T. koningiopsis	GJS 91-7	DQ313137	DQ284969
T. koningiopsis	PPRI20683	KX267820	KX267799
T. koningiopsis	GJS 04-373	DQ323437	DQ289006
T. koningiopsis	GJS 04-10	DQ323413	DQ284981
T. koningiopsis	GJS 04-314	FJ463269	FJ442655
T. koningiopsis	GJS 06-263	FJ442613	FJ467647
T. neokoningii	GJS 04-216	DQ841734	DQ841718
T. neokoningii	CBS: 120070	MH863076	KJ665620
T. viridescens	DAOM 233967	EU280137	EU280020
T. viridescens	DAOM 237554	EU280135	EU280026

Table 2.4. (continued)

Taxon	Strain no.	NCBI Gene Bank accession numbers ITS1	TEF1
T. viridescens	DAOM 234234		EU280009
T. viridescens	CIBT10	EU280104	EU279999
T. viridescens	CBC 433.34	AY380905	AY376048
T. ovalisporum	DAOM: 232077		KJ871200
T. ovalisporum	DAOM:229859	EU2801138	EU280003
T. ovalisporum	Dis 172i	DQ323438	DQ288999
T. ovalisporum	Dis 203c	DQ315458	DQ307540
T. ovalisporum	Dis 70a	AY380897	AY376037
T. viride	TR2	DQ215457	DQ307538
T. viride	CBS119327	DQ677657	DQ672617
T. viride	PPRI20672	KX267807	KX267786
T. atroviride	GJS 91-87	AF456919	AF456902
T. atroviride	STE-U6514	FJ232696	FJ232698
T. atroviride	GJS 98-134	AF456913	AF456887
T. atroviride	PPRI20685	KX267822	KX267801
T. paratroviride	CBS:136489 S385		KJ665627
T. strigosum	DAOM 166121	DQ083027	EU280019
T. strigosum	DAOM 166121	EU280120	AY937442
T. strigosellum	CPK 3604		JQ425705
T. tomentosum	DAOM:178713	AF011984	KJ871247
T. tomentosum	DAOM178713A	EU330958	EU279969
T. tomentosum	DAOM 234236	EU280083	EU279971
T. tomentosum	DAOM 171918	AY605715	AY605759
T. tomentosum	DAOM 178713a	NR 134357	AY750882
T. cerinum	DAOM 230012		KJ871242
T. cerinum	MA 3646	AJ507139	AY605823
T. ceraceum	GJS 95-159	AF275332	AY937437
T. harzianum	CBS 227.95	AF057605	AF348100
T. harzianum	T55	KX632511	KX632625
T. peberdyi	CEN 1426	MK714906	MK696664
T. peberdyi	CEN1425	MK714905	MK696663
T. tawa	DAOM 232841		EU279972
T. tawa	GJS 97-174	AY737756	AY737739
T. afroharzianum	GJS 04-186	FJ442265	FJ463301
T. afroharzianum	PPRI20674	KX267809	KX267788
T. afroharzianum	GJS 04-193	FJ442233	FJ463298

Table 2.4. (continued)

Taxon	Strains no.	NCBI Gene Bank accession numbers ITS1	TEF1
T. velutinum	MIAE00044	HM176574	HM176592
T. velutinum	38.24.06.3	KP009269	KP008910
T. velutinum	30.24.06.3	KP009268	KP008909
T. velutinum	MIAE00041	HM176571	HM176589
T. velutinum	DAOM230014	DQ083010	AY605804
T. velutinum	LESF132	KT278865	KT279019
T. velutinum	MIAE00033	HM176563	HM176581
T. velutinum	MIAE00036	HM176566	HM176584
T. velutinum	**NNC073**	**MZ677308**	**MZ826883**
T. velutinum	**NNC074**	**MZ677313**	**MZ869081**
T. velutinum	**NNC075**	**MZ677312**	**MZ869075**
T. velutinum	**NNC065**	**MZ677311**	**MZ869073**
T. velutinum	**NNC116**	**MZ677314**	**MZ869104**
T. velutinum	**NNC064**	**MZ677310**	**MZ869070**
T. velutinum	**NNC059**	**MZ677309**	**MZ869069**
T. velutinum	**NNC026**	**MZ677305**	**MZ826869**
T. velutinum	**NNC018**	**MZ677302**	**MZ816942**
T. velutinum	**NNC017**	**MZ677301**	**MZ816941**
T. velutinum	**NNC027**	**MZ677306**	**MZ826870**
T. velutinum	**NNC028**	**MZ677307**	**MZ826871**
T. velutinum	**NNC024**	**MZ677303**	**MZ826867**
T. velutinum	**NNC025**	**MZ677304**	**MZ826868**
T. rafaii	DIS 355B	FJ442663	FJ463324
T. rafaii	Dis 337F	FJ442621	FJ463321
T. camerunense	GJS 99-231	AY027783	AF348108
T. camerunense	GJS 99-230	AY027780	AF348107
T.afarasin	DIS 314F	FJ442259	FJ463400
T.afarasin	GJS 06-98	FJ442630	FJ463327
T.afarasin	GJS 99-227	AY027784	AF348093
T. crassum	DAOM:167063	AF011947	AY750892
T. crassum	CS570-5	KR911899	KR911896
T. crassum	TAMA 0238	AB856632	AB856704
T. crassum	TAMA 0232	AB856628	AB856700
T. crassum	TRS113	KP009300	KP008865
T. crassum	DAOM164916	EU280067	EU280048
T. crassum	CBS 336.93	AF011946	AF401021

Table 2.4. (continued)

Taxon	Strain no.	NCBI Gene Bank accession numbers ITS1	TEF1
T. virens	GJS 01-287	DQ083023	AY750894
T. virens	GJS 95-80	FJ442218	FJ463365
T. virens	GLI 39	AF099005	GU591800
T. virens	PPRI20676	KX267812	KX267791
T. virens	DAOM:167652	EU330955	AY750891
T. virens	GJS 06-114	FJ442632	FJ463364
T. virens	GLI 39	AF099005	AF534631
T. virens	**NNC011**	**MZ677291**	**MZ816933**
T. virens	**NNC014**	**MZ677294**	**MZ816936**
T. virens	**NNC004**	**MZ677284**	**MZ816926**
T. virens	**NNC008**	**MZ677288**	**MZ816930**
T. virens	**NNC012**	**MZ677292**	**MZ816934**
T. virens	**NNC005**	**MZ677285**	**MZ816927**
T. virens	**NNC007**	**MZ677287**	**MZ816929**
T. virens	**NNC009**	**MZ677289**	**MZ816931**
T. virens	**NNC006**	**MZ677286**	**MZ816928**
T. virens	**NNC013**	**MZ677293**	**MZ816935**
T. virens	**NNC010**	**MZ677290**	**MZ816932**
T. virens	**NNC109**	**MZ677295**	**MZ816937**
T. spirale	DAOM 183974	AF011988	AF534626
T. spirale	TRS111	KP009301	KP008963
T. spirale	DAOM183974	EU280068	EU280049
T. spirale	PAN12-65	MK322728	MK516099
T. spirale	CBS 120963	FJ442608	FJ463291
T. spirale	PPRI 20681	KX267818	KX267797
T. spirale	DAOM229883	EU280082	EU280050
T. spirale	**NNC108**	**MZ677298**	
T. spirale	**NNC111**	**MZ677299**	**MZ869100**
T. spirale	**NNC016**	**MZ677297**	**MZ816940**
T. spirale	**NNC015**	**MZ677296**	**MZ816939**
Trichoderma sp.	**NNC105**	**MZ677300**	**MZ869097**
T. longipile	DAOM 177227	NR 134354	AY937430
T. longipile	CBS 120953	FJ860770	FJ860643
T. longipile	GJS 91-93	AY737763	AY737727
T. fasciculatum	DAOM 167646	DQ087258	AY750895
T. longisporum	HMAS 248843	NR154573	KY688043
T. hunanense	HMMA 248841	NR 154571	KY688039

Table 2.4. (continued)

Taxon	Strain no.	NCBI Gene Bank accession numbers ITS1	TEF1
T. hunanense	HMMA 248867	KY687950	KY688040
T. helicum	DAOM 230016	DQ083022	EU280055
T. strictipile	DAOM 172827	AF011980	AY937451
T. azevedoi	CEN 1403	MK714880	MK696638
T. azevedoi	CEN 1422	MK714902	MK696660
Protocrea farinosa	CBS 121551	EU703910	EU703889

*****_Trichoderma_ strains isolated in this study are in boldface.

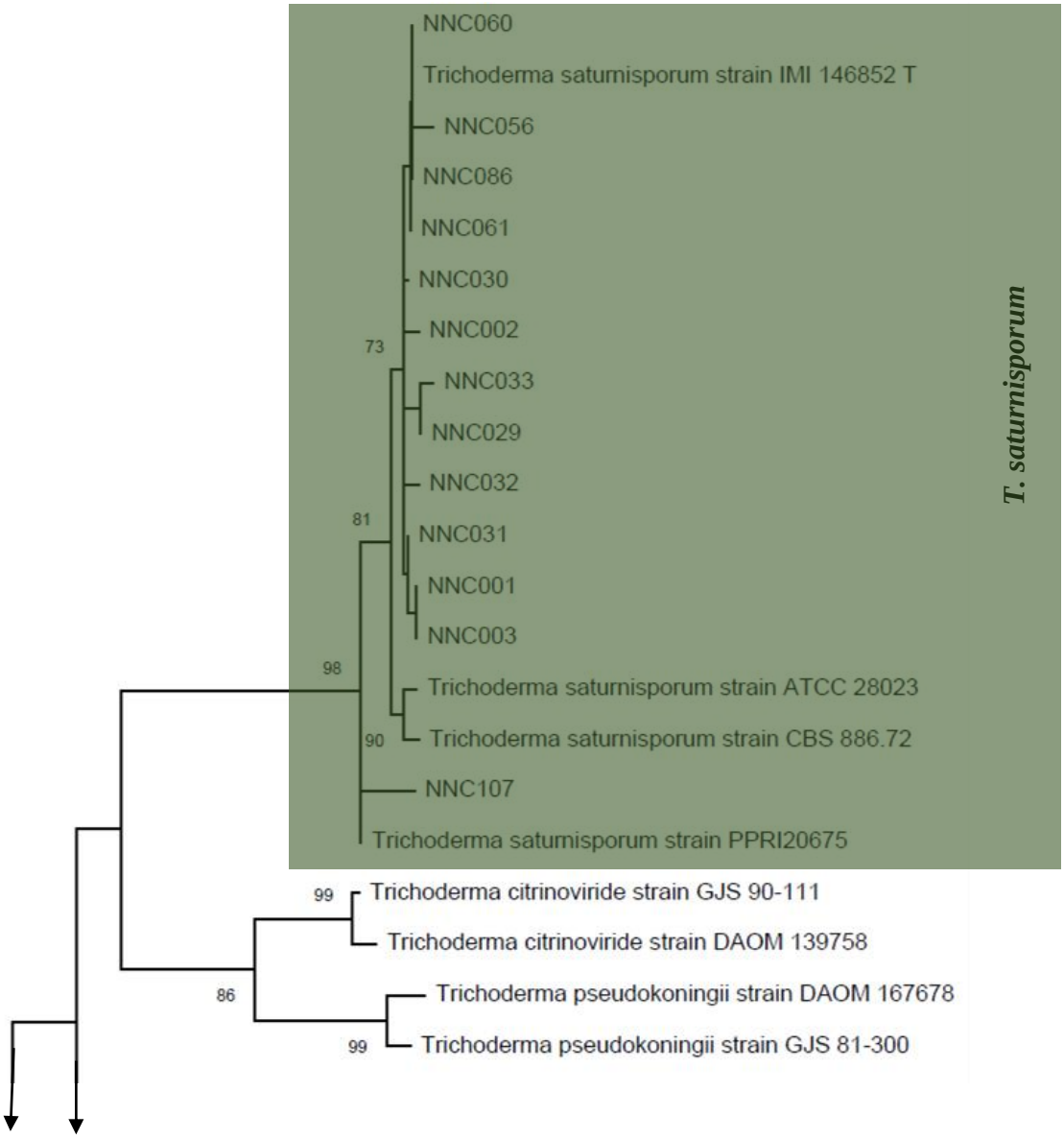


Figure 2. 5. Maximum likelihood phylogenetic tree of *Trichoderma* spp. from *T. longibrachiatum* clade, the tree was based on concatenated sequence data (ITS1 and TEF1), ex-type strains indicated by alphabet "**T**", and the tree was rooted with *Protocrea farinosa* CBS 121551. (Scale bar = 0.050)

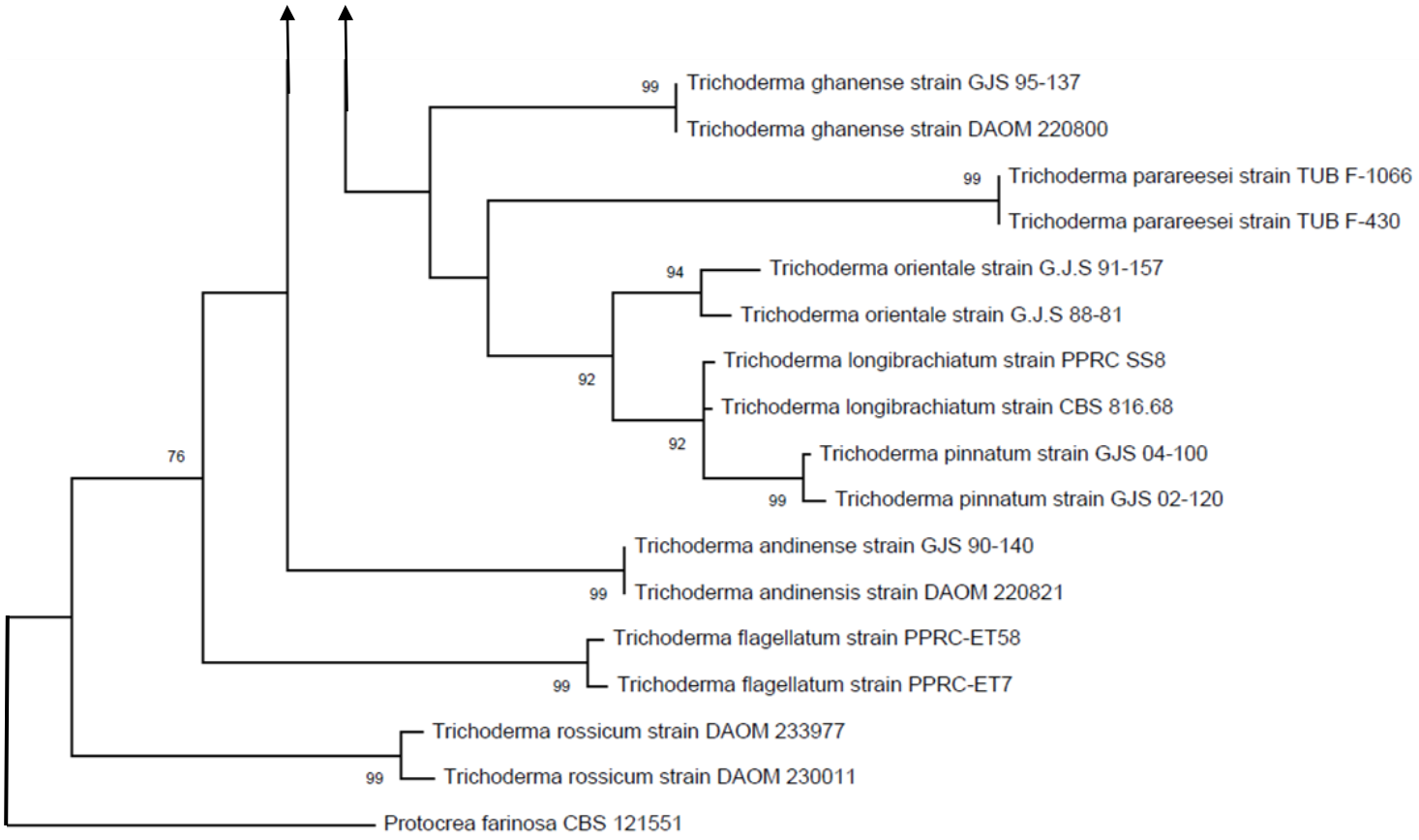


Figure 2.5. (continued)

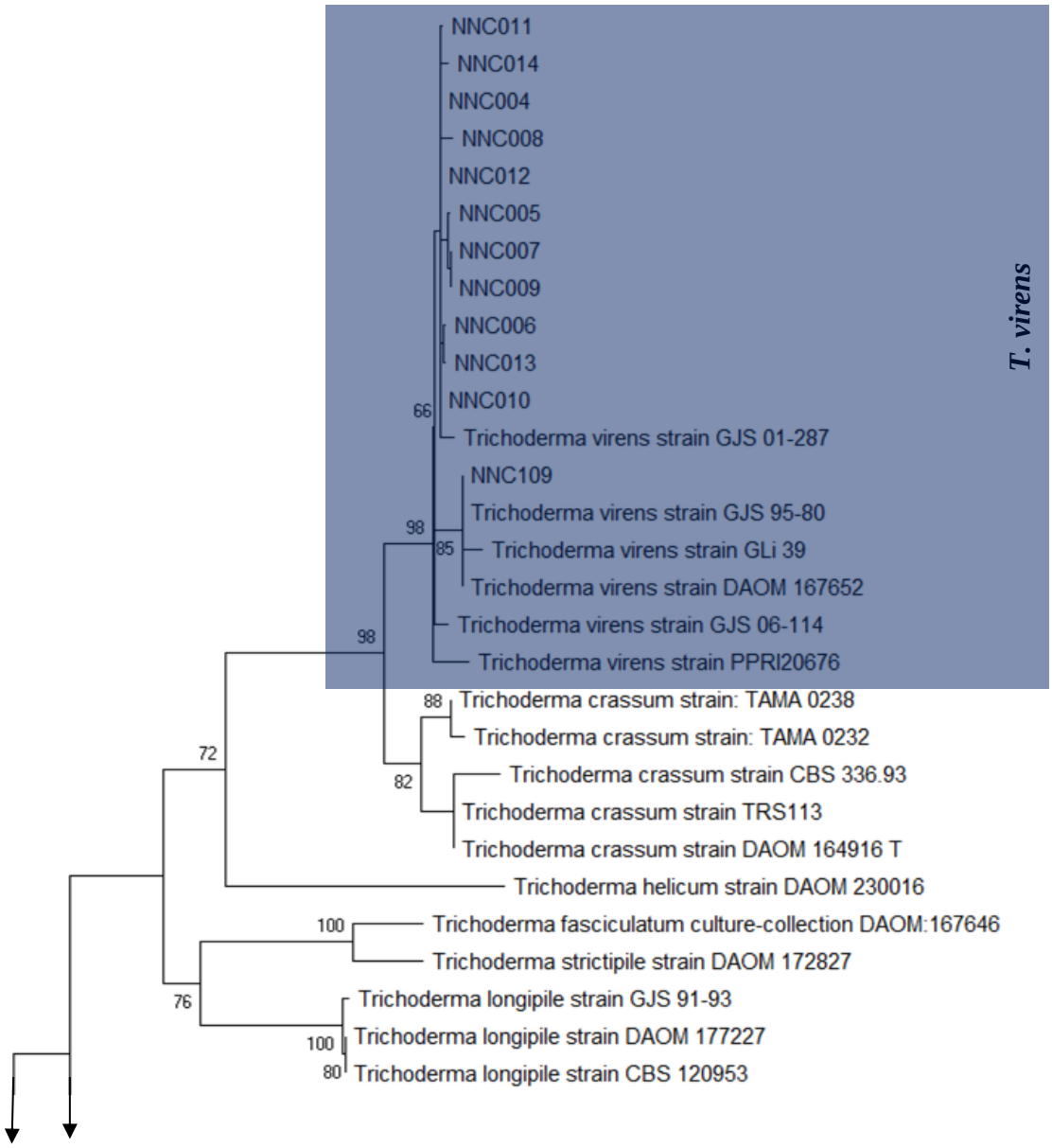


Figure 2. 6. Maximum likelihood phylogenetic tree of *Trichoderma* spp. from *T. virens* clade, the tree was based on concatenated sequence data (ITS1 and TEF1), ex-type strains indicated by alphabet "**T**", and the tree was rooted with *Protocrea farinosa* CBS 121551.

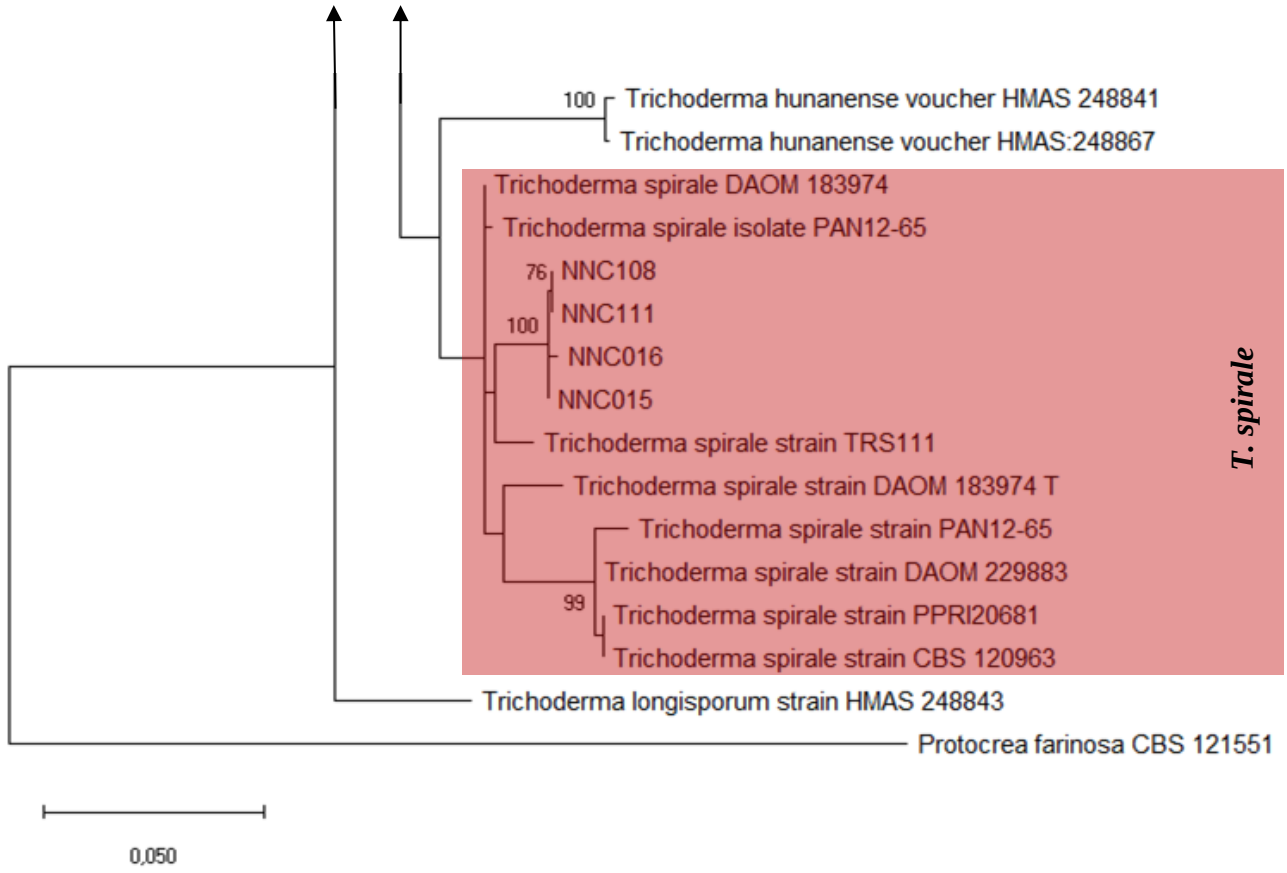


Figure 2.6. (continued)

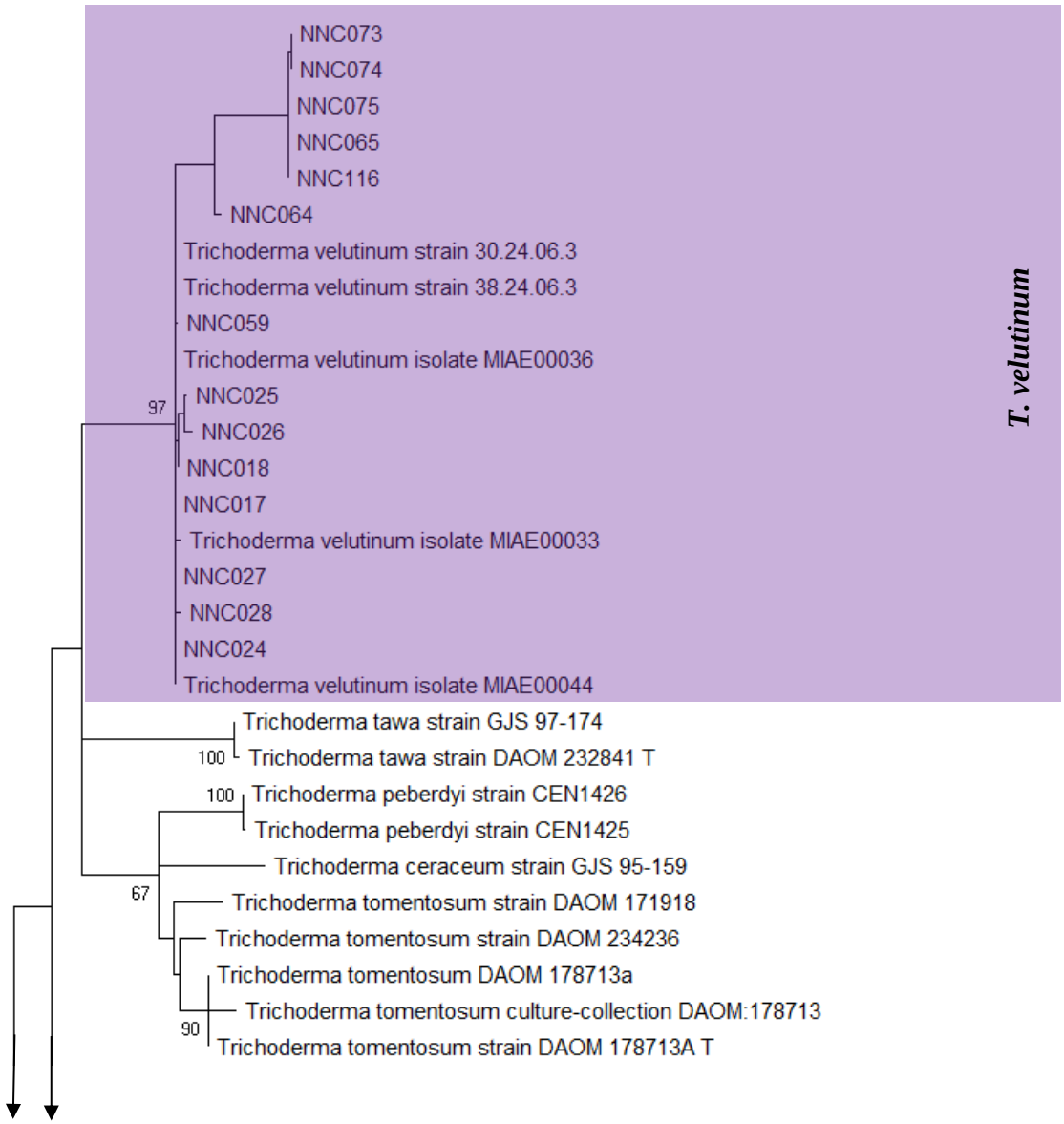


Figure 2. 7. Maximum likelihood phylogenetic tree of *Trichoderma* spp. from *T. harzianum* clade, the tree was based on concatenated sequence data (ITS1 and TEF1), ex-type strains indicated by alphabet "**T**", and the tree was rooted with *Protocrea farinosa* CBS 121551

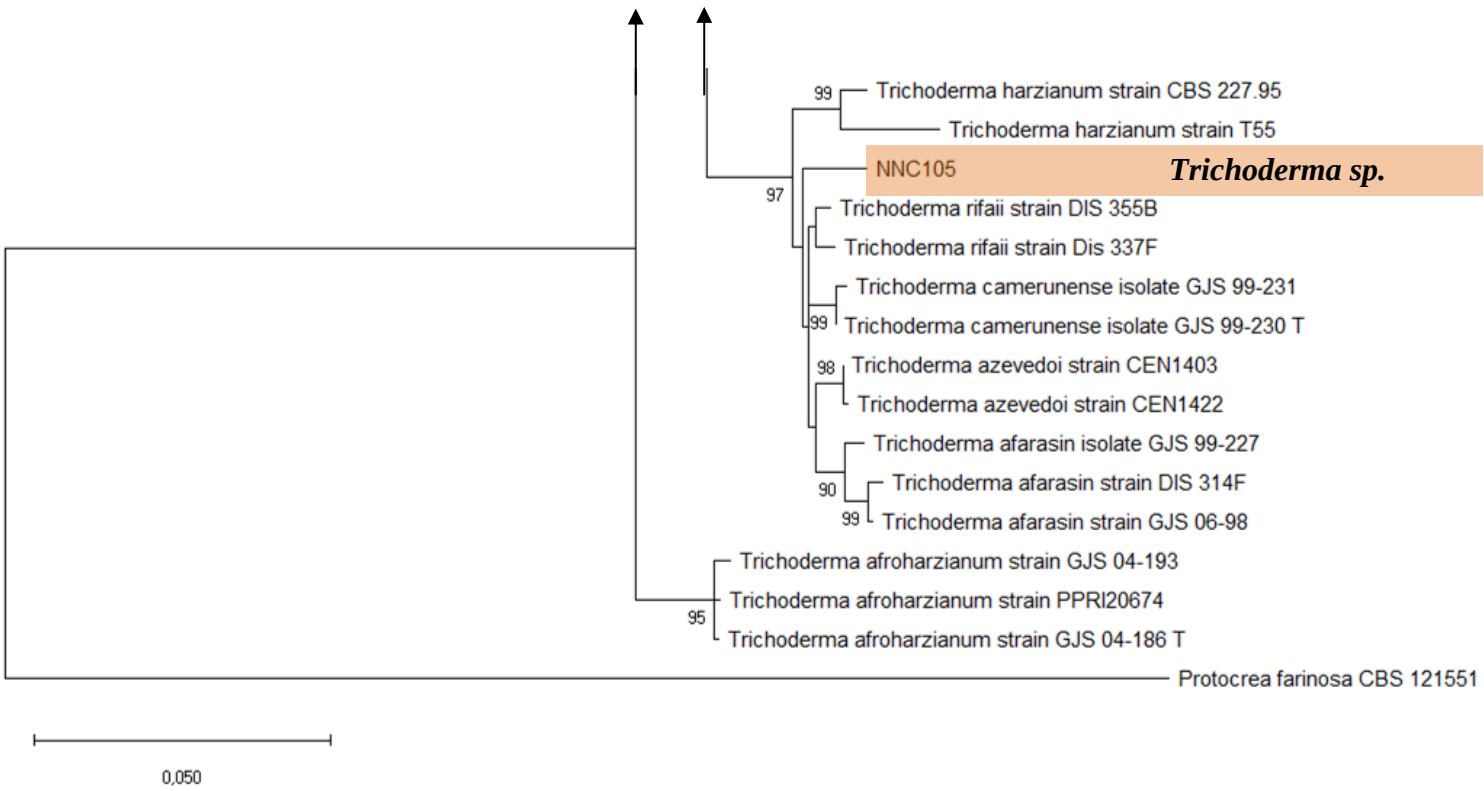


Figure 2.7. (continued)

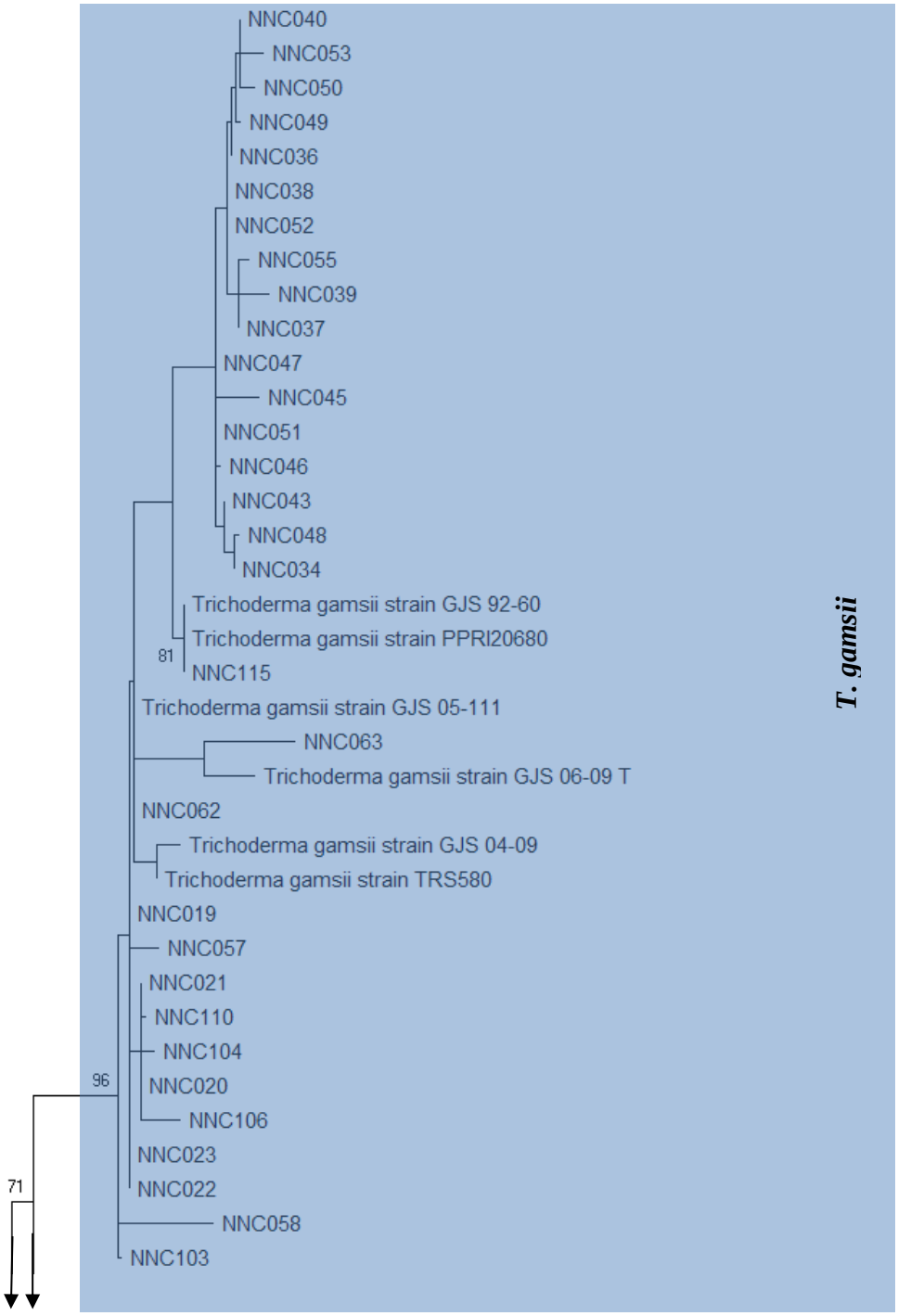


Figure 2. 8. Maximum likelihood phylogenetic tree of *Trichoderma* spp. from *T. viride* clade, the tree was based on concatenated sequence data (ITS1 and TEF1), ex-type strains indicated by alphabet "**T**", and the tree was rooted with *Protocrea farinosa* CBS 121551. (Scale bar = 0.050)

