THE EFFECT OF PHOSPHORUS FERTILIZER AND *BRADYRHIZOBIUM* INOCULATION ON GRAIN YIELD AND NUTRIENTS ACCUMULATION IN TWO CHICKPEA (*Cicer aritienum* L). GENOTYPES

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DECLARATION

I, Madzivhandila Vhulenda (student number: 11627486), hereby declare that the dissertation submitted for the degree of Master of Science (MSc.) in Agriculture (Plant Production) at The University of Venda is my original work and has not been submitted for any degree or examination at this or any other University. I further declare that all sources cited or quoted are indicated and acknowledged by means of a comprehensive list of references.

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DEDICATION

I dedicate this dissertation to the Lord Jesus Christ for the wonderful gift of life and good health throughout my study period, to my family and friends who supported me during this study and my wonderful husband, Mr Mudzanani Ndialivhuwa, for being my number one constant supporter who had faith in me even when I had lost the will to carry on, and reminding me always why I had started this project.

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ABSTRACT

Chickpea (*Cicer aritienum* L.) is an ancient crop that originated in South-Eastern Turkey and belongs to the genus *Cicer*, tribe *Cicereae*, and family Fabaceae. Chickpea has the ability to fix atmospheric nitrogen (N) for its growth. However, chickpea productivity not only depends on N_2 fixation or dry matter accumulation, but also the effectiveness of nutrient partitioning to seed, a key component to overall yield. There is a dearth of information on the effect of P with rhizobial inoculation in response of nutrients accumulation in the rhizosphere, shoots and grain of chickpea, especially when determined at different growth stages in the African continent. This study contributes knowledge on this crucial aspect which will likely lead to more other similar research reports in other settings. Therefore, the objectives of this study was to evaluate the effect of P fertilizer rates and rhizobial inoculation on yield and nutrients accumulation in two chickpea genotypes.

Field experiments were conducted in winter 2017 and 2018 at University of Venda, Thohoyandou and University of Limpopo's experiment farm, Syferkuil. Treatments consisted of a factorial combination of two rates of P fertilizer (0 and 90 kg P ha^{-1}), two desi chickpea genotypes (ACC1 and ACC5) and two rhizobial inoculation levels (with and without rhizobiam strain). The treatments were laid out in a randomized complete block design (RCBD) and replicated three times on 22 April 2017 and 11 April 2018 (Syferkuil), 13 April 2017 and 29 April 2018 (Thohoyandou). Macronutrients including P, K, Ca, Mg were determined using the citric acid method. The total N concentration were determined by the micro-Kjeldahl method in both soil, shoots and grain. Zn was extracted using a di-ammonium ethylene-diaminetetraacetic acid (EDTA) solution. The content of macronutrients (P, K, Ca, Mg, Ca, and Zn) in soil, shoots and grain was determined by first subjected to wet digestion (Mehlich, 1984). From the digest, various elements were read using relevant procedures. P contents was determined colorimetrically using a spectrophotometer. Yield and yield components were assessed at harvest maturity.

Genotypes affected the accumulation of mineral elements in rhizosphere soil, shoots, grain and yield. Accession 5 performed better in most of nutrients elements compared to accession 1 in both seasons and sites. Application of phosphorus alone, and in combination with rhizobium inoculation increased the concentration of majority of nutrients in the rhizosphere. When the test accessions were grown at the Syferkuil and Thohoyandou study location in 2017, they showed significant differences in the concentration of N, P and K while Ca, Mg and Zn were similar in the rhizosphere. The concentrations of N, P and K were markedly higher in the rhizosphere of ACC5 compared to ACC1. In fact, the concentration of P was two-fold greater in the rhizosphere of ACC5 than ACC1. Accession 5 exhibited a markedly higher shoot

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dry weight, number and dry weight of pods, 100-seed weight, grain yield and harvest index compared to ACC1. P plus rhizobium inoculation, P, rhizobium inoculation affected grain yield and yield components of chickpea genotypes.

This preliminary finding show that the combination of P and rhizobium inoculation affected the nutrients accumulation in the rhizosphere, shoots, grain, yield and yield components in both locations. Moreover, Thohoyandou had the highest nutrients accumulation on the rhizosphere, shoots, grain, yield and yield components compared to Syferkuil.

