EFFECT OF BIOFERTILIZERS ON PHOSPHORUS NUTRITION AND GRAIN YIELD OF DESI CHICKPEA (*Cicer arietinum* L) GROWN IN DIFFERENT AGRO-ECOLOGIES

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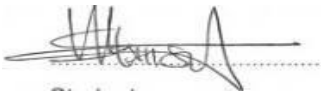
DECLARATION

I, **Sina Mashishi**, student number 11640568, hereby declare that this dissertation for the Master of Science in Agriculture (Soil Science) submitted to the Department of Plant and Soil Sciences, Faculty of Science, Engineering, and Agriculture, University of Venda, has not been submitted previously for any degree at this or any other institution. It is original in design and execution, and all references have been fully acknowledged.

Candidate:  Date: 20 / 02 / 2023

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DEDICATION

I dedicate this work to my lovely son Kabelo Zwanga Tyler Mashishi and daughter Lethabo Tshedza Daisy Mashishi for being my source of motivation. To my mother, Rosinah Raisibe Mashishi whose moral support inspired me to work harder than ever before.

ABSTRACT

South African soils are predominantly acidic and have high levels of Al and Fe, giving a higher P sorption capacity and low P levels. Consequently, soil fertility has become a major constraint in South Africa's farming, thereby threatening crop production and food security. Literature reveals, however, that biofertilizers have the potential to improve crop growth, grain yields and, acid and alkaline phosphatase activities in both the plant tissue and soil. Nonetheless, there is scant information about the biofertilizers' effects on their co-application with rhizobium inoculation as most of the studies focused on the sole application of the biofertilizers such as Bontera, Kelpak, Mycorrhiza, and Rhizobium inoculation. This study evaluated the biofertilizers' effects on the phosphorus accumulation in the rhizosphere soil, the acid and alkaline phosphatase activity in the rhizosphere soil and, the two chickpea genotypes' growth and yields under different environments.

In 2019, three field experiments were conducted in three locations, namely Thohoyandou, Syferkuil, and Sikhwahlane, and two more were done at Thohoyandou and Syferkuil in 2021. The treatments consisted of a factorial combination of six biofertilizer levels (Mycoroot, Kelpak, Rhizobium inoculation, Kelpak+Rhizobium inoculation, Mycoroot+Rhizobium inoculation, zero control) and two chickpea genotypes (Accession 3 and Accession 7). These were arranged in a three-time replicated randomised complete block design. The intercepted radiation's (IR) proportion was measured using the AccuPAR LP-80 ceptometer at Thohoyandou in 2019 and 2021. The chlorophyll content was measured at vegetative and reproductive stage using a chlorophyll meter (CCM-200 PLUS, Opti-Science) at Thohoyandou and Syferkuil in 2021. The stomatal conductance (gs) was measured using a portable porometer (AP4 DELTA-T Device) at the vegetative and reproductive stage at Thohoyandou in 2021. The normalised different vegetation index (NDVI) was measured using the portable GreenSeeker at the vegetative stage at Thohoyandou and Syferkuil in 2021. The chlorophyll fluorescence was measured at the flowering stage using the portable chlorophyll fluorometer at Thohoyandou, Syferkuil, and Sikhwahlane in 2019; and at Thohoyandou and Syferkuil in 2021. The acid and alkaline phosphatase activities were measured at the flowering stage at Thohoyandou, Syferkuil, and Sikhwahlane in 2019, and at Thohoyandou and Syferkuil in 2021. The inorganic phosphorus was measured at the flowering stage at Thohoyandou and Syferkuil in 2021. The yield and yield components (pod weight, shoot biomass, and harvest index) were measured at the harvest maturity stage at Thohoyandou, Syferkuil, and Sikhwahlane in 2019, and at Thohoyandou and Syferkuil in 2021. Data were analysed for variance (ANOVA) using the STATISTIX (2017) version 10.0. The Tukey's honestly difference (HSD) test was used to

separate the means that were significantly different (P≤0.05). The Pearson correlation was used to test the linear association's strength between the parameters studied.

The biofertilizers significantly increased the intercepted radiation's proportion (%IR) at Thohoyandou in 2019 and 2021. The Kelpak sole and in combination with the rhizobium inoculation on 61DAE (86.71) and 75DAE (85.10) recorded the highest %IR in 2019 cropping season. The rhizobium inoculation recorded the highest %IR at 47DAE (50.17) & 83DAE (80.67) and the Kelpak+Rhizobium inoculation recorded the highest %IR at 61DAE (54.33) & 75DAE (68.67) in the 2021 cropping season. The genotype had no significant effect on %IR in both seasons but the interaction between M x accession7 gave the greatest proportion of the intercepted radiation in 2019. The chlorophyll content was significantly increased by the biofertilizers at Thohoyandou (clay soil) only in 2021 with the Kelpak combined with rhizobium inoculation recording the greatest chlorophyll content (2.13-3.10) in all measurement dates. However, the genotype affected the chlorophyll content at 74DAE with accession7 recording the highest chlorophyll content compared to accession3. However, the interaction between the biofertilizer and the genotype had no significant effects on the chlorophyll content in all sites. The stomatal conductance was significantly affected by the biofertilizer application with K+R recording the highest gs at 42DAE (529.25) and M+R recording the highest gs at 56DAE (473.08) at Thohoyandou in 2021. Both the genotype and interaction between the biofertilizer and the genotype significantly affected the gs at 70DAE with accession3 recording the highest gs compared to accession7, and the Mycoroot x accession3 recording the highest gs. The normalised difference vegetative index was significantly increased by the biofertilizer application at 28DAE & 42DAE at Syferkuil only (sandy soil) with Mycoroot and K+R recording the highest NDVI at 28DAE (0.56) and rhizobium inoculation and M+R recording the highest NDVI at 42DAE (0.68). Neither the genotype nor the interaction between the biofertilizer and the genotype had a significant effect on the NDVI in all sites.

The biofertilizer's application significantly affected the acid and alkaline phosphatase activities in all sites except at Syferkuil in 2021 where the biofertilizers had no significant effect on the alkaline phosphatase activity. The co-application of K+R gave the highest APase at Syferkuil (sandy soil) and Sikhwahlane (loamy soil) in 2019, while at Thohoyandou (clay soil) the co-application of M+R gave the highest APase. In 2021, the co-application of K+R gave the highest APase at Syferkuil and Thohoyandou. The alkaline phosphatase activity was higher with the application of rhizobium inoculation at Syferkuil and Thohoyandou in 2019, and higher with the co-application of M+R (13.03) in Sikhwahlane in 2019. However, the zero control gave the highest AlkPase activity (74.14) at Syferkuil in 2021. At Thohoyandou (2021), however, the Kelpak's application recorded the highest AlkPase activity (56.81). Inorganic phosphorus was significantly affected by the biofertilizers in both sites. The co-application of K+R recorded

the highest inorganic phosphorus at Syferkuil and the sole Kelpak (15.16mg/kg) gave the highest inorganic phosphorus at Thohoyandou in 2021. The genotype had no significant effect on the APase and AlkPase activities but significantly affected the inorganic phosphorus at Syferkuil with accession3 (55.61mg/kg) recording the highest Pi compared to accession7. The interaction between the biofertilizer and the genotype affected the APase and inorganic at Thohoyandou, and only the Pi at Syferkuil in 2021.

Among the various biofertilizers used at Thohoyandou, the rhizobium inoculation recorded the highest quantum yield of PSII (0.24 to 0.33) in 2019. The biofertilizers, however, significantly affected the PSII, Fv/Fo, and Fv/Fm at Syferkuil in 2021 with the rhizobium inoculation exhibiting the highest PSII, Kelpak exhibiting the greatest Fv/Fo, and the sole Mycoroot and Mycoroot combined with the rhizobium inoculation exhibiting the strongest Fv/Fm. Among the biofertilizers administered at Thohoyandou, the Kelpak and rhizobium inoculations gave the highest PSII, and the Kelpak sole gave the greatest Fv/Fo. The genotype significantly affected the minimal fluorescence (Fo) in Sikhwahlane in 2019. It was pronounced in accession7 than in accession3 and the genotype affected the Fv/Fm at Syferkuil and was more pronounced in accession7 than in accession3. In 2019's cropping season, the biofertilizers increased the pod weight and grain yields at Thohoyandou to above-ground biomass and grain yield at Syferkuil, and pod weight to above-ground biomass and the grain yield in Sikhwahlane. In both sites in 2021, the biofertilizers increased the above-ground biomass, harvest index, and grain yield. Accession7 gave the highest value of pod weight at Syferkuil's cropping season in 2021. The interaction between the biofertilizer and the genotype had no significant effect on the grain yield and yield components in all the locations and in both cropping seasons. Therefore, this makes the biofertilizers an effective tool for increasing the chickpea's yield in these regions.

Keywords: Biofertilizers, chickpea, Kelpak, Mycoroot, rhizobium inoculum, P-enzymes.

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Al	Aluminium
AlkPase activity	Alkaline Phosphatase Activity
AMF	Arbuscular Mycorrhizal Fungi
APase activity	Acid Phosphatase Activity
BNF	Biological Nitrogen Fixation
F0	Minimal Fluorescence
FAO	Food and Agriculture Organisation
Fm	Maximal Fluorescence
Fv	Variable Fluorescence
HI	Harvest Index
Mes	Morpholinoethanesulfonic acid
NDVI	Normalized Difference Vegetative Index
OAs	Organic Acids
P	Phosphorus
PGPR	Plant Growth Promoting Regulators
Pi	Inorganic Phosphorus
PIR	Proportion of Intercepted Radiation
PSI	Maximum primary yield of photochemistry I
PSII	Effective quantum yield of photochemistry II

CHAPTER 1: INTRODUCTION AND BACKGROUND

1.1. BACKGROUND

Chickpea (*Cicer arietinum* L.) is an Asian leguminous crop by origin (Harlan, 1992). The crop has since spread throughout the world. *Cicer arietinum* L belongs to the Fabaceae family. The crop is consumed throughout the world for its nutritional benefits, particularly in Afro-Asian countries (Lokesh *et al*. 2020). At present, it is the third mostly produced pulse crop in the world after common beans and field beans (Sofi, *et al*. 2020). Asia accounts for 83%, Oceania 6% and, North America and Africa 5% each of the crop's production (FAO, 2019). In developing countries, the chickpea's production and consumption contribute about 95%, with an annual average production of about 12.1 million tonnes (FAO, 2018). The major chickpea producing countries include India (65%), Australia (14%), Ethiopia (4%), Myanmar (4%), Turkey (3%), Russia (3%), Iran (2%), Pakistan (2%), and the USA (2%), while other countries account for the remaining 1% (FAO, 2019). Like other grain leguminous crops, the chickpea's importance is that it helps improve the soil fertility by fixing the atmospheric nitrogen required by crops such as maize, chickpea, wheat, and oats (Imran, *et al*., 2015). The chickpea's productive potential is in its ability to withstand long periods of drought (Awasthi, et al. 2014). In poor households and among vegetarians, the crop is an economical source of protein (Menal-Puey, *et al*., 2018). Potentially, the chickpea has a role to play in ensuring the world's food security. That is, the crop covers India and Sub-Saharan Africa's daily food protein intake deficit (Merga & Haji., 2019).

The chickpea is a drought resistant legume but the biotic factors such as diseases and insect pests on the one hand, and the abiotic ones such as temperature, soil moisture, soil pH, poor soil fertility, and salinity decreases its productivity. Low soil fertility and the inefficient management of sub-Saharan African soils are the major challenges that hinder the smallholder farmers' crop productivity (Raimi, *et al*., 2017). Soil depletion is caused by the removal of nutrients associated with intensive cultivation and poor soil management systems (Palm, *et al*., 2001). The consequence for that are poor yields. That is, the poor soil fertility affects crop growth and reduces plants yields. Phosphorus (P) plays an important role in the leguminous crops' metabolic activities (Krishna 2017). This is particularly so regarding the chickpea's nodulation, nitrogen fixation, growth, and yields (Meena, *et al*., 2005). The chickpea's P-deficiency or its growth media, therefore, affects its root formation, nodulation, and BNF, resulting in reduced growth and yields (Ali, *et al*., 2010). For example, when the chickpea is grown in P-deficient soils, its growth and grain yields are greatly reduced (Ali, *et al*., 2010). However, legumes such as the chickpea develop mechanisms that help to improve

the P's bio-availability, solubility, and uptake in P-deficient soils (Alori, *et al*., 2017; Calvo, *et al*., 2014). These mechanisms include the cluster roots' formation (Barbas, Garcia and Manero 1999), the organic acids' exudation (Gardner, *et al.*, 1982), the acid phosphates enzymes' secretion (APase) (Mogale, *et al.*, 2018), and the symbiosis with mycorrhizal fungi (Tia, *et al*., 2012). However, these mechanisms and their effectiveness vary according to the genotype, environment (climate and soil type), and management practices (such as the type of fertilizers and planting dates) (Lusiba, *et al*., 2016; Ogola, 2015; Thangwana & Ogola, 2012).

Most smallholder crop farmers do not apply adequate quantities of fertilizers to support their crops' growth and yields. To this end, there are alternatives to improve the soil fertility such as the individual application of the biofertilizers or combined with synthetic fertilizers (Wolde-meskel *et al*., 2018; Kyei-Boahen *et al*., 2018). There are few studies conducted in South Africa about the biofertilizers' role on the chickpea's growth and yields, and hence not much is said about their effects on the crop's P nutrition. Biofertilizers have different action modes. Locally produced biofertilizers include *Bradyrhizobium Japonicum* which is utilised in the commercial crop sector for the production of legume crops. The other locally produced biostimulant is kelpak which is manufactured from the brown algae Papenfuss. Fewer published litareture show that it promotes fresh and dry weight, nodulation, water-use-efficiency, protein, carbohydrates, chlorophyll content, and nutrient accumulation in legumes (Ngoroyemoto, *et al*. 2019; Mbadlwa, *et al*. 2019). Mycoroot is also one of the locally produced biofertilizer that when it is applied to the soil, it improves soil fertility through the symbiosis process with plants in acquisition of phosphorus in the soil (Yao, *et al.* 2001). Research background has shown that their application alone or in combination with fertilizers results in improved yield-related and overall quality parameters of plants and properties of soil. Studies about biofertilizers focus on the Kelpak, Bontera, and rhizobium inoculation's individual applications (P. Macil 2018, Moloto, *et al*. 2021). There is, therefore, a dearth of information about the Kelpak and Mycoroot's effects when applied exclusively and in combination with the rhizobium inoculation. South Africa has seen an increase in companies and institutions that produce and sell biofertilizers. There is a need, therefore, for in-depth studies about different biofertilizers' roles on the chickpea's growth, yields, and P nutrition.

1.2. PROBLEM STATEMENT

The soils of South Africa are predominantly acidic and contain high concentrations of aluminium (Al) and iron (Fe), which causes high phosphorus sorption capacity, and hence low phosphorus availability. The phosphorus deficiency affects crop production as it reduces the yields' quality and quantity, thereby affecting food security. Supplementing phosphorus in the soil with inorganic fertilizers compounds environmental pollution. Although the use of biofertilizers to help improve intracellular APase activity and the chickpea plant tissue's phosphorus concentration, growth and yields are well documented, there is a dearth of information about the effects of different biofertilizers (Kelpak and Mycoroot sole and combined with rhizobium inoculation) on the extracellular APase activity in the rhizosphere and on phosphorus accumulation in the chickpea's rhizosphere soil under contrasting soil types.

1.3. JUSTIFICATION OF THE STUDY

The soil's extracellular APase solubilises its absent phosphorus into plant-available form. The biofertilizers' use helps to improve the phosphorus' availability in the soil by refining the APase activity in the rhizosphere. Doing so helps to reduce the frequent use of inorganic fertilizers to supplement the soil's phosphorus. Furthermore, there are many biofertilizer varieties with different action mechanisms in the market. In this case, understanding some of these specific biofertilizers' roles could potentially lead to their increased adaptation and judicious use. Doing so could result in improved soil fertility, increased chickpea productivity and production, and hence improve the smallholder farmers' food security and family income.

1.5. HYPOTHESIS

Biofertilizers and chickpea genotype would affect:

- P-enzyme activity and P-concentration in the rhizosphere soil.
- Quantum yield and chlorophyll fluorescence.
- Selected physiological parameters.
- Yield and yield components.

1.4. OBJECTIVES

1.4.1. Main objective

To evaluate the effects of the biofertilizer on phosphorus accumulation and, the chickpea's growth and yields in different agro-ecologies.

1.4.2. Specific objectives

To assess the effect of biofertilizer and chickpea genotype on:

- P-enzyme activity and P-concentration in the rhizosphere soil.
- Quantum yield and chlorophyll fluorescence.
- Selected physiological parameters.
- Yield and its components.

CHAPTER 2: LITERATURE REVIEW

2.1. CHICKPEA'S ORIGIN, TYPES, AND USES

The chickpea crop is native to Asia, but it is now cultivated in nearly all continents (Singh & Saxen, 1999). There are two types of chickpeas namely, kabuli and desi. The desi chickpea has purple-pink flowers while the kabuli bears white ones. The kabuli chickpea's seed is thin-coated and large, with a ram-shaped head compared to that of the desi. The desi's seed is thick-coated, small, and angular dark. The chickpea is a protein-rich legume. It is also a great source of unsaturated fatty acids and vitamins such as folate, vitamin A precursor ß-carotene, riboflavin, and thiamine (Millan, *et al*. 2015). The chickpea is one of our food crops but can also be used as animal feed (Yadav, 2017). Its leaves are eaten as leafy vegetables while its immature grains are eaten either raw or roasted to reduce digestive disorders, cardiovascular diseases, and various cancer types (Jukanti, 2012). The chickpea's dry grains are ground to either produce baking flour or to make starter meal soups.

2.2. BIOFERTILIZERS

Soil degradation refers to the decline in soil conditions due to poor agricultural management practices. For example, the frequent tillage and depletion of nutrients without supplementing them lead to poor crop production. Soil fertility is improved using organic (composts, animal manure, biochar, and plant residues) and inorganic (superphosphate, lime ammonium nitrate, and potassium chloride) fertilizers. Biofertilizers are soil amendments that could be used with other fertilizers to supplement essential nutrients to improve soil fertility. Biofertilizers are substances that improve plants' nutrition efficiency, stress tolerance, and the quality crop indifferent of their nutrient constituents (Du Jardin 2015). They consist of phytohormones such as auxins, gibberellins, cytokinins, and abscisic acids (Stirk 2004). Biofertilizers influence the physiological processes such as growth, differentiation, and development at low concentrations (Davies 2010). There are several biofertilizers in South Africa's markets. These include, *inter alia*, the Kelpak, Mycoroot, and Rhizobium inoculum.

For many years now, the rhizobium inoculants have been effective in legume production. They are used to supplement a strain of rhizobia in the soil to influence nodulation and promote plant growth (Kour *et al*., 2019). Kelpak is a commercially available sea-weed extract manufactured in Cape Town's Kelp Products International (Pty) Ltd. It is a product of brown algae *Ecklonia maxima* (Osbeck) Papenfuss, and contains auxins, gibberellins, cytokinin, abscisic acid, spermine, polyamines, and phlorotannins (Rengasamy *et al*. 2013). Mycoroot

(also commercially known as the arbuscular mycorrhizal fungi, AMF), influences the AMF in the rhizosphere soil to colonise the host plant. When Mycoroot is applied to the soil, it improves root development, increases soil microbial biodiversity and, nutrient and water uptake (Yao *et al.,* 2001). However, not much is written about these biofertilizers' effectiveness (Kelpak and Mycoroot) on the chickpea's growth and yields, and whether they might have synergistic or additive effect in combination with the rhizobium inoculation.

2.3. EFFECTS OF BIOFERTILIZERS ON P-CONCENTRATION AND P-ENZYMES ACTIVITIES IN THE RHIZOSPHERE

Phosphorus (P) is one of the major nutrients required by plants for growth and development (also essential for the formation and development of plant roots) (Saufe *et al*., 2018). Soils that are acidic with high iron and aluminium concentrations have high P absorption characteristic, and hence low P availability. P-deficiency makes plants to change their roots' morphology, leading to the formation of clustered roots to adapt to the low P conditions (Barbas *et al*., 1999). The soil's P-deficiency could be improved by applying biochar, compost, and superphosphate to maximise the P's bio-availability, solubility, and uptake.

Apart from using the inorganic and organic fertilizers to supplement the soil's P, plants use mechanisms to improve the P's solubilisation, acquisition, and use. These mechanisms include cluster roots formation, exudation of organic acids (OAs), and the acid phosphatase enzymes' secretion (Barbas *et al*., 1999; Gardner *et al*., 1982; Mogale *et al*., 2018). The extracellular acid and alkaline phosphatase enzymes produced by plant roots and/or microorganisms help liberate P from the organic sources in the rhizosphere (Maseko & Dakora, 2013; Nannipieri *et al*., 2011). These phosphatases (acid and alkaline phosphatase) are, therefore, crucial for the P nutrition in plants (Tian & Liao, 2015). The biofertilizers' application stimulates and increases the beneficial microorganisms' diversity and activity, particularly in the rhizosphere soil (Alam *et al*., 2013). It also stimulates plants and microorganisms to produce the extracellular acid phosphatases. Moloto *et al*. (2021) observed that the Bontera and Kelpak's application increased acid phosphatase in the plant tissue and the rhizosphere of soil with contrasting texture. The acid and alkaline phosphatase activities in the soil largely correlate with the accumulation of microbial biomass and total organic carbon and therefore such makes it an indicator of stress suffered by the ecosystem (Balota *et al*., 2014). Understanding the effect of the biofertilizers could help to select the appropriate biofertilizers to improve the P's availability in the rhizosphere. The biofertilizers' effects on the APase activity in the chickpea's root nodules and plant tissue are highly publicised. Yet not

much is written about the biofertilizers' effects when applied either alone or in combination with the rhizobium inoculation on the P-concentration and phosphatase enzyme activity in the rhizosphere soil and the chickpea's plant tissue.

2.4. EFFECTS OF BIOFERTILIZERS ON GAS EXCHANGE PARAMETERS

The phytohormones contained in biofertilizers enhance the plants' photosynthetic performance, including the increased stomatal conductance and water use efficiency (Li *et al*., 2018). In addition, the biofertilizers help improve the photosynthetic rate by enhancing the leaf venation and regulating the chlorophyll content (Gayathri and Aiswariya 2020). The increased stomatal conductance is widely reported (Audipudi, Bai and Sanneboyina 2021, Pandey and Gupta 2020). Several studies show that the biofertilizers affect the red beans' stomatal conductance (*Phaseolus Vulgaris* L), alfalfa (*Medicago sativa* L), and carob (*Ceratonia siliqua*) (Seyahjani, *et al*. 2020, Boutasknit, *et al*. 2021, Ben-Laouane, *et al*. 2021). The PGPR+Mycorrhiza's application increases the triticale's stomatal conductance (Arough and Sharifi 2016). Similarly, Seyahjani *et al*. (2020) observed a significant increase in the red bean's stomatal conductance when treated with the combination of rhizobium, mycorrhiza, and pseudomonas irrigated at more than 70 mm evaporation level. The biofertilizers' effect on the stomatal conductance is well documented, and these fertilizers' responses to stressful conditions such as drought, salinity, and heat are evaluated.

2.5. EFFECTS OF BIOFERTILIZERS ON QUANTUM YIELD AND CHLOROPHYLL FLUORESCENCE

Environmental stresses largely limit the chickpea's production. The chickpea requires phosphorus for its growth and development. The environmental stress that affects the chickpea production is the P-deficiency. In comparison with other legumes, the chickpea is more resistant to environmental stress. However, the P-deficiency largely reduces the chickpea's physiological and morphological characteristics (Suriyagoda, *et al*. 2010). The P-deficiency's effect on the faba bean is widely reported though (Oukaltouma, *et al*. 2021). The P-deficiency significantly reduces the chlorophyll fluorescence by disturbing the carbon dioxide assimilation since the P is needed in the fixed carbon's transportation from the chloroplast to the cytosol (Rychter, *et al*. 2018). The chlorophyll fluorescence is an integral part of the leaves' photosynthetic processes. The photosynthetic pigment absorbs the sunlight energy funnelled by a functional array of photosystem II (PSII) to generate the chemical energy used for carbon dioxide fixation in the dark (Du , *et al*. 2019). The absorbed sunlight energy that exceeds the carbon dioxide fixation's photo-chemical processes can be either dissipated

as heat or re-emitted as chlorophyll fluorescence (Haeder 2022). Preliminary results helped conclude that under simulated and natural occurring environmental stresses, the biofertilizers' application increased the chlorophyll fluorescence (Moustakas, *et al*. 2022).

The biofertilizers play an effective role in plant growth and development. They reduce the environmental stresses' effects on plants. Biofertilizers help improve the plants' tolerance of environmental stress due to hormone-like substances, plant growth regulators, and high levels of macro and micro-nutrients (Hashem, *et al*. 2019). Biofertilizers were once said to significantly increase the sesame's chlorophyll fluorescence (Kafi, *et al*. 2022). Similarly, the green compost's application combined with rhizobium inoculation and AMF enhanced the alfalfa's chlorophyll fluorescence (Ben-Laouane, *et al*. 2021). The biofertilizers' effects on the chlorophyll fluorescence for crops such as wheat and sweet pepper were investigated (ALKahtani, *et al*. 2020). Although the biofertilizers' effects on the chlorophyll fluorescence are well documented, studies focus on their effects on the chlorophyll fluorescence under salinity, drought, and heat stresses. Thus, studies to evaluate different biofertilizers' performances on the chickpea's chlorophyll fluorescence are long overdue.

2.6. EFFECTS OF BIOFERTILIZERS ON GROWTH AND YIELDS

Studies done so far show that the biofertilizers' application increases the chickpea's yields, including that of cowpeas (Singh, *et al*. 2011, Dekhane, *et al*. 2011). Such studies also prove that improving soil fertility could be achieved by applying biofertilizers to the soil instead of synthetic fertilizers. Any biofertilizer combined with synthetic fertilizer significantly improves yields while sustaining the environment (Kumar, *et al*. 2019). The rhizobium inoculation, Kelpak, arbuscular mycorrhiza, and Bontera's applications increase the chickpea's shoot biomass and yield due to the increased essential nutrients (Moloto, *et al*. 2021, Imran, *et al*. 2015). The biofertilizers and the phosphorus' applications increase the chickpea's grain yields (Kumar, *et al*. 2019). The increase in yield components such as pod weight, number of seeds, pods per plant, and shoot biomass was identified (Ditta, *et al*. 2018, Imran, *et al*. 2015, Macil 2018). The chickpea's yield and yiled components are well documented but there is not much about the biofertilizer and genotype's interactive effects on yield and yield components. Furthermore, not much has been studied about the effects of the rhizobium inoculation combined with Kelpak and Mycoroot on the chickpea's yield and its yield components. Thus, incorporating the rhizobium inoculation with Kelpak and Mycoroot might help to improve the chikpea yields.