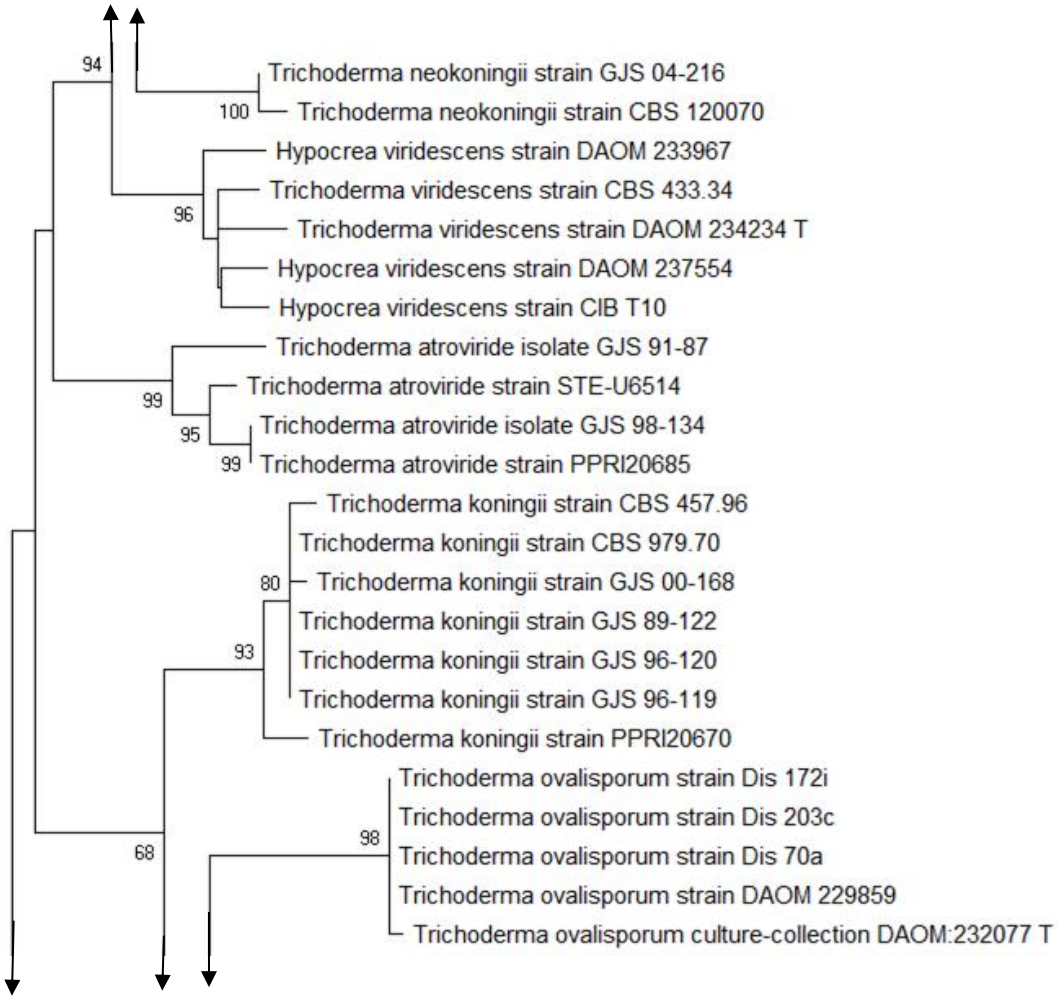


Figure 2.8. (continued)

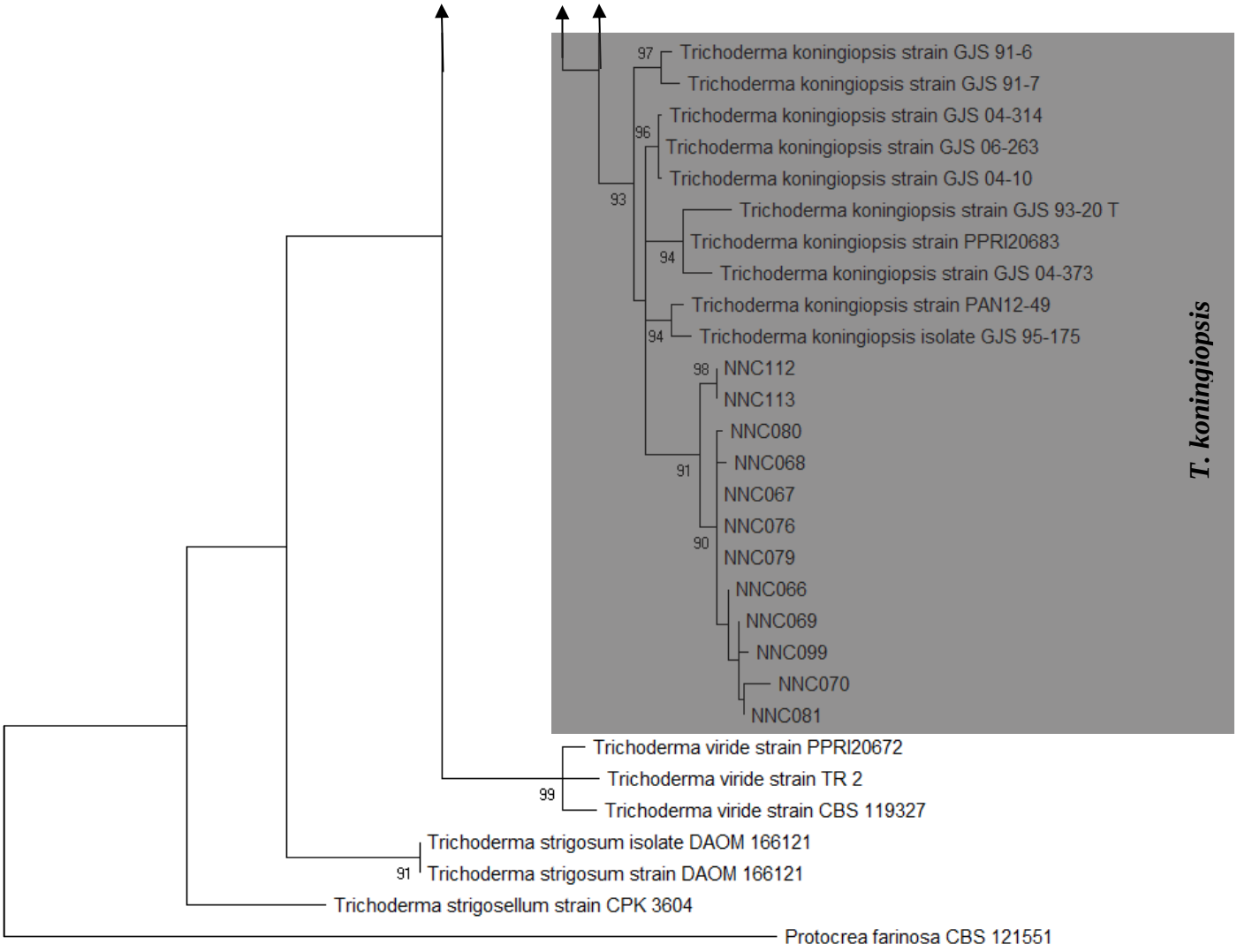


Figure 2.8. (continued)

Notes on isolated species

Trichoderma harzianum Rifai, Mycological Papers 116: 38 (1969)

Trichoderma harzianum complex species are distributed worldwide (Gherbawy *et al*., 2004; Jaklitsch *et al*., 2006; Jiang *et al*., 2016). It has been reported that at least three monophyletic lineages representing different species exist within the *T. harzianum* complex (Druzhinina *et al*., 2010). *Trichoderma* sp. NNC105 belongs to *T. harzianum* clade based on phylogenetic analysis. *Trichoderma* sp. NNC105 is phylogenetically closely related to *T. harzianum* and *T. rifaii*. Other species that belongs to this clade that have been isolated in South Africa includes *T. afroharzianum* (Du Plessis *et al*., 2018). *Trichoderma* spp. in this clade have the ability to improve plant growth and control diseases (Abdel-Fattah *et al*., 2007; Chaverri *et al*., 2015; Umadevi *et al*., 2018). In addition, these species are capable of producing various types of volatile organic compounds (VOCs) which are also useful in the process of fighting against pathogenic microorganisms (Song *et al*., 2018; Guo *et al*., 2019).

Trichoderma virens (J.H. Mill., Giddens & A.A. Foster) Arx, Beihefte zur Nova Hedwigia 87: 288 (1987)

Trichoderma virens have been isolated in South Africa and other parts of the world (Du Plessis, 2015; Jiang *et al*., 2016), and 13 strains in this study were isolated only from fields with crop rotation practices. *T. virens* have both teleomorph and anamorph states, and the anamorph strains are known to be cosmopolitan (Chaverri *et al*., 2001). In addition, *T. virens* have been widely studied for their mechanisms that they use to control various pathogenic diseases due to their common use in agriculture (Baek *et al*., 1999; Howell *et al*., 2000; Howell, 2006). A number of studies showed that strains from this species vary in terms of biocontrol activity, and were highly strain dependent (Baek *et al*., 1999; Howell *et al*., 2000; Howell, 2006). *T. virens* also have the capacity to improve plant growth (Vargas *et al*., 2009; Contreras-Cornejo *et al*., 2009).

Trichoderma spirale Bissett, Canadian Journal of Botany 69 (11): 2408 (1992)

Trichoderma spirale have been found in many studies (Bisset, 1991; Chaverri *et al*., 2003; Du Plessis, 2015; Jiang *et al*., 2016; Jang *et al*., 2017). Four strains of *Trichoderma spirale* were isolated in this study. Moreover, another study reported only two strains of *T. spirale* which were isolated from agricultural soil in East China (Jiang *et al*., 2016). In contrast, other studies found that this species was dominant when compared to other species of *Trichoderma* in China (Sun *et al*., 2012) and Republic of Korea (Oh *et al*., 2018). Furthermore, some studies investigated the ability of this species

to enhance plant growth and prevent diseases (Abdel-Monaim *et al*., 2014; Do Nascimento *et al*., 2017; Baiyee *et al*., 2019). Their findings indicated that *T. spirale* was found to be most effective in controlling *Leucoagaricus gongylophorus* and *Corynespora cassiicola* (Do Nascimento *et al*., 2017; Baiyee *et al*., 2019), in contrast to the foregoing results another study reported that *T. spirale* had a low efficacy against *Fusarium* wilt disease when compared to other *Trichoderma* spp. (Abdel-Monaim *et al*., 2014).

***Trichoderma gamsii* Samuels & Druzhin., Studies in Mycology 56: 168 (2006)**

Trichoderma gamsii was the most abundant species in this study as 34 strains were isolated. This species has been previously reported from South Africa and it is known to be distributed globally (Jaklitsch *et al*., 2006; Samuels and Druzhinina, 2006; Anees *et al*., 2010; Sun *et al*., 2012; Du Plessis *et al*., 2018). Many studies reported the potential of *T. gamsii* to improve plant growth and harness plant diseases (Rinu *et al*., 2014; Baroncelli *et al*., 2016; Chen *et al*., 2016; Zhou *et al*., 2018; Chihat *et al*., 2021), for instance *T. gamsii* isolated from lentil roots was found to have the capacity to solubilize phosphate, chitinase activity, and produce ammonia, and salicyclic acid, although the other important metabolites (e.g. Indole acetic acid and siderophores) which are also known to play an important role in plant growth improvement, were not detected (Rinu *et al*., 2014). Moreover, *T. gamsii* is known to colonize the inner plant tissues (Rinu *et al*., 2014; Chen *et al*., 2016; Sarrocco *et al*., 2020).

***Trichoderma koningiopsis* Samuels, C. Suárez & H.C. Evans, Studies in Mycology 56: 117 (2006)**

Trichoderma koningiopsis have been reported in South Africa (Du Plessis *et al*., 2018) and is known to be common in tropical America, although it has also been isolated from East Africa, Europe, and Canada (Jaklitsch *et al*., 2006; Samuels *et al*., 2006). The current study isolated twelve *T. koningiopsis* strains. This species is ubiquitous in nature and have been isolated as endophytes (Samuels *et al*., 2006; Jiang *et al*., 2016). It is well documented that *T. koningiopsis* strains can prevent pathogenic diseases and improve plant growth (Moreno *et al*., 2009; Hu *et al*., 2017; Tandon *et al*., 2020). In addition, other studies reported that this species can produce various secondary metabolites (Hu *et al*., 2017; Marik *et al*., 2018; Chen *et al*., 2019).

***Trichoderma saturnisporum* Hammill, Mycologia 62 (1): 112 (1970)**

Trichoderma saturnisporum normally found in the environments that have a high organic matter content and are also associated with warm environments (Danielson and Davey, 1973). *T. saturnisporum* was reported in Africa, Georgia, Italy, and Texas (Kuhls *et al*., 1997; Sadfi-Zouaoui *et al*., 2009; Du Plessis *et al*., 2018). *T. saturnisporum* was shown to be effective biological control agent as well as growth promotion of crop plants (Marin-Guirao *et al*., 2016; Fernando *et al*., 2018; Diánez *et al*., 2016; Sharma *et al*., 2018). In this study, 13 strains were isolated from wheat soil, two strains from monoculture fields and eleven strains from crop rotation fields. This finding supports the fact that *T. saturnisporum* is commonly found in environments with high organic matter content, as the crop residues were left on the field in the crop rotation sites, before planting.

***Trichoderma velutinum* Bissett, C.P. Kubicek & Szakacs, Canadian Journal of Botany 81 (6): 579 (2003)**

Trichoderma velutinum is a cold tolerant species that belongs to the *T. harzianum* clade (Bisset *et al*., 2003). According to our knowledge this is the first report of *T. velutinum* in South Africa, and 14 strains were isolated in this study (Fig. 2.7). The South African strains tend to have unique phialides (fusiform to papillate shape) compared to other previously isolated strains (Fig. 2.7) (Bissett *et al*., 2003). It has previously been isolated from rice agricultural soil (Jiang *et al*., 2016), and contributed to improving plant growth (Mayo *et al*., 2016; Guo *et al*., 2019). Furthermore, it is mycoparasitic on pathogenic fungi (Sharma *et al*., 2017, Matarese *et al*., 2012), and mycoparasitism was induced by the production of volatile organic compounds (VOCs) from *T. velutinum* (Guo *et al*., 2019).

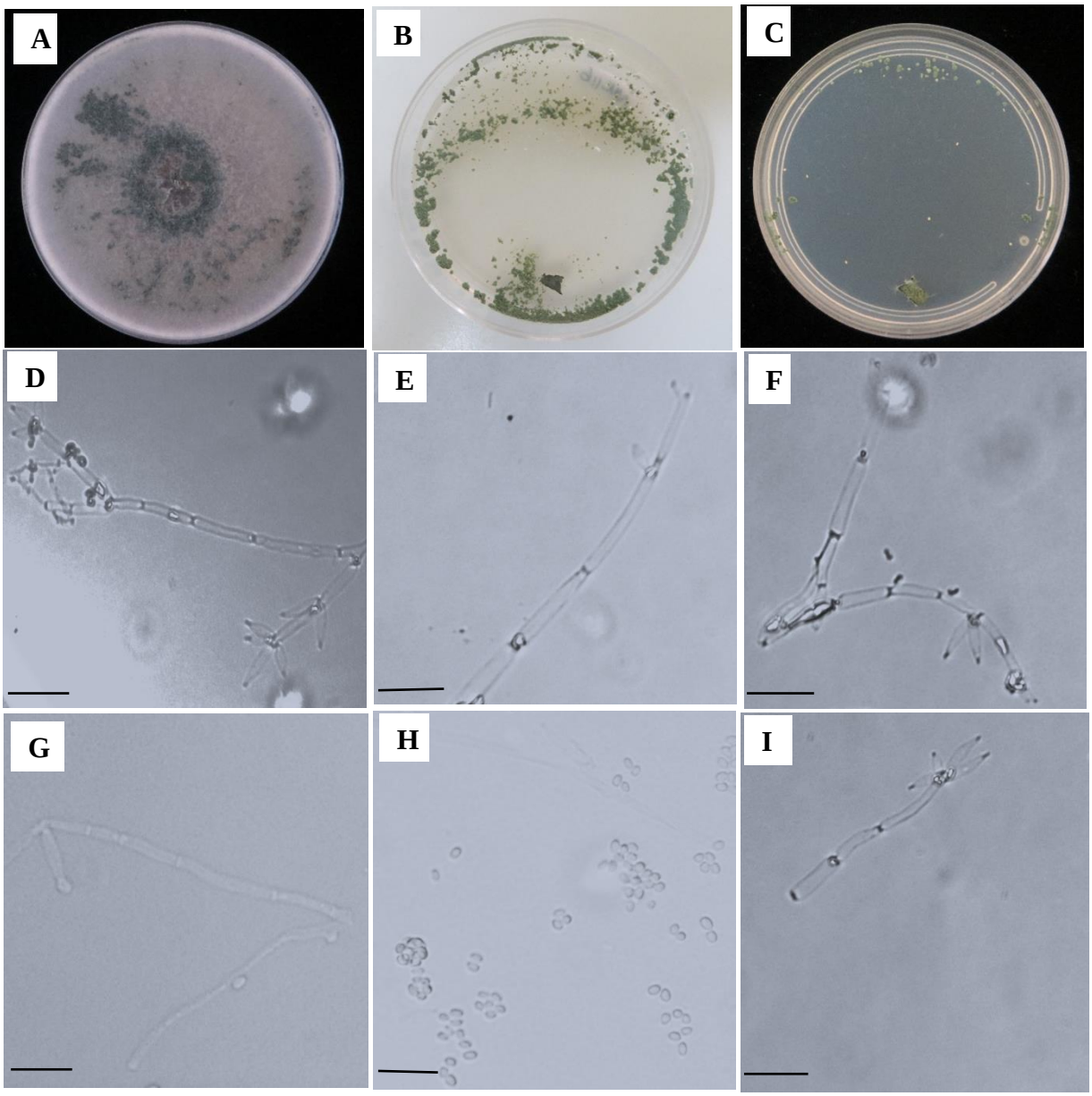


Figure 2. 9. Morphological features of *Trichoderma velutinum* (NNC116 representative), **A**. PDA, **B**. CMD, **C**. SNA all after 7 days. **D, F, I**. Phialides. **E, G**. Conidiophores. **H**. Conidia (All scale bars are 10 µm in length) (Magnification 400X)

Discussion

Trichoderma species have been explored worldwide for their beneficial impact in both controlling diseases and stimulating plant growth (Abdel-Fattah *et al*., 2007; Chaverri *et al*., 2015; Umadevi *et al*., 2018; Guo *et al*., 2019). In this study a total of seven *Trichoderma* spp. have been isolated from agricultural soil. The majority of species that were isolated in this study have been found by other researchers from agricultural soils, for example *T. velutinum*, *T. virens*, *T. spirale*, *T. saturnisporum*, and *T. koningiopsis* (Jiang *et al*., 2016). Furthermore, most of the *Trichoderma* species isolated in the current study were re-isolated since they had previously been isolated in this region (Du Plessis *et al*., 2018), but two *Trichoderma* species were discovered for the first time in this region.

Species identification in the current study was based on morphological characters and phylogenies were based on ITS and TEF markers in order to achieve a robust analysis (Jacklitsch and Voglmayr, 2015; Qiao *et al*., 2018; Maria del Carmen *et al*., 2021). This has been used in other studies as the ITS gene region does not provide sufficient resolution in this genus (Kuhls *et al*., 1997; Jacklitsch *et al*., 2006; Hatvani *et al*., 2007; Jacklitsch, 2009), and the use of TEF can effectively distinguish between closely related species (Hermosa *et al*., 2004; Lu *et al*., 2004; Overton *et al*., 2006; Samuels, 2006). However, the use of other genes such as *act*, *cal*1, and *rpb*2 could still be amplified from the *Trichoderma* species that were isolated in the current study, because recent studies have shown to also include these genes (Qiao *et al*., 2018; Ingilis *et al*., 2020; Maria del Carmen *et al*., 2021).

T. harzianum complex is the most common group isolated from soil (Druzhinina *et al*., 2010; Chaverri *et al*., 2015; Du Plessis *et al*., 2018; Ingilis *et al*., 2020). In this study, strain NNC 105 is a member of *T. harzianum* complex. In the analysis it clustered close to other strains of this clade, although it forms a separate lineage within the complex, suggesting that it may represent a novel species. We have, however, not described it, as it is represented by a single strain, where novel species should ideally be represented by more than one strain (Seifert and Rosman, 2010). Moreover, this single strain was phylogenetically related to *T. harzianum* and *T. rifaii* (Fig. 2.7). Its microscopic features were not similar to those of *T. harzianum*, but it did resemble most of the features of *T. rifaii* (Chaverri *et al*., 2015, also see Chapt. 3, Fig. 3.10).

T. gamsii and *T. saturnisporum* were isolated across all the sites in this study (Fig. 2.2). This was not surprising as these *Trichoderma* species have been reported to be cosmopolitan (Danielson and Davey, 1973; Kuhls *et al*., 1997; Jaklitsch *et al*., 2006; Samuels and Druzhinina, 2006; Sadfi-Zouaoui *et al*.,

2009; Anees *et al*., 2010; Sun *et al*., 2012). A recent study in South Africa reported that *T. saturnisporum* was found to be the second most isolated species from non-agricultural soils (Du Plessis *et al*., 2018).

The distribution of *Trichoderma* species was largely affected by plant diversity in this study. This was shown by the number of species that were isolated from monoculture sites compared to crop rotation sites. However, the comparison between monoculture and crop rotation in this study was neglected since the number of crop rotation fields were not equal to monoculture fields. In the current study only four species were isolated from monoculture sites whereas, seven species were isolated under crop rotation sites. Other studies also reported that crop rotation resulted in an increased microbial diversity compared to monoculture (Lupwayi *et al*., 1998; Zak *et al*., 2003; Venter *et al*., 2016; D'Acunto *et al*., 2018). All these studies focused on evaluating the entire microbial community rather than looking at one genus, as it was done in the current study. However, some studies showed an inverse relationship between microbial community and crop rotation, these studies were based on wheat-fallow, wheat-pea, wheat-wheat, and wheat-soybean (Yin *et al*., 2010; Reardon *et al*., 2014). Therefore, this suggest that one should consider other environmental factors that might be part of these findings, such as climate, geographical location, soil type, and soil pH. To date, no study has been conducted to evaluate the *Trichoderma* species distribution based on crop or plant diversity.

Overall, crop rotation and monoculture farming practices isolated 7 and 4 *Trichoderma* spp., respectively. *T. gamsii* was the most abundant species in both farming practices. The investigation of this genus is important since its exhibit positive functions that are vital in improving crop development (Rinu *et al*., 2014; Baroncelli *et al*., 2016; Chen *et al*., 2016; Zhou *et al*., 2018; Tandon *et al*., 2020; Chihat *et al*., 2021). Only two *Trichoderma* spp. were reported for the first time in South Africa and were identified as *T. velutinum* and *Trichoderma* sp NNC105. Future studies from other crops may reveal even more species from South Africa, expanding our knowledge on the distribution of this group. This is also vital in the development of agricultural products from local strains as bio-fertilizers, and biocontrol agents.

References

Abdel-Fattah, G.M., Shabana, Y.M., Ismail, A.E. and Rashad, Y.M., 2007. *Trichoderma harzianum*: a biocontrol agent against *Bipolaris oryzae*. *Mycopathologia*, *164*(2), pp.81-89.

Abdel-Monaim, M.F., Abdel-Gaid, M.A., Zayan, S.A. and Nassef, D.M., 2014. Enhancement of growth parameters and yield components in eggplant using antagonism of *Trichoderma* spp. against *Fusarium* wilt disease. *International Journal of Phytopathology*, *3*(1), pp.33-40.

Anees, M., Tronsmo, A., Edel-Hermann, V., Hjeljord, L.G., Héraud, C. and Steinberg, C., 2010. Characterization of field isolates of *Trichoderma* antagonistic against *Rhizoctonia solani*. *Fungal Biology*, *114*(9), pp.691-701.

Askew, D.J. and Laing, M.D., 1994. Evaluating *Trichoderma* bio-control of *Rhizoctonia solani* in cucumbers using different application methods. *J. Southern-African Soc. For Horticultural Science*, *4*, pp.25-38.

Askew, D.J. and Laing, M.D., 1994. The in vitro screening of 118 *Trichoderma* isolates for antagonism to *Rhizoctonia solani* and an evaluation of different environmental sites of *Trichoderma* as sources of aggressive strains. *Plant and Soil*, *159*(2), pp.277-281.

Baek, J.M., Howell, C.R. and Kenerley, C.M., 1999. The role of an extracellular chitinase from *Trichoderma virens* Gv29-8 in the biocontrol of *Rhizoctonia solani*. *Current Genetics*, *35*(1), pp.41-50.

Baiyee, B., Pornsuriya, C., Ito, S.I. and Sunpapao, A., 2019. *Trichoderma spirale* T76-1 displays biocontrol activity against leaf spot on lettuce (*Lactuca sativa* L.) caused by *Corynespora cassiicola* or *Curvularia aeria*. *Biological Control*, *129*, pp.195-200.

Baroncelli, R., Zapparata, A., Piaggeschi, G., Sarrocco, S. and Vannacci, G., 2016. Draft whole-genome sequence of *Trichoderma gamsii* T6085, a promising biocontrol agent of *Fusarium* head blight on wheat. *Genome Announcements*, *4*(1), pp.e01747-15.

Benitez, M.S., Osborne, S.L. and Lehman, R.M., 2017. Previous crop and rotation history effects on maize seedling health and associated rhizosphere microbiome. *Scientific Reports*, 7(1), p. 15709.

Bisby, G.R., 1939. *Trichoderma viride* Pers. ex Fries, and notes on *Hypocrea*. *Transactions of the British Mycological Society*, *23*(2), pp.149-168.

Bissett, J., 1991. A revision of the genus *Trichoderma*. III. Section *Pachybasium*. *Canadian Journal of Botany*, *69*(11), pp.2373-2417.

Bissett, J., Gams, W., Jaklitsch, W. and Samuels, G.J., 2015. Accepted *Trichoderma* names in the year 2015. *IMA fungus*, *6*(2), pp.263-295.

Bissett, J., Szakacs, G., Nolan, C.A., Druzhinina, I., Gradinger, C. and Kubicek, C.P., 2003. New species of *Trichoderma* from Asia. *Canadian Journal of Botany*, *81*(6), pp.570-586.

Cai, F. and Druzhinina, I.S., 2021. In honor of John Bissett: authoritative guidelines on molecular identification of *Trichoderma*. *Fungal Diversity*, *107*(1), pp.1-69.

Chaverri, P., Branco-Rocha, F., Jaklitsch, W., Gazis, R., Degenkolb, T. and Samuels, G.J., 2015. Systematics of the *Trichoderma harzianum* species complex and the re-identification of commercial biocontrol strains. *Mycologia*, *107*(3), pp.558-590.

Chaverri, P., Castlebury, L.A., Overton, B.E. and Samuels, G.J., 2003. *Hypocrea*/*Trichoderma*: species with conidiophore elongations and green conidia. *Mycologia*, *95*(6), pp.1100-1140.

Chaverri, P., Samuels, G.J. and Stewart, E.L., 2001. *Hypocrea virens* sp. nov., the teleomorph of *Trichoderma virens*. *Mycologia*, *93*(6), pp.1113-1124.

Chen, J.L., Sun, S.Z., Miao, C.P., Wu, K., Chen, Y.W., Xu, L.H., Guan, H.L. and Zhao, L.X., 2016. Endophytic *Trichoderma gamsii* YIM PH30019: a promising biocontrol agent with hyperosmolar, mycoparasitism, and antagonistic activities of induced volatile organic compounds on root-rot pathogenic fungi of *Panax notoginseng*. *Journal of Ginseng Research*, *40*(4), pp.315-324.

Chen, S., Li, H., Chen, Y., Li, S., Xu, J., Guo, H., Liu, Z., Zhu, S., Liu, H. and Zhang, W., 2019. Three new diterpenes and two new sesquiterpenoids from the endophytic fungus *Trichoderma koningiopsis* A729. *Bioorganic Chemistry*, *86*, pp.368-374.

Chihat, S., Aleandri, M.P., Vannini, A., Bruni, N. and Boureghda, H., 2021. Identity and biocontrol efficiency of *Trichoderma* spp. isolated from different soils and ecosystems in Algeria. *Journal of Plant Pathology*, pp.1-19.

Contreras-Cornejo, H.A., Macías-Rodríguez, L., Cortés-Penagos, C. and López-Bucio, J., 2009. *Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in Arabidopsis. *Plant Physiology*, *149*(3), pp.1579-1592.

Crous, P.W., Verkley, G.J., Groenewald, J.Z. and Samson, R.A., 2009. Fungal biodiversity. *Fungal Biodiversity*.

Cummings, N.J., Ambrose, A., Braithwaite, M., Bissett, J., Roslan, H.A., Abdullah, J., Stewart, A., Agbayani, F.V., Steyaert, J. and Hill, R.A., 2016. Diversity of root-endophytic *Trichoderma* from Malaysian Borneo. *Mycological Progress*, *15*(5), p.50.

D'Acunto, L., Andrade, J.F., Poggio, S.L. and Semmartin, M., 2018. Diversifying crop rotation increased metabolic soil diversity and activity of the microbial community. *Agriculture, Ecosystems & Environment*, *257*, pp.159-164.

Danielson, R.M. and Davey, C.B., 1973. Carbon and nitrogen nutrition of *Trichoderma*. *Soil Biology and Biochemistry*, *5*(5), pp.505-515.

De Respinis, S., Vogel, G., Benagli, C., Tonolla, M., Petrini, O. and Samuels, G.J., 2010. MALDI-TOF MS of *Trichoderma*: a model system for the identification of microfungi. *Mycological Progress*, *9*(1), pp.79-100.

Diánez Martínez, F., Santos, M., Carretero, F. and Marín, F., 2016. *Trichoderma saturnisporum*, a new biological control agent. *Journal of the Science of Food and Agriculture*, *96*(6), pp.1934-1944.

Do Nascimento, M.O., de Almeida Sarmento, R., Dos Santos, G.R., de Oliveira, C.A. and de Souza, D.J., 2017. Antagonism of *Trichoderma* isolates against *Leucoagaricus gongylophorus* (Singer) Möller. *Journal of Basic Microbiology*, *57*(8), pp.699-704.

Druzhinina, I.S., Komoń-Zelazowska, M., Kredics, L., Hatvani, L., Antal, Z., Belayneh, T. and Kubicek, C.P., 2008. Alternative reproductive strategies of *Hypocrea orientalis* and genetically close but clonal *Trichoderma longibrachiatum*, both capable of causing invasive mycoses of humans. *Microbiology*, *154*(11), pp.3447-3459.

Druzhinina, I.S., Kopchinskiy, A.G. and Kubicek, C.P., 2006. The first 100 *Trichoderma* species characterized by molecular data. *Mycoscience*, *47*(2), p.55.

Druzhinina, I.S., Kubicek, C.P., Komoń-Zelazowska, M., Mulaw, T.B. and Bissett, J., 2010. The *Trichoderma harzianum* demon: complex speciation history resulting in coexistence of hypothetical biological species, recent agamospecies and numerous relict lineages. *BMC Evolutionary Biology*, *10*(1), pp.1-14.

Du Plessis, I.L., Druzhinina, I.S., Atanasova, L., Yarden, O. and Jacobs, K., 2018. The diversity of *Trichoderma* species from soil in South Africa, with five new additions. *Mycologia*, *110*(3), pp.559-583.

Fernando, D., Milagrosa, S., Francisco, C. and Francisco, M., 2018. Biostimulant activity of *Trichoderma saturnisporum* in melon (*Cucumis melo*). *Hortscience*, *53*(6), pp.810-815.