Key words: Chickpea (*Cicer aritienum* L). Phosphorus. Rhizosphere. Rhizobial inoculation.

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LIST OF ABBREVIATION

FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistical database
BNF	Biological Nitrogen Fixation
DM	Dry matter
RI	Rhizobial Inoculation
ATP	Adenosine Diphosphate
ADP	Adenosine Triphosphate
SW	Seed Weight
ACC	Accession
RCBD	Randomized Complete Block Design

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1. INTRODUCTION

1.1 Background information

Chickpea (*Cicer arietinum* L.) belongs to genus *Cicer,* tribe Cicereae, family Fabaceae, and subfamily Papilionaceae. It is native to south eastern Turkey (Ladizinsky, 1975). The crop is largely cultivated in Asia and contributes 74% to global chickpea production. Although it is largely cultivated in Asia, chickpea is also produced in other parts of the world including in east Africa in (Ethiopia and Kenya) and southern Africa (mainly in Malawi) (Abate *et al*., 2012). In Africa, Ethiopia is the largest producer of chickpea and accounts for about 46% of the continents production, Ethiopia is also the seventh largest producer worldwide and contributes about 2% to the total world chickpea production (Kassie *et al*., 2009).

The global demand of chickpea has increased for the past three decades. For example, the number of countries importing chickpea increased from 65 to 164 between 1991 and 2011 (FAOSTAT, 2014). Moreover, the crop reached a global record of 13.3 million ha under cultivation and production of 11.75 m tons during the 2011 cropping season. In the 2013 cropping season, land under chickpea cultivation increased to 13.5 m ha and production remained at 13.1 million tons (FAOSTAT, 2015).

Given the expansion of land under chickpea production, it makes the crop one of the most important pulses. In human diet, it serves as an important source of protein for millions of people, particularly those in developing countries. It is the second leading grain legume in the world after common bean (FAOSTAT, 2015). The grain of chickpea is a good source of micronutrients (Thavarajah and Thavarajah, 2012) and it is high in carbohydrates, protein content (20-22%), and rich in fiber and minerals such as phosphorus, calcium, magnesium, iron and zinc (Abera, 2015). The crop is mainly grown for human consumption, animal feed and for medicinal purposes. Chickpea is a significant contributor to agricultural sustainability through biological N fixation and as a rotation crop allowing the diversification of agricultural production systems (FAO, 2013). Chickpea also plays an important role in maintaining soil fertility, particularly in dry areas.

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South African soils are largely acidic, with high aluminium and iron concentrations and Phosphorus absorptive capacity which results in low plant-available P in the soil solution (Buhmann *et al*., 1996). Interventions to address the low P concentrations in the country's soils should focus partly on P fertilization. Phosphorus fertilizers either supress or enhance the Zinc status of soils depending on whether the source of the P fertilizers contains Zn or not. Application of P fertilizer that does not contain Zn generally inhibits the absorption of Zn and may therefore lead to Zn deficiency in soils and in leaves or grain of crops (Alloway, 2008). In contrast, fertilization with a P source rich in Zn enhances the soil concentrations of Zn and its accumulation in above-ground organs. Overall, improving the soil P concentrations of cropping systems through P fertilizer is likely to enhance the overall grain mineral status. Specifically, plants take nutrients directly from the soil, thus, if the soils of a region is enriched in a particular mineral, families who consume food produced in such soil benefit from the relevant minerals.

Maintaining soil fertility and using plant nutrients in balanced quantities is one key component in increasing crop yield and sustaining the environment while mitigating climate change (Caliskan and Ozkaya, 2008). Under favourable conditions, symbiotic N_2 fixation can produce greater than 100 kg N ha^{-1} (Beck *et al*., 1991), and provide up to 85% of the N required by a chickpea when grown in association with effective and compatible rhizobium strain (Walley *et al*., 2005; Cheminingwa and Vessey, 2006). Moreover, chickpea is well adapted to environmental stress such as drought, high temperatures and poor soils (Siddique *et al*., 2000) and may thus be an important food security crop for smallholder resource-poor farmers in the semi-arid regions. Due to the growing awareness of the importance of chickpea as a food legume crop in this region, attention should be paid to improve its production.