CHAPTER 3: METHODOLOGY

3.1. EXPERIMENTAL SITES

The experiments were conducted in three sites, vis: University of Venda's Experimental Farm at Thohoyandou, University of Limpopo's Experimental Farm at Syferkuil in 2019 and 2021 winter seasons, and Sikhwahlane's Ikhwezi Farm, Mpumalanga Province, in 2019 winter season. The University of Venda's Experimental Farm is situated at Thohoyandou at 22°58.081 Longitude South, 30°26.411 Longitude East, and 595 m above sea level. Rainfall at the site is 500 mm per year, mostly during summer, and temperatures range between 18°C and 31°C. (Tadross, *et al*., 2006). The soils are predominantly deep red and well drained clay (Hutton soils) with an apedal structure (Soil Classification 1991).

The University of Limpopo's Experimental Farm is located at Syferkuil (1230 m above sea level with the latitude and longitude of 23°50'S and 29°40'E respectively). Average rainfall received by the area is about 451mm per annum, with the average minimum and maximum temperatures ranging between 10°C and 25°C respectively (Kutu and Asiwe 2010). This area's soil is loamy sand (Hutton soil and Glenrosa) (Soil Classification 1991).

Sikhwahlane village is located in the Nkomazi Local Municipality's Ward 16, Ehlanzeni District (Malelane), Mpumalanga Province. The temperatures in Malelane are classified as warm and temperate, with the latitude of 25º41'26.3''S and 31º43'42.5''E Longitude, and 676 m above sea level. Sikhwahlane village receives more rainfall during summer than in winter, with an average temperature of 21.7° C and an average precipitation of 750-860 mm per annum.

3.2. EXPERIMENTAL DESIGN AND TREATMENTS

The study was conducted during the 2019 and 2021 winter seasons. Treatments consisted of six biofertilizer levels (Kelpak [K], Mycoroot [M], rhizobium inoculation [R], K+R, M+R, & zero control) with two chickpea cultivars (accession3 & accession7) arranged in a randomised complete block design replicated three times. The individual plots' size was 3 m x 2 m, with 12 plots per replicate. The spacing between the plots was 50 cm and the replicates were 1 m apart (see Figure 3.1). In a mixture of rhizobium inoculum, seeds were soaked and planted immediately after inoculation. To prevent contamination, un-inoculated seeds were sown before the inoculated ones. When planting, five grams of Mycoroot were directly applied on the seed and 500 ml of Kelpak solution were applied after plant shoots. Concentrated Kelpak

was bought from Kelp products international (PTY) LTD and its solution was prepared using about 1:40 (v/v) of Kelpak to water.

rep1	T2	T6	T4	T12	T11	T5	T10	T1	T7	T9	T3	T8
rep2	T7	T10	T5	T9	T1	T3	T8	T12	T6	T2	T4	T11
rep3	T3	T12	T8	T4	T6	T2	T11	T9	T5	T10	T1	T7

Figure 3.1: Experimental layout illustrating the arrangement of treatments in the fields.

T1: R+M ACC#7	T2: R+K ACC#7	T3: R ACC#7	T4: M ACC#7	T6: Control ACC#7	T7: R+M ACC#3
T8 R+ K ACC#3	T9: R ACC#3	T10: M ACC#3	T11: K ACC#3	T12: Control ACC#3	T5: K Acc#7

3.3. CULTURAL PRACTICES

The land was prepared using a tractor. For a good seedbed, the land was disked into finer particles to allow uniform germination. The experiments were planted on the 12th of April 2019 at Syferkuil, 17th of April 2019 at Thohoyandou, and 25th of April 2019 in Sikhwahlane; and 16th of April 2021 at Thohoyandou, and 22nd of April 2021 at Syferkuil respectively. The seeds were planted manually in rows with a spacing of 30 cm x 10 cm for both inter and intra rows. All the experiments were irrigated using the micro jet sprinkler irrigation systems immediately after planting to encourage germination, emergence, and crop establishment. Supplemental irrigation was applied whenever necessary. The experiments were always weeded throughout the seasons.

3.4. DATA COLLECTION

3.4.1. Soil samples

Five soil samples were randomly collected from a depth of 30 cm prior to planting and mixed into a single homogeneous composite sample for the initial analysis of the total available phosphorus (using the Bray-1 method), organic carbon (using the Walkely -Black method), pH (using the pH meter at the ratio of 2:1 water: soil) in De-ionised water), electric conductivity (EC) (using the EC meter at a ratio of 2:1 water: soil), soil texture (using the Hydrometer method), and exchangeable cations (K, Na, Mg and Ca using the atomic absorption spectrophotometry AAS).

Table 3. 1: physical and chemical properties of soils at all the locations.

soil properties	Thohoyandou	Syferkuil	Sikhwahlane
Physical properties			
clay	61	20	9
loamy	18	21	22
sandy	21	59	78
Textural class	Clay	Sandy Loam	Sandy Loam
chemical properties			
pH	5.92	6.76	5.98
EC	28.83	65.12	13.6
SOC (%)	2.42	0.82	0.49
P (mg/kg)	11.23	15.47	29.68
Exchangeable cations (mg/kg)			
K	65	72	68
Na	140	130	142
Mg	271	389	294
Ca	724	633	798
CEC (cmol (+)/kg)	24.21	19.03	25.07

3.5. PHYSIOLOGICAL TRAITS

3.5.1 Chlorophyll content

The chlorophyll content was measured at Thohoyandou and Syferkuil in the 2021 cropping season only. For each plot, three plants from the four innermost rows were measured for chlorophyll content. Measurements were conducted on fully expanded young leaves using the portable chlorophyll content (CCM-200, PLUS, Opti-Science). Measurements were done at Syferkuil (26 DAE, 40 DAE, and 46 DAE) and Thohoyandou (50 DAE, 60 DAE, 68 DAE, and 74 DAE) at vegetative and reproductive stages.

3.5.2 Proportion of intercepted radiation

The intercepted radiation's proportion was calculated by measuring the PAR above and below the canopy using an Accu-PAR ceptometer on a clear sunny day at 14 days interval. To do so, the ceptometer was horizontally placed above the canopy to measure the PAR above canopy. To measure the PAR below, the ceptometer was placed between the rows below the ground. The equation below was used to calculate the intercepted radiation's proportion.

$(\alpha) = [1- (\frac{PAR\ below\ canopy}{PAR\ above\ canopy})]x\ 100$

3.5.3 Stomatal conductance

The stomatal conductance (g_s) was measured at Thohoyandou in the 2021 cropping season only. It was measured using the portable leaf porometer AP4 (Delta-T Devices Cambridge, UK). Measurements were taken at 42 DAE, 56 DAE, and 70 DAE at the vegetative and reproductive stages. Three plants from each of the thirty-six plots were randomly selected from four inner most rows and marked using tags. Readings were taken from the youngest fully expanded leaves. Readings were taken from the same plant on each occasion for consistence.

3.5.4 Normalized difference vegetative index

The normalised difference vegetative index (NDVI) was measured at Thohoyandou and Syferkuil during the 2021 cropping season only. It was measured using a portable GreenSeeker Handheld Crop Sensor (NDVI Meter) in the following days; 28 DAE, 42 DAE, and 52 DAE at Thohoyandou and, 28 DAE and 42DAE at Syferkuil. Three plants from four inner most rows of each plot were randomly selected and tagged for the measurements taken between 8:00 am – 12:00 pm on a clear sunny day. The same plants were used to take the readings on each occasion for consistence.

3.6. QUANTUM YIELD AND CHLOROPHYLL FLUORESCENCE (FV/FM)

The chlorophyll fluorescence was measured at Thohoyandou, Syferkuil, and Sikhwahlane in 2019 and at Thohoyandou and Syferkuil in 2021. Following Baker and Rosenqvist's (2004) proposed fluorescence nomenclature, the leaf chlorophyll fluorescence values such as the minimal fluorescence (Fo), maximum fluorescence (F_m), variable fluorescence (F_v) and the maximum photochemical efficiency of the photosystem II (F_v/F_m) were measured at the flowering stage using a PAM-2100 portable chlorophyll fluorometer (Waltz, Eiffel rich, Germany). Three plants from each of the 36 plots were randomly selected from the four inner most rows and dark-adapted using the light exclusion clips (Waltz, Eiffel rich, Germany) for 30 minutes. The chlorophyll fluorescence was measured during the day from 8:00 am on clear sunny days. The chlorophyll fluorescence's measurements were taken from the same plants for consistence.

3.7. ACID AND ALKALINE PHOSPHATASE ACTIVITY AND P CONCENTRATION IN THE RHIZOSPHERE

3.7.1. Collection and processing of rhizosphere soil

The rhizosphere soil (considered as the soil that was attached to the roots of the chickpea plants) was sampled by carefully digging out the chickpea's entire rooting system, taking special care that the attached soil remained intact. The rhizosphere soil was carefully brushed off the roots using a clean brush and put into the pre-labelled zipper plastic bags. The sample bags were placed in a cooler box with dry ice to avoid exposing that soil to direct sunlight and the possible degradation of the enzymes. The rhizosphere soil was then taken to the laboratory and temporarily stored at −4° C until the acid and alkaline phosphatase activities were analysed.

3.7.2. Bioassay of acid and alkaline phosphatase activities in the rhizosphere

The APase's activity in the rhizosphere soil was determined following Tabatabaei's (1994) method. In short, about 1 g of the soil sample was weighed using the polypropylene vials. About 4 ml of buffer (pH 6.5) was added to a 1 ml of 0.1 M phosphatase substrate and the contents were thoroughly mixed using a vortex. The mixture was incubated at 37° C for 1 h. Thereafter, 1 mL of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH were added. The mixture was filtered through the Whatman #2 filter paper. The absorbance of 2 ml of the resulting extractant was read using a UV-Visible spectrophotometer (JENWAY 7300, Bibby Scientific Ltd, Stone, Staffs, UK) at 420 nm. The filtrates' absorbance was compared with the p-nitrophenol standards. For each assay, a control was included to account for the non-enzymatic substrate hydrolysis and enzyme activity expressed as µg *p*-nitrophenol g^{-1} F wt h^{-1}.

3.7.3 Inorganic phosphorus in the rhizosphere

The extractable plant-available inorganic P in each rhizosphere soil sample was analysed following Dyer's (184) citric acid method as modified by (DuPlessis and Burger 1964). A 20 g of air-dried soil sample was extracted into 200 ml of 1% (w/v) citric acid, heated at 80° C, shaken for 2 minutes at 10 minutes intervals over an hour and filtered through the Whatman #1 filter paper. A 50 ml aliquot was heated to dryness in a water bath, digested with 5 ml of concentrated HCl and HNO_3, and evaporated to dryness in the water bath. The dry residue was then re-dissolved in 5 ml of concentrated HNO_3 and 20 ml of deionised water by heating. The sample was filtered, and the P measured by direct aspiration on a calibrated simultaneous

inductively coupled plasma-mass spectrometer (ICP-MS) (IRIS/AP HR DUO Thermo Electron Corporation, Franklin, Massachusetts, USA).

3.8. YIELD AND YIELD COMPONENTS

At harvest maturity, all plants from the inner four rows of each plot were cut at ground level at the harvest area of 2 m^2 quadrant. The pods were manually separated from the harvested plants to ascertain the pod weight per plant and the shoots were dried in an oven at the temperature of 65º C for 48 hours. The pods were threshed, and the seeds air-dried, cleaned, and weighed to determine the grain yield (kg/ha^{-1}). The Harvest Index was estimated as the proportion of grain yield to the shoot biomass.

3.9 STATISTICAL ANALYSIS

The data collected were subjected to the variance analysis (ANOVA) using a two-way factorial to test the treatments' effects on the measured parameters using the STATISTIX-10 software. The Tukey's Honestly Significant Different (HSD) test was used to separate the means that were significantly different ($P \leq 0.05$). The Pearson correlation was used to test the linear association strength between parameters studied.

CHAPTER 4: THE EFFECTS OF BIOFERTILIZERS AND GENOTYPE ON CHICKPEA'S SELECTED PHYSIOLOGICAL TRAITS

ABSTRACT

Chickpea is a seasonal crop produced in most parts of the world. It is mainly grown under rain fed conditions. The chickpea's physiological traits are affected by the environmental stresses, thus reduce its production. The study assessed the biofertilizers and genotypes' effects on the chickpea's selected physiological traits. Experiments were conducted at Syferkuil and Thohoyandou during the 2019 and 2021 winter seasons. The experiments consisted of the factorial combination of six biofertilizer levels (Kelpak [K], Mycoroot [M], rhizobium inoculation [R], K+R, M+R, and zero control) and two chickpea cultivars (accession3 and accession7) arranged in a randomised complete block design replicated three times. The intercepted radiation was measured at Thohoyandou in 2019 and 2021 only. The chlorophyll content was measured at Thohoyandou and Syferkuil in 2021. The gs was measured at Thohoyandou in 2021, and the NDVI was measured at Syferkuil and Thohoyandou in 2021. K's application revealed the highest %IR at 61DAE. The K+R gave the highest %IR at 75DAE in 2019; the R exhibited the highest %IR at 47DAE and 83DAE. The K+R exhibited the highest %IR at 61DAE and 75DAE in 2021. The genotype showed no significant effect on the IR in both seasons. The Mycoroot x accession7 exhibited the greater IR at 47DAE in the 2021 cropping season. The biofertilizers significantly affected the chlorophyll content (CHL) at Thohoyandou in all measurement dates in 2021. This ranged from 1.36-2.13 at 32DAE, 1.30-2.38 at 46DAE, 1.51-2.32 at 60DAE, and 1.38-3.10 on 74DAE. Accession7 showed a greater CHL at 74DAE but the interaction between the biofertilizers and genotypes showed no significant effects in both sites. The biofertilizers' application significantly affected the stomatal conductance at 42DAE and 56DAE. The Kelpak + Rhizobium inoculation [K+R] gave the highest increase at 42DAE. The Mycoroot + rhizobium inoculation [M+R] showed the highest increase at 56DAE on gs. Accession7 exhibited the greater gs at 70DAE including the interaction between the Kelpak and accession3 at Thohoyandou. The NDVI was only significantly affected at Syferkuil at 28DAE and 42DAE when treated with individual Mycoroot and Kelpak + rhizobium inoculation respectively. It was observed that the chickpea's increased physiological traits in this study varied with location. The conclusion is that the physiological traits' increase when responding to biofertilizers is indicative of the environment having played an important role in these traits.

Keywords: Biofertilizer, genotype, stomatal conductance (gs), chlorophyll content (cc), intercepted radiation (IR), Normalized difference vegetation index (NDVI)

4.1 INTRODUCTION

The chickpea (the garbanzo bean) is a protein-rich legume that is rich with unsaturated fatty acids (Ahmad, *et al*. 2005). It is mainly cultivated in winter after summer rainfall to use some of the soil's residual moisture (Derasirvatham & Tan, 2018). Environmental stresses were discovered to affect the chickpea crop yields by influencing its physiological traits such as the chlorophyll content, stomatal conductance, NDVI, and the intercepted radiation's proportion (Derasirvatham & Tan, 2018; Irshad *et al*., 2022). Radiation is one of the variables that influence the chickpea's crop development and yields. The reduced intercepted radiation was found to significantly impact the chickpea's chlorophyll content, resulting in lower photosynthesis, stomatal conductance, and NDVI (Rachaputi, *et al*. 2021).

The soil moisture, nutrients, and radiation's availability significant impact the stomatal conductance (Pramanik, *et al*. 2018). The opening of the stomata might indicate that the photosynthesis and transpiration rates are at peak. Heat stress can be detrimental to chickpea production, as it induces flower abortion and inhibits podding (Gau, Samineni and Varshney 2014). Early planting increased the intercepted radiation due to the increased canopy size and cover (Mubvuma, *et al*. 2021). The increase and decrease in NDVI is associated with the mineral nitrogen's availability and distribution in soil and is related to the chlorophyll content and active photosynthetic radiation (Martinez, *et al*. 2022).

There are management strategies in place to reduce the environmental stresses' effects on plants. The long-term use of the synthetic fertilizers deteriorates arable lands. The biofertilizers contain micro-organisms, phytohormones, and micro- and macro-nutrients that are beneficial to plant growth and soil fertility (Khaitov and Abdiev 2018, Igeohon and Babalola 2021). Literature shows that the biofertilizers such as rhizobium inoculation affects various plant variables and physiological traits such as intercepted radiation, chlorophyll content, and stomatal conductance (Ogola, *et al*. 2021, Fareed, *et al*. 2021). Studies about the chickpea's physiological traits are plenty but these largely focus on one type of the biofertilizers. This study assessed the effects of biofertilizer and genotype on the selected physiological parameters. Its hypothesis is that the biofertilizers and genotype would affect the selected physiological parameters.

4.2 MATERIAL AND METHODS

This study's experimental details are provided in chapter three. A concise synopsis is provided. That is, the experiments were conducted at the University of Venda's Experimental Farm (Thohoyandou) and the University of Limpopo's Experimental Farm (Syferkuil). The experiments consisted of a factorial combination of six biofertilizer levels (Mycoroot [M], Kelpak [K], rhizobium inoculation [R], M+R, K+R, & zero control) and two chickpea cultivars (accession3 & accession7) arranged in a randomised complete block design replicated three times.

The proportion of intercepted radiation was assessed by determining the proportion of the canopy's intercepted radiation. This was done by measuring the PAR above and below the canopy using an Accu-PAR ceptometer in a clear sunny day at 14 days interval. To measure PAR above the canopy, the ceptometer was placed horizontally on each occasion. It was positioned between the rows to measure the PAR below the ground. The intercepted radiation's proportion was calculated using the equation below:

$(\alpha) = [1- (\frac{PAR\ be\ canopy}{PAR\ above\ canopy})]x\ 100$

The stomatal conductance was measured at Thohoyandou in the 2021 cropping season only. We used the portable porometer (AP4 DELTA-T DEVICES) for that. Measurements were taken at vegetative and reproductive stages (42, 56, and 70DAE). Three plants from four innermost rows were randomly selected and tagged for the measurements. The chlorophyll content was measured at Thohoyandou and Syferkuil in the 2021 cropping season only. Using a chlorophyll content meter (CCM-200 Plus OPTI-SCIENCES), the chlorophyll content was measured at vegetative and reproductive stages at Thohoyandou (32, 46, 60, 74) and Syferkuil (26, 40, 54, 68). The NDVI was measured at Thohoyandou and Syferkuil in the 2021 cropping season only. Using the GreenSeeker Handheld Crop Sensor (NDVI meter), the NDVI was measured at Thohoyandou (days 28 and 42) and Syferkuil (days 28, 42, and 56). The data collected were subjected to the variance (ANOVA) analysis using a two-way factorial to test the effects of the treatments on the measured parameters using the STATISTIX-10 software. The Tukey's Honestly Significant Difference (HSD) test was used to separate the means that were significantly different ($P \leq 0.05$). The Pearson correlation was used to test the linear association strenght between parameters studied.

4.3 RESULTS

4.3.1 Intercepted radiation

The biofertilizers' application significantly affected the intercepted radiation's (IR) proportion at varying measurement dates. At 61DAE, plants supplied with the solution of the Kelpak exhibited the highest proportion of the intercepted radiation (41%) and at 75DAE, chickpea treated with K+R exhibited the greatest proportion of the intercepted radiation (91%) in 2019 (see Table 4.1). The increase in the intercepted radiation's proportion was only observed at the reproductive growth stage. In contrast, the biofertilizers significantly affected the intercepted radiation's proportion at vegetative and reproductive stages in 2021. The rhizobium inoculation exhibited more pronounced proportion of the intercepted radiation at 47DAE (118%) and 83DAE (170%). While at 61DAE (88%) and 75DAE (221%), the K+R gave the highest increase of the intercepted radiation's proportion (see Table 4.1). The genotype had no significant effect on the intercepted radiation's proportion in 2019 and 2021 (see Table 4.1). It was only at the 47DAE that the interaction between the biofertilizer and the genotype affected the intercepted radiation's proportion in 2021. The interaction between M x accession3 showed the highest intercepted radiation's proportion (see Figure 4.1).

4.3.2 Chlorophyll content

Biofertilizers significantly affected the chlorophyll content at Thohoyandou but not at Syferkuil. The chlorophyll content was affected at varying measurement dates. Plots treated with K+R exhibited the highest increase in the chlorophyll content at vegetative and reproductive growth stages (see Table 4.2). The K+R's application gave the highest increase in chlorophyll content at 32DAE (56%), 46DAE (83%), 60DAE (92%), and 74DAE (124%) respectively. The genotype affected the chlorophyll content at 74DAE at Thohoyandou only. The significant differences between the genotypes were only observed at the reproductive stage. Accession7 revealed the highest increase in chlorophyll content compared to accession3 (see Table 4.2). The interaction between the biofertilizer and the genotype had no significant effect on the chlorophyll content.

4.3.3 Stomatal Conductance (gs)

The biofertilizers' application significantly affected the stomatal conductance at 42DAE and 56DAE. An additive effect was observed on the K+R showing the highest increase in stomatal conductance (76%) at 42DAE. A synergistic effect was observed on the M+R, illustrating the highest increase in stomatal conductance (84%) at 56DAE (see Table 4.3). Both the genotype and the interaction between the biofertilizer and genotype affected the stomatal conductance at 70DAE only. The significant effect on the stomatal conductance was observed at the reproductive stage. Accession7 exhibited the highest increase in the stomatal conductance compared to accession3 (see Table 4.3). The interaction between M x accession3 indicated the greatest stomatal conductance (see Figure 4.2).

4.3.4. Normalised difference vegetation index

The biofertilizers significantly affected the NDVI at Syferkuil. The NDVI was only affected on two measurement dates. At 26DAE the sole Mycoroot and K+R displayed similar and highest increase in the NDVI (93%). At 40DAE, the sole rhizobium inoculation and the sole Mycoroot revealed similar and highest increase in the NDVI (119%) (see Table 4.3). Neither the genotype nor the interaction between biofertilizers and genotype significantly affected the NDVI both at Thohoyandou and Syferkuil.

Table 4. 1: Effects of biofertilizers and chickpea genotype on the chickpea's intercepted radiation at Thohoyandou in 2019 and 2021.

	Thohoyandou							
	2019				2021			
Biofertilizer	47DAE	61DAE	75DAE	83DAE	47DAE	61DAE	75DAE	83DAE
M+R	71.12a	65.18b	72.20a	85.86a	41.50ab	51.17a	53.33b	66.50ab
K+R	63.25a	71.40ab	85.10a	84.10a	45.33a	54.33a	68.67a	70.50a
R	62.99a	79.79ab	82.90a	76.24a	50.17a	44.67ab	54.17ab	80.67a
C	59.33a	61.47b	44.54b	64.70a	23.00b	28.83b	21.33c	29.83b
K	61.01a	86.71a	82.85a	77.67a	35.33ab	38.83ab	66.17ab	69.33a
M	63.76a	75.66ab	71.84a	83.15a	45.50a	42.17ab	53.00b	69.67a
S.E.D	12.88	6.76	8.52	11.65	6.27	6.55	4.94	12.53
Genotype								
ACC#7	65.15a	73.42a	71.45a	78.40a	41.78a	41.89a	50.00a	67.78a
ACC#3	61.10a	73.31a	74.10a	79.12a	38.50a	44.78a	55.56a	61.06a
S.E.D	7.43	3.9	4.92	6.73	3.62	3.78	2.85	7.24
P-Value								
Biofertilizer (**B**)	ns	**	***	ns	**	**	****	**
Genotype (**G**)	ns	ns	ns	ns	ns	ns	ns	ns
B*G	ns	ns	ns	ns	*	ns	ns	ns
CV (%)	35.1	15.96	20.15	25.62	27.03	26.18	16.22	33.7

Significant P≤0.05* P≤0.01** P≤0.001***, ns-not significant, Mycoroot+Rhizobium inoculum (M+R), Kelpak+Rhizobium inoculum (K+R), Rhizobium inoculum (R), Kelpak (K), Mycoroot (M), Control (C), Coefficient variation (CV), Standard error of deviation (SED)

Table 4. 2: Effects of biofertilizers and chickpea genotype on chlorophyll content at Thohoyandou and Syferkuil in 2021.