Frisvad, J.C., Møller, L.L., Larsen, T.O., Kumar, R. and Arnau, J., 2018. Safety of the fungal workhorses of industrial biotechnology: update on the mycotoxin and secondary metabolite potential of *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei*. *Applied Microbiology and Biotechnology*, *102*(22), pp.9481-9515.

Gherbawy, Y., Druzhinina, I., Shaban, G.M., Wuczkowsky, M., Yaser, M., El-Naghy, M.A., Prillinger, H.J. and Kubicek, C.P., 2004. *Trichoderma* populations from alkaline agricultural soil in the Nile valley, Egypt, consist of only two species. *Mycological Progress*, *3*(3), pp.211-218.

Guo, Y., Ghirardo, A., Weber, B., Schnitzler, J.P., Benz, J.P. and Rosenkranz, M., 2019. *Trichoderma* species differ in their volatile profiles and in antagonism toward ectomycorrhiza *Laccaria bicolor*. *Frontiers in Microbiology*, *10*, p.891.

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I. and Lorito, M., 2004. *Trichoderma* species—opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology*, *2*(1), pp.43-56.

Harris, J.L., 2000. Safe, low-distortion tape touch method for fungal slide mounts. *Journal of Clinical Microbiology*, *38*(12), pp.4683-4684.

Hatvani, L., Antal, Z., Manczinger, L., Szekeres, A., Druzhinina, I.S., Kubicek, C.P., Nagy, A., Nagy, E., Vágvölgyi, C. and Kredics, L., 2007. Green mold diseases of *Agaricus* and *Pleurotus* spp. are caused by related but phylogenetically different *Trichoderma* species. *Phytopathology*, *97*(4), pp.532-537.

Hermosa, M.R., Keck, E., Chamorro, I., Rubio, B., Sanz, L., Vizcaíno, J.A., Grondona, I. and Monte, E., 2004. Molecular characterization of biocontrol agents. *Bulletin-OILB-SROP*, *27*(8), pp.165-168.

Hosseyni-Moghaddam, M.S. and Soltani, J., 2014. Bioactivity of endophytic *Trichoderma* fungal species from the plant family *Cupressaceae*. *Annals of Microbiology*, *64*(2), pp.753-761.

Howell, C.R., 2006. Understanding the mechanisms employed by *Trichoderma virens* to effect biological control of cotton diseases. *Phytopathology*, *96*(2), pp.178-180.

Howell, C.R., Hanson, L.E., Stipanovic, R.D. and Puckhaber, L.S., 2000. Induction of terpenoid synthesis in cotton roots and control of *Rhizoctonia solani* by seed treatment with *Trichoderma virens*. *Phytopathology*, *90*(3), pp.248-252.

Hu, M., Li, Q.L., Yang, Y.B., Liu, K., Miao, C.P., Zhao, L.X. and Ding, Z.T., 2017. Koninginins RS from the endophytic fungus *Trichoderma koningiopsis*. *Natural Product Research*, *31*(7), pp.835-839.

Inglis, P.W., Mello, S.C., Martins, I., Silva, J.B., Macêdo, K., Sifuentes, D.N. and Valadares-Inglis, M.C., 2020. *Trichoderma* from Brazilian garlic and onion crop soils and description of two new species: *Trichoderma azevedoi* and *Trichoderma peberdyi*. *PloS one*, *15*(3), p.e0228485.

Jacobs, K., Bergdahl, D.R., Wingfield, M.J., Halik, S., Seifert, K.A., Bright, D.E. and Wingfield, B.D., 2004. *Leptographium wingfieldii* introduced into North America and found associated with exotic *Tomicus piniperda* and native bark beetles. *Mycological Research*, *108*(4), pp.411-418.

Jaklitsch, W.M. and Voglmayr, H., 2015. Biodiversity of *Trichoderma* (*Hypocreaceae*) in Southern Europe and Macaronesia. *Studies in Mycology*, *80*, pp.1-87.

Jaklitsch, W.M., 2009. European species of *Hypocrea* Part I. The green-spored species. *Studies in Mycology*, *63*, pp.1-91.

Jaklitsch, W.M., Samuels, G.J., Dodd, S.L., Lu, B.S. and Druzhinina, I.S., 2006. *Hypocrea rufa/Trichoderma viride*: a reassessment, and description of five closely related species with and without warted conidia. *Studies in Mycology*, *56*, pp.135-177.

Jang, S., Jang, Y., Kim, C.W., Lee, H., Hong, J.H., Heo, Y.M., Lee, Y.M., Lee, D.W., Lee, H.B. and Kim, J.J., 2017. Five New Records of Soil-Derived *Trichoderma* in Korea: *T. albolutescens, T. asperelloides, T. orientale, T. spirale, and T. tomentosum*. *Mycobiology*, *45*(1), pp.1-8.

Jiang, Y., Wang, J.L., Chen, J., Mao, L.J., Feng, X.X., Zhang, C.L. and Lin, F.C., 2016. *Trichoderma* biodiversity of agricultural fields in east China reveals a gradient distribution of species. *PLoS One*, *11*(8), p.e0160613.

Kalam, S., Das, S.N., Basu, A. and Podile, A.R., 2017. Population densities of indigenous *Acidobacteria* change in the presence of plant growth promoting rhizobacteria (PGPR) in rhizosphere. *Journal of Basic Microbiology*, 57(5), pp.376-385.

Katoh, K. and Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, *30*(4), pp.772-780.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C. and Thierer, T., 2012. *Geneious* Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*(12), pp.1647-1649.

Kredics, L., Hatvani, L., Naeimi, S., Körmöczi, P., Manczinger, L., Vágvölgyi, C. and Druzhinina, I., 2014. Biodiversity of the genus *Hypocrea*/*Trichoderma* in different habitats. In *Biotechnology and biology of Trichoderma* (pp. 3-24).

Kubicek, C.P., Komon-Zelazowska, M. and Druzhinina, I.S., 2008. Fungal genus *Hypocrea*/*Trichoderma*: from barcodes to biodiversity. *Journal of Zhejiang University Science B*, *9*(10), pp.753-763.

Kubicek, C.P., Steindorff, A.S., Chenthamara, K., Manganiello, G., Henrissat, B., Zhang, J., Cai, F., Kopchinskiy, A.G., Kubicek, E.M., Kuo, A. and Baroncelli, R., 2019. Evolution and comparative genomics of the most common *Trichoderma* species. *BMC Genomics*, *20*(1), pp.1-24.

Kuhls, K., Lieckfeldt, E., Samuels, G.J., Meyer, W., Kubicek, C.P. and Börner, T., 1997. Revision of *Trichoderma* sect. *Longibrachiatum* including related teleomorphs based on analysis of ribosomal DNA internal transcribed spacer sequences. *Mycologia*, *89*(3), pp.442-460.

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular biology and evolution*, *35*(6), p.1547.

Lieckfeldt, E., Samuels, G.J., Börner, T. and Gams, W., 1998. *Trichoderma koningii*: neotypification and *Hypocrea* teleomorph. *Canadian Journal of Botany*, *76*(9), pp.1507-1522.

Lu, B., Druzhinina, I.S., Fallah, P., Chaverri, P., Gradinger, C., Kubicek, C.P. and Samuels, G.J., 2004. *Hypocrea/Trichoderma* species with *pachybasium*-like conidiophores: teleomorphs for *T. minutisporum* and *T. polysporum* and their newly discovered relatives. *Mycologia*, *96*(2), pp.310-342.

Lupwayi, N.Z., Rice, W.A. and Clayton, G.W., 1998. Soil microbial diversity and community structure under wheat as influenced by tillage and crop rotation. *Soil Biology and Biochemistry*, *30*(13), pp.1733-1741.

María del Carmen, H.R., Evans, H.C., de Abreu, L.M., de Macedo, D.M., Ndacnou, M.K., Bekele, K.B. and Barreto, R.W., 2021. New species and records of *Trichoderma* isolated as mycoparasites and endophytes from cultivated and wild coffee in Africa. *Scientific Reports*, *11*(1), pp.1-30.

Marik, T., Tyagi, C., Racić, G., Rakk, D., Szekeres, A., Vágvölgyi, C. and Kredics, L., 2018. New 19-residue peptaibols from *Trichoderma* clade *Viride*. *Microorganisms*, *6*(3), p.85.

Marín-Guirao, J.I., Rodríguez-Romera, P., Lupión-Rodríguez, B., Camacho-Ferre, F. and Tello-Marquina, J.C., 2016. Effect of *Trichoderma* on horticultural seedlings' growth promotion depending on inoculum and substrate type. *Journal of Applied Microbiology*, *121*(4), pp.1095-1102.

Matarese, F., Sarrocco, S., Gruber, S., Seidl-Seiboth, V. and Vannacci, G., 2012. Biocontrol of *Fusarium* head blight: interactions between *Trichoderma* and mycotoxigenic *Fusarium*. *Microbiology*, *158*(1), pp.98-106.

Mayo, S., Cominelli, E., Sparvoli, F., González-López, O., Rodríguez-González, A., Gutiérrez, S. and Casquero, P.A., 2016. Development of a qPCR strategy to select bean genes involved in plant defense response and regulated by the *Trichoderma velutinum–Rhizoctonia solani* interaction. *Frontiers in Plant Science*, *7*, p.1109.

Mayo-Prieto, S., Marra, R., Vinale, F., Rodríguez-González, Á., Woo, S.L., Lorito, M., Gutiérrez, S. and Casquero, P.A., 2019. Effect of *Trichoderma velutinum* and *Rhizoctonia solani* on the Metabolome of Bean Plants (*Phaseolus vulgaris* L.). *International Journal of Molecular Sciences*, *20*(3), p.549.

McNear Jr, D.H., 2013. The rhizosphere-roots, soil and everything in between. *Nature Education Knowledge*, *4*(3), p.1.

Moreno, C.A., Castillo, F., González, A., Bernal, D., Jaimes, Y., Chaparro, M., González, C., Rodriguez, F., Restrepo, S. and Cotes, A.M., 2009. Biological and molecular characterization of the response of tomato plants treated with *Trichoderma koningiopsis*. *Physiological and Molecular Plant Pathology*, *74*(2), pp.111-120.

Mouton, M., Postma, F., Wilsenach, J. and Botha, A., 2012. Diversity and characterization of culturable fungi from marine sediment collected from St. Helena Bay, South Africa. *Microbial Ecology*, *64*(2), pp.311-319.

Mukherjee, P.K., Horwitz, B.A., Herrera-Estrella, A., Schmoll, M. and Kenerley, C.M., 2013. *Trichoderma* research in the genome era. *Annual Review of Phytopathology*, *51*, pp.105-129.

Mutawila, C., Fourie, P.H., Halleen, F. and Mostert, L., 2011. Grapevine cultivar variation to pruning wound protection by *Trichoderma* species against trunk pathogens. *Phytopathologia Mediterranea*, *50*, pp.S264-S276.

Oh, S.Y., Park, M.S., Cho, H.J. and Lim, Y.W., 2018. Diversity and effect of *Trichoderma* isolated from the roots of *Pinus densiflora* within the fairy ring of pine mushroom (*Tricholoma matsutake*). *PloS one*, *13*(11), p.e0205900.

Overton, B.E., Stewart, E.L. and Geiser, D.M., 2006. Taxonomy and phylogenetic relationships of nine species of *Hypocrea* with anamorphs assignable to *Trichoderma* section *Hypocreanum*. *Studies in Mycology*, *56*, pp.39-65.

Phua, C.K.H., Abdul Wahid, A.N. and Abdul Rahim, K., 2012. Development of Multifunctional Biofertilizer Formulation from Indigenous Microorganisms and Evaluation of Their N 2-Fixing Capabilities on Chinese Cabbage Using 15 N Tracer Technique. *Pertanika Journal of Tropical Agricultural Science*, *35*(3).

Qiao, M., Du, X., Zhang, Z., Xu, J. and Yu, Z., 2018. Three new species of soil-inhabiting *Trichoderma* from Southwest China. *MycoKeys*, (44), p.63.

Reardon, C.L., Gollany, H.T. and Wuest, S.B., 2014. Diazotroph community structure and abundance in wheat–fallow and wheat–pea crop rotations. *Soil Biology and Biochemistry*, *69*, pp.406-412.

Rinu, K., Sati, P. and Pandey, A., 2014. *Trichoderma gamsii* (NFCCI 2177): a newly isolated endophytic, psychrotolerant, plant growth promoting, and antagonistic fungal strain. *Journal of Basic Microbiology*, *54*(5), pp.408-417.

Roese, A.D., Vidal, G.S., Zielinski, E.C. and Mio, L.L.M.D., 2017. Native Trichoderma grown on oat grains controls damping-off and enhances height in soybean. *Pesquisa Agropecuária Tropical*, *47*, pp.102-109.

Roux, J. and Wingfield, M.J., 1997. Survey and virulence of fungi occurring on diseased Acacia mearnsii in South Africa. *Forest Ecology and Management*, *99*(3), pp.327-336.

Sadfi-Zouaoui, N., Hannachi, I., Rouaissi, M., Hajlaoui, M.R., Rubio, M.Á., Monte, E., Boudabous, A. and Hermosa, M.R., 2009. Biodiversity of *Trichoderma* strains in Tunisia. *Canadian Journal of Microbiology*, *55*(2), pp.154-162.

Samuels, G.J., 1996. *Trichoderma*: a review of biology and systematics of the genus. *Mycological Research*, *100*(8), pp.923-935.

Samuels, G.J., 2006. *Trichoderma*: systematics, the sexual state, and ecology. *Phytopathology*, *96*(2), pp.195-206.

Samuels, G.J., Dodd, S.L., Lu, B.S., Petrini, O., Schroers, H.J. and Druzhinina, I.S., 2006. The *Trichoderma koningii* aggregate species. *Studies in Mycology*, *56*, pp.67-133.

Sarrocco, S., Esteban, P., Vicente, I., Bernardi, R., Plainchamp, T., Domenichini, S., Puntoni, G., Baroncelli, R., Vannacci, G. and Dufresne, M., 2020. Straw competition and wheat root endophytism of *Trichoderma gamsii* T6085 as useful traits in the biocontrol of *Fusarium* head blight. *Phytopathology*, (ja).

Seifert, K.A. and Rossman, A.Y., 2010. How to describe a new fungal species. *IMA fungus*, *1*(2), pp.109-111.

Sharma, V., Salwan, R. and Shanmugam, V., 2018. Unraveling the multilevel aspects of least explored plant beneficial *Trichoderma saturnisporum* isolate GITX-Panog (C). *European Journal of Plant Pathology*, *152*(1), pp.169-183.

Sharma, V., Salwan, R., Sharma, P.N. and Kanwar, S.S., 2017. Elucidation of biocontrol mechanisms of *Trichoderma harzianum* against different plant fungal pathogens: Universal yet host specific response. *International Journal of Biological Macromolecules*, *95*, pp.72-79.

Silva, R.N., Monteiro, V.N., Steindorff, A.S., Gomes, E.V., Noronha, E.F. and Ulhoa, C.J., 2019. *Trichoderma*/pathogen/plant interaction in pre-harvest food security. *Fungal Biology*, *123*(8), pp.565-583.

Song, Y.P., Fang, S.T., Miao, F.P., Yin, X.L. and Ji, N.Y., 2018. Diterpenes and sesquiterpenes from the marine algicolous fungus *Trichoderma harzianum* X-5. *Journal of Natural Products*, *81*(11), pp.2553-2559.

Sun, R.Y., Liu, Z.C., Fu, K., Fan, L. and Chen, J., 2012. *Trichoderma* biodiversity in China. *Journal of Applied Genetics*, *53*(3), pp.343-354.

Tandon, A., Fatima, T., Shukla, D., Tripathi, P., Srivastava, S. and Singh, P.C., 2020. Phosphate solubilization by *Trichoderma koningiopsis* (NBRI-PR5) under abiotic stress conditions. *Journal of King Saud University-Science*, *32*(1), pp.791-798.

Umadevi, P., Anandaraj, M., Srivastav, V. and Benjamin, S., 2018. *Trichoderma harzianum* MTCC 5179 impacts the population and functional dynamics of microbial community in the rhizosphere of black pepper (*Piper nigrum* L.). *Brazilian Journal of Microbiology*, *49*(3), pp.463-470.

Vargas, W.A., Mandawe, J.C. and Kenerley, C.M., 2009. Plant-derived sucrose is a key element in the symbiotic association between *Trichoderma virens* and maize plants. *Plant Physiology*, *151*(2), pp.792-808.

Venter, Z.S., Jacobs, K. and Hawkins, H.J., 2016. The impact of crop rotation on soil microbial diversity: A meta-analysis. *Pedobiologia*, *59*(4), pp.215-223.

White, T.J., Bruns, T., Lee, S.J.W.T. and Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: A guide to methods and applications*, *18*(1), pp.315-322.

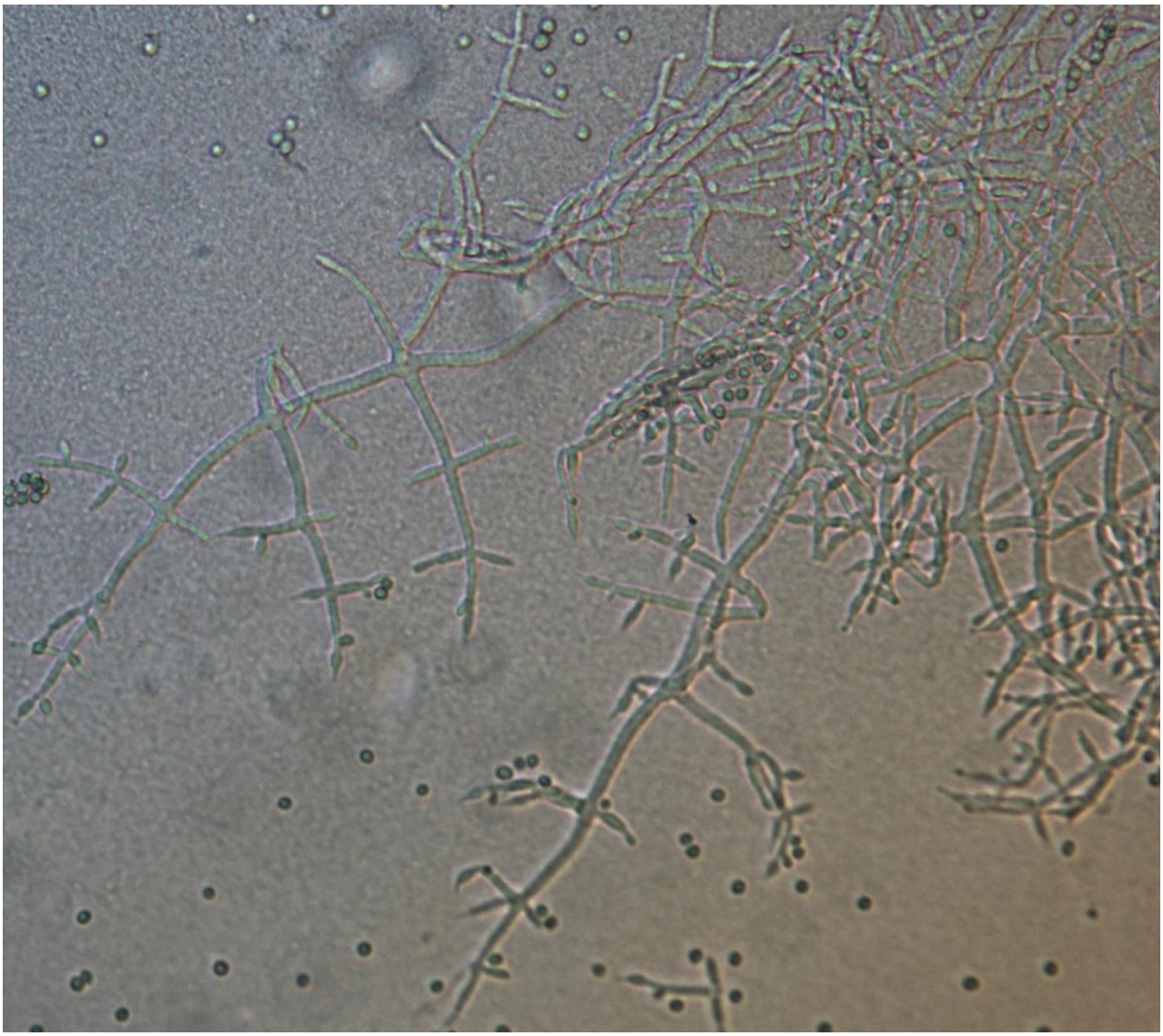
Yin, C., Jones, K.L., Peterson, D.E., Garrett, K.A., Hulbert, S.H. and Paulitz, T.C., 2010. Members of soil bacterial communities sensitive to tillage and crop rotation. *Soil Biology and Biochemistry*, *42*(12), pp.2111-2118.

Zak, D.R., Holmes, W.E., White, D.C., Peacock, A.D. and Tilman, D., 2003. Plant diversity, soil microbial communities, and ecosystem function: are there any links? *Ecology*, *84*(8), pp.2042-2050.

Zeng, Z.Q. and Zhuang, W.Y., 2019. Two new species and a new Chinese record of *Hypocreaceae* as evidenced by morphological and molecular data. *Mycobiology*, *47*(3), pp.280-291.

Zhou, D., Huang, X.F., Guo, J., dos-Santos, M.L. and Vivanco, J.M., 2018. *Trichoderma gamsii* affected herbivore feeding behaviour on Arabidopsis thaliana by modifying the leaf metabolome and phytohormones. *Microbial Biotechnology*, *11*(6), pp.1195-1206.

Chapter 3: Distribution of *Trichoderma* spp. from maize rhizosphere soil in KwaZulu-Natal and Free State, South Africa



Abstract

Trichoderma species are globally distributed and exert beneficial properties such as improving plant growth and fight against plant pathogens. Previous studies on the diversity of *Trichoderma* spp. in South Africa resulted in the identification of 28 species to date. The role of species in this group as beneficial organisms in agriculture is well known and documented. The aim of this study was to isolate and identify *Trichoderma* species from rhizosphere of maize, particularly those grown under different farming practices such as crop rotation and monocultures. The total of 337 strains were isolated from maize soil and identification was based on morphology and phylogenetic analyses. The strains grouped into eleven species which were identified as *T. asperellum, T. afroharzianum, T. gamsii, T. hamatum, T. koningiopsis, T. neokoningii, T. paratroviride, T. peberdyi, T. rifaii, T. spirale,* and *T. velutinum*. The crop rotation practice had the highest number of *Trichoderma* spp. compared to monoculture samples. *T. gamsii* and *T. hamatum* were the most abundant species isolated from maize soil. Five species identified as *T. neokoningii, T. paratroviride, T. peberdyi, T. rifaii,* and *T. velutinum* were reported for the first time in South Africa. Therefore, this study adds to our knowledge on the distribution of *Trichoderma* in South Africa and provide a pool of potential candidates for use in agriculture.

Introduction

Maize is a ubiquitous crop and serves as a food source and fodder (Byerlee and Heisey, 1997; Musokwa *et al*., 2019). It is the staple for many people and renders essential nutrients such as Ca, P, K, Na, Mg, Fe, Mn, and Zn (Devi *et al*., 2014). Maize is planted worldwide, and South Africa is among top ten countries that have the highest production (Wallington *et al*., 2012; Demeke and Di Marcantonio, 2013; FAOSTAT, 2019) with approximately 8 million tons of maize per year produced (FAOSTAT, 2019). In addition, South Africa is also recognized as being number one in the production of maize in Africa (FAOSTAT, 2019).

Yield of maize is, however, inconsistent from year to year. This is because of diseases that reduce yield (Bressan *et al*., 2008; Torres *et al*., 2014; Guadie *et al*., 2019) and environmental fluctuations such as droughts. In order to increase yields the overuse of agrochemicals are widespread, with detrimental effect on the environment (Savci, 2012; Mabe *et al*., 2017; Naeem *et al*., 2018; Badu-Apraku *et al*., 2020). Therefore, environmentally sustainable approaches are required to solve these negative effects, since the currently used methods are detrimental for soil health in the long run (Savci, 2012; Bouwman *et al*., 2013).

The rhizosphere is defined as the area around the roots of plants, and harbors important microorganisms (Curl and Truelove, 2012). Rhizosphere microorganisms have been reported to form beneficial relationships with plants (Benitez *et al*., 2017; Collinge *et al*., 2019). Most of these microorganisms have the ability to improve plant growth by making uptake of nutrients more efficient and also produce metabolites that are responsible for growth promotion (Gravel *et al*., 2007; Saber *et al*., 2017; Bader *et al*., 2020; Mendes *et al*., 2020), phosphate solubilization and the production of auxins (Zahir *et al*., 2010; Gravel *et al*., 2007; Saber *et al*., 2017).

Trichoderma spp. are ubiquitous in nature as they are predominantly found in all ecosystems including the rhizosphere (Druzhinina and Kubicek, 2005; Belayneh Mulaw *et al*., 2010; Blaszczyk *et al*., 2011; Contreras-Cornejo *et al*., 2016; Recio *et al*., 2019). Species from this genus are known for its uses in the industrial and agricultural sectors. In agriculture, they are used as plant growth stimulants (Harman *et al*., 2004; Rajankar *et al*., 2007; Saravanakumar *et al*., 2013; Mendes *et al*., 2020; Kribel *et al*., 2020) as well as for plant disease prevention (Samuels, 1996; Verma *et al*., 2007; Mukherjee *et al*., 2014; Elshahawy *et al*., 2017). In the industrial sector, they have been used to produce various secondary metabolites and some important industrial enzymes such as cellulases, and chitinases (Yao *et al*., 2015; Bischof *et al*., 2016; Waghunde *et al*., 2016).

The diversity of *Trichoderma* spp. has been widely studied across the globe, although the information for Africa is still scarce (Druzhinina *et al*., 2006; Brotman *et al*., 2013; Jaklitsch and Voglmayr, 2015; Hassan *et al*., 2019). A previous study from South Africa, focusing on non-agricultural soil, identified five novel *Trichoderma* species in addition to a number of first reports for this country (Du Plessis *et al*., 2018). Therefore, this study aim to isolate and identify *Trichoderma* spp. in maize soil under conventional and conservation agricultural practices, in order to identify naturally occurring *Trichoderma* species that could be beneficial for plant growth.

Materials and methods

Sampling and Isolation of *Trichoderma* spp.

Figure 3. 1. Map indicating the sampling sites (scale bar = 100 km)

Rhizosphere soil (fine layer of soil on the surface of roots) was collected from two farms (Zunckel Farms) in KwaZulu-Natal and two farms (Van Rooyenswoning and Uitkyk farms) in the Free State, respectively (Table 3.1). Five samples were collected in each field, which resulted in having 20 samples. At Zunckel Oats farm, we started at ZMO1 and walked to the secondary (from the middle) to sample ZMO2. We then drove around the field trying to sample in each quadrant of the field, to account for any variation in the field. At Zunckel Peas farm, we sampled ZMP1 in the outer circle, ZMP2 in the circle 3, ZMP3 in circle 4, ZMP4 in circle 5, and ZMP5 in circle 2, respectively. At VanRooyenswoning and Uitkyk farms the sampling pattern was the same, we walked 20m from the gate and established the first sampling site M1, from M1 we while looking directly away from the gate we sampled M2, 10m away from M1 starting at 9 o'clock, M3 was 10m away from M1 at 12 o'clock, M4 was 10m away from M1 at 3 o'clock and M5 was 10m away from M1 at 6 o'clock. We sampled in a clockwise direction with M1 as the center. The sampling times were as follow, October 2019 (Pre-Plant), January 2020 (Germination), and July 2020 (at Harvest) (Table 3.1). All soil samples were stored in sterile polyethene bags and kept at 4 ℃ before processing in the laboratory. Ten (10) g of soil was weighed and mixed with 100 ml saline solution in a sterile conical flask. The mixture was left for one hour on a shaker at 26 ℃, 121 rpm. The soil suspension was used for dilutions 1×10^{-1} to 1×10^{-3}, and then 0.1 ml from dilutions was spread onto PDA medium (Neogen, UK) supplemented with antibiotics 50 ppm dichloran, 50 ppm chloramphenicol and 100 ppm streptomycin (Applichem, South Africa). Plates were incubated at 26 ℃ for 7 days and thereafter, were viewed under stereo microscope (NIKON SMZ800, Japan) to identify all colonies that resemble those of *Trichoderma* spp. All colonies that resembled *Trichoderma* spp. were transferred into new PDA media (Neogen, UK) for DNA extractions.

Distribution of *Trichoderma* spp. under crop rotation and monoculture

Crop rotation and monoculture practices settings (Table 3.1) were compared to each other in regard to the prevalence of *Trichoderma* spp. In each practice we determined the number of *Trichoderma* species obtained and also the most dominant species was identified. All the analysis of diversity distribution was analysed using Microsoft Excel 2016 where the data were expressed in the form of percentages and represented in pie charts.

Table 3. 1. Sampling sites and farming practices used for isolation of *Trichoderma* spp.

Farm Names	Treatment	GPS coordinates
Van Rooyenswoning	maize after sunflower	27°54'29.8"S 28°32'08.7"E
Uitkyk	maize after maize	27°54'31.4"S 28°32'05.0"E
Zunckel Farms	maize after oats	28°38'17.5"S 29°17'09.1"E
	maize after peas	28°39'48.0"S 29°16'05.4"E

DNA extraction, PCR and Sequencing

Genomic DNA was extracted from *Trichoderma* cultures grown on PDA using bacterial/fungal DNA kit (Zymo research, USA) according to manufacturer's instructions. Polymerase chain reactions (PCRs) were conducted as described by White *et al*. (1990) using the following primers; ITS1 – ITS4 to amplify the ITS1- 5.8s- ITS2 rDNA regions and EF1F – EF2R (Jacobs *et al*., 2004; Du Plessis *et al*., 2018) to amplify the partial elongation factor 1α gene (TEF), respectively. PCR reactions were set up in 10 µl volumes, which consisted of the following, 5 µl Kapa *Taq* Ready mix (KM 1000, KAPA Biosystems), 0.2 µl of each primer (0.2mM), 0.5ng of gDNA template, and 4.1 µl milliQ H_2O. Thermal cycle for ITS were set up with an initial denaturing step at 94 ℃ for 5 minutes followed by 40 cycles consisting of 30 seconds denaturing at 94 ℃, 30 seconds annealing at 56 ℃ and 45 seconds extending at 72 ℃ and a final extension step of 7 minutes at 72 ℃ was used. The TEF thermal cycle were set up with an initial denaturing step at 96 ℃ for 5 minutes followed by 40 cycles consisting of 30 second denaturing at 94 ℃, 30 seconds annealing at 51 ℃, and 90 seconds extending step at 72 ℃, with a final extension step at 72 ℃ for 5 minutes. Sequencing reactions were set up in 10 µl volume with the following; 1 µl DNA (amplified DNA), 1.25 µl Buffer, 1 µl BigDye, and 1 µl forward primer (0.2mM) with 5.75 µl H_2O. Thermal cycle conditions were set up with an initial denaturing at 96 ℃ for 1 minute followed by 25 cycles of denaturing at 96 ℃ for 10 seconds, annealing at 50 ℃ for 10 seconds and extension step at 60 ℃ for 4 minutes. Sequence reaction products were sent to CAF (Central analytical facility, Stellenbosch University) for analyses.

Phylogenetic analyses

DNA sequences were viewed and trimmed using Chromas 2.6.6 version (Technelysium, Australia) (Available from: https://technelysium.com.au/wp/). National Center for Biotechnology Information (NCBI) database was used to blast the DNA sequences of *Trichoderma* spp. in order to compare them with the existing sequences of *Trichoderma* spp. in the database. Ex-type and reference strains were extracted from the NCBI database based on updated and recent previous studies (Bissett *et al*., 2015; Du Plessis *et al*., 2018; Inglis *et al*., 2020). MAFFT plugin from Geneious Prime 2021.03 was used for aligning all the sequences (Katoh *et al*., 2002; Kearse *et al*., 2012; Katoh and Standley, 2013). The resulting alignments were checked and refined using Geneious Prime 2021.03. Post successfully alignments, the two gene (TEF1 and ITS1) sequences were concatenated using Geneious Prime 2021.03, and all files were converted into FASTA format (Katoh *et al*., 2002; Kearse *et al*., 2012; Katoh and Standley, 2013). Thereafter, maximum likelihood phylogenetic trees were constructed using MEGA-X where defaults settings were kept unchanged and branched strengths were evaluated by using 1000 bootstrap replicates (Kumar *et al*., 2018).

Morphological characterization

Trichoderma species were grown on PDA, SNA, and CMA (Sigma-Aldrich, USA) with 2% D (+) glucose monohydrate (KIMIX, Chemicals & Lab Suppliers) for 7 days at 26 ℃. Microscopic features were observed using a compound microscope (Nikon Eclipse E800, Japan) with differential interference contrast capabilities and a CFI plain Apochromat VC 100X lens. Microscope slides were prepared using shear solution and a modified tape method was used (Harris, 2000).

Results

Isolation and distribution of *Trichoderma* species

This study resulted in the isolation and identification of eleven *Trichoderma* species which resolved in two sections namely section *Trichoderma* and section *Pachybasium* (Table 3.2). Each of these sections consisted of two clades. Section *Trichoderma* consist of *T. viride* and *T. pachybasium A*, while the section *Pachybasium* consists of *T. virens* and *T. harzianum*. The clades which obtained more species are *T. harzianum* and *T. viride* as both were represented by four species each. The clades that had the least number of species were *T. pachybasium A* and *T. virens*, represented by two and one species, respectively. Five species namely *T. paratroviride, T. velutinum, T. peberdyi, T. rifaii,* and *T. neokoningii* were reported for the first time in South Africa (Table 3.2). All other species that were isolated in the current study were previously isolated in South Africa (Jaklitsch *et al*., 2006; Kubicek *et al*., 2008; Druzhinina *et al*., 2008; Du Plessis *et al*., 2018).