The natural agricultural ecosystems depend largely on the beneficial microorganisms in the soil rhizosphere which help crops to maintain growth and sustain productivity (Rosas *et al*., 2009). These beneficial microorganisms are important components of plant productivity and soil fertility because of their participation in several key processes such as those involved in nutrient cycling and seedling establishment (Jeffries *et al*., 2003; Rosas *et al*., 2009). Recent studies show that a number of bacterial species, mostly associated with the plant rhizosphere, are beneficial for plant growth, yield and crop quality (Gaiero *et al*., 2013).

Rhizobium inoculants can be used in legumes, especially where native rhizobium population densities are low. Inoculants play an important role in ensuring nodulation, improving biological N_2 fixation and improving crop yield. Depending on the native rhizobium population in the soil, inoculation is proven worthy as it is economically cheap and environmentally and ecologically beneficial than the application of N fertilizers (Wange, 1989).

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Phosphorus is the second most critical nutrient for crops but for pulses it assumes primary importance owing to its important role in root proliferation and atmospheric N assimilation (Harisudan *et al*., 2009). Also, it plays an important role in N cycling as adenosine triphosphate is required in large quantities for legumes to undertake biological N_2 fixation (Vance, 2001). Unfortunately, low soil P availability limit crop production in the semi-arid regions (Eyasu and Scoones, 1999). Phosphorus supplementation can enhance plant growth by increasing the efficiency of biological N_2 fixation and enhancing the availability of macronutrients such as N, P, K, Ca and Mg (Makoi *et al*., 2014). Moreover, Phosphorus application influences the content of other nutrients such as Zn, Mn, Fe and B in leaves and seeds (Singh *et al*., 2011). Furthermore, microorganisms may affect the chemistry of nutrients in soils by enhancing nutrients uptake by plants. For example, Makoi *et al*. (2014) reported improved uptake of macronutrients in cowpea following inoculation with efficient strains of rhizobium. Although several studies have demonstrated the effects of inoculating chickpea with rhizobium on soil fertility improvement (Walley *et al*., 2005; Ben Romdhane *et al*., 2009; Lopez-Bellido *et al*., 2011) as well as chickpea growth and yield (Ogola, 2015) few studies have quantified the effect of inoculation on nutrients accumulation (Killham and Firestone, 1983). Therefore, it is imperative to assess the overall effect of P fertilizer and rhizobium inoculation on yield and nutrients accumulation of two chickpea genotypes. The hypothesis tested in this study was that P fertilizer rates and Bradyrhizobium inoculation affect macro and micronutrients concentration in soil, grain, shoots and yield of two chickpea genotypes. Therefore, the study evaluated the effect of P fertilizer and Bradyrhizobium inoculation on nutrient accumulation of two chickpea genotypes.

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CHAPTER 2 LITERATURE REVIEW

2.1 Description of chickpea

Chickpea (*Cicer arietinum* L.) belongs to genus *Cicer,* tribe Cicereae, family Fabaceae, and subfamily Papilionaceae. It originated in south eastern Turkey (Ladizinsky, 1975). It is grown in a wide range of environments in over 50 countries in subtropical and temperate regions of the world, mainly in the Indian subcontinent, West Asia, North Africa, the Americas and Australia (FAOSTAT, 2015). It is grown primarily for its protein-rich seeds.

Chickpea is divided into two types which are grown commercially, the desi and the kabuli type. Desi type is usually short, and its flowers range from pink to purple, and the seeds are rough coated with a variety of colours. The kabuli type has large cream round seeds with a smooth coat and its flowers are white in colour (Corp *et al.,* 2004).