	Thohoyandou 2021 Mmol cm^{-2} s^{-1}				Syferkuil 2021 Mmol cm^{-2} s^{-1}			
Biofertilizers	32DAE	46DAE	60DAE	74DAE	26DAE	40DAE	54DAE	68DAE
M+R	1.48ab	1.49b	2.10ab	1.68b	1.86a	2.26a	2.11a	2.43a
K+R	2.13a	2.38a	2.91a	3.10a	2.17a	1.81a	2.79a	2.61a
R	1.43b	1.48b	2.32ab	1.50b	1.83a	2.11a	2.28a	2.33a
C	1.36b	1.30b	1.51b	1.38b	1.64a	1.92a	2.01a	1.83a
K	1.67ab	1.38b	1.96ab	1.82b	1.92a	2.18a	1.81a	2.52a
M	1.56ab	1.45b	2.27ab	1.48b	1.99a	1.84	1.92a	2.19a
S.E. D	0.22	0.25	0.38	0.25	0.28	0.34	0.34	0.38
Genotype								
ACC#7	1.54a	1.59a	2.11a	2.02a	1.93a	2.10a	2.12a	2.31a
ACC#3	1.65a	1.56a	2.24a	1.63b	1.88a	1.98a	2.18a	2.33a
S.E. D	0.13	0.14	0.22	0.14	0.16	0.19	0.19	0.22
P-value								
Biofertilizer (B)	*	**	*	****	ns	ns	ns	ns
Genotype (G)	ns	ns	ns	**	ns	ns	ns	ns
B*G	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	23.51	26.97	30.36	24.5	25.24	29.22	27.7	29.08

Significant P≤0.05* P≤0.01** P≤0.001***, ns-not significant, Mycoroot+Rhizobium inoculum (M+R), Kelpak+Rhizobium inoculum (K+R), Rhizobium inoculum (R), Kelpak (K), Mycoroot (M), Control (C), Coefficient variation (CV), Standard error of deviation (SED)

Table4. 1**:** Effects of biofertilizers and genotype on stomatal conductance and chickpea's normalised difference vegetative index in 2021

	Thohoyandou gs			Thohoyandou NDVI		Syferkuil NDVI		
Biofertilizer	42DAE	56DAE	70DAE	32DAE	46DAE	26DAE	40DAE	54DAE
M+R	524.67a	473.08a	432.50a	0.28a	0.42a	0.54a	0.65a	0.53a
K+R	529.25a	338.58c	363.75a	0.29a	0.48a	0.56a	0.67a	0.55a
R	323.50bc	352.00bc	387.00a	0.30a	0.48a	0.51a	0.68a	0.55a
C	299.58c	256.25c	331.17a	0.27a	0.52a	0.29b	0.31b	0.52a
M	439.92ab	357.83abc	409.17a	0.26a	0.49a	0.56a	0.68a	0.56a
K	352.25bc	462.58ab	399.75a	0.28a	0.54a	0.54a	0.64a	0.54a
SED	41.21	38.40	34.02	0.02	0.05	0.04	0.03	0.04
Genotype								
ACC#3	392.83a	368.92a	408.08a	0.29a	0.49a	0.51a	0.59a	0.55a
ACC#7	430.22a	377.86a	366.36b	0.28a	0.47a	0.49a	0.62a	0.53a
SED	23.79	22.17	19.64	0.01	0.03	0.02	0.01	0.02
P-value								
Biofertilizer (B)	****	****	ns	ns	ns	****	****	ns
Genotype (G)	ns	ns	*	ns	ns	ns	ns	ns
B*G	ns	ns	**	ns	ns	ns	ns	ns
CV (%)	17.34	17.81	15.22	16.93	17.01	14.4	9.66	11.99

Significant P≤0.05* P≤0.01** P≤0.001***, ns-not significant, Mycoroot+Rhizobium inoculum (M+R), Kelpak+Rhizobium inoculum(K+R), Rhizobium inoculum(R), Kelpak(K), Mycoroot(M), Control(C), Coefficient variation (CV), Standard error of deviation (SED)

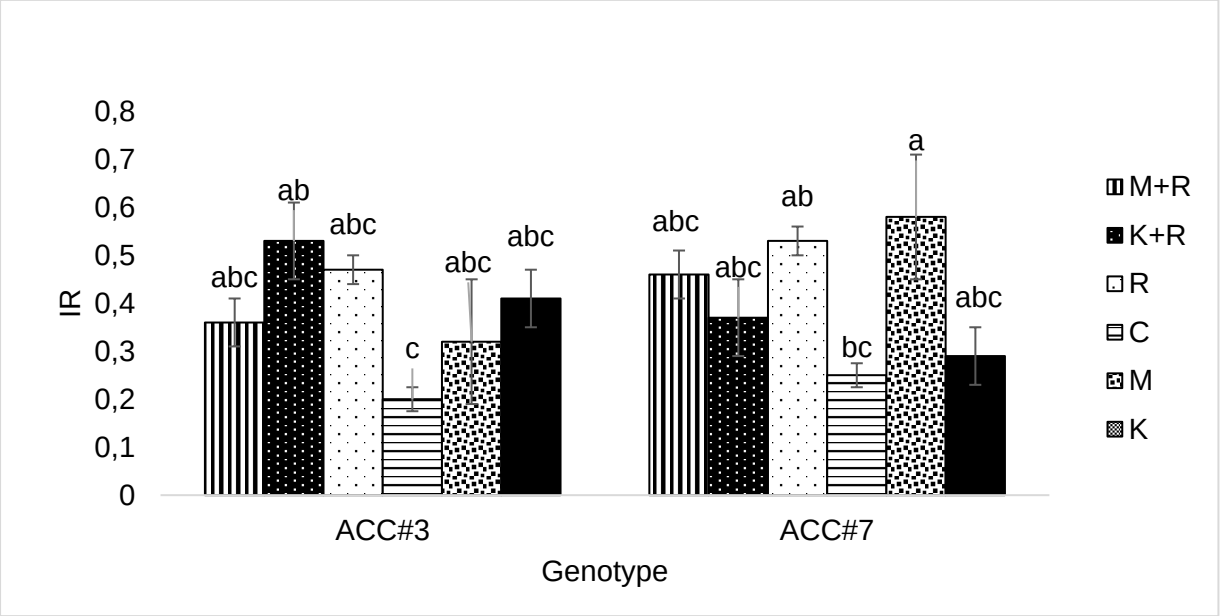

Figure 4.1: Interactive effects of the biofertilizer and genotype on the intercepted radiation' proportion at DAE47. Mean value; n=12. The different letters indicate significant differences between genotypes and biofertilizers (Tukey's honest significant difference, P≤0.000).

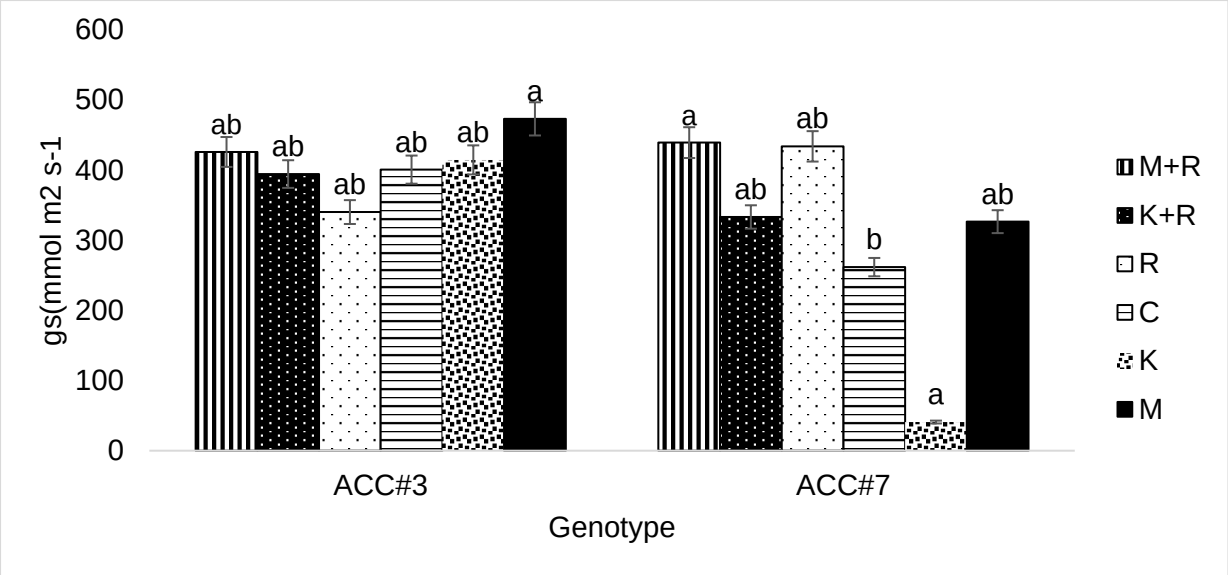

Figure 4.2: Interactive effects of biofertilizer and genotype on the chickpea's stomatal conductance (gs). Mean value; n=12. Different letters illustrate the significant differences between the genotypes and biofertilizers (Tukey's honest significant difference, P≤0.000).

4.4 DISCUSSION

4.4.1 The proportion of intercepted radiation

The crops' intercepted radiation plays an important role in determining the crop biomass accumulation and growth. The biofertilizer application significantly increased the proportion of the intercepted radiation during the reproductive stage in 2019 and, in both the vegetative and reproductive stages in 2021. The intercepted radiation's proportion was significantly greater in plots treated with the Kelpak, rhizobium inoculation, and K+R. The results suggest that the biofertilizers were more active and beneficial at the reproductive stage. In addition, more benefits were derived when the Kelpak and rhizobium inoculation were applied individually or combined. The biofertilizers' effects could be attributed to the phytohormones, macro, and micronutrients' presence, including the rhizobacteria (Szczepanek, *et al*. 2018). The phytohormones (that is, auxins, cytokinin, and gibberellins) promote cell division and elongation, resulting in enlarged leaf area, and hence the increased intercepted radiation. Several studies reveal the cytokinins' stimulating effect on plants (Taiz *et al*., 2015, Khan *et al*., 2020). The increased intercepted radiation due to the rhizobium inoculation is associated with the increased mineral nitrogen's availability (Solaiman *et al*., 2010). Mineral nitrogen increases the shoot biomass, resulting in the crops' increased intercepted radiation (Lemus, *et al*. 2008). Studies show that the rhizobium inoculation is an excellent source of nitrogen and helps increase the number of leaves and canopy size, thereby increasing the intercepted radiation (Yousefi *et al*., 2021). Macil *et al*. (2020) found that the rhizobium inoculation significantly increases the intercepted radiation and that it prevents the chickpea's early senescence by improving soil nitrogen. The increased leaf area index and leaf area duration were shown to significantly increase the intercepted radiation's proportion (Aram *et al*., 2018; Yousefi *et al*., 2021). The increased intercepted radiation due to the biofertilizer application was discovered on the chickpea, mung bean, and sesame, among other crops (Ogola *et al*., 2021; Yousefi *et al*., 2021; Jahan *et al*., 2013). This study's results show that radiation capture could be improved by combining Kelpak and rhizobium inoculation.

The intercepted radiation at Thohoyandou was not significantly affected by the genotype in 2019 and 2021 cropping seasons. The non-significant results might be explained by the two genotypes' similar canopy structure and growth patterns. Despite the non-significant results, the generally intercepted radiation in genotype increased with growth stages. Ogola *et al*. (2021) observed an increase in the proportion of the intercepted radiation with the growth stage's increase. Honnaiah *et al*. (2021) reported a significant increase in the intercepted

radiation in genotype due to the increased leaf area index resulting from the guar's increased number of leaves and branches. A genotype with greater lateral growth intercepts more radiation than a genotype with a smaller canopy range.

The interaction between the biofertilizers and genotype was significant on 47DAE in 2021. It was greater at M x accession3. An increased IR was observed at the vegetative growth stage. This might be linked to its growth rate. Plant growth is improved by the biofertilizers' application. This is accomplished by increasing the availability of nutrients for the plants' uptake. The Mycoroot improves root development, nutrient uptake, and water absorption. Depending on the genotype, the strain compatibility could significantly vary. The Mycoroot strains' selection might improve the crop's growth and yields.

4.5. CHLOROPHYLL CONTENT

The biofertilizers' application significantly affected the chlorophyll content at Thohoyandou only. The Kelpak combined with rhizobium inoculation had the greatest chlorophyll content at the vegetative and reproductive stages at Thohoyandou in 2021. The biofertilizers' effects on the chlorophyll content might be attributed to the increased nodulation (Appendix 5b). For instance, K+R had the greatest nodulation at the reproductive stage. This indicates the mineral nitrogen's availability due to biological nitrogen fixation. The results suggest that the K+R demonstrated the highest availability of nutrients for the chlorophyll's biosynthesis in leaves. In the same location, Ogola *et al*. (2021) found that the rhizobium inoculation significantly increased the chlorophyll content. This might be attributed to the mineral nitrogen, which improves nodulation in legumes, thus improving the crop's chlorophyll content. Aremu *et al*. (2022) discovered a significant increase in the *Raphanus sativus'* chlorophyll content when treated with Kelpak. Kelpak is a brown seaweed extract rich in growth hormones that influences the crop nutrient translocation, absorption, and retention (Kocira *et al*., 2020; Battacharyya *et al*., 2015). In addition, the *Ecklonia maxima* compounds increase malondialdehyde (MDH) activity and secondary metabolite content to enhance the plants' abiotic stress tolerance (Rengasamy *et al*., 2015). The metabolites' presence enhances the enzymes' production and activities that are involved in biological processes like nitrogen assimilation (Mahmoud, *et al*. 2019). The biofertilizers' application increases the chickpea's chlorophyll content, including that of the mung bean crop (Rashidi, *et al*. 2018). The increased chlorophyll content in this study could be associated with the increased nitrogen in leaves, even though we did not measure the leaves' nitrogen content.

At Thohoyandou, accession7 showed a greater chlorophyll content when compared with accession3. The increased chlorophyll content and the accession7's adaptability is associated, owing to the chickpea's heat tolerant trait (Makonya *et al*., 2019). Karimpour (2019) discovered that the heat resistant wheat genotypes presented the highest chlorophyll content. The increase in chlorophyll content was also observed in crops such as cowpea, common bean, and mung bean (Jha and Trivedi 2021, Rafat and Mostafa 2022, Khangarot, *et al*. 2022). The increased chlorophyll content in leaves was due to the rapid plant growth and the new leaves' development, as they were photosynthetically more active (Mi, *et al*. 2020). In addition, it is likely that the mineral nitrogen was not limited at reproductive stage. Golkar *et al*. (2009) illustrated a significant increase in the safflower genotype chlorophyll content due to the increased nitrogen content in the plant leaves.

The interaction between biofertilizers and genotypes had no significant effect in all measurement dates at Thohoyandou and Syferkuil in 2021. It is not clear why there was no significant variation as the possible cases were not measured. These results suggest that the genotype's response to biofertilizers did not vary despite the different levels and sites. Furthermore, the non-significant response could be attributed to the synchronized or similarities in growth patterns. Moreover, the genotype did not affect any of the growth parameters across the study, which might have influenced the non-significant results.

4.6 STOMATAL CONDUCTANCE

The stomatal conductance (gs) was significantly affected by the biofertilizer application at vegetative and reproductive stages. The individual Kelpak and M+R's applications significantly increased the stomatal conductance at 42DAE and 56DAE respectively (see Table 4.3). This study's findings suggest that the co-application of Mycoroot and rhizobium inoculation might have effectively improved the crop's nutrient and water uptake. The beneficial microorganisms found in the biofertilizers might have increased the production of cytokinins and enhanced the stomatal opening. The cytokinins hormones are the mycorrhizal fungi and the PGPR's common trait as they ameliorate stress (Dodd, *et al*., 2010). The biofertilizers significantly increase the plant nutrient status, nitrogen fixation, and phytohormones that play a vital role in plant growth (Bhardwaj, *et al.,* 2014). Boutasknit *et al*. (2021) indicated that the arbuscular mycorrhizal fungi combined with compost increased the soil moisture while the arbuscular mycorrhizal fungi improved the plant root performance by increasing the hydraulic conductivity. The inoculation with AMF significantly extends the roots' network, which helps plants to capture phosphorus and water from a wide range (Mehrvarz, *et al*., 2008).

Sangakkara *et al*. (2000) observed that the moringa leaf extract's foliar application increased the stomatal conductance due to the nutrient availability, growth hormones (increase enzymatic activity), photosynthesis, and various biochemical activities.

The stomatal conductance was greater in accession3 than in accession7 at 70DAE at Thohoyandou in 2021. The genotypic differences were observed during the reproductive stage. It is during this stage that the BNF reaches its peak level. This study's finding suggests that the accession3's root architecture was better enhanced than that of accession7, which might have improved the access to moisture and nutrients. The stomata are influenced by the root signal rather than the leaf water (Tardieu, *et al*., 1991). It is, therefore, likely that accession3's root physiological characteristics were more pronounced than that of accession7. However, Makonya *et al*. (2019) noted that accession3 at Thohoyandou had a low stomatal conductance in contrast to accession7. This might have been caused by a double stress effect at the time of flowering. That is, accession7 was more heat tolerant compared to accession3 (Makonya *et al*., 2019). The likelihood is that accession3 had better water and nutrients partitioning that allowed it to transpire better than accession7.

The biofertilizers and genotype's interactive effects on the stomatal conductance were significant at 70DAE. The Mycoroot x accession3 recorded the highest stomatal conductance at the reproductive stage. The results suggest that accession3's response to Mycoroot was possibly due to the improved soil aggregation and aeration to enhance the root development. The increase in stomatal conductance might be associated with nutrient availability and uptake (Al-Amri 2021).

4.7 NORMALISED DIFFERENCE VEGETATION INDEX (NDVI)

This study's results show the biofertilizers' positive effects on NDVI at Syferkuil as opposed to Thohoyandou. The K+R, Mycoroot, and rhizobium inoculation's applications provided the highest NDVI. The Kelpak + rhizobium inoculation's positive effects on NDVI could be associated with the phytohormones' presence, especially the cytokinins. The cytokinins and auxins regulate many physiological processes, including those that are responsible for plant growth and development (Rivas, *et al*., 2022). The cytokinin also plays a vital role in the plant's resistance to stress (Liu, *et al*., 2020). The Kelpak improves the nutrients and water's uptake, stimulates plant growth and development, and hence contributes to increased yields. This is similar to the application of Mycoroot on NDVI. The NDVI's increase due to Mycoroot could be attributed to an increase in particular of P given that Mycoroot contains *Rhizophagus clarus*,

which enhances the uptake of phosphorus (Ferreira *et al*., 2018). Also, there could have been an improvement in the uptake of nitrogen acquired through the symbionts association the AMF has with plants. When host plants are exposed to the AMF, a symbiotic mutualism prevails (Kaur, *et al*. 2022). Arbuscular mycorrhizal fungi grow in association with the plant through hyphae, enhancing nutrient acquisition and water uptake, thus improve plant growth (Bowles, *et al*., 2016). The biofertilizers' application increased the NDVI (Elgaml, *et al*., 2022). Linear regressions were found to fit the association between the NDVI and shoot biomass. A substantial correlation coefficient in the current study (r=) was found between the NDVI and shoot biomass. This suggests that the NDVI (GreenSeeker) might be the best tool to predict plant health and nitrogen content. The increased NDVI for crops such as lucerne and maize was discovered to be due to inoculation (Tang *et al*., 2022; Shirkhani & Nasrdahzadeh, 2016).

The genotype's effect on NDVI was not significant in both locations. The NDVI's non-significant response on genotype might be attributed to the growth stage and growth conditions. The NDVI depends on several factors that include colour, percent live colour, shoot density, and shoot injury (Bell *et al*., 2002). Generally, poor growth and development due to injury or low nitrogen in leaves reduces the crop NDVI and this result in poor plant vigour. Similarly, the chlorophyll content and nodulation were not affected by the genotype. That might explain the NDVI's similarities at all growth stages. The results suggest that the canopy greenness and, accession3 and accession7's densities were likely stable across the different growth stages. The strong correlation between the NDVI and the number of nodules (r=0.40), and the nodules' fresh weight (r=0.34) were observed. The positive correlation indicates that the NDVI could be used as an indirect selection criterion to identify the physiologically superior and high yielding chickpea genotypes (Appendix 7). (Khadka, *et al*., 2021) pointed out that the NDVI might be affected by the plant's waxiness.

The biofertilizer and genotype's interactive effect had no significant effect on the NDVI. There is no explanation why there was no significance. The biofertilizers improve plant growth and nutrients. This study's results suggest that accession3 and accession7 responded similarly to the biofertilizers' effects. This could be explained by the genotype's non-significant effect on the intercepted radiation, chlorophyll content, acid, and alkaline phosphatase activity across the experiments. No interaction between the biofertilizer and genotype was observed on the chlorophyll content.

4.8 CONCLUSION

The biofertilizers increased the chickpea's physiological traits with the rhizobium inoculation, K+R, and the Mycoroot, exhibiting the highest proportion of the intercepted radiation, stomatal conductance, and NDVI. The biofertilizers' effects on the chlorophyll content varied with locations, while it varied with seasons on the intercepted radiation. The intercepted radiation was higher in the second season. For example, at Thohoyandou the biofertilizer application increased the stomatal conductance, intercepted radiation, and chlorophyll content, while at Syferkuil only the NDVI increased due to the biofertilizer's application. The results suggest that the biofertilizers' efficacy was influenced by the soil type. The physiological parameters were greater at Thohoyandou's clay soil than at Syferkuil's sandy-loam soil. However, further research is required to better understand the biofertilizers effects on various chickpea genotypes' physiological parameters under various environmental conditions.

CHAPTER 5: EFFECTS OF BIOFERTILIZERS AND GENOTYPE ON PHOSPHORUS CONCENTRATION AND P-ENZYMES ACTIVITY IN THE RHIZOSPHERE SOIL

ABSTRACT

The phosphatases are widely recognised for their potential to improve availability of organic and inorganic P that is sorbed onto soil particles for improved uptake by roots especially soil deficient soils. Although the grain legumes grown in nutrient impoverished soils adopt various mechanisms to solubilize unavailable nutrients for uptake, the nutrients might not be sufficient to meet the crop's requirements. This study assessed the effect of adding biofertilizers and genotypes on the acid and alkaline phosphatase activity and phosphorus accumulation in the rhizosphere of chickpea. The field experiments were carried out in three locations namely, Syferkuil, Thohoyandou, and Sikhwahlane in 2019, and two locations vis: Syferkuil and Thohoyandou in 2021. The experiments consisted of a factorial combination of six biofertilizer levels (Kelpak [K], Mycoroot [M], rhizobium inoculation [R], K+R, M+R) and two chickpea cultivars (accession3 and accession7) arranged in a randomized block design replicated three times. The activity of acid (APase) and alkaline (AlkPase) phosphatase as well as concentration of inorganic phosphorus were determined in the rhizospheric soil during the flowering stage. The biofertilizers affected the rhizosphere phosphatase activity in all sites and seasons. In the first season, the rhizobium inoculation gave the highest AlkPase activity at Syferkuil and Thohoyandou, while the M+R revealed the greatest AlkPase activity in Sikhwahlane. In contrast, the APase activity was higher with the application of K+R at Syferkuil and Sikhwahlane, while the M+R gave the highest APase activity at Thohoyandou. In the second season, the K+R showed greater APase activity at Syferkuil and Thohoyandou. The control gave the highest AlkPase activity at Syferkuil and the Kelpak displayed the greatest AlkPase activity at Thohoyandou. The combination of K+R gave the highest inorganic phosphorus (Pi) at Syferkuil, and the Kelpak provided the highest Pi at Thohoyandou. Accession3 exhibited the greater Pi compared to accession7 at Syferkuil. The interaction between the M+R x accession7 revealed the highest APase activity at Thohoyandou in 2021. The interaction between R x accession3 showed the highest Pi at Thohoyandou in 2021. The K+R x accession3 and M x accession3 displayed the highest inorganic P at Syferkuil in 2021. The preliminary results suggested that the acid and alkaline phosphatase activity including the phosphorus accumulation might be enhanced by the biofertilizers irrespective of the soil type and environment.

Keywords: Phosphatase enzyme, chickpea, inorganic phosphorus, biofertilizer, rhizosphere.

5.1 INTRODUCTION

The chickpea is a nitrogen-fixing food crop. It is currently the second most widely grown crop in the world after common beans and is a good source of carbohydrates and proteins (Yousef, *et al*. 2020). Previous studies show that the chickpea could solubilize especially organic P into inorganic P as well as catalyse the solubility of the latter in the soil through morphological changes in the roots such as increasing root branching, length, and surface area (Pang, Bansal, et al., The carboxylate-releasing phosphorus mobilizing strategy can be proxied by foliar manganese concetration in a large set of chickpea germplasm under low phosphorus supply 2019, Sun , Gao and Lynch 2018). The chickpea can also secrete acid phosphatases that hydrolyse organic P in P deficient soils (Kaur, *et al*. 2022). The acid and alkaline phosphatase's secretion appears to be affected by cultural practices such as planting density and genotype (Makoi, *et al*. 2010). The activity of alkaline phosphatase (AlkPase) in the rhizosphere is reportedly greater in soil with alkaline pH and is linked to microbial activity and population and is decreased by high carbon concentrations rather than phosphorus concentrations (Spohn, *et al*. 2015). The alkaline phosphatase activity's role in the rhizosphere is partly to mineralise the fixed or unavailable phosphorus into a usable form such as the orthophosphate (Singh and Satyanarayana 2011).

The acid phosphatases and alkaline phosphatases are convoluted in the following rhizosphere constituents, soil, roots, and microorganisms. The chickpea's phosphatase production is yet to be highly studied in South Africa (Moloto, *et al*. 2021, Mogale, Lengwati, *et al*. 2018). For example, Mogale *et al*. (2018) focused on the acid phosphatase produced by the Kabuli and desi-type chickpea root nodules and leaves. On the other hand, Moloto *et al*. (2021) showed that the application of Kelpak increased the tissue and rhizosphere APase activity in the desi-type chickpea when grown in soils with different textures. However, none of these studies investigated the relationship between the APase and AlkPase activities and the accumulation of P-concentration in the rhizosphere soil. Furthermore, the extracellular phosphatases occur in the root and suspension cell cultures, where they might be embedded in the cell wall and secreted into the rhizosphere (Gilbert, *et al*. 2002). The extracellular phosphatases' role is suspected to be the mobilisation of organic P into the bioavailable orthophosphate. It is greatly influenced by the soil physicochemical parameters such as pH, moisture content (MC), organic carbon (OC), and nitrogen (N) (Arenberg and Arai 2019, Fu, *et al*. 2020).

The phosphorus is one of the most limiting nutrients in South Africa. That is, most soils in the semi-arid and arid regions are highly acidic, resulting in the high P adsorption potential. The soil's acidity could be caused by the parent material's resistance and the prevalence of high

P-fixing cations and complexes that make the available P to be unavailable for plant use (Nziguheba 2007). The easiest way to replenish the phosphorous is to apply inorganic phosphorus instead of nitrogen, which can be obtained through biological fixation. The phosphorus is important in plants for the maintenance of root development, including the biological nitrogen fixation process in legumes, grain yields, and accelerated crop maturity (Mitran , *et al*. 2018). Although attention is given to controlling the P deficiency in soil using organic and inorganic fertilizers, most of these organic fertilizers have insufficient P (Gowami, Maurya and Dubey 2019, Nziguheba 2007), and hence cannot meet the legumes' P requirements.

Legumes with P-deficiency are deprived of other essential nutrients such as nitrogen, resulting in retarded plant growth, and hence reduced crop yields (Singh, *et al*. 2018). Plants can only take up phosphorus in an inorganic form (orthophosphate) for their physiological upkeep. It is important to improve the agricultural soils' nutrient status with fertilizers. In doing so, it is critical to use fertilizers that would equally sustain the environment while enhancing the soil's nutrients and, the crops' growth and yields. The phosphatase activity in the rhizosphere soils increased with the P-deficiency. Thus, the inorganic P's application could suppress the acid phosphatase activity while the carbon's high concentration might suppress the alkaline phosphatase activity (Spohn and Kuzyakov, 2013, Spohn, *et al*. 2015). This study evaluated the biofertilizers' effects on the chickpea's P-nutrition by measuring the rhizosphere soils' phosphatase activity and P concentration under different field conditions in South Africa's North-Eastern regions.

5.2 MATERIALS AND METHODS

5.2.1 Experimental design and management

Three field experiments were successfully conducted at Thohoyandou and Syferkuil in the Limpopo Province and in the Mpumalanga Province's Sikhwahlane. This was done in the first growing season in 2019 and in the second growing season in 2021 at Syferkuil and Thohoyandou. The experiments consisted of a factorial combination of six biofertilizer levels (Mycoroot [M], Kelpak [K], Rhizobium inoculation [R], M+R, K+R, zero control) and two chickpea cultivars (accession3 and accession7) arranged in a randomized complete block design replicated three times. The chickpea was planted manually, and the plot size was 3 m x 2 m with 12 plots per replicate. Each plot had six rows with intra and inter-row spacing of 10 cm and 30 cm.