Different farming treatments resulted in different numbers of *Trichoderma* spp. isolated (Fig. 3.4). According to the data, maize after maize resulted in seven *Trichoderma* spp., which was comparable to the maize after peas treatment. Among all treatments, maize after oats resulted in the largest number of *Trichoderma* spp. (9), whereas maize after sunflower resulted in the least number of species (6) (Fig. 3.4). Moreover, in terms of geographical location it was revealed that higher number of *Trichoderma* species were isolated from the farms in KwaZulu-Natal (KZN) compared to the sites in the Free State (FS). All *Trichoderma* spp. isolated in FS were also found in KZN, with the exception of *T. paratroviride, T. asperellum*, and *T. neokoningii* which were exclusively found in KZN.

A total of 337 *Trichoderma* strains were isolated from maize soil. Sixty (18%) strains were isolated from monoculture sites, whereas 277 (82%) strains were isolated from sites under crop rotation. In the monoculture system, 7 species were identified as *T. spirale, T. gamsii, T. koningiopsis, T. hamatum, T. afroharzianum, T. rifaii,* and *T. peberdyi* (Table 3.2 and Fig. 3.2), respectively. *T. hamatum* was the most isolated species while the *T. koningiopsis* and *T. spirale* were the least isolated species in monoculture systems. These findings are in contrast to fields under crop rotation, where 11 species were identified as *T. gamsii, T. paratroviride, T. koningiopsis, T. spirale, T. asperellum, T. hamatum, T. afroharzianum, T. velutinum, T. peberdyi, T. rifaii* and *T. neokoningii* (Table 3.3 and Fig. 3.3), respectively. *T. gamsii* and *T. hamatum* were represented by 84 and 64 strains, respectively and were the most abundant species isolated in this study while *T. neokoningii* was represented by only one strain isolated. Four species were

only isolated from fields under crop rotation and were identified as *T. paratroviride*, *T. asperellum*, *T. velutinum* and *T. neokoningii*.

Table 3. 2. *Trichoderma* species that were obtained from maize soil

Sections	Clades	Species	First report in South Africa
Trichoderma	*Viride*	*T. paratroviride*	YES
		T. gamsii	NO
		T. koningiopsis	NO
		T. neokoningii	YES
	Pachybasium	*T. hamatum*	NO
		T. asperellum	NO
Pachybasium	*Virens*	*T. spirale*	NO
	Harzianum	*T. afroharzianum*	NO
		T. velutinum	YES
		T. peberdyi	YES
		T. rifaii	YES

Table 3. 3. *Trichoderma* isolates from monoculture agricultural practice

***Trichoderma* species**	**Number of strains under monoculture**
T. gamsii	10
T. koningiopsis	1
T. spirale	1
T. hamatum	18
T. afroharzianum	13
T. peberdyi	4
T. rifaii	13

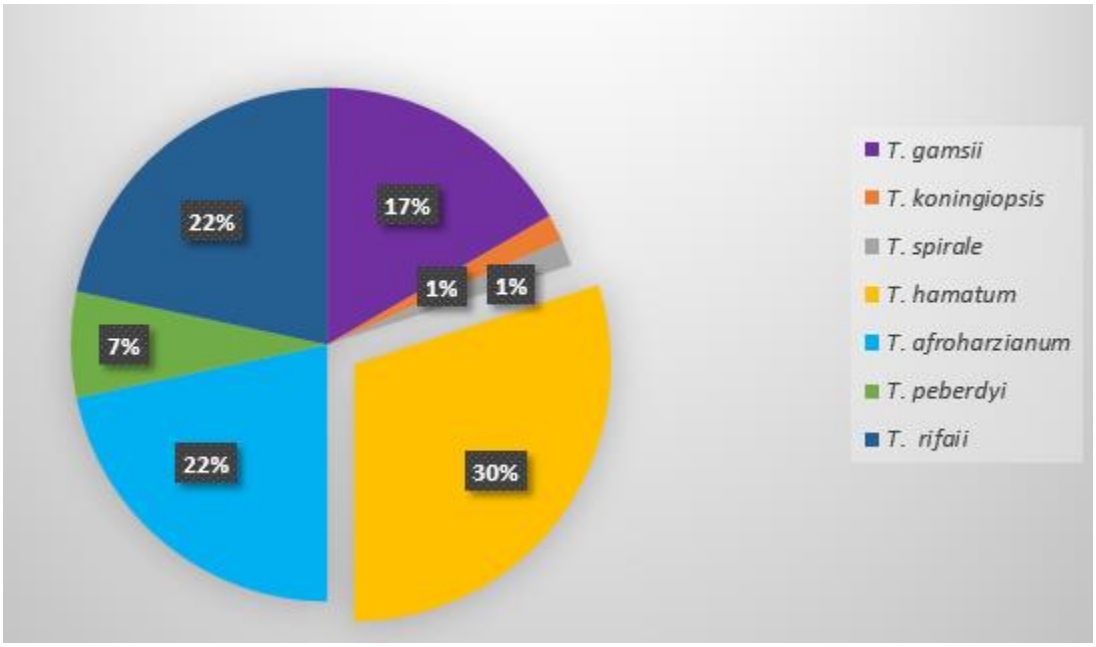


Figure 3. 2. *Trichoderma* diversity under maize monoculture practice

Table 3. 4. *Trichoderma* strains from crop rotation agricultural practice

***Trichoderma* species**	**Number of strains under crop rotation**
T. gamsii	74
T. neokoningii	1
T. paratroviride	8
T. koningiopsis	32
T. spirale	21
T. asperellum	31
T. hamatum	46
T. afroharzianum	5
T. velutinum	9
T. peberdyi	33
T. rifaii	17

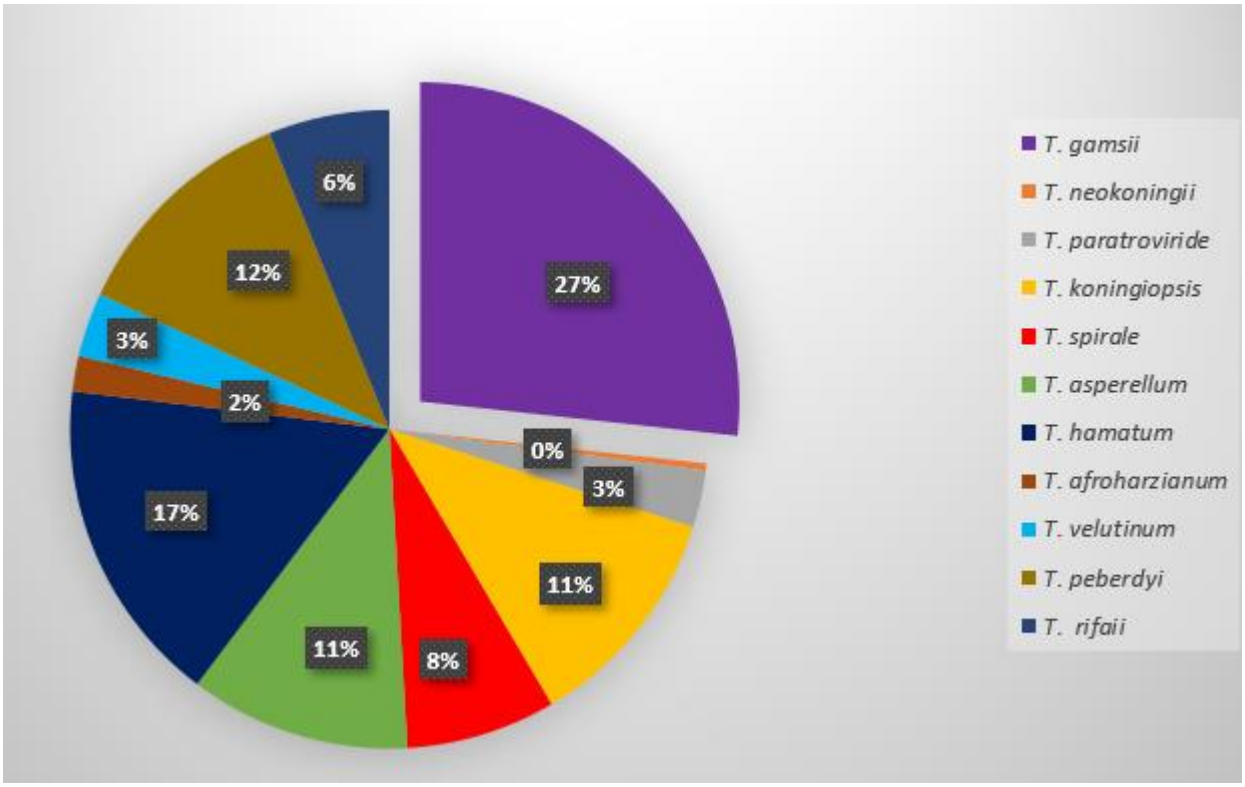


Figure 3. 3. *Trichoderma* diversity under crop rotation practice

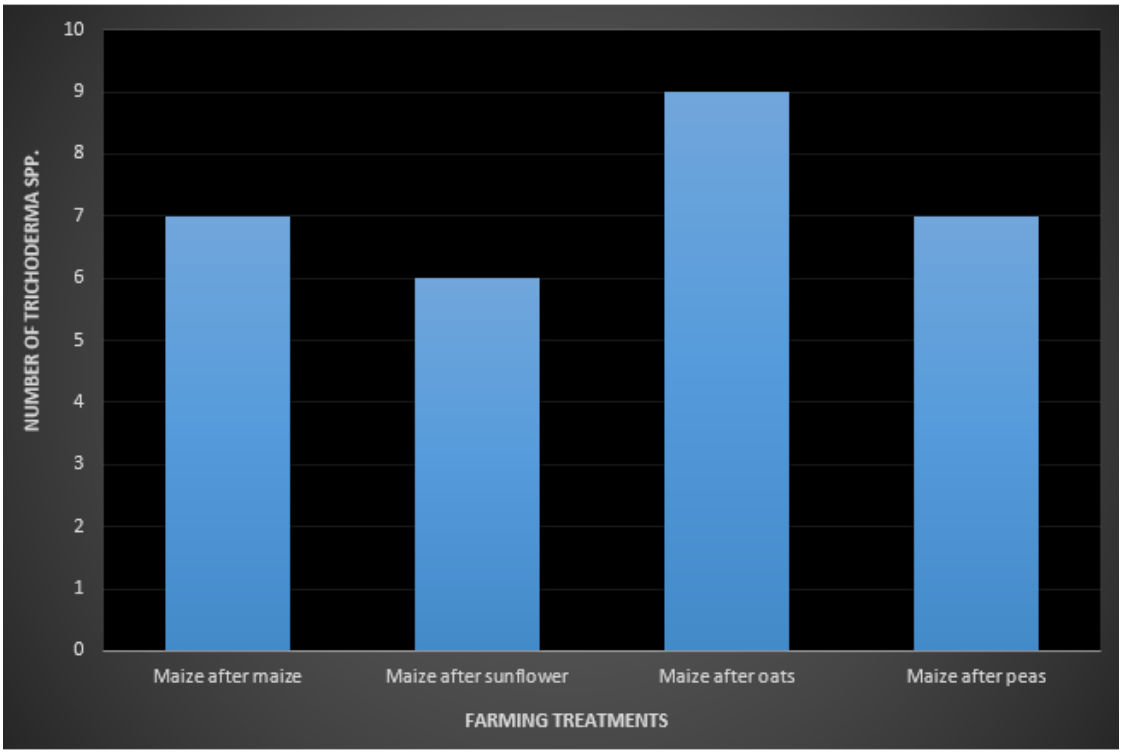


Figure 3. 4. Total number of *Trichoderma* spp. under different farming treatments

Phylogenetic analyses

PCR reactions resulted in amplicons of 540 and 700 bp for ITS1 and TEF1, respectively. All strains isolated in this study clustered with four clades, namely the *T. harzianum* clade, *T. virens* clade, *T. viride* clade, and *T. pachybasium A* clade. All of the clades obtained were well supported by the bootstrap analysis.

***Pachybasium A* clade**

NN192 is a representative which belongs to *T. pachybasium A* clade, under subclade *T. asperellum*. This representative grouped with *T. asperellum* strains identified in previous studies (Table 3.5 & Fig. 3.5). *T. asperellum* is phylogenetically related to *T. yunnanense* and *T. asperelloides*. Furthermore, ex-type strain *T. kumningense* used in this study was found grouping with other strains of *T. asperellum*.

NN13 is a representative that forms a cluster with *T. hamatum*. This species belongs to the *Pachybasium A* clade, under *T. hamatum* subclade. *T. hamatum* is phylogenetically closely related to *T. pubescens* (Fig. 3.5).

Harzianum **clade**

NN15 is a representative belonging to the *Harzianum* clade that was obtained in the study and found to phylogenetically grouped with other *T. rifaii* strains that were identified previously (Fig. 3.6). This species resembles *T. rifaii*, for instance, fast growth on SNA media, small globose conidia, phialides and conidiophores. All these features of *T. rifaii* did not diverge from those that were previously identified for this species (Fig. 3.10). *T. azevedoi* is phylogenetically closely related to *T. rifaii*.

NN25 grouped with other *T. afroharzianum* strains that were obtained in previous studies and this was supported by 86% bootstrap value, which also make this node significant (Fig. 3.6). Morphological features of NN25 strain were similar to these strains classified previously.

NN102 belongs to the *T. velutinum* as it clustered with other previously identified *T. velutinum* (Fig. 3.6). *T. velutinum* is phylogenetically related to *T. tomentosum*. This species is to be reported for the first time is South Africa.

NN70 is a representative of *T. peberdyi* that grouped with strains of *T. peberdyi* that were previously isolated (Fig. 3.6). This species is recently discovered in garlic and onion soil in Brazil. They descendent from the same ancestor and is supported by 99% bootstrap value. NN70 showed features that were similar to previously identified strains. However, other features diverge from identified strains such as the color of colony on PDA media (Fig. 3.11), which resembles a green mycelium in the current study whereas whitish mycelium was observed in previous study (Inglis *et al*., 2020).

Virens **clade**

NN321 representative clustered with *T. spirale* which were identified in previous studies (Fig. 3.7). Morphological features were similar to those identified previously, with one exception: yellowish reverse pigment on PDA media, which had not been detected in prior studies (see Chap 1). *T. longisporum and T. hunanense* were phylogenetically related to *T. spirale* in this study, and this relationship was also shown by Chen and Zhuang. (2017).

Viride clade

NN311 is a representative which grouped with *T. gamsii* strains that were previously identified (Fig. 3.8, Chap 2). *T. gamsii* is phylogenetically close to *T. neokoningii*, and in this study this was also observed. Features that were obtained from previously identified *T. gamsii* strains did not differ from the NN311 representative.

NN191 is the only strain that grouped separately within the clade, and it was observed that it is phylogenetically related to *T. viridescens* and *T. gamsii* strains (Fig. 3.8). However, this strain was identified as *T. neokoningii* due to non-significant bootstrap value and it grouped with other *T. neokoningii* strains.

NN275, this representative belongs to the *viride* clade and was identified as *T. paratroviride*, due to grouping with other *T. paratroviride* strains. This species is reported for the first time in SA and is known to be closely related to *T. atroviride* (Fig. 3.8).

NN312 represent the *T. koningiopsis* which belongs to the *viride* clade. NN312 was found to group with other *T. koningiopsis* strains that were identified previously (Fig. 3.8, Chap 2). *T. koningiopsis* and *T. ovalisporum* are phylogenetically related to each other, with a high bootstrap support.

Table 3. 5. *Trichoderma* strains used to construct maximum likelihood phylogenetic tree of *T. pachybasium A*, *T. virens*, *T. viride*, and *T. harzianum* clades

Taxon	Strain number	GeneBank accession numbers ITS1	TEF1
T. asperellum	GJS 91-162	FJ442224	FJ463285
T. asperellum	ATCC 204424 CBC 433.97		AF456907
T. asperellum	PPRI20669	KX267802	KX267781
T. asperellum	GJS 01-294	EU856297	EU856323
T. asperellum	**NN03**	**MZ708967**	
T. asperellum	**NN192**	**MZ708753**	
T. asperellum	**NN27**	**MZ708988**	
T. asperellum	**NN281**	**MZ708764**	
T. asperellum	**NN28**	**MZ708989**	
T. hamatum	GJS 04-203	EU883567	EU883565
T. hamatum	GJS 05-18	EU856290	EU856315
T. hamatum	DAOM 167057	NR 134371	EU279965
T. hamatum	PPRI20673	KX267808	KX267787
T. hamatum	**NN284**	**MZ708766**	
T. hamatum	**NN245**	**MZ708757**	
T. hamatum	**NN29**	**MZ708990**	
T. hamatum	**NN310**	**MZ708771**	
T. hamatum	**NN55**	**MZ709002**	
T. hamatum	**NN44**	**MZ708999**	
T. hamatum	**NN72**	**MZ709007**	
T. hamatum	**NN13**	**MZ708975**	
T. hamatum	**NN316**	**MZ708777**	
T. kumningense	YMF 1.02659	NR 165846	KJ742802
T. yunnanense	YMF 1.01694	AY941823	AY941825
T. yunnanense	CBS 121219	GU198302	GU198243
T. asperelloides	GJS 99-6	DQ315464	DQ109550
T. asperelloides	GJS 04-116	GU198301	GU248412
T. asperelloides	PPRI20679	KX267816	KX267795
T. paucisporum	GJS 03-69	DQ109527	DQ109541
T. paucisporum	GJS 01-13	DQ109526	DQ109540
T. theobromicola	Dis 376f	EU856296	EU856322
T. theobromicola	Dis 85f	DQ109525	EU856321
T. neorufum	GJS 96-135	AF487653	AF487670
T. lieckfeldtiae	GJS 00-14	DQ109528	EU856326

Table 3.5. (continued)

Taxon	Strain number	GeneBank accession numbers ITS1	TEF1
T. lieckfeldtiae	GJS 05-01	EU856301	EU856328
T. evansii	Dis 380a	EU856295	EU856320
T. evansii	DIS 341HI	EU883568	EU883566
T. flaviconidium	GJS 99-49	DQ023301	DQ020001
T. pubescens	DAOM 166162	NR 077179	AY750887
T. pubescens	CBS 345.93	MH862413	AY665704
T. restrictum	PPRI19367	KX267815	KX267794
T. koningii	CBS 457.96	MH862585	AF456909
T. koningii	CBS 979.70	DQ323410	DQ288994
T. koningii	GJS 00-168	DQ323427	DQ307571
T. koningii	GJS 89-122	AY380902	AY376045
T. koningii	GJS 96-120	DQ109536	DQ109548
T. koningii	GJS 96-119	DQ323424	DQ289003
T. koningii	PPRI20670	KX267804	KX267783
T. neokoningii	GJS 04-216	DQ841734	DQ841718
T. neokoningii	CBS: 120070	MH863076	KJ665620
T. viridescens	DAOM 233967	EU280137	EU280020
T. viridescens	DAOM 237554	EU280135	EU280026
T. viridescens	DAOM 234234		EU280009
T. viridescens	CIBT10	EU280104	EU279999
T. viridescens	CBC 433.34	AY380905	AY376048
T. ovalisporum	DAOM: 232077		KJ871200
T. ovalisporum	DAOM:229859	EU2801138	EU280003
T. ovalisporum	Dis 172i	DQ323438	DQ288999
T. ovalisporum	Dis 203c	DQ315458	DQ307540
T. ovalisporum	Dis 70a	AY380897	AY376037
T. viride	TR2	DQ215457	DQ307538
T. viride	CBS119327	DQ677657	DQ672617
T. viride	PPRI20672	KX267807	KX267786
T. strigosum	DAOM 166121	DQ083027	EU280019
T. strigosum	DAOM 166121	EU280120	AY937442
T. strigosellum	CPK 3604		JQ425705

Table 3.5. (continued)

Taxon	Strain number	GeneBank accession numbers ITS1	TEF1
T. koningiopsis	GJS 91-6	DQ313135	DQ307539
T. koningiopsis	GJS 95-175	AF456923	AF456910
T. koningiopsis	PAN12-49	MK322716	MK16070
T. koningiopsis	GJS 93-20	DQ313140	DQ284966
T. koningiopsis	GJS 91-7	DQ313137	DQ284969
T. koningiopsis	PPRI20683	KX267820	KX267799
T. koningiopsis	GJS 04-373	DQ323437	DQ289006
T. koningiopsis	GJS 04-10	DQ323413	DQ284981
T. koningiopsis	GJS 04-314	FJ463269	FJ442655
T. koningiopsis	GJS 06-263	FJ442613	FJ467647
T. koningiopsis	**NN312**	**MZ708773**	
T. koningiopsis	**NN09**	**MZ708972**	
T. koningiopsis	**NN272**	**MZ708761**	
T. koningiopsis	**NN202**	**MZ708756**	
T. koningiopsis	**NN82**	**MZ708747**	
T. paratroviride	ES12	KY764429	KY764529
T. paratroviride	T11	KY764431	KY764531
T. paratroviride	ES11	KY764428	KY764528
T. paratroviride	S489		KJ665628
T. paratroviride	SFC102188	MF186130	MF185938
T. paratroviride	S385		KJ665627
T. paratroviride	CS107-5	KT153590	KT153586
T. paratroviride	**NN275**	**MZ708763**	
T. paratroviride	**NN274**	**MZ708762**	
T. paratroviride	**NN197**	**MZ708755**	
T. paratroviride	**NN207**		
T. paratroviride	**NN199**		
T. paratroviride	**NN264**		
T. paratroviride	**NN273**		
T. paratroviride	**NN261**		
T. atroviride	S384		KJ665423
T. atroviride	S360		KJ665419
T. atroviride	S383		KJ665422
T. atroviride	S127		KJ665415
T. atroviride	S508		KJ665425
T. atroviride	GJS 98-134	AF456913	AF456887
T. atroviride	PPRI20685	KX267822	KX267801

Table 3.5. (continued)

Taxon	Strain number	GeneBank accession numbers ITS1	TEF1
T. velutinum	MIAE00044	HM176574	HM176592
T. velutinum	38.24.06.3	KP009269	KP008910
T. velutinum	30.24.06.3	KP009268	KP008909
T. velutinum	MIAE00041	HM176571	HM176589
T. velutinum	DAOM230014	DQ083010	AY605804
T. velutinum	LESF132	KT278865	KT279019
T. velutinum	MIAE00033	HM176563	HM176581
T. velutinum	MIAE00036	HM176566	HM176584
T. velutinum	**NN102**	**MZ708748**	
T. velutinum	**NN323**	**MZ708784**	
T. peberdyi	CEN 1426	MK714906	MK696664
T. peberdyi	CEN1425	MK714905	MK696663
T. peberdyi	**NN308**	**MZ708769**	
T. peberdyi	**NN271**	**MZ708760**	
T. peberdyi	**NN70**	**MZ709006**	
T. peberdyi	**NN31**	**MZ708992**	
T. peberdyi	**NN30**	**MZ708991**	
T. peberdyi	**NN01**	**MZ708966**	
T. peberdyi	**NN67**	**MZ709004**	
T. peberdyi	**NN21**	**MZ708982**	
T. peberdyi	**NN309**	**MZ708770**	
T. peberdyi	**NN317**	**MZ708778**	
T. afroharzianum	GJS 04-186	FJ442265	FJ463301
T. afroharzianum	PPRI20674	KX267809	KX267788
T. afroharzianum	GJS 04-193	FJ442233	FJ463298
T. afroharzianum	**NN25**	**MZ708986**	
T. afroharzianum	**NN10**	**MZ708973**	
T. afroharzianum	**NN160**	**MZ708751**	
T. afroharzianum	**NN32**	**MZ708993**	
T. camerunense	GJS 99-231	AY027783	AF348108
T. camerunense	GJS 99-230	AY027780	AF348107
T.afarasin	DIS 314F	FJ442259	FJ463400
T.afarasin	GJS 06-98	FJ442630	FJ463327
T.afarasin	GJS 99-227	AY027784	AF348093
T. tawa	DAOM 232841		EU279972
T. tawa	GJS 97-174	AY737756	AY737739

Table 3.5. (continued)

Taxon	Strain number	GeneBank accession numbers ITS1	TEF1
T. gamsii	TRS 580	KP009332	KP008925
T. gamsii	GJS 05-111	DQ841730	DQ841722
T. gamsii	GJS 06-09		KT028598
T. gamsii	GJS 92-60	DQ315448	DQ307529
T. gamsii	PPRI 20680	KX267817	KX267796
T. gamsii	GJS 04-09	DQ315459	DQ307541
T. gamsii	**NN73**	**MZ695286**	
T. gamsii	**NN40**	**MZ695265**	
T. gamsii	**NN02**	**MZ695263**	
T. gamsii	**NN45**	**MZ695269**	
T. gamsii	**NN50**	**MZ695272**	
T. gamsii	**NN58**	**MZ695276**	
T. gamsii	**NN65**	**MZ695282**	
T. gamsii	**NN75**	**MZ695288**	
T. gamsii	**NN76**	**MZ695289**	
T. gamsii	**NN307**	**MZ708768**	
T. gamsii	**NN41**	**MZ695266**	
T. gamsii	**NN196**	**MZ708754**	
T. gamsii	**NN320**	**MZ708781**	
T. gamsii	**NN43**	**MZ695268**	
T. gamsii	**NN319**	**MZ708780**	
T. gamsii	**NN46**	**MZ695270**	
T. gamsii	**NN42**	**MZ695267**	
T. gamsii	**NN52**	**MZ695274**	
T. gamsii	**NN38**	**MZ695264**	
T. gamsii	**NN311**	**MZ708772**	
T. gamsii	**NN57**	**MZ695275**	
T. gamsii	**NN74**	**MZ695287**	
T. gamsii	**NN122**	**MZ708749**	
T. gamsii	**NN314**	**MZ708775**	
T. gamsii	**NN66**	**MZ695283**	
T. gamsii	**NN123**	**MZ708750**	
T. gamsii	**NN62**	**MZ695279**	
T. gamsii	**NN51**	**MZ695273**	
T. gamsii	**NN49**	**MZ695271**	
T. gamsii	**NN64**	**MZ695281**	
Trichoderma sp.1	**NN191**	**MZ708752**	

Table 3.5. (continued)

Taxon	Strain number	GeneBank accession numbers ITS1	TEF1
T. tomentosum	DAOM:178713	AF011984	KJ871247
T. tomentosum	DAOM178713A	EU330958	EU279969
T. tomentosum	DAOM 234236	EU280083	EU279971
T. tomentosum	DAOM 171918	AY605715	AY605759
T. tomentosum	DAOM 178713a	NR 134357	AY750882
T. cerinum	DAOM 230012		KJ871242
T. cerinum	MA 3646	AJ507139	AY605823
T. ceraceum	GJS 95-159	AF275332	AY937437
T. harzianum	CBS 227.95	AF057605	AF348100
T. harzianum	T55	KX632511	KX632625
T. azevedoi	CEN 1403	MK714880	MK696638
T. azevedoi	CEN 1422	MK714902	MK696660
T. rifaii	DIS 355B	FJ442663	FJ463324
T. rifaii	Dis 337F	FJ442621	FJ463321
T. rifaii	**NN16**	**MZ708977**	
T. rifaii	**NN35**	**MZ708996**	
T. rifaii	**NN318**	**MZ708779**	
T. rifaii	**NN34**	**MZ708995**	
T. rifaii	**NN26**	**MZ708987**	
T. rifaii	**NN20**	**MZ708981**	
T. rifaii	**NN15**	**MZ708976**	
T. rifaii	**NN37**	**MZ708998**	
T. rifaii	**NN22**	**MZ708983**	
T. rifaii	**NN270**	**MZ708759**	
T. rifaii	**NN23**	**MZ708984**	
T. rifaii	**NN06**	**MZ708969**	
T. rifaii	**NN18**	**MZ708979**	
T. rifaii	**NN07**	**MZ708970**	
T. rifaii	**NN05**	**MZ708968**	
T. rifaii	**NN19**	**MZ708980**	
T. rifaii	**NN33**	**MZ708994**	
T. rifaii	**NN17**	**MZ708978**	
T. rifaii	**NN08**	**MZ708971**	
T. rifaii	**NN11**	**MZ708974**	
T. rifaii	**NN36**	**MZ708997**	
T. rifaii	**NN24**	**MZ708985**	

Table 3.5. (continued)

Taxon	Strain number	GeneBank accession numbers ITS1	TEF1
T. virens	GJS 01-287	DQ083023	AY750894
T. virens	GJS 95-80	FJ442218	FJ463365
T. virens	GLI 39	AF099005	GU591800
T. virens	PPRI20676	KX267812	KX267791
T. virens	DAOM:167652	EU330955	AY750891
T. virens	GJS 06-114	FJ442632	FJ463364
T. virens	GLI 39	AF099005	AF534631
T. crassum	DAOM:167063	AF011947	AY750892
T. crassum	CS570-5	KR911899	KR911896
T. crassum	TAMA 0238	AB856632	AB856704
T. crassum	TAMA 0232	AB856628	AB856700
T. crassum	TRS113	KP009300	KP008865
T. crassum	DAOM164916	EU280067	EU280048
T. crassum	CBS 336.93	AF011946	AF401021
T. spirale	DAOM 183974	AF011988	AF534626
T. spirale	TRS111	KP009301	KP008963
T. spirale	DAOM183974	EU280068	EU280049
T. spirale	PAN12-65	MK322728	MK516099
T. spirale	CBS 120963	FJ442608	FJ463291
T. spirale	PPRI 20681	KX267818	KX267797
T. spirale	DAOM229883	EU280082	EU280050
T. spirale	**NN321**	**MZ708782**	
T. spirale	**NN322**	**MZ708783**	
T. spirale	**NN247**	**MZ708758**	
T. spirale	**NN282**	**MZ708765**	
T. spirale	**NN56**	**MZ709003**	
T. longipile	DAOM 177227	NR 134354	AY937430
T. longipile	CBS 120953	FJ860770	FJ860643
T. longipile	GJS 91-93	AY737763	AY737727
T. fasciculatum	DAOM 167646	DQ087258	AY750895
T. longisporum	HMAS 248843	NR154573	KY688043
T. hunanense	HMMA 248841	NR 154571	KY688039
T. hunanense	HMMA 248867	KY687950	KY688040
T. helicum	DAOM 230016	DQ083022	EU280055
T. strictipile	DAOM 172827	AF011980	AY937451

Trichoderma strains isolated in this study are in boldface.

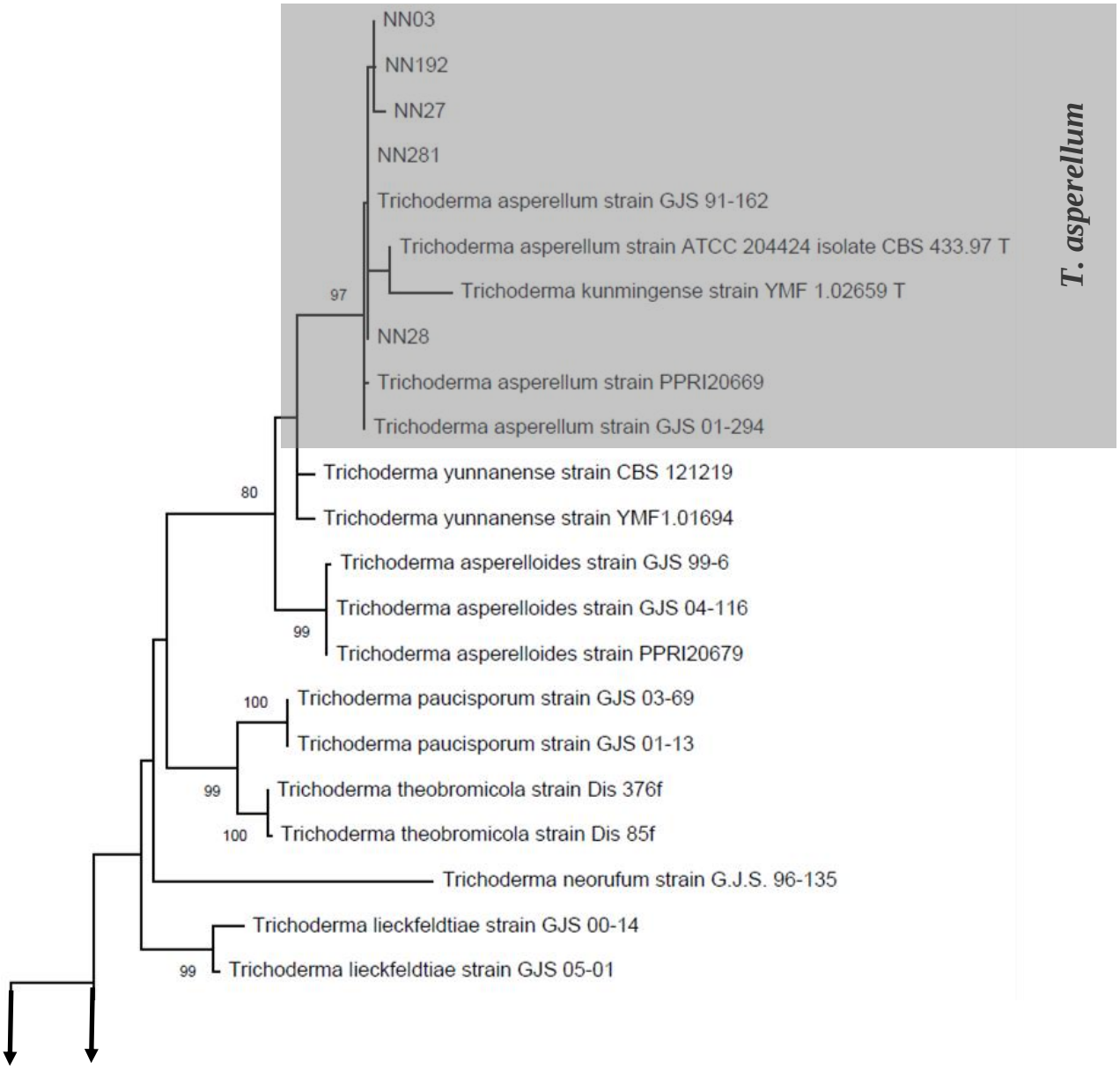


Figure 3. 5. Maximum likelihood phylogenetic tree of *Trichoderma* spp. from *T. pachybasium A* clade, the tree was based on concatenated sequence data (ITS1 and TEF1), ex-type strains indicated by alphabet "**T**", and the tree was rooted with *Protocrea farinosa* CBS 121551.