Under favourable conditions, symbiotic N_2 fixation can produce greater than 100 kg N ha^{-1} (Beck *et al*., 1991), and provide up to 85% of the N required by a chickpea when grown in association with effective and compatible rhizobium strain (Walley *et al*., 2005; Cheminingwa and Vessey, 2006)

2.2 Nutritional value of chickpea

Chickpea is a good source of carbohydrates and proteins, which together constitute about 80% of the total dry seed mass. The starch content of chickpea cultivars has been reported to vary from 41% to 50%. The kabuli type contains more soluble sugars (Jambunathan and Singh, 1980). The unavailable carbohydrate content is higher in chickpea than other legumes (Kamath and Belavady, 1980), and chickpea carbohydrate has a lower digestibility than that of other pulses (Rao, 1969). Chickpeas contain about 6% fat that is important in the vegetarian diets of resource-poor consumers. Although lipids are present in low amounts, chickpea is rich in nutritionally important unsaturated fatty acids such as linoleic and oleic acids. Ca, Mg, P and, especially, K are also present in chickpea seeds.

Chickpea is a good source of important vitamins such as riboflavin, niacin, thiamine, folate and the vitamin A precursor β-carotene. Chickpea has several potential health benefits, and, in combination with other pulses and cereals, it could have beneficial effects on some of the important human diseases such as, Type 2 diabetes, digestive diseases and some cancers. (Jukanti, 2012). Chickpea contains nutritionally important minerals, notably calcium and iron, and the availability of iron is reported to be good (Murty *et al*., 2010). Immature green chickpea seeds are reported to contain 2.2mg thiamine (100 g)-1 and .7 mg riboflavin (100 g)-1(Geervani and Uma Devi, 1989). Overall, chickpea is an important pulse crop with a diverse array of potential nutritional and health benefits (Segev,2010).

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2.3 Effect of Phosphorus in soils

Phosphorus is an important macronutrient needed by plants for their growth and development and required by humans and animals who acquire it largely through consuming crops and plants. The main source of the P used in fertilisation of crops is rock phosphate, a non-renewable resource.

The need and use of phosphate, a product of rock phosphate is in high demand. About 75% of the rock phosphate comes from Morocco/Western Sahara and the rest is mined in China, Algeria, Syria, South Africa, Russia, Jordan, USA and Australia (USGS, 2015). However, it is predicted that rock phosphate will be depleted in a few decades (Cordell et al., 2009).

Chickpea is a symbiotic grain legume that fixes atmospheric N and, in the process, contributes N into cropping systems. This has been demonstrated in South Africa under contrasting agroecologies of the Mpumalanga Province (Dakora *et al*., 2015). However, the N_2 fixation process is hardly successful and beneficial where the crop is established in soils containing low P. This, therefore, necessitates the use of fertiliser P.

2.4 Effect of phosphorus fertilizer in crop production

Phosphorus is the second most limiting soil nutrient in crop production. Phosphorus is vital for development of new tissue and the transfer of the genetic information within the plant from one cell to another during cell formation (Bucher *et al*., 2001). It can be applied into the soil in form of phosphate fertilizer (P_20_5). Crop need P for growth, utilization of sugar and starch, photosynthesis, nucleus formation and cell division. Moreover, P compounds are involved in the transfer and storage of energy within plants. Energy from photosynthesis and the metabolism of carbohydrates is stored in phosphate compounds for later use in growth and reproduction. P is readily translocated within plants, moving from older to younger tissues as the plant forms cells and develops roots, stems and leaves. Thus, adequate P is associated with an increase of root growth, rapid growth and enhancing the quality of vegetative growth. Therefore, most crops require significant quantities of P during the early stages of growth and development (Sessitsch *et al*., 2002).

In legume crops, P plays a role in symbiotic N_2 fixation, hence nodules formation and N_2 fixation are generally limited by low P fertilizer. Although crop response to P fertilization depends on soil available P as well as genotype, in general, P fertilization promotes root growth which in turn will accommodate a large number of nodules, improves the growth efficiency of crops, and enhances nutrient and water-use efficiency and increases yields (Crews, 1993).

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Legumes that depend on symbiotic N fixation have high ATP requirements for nodule development, thus P will convert ADP to ATP for nodulation process (Ribet and Drevon, 1996).

Crops need P for obtaining good quality yield. In view of this, Zafar *et al*. (2003) designed a study to see the effect of different levels of P on lentil yield under irrigated conditions. The study showed that the application of P increased the number of seeds per pod, 100-seed weight (g) and the number of pods per plant because P encouraged flowering and fruiting.