5.2.2 Soil collection and analysis

Soil samples were collected at the flowering stage in all 36 plots as described in chapter 3. The soil samples were collected from each of the plant's rhizosphere using a hand shovel. The soil samples were placed in plastic-zip-bags and kept in the refrigerator at 4°C until they were analysed for acid and alkaline phosphatase activities.

The soil's acid and alkaline phosphatase activities were measured using Tabatabaei's (1994) method. That is, about 1 g of the soil sample was weighed in polypropylene vials, and 4 ml of buffer (pH 6.5) was added. Then, 1 ml of 0.1 M phosphatase substrate was added, and the contents thoroughly mixed using a vortex. The mixture was incubated at 37° C for 1 h, and 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH were added. The mixture was then filtered through the Whatman #2 filter paper. The absorbance of 2 ml of the resulting extractant was read using the UV-Visible spectrophotometer (JENWAY 7300, Bibby Scientific Ltd, Stone, Staffs, UK) at 420 nm. The filtrates' absorbance was compared with the *p*-nitrophenol standards. For each assay, a control was included to account for the non-enzymatic substrate hydrolysis and enzyme activity expressed as µg *p*-nitrophenol g^{-1} F wt. h^{-1}.

5.3 STATISTICAL ANALYSIS

Data were subjected to the variance (ANOVA) analysis using the STATISTIX (2017) version 10.0. The Tukey's Honestly Significant Difference (HSD) test was used to separate the means that were significantly different ($P \leq 0.05$). The Pearson correlation was used to test the linear association's strength between the parameters.

5.4 RESULTS

5.4.1 Acid phosphatase and alkaline phosphatase activities

The biofertilizers significantly increased the extracellular acid phosphatase activity in the rhizosphere soil in all the sites and in both cropping seasons except for the rhizobium inoculation at Syferkuil and the Mycoroot at Thohoyandou (see Tables 5.1 & 5.2). At Syferkuil, the K+R gave the highest increase of the APase (76.36%) but showed a significant decrease under R APase. At Thohoyandou, the M+R gave the highest increase in the APase (48.63%). In Sikhwahlane, the K+R gave the highest increase in the APase (72.87%) compared to the control. In the second cropping season in 2021, the K+R gave the highest increase in the

APase at Syferkuil (78.39%) and Thohoyandou (34.21%) compared to its controls. The genotype showed no significant effect on the APase in both locations. Only at Thohoyandou was the APase significantly affected by the interaction between the biofertilizer and the genotype (see Figure 5.2). The interaction between M+R x accession 7 significantly affected acid phosphatase activity of chickpea at flowering stage in Thohoyandou (see Figure 5.1).

The variations between the extracellular alkaline phosphatase activities at Thohoyandou, Syferkuil, and Sikhwahlane in 2019 are presented in Table 5.1. Similar trends were observed in 2021 at Thohoyandou and Syferkuil (see Table 5.2). In the 2019 cropping season, the rhizobium inoculation resulted in the highest increase in the AlkPase in the rhizosphere at Syferkuil (83.03%) and at Thohoyandou (72.64%). Meanwhile, the Mycoroot+rhizobium inoculation resulted in the highest increase in the AlkPase (60.55%) in Sikhwahlane compared to the control. In contrast, the control showed the highest AlkPase activity at Syferkuil and the Kelpak gave the highest increase in AlkPase (34.78%) at Thohoyandou in the 2021 cropping season. Neither the genotype nor the interaction between the biofertilizer and the genotype showed a significant effect on the alkaline phosphatase activity.

The biofertilizers significantly affected the inorganic phosphorus at Syferkuil and Thohoyandou in the 2021 cropping season (see Table 5.2). At Syferkuil, the K+R gave the highest increase in the inorganic P (12.60%) but the M+R showed the inorganic phosphorus' significant decrease. At Thohoyandou, the Kelpak gave the highest increase in the inorganic P (14.24%) compared to the control but there was a significant decrease under M. The genotype showed a significant variation at Syferkuil only, with accession3 exhibiting the highest increase (3%) in the inorganic P compared to the control. The interaction between the biofertilizer and the genotype had a significant effect in both locations. The K+R x accession3 and M x accession3 gave the highest inorganic P at Syferkuil (see Figure 5.3). The R x accession3 gave the highest inorganic P at Thohoyandou (see Figure 5.3).

Table 5. 1**:** Effects of biofertilizers on acid and alkaline phosphatase activity (µg p-nitrophenol. $g^{-1}Fwt.h^{-1}$) in the chickpea's rhizosphere under different agro-ecologies.

	Syferkuil		Thohoyandou		Sikhwahlane	
	µg p-nitrophenol. g-1Fwt.h-1					
Biofertilizer	APase	AlkPase	APase	AlkPase	APase	AlkPase
M+R	17.76b	13.96b	25.33a	16.91b	14.89b	13.03a
K+R	22.09a	8.20d	15.92bc	17.41b	20.90a	8.13b
R	15.50b	15.86a	20.31ab	22.15a	15.53b	5.60c
C	5.22d	2.69e	13.01c	6.06e	5.67d	5.14c
K	7.61cd	9.82cd	19.85ab	13.19c	10.56c	9.54b
M	9.60c	10.10c	18.03bc	8.56d	15.51b	9.73b
SED	2.85	1.88	5.55	2.14	1.95	2.46
Genotype						
ACC#3	13.61a	9.77a	19.19a	13.74a	14.10a	9.02a
ACC#7	12.26a	10.44a	18.20a	14.36a	13.59a	8.03a
SED	1.64	1.08	3.20	1.23	1.13	1.42
P-value						
Biofertilizer(B)	***	***	***	***	***	***
Genotype (G)	ns	ns	ns	ns	ns	ns
B*G	ns	ns	ns	ns	ns	ns
CV (%)	18.35	15.58	24.75	12.74	11.80	24.08

The means with different letters in the same column are significantly different from each other, that is, P≤0.05* P≤0.01** P≤0.001***, ns-not significant. Mycoroot+Rhizobium inoculum (M+R), Kelpak+Rhizobium inoculum (K+R), Rhizobium inoculum (R), Kelpak (K), Mycoroot (M), Control (C), Coefficient variation (CV), Standard error of deviation (SED), Acid Phosphatase Activity (APase), Alkaline Phosphatase activity (AlkPase activity).

Table 5. 2**:** The effects of biofertilizer and genotype on the activity of rhizospheric acid and alkaline phosphatase as well as inorganic phosphorus.

	Syferkuil			Thohoyandou		
	µg p-nitrophenol. g-1Fwt.h-1					
Biofertilizer	APase	AlkPase	Pi(mg/g))	APase	AlkPase	Pi(mg/g)
M+R	28.24b	53.36b	50.33c	34.01b	49.41a	11.00e
K+R	43.24a	59.40ab	59.50a	40.62a	47.61ab	13.50c
R	8.46c	63.74a	56.66b	9.90e	47.91ab	14.50b
C	9.34c	74.14a	52.00c	26.72c	37.05b	13.00d
K	13.67c	53.53b	52.50c	10.16e	56.81a	15.16a
M	25.95b	35.84c	57.66ab	22.18d	22.47c	10.83e
SED	6.32	17.05	2.64	1.84	12.26	0.44
Genotype						
ACC#3	21.06a	58.13a	55.61a	23.75a	41.43a	13.00a
ACC#7	21.90a	52.21a	53.94b	24.32a	45.65a	13.00a
SED	3.65	9.84	1.01	1.06	7.07	0.17
P-value						
Biofertilizer	***	***	***	***	***	***
Genotype	ns	ns	**	ns	ns	ns
B*G	ns	ns	***	**	ns	***
CV (%)	24.58	25.13	2.69	6.39	23.51	1.89

The means with different letters in the same column are significantly different from each other, that is, P≤0.05* P≤0.01** P≤0.001***, ns-not significant. Mycoroot+Rhizobium inoculum (M+R), Kelpak+Rhizobium inoculum (K+R), Rhizobium inoculum (R), Kelpak (K), Mycoroot (M), Control (C), Coefficient variation (CV), Standard error of deviation (SED), Acid Phosphatase Activity (APase), Alkaline Phosphatase activity (AlkPase activity), Pi (inorganic phosphorus).

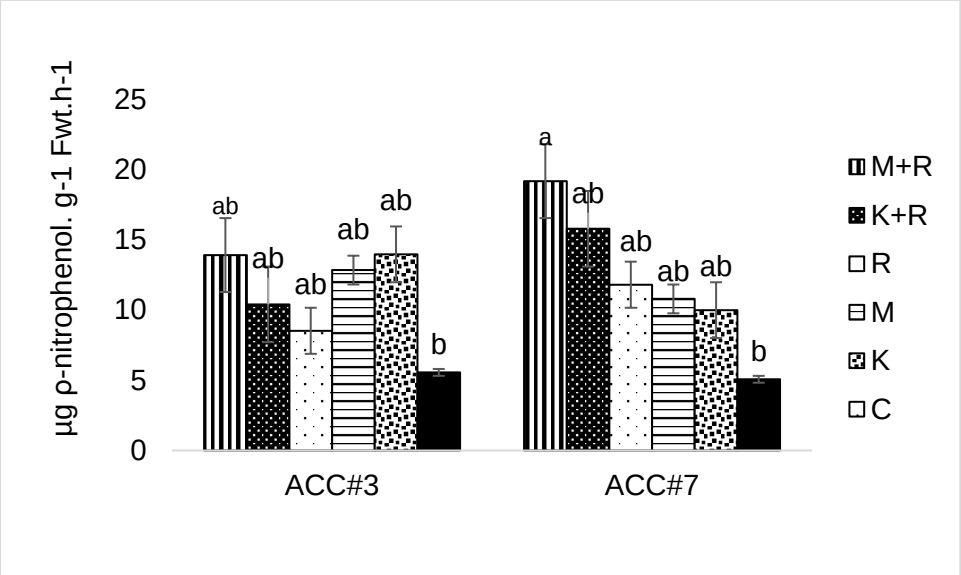

Figure 5.1: Interaction of the biofertilizer and genotype on acid phosphatase activity at the chickpea's flowering stage at Thohoyandou's 2021 cropping season. Mean value; n=12. The different letters indicate significant differences between the genotypes and the biofertilizers (Tukey's honest significant difference; P≤0.001).

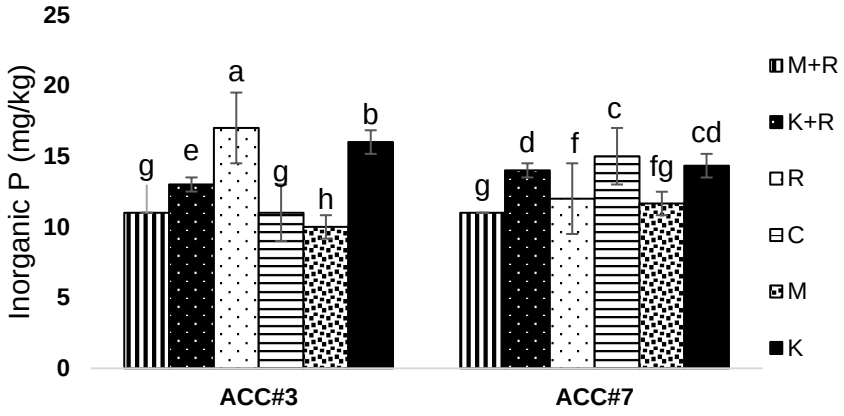

Figure 5.2: Interaction of the biofertilizer and genotype on the rhizosphere inorganic phosphorus at the chickpea's flowering stage at Thohoyandou's 2021 cropping season. Mean value; n=12. The different letters indicate the significant differences between the genotypes and the biofertilizers (Tukey's honest significant difference; P≤0.000).

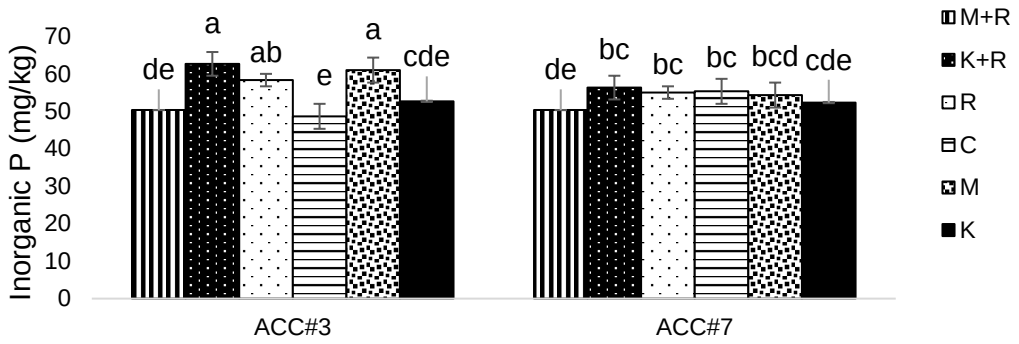

Figure 5.3: Interaction of the biofertilizer and genotype on the rhizosphere inorganic phosphorus at the chickpea's flowering stage at Syferkuil's 2021 cropping season. Mean value; n=12. The different letters indicate the significant differences between the genotypes and biofertilizers (Tukey's honest significant difference; P≤0.000).

5.5 DISCUSSION

Chickpeas produce phosphatase and it varies between the desi and kabuli type, genotypes of each type, and soil texture (Mogale, *et al*., 2018, Moloto, *et al*. 2021). A review of published reveal that in general, the efficacy of biofertilizers is affected by the texture, pH, and fertility of soils (Schutz *et al*., 2018). Biofertilizers significantly affected the activity of the extracellular acid and alkaline phosphatase in the chickpea's rhizosphere soil in all sites and both seasons. The acid phosphatase activity was significantly higher with the K+R's application at Syferkuil and Sikhwahlane, and the M+R at Thohoyandou in 2019; and with the K+R at Thohoyandou and Syferkuil in 2021. These results show that the Kelpak and rhizobium inoculation's combined application and the Mycoroot's combination with the rhizobium inoculation provide a beneficial effect on the acid phosphatase activity in the rhizosphere soil. The significant result might be attributed to the biofertilizer solubilising and mineralising the unavailable mineral nutrients in the rhizosphere, thereby releasing the organic and inorganic acids that include the phosphatase enzymes (Kalayu 2019). The increased acid phosphatase activity might coincide with the high nodule phosphorus requirement and the depletion of the vacuole inorganic phosphorus pool (Lazali & drevon, 2021). The biofertilizers used in this study are characterised as phosphorus-solubilising bacteria. This could have contributed to the increased acid phosphatase activity in the rhizosphere. A significant increase in the acid phosphatase activity in the rhizosphere soil was observed (Moloto, *et al*. 2021, Shravani, *et al*. 2019). Moloto *et al*. (2021) stated that the biofertilizer Bontera's application gave the highest acid phosphatase activity in the rhizosphere soil compared to the Kelpak. This study noted that when the Kelpak was applied alone, it performed the least when compared to the rest of the treatments.

The extracellular alkaline phosphatase activity was affected in all sites in 2019. A similar trend was observed in 2021 at Thohoyandou alone. In 2019, the rhizobium inoculation gave the highest alkaline phosphatase activity at Syferkuil and Thohoyandou. The M+R gave the highest alkaline phosphatase activity in Sikhwahlane. In 2021, the Kelpak gave the highest alkaline phosphatase activity at Thohoyandou and at Syferkuil, the alkaline phosphatase activity was the highest in the control plots. The results showed that when the rhizobium inoculation is applied alone or in combination with the Mycoroot, it was more effective and beneficial, thus giving a synergistic effect. The increased alkaline phosphatase activity in the rhizosphere is attributed to the fact that the biofertilizer application (the rhizobium and Mycoroot) influences the root colonisation and an increased microbial activity in the rhizosphere, increasing the nutrients in the process. Spohn and kuzyakov (2013) asserted that the alkaline phosphatase activity is regulated by the microbial phosphorus demand and

microbial need for carbon, unlike the acid phosphatase activity, which is influenced by plant root nodules' need for phosphorus. At Syferkuil, it was observed that the control gave the highest alkaline phosphatase activity in the rhizosphere. An explanation for this could be that the carbon might have been very low. As such, the microbial activity was also very low. Syferkuil soil is sandy and sandy soils do not hold much of the nutrients due to leaching. Such soils also have little organic carbon/matter. Lastly, most other published literature reported higher alkaline phosphatase activity in soil with neutral pH range such a that at Syferkuil. Spohn and Kuzyakov (2013) showed a significant increase in the alkaline phosphatase activity in the rhizosphere under control plots. To acquire phosphorus, the plant-microbial relationship might be beneficial and competitive (Spohn & Kuzyakov, 2013).

The Kelpak's individual application and in combination with the rhizobium inoculation provided the rhizosphere's highest inorganic phosphorus accumulation at Syferkuil and Thohoyandou. The biofertilizers' application was observed to solubilise about 76.60-88.21% of the fixed phosphorus in the soil through organic and inorganic acids (Rahman *et al.,* 2021). The increased acid and alkaline phosphatase activities in the rhizosphere significantly increased the inorganic phosphorus (Nannipieri, *et al*. 2011) (Lazali and Drevon 2021) (Spohn and Kuzyakov, 2013). The extracellular enzymes release phosphorus from the organic compounds. Iron is made more available through chelation by molecules like defexamine and siderophore (Dakora & Phillip, 2002). Sonivaldo *et al.* (2019) noted that the AMF inoculation's application in Lamiaceae increased the phosphorus accumulation in the rhizosphere soil. In this study, it was noted that the Mycoroot increased the inorganic phosphorus by 9.6% at Syferkuil in 2019. The results suggest that inoculation with Mycoroot might have colonised roots to form a symbiotic relation with the crop to solubilise the unavailable phosphorus into usable forms. The phosphorus' increase through the AMF is widely reported (Dobo 2022, Kavadia, *et al*. 2020).

The genotype had no significant effect on the acid and alkaline phosphatase activity in the rhizosphere in all sites and seasons. The non-significant results could be attributed to the phosphorus, carbon, and microbial population and activity levels (Spohn and Kuzyakov, 2013). In contrast, Moloto *et al*. (2021) related a significant increase in the acid phosphatase activity in the rhizosphere (of ICCV92944) associated with the genotype as an early maturing cultivar. Kaur *et al*. (2022) observed a higher acid phosphatase activity in tolerant cultivars compared to their control plants under the phosphorus-deficient conditions. However, a similar activity was not noted in this study. Nonetheless, the genotype affected the inorganic phosphorus in the rhizosphere soil at Syferkuil in the 2021 cropping season. Accession3 recorded the greater inorganic phosphorus compared to accession7. The increase in the inorganic phosphorus could be due to mechanisms such as the cluster roots formation,

production of organic and inorganic acids, and the mycorrhizal infection that enhanced the solubilisation of the soil's inorganic phosphorus (Gunes *et al*., 2007; Schweiger *et al.*, 2007). The results indicate that accession3 is a phosphorus efficient genotype than accession7. The inorganic phosphorus' genotypic variation was noted by Zhang *et al*. (2009). These authors observed that the genotype 102 had a higher phosphorus concentration than the genotype 105 associated with the root excreted acid anions.

The interaction between the biofertilizer and the genotype had a significant effect on the acid phosphatase activity and inorganic phosphorus at Thohoyandou and only inorganic phosphorus at Syferkuil in 2021. The interaction between the M+R x accession7 had the highest effect on the acid phosphatase activity in the rhizosphere soil. The increased interaction was due to the rhizobia and mycorrhiza's effects on nutrients. The rhizobia and mycorrhiza colonise the rhizosphere and increase the BNF through the increased nodulation and nutrient uptake. The K+R x accession3's interaction and that of the Mycoroot x accession3's was similar. They delivered the highest effect on the inorganic phosphorus at Syferkuil. R x accession3 provided the highest effect at Thohoyandou. The significant effect was due to the rhizobium inoculation solubilising the soil's phosphorus, thereby increasing the activity by colonising the host plant. The rhizosphere soil's improved phosphorus could be because of the mycorrhizal fungi symbiosis with the host plants. That is, the mycorrhizal fungi play a significant role in the phosphorus availability.

5.5 CONCLUSION

The biofertilizers significantly affected the production of extracellular phosphatase by chickpea as well as its activity. Also, the bio-inputs affected the concentration of chickpea's rhizospheric inorganic phosphorus. The rhizobium inoculation's combination with Kelpak and Mycoroot revealed a synergistic effect on the phosphatases and inorganic phosphorus. This suggests that the rhizobium inoculation increases the Kelpak and Mycoroot's performances. The acid phosphatase activity was the highest in the Syferkuil's sandy loam soils than at Thohoyandou's clay soils. However, at Syferkuil's 2021 cropping season, the biofertilizers did not affect the alkaline phosphatase activity in the rhizosphere soil but did so at Thohoyandou's clay soils. The effects were greater in the second season than in the first one. Although the genotype did not affect the extracellular phosphatase activity in the rhizosphere, a genetic variation was observed for the inorganic phosphorus at Syferkuil. Accession3 out-performed accession7. The study results show that the biofertilizers were more pronounced in the sandy loam soils than in the clay soils. However, more research is needed to provide concrete agronomic recommendations.

CHAPTER 6: EFFECTS OF BIOFERTILIZERS AND CHICKPEA GENOTYPE ON QUANTUM YIELD AND CHLOROPHYLL FLUORESCENCE

ABSTRACT

The chlorophyll fluorescence's method is globally used to detect the plants' physiological status under different abiotic stresses. The chlorophyll fluorescence parameter (Fv/Fm) is the quantitative measure of the photosystem II's photochemical efficiency. The Fv/Fm's reduced values indicate a damaged proportion of the PSII reaction centre. The increased plant stress tolerance with the biofertilizers is well documented. Nevertheless, there is a dearth of information on the biofertilizer's effect on the chlorophyll fluorescence despite the abundance of information about the former's uses. This study evaluated the biofertilizers' effects on the chlorophyll fluorescence and the two chickpea cultivars' quantum yields. A field experiment was conducted at Thohoyandou, Syferkuil, and Sikhwahlane in 2019, and at Thohoyandou and Syferkuil in 2021. The experiment was a factorial combination of six biofertilizer levels (Mycoroot [M], Kelpak [K], rhizobium inoculation [R], M+R, K+R, & zero control) and two chickpea cultivars (accession3 and accession7) arranged in a randomised complete block design replicated three times. Accession3 and accession7 are heat tolerant cultivars. The leaf chlorophyll fluorescence was measured at the flowering stage using a portable chlorophyll fluorometer (PAM-2100). The biofertilizers significantly affected the ΦPSII at Thohoyandou in 2019, and the Fv/Fm, Fv/Fo, and ΦPSII at Syferkuil and Thohoyandou in 2021. The rhizobium inoculation showed the highest ΦPSII (0.33). The genotype affected accession7's minimal fluorescence (Fo), recording the highest Fo in the process. The interaction between R x accession3 provided the highest ΦPSII at Thohoyandou, while R x accession7 led to the highest Fv/Fo at Syferkuil in 2019. The individual Kelpak and in combination with the rhizobium inoculation recorded the highest effects on the Fv/Fm (0.72-0.77), ΦPSII (0.25-0.33), and Fv/Fo (2.43-3.66) at Syferkuil and Thohoyandou. However, at Thohoyandou, the Fv/Fm was not significantly affected. Only at Syferkuil did accession7 record the highest effect on the Fv/Fm compared to accession3. The interactive effect of the R x accession3 displayed the highest ΦPSII at Thohoyandou. Thus, the Fv/Fo and Fv/Fm were consistently higher at Thohoyandou than at Syferkuil. The biofertilizers' positive effects on the chlorophyll fluorescence parameters' functional activity suggest that the biofertilizers' individual application or in combination had a profound influence.

Keywords: Chlorophyll fluorescence, biofertilizers, chickpea, genotype, effect.

6.1 INTRODUCTION

The chickpea's production is threatened by the climate change that include heat stress, a major threat to the current chickpea production (Batra, *et al*. 2020). From 2020 to 2080, the global air temperature is expected to rise by 6.8° C (Jiang *et al*., 2017). This might result in crop damages. That is, the crops' thylakoid membrane is easily damaged by the thermal stress, resulting in reduced photophosphorylation (Nishimura, *et al*. 2021). This affects the PSII reaction's activation. Worse more, the chlorophyll biosynthesis is restricted as the enzyme's activities stop due to the thylakoid's heat stress damage (Hu *et al*., 2020). The photosystem II (PSII) is a membrane protein complex that catalyses the light-induced water oxidation in oxygenic photosynthesis. It exists as dimers in the grana stacks' thylakoid membranes (Kawakami & Shen, 2018). The heat stress at the legumes' terminal stage is one of the major factors that cause drastic yield loss.

The heat stress affects the chickpeas and other leguminous crops' sexual development. With higher temperatures, the chickpea and lentil reduce podding and biomass (Bhandari, *et al*. 2020). The chlorophyll fluorescence's measurement shows the crops' damage under challenging environmental conditions before symptoms appear. The chlorophyll fluorescence is a measure for the PSII's full quantum efficiency in chickpeas, warm, and cold conditions. There is evidence to the effect that cold temperatures significantly reduce the photosystem (PSII). This might be related to the damage to the photoreaction complexes due to cold temperatures (Baker and Rosenqvist 2004). In plants, the photo-inhibition damages the chlorophyll's molecules and deactivates the PSII enzymes, thereby decreasing the plant's carbon dioxide assimilation (Auge 2000).

Good management practices might help the crops to cope with stressful environments. The biofertilizers significantly improved the chickpea plants' stomatal conductance and their chlorophyll content by promoting their transpiration activity**.** The stomatal conductance's improvement significantly impacts the photosynthetic efficiency and phosphorus use and, consequently, the biomass accumulation and nutrient uptake (Ben-Laouane, *et al*. 2021). Most agricultural lands are situated in the semi-arid and arid regions, which calls for the suitable strategies to improve the crops' resistance to stressful conditions. Studies about the chickpea's chlorophyll fluorescence abound but not much has been studied about the biofertilizers' effects. A recent study about the chlorophyll fluorescence conducted in a similar location assessed the desi type genotypes' performance or stress tolerance. The study evaluated the biofertilizers' effects on the chickpea's chlorophyll fluorescence under contrasting soil types and temperature regimes.

6.2 MATERIALS AND METHODS

6.2.1 Experimental design

The experimental design's detailed description was done in chapter 3, but its summary follows. The experiments were done at Thohoyandou, Syferkuil, and Sikhwahlane in 2019, and at Thohoyandou and Syferkuil in 2021. The treatment consisted of a factorial combination of six biofertilizer levels (Mycoroot [M], Kelpak [K], rhizobium inoculation [R], M+R, K+R & zero control) and two chickpea cultivars (accession3 & accession7) arranged in a randomised complete block design replicated three times.