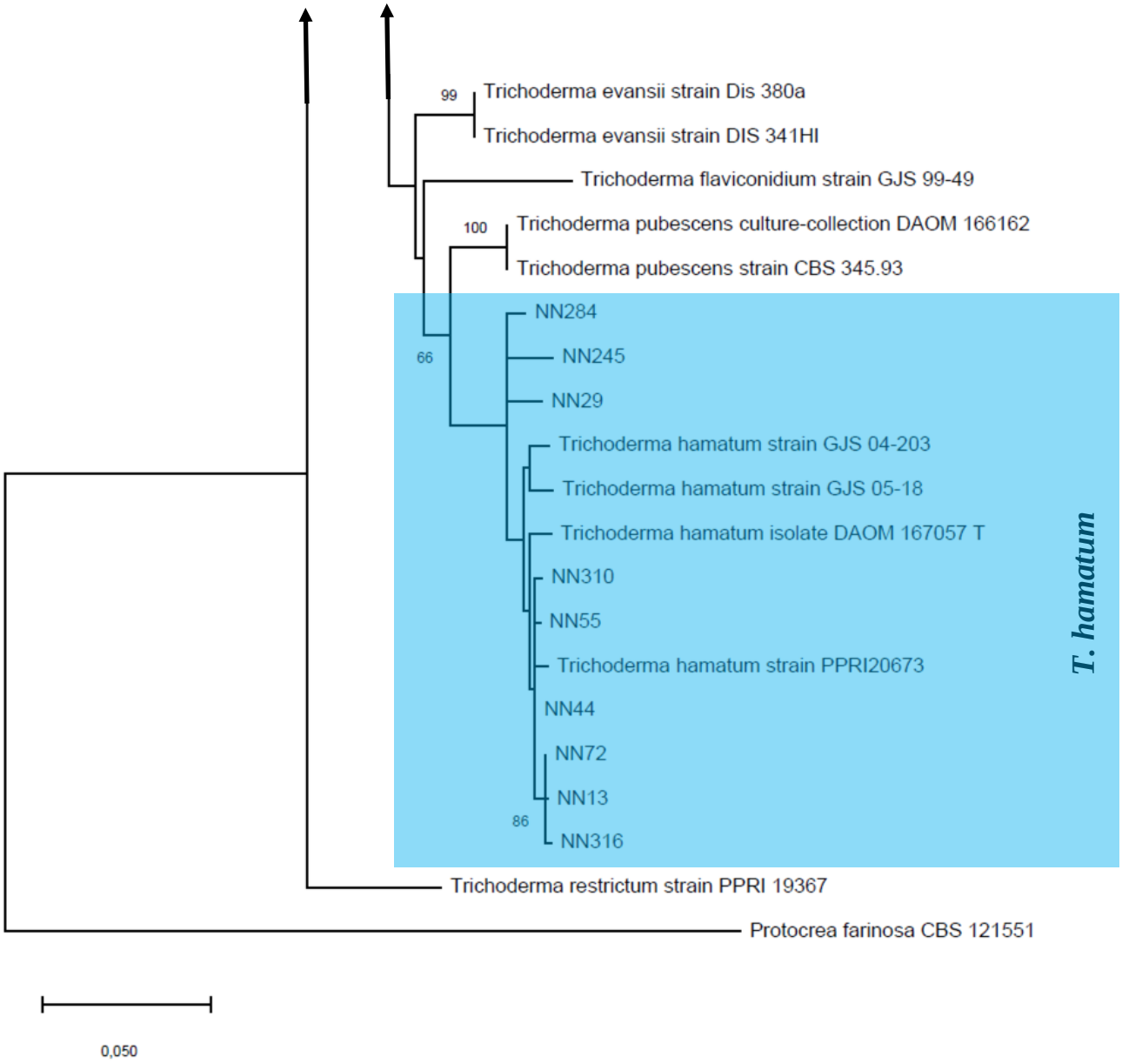


Figure 3.5. (continued)

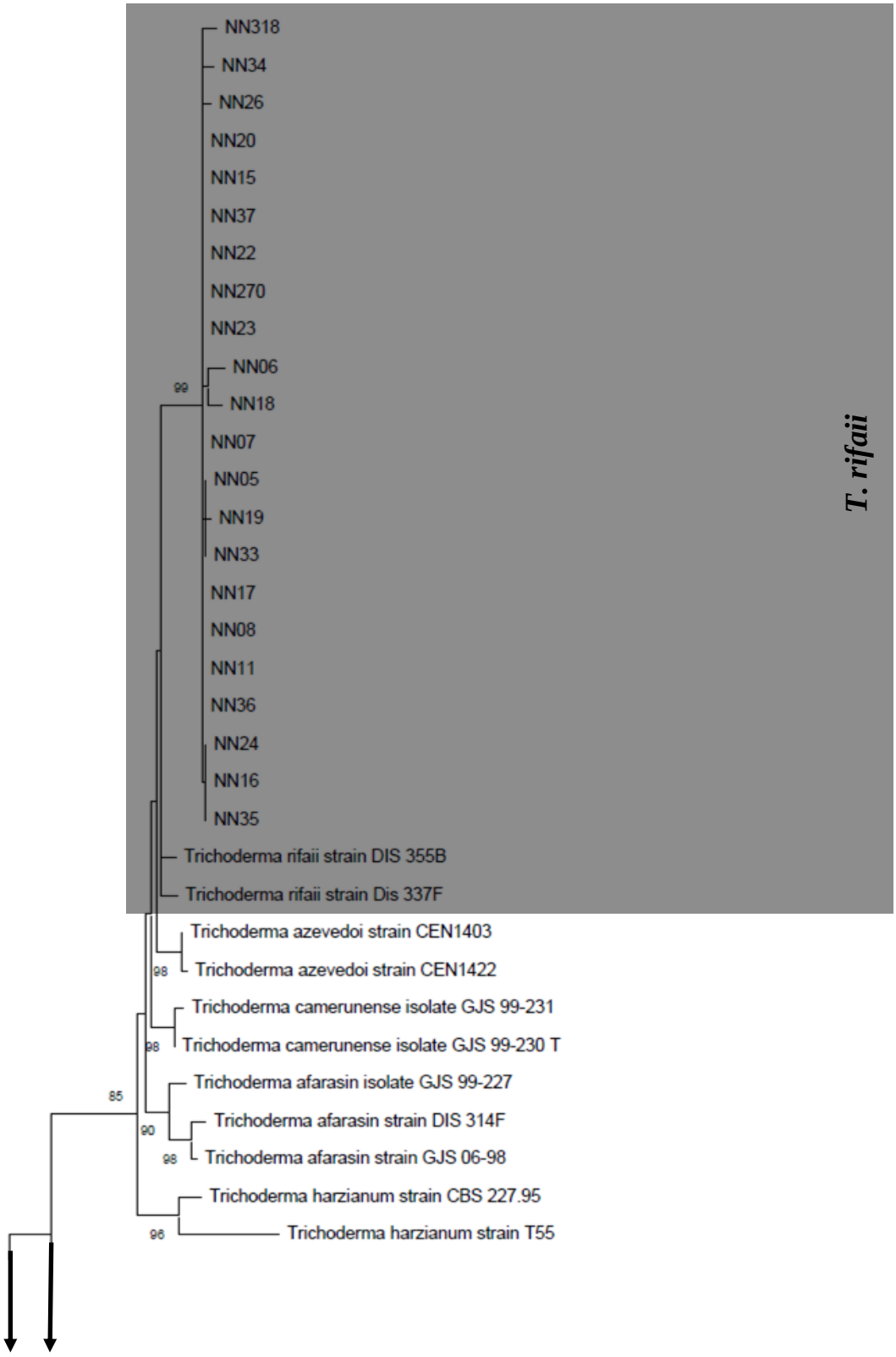


Figure 3. 6. Maximum likelihood phylogenetic tree of *Trichoderma* spp. from *T. harzianum* clade, the tree was based on concatenated sequence data (ITS1 and TEF1), ex-type strains indicated by alphabet "**T**", and the tree was rooted with *Nectria eustromatica* CBS 125578. (Scale bar = 0.050)

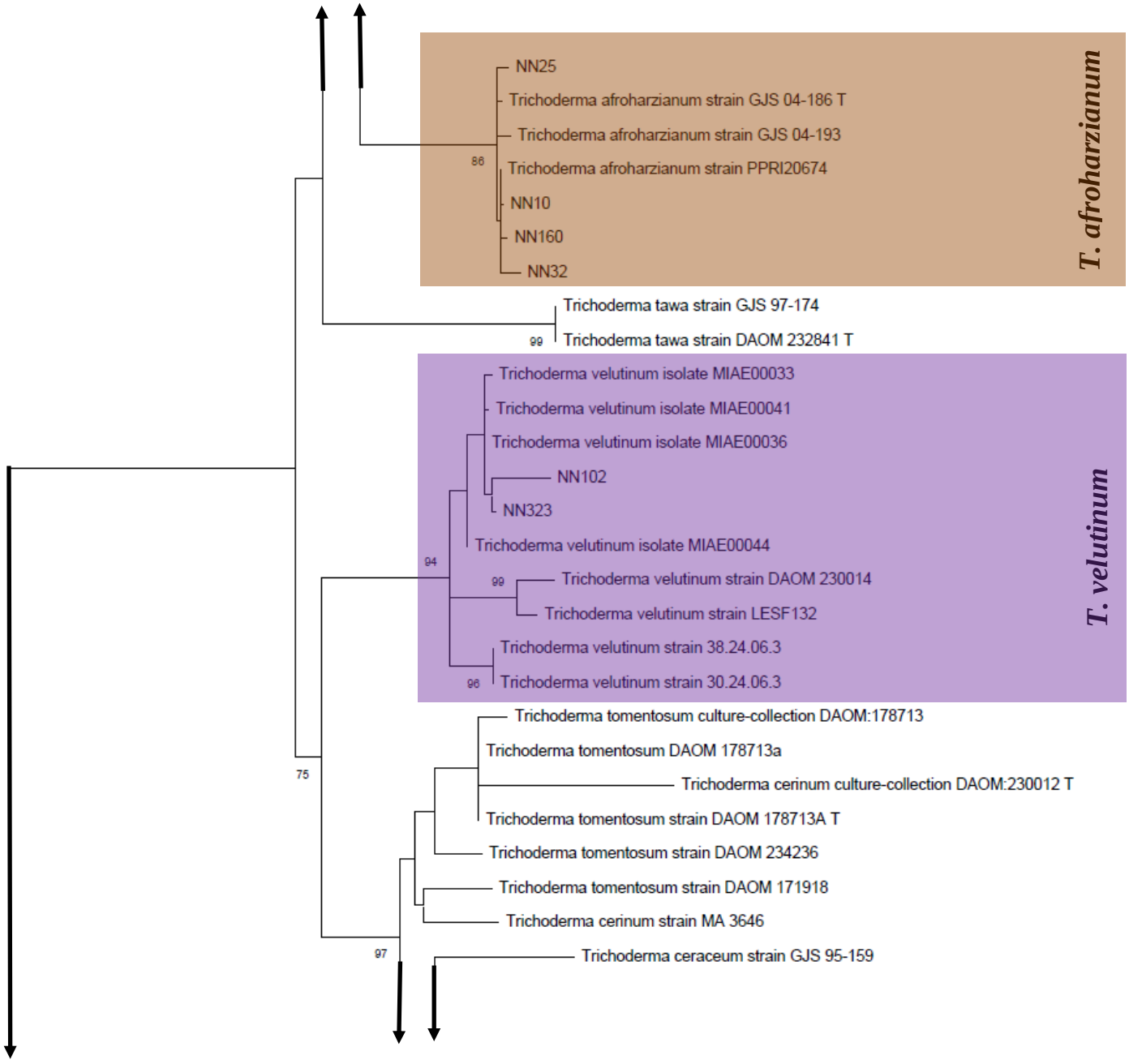


Figure 3.6. (continued)

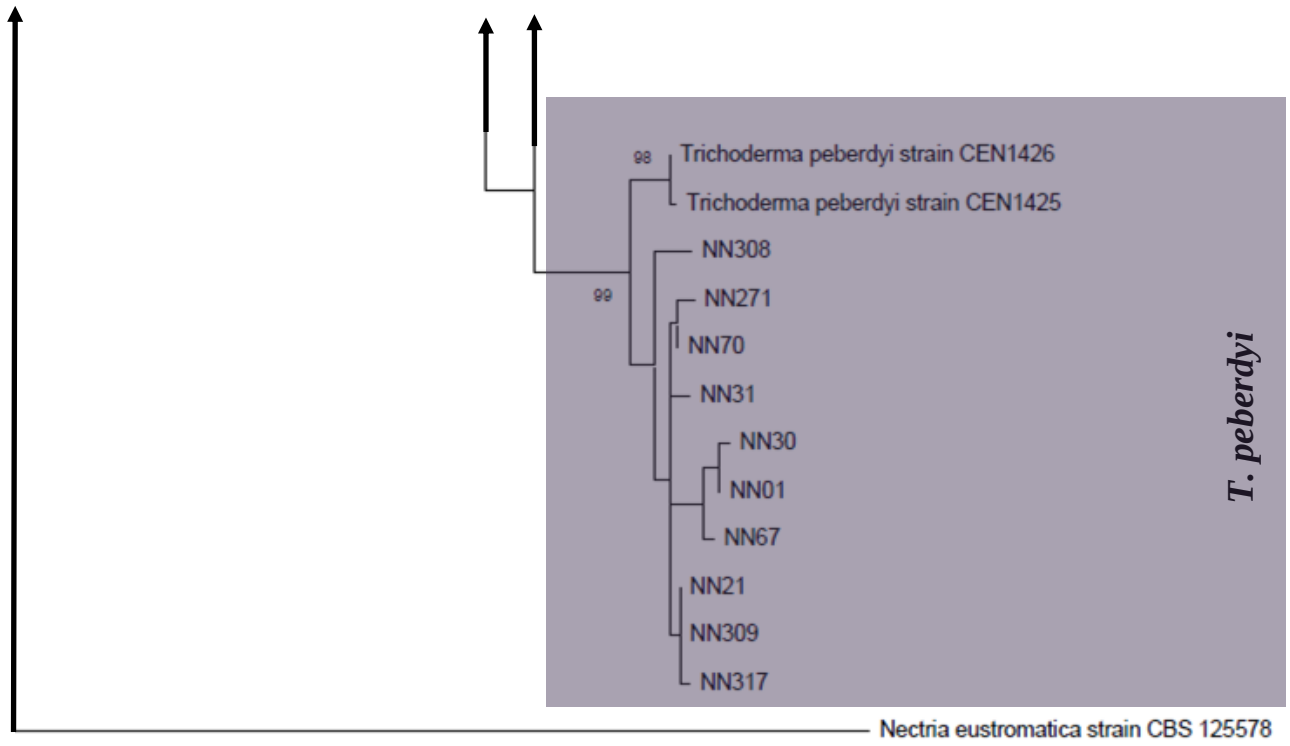


Figure 3.6. (continued)

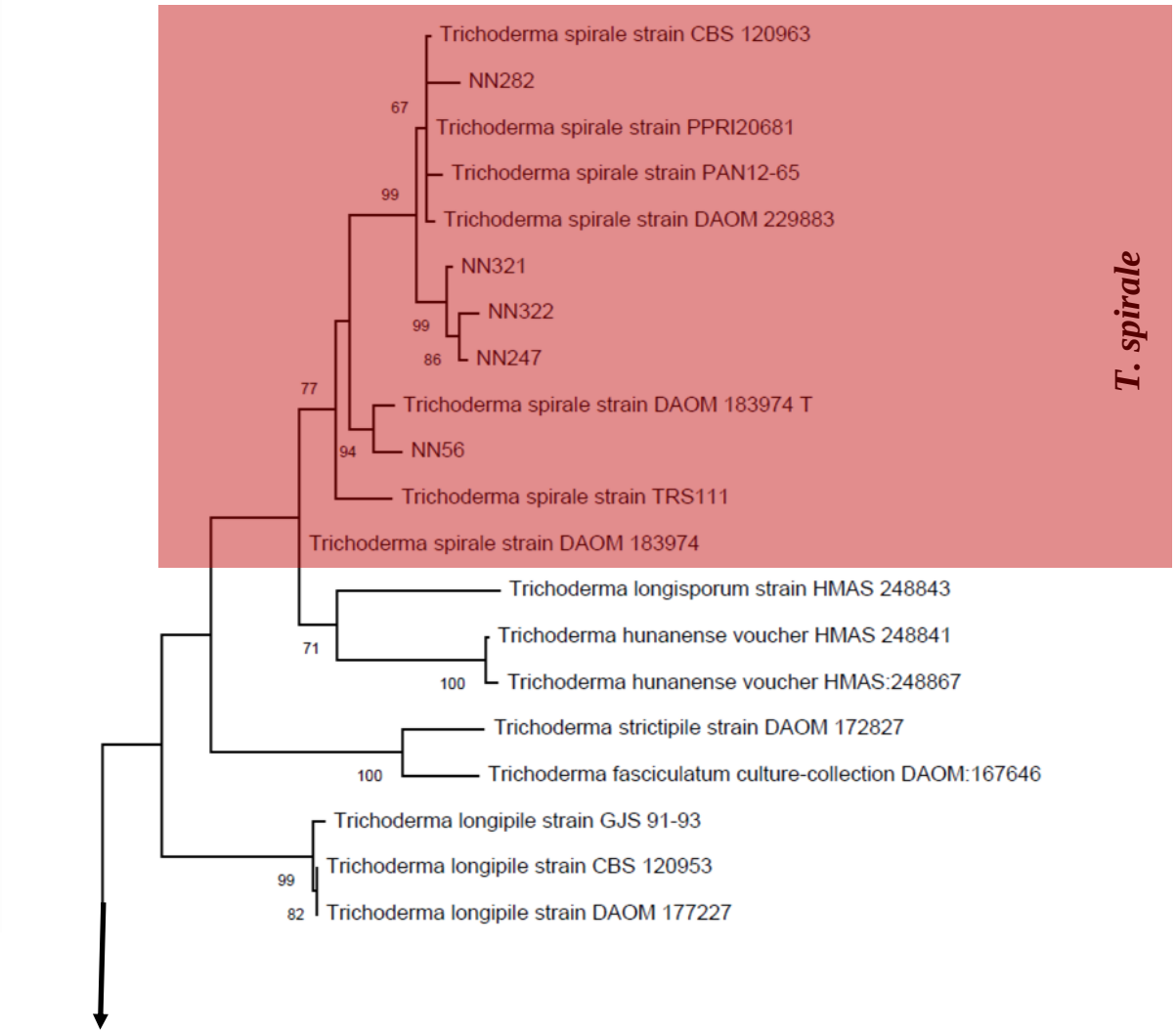


Figure 3. 7. Maximum likelihood phylogenetic tree of *Trichoderma* spp. from *T. virens* clade, the tree was based on concatenated sequence data (ITS1 and TEF1), ex-type strains indicated by alphabet "**T**", and the tree was rooted with *Protocrea farinosa* CBS 121551.

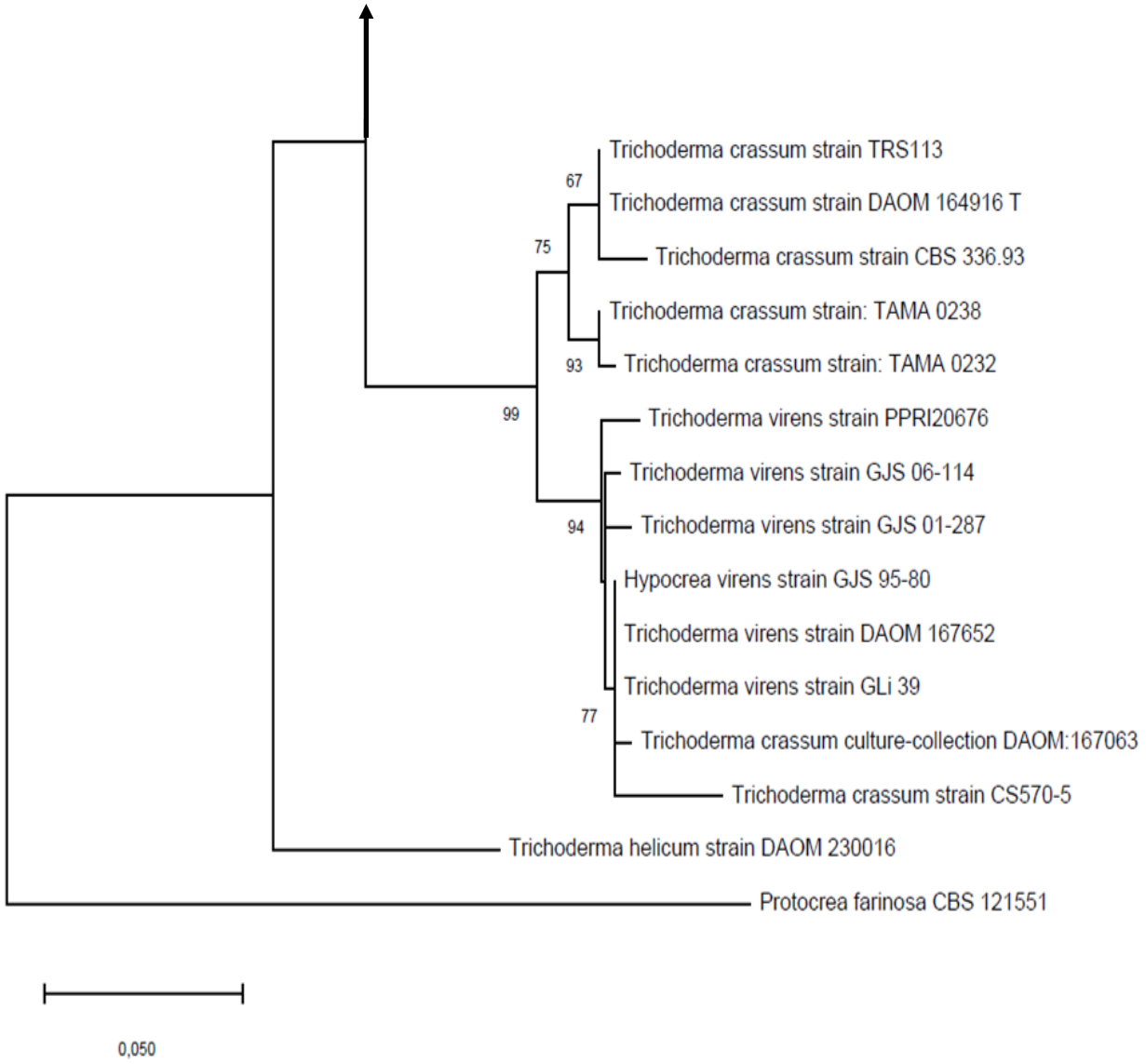


Figure 3.7. (continued)

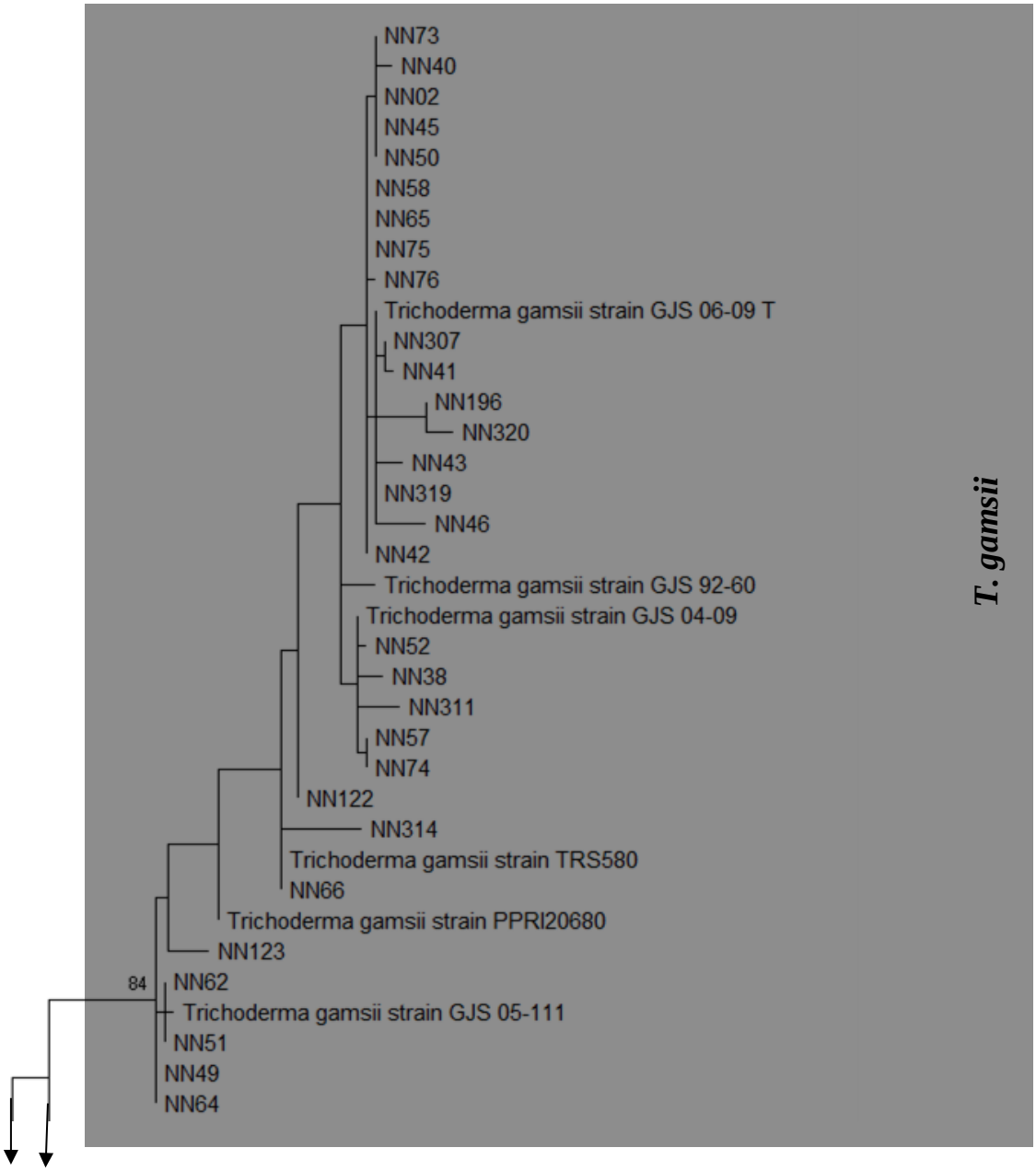


Figure 3. 8. Maximum likelihood phylogenetic tree of *Trichoderma* spp. from *T. viride* clade, the tree was based on concatenated sequence data (ITS1 and TEF1), ex-type strains indicated by alphabet "**T**", and the tree was rooted with *Protocrea farinosa* CBS 121551. (Scale bar = 0.050)

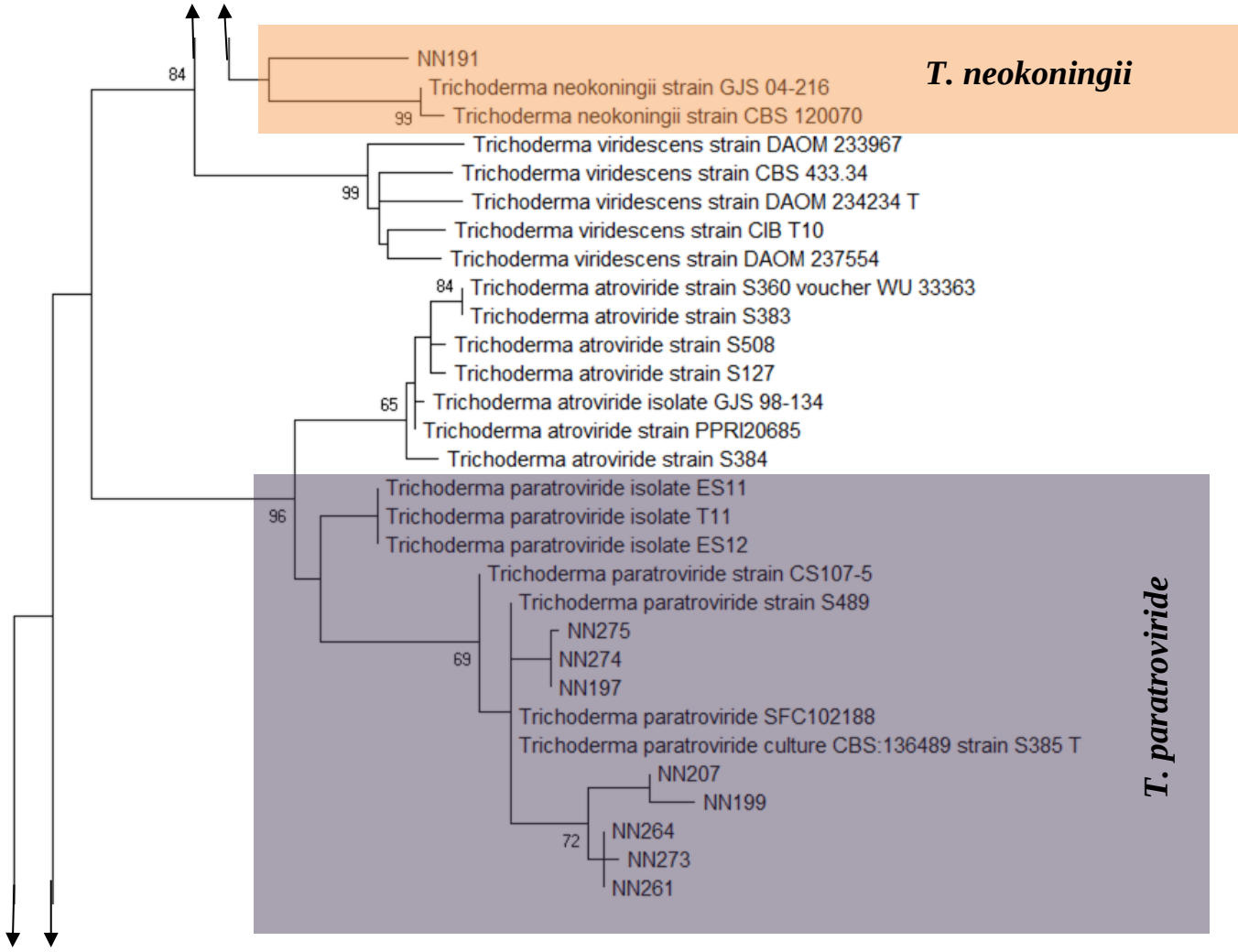


Figure 3.8. (continued)

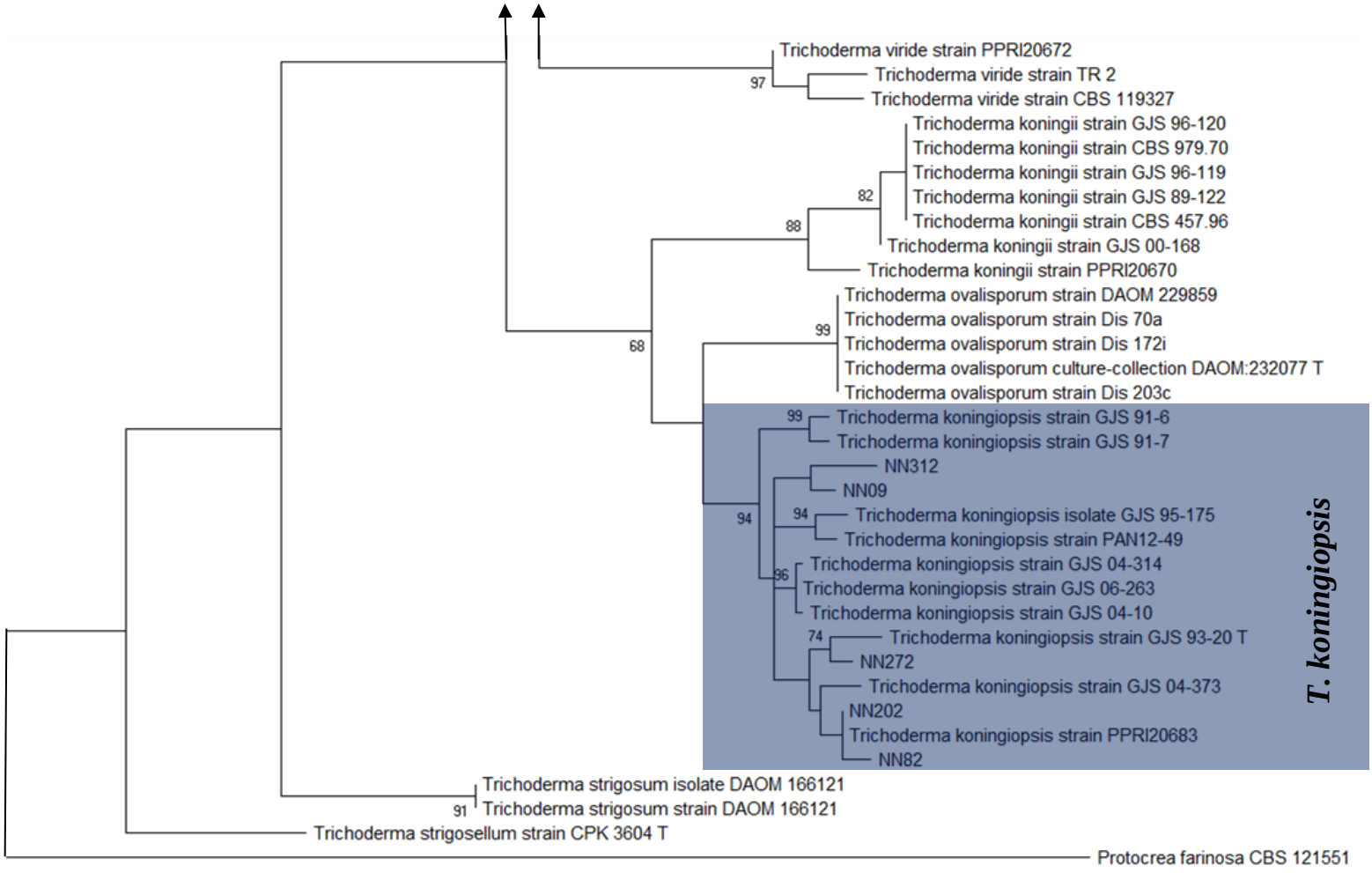


Figure 3.8. (continued)

Notes on isolated *Trichoderma* species

This section is providing the notes about all the *Trichoderma* species that were isolated in this study. Although other *Trichoderma* species will not be discussed here because they were also isolated and discussed namely *T. gamsii, T. koningiopsis, T. spirale,* and *T. velutinum* (see Chapter 2).