Moreover, Madzivhandila *et al.* (2012), reported greater crop biomass at 90 kg P ha^{-1} in winter sowing compared to lower rate of P fertilizer. Furthermore, Lusiba *et al*. (2017) reported significantly increased biomass, grain yield and water-use efficiency of biomass production at 90 kg P ha^{-1}. However, in the Limpopo region, there is no documented information concerning the effect of P fertilizer on dry matter and N accumulation, partitioning and translocation in respect of chickpea genotypes.

2.5 Effect of rhizobial inoculation on chickpea production

Most cropping fields cultivated by smallholder farmers in South Africa experience inadequate replacement of nutrients. In advocating sustainable production of chickpea in South Africa, the approach should include environmentally friendly mechanisms. Successful growth, development and biological N_2 fixation by chickpea depends on availability of compatible soil rhizobia. The crop hardly contributes much N to cropping systems especially were cultivated in soils where compatible soil rhizobial strains are absent or inefficient. This necessitates the use of rhizobial inoculation. The success of inoculation, however, depends on the environmental conditions, soil richness, number and application method of effective rhizobial cells, presence of high population of competing strains of rhizobia and plant genotype (O'Callaghan, 2016).

Generally, rhizobial inoculation can lead to the establishment of large rhizobial population in the rhizosphere to improve nodulation and N-fixation even under adverse soil conditions (Peoples et al., 1995). Chickpea can obtain a significant portion (4–85%) of its N requirement through symbiotic N fixation when grown in association with effective and compatible rhizobium strains (Cheminingwa and Vessey, 2006). Moreover, chickpea that is associated with compatible rhizobium produces up to 176 kg N ha^{-1} depending on the genotypes and environmental factors (Ogutcu *et al*., 2008). Inoculating the seeds with rhizobium is known to increase nodulation, crop biomass and grain yield of chickpea (Ogola, 2015).

Rhizobial inoculation of crops has positive effect in that the rhizobia produce plant growth regulators that are able to solubilise nutrients including N, P, Fe and K, all which enhance

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plant growth (Caravaca *et al*., 2003). Where crops are inoculated under low-P conditions, the effectiveness of rhizobial inoculants in solubilising particle-bound P (in low-P soils) can be affected by whether the strain is applied in a liquid or a solid form (Marra *et al*., 2011). Moreover, the effectiveness of rhizobia depends on the form of chemical complexation, such as Al-P, Fe-P or Ca-P (Marra *et al*, 2011). Ogola (2015) reported that the number and dry weight of nodules were greater with inoculation compared with the control. However, the impact of Bradyrhizobial inoculants on dry matter and nutrient accumulation by chickpea genotypes is not well documented.

2.6 Effect of Rhizobium inoculants with phosphorus supply on grain yield of chickpea.

Limiting factors such as diversity or scarcity of native rhizobium population in soil can affect legume performance and grain yield (O'Hara *et al*., 2002). Supplementing chickpea legumes with nutrients has great potential for increasing yield and enhancing symbiotic establishment for increased N fixation (Salvagiotti *et al.,* 2008). Chickpea grown with the application of P increased shoot dry weight, formed more nodules, and had higher nodule dry weight, enhanced N fixation and grain yield (Yahiya *et al*., 1995).

The effects of *Rhizobium* inoculants are well documented. For example, Nyoki and Ndakidemi (2014) reported that *Rhizobium* inoculation significantly improved the yield and yield components such as the number of pods per plant, number of seeds per pod, number of seeds per plant, 100-seed weight, and seed yield of climbing bean relative to control. Moreover, Giller *et al* (1998) reported that the chickpea plants which were inoculated with rhizobia gave significantly higher stover and seed yield compared with un-inoculated control (Panwar *et al*., 2011).

Phosphorus availability in soils was found to have a positive influence on survival and efficiency of rhizobium bacteria (Pramanick *et al*., 2013). Many researchers have reported a significant performance of legumes when supplied with P (Ndakidemi *et al*., 2006; Madzivhandila et al., 2012; Macil *et al*., 2017; Lusiba *et al*., 2017). P significantly increased dry matter yield, yield components, leaf area, number of branches per plant and seed yield on cowpea (Karikari *et al*., 2015). The effect of P fertilizer on yield and yield components of chickpea is well documented (Madzivhandila *et al*., 2012; Turuko *et al*. 2014). However, the effect of inoculation and P fertilizer in combination and their influence on the yield is still limited in literature.