6.2.2 Data collection

Using the PAM-2100 portable chlorophyll fluorometer (Walz, Eiffeltrich, Germany) and Baker and Rosenqvist's (2004) fluorescence nomenclature, the leaf chlorophyll fluorescence values, including the minimal fluorescence (Fo), the maximum fluorescence (Fm), the variable fluorescence (Fv), and the photosystem II's (Fv/Fm) maximum photochemical efficiency, were measured during the flowering stage. Three plants from each of the 36 plots were randomly chosen from the four innermost rows and dark-adapted for 30 minutes using the light exclusion clips (Walz, Eiffeltrich, Germany). The measurements were taken in the morning of a clear sunny from 8:00 am to 12:00 noon.

6.2.3 Statistical

Data were subjected to the variance (ANOVA) analysis using the STATISTIX (2017) version 10.0. The Tukey's Honestly Significant Difference (HSD) test was used to separate the means that were significantly different ($P \leq 0.05$)

6.3 RESULTS

The biofertilizers significantly affected the photochemistry II's (PSII) effective quantum yield (about 12%) at Thohoyandou in 2019 but did not have a significant effect on the chlorophyll fluorescence parameters at Syferkuil and Sikhwahlane (see Table 6.1). Only the rhizobium inoculation increased the effective quantum yield (13.79%) compared to the rest of the treatments (see Table 6.1). It was only at Sikhwahlane that the genotype affected the minimal fluorescence (Fo), with accession7 recording the higher Fo (13.33%) compared to accession3. The interaction between the biofertilizer and the genotype was only significant at Thohoyandou and Syferkuil for the photochemistry II and maximum primary yield of photochemistry I's (Fv/Fo) effective quantum yield respectively. The R x accession3 gave the highest ΦPSII at Thohoyandou (see Figure 6.1) and the R x accession7 provided the highest Fv/Fo at Syferkuil (see Figure 6.2).

In 2021, the biofertilizers significantly affected the ΦPSII and Fv/Fo at Thohoyandou and the Fo, ΦPSII, and Fv/Fm at Syferkuil (see Table 6.2). At Thohoyandou, the K gave the highest increase of the Fv/Fo (0.50%). The genotype did not affect any of the chlorophyll fluorescence parameters. The interaction between the biofertilizer and the genotype affected the ΦPSII, with M+R x accession7 recording the highest ΦPSII (see Figure 6.2). At Syferkuil, the biofertilizers did not affect the Fo. The highest Fo was observed in control plots (see Table 6.2). The rhizobium inoculation and the Kelpak recorded the highest ΦPSII of 22.22%. The Kelpak gave the highest Fv/Fo increase, and the Mycoroot and M+R led to the highest Fv/Fm (see Table 6.2). The genotype only affected the Fv/Fm and, accession7 recorded the highest Fv/Fm increase (see Table 6.2). The interaction between the biofertilizer and the genotype had significant effect only on effective quantum yield of the chlorophyll fluorescence parameters. Only at Syferkuil the interaction between rhizobium inoculum x accession 7 showed greater effect on the photochemistry I (Fv/Fo) (see Figure 6.3).

In Sikhwahlane, there was no significant effect observed on the chlorophyll fluorescence. The minimal fluorescence was significantly affected by the genotype, with accession7 exhibiting the highest Fo (13.33%). The interaction between the biofertilizer and the genotype showed no significant effect on the chlorophyll fluorescence.

Table 6. 1**:** Effects of biofertilizers and genotype on chickpea leaves' chlorophyll fluorescence parameters (light and dark-adapted) in 2019.

	Thohoyandou				Syferkuil				Sikhwahlane			
Biofertilizer	Minimal Fluorescence (Fo)	Maximum primary yield of PSI (Fv/Fo)	Maximal quantum yield of PSII (Fv/Fm)	Effective quantum yield of PSII (ΦPSII)	Minimal Fluorescence (Fo)	Maximum primary yield of PSI (Fv/Fo)	Maximal quantum yield of PSII (Fv/Fm)	Effective quantum yield of PSII (ΦPSII)	Minimal Fluorescence (Fo)	Maximum primary yield of PSI (Fv/Fo)	Maximal quantum yield of PSII (Fv/Fm)	Effective quantum yield of PSII (ΦPSII)
M+R	457.95a	3.22a	0.76a	0.27ab	683.87a	2.35a	0.71a	0.29a	552.90a	2.83a	0.75a	0.38a
K+R	354.78a	3.32a	0.77a	0.28ab	575.55a	2.38a	0.74a	0.30a	526.63a	2.72a	0.74a	0.39a
R	447.03a	3.33a	0.77a	0.33a	591.18a	2.61a	0.73a	0.27a	617.13a	2.64a	0.74a	0.33a
C	416.38a	3.44a	0.79a	0.29ab	649.25a	2.43a	0.71a	0.25a	598.75a	2.74a	0.74a	0.38a
M	395.62a	3.44a	0.77a	0.24b	654.07a	2.46a	070a	0.28a	547.05a	3.07a	0.75a	0.35a
K	420.48a	3.34a	0.77a	0.28ab	618.17a	2.62a	0.72a	0.30a	586.40a	2.66a	0.74a	0.34a
SED	70.76	0.79	0.06	0.02	54.10	0.14	0..04	0.03	52.76	0.88	0.05	0.04
Genotype												
ACC#3	403.49a	3.38a	0.76a	0.28ab	626.97a	2.48a	0.71a	0.28a	535.76b	2.80a	0.75a	0.37a
ACC#7	427.16a	3.31a	0.77a	0.29ab	630.39a	2.47a	0.72a	0.28a	607.19a	2.75a	0.74a	0.34a
SED	40.85	0.30	0.02	0.02	31.23	0.07	0.02	0.02	30.46	0.33	0.02	0.03
P-value												
Biofertilizer(B)	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns
Genotype (G)	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns
B*G	ns	ns	ns	**	ns	**	ns	ns	ns	ns	ns	ns
CV (%)	13.06	13.25	4.03	18.32	14.90	9.21	3.49	18.51	13.06	13.11	4.10	22.52

Mean values with different letters show a significant difference as follows; P≤0.05* P≤0.01** P≤0.001***, ns-not significant, Mycoroot+Rhizobium inoculum (M+R), Kelpak+Rhizobium inoculum (K+R), Rhizobium inoculum (R), Kelpak (K), Mycoroot (M), Control (C), Coefficient variation (CV)

Table 6. 2**:** Effect of biofertilizers and genotype on the chickpea leaves' chlorophyll fluorescence parameters (light and dark-adapted) in 2021

	Thohoyandou				Syferkuil			
Biofertilizer	Minimal Fluorescence (Fo)	Effective quantum yield of PSII (ΦPSII)	Maximum primary yield of photochemistry I (PSI) (Fv/Fo)	Maximal quantum yield of PSII (Fv/Fm)	Minimal Fluorescence (Fo)	Effective quantum yield of PSII (ΦPSII)	Maximum primary yield of PSI (Fv/Fo)	Maximal quantum yield of PSII (Fv/Fm)
M+R	452.95a	0.32a	3.22b	0.79a	533.9b	0.32a	2.82ab	0.77a
K+R	354.78a	0.33a	3.32ab	0.79a	522.2b	0.32a	2.67ab	0.76ab
R	430.37a	0.31a	3.50ab	0.78a	536.2b	0.33a	3.06a	0.75ab
C	449.72a	0.25b	2.43c	0.78a	645.9a	0.27b	2.37b	0.72b
M	395.62a	0.30a	3.61ab	0.77a	517.4b	0.32a	2.63ab	0.77a
K	387.15a	0.31a	3.66a	0.79a	534.8b	0.33a	3.12a	0.76ab
SED	230.04	0.05	0.44	0.06	87.34	0.04	0.50	0.04
Genotype								
ACC#3	392.48a	0.29a	3.23a	0.78a	568.10a	0.31a	2.81a	0.74b
ACC#7	431.05a	0.31a	3.38a	0.79a	528.70a	0.31a	2.47a	0.77a
SED	88.47	0.03	0.26	0.02	50.42	0.02	0.29	0.01
P-value								
Biofertilizer (B)	ns	*	****	ns	*	*	*	*
Genotype (G)	ns	ns	ns	ns	ns	ns	ns	***
B*G	ns	*	ns	ns	ns	ns	ns	ns
CV (%)	31.06	12.43	11.24	4.09	13.30	11.03	15.16	3.37

Mean values with different letters show a significant difference. That is, *P≤0.05, **P≤0.01, ***P≤0.001, ns-not significant. M+R= Mycoroot +Rhizobium inoculum, K+R= Kelpak+Rhizobium inoculum, R= Rhizobium inoculum, C= control, K= Kelpak, M=Mycoroot. SED= standard error of deviation, CV= coefficient variance.

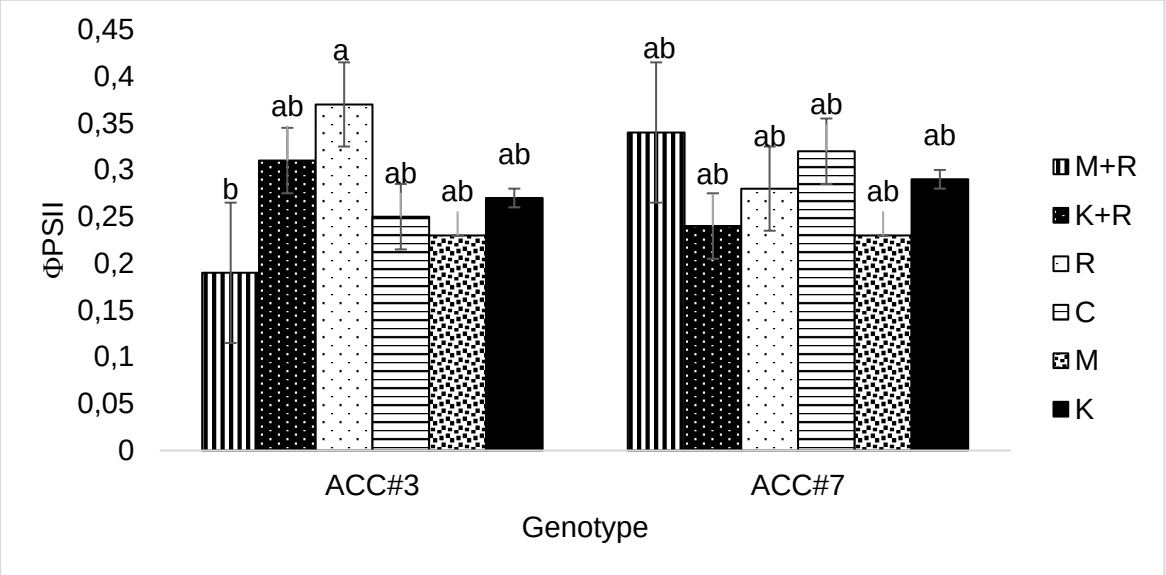

Figure 6.1: Interactive effect of biofertilizer and genotype on the chickpea's effective quantum yield of photochemistry II (ΦPSII) at flowering stage at Thohoyandou in 2019. Mean value; n=12. Different letters indicate significant differences between the biofertilizers and genotypes (Tukey's honest significant differences, P≤0.01).

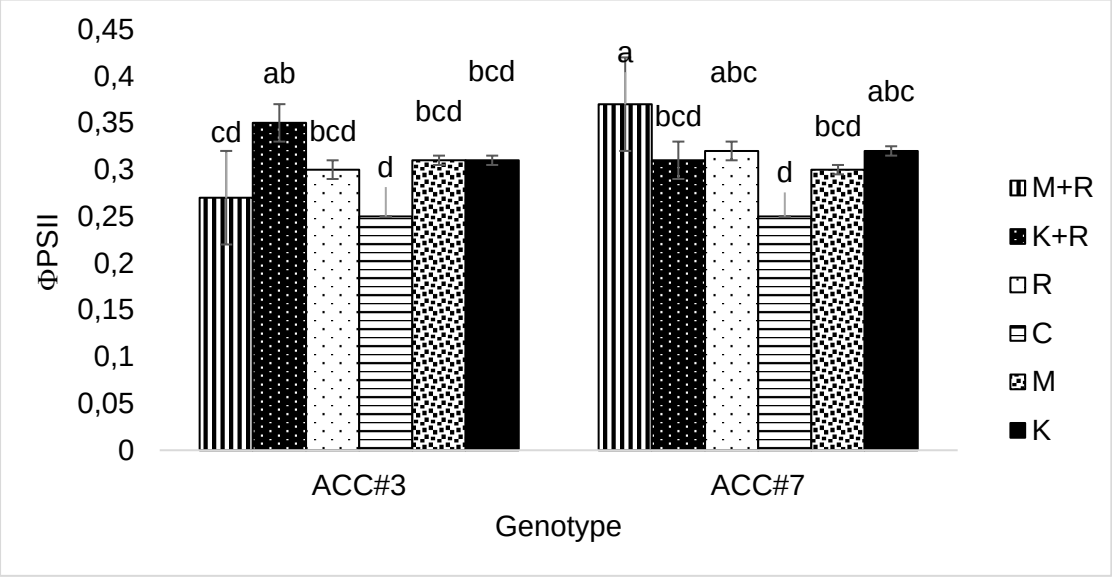

Figure 6.2: Interactive effect of the biofertilizer and genotype on the effective quantum yield of the chickpea's photochemistry II (ΦPSII) at flowering stage at Thohoyandou in 2021. Mean value; n=12. Different letters indicate the significant differences between biofertilizers and genotypes (Tukey's honest significant differences, P≤0.01).

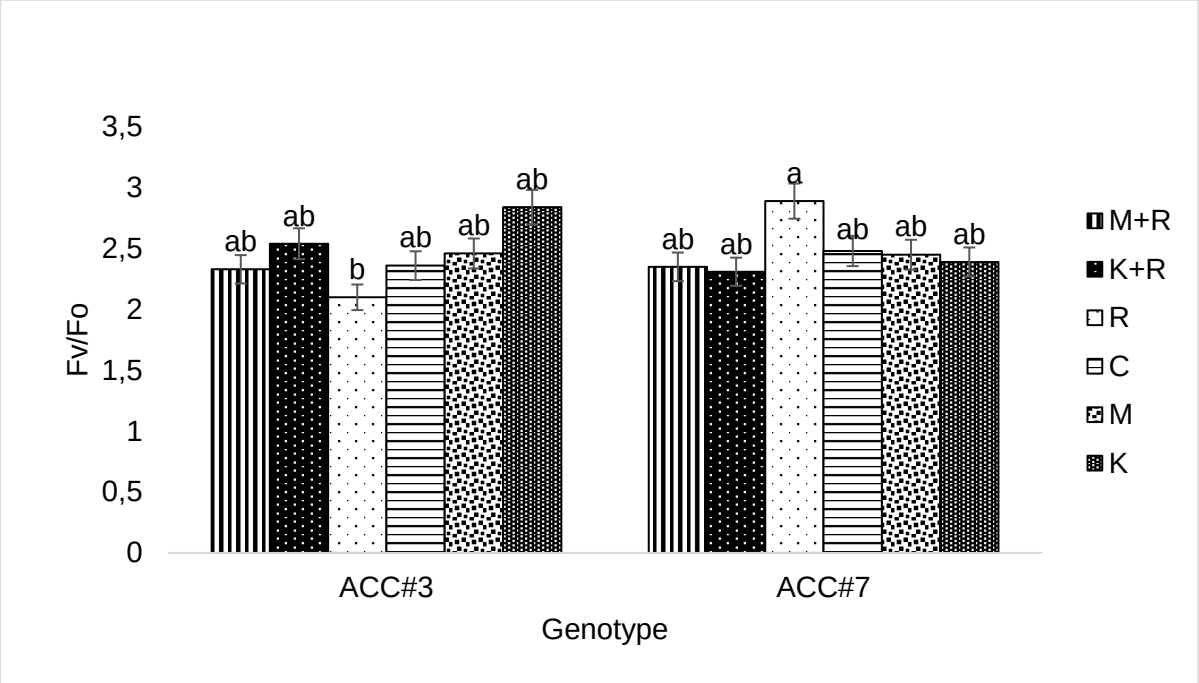

Figure 6.3: Interactive effect of the biofertilizer and genotype on the maximum primary yield of the photochemistry I (Fv/Fo) of the chickpea at the flowering stage at Syferkuil. Mean; n=1. Different letters indicate the significant differences between the biofertilizers and the genotypes (Tukey's honest significant differences, P≤0.03).

6.4 DISCUSSION

This study's results indicate that the biofertilizers had a significant effect on the chlorophyll fluorescence parameters. These results are consistent with Sharifi *et al*.'s (2020) findings that the biofertilizer's application increases the wheat's chlorophyll fluorescence parameters. Shinde and Hunje (2019) noted that the biofertilizers constitute essential micronutrients that act as the main components of the antioxidants' enzymes involved in the protection of the chloroplast. The proper distribution of the essential nutrients in many crops prevent senescence by improving the physiological, metabolic, and biochemical processes that affect the crops' pigment content and photosynthetic activity.

In this study, the chlorophyll fluorescence parameters varied among the treatments applied. That is, at Thohoyandou, the ΦPSII was greater with the rhizobium inoculation in 2019, and with K+R in 2021, and the Fv/Fo was greater with the Kelpak. At Syferkuil, the ΦPSII was greater with the rhizobium inoculation and the Kelpak, the Fv/Fo was greater with the Kelpak, and the Fv/Fm was greater with the M+R and Mycoroot. These results could be attributed to the increased availability of the micronutrients and their positive effects on the growth attributes. Shool and Shamshiri (2014) indicated that the use of the biofertilizer containing the phosphate-solubilising bacteria increased the chlorophyll fluorescence parameters' (such as Fv/Fm, ΦPSII, and Fv'/Fm) values. This is in line with this study's results. Kaschuk *et al*. (2009) discovered that the AMF inoculated plants showed 1.3% increase in the Fv/Fm. Fluorescence of chlorophyll increased as a result of photosynthesis being stimulated by AMF. A variety of factors affect the photosystem II, including the concentration of phosphorus (P) and nitrogen (N), the content of both P and N in the shoot, the root length, the area of the leaf, the number of leaves, and the electrical conductivity. Anli *et al*. (2020) claimed that the biofertilizers benefit plant growth by increasing their growth parameters.

This study's increased chlorophyll fluorescence parameters could be associated with the biofertilizer's benefits to the host plant that improved the nutrient uptake, osmotic adjustment, and better reproductive adjustment. We also noted that this study's Fv/Fm values were above 0.72. According to Critchley (1998), this indicates that there was no photoinhibition. The high minimal fluorescence (Fo) at Syferkuil in 2021 was observed in the none control plots. According to Sun *et al*. (2019), the increased minimal fluorescence indicates the occurrence of the photo-inhibitory damage. These results suggest that none control plots experienced stress that affected the photoreaction complexes (Baker & Resenqvist, 2004).

The genotype affected the minimal fluorescence in Sikhwahlane in 2019, and the Fv/Fm at Syferkuil in 2021. Accession7 provided the highest minimal fluorescence and the Fv/Fm. The

variation might have been caused by their ability to adaptation to changes in the environmental conditions. Similar results were observed by Makonya *et al.* (2019), where accession7 recorded the highest chlorophyll fluorescence (Fv/Fm) in Polokwane (cooler site) without any decrease at Thohoyandou (warmer site). Makonya *et al*. (2019) added that the increased chlorophyll fluorescence could be attributed to accession7 better acclimating to stress. They identified accession7 as the heat tolerant cultivar. This study's results suggest that accession7 is more tolerant to stress than accession3. The results are in line with Makonya *et al*.'s (2019) observations as well.

The interaction between the biofertilizer and genotype had a significant effect on the ΦPSII and Fv/Fo. The highest ΦPSII was observed in R x accession3 in 2019. M+R x accession7 and Fv/Fo were highest in R x accession7. The significant response by the biofertilizer and genotype's interactive effect on ΦPSII and Fv/Fo were due to the biofertilizers' effects on ΦPSII and FV/Fo. This was despite the genotype not affecting the chlorophyll fluorescence parameters. The biofertilizers boost the soil's physical, chemical, and biological properties, thus improving crop growth. For instant, the rhizobium inoculation and Mycoroot are important in improving natural processes were the rhizobia and AMF form a symbiotic relation with the host plant, thereby improving the BNF and, nutrient and water uptake. The results suggest that the biofertilizers and genotypes' interactive effect help to identify the biofertilizers that are suitable for increasing the crops' stress tolerance, including genotypes that are resistant to stress.

6.5 CONCLUSION

The chlorophyll fluorescence parameters increased with the biofertilizer application. The chlorophyll fluorescence responded to the biofertilizers in the second season compared to the first season in all sites. The higher chlorophyll fluorescence parameters were observed in the warmer Thohoyandou site than in the cooler Syferkuil one. Furthermore, the rhizobium inoculation, Kelpak, K+R, and M+R resulted in the higher chlorophyll fluorescence. We can conclude, therefore, that these were the best performing treatments among the rest. The chlorophyll fluorescence's genetic variation among the chickpea genotypes was significant at Syferkuil in 2021. Accession7 revealed the greater Fv/Fm than accession3. Although accession3's Fv/Fm was lower than 4% to that of accession7, both genotypes could be said to be heat tolerant. Nonetheless, further trials are required before objective agronomic recommendations are made. Furthermore, further research is needed to better understand the chlorophyll fluorescence when treated with biofertilizers to ensure improved yields.

CHAPTER 7: EFFECTS OF BIOFERTILIZERS ON YIELD AND YIELD COMPONENTS OF CHICKPEA UNDER DIFFERENT AGRO ECOLOGIES

ABSTRACT

The chickpea is a versatile legume ranked the third most consumed legume after the common bean and pea. The chickpea yields are, however, greatly affected by several abiotic factors such as thermal heat, drought (intermittent and terminal), and poor soil fertility. Nutrient deficiency such as nitrogen (N), phosphorus (P), zinc (Zn), and boron (B) affects the chickpea production. That is, they cause serious disturbances to the plant's physiology, thus affecting its grain yield. The biofertilizers ameliorate the abiotic stresses' adverse effects to improve the chickpea production. However, not much has been studied about the different biofertilizers' effects on the chickpea's productivity under contrasting environments. This study evaluated the biofertilizers' effects on the chickpea's yield and yield components in different agro-ecologies. The field experiments were piloted at Thohoyandou, Syferkuil, and Sikhwahlane in 2019, and at Thohoyandou and Syferkuil in 2021. The treatments consisted of a factorial combination of six biofertilizer levels (Kelpak [K], Mycoroot [M], Rhizobium inoculum [R], M+R, K+R, and zero control) and two chickpea cultivars (accession3 and accession7) arranged in a randomised complete block design replicated three times. The shoot biomass, pod weight, harvest index, and grain yields were determined at harvest maturity. In the 2019 cropping season, the biofertilizers increased the pod weight and grain yields at Thohoyandou. In the same season, the above-ground biomass and grain yields increased at Syferkuil, while in Sikhwahlane, the pod weight, above-ground biomass, and grain yield also increased in the same season. In the 2021 cropping season, the biofertilizers increased the above-ground biomass, harvest index, and grain yield at Thohoyandou and Syferkuil. Accession7 provided the highest value of the pod weight at Syferkuil in the 2021 cropping season. The interaction between the biofertilizer and the genotype did not affect the chickpea's grain yield and yield components in all sites in both cropping seasons. The study results showed that different biofertilizers with different modes of action increase the chickpea yields when applied exclusively. Nonetheless, agronomic recommendations could only be made after more field trials.

Keywords: Harvest index, chickpea, biofertilizers, grain yield, shoot biomass.

7.1 INTRODUCTION

The chickpea is a member of the Fabaceae family. Its cultivation has become popular around the world. The environmental stresses significantly impact food quality, thereby affecting global food security. Climate change and environmental changes such as heat stress, poor soil fertility, and soil moisture significantly impact crop yields. The chickpea is severely affected by temperatures above 35° C that inhibit its productivity (Kumari, *et al*. 2020). Heat stress reduces the chickpea yields by affecting its sexual productivity when flowering, resulting in fewer pods set per chickpea plant (Gaur, *et al*. 2008). Furthermore, when plants acclimate to changes in temperature, growth and yields are negatively affected. The chickpea is primarily grown on residual moisture, which affects yields by significantly reducing them to 16.5%, and affecting pod filling due to moisture stress (Kaloki, *et al*. 2019). High temperatures result with the chickpea developing root diseases as pathogens invade the plant and cause damage, thus reducing its nutrient uptake (Kendig, *et al*. 2000).

Low soil fertility, particularly phosphorus and nitrogen deficiency, also reduces the chickpea yields. The chickpea can fix the atmospheric nitrogen for itself and other subsequent crops. The former, however, suffers greatly from the N deficiency in its early developmental stages (Gopalakrishnan, *et al*. 2018). Nitrogen deficiency stunts plant growth by inhibiting its biomass production, flowering, and pod formation, resulting in low yields (Mu and Chen 2021). Phosphorus is one of the most important soil mineral nutrients that promotes high crop yields (Bindraban *et al*. 2020). Phosphorus deficiency is difficult to visually detect. In legumes, phosphorus influences the nodule formation and growth among other functions (Bargaz, *et al*. 2012). Effective management practices have been implemented to improve the soil fertility (particularly the P) but these have not been effective to provide sufficient P to plants due to the macro and micronutrient's low concentrations. Furthermore, the excessive use of chemical fertilizers to high legacy nutrient soils has also caused soil degradation in the long term and this leads to the rock phosphate reserves' depletion (Pang, *et al*. 2018).

The biofertilizers are eco-friendly as they produce a lot for the number of resources used. The chickpea yields significantly improved with the biofertilizers' exclusive application or in combination with inorganic fertilizers (Reddy, *et al*. 2022). The biofertilizers such as the rhizobium inoculation are noted to improve yields and have many economic benefits. Yet not much has been studied about their effects on the chickpea yields grown in diverse soils in South Africa. For instance, the application of the rhizobium inoculation with micronutrients fertilizers significantly increases the chickpea grain yields (Kumari, *et al*. 2019). However, there is a variation in the biofertilizers' effects on agronomic performances. These are strongly

influenced by each biofertilizer's physical and chemical characteristics, which influence the mode of action. Few studies have been done on the biofertilizers' effects on the chickpea's growth and yields when singularly applied or in combination with the rhizobium inoculation in South Africa, and hence the need for this study. This study evaluated the biofertilizers and genotypes' effects on the chickpea's growth and yields under contrasting environments.