***Trichoderma neokoningii* Samuels & Sober., Studies in Mycology 56: 172 (2006)**

T. neokoningii is originally known to be isolated from *pseudostroma* of *Monilophthora roreri* infecting a pod of *Theobroma cacao* in tropical region of Peru. It belongs to the *T. viride* clade of *Trichoderma. T. neokoningii* is morphologically and phylogenetically closely related to *T. gamsii* (Jaklitsch *et al*., 2006). For instances, *T. gamsii* and *T. neokoningii* produced abundant of chlamydospores. The notable unique character that can be recognized between the two is the large size of conidia that are exhibited by *T. gamsii*. In addition, *T. neokoningii*, *T. koningii*, and *T. koningiopsis* are morphologically difficult to distinguish. It can be concluded that the best and accurate method of differentiating closely related species in regard to their morphology is to employ phylogenetic analysis where two or more concatenated markers are used.

***Trichoderma hamatum* (Bonord.) Bainier, Bulletin de la Société Mycologique de France 22: 131 (1906)**

T. hamatum is a cosmopolitan species that was firstly discovered by Bonorden in 1851. Bainier re-described the species and rendered detailed illustrations, however that was not the specimen that was originally collected by Bonorden (Bissett 1991). Thereafter, Bissett (1991) neotypified the species, which then was fully described in detail by Chaverri *et al*. (2003). *T. hamatum* has been found in various habitats such as soil, wood, and herbaceous tissues. It is known of being identical to *T. pubescens*, although there are slightly differences between them which includes growth rate, dimension of conidia and phialides. In the current study 64 strains of *T. hamatum* were identified. A previous study that was conducted in South Africa isolated eleven strains of *T. hamatum* from non-agricultural soil in the Western Cape province (Du Plessis, 2015).

***Trichoderma asperellum* Samuels, Lieckf. & Nirenberg, Sydowia 51: 81 (1999)**

T. asperellum was firstly described in 1999, and it is common in many nations such as South Western Asia, Africa and Peru (Samuels *et al*., 1999). It is widely distributed due to the fact that it was reported to be an endophyte and occur in soil. This species belongs to the *T. pachybasium A* clade. It is known of being phenotypically indistinguishable to *T. asperelloides* (Samuels *et al*., 2010). The 12 strains have been documented on previous study from non-agricultural soil in Western Cape, South Africa (Du Plessis, 2015). In contrast 31 strains of *T. asperellum* were obtained in this study. In addition, it is also used as a biostimulant or biocontrol (Kumar *et al*., 2017; Fu *et al*., 2021).

***Trichoderma afroharzianum* P. Chaverri, F.B. Rocha, Degenkolb & Druzhin., Mycologia 107 (3): 568 (2015)**

Trichoderma afroharzianum was first recognized in 2010 and it was fully described in 2015 (Druzhinina *et al*., 2010; Chaverri *et al*., 2015). This species belongs to the *T. harzianum* complex and is widely distributed (Druzhinina *et al*., 2010; Du Plessis *et al*., 2018). A previous study from South Africa isolated 10 strains of *T. afroharzianum* (Du Plessis, 2015), whereas 18 strains were obtained in this study. It is mostly known to occur in soil and has been reported as biocontrol agent, this was due to the lytic enzymes produced by *T. afroharzianum* (Sawant *et al*., 2017; Liu *et al*., 2020; Tchameni *et al*., 2020).

***Trichoderma paratroviride* Jaklitsch & Voglmayr, Studies in Mycology 80: 75 (2015)**

Trichoderma paratroviride was isolated from wood and bark of trees and shrubs in Spain (Jaklitsch and Voglmayr, 2015). Furthermore, *T. paratroviride* is suspected to be from shiitake mushroom farms in Korea (Kim *et al*., 2012; Jaklitsch and Voglmayr, 2015). *T. paratroviride* was isolated from maize soil in this study, which is the first to report of its presence in South Africa. It is known that *T. paratroviride* and *T. atroviride* are not distinct in terms of their microscopic features, although they show unique colony characters. The NN275 representative resembled the phenotypic characters that are similar to the previously described *T. paratroviride* strains (Jaklitsch and Voglmayr, 2015). However, the colony growth rate was faster on CMD plate at 25 ℃ for this strain compared to those previously identified (Jaklitsch and Voglmayr, 2015).

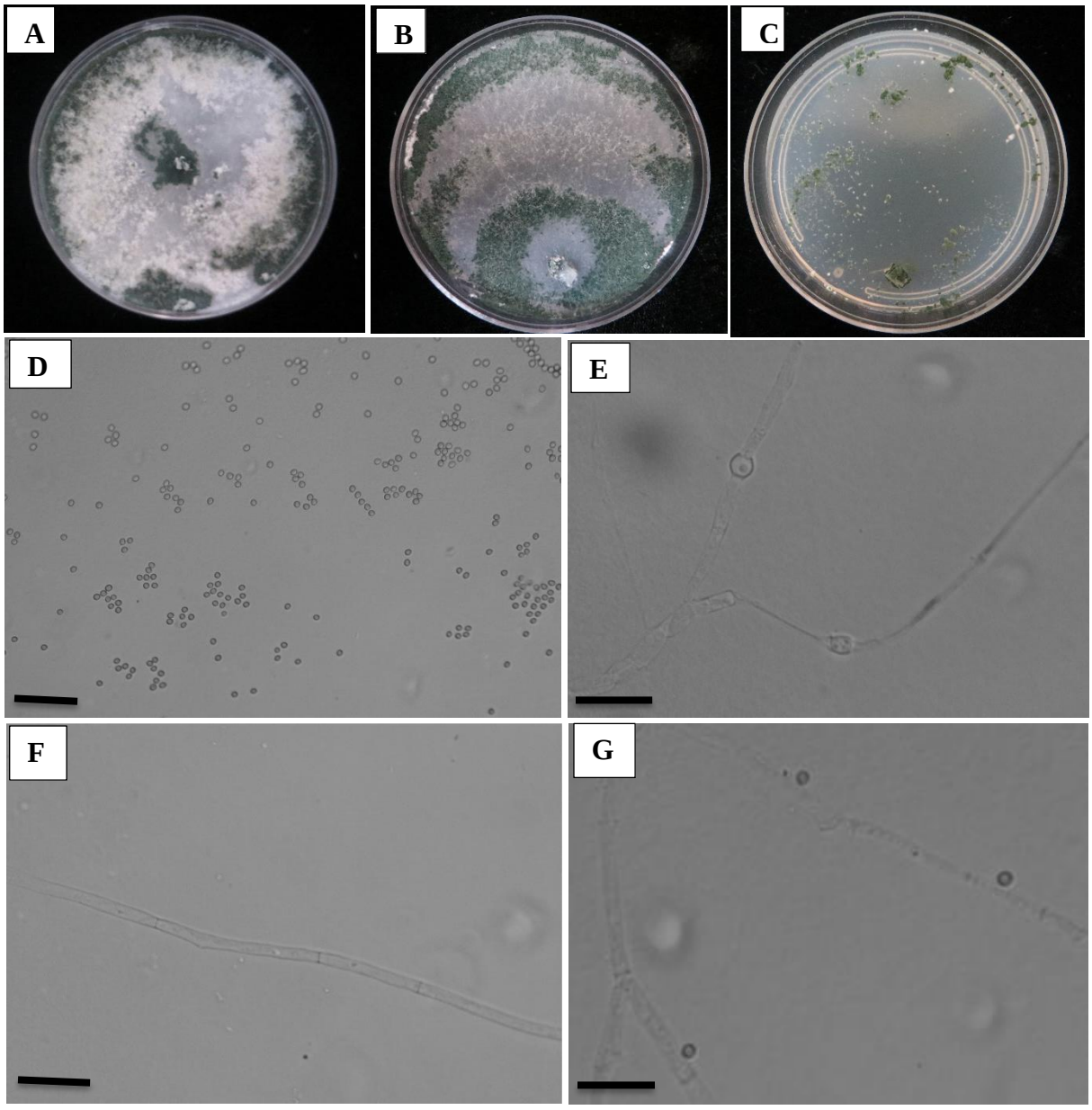


Figure 3. 9. Morphological features of *Trichoderma paratroviride* (NN275) on **A**. PDA, **B**. CMD, and **C**. SNA. **D**. Conidia, **E**. Chlamydospores, **F-G**. Hyphae (All scale bars = 20 µm) (Magnification 400X)

***Trichoderma rifaii* F.B. Rocha, P. Chaverri & Samuels, Mycologia 107 (3): 586 (2014)**

T. rifaii is a member of *T. harzianum* complex and phylogenetically related to *T. azevedoi* (Chaverri *et al*., 2015; Ingilis *et al*., 2020). *T. rifaii* is the first time to be reported in South Africa as it is only known to occur in tropical South America. The 30 strains were attained in this study. This species is commonly known only as endophytes in leaves and stems of tropical trees, although it was isolated from agricultural soil in the current study. Morphological features of the representative strain are similar with other strains that were previously identified, such as fast growth on SNA, ampuliform to lageniform phialides, and phialides arising in whorls at the tips of secondary branches, and conidial pustules usually not well formed.

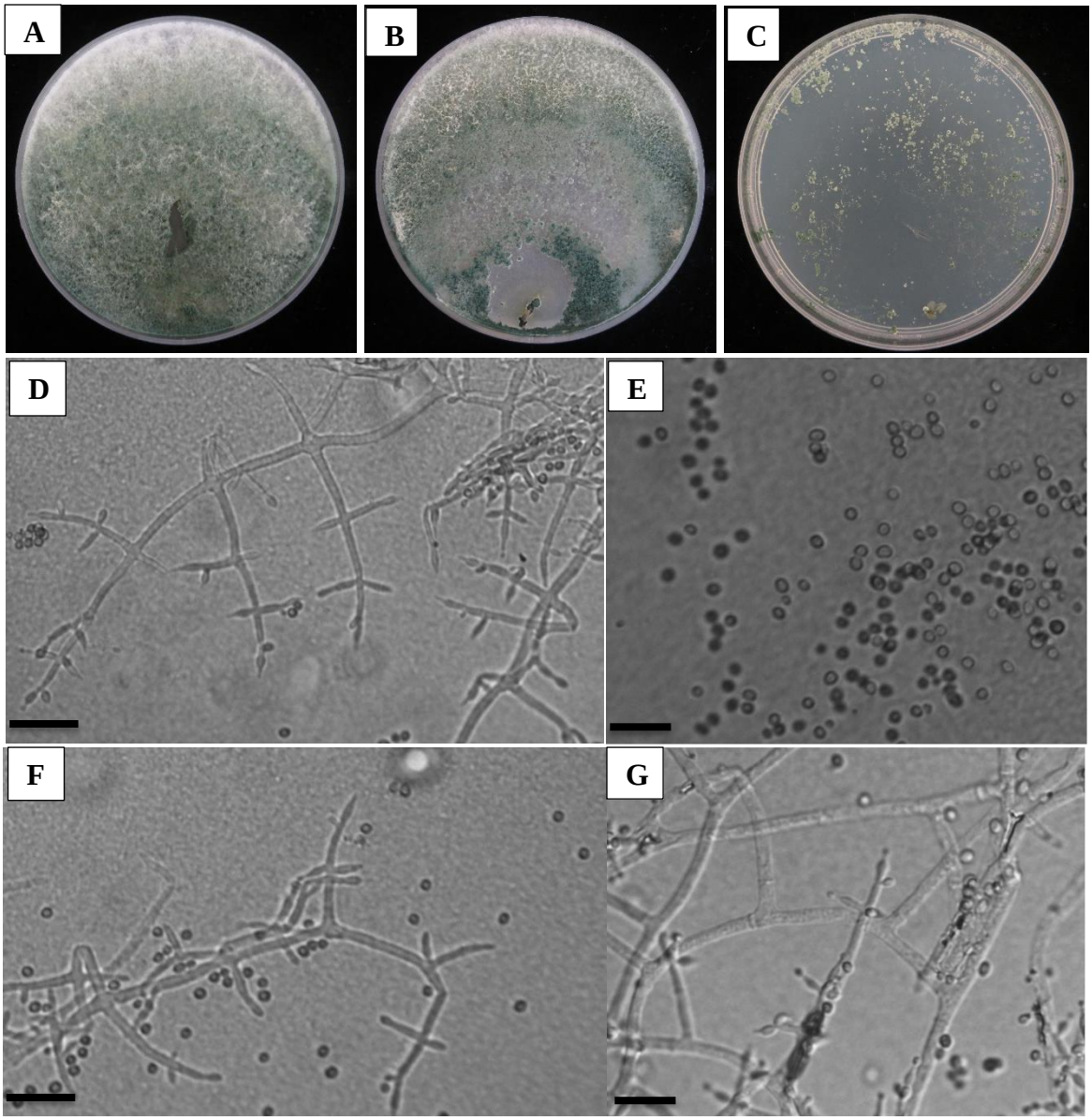


Figure 3. 10. Morphological features of *Trichoderma rifaii* (NN15) on **A**. PDA, **B**. CMD, and **C**. SNA. **D**-**F**. Phialides, **E**. Conidia, **G**. Conidiophores (All scale bars = 80 µm) (Magnification 400X)

***Trichoderma peberdyi* M.C. Valadares-Inglis & P.W. Inglis, PLoS One 15 (3): 12 (2020)**

This species was firstly isolated in garlic and onion soil in Brazil (Ingilis *et al*., 2020). It belongs to *T. harzianum* clade and has been reported that it is closely related to *T. tomentosum* and *T. ceraceum*. This is the first study to isolate this species in South Africa and 37 strains of this species were attained. Moreover, to date no studies have been conducted to evaluate it potential applications. Therefore, this species still needs to be explored for various applications that are currently known to be exhibited by other *Trichoderma* species (Rudresh *et al*., 2005; Mukherjee *et al*., 2014; Carvalho *et al*., 2017; Khoshmanzar *et al*., 2020).

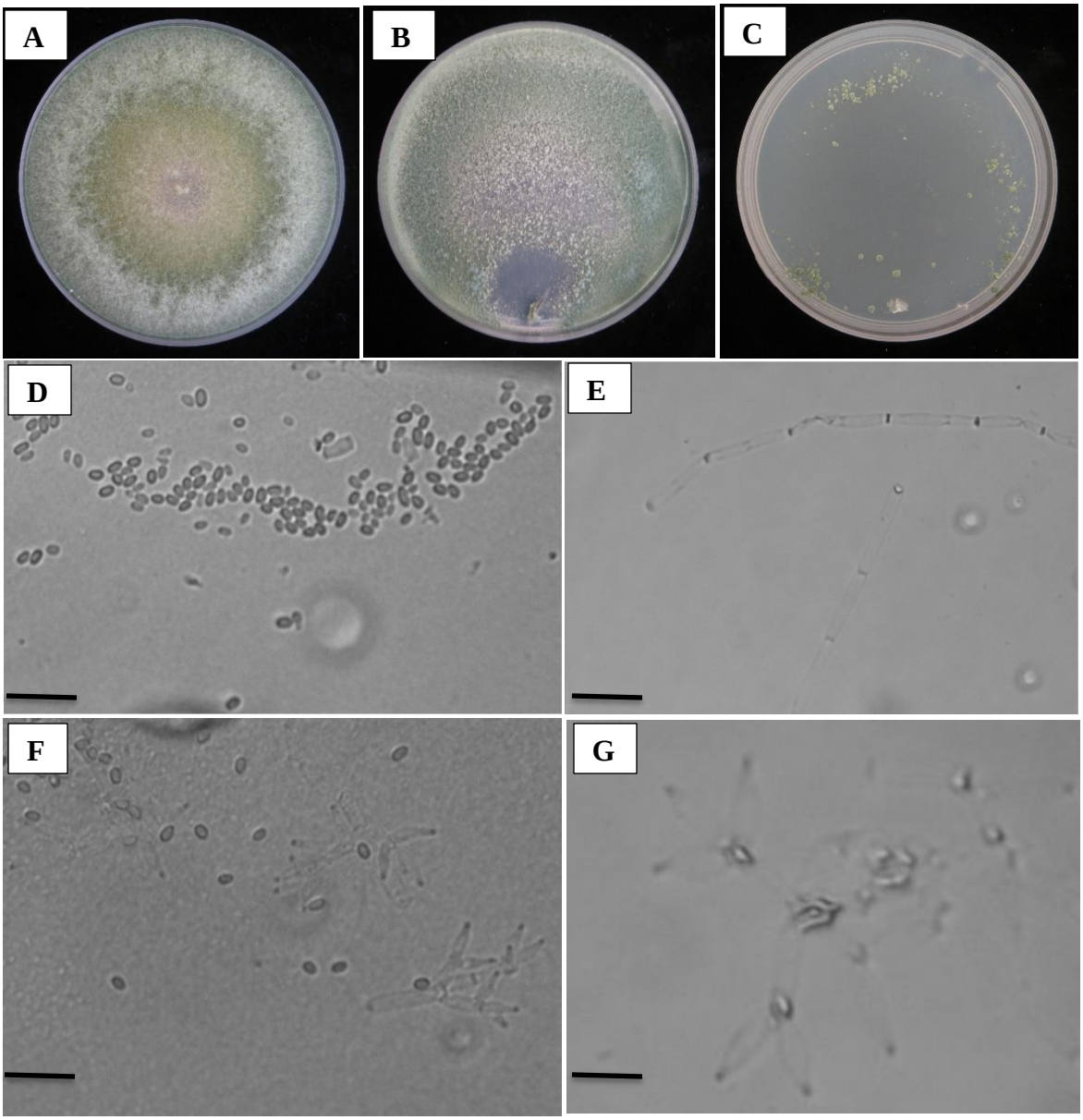


Figure 3. 11. Morphological features of *Trichoderma peberdyi* (NN70) on **A**. PDA, **B**. CMD, and **C**. SNA, **D**. Conidia, **E**. Hyphae, **F-G**. Phialides and Conidiophores (All scale bars = 80µm) (Magnification 400X)

Discussion

The correct identification and classification of *Trichoderma* spp. is crucial as this genus has various beneficial impacts on our daily lives (Rudresh *et al*., 2005; Mukherjee *et al*., 2014; Waghunde *et al*., 2016; Bischof *et al*., 2016; Khoshmanzar *et al*., 2020). One example of this is where *Trichoderma* is used as plant growth promoting agent and as well as biological control agent (Harman *et al*., 2004; Saravanakumar *et al*., 2013; Zhang *et al*., 2016) in food production. In addition, its ability to produce various enzymes is well studied (Mander *et al*., 2014; Mostafa *et al*., 2014). These enzymes are useful in biotechnology industries for different purposes (Saravanakumar and Kathresan, 2014; Carvalho *et al*., 2017). Having a larger pool of local potential beneficial strains can enhance crop production in South Africa.

Maize is regarded as a cosmopolitan crop and one investigation reported that the same *Trichoderma* spp. were isolated from several maize sites (Zachow *et al*., 2016). *T. koningii*, *T. koningiopsis*, *T. harzianum,* and *T. hamatum* are among the maize associated species (Zachow *et al*., 2016). The current investigation confirms earlier findings, as two of these species, *T. koningiopsis* and *T. hamatum,* were isolated in this study. Jiang *et al*. (2016) reported nine *Trichoderma* spp. in maize soil, whereas eleven *Trichoderma* spp. were isolated in this study. This might be as a result of differences in geographical locations and other environmental factors (Danielson and Davey, 1973; Hermosa *et al*., 2004; Jaklitsch *et al*., 2006). In this study, we isolated three species (*T. koningiopsis*, *T. asperellum,* and *T. hamatum*), that was also identified by Jiang *et al*. (2016) from maize associated soils.

Some of the species that are found in the current study were also isolated from wheat soil (see Chapter 2) in the Western Cape. These species were *T. gamsii*, *T. koningiopsis*, *T. spirale,* and *T. velutinum* (see Chapter 2 for full description). This might suggest that these particular species are generalists, and are commonly found in agricultural soil in South Africa regardless of differences in biogeography, environmental conditions or crops. Two species, *T. asperellum* and *T. gamsii*, were isolated from this study, both of which are known to be abundant in Mediterranean climates (Hermosa *et al*., 2004; Jaklitsch *et al*., 2006). It's not a surprise that these species may be found in non-Mediterranean climate, given their widespread distribution (Samuels *et al*., 1999; Samuels *et al*., 2010; Chen *et al*., 2016; Zhou *et al*., 2018).

Crop rotation and monoculture agricultural practices had an apparent impact in the distribution of *Trichoderma* species. More species and strains were isolated from fields under crop rotation compared to species and strains isolated from fields under monoculture (Fig. 3.4). Diversification of plants is normally known to have a direct result on the microbial diversity which also shows an increase in diversity (Zak *et al*., 2003; Venter *et al*., 2016). This is, however, not always the case, and some studies showed that monoculture performed better compared to some crop rotation practices (Yin *et al*., 2010; Gałązka *et al*., 2017). In this study the monoculture resulted in a higher number of *Trichoderma* spp. compared to maize after sunflower rotation (Fig. 3.4). There may be a variety of reasons for this, including environmental conditions, soil types, geographical location, moisture, seasons and this warrants further investigation (Hermosa *et al*., 2004; Jaklitsch *et al*., 2006; Marais *et al*., 2012; Reardon *et al*., 2014).

The diversity of *Trichoderma* spp. from agricultural habitats has only been documented in a few studies (Zachow *et al*., 2016; Jiang *et al*., 2016), and no diversity study has been undertaken on *Trichoderma* from maize soil in South Africa. Furthermore, most *Trichoderma* spp. used to promote maize crop growth and development, have been isolated from habitats other than maize soil (Okoth *et al*., 2011; Kumar *et al*., 2017; Nepali *et al*., 2020; Fu *et al*., 2021). Thus, in the current study we focused on isolating and identifying *Trichoderma* spp. that are already associated with maize crop. These strains could potentially have a good interaction with maize when applied as bio-stimulants or biological control agents because they have been accustomed to the maize soil environments.

T. kunmingense is a recently described species (Qiao *et al*., 2018) and group with all *T. asperellum* strains, including the type strains and strains isolated in this study (Fig. 3.5). However, the validity of this species may be questioned because Qiao *et al*. (2018) based their analyses solely on a single strain. Based on this, we argue that *T. kunmingense* be considered a synonym of *T. asperellum.*

The strains from this study that have been identified as *T. rifaii* (Fig. 3.6), could potentially represent a novel species as it formed a monophyletic clade within *T. rifaii*, and is significantly supported by bootstrap value of 99%. However, all the phenotypic characters resembled that of *T. rifaii* (Chaverri *et al*., 2015). A more detailed study, including additional strains and gene markers should be included to determine the validity of a novel species.

Overall, findings showed that eleven *Trichoderma* spp. were isolated in this study, with five species reported for the first time in South Africa. *T. gamsii* and *T. hamatum* were the most abundant species in crop rotation and monoculture practices, respectively. Both of these species were reported to be cosmopolitan (Bissett, 1991; Chaverri *et al*., 2003; Hermosa *et al*., 2004; Jaklitsch *et al*., 2006). The distribution of *Trichoderma* spp. in this study showed that crop rotation farming should be adopted since it consitsts of higher number of *Trichoderma* species, although this was biased when we looked at it in comparison with monoculture fields since there were more fields of crop rotation than monoculture fields. This is essential because *Trichoderma* spp. have the beneficial functions in agriculture including the improvement of plant growth as well as prevention of plant diseases. This study will also reinforce the knowledge of *Trichoderma* in SA and increases the pool of locally *Trichoderma* strains that could be used in agriculture.

References

Bader, A.N., Salerno, G.L., Covacevich, F. and Consolo, V.F., 2020. Native *Trichoderma harzianum* strains from Argentina produce indole-3 acetic acid and phosphorus solubilization, promote growth and control wilt disease on tomato (*Solanum lycopersicum L.*). *Journal of King Saud University-Science*, *32*(1), pp.867-873.

Badu-Apraku, B., Adewale, S., Paterne, A.A., Gedil, M., Toyinbo, J. and Asiedu, R., 2020. Identification of QTLs for grain yield and other traits in tropical maize under Striga infestation. *PloS one*, *15*(9), p.e0239205.

Belayneh Mulaw, T., Kubicek, C.P. and Druzhinina, I.S., 2010. The rhizosphere of *Coffea arabica* in its native highland forests of Ethiopia provides a niche for a distinguished diversity of *Trichoderma*. *Diversity*, *2*(4), pp.527-549.

Benitez, M.S., Osborne, S.L. and Lehman, R.M., 2017. Previous crop and rotation history effects on maize seedling health and associated rhizosphere microbiome. *Scientific Reports*, 7(1), p. 15709.

Bischof, R.H., Ramoni, J. and Seiboth, B., 2016. Cellulases and beyond: the first 70 years of the enzyme producer *Trichoderma reesei*. *Microbial Cell Factories*, *15*(1), p.106.

Bissett, J., 1991. A revision of the genus Trichoderma. III. Section Pachybasium. *Canadian Journal of Botany*, *69*(11), pp.2373-2417.

Bissett, J., 1992. *Trichoderma atroviride*. *Canadian Journal of Botany*, *70*(3), pp.639-641.

Bissett, J., Gams, W., Jaklitsch, W. and Samuels, G.J., 2015. Accepted *Trichoderma* names in the year 2015. *IMA fungus*, *6*(2), pp.263-295.

Błaszczyk, L., Popiel, D., Chełkowski, J., Koczyk, G., Samuels, G.J., Sobieralski, K. and Siwulski, M., 2011. Species diversity of *Trichoderma* in Poland. *Journal of applied genetics*, *52*(2), pp.233-243.

Bouwman, L., Goldewijk, K.K., Van Der Hoek, K.W., Beusen, A.H., Van Vuuren, D.P., Willems, J., Rufino, M.C. and Stehfest, E., 2013. Exploring global changes in nitrogen and phosphorus cycles in agriculture induced by livestock production over the 1900–2050 period. *Proceedings of the National Academy of Sciences*, *110*(52), pp.20882-20887.

Bressan, W., Bressan, W., Figueiredo, J.E.F. & Figueiredo, J.E.F. 2008. Efficacy and dose–response relationship in biocontrol of *Fusarium* disease in maize by *Streptomyces* spp. *European journal of plant pathology*. 120(3), pp.311–316.

Brotman, Y., Landau, U., Cuadros-Inostroza, Á., Takayuki, T., Fernie, A.R., Chet, I., Viterbo, A. and Willmitzer, L., 2013. *Trichoderma*-plant root colonization: escaping early plant defense responses and activation of the antioxidant machinery for saline stress tolerance. *PLoS Pathogens*, *9*(3), p.e1003221.

Brunner, K., Zeilinger, S., Ciliento, R., Woo, S.L., Lorito, M., Kubicek, C.P. and Mach, R.L., 2005. Improvement of the fungal biocontrol agent Trichoderma atroviride to enhance both antagonism and induction of plant systemic disease resistance. *Applied and environmental microbiology*, *71*(7), pp.3959-3965.

Byerlee, D. and Heisey, P.W., 1997. Evolution of the African maize economy. *Africa's Emerging Maize Revolution*, pp .9-22.

Cai, F. and Druzhinina, I.S., 2021. In honor of John Bissett: authoritative guidelines on molecular identification of *Trichoderma*. *Fungal Diversity*, *107*(1), pp.1-69.

Carvalho, F.P., 2017. Pesticides, environment, and food safety. *Food and Energy Security*, *6*(2), pp.48-60.

Chaverri, P., Branco-Rocha, F., Jaklitsch, W., Gazis, R., Degenkolb, T. and Samuels, G.J., 2015. Systematics of the *Trichoderma harzianum* species complex and the re-identification of commercial biocontrol strains. *Mycologia*, *107*(3), pp.558-590.

Chaverri, P., Castlebury, L.A., Overton, B.E. and Samuels, G.J., 2003. *Hypocrea*/*Trichoderma*: species with conidiophore elongations and green conidia. *Mycologia*, *95*(6), pp.1100-1140.

Chen, J.L., Sun, S.Z., Miao, C.P., Wu, K., Chen, Y.W., Xu, L.H., Guan, H.L. and Zhao, L.X., 2016. Endophytic *Trichoderma gamsii* YIM PH30019: a promising biocontrol agent with hyperosmolar, mycoparasitism, and antagonistic activities of induced volatile organic compounds on root-rot pathogenic fungi of *Panax notoginseng*. *Journal of Ginseng Research*, *40*(4), pp.315-324.

Chen, K. and Zhuang, W.Y., 2017. Discovery from a large-scaled survey of *Trichoderma* in soil of China. *Scientific Reports*, *7*(1), pp.1-37.

Collinge, D.B., Jørgensen, H.J.L., Latz, M., Manzotti, A., Ntana, F., Rojas Tayo, E.C. and Jensen, B., 2019. Searching for novel fungal biological control agents for plant disease control among endophytes. *Endophytes for a Growing World*, p. 25.

Contreras-Cornejo, H.A., Macías-Rodríguez, L., del-Val, E. and Larsen, J., 2016. Ecological functions of *Trichoderma* spp. and their secondary metabolites in the rhizosphere: interactions with plants. *FEMS Microbiology Ecology*, *92*(4), p.fiw036.

Curl, E.A. and Truelove, B., 2012. *The Rhizosphere* (Vol. 15). Springer Science & Business Media.

Danielson, R.M. and Davey, C.B., 1973. Carbon and nitrogen nutrition of *Trichoderma*. *Soil Biology and Biochemistry*, *5*(5), pp.505-515.

Demeke, M. and Di Marcantonio, F., 2013. Understanding the performance of food production in sub-Saharan Africa and its implications for food security. *Journal of Development and Agricultural Economics*, *5*(11), pp.425-443.

Devi, P.B., Vijayabharathi, R., Sathyabama, S., Malleshi, N.G. and Priyadarisini, V.B., 2014. Health benefits of finger millet (*Eleusine coracana* L.) polyphenols and dietary fiber: a review. *Journal of Food Science and Technology*, *51*(6), pp.1021-1040.

Dodd, S.L., Lieckfeldt, E. and Samuels, G.J., 2003. *Hypocrea atroviridis* sp. nov., the teleomorph of *Trichoderma atroviride*. *Mycologia*, *95*(1), pp.27-40.

Druzhinina, I. and Kubicek, C.P., 2005. Species concepts and biodiversity in *Trichoderma* and *Hypocrea*: from aggregate species to species clusters?. *Journal of Zhejiang University. Science. B*, *6*(2), p.100.

Druzhinina, I.S., Kopchinskiy, A.G. and Kubicek, C.P., 2006. The first 100 *Trichoderma* species characterized by molecular data. *Mycoscience*, *47*(2), p.55.

Druzhinina, I.S., Kubicek, C.P., Komoń-Zelazowska, M., Mulaw, T.B. and Bissett, J., 2010. The *Trichoderma harzianum* demon: complex speciation history resulting in coexistence of hypothetical biological species, recent agamospecies and numerous relict lineages. *BMC Evolutionary Biology*, *10*(1), pp.1-14.

Du Plessis, I.L., 2015. The diversity of *Trichoderma* spp. in South Africa, MSc Thesis, Stellenbosch University, South Africa.

Du Plessis, I.L., Druzhinina, I.S., Atanasova, L., Yarden, O. and Jacobs, K., 2018. The diversity of *Trichoderma* species from soil in South Africa, with five new additions. *Mycologia*, *110*(3), pp.559-583.

Elshahawy, I.E., Saied, N., Abd-El-Kareem, F. and Morsy, A., 2017. Biocontrol of onion white rot by application of *Trichoderma* species formulated on wheat bran powder. *Archives of Phytopathology and Plant Protection*, *50*(3-4), pp.150-166.

Fu, J., Xiao, Y., Wang, Y.F., Liu, Z.H. and Yang, K., 2021. Saline–alkaline stress in growing maize seedlings is alleviated by *Trichoderma asperellum* through regulation of the soil environment. *Scientific Reports*, *11*(1), pp.1-11.

Gałązka, A., Gawryjołek, K., Perzyński, A., Gałązka, R. and Księżak, J., 2017. Changes in Enzymatic Activities and Microbial Communities in Soil under Long-Term Maize Monoculture and Crop Rotation. *Polish Journal of Environmental Studies*, *26*(1).

Gravel, V., Antoun, H. and Tweddell, R.J., 2007. Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole acetic acid (IAA). *Soil Biology and Biochemistry*, *39*(8), pp.1968-1977.

Guadie, D., Knierim, D., Winter, S., Tesfaye, K. and Abraham, A., 2019. Survey for the identification and geographical distribution of viruses and virus diseases of maize (*Zea mays* L.) in Ethiopia. *European Journal of Plant Pathology*. 153(1), pp.429–439.

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I. and Lorito, M., 2004. *Trichoderma* species—opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology*, *2*(1), p.43.

Harris, J.L., 2000. Safe, low-distortion tape touch method for fungal slide mounts. *Journal of Clinical Microbiology*, *38*(12), pp.4683-4684.

Hassan, M.M., Farid, M.A. and Gaber, A., 2019. Rapid identification of *Trichoderma koningiopsis* and *Trichoderma longibrachiatum* using sequence-characterized amplified region markers. *Egyptian Journal of Biological Pest Control*, *29*(1), p.13.

Hermosa, M.R., Keck, E., Chamorro, I., Rubio, B., Sanz, L., Vizcaíno, J.A., Grondona, I. and Monte, E., 2004. Molecular characterization of biocontrol agents. *Bulletin-OILB-SROP*, *27*(8), pp.165-168.

Inglis, P.W., Mello, S.C., Martins, I., Silva, J.B., Macêdo, K., Sifuentes, D.N. and Valadares-Inglis, M.C., 2020. Trichoderma from Brazilian garlic and onion crop soils and description of two new species: *Trichoderma azevedoi* and *Trichoderma peberdyi*. *PloS one*, *15*(3), p.e0228485.

Jacobs, K., Bergdahl, D.R., Wingfield, M.J., Halik, S., Seifert, K.A., Bright, D.E. and Wingfield, B.D., 2004. *Leptographium wingfieldii* introduced into North America and found associated with exotic *Tomicus piniperda* and native bark beetles. *Mycological Research*, *108*(4), pp.411-418.

Jaklitsch, W.M. and Voglmayr, H., 2015. Biodiversity of *Trichoderma* (*Hypocreaceae*) in Southern Europe and Macaronesia. *Studies in Mycology*, *80*, pp.1-87.

Jaklitsch, W.M., Samuels, G.J., Dodd, S.L., Lu, B.S. and Druzhinina, I.S., 2006. *Hypocrea rufa/Trichoderma viride*: a reassessment, and description of five closely related species with and without warted conidia. *Studies in Mycology*, *56*, pp.135-177.

Jiang, H., Zhang, L., Zhang, J.Z., Ojaghian, M.R. and Hyde, K.D., 2016. Antagonistic interaction between *Trichoderma asperellum* and *Phytophthora capsici* in vitro. *Journal of Zhejiang University-Science B*, *17*(4), pp.271-281.

Katoh, K. and Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, *30*(4), pp.772-780.

Katoh, K., Misawa, K., Kuma, K.I. and Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic acids research*, *30*(14), pp.3059-3066.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C. and Thierer, T., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*(12), pp.1647-1649.

Khoshmanzar, E., Aliasgharzad, N., Neyshabouri, M.R., Khoshru, B., Arzanlou, M. & Asgari Lajayer, B. 2020. Effects of *Trichoderma* isolates on tomato growth and inducing its tolerance to water-deficit stress. *International journal of environmental science and technology* (Tehran). 17(2), pp.869–878.

Kim, C.S., Park, M.S., Kim, S.C., Maekawa, N. and Yu, S.H., 2012. Identification of *Trichoderma*, a competitor of shiitake mushroom (*Lentinula edodes*), and competition between *Lentinula edodes* and *Trichoderma* species in Korea. *The Plant Pathology Journal*, *28*(2), pp.137-148.

Kribel, S., Qostal, S., Ouazzani Touhami, A., Selmaoui, K., Chliyeh, M., Benkirane, R. and Achbani, E.H., 2020. Effects of *Trichoderma* on growth and yield of wheat and barley and its survival ability on roots and amended rock phosphate growing substrates. *Current Research in Environmental & Applied Mycology (Journal of Fungal Biology)*, *10*(1), pp.400-416.

Kubicek, C.P., Mach, R.L., Peterbauer, C.K. and Lorito, M., 2001. Trichoderma: from genes to biocontrol. *Journal of Plant Pathology*, pp.11-23.

Kumar, K., Manigundan, K. and Amaresan, N., 2017. Influence of salt tolerant *Trichoderma* spp. on growth of maize (*Zea mays*) under different salinity conditions. *Journal of Basic Microbiology*, *57*(2), pp.141-150.

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular biology and evolution*, *35*(6), p.1547.

Liu, B., Ji, S., Zhang, H., Wang, Y. and Liu, Z., 2020. Isolation of Trichoderma in the rhizosphere soil of Syringa oblata from Harbin and their biocontrol and growth promotion function. *Microbiological Research*, *235*, p.126445.

Mabe, F.N., Talabi, K. & Danso-Abbeam, G. 2017. Awareness of Health Implications of Agrochemical Use: Effects on Maize Production in Ejura-Sekyedumase Municipality, Ghana. Advances in agriculture (Hindawi Publishing Corporation). 2017, pp.1–11.

Mander, P., Choi, Y.H., Pradeep, G.C., Choi, Y.S., Hong, J.H., Cho, S.S. and Yoo, J.C., 2014. Biochemical characterization of xylanase produced from *Streptomyces* sp. CS624 using an agro residue substrate. *Process Biochemistry*, *49*(3), pp.451-456.

Marais, A., Hardy, M., Booyse, M. and Botha, A., 2012. Effects of monoculture, crop rotation, and soil moisture content on selected soil physicochemical and microbial parameters in wheat fields. *Applied and Environmental Soil Science*, *2012*.

Mendes, J.B.S., da Costa Neto, V.P., de Sousa, C.D.A., de Carvalho Filho, M.R., Rodrigues, A.C. and Bonifacio, A., 2020. *Trichoderma* and *bradyrhizobia* act synergistically and enhance the growth rate, biomass and photosynthetic pigments of cowpea (*Vigna unguiculata*) grown in controlled conditions. *Symbiosis*, *80*(2), pp.133-143.

Mostafa, F.A., El Aty, A.A.A. and Wehaidy, H.R., 2014. Improved Xylanase production by mixing low cost wastes and novel co-culture of three marine-derived fungi in solid state fermentation. *Int J Curr Microbiol App Sci*, *3*, pp.336-349.

Mukherjee, A.K., Kumar, A.S., Kranthi, S. and Mukherjee, P.K., 2014. Biocontrol potential of three novel *Trichoderma* strains: isolation, evaluation and formulation. *3 Biotech*, *4*(3), pp.275-281.

Musokwa, M., Mafongoya, P. and Lorentz, S., 2019. Evaluation of agroforestry systems for maize (*Zea mays*) productivity in South Africa. *South African Journal of Plant and Soil*, 36(1), pp. 65-67.

Naeem, M.A., Khalid, M., Aon, M., Abbas, G., Amjad, M., Murtaza, B., Khan, W.-D. & Ahmad, N. 2018. Combined application of biochar with compost and fertilizer improves soil properties and grain yield of maize. *Journal of Plant Nutrition*. 41(1), pp.112–122.

Nepali, B., Subedi, S., Bhattarai, S., Marahatta, S., Bhandari, D. and Shrestha, J., 2020. Bio-fertilizer activity of *Trichoderma viride* and *Pseudomonas fluorescens* as growth and yield promoter for maize. *Agraarteadus*, *31*(2).

Okoth, S.A., Otadoh, J.A. and Ochanda, J.O., 2011. Improved seedling emergence and growth of maize and beans by *Trichoderma harziunum*. *Tropical and subtropical agroecosystems*, *13*(1), pp.65-71.

Qiao, M., Du, X., Zhang, Z., Xu, J. and Yu, Z., 2018. Three new species of soil-inhabiting *Trichoderma* from Southwest China. *MycoKeys*, (44), p.63.

Rajankar, P.M., Tambekar, P.R.D. and WATE, S., 2007. Study of phosphate solubilization efficiencies of fungi and bacteria isolated from saline belt of Puma river basin. *Research Journal of Agriculture and Biological Sciences*, *3*(6), pp.701-703.

Reardon, C.L., Gollany, H.T. and Wuest, S.B., 2014. Diazotroph community structure and abundance in wheat–fallow and wheat–pea crop rotations. *Soil Biology and Biochemistry*, *69*, pp.406-412.

Recio, R., Meléndez-Carmona, M.Á., Martín-Higuera, M.C., Pérez, V., López, E., López-Medrano, F. and Pérez-Ayala, A., 2019. Trichoderma longibrachiatum: an unusual pathogen of fungal pericarditis. *Clinical Microbiology and Infection*, *25*(5), pp.586-587.

Rudresh, D.L., Shivaprakash, M.K. and Prasad, R.D., 2005. Tricalcium phosphate solubilizing abilities of *Trichoderma* spp. in relation to P uptake and growth and yield parameters of chickpea (*Cicer arietinum* L.). *Canadian Journal of Microbiology*, *51*(3), pp.217-222.

Saber, W.I., Ghoneem, K.M., Rashad, Y.M. and Al-Askar, A.A., 2017. *Trichoderma Harzianum* WKY1: an indole acetic acid producer for growth improvement and anthracnose disease control in sorghum. *Biocontrol Science and Technology*, *27*(5), pp.654-676.

Samuels, G.J., 1996. *Trichoderma*: a review of biology and systematics of the genus. *Mycological Research*, *100*(8), pp.923-935.

Samuels, G.J., Dodd, S.L., Gams, W., Castlebury, L.A. and Petrini, O., 2002. *Trichoderma* species associated with the green mold epidemic of commercially grown *Agaricus bisporus*. *Mycologia*, *94*(1), pp.146-170.

Samuels, G.J., Ismaiel, A., Bon, M.C., De Respinis, S. and Petrini, O., 2010. *Trichoderma asperellum sensu lato* consists of two cryptic species. *Mycologia*, *102*(4), pp.944-966.

Samuels, G.J., Lieckfeldt, E. and Nirenberg, H.I., 1999. *Trichoderma asperellum*, a new species with warted conidia, and redescription of *T. viride*. *SYDOWIA-HORN-*, *51*, pp.71-88.

Saravanakumar, K. and Kathiresan, K., 2014. Bioremoval of the synthetic dye malachite green by marine *Trichoderma* sp. *SpringerPlus*, *3*(1), p.631.

Saravanakumar, K., Arasu, V.S. and Kathiresan, K., 2013. Effect of *Trichoderma* on soil phosphate solubilization and growth improvement of *Avicennia marina*. *Aquatic Botany*, *104*, pp.101-105.

Savci, S., 2012. Investigation of effect of chemical fertilizers on environment. *Apcbee Procedia*, *1*, pp.287-292.

Sawant, I.S., Wadkar, P.N., Ghule, S.B., Rajguru, Y.R., Salunkhe, V.P. and Sawant, S.D., 2017. Enhanced biological control of powdery mildew in vineyards by integrating a strain of *Trichoderma afroharzianum* with sulphur. *Biological Control*, *114*, pp.133-143.

Seifert, K.A. and Rossman, A.Y., 2010. How to describe a new fungal species. *IMA fungus*, *1*(2), pp.109-111.

Tchameni, S.N., Cotârleț, M., Ghinea, I.O., Bedine, M.A.B., Sameza, M.L., Borda, D., Bahrim, G. and Dinică, R.M., 2020. Involvement of lytic enzymes and secondary metabolites produced by *Trichoderma* spp. in the biological control of *Pythium myriotylum*. *International Microbiology*, *23*(2), pp.179-188.

Torres, M.F., Cuadros, D.F. & Vaillancourt, L.J. 2014. Evidence for a diffusible factor that induces susceptibility in the *Colletotrichum*–maize disease interaction. *Molecular Plant Pathology*. 15(1), pp.80–93.

Venter, Z.S., Jacobs, K. and Hawkins, H.J., 2016. The impact of crop rotation on soil microbial diversity: A meta-analysis. *Pedobiologia*, *59*(4), pp.215-223.

Verma, M., Brar, S.K., Tyagi, R.D., Surampalli, R.Y. and Valero, J.R., 2007. Antagonistic fungi, *Trichoderma* spp.: panoply of biological control. *Biochemical Engineering Journal*, *37*(1), pp.1-20.

Waghunde, R.R., Shelake, R.M. and Sabalpara, A.N., 2016. *Trichoderma*: A significant fungus for agriculture and environment. *African Journal of Agricultural Research*, *11*(22), pp.1952-1965.

Wallington, T.J., Anderson, J.E., Mueller, S.A., Kolinski Morris, E., Winkler, S.L., Ginder, J.M. and Nielsen, O.J., 2012. Corn ethanol production, food exports, and indirect land use change. *Environmental Science & Technology*, *46*(11), pp. 6379-6384.

White, T.J., Bruns, T., Lee, S.J.W.T. and Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: A guide to methods and applications*, *18*(1), pp.315-322.

Yao, L., Teng, Y., Luo, Y., Christie, P., Ma, W., Liu, F., Wu, Y., Luo, Y. and Li, Z., 2015. Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by *Trichoderma reesei* FS10-C and effect of bioaugmentation on an aged PAH-contaminated soil. *Bioremediation Journal*, *19*(1), pp.9-17.

Yin, C., Jones, K.L., Peterson, D.E., Garrett, K.A., Hulbert, S.H. and Paulitz, T.C., 2010. Members of soil bacterial communities sensitive to tillage and crop rotation. *Soil Biology and Biochemistry*, *42*(12), pp.2111-2118.

Zachow, C., Berg, C., Müller, H., Monk, J. and Berg, G., 2016. Endemic plants harbour specific *Trichoderma* communities with an exceptional potential for biocontrol of phytopathogens. *Journal of Biotechnology*, *235*, pp.162-170.

Zahir, Z.A., Yasin, H.M., Naveed, M., Anjum, M.A. and Khalid, M., 2010. L-tryptophan application enhances the effectiveness of rhizobium inoculation for improving growth and yield of mungbean (*Vigna radiata* (L.) Wilczek). *Pak J Bot*, *42*(3), pp.1771-1780.

Zak, D.R., Holmes, W.E., White, D.C., Peacock, A.D. and Tilman, D., 2003. Plant diversity, soil microbial communities, and ecosystem function: are there any links? *Ecology*, *84*(8), pp.2042-2050.

Zhang, S., Gan, Y. and Xu, B., 2016. Application of plant-growth-promoting fungi *Trichoderma longibrachiatum* T6 enhances tolerance of wheat to salt stress through improvement of antioxidative defense system and gene expression. *Frontiers in Plant Science*, *7*, p.1405.

Zhou, D., Huang, X.F., Guo, J., dos-Santos, M.L. and Vivanco, J.M., 2018. *Trichoderma gamsii* affected herbivore feeding behaviour on *Arabidopsis thaliana* by modifying the leaf metabolome and phytohormones. *Microbial Biotechnology*, *11*(6), pp.1195-1206.

Chapter 4: Assessing the solubilization of phosphate and the production of indole acetic acid (IAA) by *Trichoderma* species

Abstract

Plant growth is mostly dependant on nutrients and hormones. Phosphate is one of the essential nutrients needed by plants for their growth and development. Soil is known to have an abundance of insoluble phosphate that is inaccessible to plants. Therefore, solubilized phosphate is needed to improve the growth of plants. Indole acetic acid (IAA) is an auxin hormone which is normally produced by plants and microorganisms, and exogenous IAA is needed for plant root growth at a specific stage of development. *Trichoderma* spp. is a cosmopolitan genus that has the capacity to solubilize phosphate and produce indole acetic acid. In this study, at least one strain as a representative of the *Trichoderma* species that were previously identified were screened for their ability to solubilize phosphate and produce IAA. All the screening methods were quantitative since they are known to give accurate results as compared to qualitative assays. Findings showed that most *Trichoderma* strains solubilized varied amounts of phosphate. *T. koningiopsis* NNC066 (187 µg/ml) solubilized the highest amount of phosphate while the least amount was obtained with *Trichoderma* sp. K4 (0.83 µg/ml), a commercial strain. Other *Trichoderma* strains that were able to solubilize phosphate were *T. gamsii* NN42 (104.60 µg/ml), *T. koningiopsis* NNC113 (154.60 µg/ml), and *T. koningiopsis* NN266 (132.50 µg/ml) from the *T. viride* clade. All *Trichoderma* species that were used in the study were unable to produce IAA in the absence of L-tryptophan. However, all strains produced IAA when tryptophan was supplemented. *T. gamsii* NNC019 produced the highest amount of IAA (41.90 µg/ml), and *Trichoderma* sp. K1 produced the lowest amount of IAA (0.30 µg/ml). Other *Trichoderma* strains that produced high amounts of IAA were *T. paratroviride* NN275 (28.50 µg/ml), *T. paratroviride* NN207 (33.70 µg/ml), *T. gamsii* NNC037 (29.00 µg/ml), and *T. koningiopsis* NNC113 (21.40 µg/ml) from the *T. viride* clade. The solubilization of phosphate and production of IAA appeared to be strain specific. These strains can be further evaluated for their performances in green house or field trials to observe their ability to enhance crop growth. This enlarges the pool of locally isolated strains for incorporation into biological products aimed at the local market.

Introduction

Metabolic factors including phosphate solubilization and auxin production, are known to be responsible for the growth regulation in different plants (Altomare *et al*., 1999; Kotasthane *et al*., 2015; Saber *et al*., 2017; Sanchez-Montesinos *et al*., 2020). Organic phosphate and auxins are also commonly known for their abilities to enhance the growth in various crops (Zahir *et al*., 2010; Saravanakumar *et al*., 2013; Naveed *et al*., 2015; Kotasthane *et al*., 2015; Bader *et al*., 2020). Regardless of these metabolic factors being known to promote plant growth, other studies showed that some of the *Trichoderma* strains were not able to improve plant growth even though they exhibit some metabolic factors that are essential for plant growth development (Hoyos-Carvajal *et al*., 2009; Kotasthane *et al*., 2015). However, to date these metabolic factors (phosphate solubilization, and auxin production) are primary parameters to be evaluated prior to their application as bio-stimulants to improve plant growth.

Phosphorus (phosphate) is one of the essential nutrients that is required for plant growth (Grant *et al.*, 2001; Richardson, 2001; Kapri and Tewari, 2010; Saravanakumar *et al*., 2013). Soil may contain high amounts of phosphate although a large portion of it is in the insoluble form such as $Ca_3(PO_4)_2$, $FePO_4$ or $AlPO_4$, and cannot be absorbed by the plants (Grant *et al.*, 2001; Kapri & Tewari, 2010; Kudoyarova *et al.*, 2017). Microorganisms are able to make phosphate available to plants by solubilizing it via the production of organic acids and phosphatases (Dechassa & Schenk, 2004; Tandon *et al.*, 2020).

Organic acids can be produced by microorganisms and plants (Richardson, 2001; Tandon *et al.*, 2020). Usually organic acids serve as anions during the process of displacing phosphate (ligand exchange reactions) (Raghothama and Karthikeyan, 2005; Tandon *et al.*, 2020). Organic acids that consist of more hydroxyl groups such as citric acid tend to exhibit better efficiency in phosphate mobilization compared to those containing less hydroxyl groups such as lactic, and acetic acids (Raghothama and Karthikeyan, 2005; Alori *et al*., 2017). In addition, production of organic acids may result in the reduction of pH in a solution (Saravanakumar *et al*., 2013; Zuniga-Silgado *et al*., 2020).

Indole acetic acid (IAA) is a type of auxin which it is a plant hormone that plays a significant role in the plant growth (Saber *et al*., 2017; Mehmood *et al*., 2018; Bader *et al*., 2020). IAA has been known to be produced by plants through a mechanism that involves gravity and light (Rashotte *et al*., 2000; Buer and Muday, 2004). Microorganisms can also produce IAA since it is needed by plants at their certain stage of development (Gravel *et al*., 2007; Hussein and Joo, 2015; Herrera-Jaminez *et al*., 2018; Bader *et al*., 2020; Mendes *et al*., 2020). Moreover, microorganisms can synthesize IAA to alter physiological precesses of the host for various purposes (Waqas *et al*., 2012; Mohite, 2013). For instances, it was

reported that the process of nodule formation could also be the results of IAA produced by microorganisms (Badenochjones *et al*., 1984; Basu and Ghosh, 1998; Theunis *et al*., 2004; Ghosh and Basu, 2006). Therefore, this can suggest that IAA serves as a molecule that keep the interaction between plants and microorganisms. Furthermore, its potential in the improvement of plant growth have been widely reported (Patten and Glick, 2002; Gravel *et al*., 2007; Hussein and Joo, 2015; Saber *et al*., 2017; Bader *et al*., 2020; Mendes *et al*., 2020).

Various microorganisms including *Trichoderma* spp. can be either L-tryptophan dependent or independent, and sometimes can produce IAA in both presence and absence of L-tryptophan (Sarwar *et al*., 1992; Naveed *et al*., 2015; Palacios *et al*., 2016; Saber *et al*., 2017). L-Tryptophan is a precursor of IAA; therefore, the presence of tryptophan could potentially result in the production of IAA (Davies, 2004; Khalid *et al*., 2006; Naveed *et al*., 2015). Previous studies indicated that amendments of L-tryptophan helped the microorganisms to produce higher amounts of IAA and ultimately improved the plant growth (Zahir *et al*., 2010; Naveed *et al*., 2015; Saber *et al*., 2017). There are limited studies that evaluated the phosphate solubilization and IAA production on locally isolated *Trichoderma* strains. As a result, this study sought to assess the potential of selected *Trichoderma* strains isolated from South African agricultural soil to solubilize phosphate and produce indole acetic acid.

Materials and methods

Solubilization of phosphate

The modified method by Saravanakumar *et al.* (2013) was used with National Botanical Research Institute's Phosphate (NBRIP) broth medium (g/l) (glucose 10g, tricalcium phosphate 5g, Magnesium chloride 5g, Magnesium sulphate 0.25g, Potassium chloride 0.2g, Ammonium sulphate 0.1g). The 250 ml Erlenmeyer flasks containing 100 ml of broth was inoculated with 4 agar discs (5 mm diameter) of active growing cultures of *Trichoderma* strains. Flasks were incubated at 26 ℃ in a shaker at 121 rpm for 7 days. The samples were then centrifuged at 5000 rpm for 10 minutes and 750 µl supernatant was mixed with 750 µl colour reagent containing ammonium molybdate 1.5 % (w/v); sulphuric acid solution 5.5 % (v/v) and ferrous sulphate solution 2.7 % (w/v). Experiments were done in triplicate for each strain and the absorbance was determined using a spectrophotometer at 595 nm (BioRad iMark Microplate Reader, Lasec). The concentration of phosphate was determined by using a standard graph of K_2HPO_4 (SAARCHEM, SA) and expressed in µg/ml.

Screening of indole acetic acid (IAA)

An indole acetic acid assay was conducted following modified methods described in the literature (Loper and Scroth, 1986; Brick *et al*., 1991; Patten and Glick, 2002). *Trichoderma* strains were grown in Czapek broth (g/l) (containing sucrose 30g, sodium nitrate 3g, dipotassium phosphate 1g, magnesium sulphate 0.5g, potassium chloride 0.5g, and ferrous sulphate 0.01g) with and without L-tryptophan (1%) (Sigma-Aldrich, USA). The 250 ml Erlenmeyer flasks containing 100 ml of broth was inoculated with 4 agar discs (5 mm diameter) of actively growing *Trichoderma* strains and incubated on a shaker at 26℃ at 121 rpm for 7 days (Qiang *et al*., 2019). After incubation, the culture was centrifuged for 30 min at 3000 rpm. The supernatant (1ml) was collected into test tubes and 2 – 3 drops of ortho-phosphoric acid and 2 ml of Salkowski's reagent was added and incubated at 26℃ for 30 min in a dark room. Development of pink or red colour from the mixture indicated the production of IAA. Samples were pipetted on a sterile flat bottom 96-well microliter plate, and absorbance was measured at 540 nm using a spectrophotometer. The concentration of IAA was calculated using an Indole-3-acetic acid (Merck) standard curve (10 -100 µg/ml). The IAA produced by each strain was measured in triplicate.

Statistical Analysis

For all data, mean and standard deviation values were determined. Data were subjected to one-way analysis of variance (ANOVA) using GeneStat (12th edition) and GraphPad Prism 9 (Available from: https://www.graphpad.com/scientific-software/prism/) to determine whether the means differences are significant or not, where a significance level of $p < 0.05$ was used. All means values were compared to each other using multiple comparison test (Tukey's method), this was done after confirming that the means difference is significant. GraphPad Prism was used to construct the graphs.

Table 4. 1. *Trichoderma* strains used for screening of phosphate and indole acetic acid (IAA)

***Trichoderma* species**	**Strain number**	**Strain source**
T. afroharzianum	NN32	Maize soil
T. asperellum	NN209	Maize soil
T. asperellum	NN198	Maize soil
T. asperellum	NN194	Maize soil
T. atroviride	N/A	Commercial strain
T. gamsii	NNC019	Wheat soil
T. gamsii	NNC037	Wheat soil
T. gamsii	NNC106	Wheat soil
T. gamsii	NN311	Maize soil
T. gamsii	NN42	Maize soil
T. hamatum	NN13	Maize soil
T. hamatum	NN150	Maize soil
T. koningiopsis	NN312	Maize soil
T. koningiopsis	NN244	Maize soil
T. koningiopsis	NN266	Maize soil
T. koningiopsis	NNC081	Wheat soil
T. koningiopsis	NNC113	Wheat soil
T. koningiopsis	NNC066	Wheat soil
T. neokoningii	NN191	Maize soil
T. paratroviride	NN207	Maize soil
T. paratroviride	NN275	Maize soil
T. peberdyi	NN308	Maize soil
T. peberdyi	NN130	Maize soil
T. rifaii	NN318	Maize soil
T. rifaii	NN112	Maize soil
T. rifaii	NNC105	Wheat soil
T. saturnisporum	NNC001	Wheat soil
T. saturnisporum	NNC107	Wheat soil
T. spirale	NN322	Maize soil
T. spirale	NN321	Maize soil
T. spirale	NN100	Maize soil
T. spirale	NNC111	Wheat soil
T. velutinum	NN263	Maize soil
T. velutinum	NNC018	Wheat soil
T. velutinum	NNC116	Wheat soil
T. virens	NNC012	Wheat soil
T. virens	NNC109	Wheat soil
Trichoderma sp.	K1	Commercial strain
Trichoderma sp.	K2	Commercial strain
Trichoderma sp.	K3	Commercial strain
Trichoderma sp.	K4	Commercial strain

*****T. atroviride* and all other strains with "**K**" are used in commercial products, and the origin of the strains is confidential.

Results

Screening of *Trichoderma* strains to solubilize phosphate

Trichoderma strains varied in terms of phosphate solubilization and ranged from 0.83 µg/ml to 187.80 µg/ml (Fig. 4.1 and Table 4.2). *T. koningiopsis* NNC066 showed the highest phosphate concentration of 187.80 µg/ml whereas *Trichoderma* sp. K4 solubilized the least amount of 0.83 µg/ml (Fig. 4.1 and Table 4.2). It was noticeable that other strains such as *T. gamsii* NN42, *T. koningiopsis* NNC113, and *T. koningiopsis* NN266 that solubilized 104.60 µg/ml, 154.60 µg/ml, and 132.50 µg/ml, respectively are better candidates for phosphate solubilization than the commercial strains (Fig. 4.1 and Table 4.2). However, four strains including *T. asperellum* NN198, *T. hamatum* NN150, *T. saturnisporum* NNC001 and *T. virens* NNC109 were unable to solubilize phosphate (Fig. 4.1 and Table 4.2). Of the 41 strains evaluated in this study only ten strains had the capacity to solubilize phosphate above 50 µg/ml (Table 4.2).

This study showed that the pH values of the environment have an inverse proportion compared to the phosphate concentration solubilized; as the pH values decrease, the concentration of phosphate solubilized increases (Fig. 4.2). The pH values of the strains, *T. koningiopsis* NNC113, *T. koningiopsis* NNC066, *T. gamsii* NNC019, *T. koningiopsis* NN312, *T. koningiopsis* NN266, *T. paratroviride* NN275, *T. gamsii* NN311, *T. gamsii* NNC037, *T. gamsii* NN42, *T. gamsii* NNC106, and *T. paratroviride* NN207 were recorded as 4.56, 4.36, 5.97, 4.91, 4.65, 4.92, 5.20, 5.34, 4.80, 5.00, and 5.14, respectively (Fig. 4.2). A decrease in pH from the initial pH of 8.90 was observed. In strains that did not solubilize phosphate, the pH values remained the same or there was only a slight change (Fig. 4.2).

Assessing the ability of *Trichoderma* strains to produce indole acetic acid (IAA)

All the strains that were used in this study did not produce IAA in the absence of L-tryptophan. This was also supported by the lack of red or pink colour development when Salkowski's reagent and supernatant of strain were mixed. However, in the presence of L-tryptophan all strains produce a certain amount of IAA (Fig. 4.3 and Table 4.2). The maximum amount of IAA produced was 41.90 µg/ml by *T. gamsii* NNC019 belonging to *Trichoderma* section, *T. viride* clade while the minimum amount was at 0.30 µg/ml by *Trichoderma* sp. K1 (Fig. 4.3 and Table 4.2).

The other nine strains were also found to produce good amount of IAA (greater or equal to 10 µg/ml) namely, *T. spirale* NNC111, *T. koningiopsis* NNC066, *T. gamsii* NNC037, *T. gamsii* NNC106, *T. paratroviride* NN275, *T. koningiopsis* NNC113, *T. gamsii* NN311, *T. koningiopsis* NNC266, and *T. paratroviride* NN207 obtained 17.40 µg/ml, 16.60 µg/ml, 29.00 µg/ml, 15.80 µg/ml, 28.50 µg/ml, 21.40 µg/ml, 11.00 µg/ml, 12.10 µg/ml, and 33.70 µg/ml, respectively (Fig. 4.3 and Table 4.2). Most of the *Trichoderma* strains had the significant difference when compared with the control (uninoculated media). Only two strains, *Trichoderma* sp. K1 and *T. rifaii* NNC105, showed to have a non-significant difference when compared to control as all of them have the same letter.

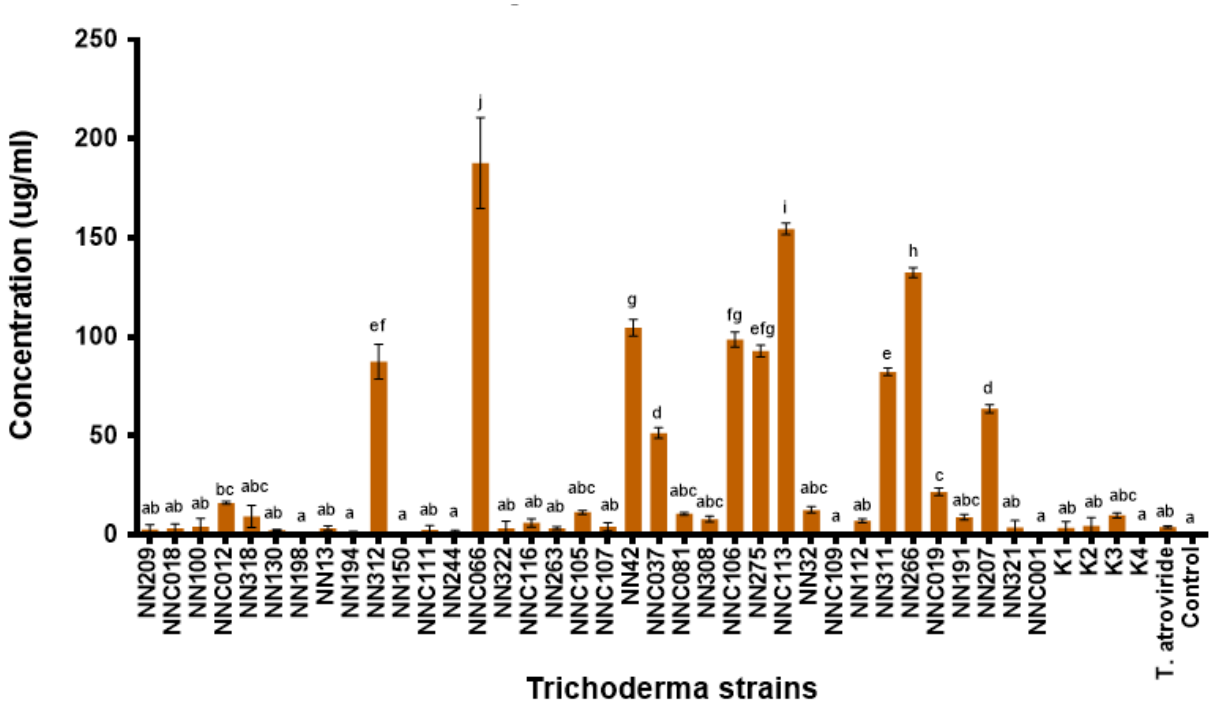


Figure 4. 1. The representation of *Trichoderma* strains showing their capacity to solubilize phosphate concentration measured in µg/ml. Error bars represent standard deviation. Multiple comparison test (Tukey's method) was done. Different letters indicate significant differences results where $p<0.05$.