7.2 MATERIALS AND METHODS

7.2.1 Yield and yield components

Chapter three illustrated this study's experimental details, but a concise synopsis is provided here. The experiments were conducted in three locations namely, Thohoyandou, Syferkuil, and Sikhwahlane in 2019, and two locations, Thohoyandou and Syferkuil in 2021. The treatments consisted of a factorial combination of six biofertilizer levels (Kelpak [K], Mycoroot [M], rhizobium inoculation [R], K+R, M+R, & zero control) and two chickpea cultivars (accession3 and accession7) arranged in a randomized complete block design replicated three times. When the crops were harvest mature, all plants from the inner 4 rows of each plot were cut at ground level within the harvest area of 1.5 m^2. The pods were manually removed from the harvested plants to determine their weight per plant. All pods were threshed, and seeds air-dried, cleaned, and weighed to determine the grain yield (kg/ha^{-1}). The shoot biomass was oven dried at 65º C for 48 hours to determine their biomass. The harvest index was determined as the grain yield ratio to the total above-ground biomass.

7.2.2 Statistical analysis

Data were variance (ANOVA) analysed using the STATISTIX (2017) version 10.0. The Tukey's Honestly Significant Difference (HSD) test was used to separate the means that were significantly different ($P \leq 0.05$). The Pearson correlation was used to test the strength of the linear association between the parameters studied.

7.3 RESULTS

The biofertilizers' application significantly affected the grain yield and the selected yield attributes in 2019 (see Table 7.1). At Thohoyandou, all the biofertilizers affected the grain yield compared to the control. The highest grain yield (813.06kg/ha) was obtained with the application of the Mycoroot (see Table 7.1). Similarly, the pod weight (343.35g/m^2) was the highest when the Mycoroot was applied. Neither the genotype nor the interaction between the biofertilizer and the genotype affected the grain yield and yield attributes (see Table 7.1). In 2021, the biofertilizers significantly affected the shoot biomass, harvest index, and grain yields. The shoot biomass was highest (3346.70kg/ha) with the Mycoroot's application. The harvest index (52.88%) was the highest with the rhizobium inoculation's application but decreased under K+R, M+R, M, & K. (see Table 7.2). The grain yield (1570.20kg/ha) was the highest with the Mycoroot's application. Neither the genotypes nor the interaction between the biofertilizer and the genotypes affected the grain yield and yield attributes (see Table 7.2).

At Syferkuil, the biofertilizers significantly affected the shoot biomass and grain yield only. The highest shoot biomass (2353.50kg/ha) was obtained in plots treated with the rhizobium inoculation. Similarly, the grain yield of 1390.10kg/ha was the highest with the rhizobium inoculation's application (see Table 7.1). In 2021, the biofertilizers' application significantly affected the shoot biomass, harvest index, and grain yields. The shoot biomass of 3505.40kg/ha was the highest in plots treated with the Mycoroot. The grain yield (1550.60kg/ha) and harvest index (48.72%) were the highest with the Mycoroot and rhizobium inoculation's co-application (see Table 7.2). However, the grain yield showed a significant decrease when treated with K+R, K, & R, and increased by 13.47% when treated with M+R, and by 2% under M. The harvest index decreased by 0.81% under M. The genotype affected the pod weight and was the highest with accession7 (343.49g/m^2). The interaction between the biofertilizer and the genotype had no effect on the grain yield and yield attributes.

The grain yield and yield attributes were only measured in the 2019 season in Sikhwahlane. The biofertilizers significantly affected the pod weight, shoot biomass, and grain yields (see Table 7.1). The Mycoroot and rhizobium inoculation's co-application provided the highest pod weight of 308.62g/m^2. The shoot biomass was the highest (1650.60kg/ha) in plots treated with the rhizobium inoculation. The grain yield of 511.12kg/ha was the highest in plots treated with the rhizobium inoculation (see Table 7.1).

Table 7. 1**:** Effect of biofertilizers and genotype on chickpea yield and yield components grown under different agro-ecologies in 2019.

	Thohoyandou				Syferkuil				Sikhwahlane			
Biofertilizer	Pod Weight (g m)	Shoot biomass (Kg ha)	Harvest index (%)	Grain yield (Kg ha)	Pod Weight (g m)	Shoot biomass (Kg ha)	Harvest index (%)	Grain yield (Kg ha)	Pod Weight (g m)	Shoot biomass (Kg ha)	Harvest index (%)	Grain Yield (Kg ha)
M+R	268.30b	1681.60a	43.66a	719.31ab	323.42a	1956.40ab	68.31a	1328.30a	308.62a	1169.60ab	39.94a	459.37ab
K+R	264.25b	1613.90a	48.73a	787.22ab	355.85a	1782.80b	67.68a	1189.00ab	266.82ab	917.70b	39.41a	363.75bc
R	312.13ab	1637.20a	44.40a	712.71ab	381.95a	2353.50a	59.32a	1390.10a	307.25a	1650.60a	32.35a	511.12a
C	286.68b	1565.10a	38.16a	591.67b	310.20a	1720.40b	63.62a	1072.80b	249.33b	747.80b	41.99a	308.34c
M	343.35a	1675.10a	48.48a	813.06a	385.05a	1897.60ab	71.86a	1362.40a	255.02b	1170.30ab	34.57a	396.52abc
K	293.73ab	1712.30a	43.41a	671.67ab	361.58a	1946.70ab	69.68a	1340.70a	254.68b	1217.40ab	41.93a	494.90ab
SED	54.35	932.60	14.69	221.33	81.92	515.70	20.56	223.17	44.34	583.05	16.23	133.12
Genotype												
ACC#3	296.93a	1660.40a	45.03a	718.43a	346.98a	1945.60a	67.04a	1292.60a	268.07a	1209.10a	35.91a	421.74a
ACC#7	292.56a	1634.60a	43.92a	713.45a	359.03a	1940.20a	66.45a	1268.50a	279.17a	1082.10a	40.82a	442.93a
SED	31.38	358.65	5.64	127.78	47.29	198.32	7.90	128.85	25.60	224.22	6.24	76.85
P-Value												
Biofertilizer (B)	*	ns	ns	*	ns	**	ns	*	*	***	ns	*
Genotype (G)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
B*G	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	15.40	31.47	18.36	25.82	19.38	14.76	17.54	14.56	13.53	28.30	23.52	26.33

Mean values with different letters show a significant difference as follows; *P≤0.05, **P≤0.01, ***P≤0.001, ns-not significant. M+R= Mycoroot +Rhizobium inoculum, K+R= Kelpak+Rhizobium inoculum, R= Rhizobium inoculum, C= control, K= Kelpak, M=Mycoroot. SED= standard error of deviation, CV= coefficient variance.

Table 7. 2**:** Effect of biofertilizers and genotype on chickpea yield and yield components grown under different agro-ecologies in 2021.

	Thohoyandou				Syferkuil			
Biofertilizer	Pod Weight (g m)	Shoot biomass (Kg ha)	Grain yield (Kg ha)	Harvest Index (%)	Pod weight (g m)	Shoot Biomass (Kg ha)	Grain Yield (Kg ha)	Harvest Index (%)
M+R	291.00a	3144.10a	1128.00ab	36.10c	346.39a	3202.00ab	1550.60a	48.72a
K+R	324.65a	2753.70ab	1148.50ab	42.44bc	338.56a	3045.80ab	1362.60b	45.39a
R	340.62a	2532.50ab	1291.60ab	52.88a	290.56a	2779.20b	1272.0c	46.41a
C	290.15a	1818.30b	913.20b	50.37ab	304.69a	3204.00ab	1366.50b	42.32b
M	371.67a	3346.70a	1570.20a	47.21ab	340.69a	3505.40a	1395.80b	41.98b
K	322.61a	3048.20ab	1312.00ab	42.36bc	344.92a	3123.80ab	1313.70b	43.18b
SED	45.33	396.09	210.42	8.90	43.00	472.35	123.56	4.50
Genotype								
ACC#3	320.68a	2787.50a	1190.40a	43.15a	311.75b	3077.20a	1358.10a	45.47a
ACC#7	326.22a	2760.50a	1264.10a	47.29a	343.49a	3209.60a	1375.60a	43.86a
SED	26.17	228.68	121.49	5.14	24.74	272.71	71.33	2.59
P-value								
Biofertilizer								
(B)	ns	**	*	**	ns	*	**	*
Genotype (G)	ns	ns	ns	ns	*	ns	Ns	ns
B*G	ns	ns	ns	ns	ns	ns	Ns	ns
CV (%)	24.28	24.73	29.70	16.44	22.65	26.03	15.66	17.44

7.4 DISCUSSION

7.4.1 Shoot biomass

The results show that the shoot biomass was the highest in plots treated with the rhizobium inoculation and Mycoroot at Syferkuil in both cropping seasons. In Sikhwahlane, the shoot biomass was greater with rhizobium inoculation in 2019. The shoot biomass was the highest at Syferkuil in both seasons compared to Thohoyandou and Sikhwahlane. It is possible that the shoot biomass response to the rhizobium inoculation and Mycoroot could be due to the leaves' high nitrogen content as well as the phosphorus through the AMF and Rhizobia's natural association in the soil (Gao, *et al*. 2019). Similarly, the biofertilizers' application increased the grain yield and pod dry weight and harvest index. In this study, the significant increase in the shoot biomass could be partly attributed to the leaves' high N content, measured by the chlorophyll content and NDVI's increase. The shoot biomass' increase with the rhizobium inoculation's application is related to the possible N fixation, ammonia excretion, phosphate solubilisation, siderophore, and growth hormone production mechanisms (Li, *et al*. 2020). The nitrogen's importance to plant growth and development is well known. Increasing N in the soil, therefore, leads to larger crop yields. The shoot biomass' increase after the Mycoroot's application is attributed to the improvements in the root system and hydraulic conductivity due to the AMF (Kavadia, *et al*. 2020). Similar results were realised by Dobo *et al*. (2022). That is, inoculation with the rhizobium inoculation and mycorrhiza produces greater biomass due to the nutrient supply acquired through the symbiotic relation AMF and rhizobia formed with the plant's roots (Dobo *et al*., 2022). Inoculated plants produce greater shoot biomass compared to uninoculated ones, which is associated with the increased BNF influenced by the increased nodulation (Macil *et al*., 2020, Imran *et al*., 2015). The biofertilizers use microorganisms to improve soil fertility through nitrogen fixation and solubilising phosphorus' natural processes, and this stimulates plant growth due to growth promoting substances (Suhag, 2016).

The genotype had no significant effect on the shoot biomass in all sites and seasons. The genotypes used in this study showed no variation on any of the growth parameters across the study. These results suggest that both genotypes growth was in sync. Makonya *et al*. (2019) discovered that both accession3 and accession7 are similar and identify as heat tolerant cultivars. The increased shoot biomass in genotypes is associated with high nodulation, chlorophyll content, number of branches, and other growth parameters (Macil *et al*., 2020; Imran *et al*., 2015). The genetic variations are influenced by many factors. These factors include environmental conditions, psychological and physiological factors, and are

interconnected. Chaudhry and Sidhu (2022) found that the environmental conditions significantly influence different genotypes' growth. The interaction between the biofertilizer and genotype had no significant effect on the shoot biomass in all sites and in both seasons. Similarly, the genotype had no significant effect on the chlorophyll content and NDVI in this study. Furthermore, the growth parameters were not affected by the genotype and biofertilizers.

7.4.2 Grain yield

The biofertilizer application is currently an innovative management practice used to mitigate poor soil fertility and increase grain yield while preserving the environment (Yadav and Sarkar 2019). Biofertilizers significantly affected the chickpea grain yields in all sites and in both seasons. The chickpea grain yields varied among the treatments and seasons. The grain yields were highest at Syferkuil compared to Thohoyandou and Sikhwahlane. Syferkuil also realised the greatest pod dry weight. This indicates that the pod dry weight could be used to estimate the chickpea's grain yield. The results also suggest that Syferkuil provides favourable conditions for the biofertilizers to help the chickpea growth compared to Thohoyandou and Sikhwahlane. The grain yields' response to biofertilizers could be associated with the higher shoot biomass (r= 0.49) and pod weight (r=0.64), indicating a significant linear correlation. The results revealed that the rhizobium inoculation's application showed the highest increased in grain yields at Syferkuil and Sikhwahlane, and the Mycoroot at Thohoyandou in 2019; and Mycoroot at Thohoyandou and M+R at Syferkuil in 2021. Based on these findings, we observed that the biofertilizers are effective and that more benefits could be obtained through the combined use of the rhizobium inoculation and Mycoroot. The increased grain yields in the rhizobium-inoculated plots were a result of positive effects on crop nitrogen due to the improved rhizobium's biological nitrogen fixation, ammonia excretion, phosphate solubilisation, and growth hormone production provided (De Souza-Torres, *et al*. 2021, Kizilkaya 2018).

In plots treated with Mycoroot, the grain yields were increased due to the symbiotic relation the arbuscular mycorrhizal fungi formed with the roots in the rhizosphere to improve the moisture and nutrients uptake (Habibzadeh, *et al*. 2015). Ogola *et al*. (2021) reported that the rhizobium inoculation increased the grain yields, number of pods, number of seeds per plant, and the chickpea's harvest index compared to the uninoculated plots. Das *et al*. (2013) asserted that the phosphorus solubilising bacteria's application combined with the rhizobium inoculation increased the chickpea's grain yields. A possible explanation for this study's increased grain yields in plots treated with the biofertilizers include the biofertilizers' effects to

improve and facilitate the micro and macronutrients uptake. By combining other biofertilizers with rhizobium inoculation, you are increasing soil nutrients, improving nutrient availability, and improving plant growth, resulting in greater grain production (Ditta *et al*., 2018).

The genotype had no significant effect on grain yields but affected the pod dry weight at Syferkuil in 2021. Accession7 exhibited the highest pod dry weight compared to accession3. There was a positive linear correlation between the pod dry weight and the shoot biomass (r=0.79), and grain yields (r=0.64). However, there was a significant negative correlation between the pod dry weight and the harvest index (r=-0.49). It is noteworthy that the genotype did not affect the growth parameters, extracellular acid, alkaline phosphatases, and inorganic phosphorus. This might have influenced the results. In contrast, Ogola *et al*. (2021) reported that the genotype affects the chickpea's grain yield and yield components. The interaction between the biofertilizer and genotype had no significant effect on the chickpea's grain yield and yield components in all sites and in both seasons.

7.5 CONCLUSION

The biofertilizer's application significantly increased the grain yields. The highest grain yields were realised with the Mycoroot, rhizobium inoculation, and M+R applications. The highest were observed with the rhizobium inoculation and Mycoroot at Syferkuil, and with the rhizobium inoculation in Sikhwahlane in 2019; and the Mycoroot exhibited the highest grain yields at Thohoyandou and Syferkuil in 2021 cropping season. The genetic variation was not significant for the grain yields and was only observed for the pod dry weight at Syferkuil with accession7 giving the highest increase. These results indicate that the biofertilizers' individual application or in combination with the rhizobium inoculation gave the maximum results in most parameters. Furthermore, the highest grain yields were observed at Syferkuil in both seasons, suggesting that Syferkuil is the best site for the chickpea production. However, for factual agronomic recommendations to be made, more research on these is required.

CHAPTER 8: GENERAL DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

8.1 GENERAL DISCUSSION

The chickpea crop is a drought tolerant legume high in proteins. It has beneficial characteristics such as its ability to fix atmospheric nitrogen into usable forms. However, it is vulnerable to biotic and abiotic stresses, which are aggravated by climate change. Poor soil fertility and heat stress significantly reduce the chickpea yields throughout the world. These pose challenges to farmers as they affect food security. Heat and drought stresses are both caused by high temperatures and reduce chickpea production by affecting its flowering and podding abilities (making chickpeas to lose flowers and pods) (Rani, *et al*. 2020). Studies about the chickpea's responses to organic and inorganic fertilizers for improved growth and yield (Imran, *et al*. 2015, Ogola ,*et al*. 2021) and its response to drought and heat stresses are plenty (Makonya, *et al*. 2019, Makonya, *et al*. 2020).

Numerous studies illustrate the biofertilizers' ability to increase agricultural production while preserving the environment. Literature demonstrates how biofertilizers significantly affect the phosphatase activity in bulk soil, rhizosphere soil, and root nodule tissue (Mogale, *et al*. 2018, Moloto, *et al*. 2021). However, few studies done so far use one or two types of biofertilizers applied exclusively. This study, therefore, assessed the biofertilizers' effects (single and in combination) on the selected physiological traits, acid and alkaline phosphatases, quantum yield and chlorophyll fluorescence, and the chickpea's yield and yield components grown under varying conditions.

The biofertilizers were observed to increase certain physiological attributes during this study. At Thohoyandou, the intercepted radiation's proportion in 2019 and 2021, the chlorophyll content and stomatal conductance at Thohoyandou in 2021, and the NDVI at Syferkuil in 2021 were significantly increased by the biofertilizer application. The enlarged canopy cover and size significantly increased the intercepted radiation's proportion. The increased radiation contributed to the higher stomatal conductance. More chlorophyll content increased the intercepted radiation's proportion. The green pigment oversees light harvesting, thereby increasing the photosynthesis process. The biofertilizers were observed to significantly improve the physiological plant parameters by influencing the chickpea's N-fixation (Mapope & Dakora, 2016).

The biofertilizers had a significant effect on the extracellular acid and alkaline phosphatase enzymes, including the inorganic phosphorus in the rhizosphere in all locations in 2021. The

plants' symbiotic relationships with the microorganisms such as the fungi and bacteria help explain the increased phosphatase activity in the rhizosphere. The biofertilizers are microorganisms that contain micro and macronutrients that are starters for growth and development, while the microorganisms colonise the roots to recycle nutrients. The increased acid phosphatase activity in the rhizosphere is significantly influenced by the phosphorus deficiency, and the alkaline phosphatase activity is significantly influenced by the microbe population and its activity (Moscatelli, et al. 2018). These findings are consistent with the biofertilizers' ability to secrete the organic acid and increase the microbial population to solubilise the unavailable phosphorus. Clusters of acid phosphatase and alkaline phosphatase activity are associated with soil CP (Luo, et al. 2019). Plants accumulate inorganic phosphorus from the rhizosphere as a result of increased phosphatase activity (Spohn, *et al*. 2015). Accession3 significantly impacted the inorganic phosphorus in the rhizosphere soil. This could be attributed to legumes forming cluster roots to release organic acids and the phosphatase to solubilize phosphorus for uptake. The phosphorus accumulation is significantly influenced by the plant's need for phosphorus, including other essential mineral nutrients for growth and development.

This study also revealed that the biofertilizers are associated with the significant changes in the chlorophyll fluorescence and its parameters. The quantum yield and chlorophyll fluorescence's responses to the biofertilizers were due to the later's effects on the plant nutrient's status. The biofertilizers used in this study contained adequate nutrients. Due to their different modes of action, these fertilisers were classified as PGPR and PSB. Their responsibility is to solubilise and mineralise nutrients. Kafi *et al*. (2022) reported a significant increase in the sesame seeds' Fv/Fm due to the pigments and NP's increased productions after the application of biofertilizers and potassium, silicon, and calcium combined. Javanmarf *et al*. (2022) revealed the highest chlorophyll fluorescence obtained at 90% field capacity treated with Mycoroot + chitosan nanoparticles (Cs-NPs). The biofertilizers' effects on stresses were found to be common (Hoque, *et al*. 2022). Furthermore, the biofertilizers mitigate stress in plants by impacting water homeostasis and photosynthetic responses (Javanmard, *et al*. 2022).

Through the rhizobium inoculation, the biological nitrogen fixation could be improved by increasing the root nodulation. The effective root nodulation increases nitrogen through BNF, which leads to the reduction in plant senescence. There was a genotypic variation Fv/Fm, with accession7 exhibiting the greater chlorophyll fluorescence (Fv/Fm) at Syferkuil in 2021. At Thohoyandou's 2019 and 2021 cropping seasons, the interaction between the biofertilizer and genotype was significant, as were the differences between the PSI's maximum primary yields (Fv/Fo) at Syferkuil in 2019.

The biofertilizers' effects on the chickpea yield and selected yield components, shoot biomass, harvest index, and pod weight were significant in all locations and both seasons. This was due to the biofertilizers saturating and mineralizing nutrients in the rhizosphere in preparation for the plant uptake (nitrogen and phosphorus). The biofertilizers' application to the soil significantly improves the root colonization to enhance nutrient cycling and their uptake by plants for growth and development (thus improving yields) (Chaechian, *et al*. 2022, Gao, *et al*. 2022).

This study's findings are consistent with Ogola *et al*. (2021) observations that the rhizobium inoculation significantly increased the chickpea's yield and yield components at Thohoyandou. The grain yields' response to the biofertilizers was due to the increased yield and yield components, which had a positive correlation with the pod-weight (r=0.64) and the shoot biomass (r=0.49) at Syferkuil, and pod-weight (r=0.75), shoot biomass (r=0.75), and harvest index (r=0.32) at Thohoyandou in 2021. The grain yield is associated with the yield components such as the shoot biomass, pod count, seed weight, and harvest index. An increase in the yield components significantly improves grain yields (Al-Amri, 2021; Ogola, *et al*., 2021).

However, the grain yield negatively correlated with the alkaline phosphatase activity (r=-0.34) at Syferkuil in 2021. We did not test for the microbial efficacy, but the negative correlation could be attributed to the reduced microbial population in the rhizosphere. This resulted in microbes utilising the available nutrients for their upkeep, and thus reduced the P uptake and P use efficiency (PUE). In contrast**,** a positive correlation between the grain yield and alkaline phosphatase activity in rice due to the enhanced P uptake and PUE, which positively correlated with AlkPase activity was observed. The shoot biomass negatively correlated with the nodule fresh weight (r=-0.005) at Thohoyandou in 2021.

8.2 CONCLUSION

There was a positive effect of the biofertilizer application on acid and alkaline phosphatase activity, inorganic phosphorus, quantum yield, chlorophyll fluorescence, stomatal conductance, NDVI, chlorophyll content, proportion of intercepted radiation and, grain yields and components of grain yields. As a result of the increased phosphatase activity in the rhizosphere and the increased inorganic phosphorus levels, the chickpea grain yields significantly increased. The genotypes used in this study were previously identified as heat tolerant. Accession3 and accession7 showed some similarities in their performance and variation in this study. Although accession3 and accession7's performances had similarities,

accession7 showed the higher Fv/Fm at Syferkuil in 2021 and Fo in Sikhwahlane in 2019. Based on this study's findings, the conclusion is that biofertilizers have the potential to increase the chickpea yields, soil fertility, and substantially contribute to the environment's sustainability.

8.3 RECOMMENDATIONS

- There is potential for the biofertilizers to improve soil fertility and increase crop yields, and where accessible and affordable, they may be feasible to use by smallholder crop farmers to replace synthetic fertilizers.
- Our recommendation is that further studies should be done to assess the influence of other biofertilizers on the activity of phosphatase and other important enzymes, concentration of essential mineral nutrients in the soil and plant tissue, and relationship with soil.
- Quantum yield and chlorophyll fluorescence were tested on the genotypes that were previously identified as heat tolerant (Makonya, *et al*. 2019). We further recommend that more research should be conducted about accessions that are susceptible to heat stress to evaluate the biofertilizers' efficacy to improve plant acclimation.
- Association between acid and alkaline phosphatase activity with inorganic phosphorus, physiological traits, growth, and yield parameters should be further investigated.

LIST OF REFERENCES

1. Ahmad, F., Gaur, P. M. & Croser, J., 2005. Chickpea (*Cicer arietinum* L). *Genetic Resources, Chromosome Engineering, and Crop Improvement-grain Legumes,* Volume 1, pp. 187-217.
2. Alam, M. Z., Braun, G., Norrie, J. & Hodges, D. M., 2013. Effect of Ascophyllum extract application on plant growth, fruit yield and soil microbial communities of strawberry. *Canada Journal of Plant Science.,* Volume 93, pp. 23-36.
3. Al-Amri, S. M., 2021. Application of biofertilizers for enhencing growth and yield of common bean plants grown under water stress conditions. *Saudi Journal of Biological Sciences,* Volume 28, pp. 3901-3908.
4. ALKahtani, M. D. F., Attia, K. A., Hafez, Y. M., Khan, N., Eid, A. M., Ali, M. A. M. & Abdelaal, K. A. A., 2020. Chlorophyll fluorescence parameters and antioxidant defense system can display salt tolerance of salt acclimated sweet pepper plants treated with chotosan and plant growth promoting rhizobacteria. *Agronomy,* Volume 10.
5. Alori, E. T., Glick, B. R. & Babolola, O. O., 2017. Microbial phosphorus solubilisation and its potential for use in sustainable agriculture. *Journal of Frontiers in Microbiology.,* Volume 10, p. 971.
6. Aram, S. V., Ahmadvand, G. & Hajinia, S., 2018. Effect of biological and chemical phosphorus fertilizers on radiation use efficiency, P concentration and yield of wheat cultivar (Pishgam). *Journal of Crop Ecophysiology,* Volume 12, pp. 171-190.
7. Arenberg, M. R. & Arai, Y., 2019. Uncertainities in soil physicochemical factors controlling phosphorus mineralisation and immobilisation processes. *Advanced Agronomy,* Volume 154, pp. 153-200.
8. Arough, Y. K. & Sharifi, R. S., 2016. Biofertilizers and Zinc effects on some physiological parameters of triticale under water limiting conditions. *Journal of Plant Interactions,* Volume 11, pp. 167-177.
9. Audipudi, A. V., Bai, V. T. & Sanneboyina, N., 2021. Impact of pseudomonas plecoglossicida AVP1, plant growth promoting rhizobacteria on growth and physiological attributes in chilli. *International Journal of Modern Agriculture,* Volume 10, pp. 247-257.
10. Auge, R. M., 2000. Stomatal behavior of arbuscular myacorrhizal plant. In: Y. Kapulnik & D. D. Douds, eds. *Arbuscular Mycorrhizal: Physiological and Function.* Dordrecht: Springer, pp. 201-237.
11. Awasthi, R., Kaushal, N., Vadez, V., Turner,N. C., Berger, J., Siddique, K. H. M. & Nayyar, H., 2014. Individual and combined effects of transient drought and heat

stress on carbon assimilation and seed filling in chickpea. *Functional Plant Biology,* Volume 41, pp. 1148-1167.