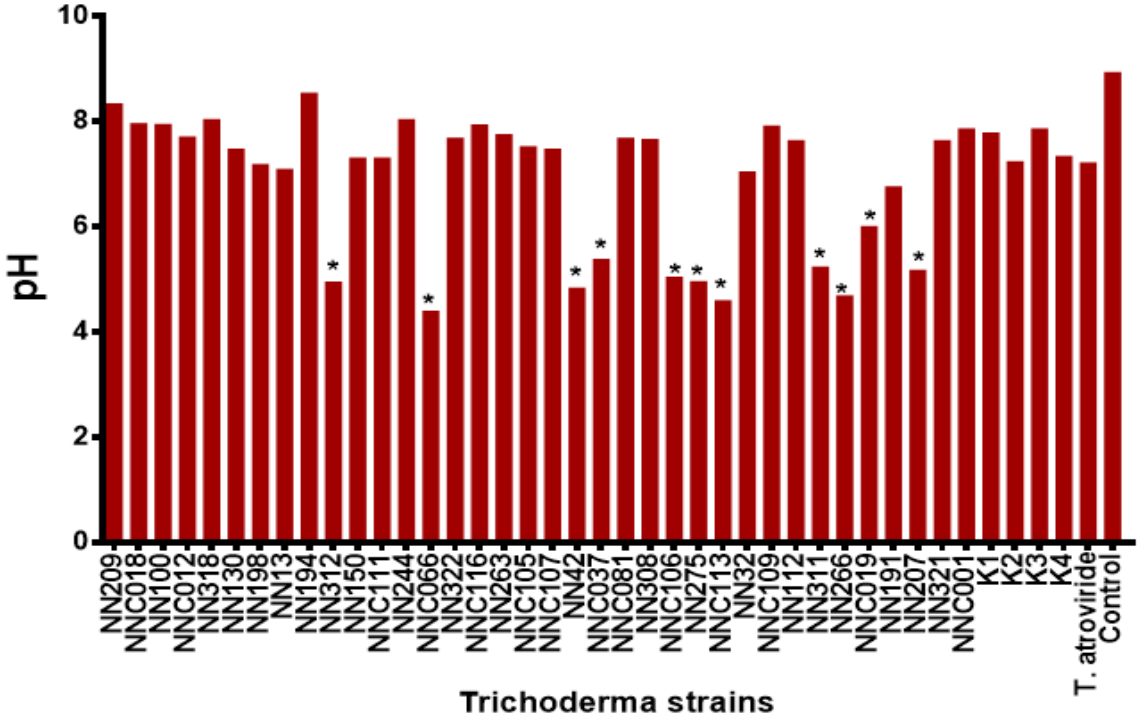


Figure 4. 2. The representation of NBRIP broth pH after 7 days incubation period with *Trichoderma* strains and control (uninoculated media). pH values less than 6.0 are represented by asterisks (*****).

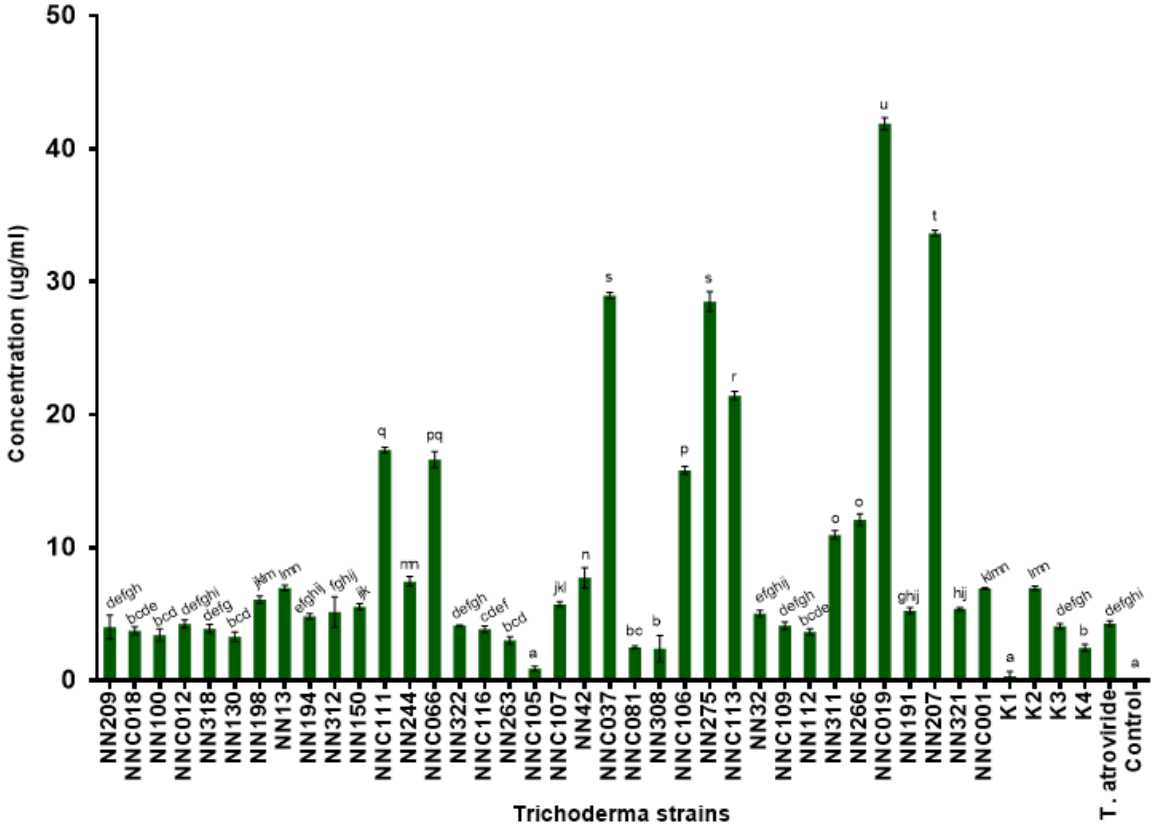


Figure 4. 3. The illustration of *Trichoderma* strains showing their ability to produce indole acetic acid (IAA) in the presence of tryptophan (1% L-TRP). Error bars represent standard deviation. Multiple comparison test (Tukey's method) was done. Different letters indicate significant differences results, $p<0.05$.

Table 4. 2. *Trichoderma* strains and their potential to produce IAA and solubilize phosphate.

***Trichoderma* species**	**Strain number**	**Phosphate concentration (µg/ml)**	**IAA concentration (µg/ml)**
T. afroharzianum	NN32	12.70	3.70
T. asperellum	NN209	2.80	4.00
T. asperellum	NN198	0.00	6.10
T. asperellum	NN194	0.97	4.80
T. atroviride	N/A	4.00	4.30
T. gamsii	NNC019	21.80	41.90
T. gamsii	NNC037	51.50	29.00
T. gamsii	NNC106	98.70	15.80
T. gamsii	NN311	82.40	11.00
T. gamsii	NN42	104.60	7.70
T. hamatum	NN13	3.40	6.90
T. hamatum	NN150	0.00	5.60
T. koningiopsis	NN312	87.50	5.20
T. koningiopsis	NN244	0.84	7.50
T. koningiopsis	NN266	132.50	12.10
T. koningiopsis	NNC081	10.61	2.50
T. koningiopsis	NNC113	154.60	21.40
T. koningiopsis	NNC066	187.80	16.60
T. neokoningii	NN191	9.00	5.30
T. paratroviride	NN207	63.70	33.70
T. paratroviride	NN275	92.90	28.50
T. peberdyi	NN308	8.10	2.40
T. peberdyi	NN130	2.50	3.30
T. rifaii	NN318	9.30	3.90
T. rifaii	NN112	7.10	3.70
T. rifaii	NNC105	11.40	0.90
T. saturnisporum	NNC001	0.00	6.90
T. saturnisporum	NNC107	4.10	5.70
T. spirale	NN322	3.50	4.10
T. spirale	NN321	3.90	5.40
T. spirale	NN100	4.20	3.40
T. spirale	NNC111	2.50	17.40
T. velutinum	NN263	3.40	3.00
T. velutinum	NNC018	3.40	3.80
T. velutinum	NNC116	6.10	3.90
T. virens	NNC012	16.20	4.30
T. virens	NNC109	0.00	4.10
Trichoderma sp.	K1	3.60	0.30
Trichoderma sp.	K2	4.60	7.00
Trichoderma sp.	K3	9.80	4.10
Trichoderma sp.	K4	0.47	2.50

Discussion

Sufficient plant growth is dependent on various nutrients and minerals, and phosphate is the second most important nutrient for plant growth. Phosphate is predominantly abundant in soil, however not always in form that can be utilized by plants (Kapri and Tewari, 2010; Kudoyarova *et al.*, 2017). *Trichoderma* strains are known to convert insoluble phosphate into soluble phosphate (Altomare *et al*., 1999; Saravanakumar *et al*., 2013; Kotasthane *et al*., 2015; Zuniga-Silgado *et al*., 2020). This was also observed in the current study where most tested *Trichoderma* strains were able to solubilize phosphate.

Phosphate concentrations varied among tested strains in this study with a maximum amount of phosphate of 187.80 µg/ml and a minimum amount of 0.83 µg/ml. This variation has also been observed in previous studies (Kotasthane *et al*., 2015; Khoshmanzar *et al*., 2020; Gomez-Ramirez and Uribe-Velez, 2021). For instance, Saravanakumar *et al*. (2013) found that solubilized phosphate concentrations between different strains ranged from 139 µg/ml to 301 µg/ml. In contrast, other studies found very low concentrations of solubilized phosphate with a maximum of 25 µg/ml (Rudresh *et al*., 2005; Chagas *et al*., 2016). This suggests that the solubilization of phosphate is strain dependent as different strains from the same species solubilized different concentrations of phosphate.

Strains of *T. koningiopsis* and *T. gamsii* were the best phosphate solubilizers in the current study (Fig. 4.1 and Table 4.2). Despite these species being commonly used as biocontrol agents (Moreno *et al*., 2009), other studies also reported that *T. koningiopsis* (Saxena *et al*., 2015; Tandon *et al*., 2020) and *T. gamsii* (Rinu *et al*., 2014) have the capacity to solubilize phosphate. *Trichoderma* strains that have a potential in solubilizing phosphate display a lowest pH than others with no phosphate activity, these findings agree with the results reported by Rinu *et al*. (2014).

This reduction of pH plays a major part in solubilization of phosphate, which might suggest that organic acids are being produced in this process (Dechassa and Schenk, 2004; Tandon *et al.*, 2020; Zuniga-Silgado *et al*., 2020). The pH values in this study were in inverse proportion to the phosphate concentration as pH decreases the concentration of phosphate increases. The strains that had no, or low phosphate concentrations showed no or negligible changes in the pH values. These findings are in agreement with Saravanakumar *et al*. (2013), and Zuniga-Silgado *et al*. (2020) as they also reported pH values that were inversely proportional to the amount of phosphate in the solution, which was also supported by the production of organic acids in these studies. However, in the current study the production of organic acids was not assessed, therefore we would not conclude that the main contribution in the reduction of pH was because of the organic acids production.

Results showed that the strains that exhibit the lowest pH in the medium (lower than 6) were the best phosphate solubilizers. In contrast, Altomare *et al*. (1999); Rudresh *et al*. (2005); and Chagas *et al*. (2016) found no correlation between the reduction of pH in the solubilization of phosphate. This could be as a result of the inherent differences in strains.

The production of auxins such as indole acetic acid (IAA) is one of the properties that has a significant role in plant growth. *Trichoderma* spp. have been widely reported for their ability to produce IAA (Gravel *et al*., 2007; Naveed *et al*., 2015; Saber *et al*., 2017; Sanchez-Montesinos *et al*., 2020; Bader *et al*., 2020). None of the strains used in this study produce IAA when L-tryptophan (IAA precursor) was absent. This is similar to Hoyo-Carvajal *et al*. (2009), which also reported that no IAA was produced by *Trichoderma* strains when L-tryptophan was not supplemented in the medium. This could be an indication that these strains solely rely on one pathway for IAA production. Moreover, various studies showed that medium amended with L-tryptophan can increase the IAA amount (Gravel *et al*., 2007; Hoyo-Carvajal *et al*., 2009; Zahir *et al*., 2010; Saber *et al*., 2017; Sanchez-Montesinos *et al*., 2020). When L-tryptophan was added to the medium, all strains used in this study were able to produce IAA. Of the 41 strains evaluated, only *T. paratroviride* NN275 (28.50 µg/ml), *T. koningiopsis* NNC113 (21.40 µg/ml), *T. paratroviride* NN207 (33.70 µg/ml), *T. gamsii* NNC037 (29.00 µg/ml), and *T. gamsii* NNC019 (41.90 µg/ml) were categorized as the best producers of IAA.

The results of this study are in concordance with other previous studies, for instance, some strains had the ability to increase the production of IAA substantially when L-tryptophan was added as a precursor (Gravel *et al*., 2007; Zahir *et al*., 2010; Saber *et al*., 2017; Sanchez-Montesinos *et al*., 2020). Furthermore, the *Trichoderma* species that were categorized as best IAA producers in the current study have been reported by other studies to produce IAA including *T. koningiopsis* (Saxena *et al*., 2015; You *et al*., 2016; Ortuno *et al*., 2017), and *T. gamsii* (Bader *et al*., 2020). In contrast some studies have found that *T. gamsii* does not produce IAA (Rinu *et al*., 2014; Zhou *et al*., 2018). It was interesting to note that *T. paratroviride,* a sister species of *T. atroviride,* which is well documented to produce IAA (Gravel *et al*., 2007; Salas-Marina *et al*., 2011; Contreras-Cornejo *et al*., 2014; Colla *et al*., 2015; Chen *et al*., 2021), were also able to produce IAA. This is the first report that shows *T. paratroviride* able to produce IAA.

The majority of *Trichoderma* strains evaluated in this study showed significant differences when compared to control (uninoculated medium) in terms of IAA production. Only two strains, *Trichoderma* sp. K1 and *Trichoderma* sp. NNC105, were not significant compared to the control. The IAA production ranged from 0.30 to 41.90 µg/ml. Kotasthane *et al*. (2015) and Bader *et al*. (2020) also obtained similar values as they reported between 1.08 to 30.80 µg/ml and 7.19 to 21.40 µg/ml, respectively in their studies.

Conclusion

Trichoderma strains that have the capacity to produce indole acetic acid and solubilize phosphate could be potentially useful for improving plant growth (Rudresh *et al*., 2005; Saravanakumar *et al*., 2013; Chagas *et al*., 2016; Khoshmanzar *et al*., 2020). All *Trichoderma* strains evaluated were L-tryptophan dependent when comes to producing indole acetic acid (IAA). Not all *Trichoderma* strains were able to solubilize phosphate, as four strains were tested negative for phosphate solubilization. All of the *Trichoderma* strains that were better at solubilizing phosphate and producing IAA belonged to the *T. viride* clade. Therefore, further studies are required to evaluate the strains for optimal IAA production and phosphate solubilization under greenhouse and field conditions for their potential to increase growth of various crops. These strains will increase the pool of potential biological agents to be included in biofertilizers aimed at the local market.

References

Alori, E.T., Glick, B.R. and Babalola, O.O., 2017. Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Frontiers in microbiology*, *8*, p.971.

Altomare, C., Norvell, W.A., Björkman, T. and Harman, G.E., 1999. Solubilization of phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22. *Applied and Environmental Microbiology*, *65*(7), pp.2926-2933.

Badenoch-Jones, J., Summons, R.E., Rolfe, B.G. and Letham, D.S., 1984. Phytohormones, Rhizobium mutants, and nodulation in legumes. IV. Auxin metabolites in pea root nodules. *Journal of Plant Growth Regulation*, *3*(1), pp.23-39.

Bader, A.N., Salerno, G.L., Covacevich, F. and Consolo, V.F., 2020. Native *Trichoderma harzianum* strains from Argentina produce indole-3 acetic acid and phosphorus solubilization, promote growth and control wilt disease on tomato (*Solanum lycopersicum* L.). *Journal of King Saud University-Science*, *32*(1), pp.867-873.

Basu, P.S. and Ghosh, A.C., 1998. News & Notes Indole Acetic Acid and Its Metabolism in Root Nodules of a Monocotyledonous Tree Roystonea regia. *Current microbiology*, *37*(2), pp.137-140.

Brick, J.M., Bostock, R.M. and Silverstone, S.E., 1991. Rapid in situ assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. *Applied and Environmental Microbiology*, *57*(2), pp.535-538.

Buer, C.S. and Muday, G.K., 2004. The transparent testa4 mutation prevents flavonoid synthesis and alters auxin transport and the response of Arabidopsis roots to gravity and light. *The Plant Cell*, *16*(5), pp.1191-1205.

Chagas, L.F.B., De Castro, H.G., Colonia, B.S.O., De Carvalho Filho, M.R., Miller, L.D.O. & Chagas, A.F.J. 2016. Efficiency of *Trichoderma* spp. as a growth promoter of cowpea (*Vigna unguiculata*) and analysis of phosphate solubilization and indole acetic acid synthesis. *Revista brasileira de botânica*, 39(2), pp.437–445.

Chen, D., Hou, Q., Jia, L. and Sun, K., 2021. Combined Use of Two *Trichoderma* Strains to Promote Growth of Pakchoi (*Brassica chinensis* L.). *Agronomy*, *11*(4), p.726.

Colla, G., Rouphael, Y., Di Mattia, E., El-Nakhel, C. and Cardarelli, M., 2015. Co-inoculation of Glomus intraradices and *Trichoderma atroviride* acts as a biostimulant to promote growth, yield and nutrient uptake of vegetable crops. *Journal of the Science of Food and Agriculture*, *95*(8), pp.1706-1715.

Contreras-Cornejo, H.A., Macías-Rodríguez, L., Alfaro-Cuevas, R. and López-Bucio, J., 2014. *Trichoderma* spp. improve growth of Arabidopsis seedlings under salt stress through enhanced root development, osmolite production, and Na+ elimination through root exudates. *Molecular Plant-Microbe Interactions*, *27*(6), pp.503-514.

Davies, P.J. ed., 2004. Plant hormones: biosynthesis, signal transduction, action!. *Springer Science & Business Media*.

Dechassa, N. & Schenk, M.K. 2004. Exudation of organic anions by roots of cabbage, carrot, and potato as influenced by environmental factors and plant age. *Journal of Plant Nutrition and Soil Science*. 167(5), pp.623–629.

Fernando, D., Milagrosa, S., Francisco, C. and Francisco, M., 2018. Biostimulant activity of *Trichoderma saturnisporum* in melon (*Cucumis melo*). *Hortscience*, *53*(6), pp.810-815.

Ghosh, S. and Basu, P.S., 2006. Production and metabolism of indole acetic acid in roots and root nodules of Phaseolus mungo. *Microbiological research*, *161*(4), pp.362-366.

Gomez-Ramirez, L.F. & Uribe-Velez, D. 2021. Phosphorus Solubilizing and Mineralizing *Bacillus* spp. Contribute to Rice Growth Promotion Using Soil Amended with Rice Straw. *Current Microbiology*. 78(3), pp.932–943.

Grant, C.A., Flaten, D.N., Tomasiewicz, D.J. & Sheppard, S.C. 2001. The importance of early season phosphorus nutrition. *Canadian Journal of Plant Science*. 81(2), pp.211–224.

Gravel, V., Antoun, H. and Tweddell, R.J., 2007. Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole acetic acid (IAA). *Soil Biology and Biochemistry*, *39*(8), pp.1968-1977.

Hoyos-Carvajal, L., Orduz, S. and Bissett, J., 2009. Growth stimulation in bean (*Phaseolus vulgaris* L.) by *Trichoderma*. *Biological Control*, *51*(3), pp.409-416.

Hussein, K.A. and Joo, J.H., 2015. Isolation and characterization of rhizomicrobial isolates for phosphate solubilization and indole acetic acid production. *Journal of the Korean Society for Applied Biological Chemistry*, *58*(6), pp.847-855.

Kapri, A. & Tewari, L. 2010. Phosphate solubilization potential and phosphatase activity of rhizospheric *Trichoderma* SPP. *Brazilian Journal of Microbiology*. 41(3).

Khalid, A., Arshad, M. and Zahir, Z.A., 2006. Phytohormones: Microbial production an applications. In *Biological approaches to sustainable soil system* (pp. 207-220).

Khoshmanzar, E., Aliasgharzad, N., Neyshabouri, M.R., Khoshru, B., Arzanlou, M. & Asgari Lajayer, B. 2020. Effects of *Trichoderma* isolates on tomato growth and inducing its tolerance to water-deficit stress. *International journal of environmental science and technology* (Tehran). 17(2), pp.869–878.

Kotasthane, A., Kotasthane, A., Agrawal, T., Agrawal, T., Kushwah, R., Kushwah, R., Rahatkar, O.V. & Rahatkar, O.V. 2015. In-vitro antagonism of *Trichoderma* spp. against *Sclerotium rolfsii* and *Rhizoctonia solani* and their response towards growth of cucumber, bottle gourd and bitter gourd. *European Journal of Plant Pathology*. 141(3), pp.523–543.

Kudoyarova, G.R., Vysotskaya, L.B., Arkhipova, T.N., Kuzmina, L.Y., Galimsyanova, N.F., Sidorova, L. V., Gabbasova, I.M., Melentiev, A.I., et al. 2017. Effect of auxin producing and phosphate solubilizing bacteria on mobility of soil phosphorus, growth rate, and P acquisition by wheat plants. *Acta Physiologiae Plantarum*. 39(11), pp.1–8.

Loper, J.E. and Schroth, M.N., 1986. Influence of bacterial sources of indole-3-acetic acid on root elongation of sugar beet. *Phytopathology*, *76*(4), pp.386-389.

Mehmood, A., Hussain, A., Irshad, M., Khan, N., Hamayun, M., Ismail, Afridi, S.G. and Lee, I.J., 2018. IAA and flavonoids modulates the association between maize roots and phytostimulant endophytic *Aspergillus fumigatus* greenish. *Journal of Plant Interactions*, *13*(1), pp.532-542.

Mendes, J.B.S., da Costa Neto, V.P., de Sousa, C.D.A., de Carvalho Filho, M.R., Rodrigues, A.C. and Bonifacio, A., 2020. *Trichoderma* and *bradyrhizobia* act synergistically and enhance the growth rate, biomass and photosynthetic pigments of cowpea (*Vigna unguiculata*) grown in controlled conditions. *Symbiosis*, *80*(2), pp.133-143.

Mohite, B., 2013. Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. *Journal of soil science and plant nutrition*, *13*(3), pp.638-649.

Moreno, C.A., Castillo, F., González, A., Bernal, D., Jaimes, Y., Chaparro, M., González, C., Rodriguez, F., Restrepo, S. and Cotes, A.M., 2009. Biological and molecular characterization of the response of tomato plants treated with *Trichoderma koningiopsis*. *Physiological and Molecular Plant Pathology*, *74*(2), pp.111-120.

Naveed, M., Qureshi, M.A., Zahir, Z.A., Hussain, M.B., Sessitsch, A. and Mitter, B., 2015. L-Tryptophan-dependent biosynthesis of indole-3-acetic acid (IAA) improves plant growth and colonization of maize by *Burkholderia phytofirmans* PsJN. *Annals of Microbiology*, *65*(3), pp.1381-1389.

Ortuño, N., Castillo, J.A., Miranda, C., Claros, M. and Soto, X., 2017. The use of secondary metabolites extracted from *Trichoderma* for plant growth promotion in the Andean highlands. *Renewable Agriculture and Food Systems*, *32*(4), pp.366.

Palacios, O.A., Gomez-Anduro, G., Bashan, Y. and de-Bashan, L.E., 2016. Tryptophan, thiamine and indole-3-acetic acid exchange between Chlorella sorokiniana and the plant growth-promoting bacterium *Azospirillum brasilense*. *FEMS Microbiology Ecology*, *92*(6), p.fiw077.

Patten, C.L. and Glick, B.R., 2002. Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system. *Applied and Environmental Microbiology*, *68*(8), pp.3795-3801.

Qiang, X., Ding, J., Lin, W., Li, Q., Xu, C., Zheng, Q. and Li, Y., 2019. Alleviation of the detrimental effect of water deficit on wheat (*Triticum aestivum* L.) growth by an indole acetic acid-producing endophytic fungus. *Plant and Soil*, *439*(1), pp.373-391.

Raghothama, K.G. & Karthikeyan, A.S. 2005. Phosphate acquisition. *Plant and Soil*. 274(1–2):37–49.

Rashotte, A.M., Brady, S.R., Reed, R.C., Ante, S.J. and Muday, G.K., 2000. Basipetal auxin transport is required for gravitropism in roots of Arabidopsis. *Plant Physiology*, *122*(2), pp.481-490.

Richardson, A.E. 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. *Australian Journal of Plant Physiology*. 28(9), pp.897–906.

Rinu, K., Sati, P. and Pandey, A., 2014. *Trichoderma gamsii* (NFCCI 2177): a newly isolated endophytic, psychrotolerant, plant growth promoting, and antagonistic fungal strain. *Journal of Basic Microbiology*, *54*(5), pp.408-417.

Rudresh, D.L., Shivaprakash, M.K. & Prasad, R.D. 2005. Tricalcium phosphate solubilizing abilities of *Trichoderma* spp. in relation to P uptake and growth and yield parameters of chickpea (*Cicer arietinum* L.). *Canadian Journal of Microbiology*. 51(3), pp.217–222.

Saber, W.I., Ghoneem, K.M., Rashad, Y.M. and Al-Askar, A.A., 2017. Trichoderma Harzianum WKY1: an indole acetic acid producer for growth improvement and anthracnose disease control in sorghum. *Biocontrol Science and Technology*, *27*(5), pp.654-676.

Salas-Marina, M.A., Silva-Flores, M.A., Uresti-Rivera, E.E., Castro-Longoria, E., Herrera-Estrella, A. and Casas-Flores, S., 2011. Colonization of Arabidopsis roots by *Trichoderma atroviride* promotes growth and enhances systemic disease resistance through jasmonic acid/ethylene and salicylic acid pathways. *European Journal of Plant Pathology*, *131*(1), pp.15-26.

Sanchez-Montesinos, B., Dianez, F., Moreno-Gavira, A., Gea, F.J. and Santos, M. (2020) 'Role of *Trichoderma aggressivum* f. europaeum as Plant-Growth Promoter in Horticulture', *Agronomy*,10(7),1ch+,available:https://link.gale.com/apps/doc/A636778004/AONE?u=27uos&sid=AONE&xid=fdc2e9fd [accessed 16 Mar 2021].

Saravanakumar, K., Shanmuga Arasu, V. & Kathiresan, K. 2013. Effect of *Trichoderma* on soil phosphate solubilization and growth improvement of Avicennia marina. *Aquatic Botany*. 104, pp.101–105.

Sarwar, M., Arshad, M., Martens, D.A. and Frankenberger, W.T., 1992. Tryptophan-dependent biosynthesis of auxins in soil. *Plant and Soil*, *147*(2), pp.207-215.

Saxena, A., Raghuwanshi, R. and Singh, H.B., 2015. *Trichoderma* species mediated differential tolerance against biotic stress of phytopathogens in *Cicer arietinum* L. *Journal of Basic Microbiology*, *55*(2), pp.195-206.

Tandon, A., Fatima, T., Shukla, D., Tripathi, P., Srivastava, S. and Singh, P.C., 2020. Phosphate solubilization by *Trichoderma koningiopsis* (NBRI-PR5) under abiotic stress conditions. *Journal of King Saud University-Science*, *32*(1), pp.791-798.

Theunis, M., Kobayashi, H., Broughton, W.J. and Prinsen, E., 2004. Flavonoids, NodD1, NodD2, and nod-box NB15 modulate expression of the y4wEFG locus that is required for indole-3-acetic acid synthesis in Rhizobium sp. strain NGR234. *Molecular Plant-Microbe Interactions*, *17*(10), pp.1153-1161.

Waqas, M., Khan, A.L., Kamran, M., Hamayun, M., Kang, S.M., Kim, Y.H. and Lee, I.J., 2012. Endophytic fungi produce gibberellins and indoleacetic acid and promotes host-plant growth during stress. *Molecules*, *17*(9), pp.10754-10773.

You, J., Zhang, J., Wu, M., Yang, L., Chen, W. and Li, G., 2016. Multiple criteria-based screening of *Trichoderma* isolates for biological control of *Botrytis cinerea* on tomato. *Biological Control*, *101*, pp.31-38.

Zahir, Z.A., Yasin, H.M., Naveed, M., Anjum, M.A. and Khalid, M., 2010. L-tryptophan application enhances the effectiveness of rhizobium inoculation for improving growth and yield of mungbean (*Vigna radiata* (L.) Wilczek). *Pak J Bot*, *42*(3), pp.1771-1780.

Zhou, D., Huang, X.F., Guo, J., dos-Santos, M.L. and Vivanco, J.M., 2018. *Trichoderma gamsii* affected herbivore feeding behaviour on *Arabidopsis thaliana* by modifying the leaf metabolome and phytohormones. *Microbial Biotechnology*, *11*(6), pp.1195-1206.

Zúñiga-Silgado, D., Rivera-Leyva, J.C., Coleman, J.J., Sánchez-Reyez, A., Valencia-Díaz, S., Serrano, M., de-Bashan, L.E. & Folch-Mallol, J.L. 2020. Soil Type Affects Organic Acid Production and Phosphorus Solubilization Efficiency Mediated by Several Native Fungal Strains from Mexico. *Microorganisms* (Basel). 8(9), pp.1337.

Conclusion and Recommendation for future research

Trichoderma species have been found to have a variety of beneficial properties. *Trichoderma* spp. are known for their ability to promote plant growth and development as well as to reduce pathogens. Other positive functions of this genus have also been discovered, including the production of enzymes and antibiotic compounds. The main aim of this study was to isolate and identify naturally occurring *Trichoderma* spp. from wheat and maize agricultural soil using taxonomic and molecular methods.

Trichoderma is one of the least studied, but most used fungal groups in South Africa. Most research from South Africa seem to be more concerned with bioprospecting of this genus than with understanding its diversity. The knowledge of *Trichoderma* spp. diversity in South Africa is needed because of its ecological importance. To date 28 *Trichoderma* species have been reported from South Africa. As a result of this research, we now have increased this to 33 species known from South Africa.

A total number of 91 strains from wheat rhizosphere soil were isolated and resolved in seven species which were assigned to *T. gamsii, T. velutinum, T. saturnisporum, T. virens, T. spirale, T. koningiopsis,* and *Trichoderma* sp. NNC105. Some species have been reported before, while *T. velutinum* was the first report for South Africa. Different agricultural practices also yielded different results as monoculture and crop rotation isolated 4 and 7 species, respectively. However, when evaluating *Trichoderma* species in terms of treatments it showed that both wheat after wheat (monoculture) and wheat after canola (crop rotation) resulted in the same number of *Trichoderma* species (4), whereas wheat after medic (crop rotation) produced the highest number of *Trichoderma* species (6) than the other two treatments. Moreover, *T. gamsii* and *T. saturnisporum* species were isolated in all treatments. These two species are widely distributed in nature, and in addition *T. gamsii* was the most abundant species isolated in wheat soil.

In maize soil a total of 337 strains were isolated and resulted in the classification of 11 species which were assigned to *T. koningiopsis, T. gamsii, T. velutinum, T. rifaii, T. hamatum, T. spirale, T. peberdyi, T. asperrelum, T. hamatum, T. paratroviride* and *T. neokoningii.* In terms of agricultural practices, crop rotation isolated 11 *Trichoderma* spp. and monoculture practice isolated 7 *Trichoderma* spp. Five *Trichoderma* species were reported for the first time in South Africa namely *T. velutinum, T. rifaii, T. peberdyi, T. paratroviride,* and *T. neokoningii*. The most dominant species under monoculture was *T. hamatum,* whereas in crop rotation it was *T. gamsii*. Overall, *T. gamsii* was the most abundant species isolated in maize soil.

Trichoderma species isolated from wheat and maize soil varied, although *T. spirale, T. velutinum, T. gamsii,* and *T. koningiopsis* were isolated in both crops. This study also indicated that *T. gamsii* was most abundant species in both crops. These findings were not surprising since *T. gamsii* is commonly known to be cosmopolitan. The differences in *Trichoderma* distribution in both crops could be due to the different treatments that were used on the farms. In addition, parameters such as soil type, soil pH, geographical locations, and climate are other contributing factors that one should also consider. Furthermore, the distribution of *Trichoderma* spp. in three provinces varied because KZN, FS, and WC isolated 11, 8, and 7 species respectively. This could be due to different factors such as soil types, farming practice, and sampling procedures.

The secondary objective of this study was to screen the ability to solubilize phosphate of selected strains. Plants require nutrients such as nitrogen (N), phosphorus (P), and potassium (K) for their optimum growth. Phosphate (phosphorus) is one of the second most important nutrient after nitrogen that is needed by plants. However, soil is known to have an abundance of phosphorus which is in the form of rock phosphate under alkaline environments and ferric or aluminum phosphate under acidic environments. Plants are unable to access this phosphate in the soil because it is insoluble. Therefore, in this study we screened for phosphate solubilization potential from *Trichoderma* strains isolated on maize and wheat soil. Findings shows that *T. koningiopsis* NNC066 solubilized the highest amount of phosphate (187.80 µg/ml).

The last objective of this study was to determine if selected *Trichoderma* species have the capacity to produce indole acetic acid (IAA). IAA is a plant hormone that is required by plants for their root development. Plants produce IAA, although it is insufficient for the plant's optimal growth and development. It has been reported that exogenous IAA is helpful in some developmental stages of plants. No strains produce IAA in the absence of L-tryptophan. However, all *Trichoderma* strains were able to produce IAA in the presence of L-tryptophan. The strain that produced highest IAA was *T. gamsii* NNC019 (41.90 µg/ml).

Trichoderma strains that were used to screen phosphate solubilization and production of IAA were presented by various *Trichoderma* species namely, *T. spirale, T. virens, T. gamsii, T. asperellum, T. hamatum, T. koningiopsis, T. paratroviride, T. saturnisporum, T. neokoningii, T. afroharzianum, T. velutinum, T. peberdyi,* and *T. rifaii*. This study found that most of *Trichoderma* strains identified as the best phosphate solubilizers and IAA producers belonged to *T. viride* clade. However, these functions were shown to be strain specific most of the time.

Trichoderma species is still not thoroughly explored in South Africa, therefore additional research is required to gain a better understanding of *Trichoderma* diversity in South Africa. Moreover, *Trichoderma* spp. that were isolated in this study will add to the pool of *Trichoderma* spp. that could be used in agriculture as bio-stimulants or biological control agents since they have been accustomed to the South African environments. *Trichoderma* strains that showed ability to solubilize phosphate and produce IAA under *in vitro* assays can be further evaluated for their potential to enhance crop development under green house or field conditions.