12. Baker, N. R. & Rosenqvist, E., 2004. Application of chlorophyll fluorescence can improve crop production strategies:an exaination of future possibilities. *Journal of Experimental Botany,* Volume 55, pp. 1607-1621.
13. Balota, E. L., Machineski, O., Hamid, K. I. A., Yada, I. F. U., Barbosa, G. M. C., Nakatani, A. S. & Coyne, M. S., 2014. Soil microbial properties after long-term swine slurry application to conventional and no-tillage systems in Brazil. *Journal of Science Total Environmental,* Volume 490, pp. 397-404.
14. Barbas, C., Garcia, J. A. & Manero, I. J., 1999. Separation and identification of organic acids in root exudates of *Lupinus leteus* by capillary zone electrophoresis. *Journal of Phytochemistry Analysis.,* Volume 10, pp. 55-59.
15. Bargaz, A., Faghire, M., Abdi, N., Farissi, M., Sifi, B., Drevon, J.J., Ikbal, M.C, & Ghoulam C., 2012. Low soil phosphorus avalaibility increases acid phosphatases activities and affects P partitioning in nodules, seeds, and rhizosphere of *Phaseolus vulgaris*. *Journal of Agriculture,* Volume 2, pp. 139-153.
16. Batra, P., Barkodia, M., Ahlawat, U., Sansanwal, R., Wati, L.,2020. Effect of compatible and incompatible endophytic bacteria on growth of chickpea plant. *Journal of Defence Life Science.* Volume 5, pp. 45-48.
17. Bell, G. E., Martin, D. L., Wiese, S. G., Dobson, D. D., Smith, M. W., Stone, M. L. & Solie, J. B., 2002. Vehicle-mounted optical sensing: An objective means for evaluating turf quality. *Crop Science,* Volume 42, pp. 197-201.
18. Ben-Laouane, R., Meddich, A., Bechtaoui, N., Oufdou, K. & Wahbi, S., 2019. Effects of Arbuscular Mycorrhizal Fungi and Rhizobia Symbiosis on the Tolerance of *Nedicago Sativa* to Salt Stress. *Journal of Frontiers in Plant Science,* Volume 71, pp. 135-146.
19. Ben-Laouane, R., Ait El-Mokhtar, M., Anli, M., Boutasknit, A., Ait Rahou, Y., Raklami, A., Oufdou, K., Wahbi, S. & Meddich, A., 2021. Green compost combined with mycorrhizae and rhizobia: A strategy for improving Alfalfa growth and yield under field conditions. *Gesunde pflanzen,* Volume 73, pp. 193-207.
20. Bhandari, K., Sita, K., Sehgal, A., Bhardwaj, A., Gaur, P., Kumar, S., Singh, S., Siddique, K. H. M., Prasad, V.V., Jha, U. & Nayyar,m H., 2020. Differential heat sensitivity of two cool-season legumes, chickpea, and lentil, at the reproductive stage, is associated with response in pollen function, photosynthetic ability, and oxidative damage. *Journal of Agronomy and Crop Science,* Volume 206, pp. 734-758.

21. Bhardwaj, D., Ansari, M. W., Sahoo, R. K. & Tuteja, N., 2014. Biofertilizers fucntion as key player in sustainable agriculture by improving soil fertility, plant tolerance, and crop production. *Microbial Cell Factories,* Volume 66.
22. Bindraban, P. S., Dimkpa, C. O. & Pandey, R., 2020. Exploring phosphorus fertilizers snd fertilization stratergies for improved human and envrionmental health. *Biology and Fertility of Soils,* Volume 56, pp. 299-317.
23. Boutasknit, A., Baslam, M., Anli, M., Ait-EL-Mokhtar, M., Ben-Laorone, R., Ait-Rahou, Y., El-Modafar, C., Douira, A., Wahbi, S. & Meddich, A., 2021. Impact of arbuscular mycorrhizal fungi and compost on the growth, water status, and photosynthesis of carob (Ceratonia siliqua) under drought stress and recovery, plant biosysntems. *International Journal of Plant Biology,* Volume 10, p. 1080.
24. Bowles, T. M., Jackson, L. E., Loeher, M. & Cavagnaro, T. R., 2016. Ecological intensification and arbuscular mycorrhizas: a meta-analysis of tillage and cover crop effects. *Journal of Applied Ecology,* Volume 54, pp. 1785-1793.
25. Calvo, P., Nelson, L. & Kloepper, J. W., 2014. Agricultural uses of plant biostimulant. *Journal of Plant and Soil,* Volume 383, pp. 3-41.
26. Chaechian, F., Pasari, B., Sabaghpour, H. S., Rokhzadi, A.& Mohammadi, K., 2022. Yield,yield components and evaluation indices in wheat Chickpea intercropping as affected by different sowing methods and ratios and biofertilizer inoculation. *Gesunde Pflanzen,* pp. 1-11.
27. Dakora, F. D. & Phillips, D. A., 2002. Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant and Soil,* Volume 245, pp. 35-47.
28. Das, S., Pareek, B. I. & Dhikwal, S. R., 2013. Effect of phosphorus and biofertilizers on productivity of chickpea (Cicer arietinum L) in North Western Rajasthan, India. *Legume Research,* Volume 36, pp. 511-514.
29. Davies, P., 2010. Their Nature, Occurrence, and Functions. In: P. Davies, ed. *The Plant Hormones.* Ithaca, USA: Springer Dordrecht, pp. 1 - 15.
30. De Souza-Torres, A., Alcaide, E. G., Padilla, E. G., Masunaga, S. H., Effenberger, F. B., Rossi, L. M., Lopez-Sanchez, R. & Jardim, R. F., 2021. Fe3O4 nanoparticles and rhizobium inoculation enhence nodulation, nitrogen fixation and growth of common bean plants grown in soil. *Rhizosphere,* Volume 17, p. 100275.
31. Dekhane, S. S., Khafi, H. R., Raj, A. D. & Parmar, R. M., 2011. Effect of biofertilizer and fertility levels on yield, protein content, and nutrient uptake of cowpea (*Vigna unguiculata* L. walp). *Legume Research-An International Journal,* Volume 34, pp. 51-54.

32. Devasirvatham, V. & Tan, P., 2018. Impact of high temperatures and drought stress on chickpea production. *Journal of Agronomy,* Volume 8, p. 145.
33. Ditta, A., Imtiaz, M., Mehmood, S., Rizwan, M. S., Mubeen, F., Aziz, O., Zheng, Q., Ijaz, R. & Shuxin, T., 2018. Rock phosphate-enriched organic fertilizers with phosphate solubilising microorganisms improves nodulation, growth, and yield of legumes. *Communication Soil Science Plant Anals,* Volume 49, pp. 2715-2725.
34. Dobo, B., 2022. Arbuscular Mycorrhizal Fungi (MAF) and Rhizobium inoculation on growth and yield of *Glycine max* L vaerities. *International Journal of Agronomy,* Volume 2022, p. 10.
35. Dodd, I. C., Zinovkina, N. Y., Safronova, V. I. & Belimov, A. A., 2010. Rhizobzterial mediation of plant hormone status. *Annals of Applied Biology,* Volume 157, pp. 361379.
36. Du Jardin, P., 2015. Plant biostimulants: definition, concept, main categories, and regulation. *Journal of Scientia Hoticulturae,* Volume 196, pp. 3 - 14.
37. DuPlessis, S, and R Burger. "A comparison of `chemical extraction methods for the evaluation of phosphate availability of topsoil." *South African Journal of Agricultual Science* 8 (1964): 11-13.
38. Du, S., Liu, L., Liu, X., Guo, J., Hu, J., Wang, S. & Zhang, Y., 2019. SIFSpec: Measuring solar induced chlorophyll fluorescence observations for remote sensing of photosynthesis. *Sensor,* Volume 19, p. 3009.
39. Elgaml, N. M., Salama, A. B., Shehata, H. S. & Abdelhamid, M. T., 2022. Effective microorganisms improve growth, nutrient uptake, normalised difference vegetation index, photoystem II, and essential oil while reducing canopy temperature in water stressed *Salnia sclarea* plants. *International Journal of Agronomy,* Volume 2022.
40. FAO, 2018. *FAOSTAT. Food and Agriculture Organization of the United Nations.* Rome, Italy, s.n.
41. FAO, 2019. *FAOSTAT Statistical Database of the United Nation Food and Agriculture Organisation (FAO) statistical division.* Rome, s.n.
42. Fareed, H. M. R., Matloob, A. & Hussain, M. B., 2021. Rhizobial inoculation in soil improves growth, yield and economic returns of irrigated chickpea. *Soil and Environment,* Volume 40, pp. 183-193.
43. Farhangi-Arbriz, S. & Torabian, S., 2018. Biochar improved nodulation and nitrogen metabolism of soybean under salt stress. *Symbiosis,* Volume 74, pp. 215-223.
44. Fernandez-Tizon, M., Emmenegger, T., Perner, J. & Hahn, S., 2020. Athropod biomass increase in spring correlates with NDVI in grassland habitat. *The Science of Nature,* Volume 107, p. 42.

45. Fu, D., Wu, X., Duan, C., Chadwick, D. R. & Jones, D. L., 2020. Response of soil phosphorus fractions and fluxes to different vegetation restoration types in a subtropical mountain ecosystem. *CATENA,* Volume 193, p. 104663.
46. Gao, F., Che, X., Yu, F. & Li, J., 2019. Cascading effects of nitrogen, rhizobia, and parasitism via a host plant. *Flora,* Volume 251, pp. 62-67.
47. Gao, N., Zhang, H., Xiong, R., Fang, L., Shen, W. & Senoo, K., 2022. Different strategies for colonizition and prevalence after inoculation with plant growth-promoting rhizobacteria revealed by a monitoring method. *Soil Science and Plant Nutrition,* pp. 1-12.
48. Gardner, W. K., Parbery, D. G. & Barber, D. A., 1982. The acquisition of phosphorus by *Lupinus albus* L. I. Some charecteristics of the soil/root interface. *Journal of Plant Soil,* Volume 68, pp. 19-32.
49. Gau, P. M., Samineni, S. & Varshney, R. K., 2014. Drought and heat tolerance in chickpea. *Legumes Pespectives,* Volume 3, p. 15.
50. Gaur, P. M., Krishnamurthy, L. & Kashiwagi, J., 2008. Improving drought-avoidance root traits in chickpea (*Cicer arietinum* L)-current status of research at ICRISAT. *Journal of Plant Production Science,* Volume 11, pp. 3-11.
51. Gherardi, M. & Rengel, Z., 2004. The effect of manganese supply on exudation of carboxylates by roots of lucerne (*Medicago Sativa*). *Journal of Plant and Soil.,* Volume 260, pp. 271 - 282.
52. Gilbert, G. A., Knight, J. D., Vance, C. P. & Allan, D. L., 2002. Acid phosphatase activity in phosphorus deficient white lupin roots. *Journal of Plant, Cell and Environment,* Volume 22, pp. 801-810.
53. Golkar, P., Arzani, A., Rezaei, A. M., Yarali, Z. & Yousefi, M., 2009. Genetic variation of leaf antioxidants and chlorophyll content in safflower. *African Journal of Agricultural Research,* Volume 4, pp. 1475-1482.
54. Gopalakrishnan, S., Srinivas, V., Vemula, A., Samineni, S. & Rathore, A., 2018. Influence of diazotropic bacteria on nodulation, nitrogen fixation, growth promotion and yield traits in five cultivars of chickpea. *Journal of Biocatalysis and Agricultural Biotechnology,* Volume 15, pp. 35-42.
55. Gowami, S. P., Maurya, B. R. & Dubey, A. N., 2019. Role of phosphorus solubilizing microorganisms and dissolution of insoluble phosphorus in soil. *International Journal of Chemical Studies,* Volume 7, pp. 3905-3913.
56. Habibzadeh, Y., Jalilian, J., Zardashti, M. R., Pirzad, A. & Eini, O., 2015. Some morpho physiological charecteristics of mung bean mycorrhizal plant and different

irrigation regimes in field conditions. *Journal of Plant Nutrition,* Volume 38, pp. 1754-1767.

57. Haeder, D., 2022. Photosynthesis in plants and algae. *Anticancer Research,* Volume 42, pp. 5035-5041.
58. Harlan, J., 1992. Crops and Man. In: *American Society of Agronomy, Crop Science Society of America.* s.l.:Madison Wisconsin, pp. 63 - 262.
59. Hashem, H. A., Mansour, H. A., El-Khawas, S. A. & Hassanein, R. A., 2019. The potentiality of marine micro-algae as biofertilizer to improve the productivity and salt stress tolerance of canola (*Brassica napus* L) plants. *Agronomy,* Volume 9, p. 146.
60. Honnaiah, P. A., Sridhara, S., Gopakkali, P., Ramesh, N., Mahmoud, E. A., Abdelmohsen, S. A. M., [+2] & Elansary, H. O., 2021. Influence of sowing windows and genotypes on growth, radiation interception, conversion efficiency, and yield of guar. *Saudi Journal of Biological Sciences,* Volume 28, pp. 3453-3460.
61. Hoque, M. N., Imran, S., Hannan, A., Paul, N. C., Muhamud, A., [+4] & Rhaman, M. S., 2022. Organic amendments for mitigation of salinity stress in plants: A review. *Life,* Volume 12, p. 1632.
62. Hu, S., Ding, Y. & Zhu, C., 2020. Sensitivity and response of chloroplasts to heat stress in plants. *Journal of Frontiers in Plant Science,* Volume 11, p. 375.
63. Hyyrylainen, A., Rautio, P., Turunen, M. & Huttunen, S., 2015. Seasonal and interannual variation in the chlorophyll content of three co-existing Sphagnum species exceeds the effect of solar UV radiation in a Subarctic peat land. *Journal of SpringerPlus,* Volume 4, p. 478.
64. Igeohon, O. N. & Babalola, O., 2021. Rhizobium and mycorrhizall fungal species improved soybean yield under drought stress conditions. *Journal of Current Microbiology,* Volume 78, pp. 1615-1627.
65. Imran, A., Mirza, M.S., Shah, T.M., Malik, T.M. &Hafeez, F.Y., 2015. Differential response of kabuli and desi chickpea genotypes towards inoculation with PGPR in different soils. *Journal of Frontiers in Microbiology,* Volume 6, p. 859.
66. Irshad, S., Matloob, A., Igbal, S., Ibar, D., Hasnain, Z., Khan, S., Rashid, N., [+5] & Diao, Z., 2022. Foliar application of potassium and moringa leaf extract improves growth, physiology, and productivity of kabuli chickpea grown under varying sowing regimes. *Journal of PLOS ONE,* Volume 17.
67. Jahan, M., Mahallati, M. N., Amiri, M. B. & Ehyayi, H. R., 2013. Radiation absorption and use efficiency of sesame as affected by biofertilizers inoculation in a low input cropping system. *Industrial Crops and Products,* Volume 43, pp. 606-611.

68. Javanmard, A., Ashrafi, M., Morshedloo, M.R., Machiani, M.A., Rasouli, F. &Maggi, F., 2022. Optimizing phytochemical and physiological charecteristics of Balangu (*lallemantia iberica*) by foliar application of chitosan nanoparticles and mycoroot inoculation under water supply restrictions. *Journal of Horticulturae,* Volume 8, p. 695.
69. Jha, B. & Trivedi, J., 2021. Effect of different biofertilizer and fertility level on growth, quality, and yield attributes of cowpea (*Vigna unguiculata* L Walp). *The Pharma Innovation,* Volume 10, pp. 247-249.
70. Jiang, R., Gan, T.Y., Xie, J., Wang, N. & Kuo, C.C., 2017. Histirocal and potential changes of precipitation and temperature of Alberta subjected to climate change impact:1900-2100. *Journal of Theoretical and Applied Climatology,* Volume 127, pp. 725-739.
71. Jukanti, 2012. nutritional quality and health benefits of chickpea (*Cicer arientum* L). *Journal of nutrition,* Issue 108(S1), pp. 11-26.
72. Kafi, M., Nabati, J., Rezazadeh, E.B., Oskoueian, A. & Soureshjani, H.K., 2022. Single and poly capsule sesame (*Sesame indicum* L) productivity in response to plant growth-promoting rhizobacteria and foliar application of silicon, potassium, & calcium. *Acta Physiologiae Planturum,* Volume 44, p. 103.
73. Kalayu, G., 2019. Phosphate solubilising microorganisms: Promising approach as biofertilizers. *International journal of Agronomy,* Volume 2019, pp. 1-7.
74. Kaloki, P., Luo, Q., Trethowan, R. & Tan, D. Y., 2019. Can the development of droughttolerant ideotype sustain Australian chickpea yield? *International Journal of Biometeorology,* Volume 63, pp. 393-403.
75. Karimpour, M., 2019. Effect of drought stress on RWC and chlorophyll content on wheat (*Triticum Durum* L) genotypes. *World Essay Journal,* Volume 7, pp. 52-56.
76. Kaschuk, G., Alberton, O. & Hungria, M., 2011. Quantifying effects of different agricultural uses on soil microbial biomass and activity in Brazilian biomes: inferences to improve soil quality. *Journal of Plant and Soil,* Volume 338, pp. 467-481.
77. Kaur, S., Campbell, B. J. & Suseela, V., 2022. Root metabolome of plant-arbuscular mycorrhizal symbiosis mirrors the mutualistic or parasitic mycorrhizal phenotype. *New Phytologist,* Volume 234, pp. 672-687.
78. Kaur, S., Grewal, S. K., Singh, S. & Virk, H. K., 2022. Impact of phosphorus- deficit conditions on morpho-physiological traits and phosphorus metabolism in chickpea genotypes. *Protoplasma,* Volume 259, pp. 775-788.
79. Kavadia, A., Omirou, M., Fasoula, D., Trajanoski, S., Andreou, E. & Loannides, I.M., 2020. Genotype and soil water availability shape the composition of MAF

communities at chickpea early growth stages. *Applied Soil Ecology,* Volume 50, p. 103443.

80. Kawakami, K. & Shen, J. R., 2018. Chapter one-purification of fully active and crystalizable photosystem II from thermophilic cyanobacteria. *Journal of Methods in Enzymology,* Volume 613, pp. 1-6.
81. Kendig, S. R., Rupe, J. C. & Scott, H. D., 2000. Effect of irrigation and soil water stress on densities of Macrophomina phaseolina in soil and roots of two soybean cultivars. *Journal of Plant Diseases,* Volume 84, pp. 895-900.
82. Khaitov, B. & Abdiev, A., 2018. Perfomance of chickpea (*Cicer arietinum* L) to biofertilizer and nitrogen appliaction in arid condition. *Journal of Plant Nutrition,* Volume 41, pp. 1980-1987.
83. Khangarot, A. K., Yadav, S. S., Jakhar, R. & Rajesh, 2022. Physiological parameters of mungbean (*Vigna radiata* L. Wilczek) as influenced by application of prom and microbial inoculants. *The Pharma Innovation,* Volume 11, pp. 774-777.
84. Khan, N., Bano, A. & Cura, J. A., 2020. Role of beneficial microorganisms and salicylic acid in improving rainfed agriculture and future food safety. *Journal of Microorganisms and Plant Nutrition,* Volume 8, p. 1018.
85. Khan, N., Bano, A. & Cura, J. A., 2020 "Role of beneficial microorganisms and salicylic acid in improving rainfed agriculture and future food safety." *Journal of Microorganisms and Plant Nutrition* 8: 1018.
86. Kizilkaya, R., 2018. Yield response and nitrogen concentration of spring wheat (*Triticum aestivum*) inoculation with *Azobacter chroococcum* strains. *Ecological Engineering,* Volume 33, pp. 150-160.
87. Kour, D., Rana, K.L., Yadav, A.N., Yadav, N., Kumar, M., Kumar, V., Vyas, P., Dhaliwal, H.S. & Saxena, A.K., 2019. Microbial biofertilizers bioresources and eco-friendly technologies for agricultural and environmental sustanability. *Journal of Biocatalysis and Agricultural Biotechnoogy.,* Volume 23, p. 101487.
88. Koyama, T., Adachi, K. & Suzuki, T., 2019. Response of soybean plants to two inoculation methods with arbuscular mycorrhizal fungus of *Glomus* sp. strain R-10 under field condition. *Journal of Plant Production Science,* Volume 22, pp. 215 - 219.
89. Krishna, G., 2017. Effect of conserved soil moisture, phosphorus levels and bacterial inoculation on dry matter production and uptake pattern of phosphorus by chickpea. *Indian Journal of Pulses Research,* Volume 18, pp. 32-35.
90. Kumari, N., Mondal, S., Mahapatra, P., Meetei, TT. & Den, YB., 2019. Effect of Biofertilizer and Micronutrients on Yield of Chickpea. *International Journal of Current Microbiology and Applied Sciences,* Volume 8, pp. 2389-2397.

91. Kumari, P., Rastogi, A. & Yadav, S., 2020. Effects of heat stress and molecular mitigation approaches in orphan legume, chickpea. *Journal of Molecular Biology Reports,* Volume 47, pp. 4659-4670.
92. Kumar, J., Kumar, S. & Prakash, V., 2019. Effect of biofertilizers and phosphorus levels on soil fertility, yield and nodulation in chickpea (*Cicer arietinum* L). *Journal of the Indian Society of Soil Science,* Volume 67, pp. 199-203.
93. Kutu, F. R. & Asiwe, J. A. N., 2010. Assessment of maize and dry bean productivity under different intercrop systems and fertilization regimes. *African Journal Agricultural Research,* Volume 5, pp. 1627-1630.
94. Kuwada, K., Wamocho, L.S., Utamura, M., Matsushita, M. & Ishii, T., 2006. Effect of red and green algal extracts on hyphal growth of arbuscular mycorrhizal fungi and on mycorrhizal development and growth of papaya and passion fruit. *Agronomy Journal,* Volume 98, pp. 1340-1344.
95. Kyei-Boahen, S., Savala, C., Chikoye, D. & Abaidoo, R., 2017. Growth and yield response of cowpea to inoculation and phosphorus fertilization in different environments. *Journal of Frontiers in Plant Science,* p. 00646.
96. Lazali, M. & Drevon, J. J., 2021. Role of acid phosphatase in the tolerance of the rhizobial symbiosis with legumes to phosphorus deficiency. *Springer Nature,* Volume 52, pp. 1469-1483.
97. Lemus, R., Parrish, D. J. & Abaye, O., 2008. Nitrogen-use dynamics in switchgrass grown for biomass. *Bioenergy Research,* Volume 1, pp. 153-162.
98. Li, R., Chen, H., Yang, Z. & Yuan, S., 2020. Research status of soybean symbiosis nitrogen fixation. *Oil Crop Science,* Volume 5, pp. 6-10.
99. Liu, Y., Zhang, M., Meng, Z., Wang, B. & Chen, M., 2020. Research progress on the roles of cytokinin in plant response to stress. *International Journal of Molecular Sciences,* Volume 21, p. 6574.
100. Lokesh, R., Rakholiya, K.B. & Thesiya, M. R., 2020. Evaluation of different fungicides against macrophomina phaseolina (Tassi) goid. causing dry root rot of chickpea (*Cicer arietinum* L) invitro. *International Journal of Current Microbiology and Applied Science,* Volume 9, pp. 901911.
101. Luo, G., Sun, B., Li, L., Li, M., Zhu, Y., Guo, S., Ling, N. & Shen, Q., 2019. Understanding how long-term organic amendments increase soil phosphatase activities: insight into phoD- and phoC-harboring functional microbial populations. *Soil Biological Biochemistry,* Volume 139, p. 107632.

102. Lusiba, S., Odhiambo, J. & Ogola, J., 2016. Effect of biochar and phosphorus fertilizer application on soil fertility: soil physical and chemical properties. *Archives of Agronomy and Soil Science,* Volume 63, pp. 1-13.

103. Macil, P., 2018. *Effect of biochar and rhizobium inoculation on nodulation, chlorophyll content, growth and yield of chickpea (Cicer arietinum L).* Thohoyandou; South Africa, Masters Dessertation, University of Venda.

104. Macil, P. J., Ogola, J. B. O. & Odhiambo, J. J. O., 2020. Response on soil pH and nodulation of three chickpea genotypes to biochar and rhizobium inoculation. *Journal of communications in soil science and Plant analysis,* Volume 51, pp. 23772387.

105. Mahmoud, S. H., Salama, D. M., El-Tanahy, A. M. M. & El-Samad, E. H. A., 2019. Utilization of seaweed (*Sargassum vulgare*) extract to enhence growth, yield and nutritional quality of red radish plants. *Annals of Agricultural Science,* Volume 64, pp. 167-175.

106. Makoi, J. H. J. R., Chimphango, S. B. M. & Dakora, F. D., 2010. Elevated levels of acid and alkaline phosphatase activity in roots and rhizosphere of cowpea (*Vigna unguiculata* L. Walp) genotype grown in mixed culture and at different densities with sorghum (*Sorghum bicolor* L). *Journal of Crop and Pasture Science,* Volume 61, pp. 279-286.

107. Makonya, G. M., Ogola, J. B. O., Muasya, A. M., Crespo, O., Maseko, S., Valentine, A. J., Ottosen, C., Rosenqvist, E. &Chimphango, S. B. M., 2020. Intermittent moisture supply induces drought priming responses in some heat-tolerant chickpea genotypes. *Crop Science,* Volume 60, pp. 2527-2542.

108. Makonya, G. M., Ogola, J. B. O., Muasya, A. M., Crespo, O., Maseko, S., Valentine, A. J., Ottosen, C., Rosenqvist, E. &Chimphango, S. B. M., 2019. Chlorophyll fluorescence and carbohydrate concentration as field selection traits for heat-tolerant chickpea genotypes. *Journal of Plant Physiology and Biochemistry,* Volume 141, p. 1016.

109. Martinez, F. D. G., Castorena, E. V. G., Uribe, V. V. E., Alvarado, R. E. V. & Saenz, E. O., 2022. Sustainability of the soil resource in intensive production with organic contributions. *Agronomy,* Volume 12, p. 67.

110. Maseko, S. T. & Dakora, F. D., 2013. Plant Enzymes, Root Exudates, Cluster Roots and Mycorrhizal Symbiosis are the drivers of P Nutrition in Native Legumes Growing in P Deficient Soil of the Cape Fynbos in South Africa. *Journal of Agricultural Science and Technology A.,* Volume 3, pp. 331-340.

111. Meena, L. R., Singh, R. K. & Gautam, R. C., 2005. effect of conserved soil moisture, phosphorus levels and bacterial inoculation on dry matter production and uptake pattern of phosphorus by chickpea. *Indian Journal of Pulses Research,* Volume 18, pp. 32-35.

112. Mehrvarz, S., Chaichi, M. R. & Alikhani, H. A., 2008. Effect of phosphate solubilising microorganisms and phosphorus chemical fertilizer on yield and yield components of barley (*Hordeum Vulgare* L). *Am-Eurasian Journal of Agriculture Environment Science,* Volume 3, pp. 822-828.

113. Menal-Puey, S., Martinez-Biarge, M. & Marques-Lopez, I., 2018. Developing a Food Exchange System for Meal Planning in Vegan Children and Adolescents. *Journal of Nutrients,* Volume 11, p. 43.

114. Merga, B, and J Haji. "Economic importance of chickpea:production, value, and world trade." *Journal of Cogent Food Agriculture* 5 (2019): 1615718.

115. Mi, J., Agrawal, S., Kossler, S., Tureckova, V., Tarkowska, D., Thiele, W., Al-Babili, S., Bock, R. & Schottler, M. A., 2020. Expression of a carotenogenic gene allows faster biomass production by redesigning plant architecture and improving photosynthetic efficiency in Tobacco. *The Plant Journal,* Volume 103, pp. 1956-1984.

116. Millan, T., Madrid, E., Castro, P., Gil, J. & Rubio, J., 2015. Handbook of Plant Breeding. In: A. M. D. Ron, ed. *Grain Legumes.* New York: Springer Science +Business Media, pp. 85-103.

117. Mitran, T., Meena, R. S., Lal, R., Layek, J., Kumar, S. &Datta, R., 2018. Role of phosphorus on legume production. In: R. Meena, A. Das, G. Yadav & R. Lal, eds. *Legumes for soil health and sustainable management.* Singapore: Springer, pp. 487-510.

118. Mogale, B., Lengwati, M. D., Soundy, P., Dakora, F. D. & Maseko, S. T, 2018. Effect of P and biostimulants appication on growth and tissue acid phosphatase activity of desi and kabuli at Klipplaatdrift, South Africa. *Journal of Botany.,* Volume 115, p. 299.

119. Moloto, R. M., Dakora, F. D., Soundy, P. & Maseko, S. T., 2021. Effects of biostimulants on tissue and rhizospheric acid phosphatase activity of chickpea genotypes. *South African Journal of Plant and Soil,* Volume 38, pp. 180-183.

120. Moscatelli, M. C., Secondi, L., Marabottini, R., Papp, R., Stazi, S. R., Mania, E. & Marinari, s., 2018. Assessment of soil microbial functional diversity: land use and soil properties affect CLPP-MicroResp and enzyme responses. *Pedobiologia,* Volume 66, pp. 36-42.

121. Moustakas, M., Sperdouli, L. & Moustakas, J., 2022. Early drought stress warning in plants: color pictures of photosystem II photochemistry. *Climate,* Volume 10, p. 179.

122. Mubvuma, M. T., Ogola, J. B. O. & Mhizha, T., 2021. Effect of planting date and genotype on intercepted radiation and radiation use efficiency in chickpea crop (*Cicer arietinum* L). *journal of Cogent Food & Agriculture,* Volume 7.

123. Mu, X. & Chen, Y., 2021. The physiological response of photosynthesis to nitrogen deficiency. *Journal of Plant Physiology and Biochemistry,* Volume 158, pp. 76-82.

124. Nannipieri, P., Giagnoni, L., Landi, L. & Renella, G., 2011. *Role of phosphatase Enzymes in soil.In:Bunemann E., Oberson A., Frossard E. (eds) phosphorus in Action.,* Berlin,Heidelberg.: Springer.

125. Nosheen, S., Ajmal, I. & Song, Y., 2021. Microbes as biofertilizers, a potential approach for sustainable crop production. *Journal of Sustainability,* Volume 13, p. 1868.

126. Nziguheba, G., 2007. Overcoming phosphorus deficiency in soils of Eastern Africa: recent advances and challenges. In: A. Bationo, B. Waswa, J. Kihara & J. Kimetu, eds. *Advances in Integrated Soil Fertility Management in sub-Saharan Africa: Challenges and Opportunities.* s.l. :Springer,Dordrecht, pp. 149-160.

127. Ogola, J. B. O., Macil, P. J. & Odhiambo, J. J. O., 2021. Biochar application and rhizobium inoculation increased intercepted radiation and yield of chickpea in contrasting soil types. *International Journal of Plant Production,* Volume 15, pp. 219229.

128. Ogola, J. O., 2015. Growth and yield response of chickpea to Rhizobium inoculation: productivity in relation to interception of radiation. *Journal of Legume Research,* Volume 38, pp. 837-843.

129. Ogola, J., Wheeler, T. R. & Harris, P. M., 2002. Water use of maize crop in response to nitrogen and irrigation. *Journal of Field Crops Research,* Volume 78, pp. 105-117.

130. Ort, D. R., Merchant, S. S., Alric, J., Barkan, A., Blankenship, R. E., Bock, R., Croce, R., Hanson, M. R. & Zhu, G. X., 2015. Redesigning photosynthesis to sustainably meet global food and bioenergy demand. *Proceedings of the National Academy of Sciences of the Unites State of America,* Volume 112, pp. 8529-8536.

131. Oukaltouma, K., El-Moukhtari, A., Lahrizi, Y., Mouradi, M., Farissu, M., Willems, A., Qaddourg, A., Bekkaoui, F. & Ghoulam, C., 2021. Phosphorus deficiency

enhences water deficit impact on some morphological and physiological traits in four faba bean (*Vicia faba* L) varieties. *Journal of Agronomy,* Volume 16.

132. Palm, C. A., Ganchengo, C. N., Delve, R. J., Cadisch, G. & Giller, K. E., 2001. Organic inputs for soil fertility management in tropical agrosystems: application of an organic resource database. *Journal of Agricultural Ecosystems Environments.,* Volume 83, pp. 27-42.

133. Palta, J. P., 1990. Leaf chlorophyll content. *Remote sensing reviews,* Volume 5, pp. 207-213.

134. Pandey, S. & Gupta, S., 2020. Diversity analysis of ACC deaminase producing bacteria associated with rhizosphere of coconut tree (*Cocos nucifera* L) grown in Lakshadweep islands of India and their ability to promote plant growth under saline conditions. *Journal of Biotechnology,* Volume 324, pp. 183-197.

135. Pang, J., Bansal, R., Zhao, H., Bohuon, E., Lambers, H., Ryan, M. H., Ranathunge, K. & Siddique, K. H. M., 2019. The carboxylate-releasing phosphorus mobilizing strategy can be proxied by foliar manganese concentration in a large set of chickpea germplasm under low phosphorus supply. *Journal of New Phytologist Foundation,* Volume 219, pp. 518-529.

136. Poorter, H. & Nagel, O., 2000. The role of biomass growth in the growth response of plants to different levels of light, CO2, nutrients, and water: a quantitative review. *Australian Journal of Plant Physiology,* Volume 27, pp. 595-607.

137. Pramanik, P., Chakrabarti, B., Bhatia, A., Singh, S. P., Maity, A., Aggrwal, P. & Krishnan, P., 2018. Effects of elevated temperature on soil hydrothermal regimes and growth of wheat crop. *Environmental Monitoring and Assessment,* Volume 190, pp. 1-10.

138. Rachaputi, R. C. N., Sands, D., McKenzie, K., Chauhan, Y., Bell, K., Seyoum, S., Agius, P., Krosch, S. & Lehane, J., 2021. Can partial reduction of shoot biomass during early vegetative phase of chickpea save subsoil water for reproductive and pod filling. *Agricultural Water Management,* Volume 247, p. 106704.

139. Rafat, A. & Mostafa, D., 2022. Differential responses of common bean, (*Phaseolus vulgaris* L) to the interactive effects of ascorbic acid and Trichoderma harzianum under salinity stress. *Egyptian Academic Journal of Biological Sciences, H. Botany,* Volume 13, pp. 11-29.

140. Raimi, A., Adeleke, R. & Roopnarain, A., 2017. Soil fertility challenges and biofertilizers as a viable alternative for increasing smallholder farmer crop productivity in Sub-Saharan Africa. *Journal of Cogent Food and Agriculture.,* Volume 3, p. 140093.

141. Rani, A., Devi, P., Jha, U. C., Sharma, K. D., Siddique, K. H. M. & Nayyar, H., 2020. Developing climate-resilient chickpea involving physiological molecular approaches with a focus on temperature and drought stresses. *Frontiers in Plant Sciences,* Volume 10, p. 1759.

142. Rashidi, M., Abbasi, N. O. & Zarea, M., 2018. Effect of phosphorus biofertilizers and chemical on element accumulation, chlorophyll content, seed yield and root growth of three local mung bean (*Vigna radiata* L) populations. *Journal of Crop Ecophysiology,* Volume 12, pp. 631-650.

143. Reddy, M. M., Mehera, B. & Thodkar, B. E., 2022. Effect of biofertilizers and micronutrients (Zn &B) on growth, yield, and economics of chickpea (*Cicer arietinum* L). *The Pharma Innovation,* Volume 11, pp. 367-370.

144. Rengasamy, K. R. R., Aderogba, M. A., Amoo, S., Stirk, W. A. & Van Staden, J., 2013. Potential antiradical and alpha-glucosidaoe inhibitors from Ecklonia maxima (Obseck) Papenfuss. *Food Chemistry,* Volume 141, pp. 1412-1415.

145. Rengasamy, K. R. R., Kulkarni, M. G., Stirk, W. A. & Van Staden, J., 2015. Eckol-a new plant growth stimulant from the brown seaweed *Ecklonia maxima*. *Journal of Environmental Biology and Fishes,* Volume 27, pp. 581-587.

146. Rivas, M. A., Friero, I., Alarcon, M. V. & Salguero, J., 2022. Auxin-cytokinin balance shapes maize root architecture by controlling primary root elongation and lateral root development. *Frontiers in Plant Science,* Volume 13, p. 836592.

147. Rychter, A. M., Rao, I. M. & Cardoso, J. A., 2018. Role of phosphorus in photosynthetic carbon assimilation and partitioning. In: M. Pessarakli, ed. *Handbook of photosynthesis.* Boca Raton: CRC Press, pp. 603-625.

148. Sangakkara, U., Frehner, M. & Nosberger, J., 2000. Effect of soil moisture and potassium fertilizer on shoot water potential, photosynthesis and partitioning of carbon in mung bean and cowpea. *Journal of Agronomy and Crop Science,* Volume 185, pp. 201-207.

149. Senger, M, Moresco, E., Dalbosco, M., Santin, R., Inderbitzin, P. & Barrocas, E. N., 2022. Methods to quantify Bacillus Simplex-based inoculant and its effect as a seed treatment on field-grown corn and soybean in Brazil. *Journal of Seed Science,* Volume 44, pp. 1-13.

150. Seyahjani, E. A., Yamia, M., Farahvash, F., Benam M. B. K. & Rahmani, H. A., 2020. Influence of rhizobium, pseudomonas ans mycorrhiza on some physiological traits of red bean (*Phaseolus vulgaris* L) under different irrigation conditions. *Legume Research-An International Journal,* Volume 43, pp. 81-86.

151. Sharifi, R. S., Khalilzadeh, R., Pirzad, A. & Anwar, S., 2020. Effect of biofertilizers and nano zinc-iron oxide on yield and physicochemical properties of wheat under water deficit conditions. *Communications in Soil Science and Plant Analysis,* Volume 51, pp. 2511-2524.

152. Shinde, P. & Hunje, R., 2019. Influence of soil application of organic manures and foliar spray of liquid biofertilizer on growth and seed yield of Kabuli chickpea (*Cicer arietinum* L) varieties. *Journal of Agricultural Reasearch Communication Centre,* Volume 4193, pp. 1-6.

153. Shirkhani, A. & Nasrolahzadeh, S., 2016. Vermicompost and Azotobacter as an ecological pathway to decrease chemical fertilizers in the maize, *Zea mays*. *Bioscience Biotectnology Research Communications,* Volume 9, pp. 382-390.

154. Shravani, K., Triveni, S., Latha, P. C. & Chari, D., 2019. Influence of biofertiizers under drip fertigation system on soil microbial population and enzyme activities in the rhizosphere of mungbean. *Asian Journal of Microbiology and Biotechnology environmental Science,* Volume 21, pp. 67-72.

155. Singh, B. & Satyanarayana, T., 2011. Microbial phyatases in phosphorus acquisition and plant growth promotion. *Physiology and Molecular Biology of Plants,* Volume 17, pp. 93-103.

156. Singh, G., Sekhon, H. S. & Sharma, P., 2011. Effect of irrigation and biofertilizer on water use, nodulation, growth, and yield of chickpea (*Cicer arietinum* L). *Journal of Archives Agronomy Soil Science.,* Volume 57, pp. 715-726.

157. Singh, K. & Saxena, M., 1978. *The Chickpea.* Wellingford, Oxon, UK: Commonwealth Agricultural Bureaux International.

158. Singh, R., Pratap, T., Singh, D., Singh, G. & Singh, A. K., 2018. Effect of phosphorus, Sulphur, and biofertilizers on growth attributes and yield of chickpea (*Cicer arietinum* L). *Journal of Pharmacognosy and Phytochemistry,* Volume 7, pp. 3871-3875.

159. Sofi, S. A. Muzaffar, K., Ashraf, S., Gupta, I. & Mir, S. A., 2020. Chickpea. In: A. Manickavasagan & P. Thirunathan, eds. *Pulses.* Switzerland AG: Springer, Cham, pp. 55-76.

160. Soil Classification, 1991. *A Taxonomic System for South Africa.* Second Edition ed. Pretoria: The Department of Agricultural Development Republic of South Africa.

161. Solaiman, A. R. M., Talukder, M. S. & Rabbani, M. G., 2010. Influence of some rhizobium strains on chickpea nodulation, dry matter yield, and N uptake. *Bangladesh Journal of Microbiology,* Volume 27, pp. 61-64.

162. Sonivaldo, R B, M S C Rayane, L B L Emerson, and A Odair. "Meta-analysis of Lamiaceae and Euphorbiaceae medicinal plants inoculated with arbuscular mycorrhizal fungi." *Australian Journal of Crop Science* 13 (2019): 588-598.

163. Spohn, M. & Kuzyakov, Y., 2013. Phosphorus mineralization can be driven by the microbial need for carbon. *Journal of Soil Biology and Biochemistry,* Volume 61, pp. 60-75.

164. Spohn, M. Treichel, N. S., Comann, M., Schloter, M. & Fischer, D., 2015. Distribution of phosphatase activity and various bacterial phyla in the rhizosphere of *Hordeum vulgare* L depending on P availability. *Journal of Soil Biology and Biochemistry,* Volume 89, pp. 44-51.

165. Stirk, 2004. changes in cytokinin and auxin concentration in seaweed concentrates when stored at an elevated temperature. Volume 16, pp. 31-39.

166. Sun, B. R., Gao, Y. Z. & Lynch, J. P., 2018. Large ceown root number improves topsoil foraging and phosphorus acquisition. *Journal of Plant Physiology,* Volume 177, pp. 90-104.

167. Sun, L., Xu, H., Hao, H., An, S., Lu, C., Wu, R. & Su, W., 2019. Effects of bensulfuron-methyl resisude on photosynthesis and chlorophyll fluorescence in leaves of cucumber seedlings. *PLoS ONE,* Volume 14.

168. Suriyagoda, L. D. B., Ryan, M. H., Renton, M. & Lambers, H., 2010. Multiple adaptative responses of Australian native perennial legumes with pasture potential to grow in phosphorus- and moisture-limited environments. *Annals of Botany,* Volume 105, pp. 755-767.

169. Szczepanek, M., Wszelaczynska, E. & Poberenzy, J., 2018. Effect of seaweed biostimulant application in spring wheat. *Agrolife Scientific Journal,* Volume 7, pp. 131136.

170. Tabatabai, M., 1994. Soil Enzymes. In: *Methods of soil analysis part 2; Microbiological and biochemical properties.* s.l.:Soil Science Society of America Book., pp. 77 - 859.

171. Tadross, M. A., Gutowski, W. J., Hewitson, B. C., Jack, C. & New, M., 2006. MM5 simulation of interannual change and the diurnal cycle of Southern African regional climates. *Journal of Theoratical and Applied Climatology,* Volume 86, pp. 63-80.

172. Takele, E., Mekbib, F. & Mekonnen, F., 2022. Genetic variability and characters association for yield, yield attribiuting traits and protein content of lentil (*Lens Culinaris Medikus*) genotype in Ethiopia. *CABI Agriculture and Bioscience,* Volume 3, pp. 1-14.

173. Tardieu, F., Katerji, N., Bethenod, O., Zhang, J. & Davies, W. J., 1991. Maize stomatal conductance in the field: its relationship with soil and plant water potentials, mechanical constraints, and ABA concentration in the xylem sap. *Plant Cell Environment,* Volume 14, pp. 121-126.

174. Thangwana, N. M. & Ogola, J. O., 2012. Yield and yield components of chickpea (*Cicer arietinum* L): Response to genotype and planting density in summer and winter sowings. *Journal of Food, Agriculture& Environment,* Volume 10, pp. 710715.

175. Tian, J. & Liao, H., 2015. The role of intracellular and secreted purple acid phosphatase in plant phosphorus scavenging and recycling. *Annual Plant Reviews Online,* Volume 48, pp. 265-287.

176. Wolde-Meskel, E., Van Heerwaarden, J., Abdulkadir, B., Kassa, S., Aliyi, I., Deyefu, T., Wakweya, K., Kanampiu, F. & Giller, K. E., 2018. Addictive yield response of chickpea (*Cicer arientinum* L.) to rhizobium inoculation and phosphorus fertiliser across smallholder farms in Ethopia. *Journal of Agriculture, Ecosystems and Environment.,* Volume 261, pp. 144-152.

177. Yadav, 2017. Compatible rhizosphere-competent microbial consortium adds value to the nutritional quality in edible parts of chickpea. *Agricultural Food Chemistry,* Volume 65, pp. 22-30.

178. Yadav, K. K. & Sarkar, S., 2019. Biofertilizers, impact on soil fertility and crop productivity under sustainable agriculture. *Journal of Environmental and Ecology,* Volume 37, pp. 89-93.

179. Yang, J., Kloepper, J. W. & Choong-Min, R., 2009. Rhizosphere bacteria help plants tolerate abiotic stress. *Trends in Plant Science,* Volume 14, pp. 1-4.

180. Yao, Q., Li, X., Feng, G. & Christie, P., 2001. Mobilization of sparingly soluble inorganic phosphates by external mycelium of an abuscular mycorrhizal fungus. *Journal of Plant and Soil.,* Volume 230, pp. 279 - 285.

181. Yousef, F., Shafique, F., Ali, Q. & Malik, A., 2020. Effcet of salt stress on the growth traits of chickpea (*Cicer arietinum* L) and pea (*Pisum sativum* L) seedlings. *Journal of Biological and Clinical Sciences Reaserch,* Volume 2020, pp. 1-6.

182. Yousefi, A., Mirzaeitalarposhti, R., Nabati, J. & Soufizadeh, S., 2021. Evaluation radiations use efficiency and growth indicators on two mung bean (*Vigna radiata* L) genotypes under the influence of biological fertilizers. *Journal of Plant Nutrition,* Volume 44, pp. 1095-1106.

183. Zhang, X. Y., Chen, S., Sun, H., Wang, Y. & Shao, L., 2009. Root size distribution and soil water depletion as affected by cultivars and environmental factors. *Journal of Field Crop Research,* Volume 114, pp. 75-83.

APPENDICES

Appendix 1: Correlation between APase, AlkPase activity, Pi, and grain yield for (a) Thohoyandou and (b) Syferkuil

(a)

Correlations

	APase	AlkPase activity	Pi
AlkPase activity	-0,128		
Pi	0,051	0,194	
GY	-0,225	-0,035	-0,018

Pairwise Pearson Correlations

Sample 1	Sample 2	Correlation	95% CI for ρ	P-Value
AlkPase activity	APase	-0,128	(-0,438. 0,210)	0,458ns
Pi	APase	0,051	(-0,282. 0,373)	0,768ns
GY	APase	-0,225	(-0,515. 0,112)	0,187ns
Pi	AlkPase activity	0,194	(-0,144. 0,491)	0,258ns
GY	AlkPase activity	-0,035	(-0,360. 0,297)	0,839ns
GY	Pi	-0,018	(-0,344. 0,313)	0,918ns

(b)

	APase	AlkPase activity	Pi
AlkPase activity	-0,110		
Pi	0,044	0,125	
GY	0,279	-0,344	-0,017

Pairwise Pearson Correlations

Sample 1	Sample 2	Correlation	95% CI for ρ	P-Value
AlkPase activity	APase	-0,110	(-0,423. 0,227)	0,525ns
Pi	APase	0,044	(-0,289. 0,367)	0,799ns
GY	APase	0,279	(-0,055. 0,556)	0,100ns
Pi	AlkPase activity	0,125	(-0,213. 0,435)	0,469ns
GY	AlkPase activity	-0,344	(-0,604. -0,018)	0,040*
GY	Pi	-0,017	(-0,344. 0,313)	0,921ns

Appendix 2: Correlation between grain yield and yield components for (a) Thohoyandou and (b) Syferkuil 2021.

(a)

	BMU	GYU	HIU
GYU	0,755		
HIU	-0,337	0,328	
PDU	0,540	0,753	0,237

Pairwise Pearson Correlations

Sample 1	Sample 2	Correlation	95% CI for ρ	P-Value
GYU	BMU	0,755	(0,568. 0,868)	0,000***
HIU	BMU	-0,337	(-0,599. -0,010)	0,044*
PDU	BMU	0,540	(0,257. 0,738)	0,001***
HIU	GYU	0,328	(-0,000. 0,593)	0,051*
PDU	GYU	0,753	(0,563. 0,867)	0,000***
PDU	HIU	0,237	(-0,100. 0,524)	0,165ns

(b)

Correlations

	GYL	BYL	HIL
BYL	0,491		
HIL	0,106	-0,772	
PDL	0,646	0,799	-0,496

Pairwise Pearson Correlations

Sample 1	Sample 2	Correlation	95% CI for ρ	P-Value
BYL	GYL	0,491	(0,193. 0,705)	0,002**
HIL	GYL	0,106	(-0,230. 0,420)	0,538ns
PDL	GYL	0,646	(0,403. 0,804)	0,000***
HIL	BYL	-0,772	(-0,878. -0,594)	0,000***
PDL	BYL	0,799	(0,639. 0,893)	0,000***
PDL	HIL	-0,496	(-0,709. -0,200)	0,002**

Appendix 3: Correlation between number of nodules, nodule fresh weight, nodule dry weight, shoot biomass, and inorganic phosphorus (Pi).

Method

Correlations

	Fresh (W)	Dry (W)	No(nodules)	BMU
Dry nodules	0,596			
No(nodules)	0,431	0,322		
Shoot Biomass	-0,005	0,002	0,296	
Pi	0,233	-0,059	0,135	-0,247

Pairwise Pearson Correlations

Sample 1	Sample 2	Correlation	95% CI for ρ	P-Value
Dry nodules	Fresh nodules	0,596	(0,332. 0,773)	0,000***
No(nodules)	Fresh nodules	0,431	(0,119. 0,665)	0,009**
Shoot Biomass	Fresh nodules	-0,005	(-0,333. 0,324)	0,978ns
Pi	Fresh nodules	0,233	(-0,103. 0,522)	0,171ns
No(nodules)	Dry nodules	0,322	(-0,007. 0,588)	0,056ns
Shoot Biomass	Dry nodules	0,002	(-0,327. 0,330)	0,991ns
Pi	Dry nodules	-0,059	(-0,380. 0,275)	0,732ns
Shoot Biomass	No(nodules)	0,296	(-0,037. 0,569)	0,080ns
Pi	No(nodules)	0,135	(-0,202. 0,444)	0,431ns
Pi	Shoot Biomass	-0,247	(-0,532. 0,089)	0,146ns

Appendix 4: The analysis of variance (ANOVA)

source of variance	degrees of freedom

Replication	r-1=2
Genotype (G)	a-1=1
Biofertilizer (B)	b-1=5
G x B	(a-1) (b-1) =5
Error	22
Total	35

Appendix 5: Response of number of nodules and dry weight per plant to genotype and biofertilizer at Thohoyandou (a) 2019 & (b) 2021

(Nemaembeni, 2019)

	No of nodules per plant	Nodule dry weight (g)/plant
Genotype		
ACC#7	22.778^{a}	0.9339^{a}
ACC#3	16.667^{b}	0.7278^{b}
S.E.D	1.1345	1.9650
Biofertilizer		
Rhizobium+Kelpak	38.333^{a}	1.4600^{a}
Kelpak	20.000^{b}	0.8383^{b}
Rhizbium+Mycoroot	18.167^{bc}	0.7767^{bc}
Rhizobium	15.833^{bc}	0.6983^{bc}
Mycoroot	13.833^{c}	0.6333^{bc}
Control	12.167^{c}	0.5783^{c}
S.E.D	0.0388	0.0672
P-value		
Genotype(G)	**	**
Biofertilizer(B)	**	**
G x B	ns	ns
CV (%)	17.26	14.01

**highly significant (P≤0.001), significant *(P≤0.05) and ns (non-significant). Means in the same column followed by the same letter are not significantly different, SE (Standard error) and CV (coefficient of variation).

(Kgaditse,2021)

	fresh (W)	Dry(W)	no of nodules.
Biofertilizer			
M+ R	3,84 ab	1,52 ab	1,59abc
K+R	3,47 ab	2,18a	2,48 a
R	2,96 ab	0,85bcd	1,00abc
K	2,13bc	0,64cd	0,77bc
M	1,12 c	1,41 c	0,53 c
C	3,97 a	1,32 bc	2,13 ab
S.E. D	0,56	0,23	0,50
Genotype			
ACC#7	3,14 a	1,12 a	1,52 a
ACC#3	2,69 a	1,19 a	1,32 a
S.E. D	0,32	0,13	0,29
P value			
Biofertilizer (B)	***	***	**
Genotypes (G)	ns	ns	ns
B*G	ns	ns	ns
CV (%)	32,27	33,46	59,57

Means followed by the same letter are not significantly different, *** p (<0,001), **p (<0,01), *p (<0,05) and CV (coefficient of variation)

Appendix 6: correlation between number of nodules, fresh nodules, biomass, and NDVI at 26, 40, and 54DAE at Syferkuil.

Correlations

	26DAE	40DAE	54DAE	Fresh (W)	Dry (W)	No(nodules)
40DAE	0,281					
54DAE	0,370	0,703				
Fresh (W)	0,341	-0,030	0,084			
Dry (W)	0,178	-0,172	0,100	0,596		
No(nodules)	0,402	0,264	0,514	0,431	0,322	
Biomass	0,016	0,357	0,361	-0,005	0,002	0,296

Pairwise Pearson Correlations

Sample 1	Sample 2	Correlation	95% CI for ρ	P-Value
40DAE	26DAE	0,281	(-0,052. 0,558)	0,096
54DAE	26DAE	0,370	(0,047. 0,623)	0,026*
Fresh (W)	26DAE	0,341	(0,015. 0,602)	0,042*
Dry (W)	26DAE	0,178	(-0,160. 0,478)	0,299
No(nodules)	26DAE	0,402	(0,084. 0,645)	0,015*
Biomass	26DAE	0,016	(-0,315. 0,342)	0,928
56DAE	40DAE	0,703	(0,487. 0,838)	0,000***