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2.7 Effect of rhizobium inoculants with phosphorus fertilizer on nutrient uptake

Nutrient uptake by leguminous plants depends mainly on the quantity, concentration and activities in the root zone soil as well as the capacity of soil to replenish the concerned nutrients in the soil solution (Killham and Firestone, 1983). Soil microorganisms such as rhizobium are known to have influence on the chemistry of soil nutrients and thus promote nutrients uptake (Dobbelaere *et al*., 2003; Bais *et al*., 2006; Lugtenberg and Kamilova, 2009).

Currently, microbial inoculants have become promising components for integrated solution to environmental problems due to the fact that inoculants possess the capacity to promote plant growth, enhance nutrient availability and uptake, and ultimately support the health of plant for better yield (Mmbaga and Friesen, 2003). The uptake of primary (N, P, K) and secondary (Ca, Mg) macronutrients in different plant organs greatly depend on fauna activities in the rhizosphere soil (Makoi *et al*., 2014). P supplementation can enhance plant growth by increasing the efficiency of biological N fixation, enhancing the availability of macronutrients such as N, P, K, Ca and Mg (Makoi *et al*., 2014; Sa and Israel, 1991). It has been reported that P application influences the content of other nutrients such as Zn, Mn, Fe and B in leaves and seeds (Singh *et al*., 2011).

Microorganisms such as *Bradyrhizobium* inoculants may affect the chemistry of nutrients in soils by enhancing nutrients uptake by plants. For example, Makoi *et al*. (2014) reported improved uptake of macronutrients following inoculation with efficient strains of rhizobium. Eutropia *et al*. (2014) reported that inoculation with *B. japonicum* and P supplementation significantly increased the uptake of N, P, K, Ca and Mg in roots, shoots, pods and the whole soybean plant. Furthermore, the application of P fertilizers in combination with organic matter improved soil physical and chemical properties by enhancing biological activity and soil organic carbon accumulation thus helping in the uptake of nutrients (Aneto and Akinrinde, 2006). Another study reported enhanced nitrate absorption and reduction to amino acids and increased the protein formation in plants following rhizobium inoculation. Growth and yield response of chickpea to rhizobium inoculation at Thohoyandou (Limpopo province) has been documented (Ogola, 2015). However, the uptake of nutrients in chickpea supplied with rhizobium and P supplements is not well established and needs further investigation.

2.8 Effect of rhizobium inoculation and phosphorus on rhizosphere soil

In low-P soils, addition of phosphate will enhance the soil solution P, solubility, and availability of Ca, Mg and S and the exudation of organic acids by the P-fertilised plant (Zhang *et al*., 2015). The concentration of macro and micronutrients is generally higher in the rhizosphere relative to that in the non-rhizospheric bulk soil of both agricultural and terrestrial legumes

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(Makoi *et al.,* 2014; Maseko and Dakora, 2013). Legumes exhibit a variety of mechanisms which enable the markedly greater nutrient content in their rhizosphere. During the symbiotic N_2 fixation process by symbiotic legumes, legumes typically take up excess cations compared to anions, thus, creating an imbalance in cation/anion in the rhizosphere (Dakora and Philips, 2002; Latati *et al*., 2014). The protons released as a result of the imbalance in cation and anion uptake leads to acidification of the rhizosphere, that is, a decrease in rhizosphere pH.

The resulting lower pH in rhizosphere of legume plants increases the dissolution of mineral elements such as P, Mn, Zn, Fe and Cu (Treeby *et al*., 1989; Neumann and Romheld, 2002; Richardson *et al*., 2009). Once solubilized through symbiotic N_2 fixation, mineral nutrients such as P, Mn, Zn, Fe and Cu become readily available for uptake by the leguminous plants, leading to their increased accumulation of mineral nutrients in the rhizosphere, roots, shoots, pods and grains (Howell, 1998; Makoi *et al*.,2013; Belane *et al.,* 2014). This, therefore, creates a positive relationship between rhizosphere nutrient availability and N fixation as well as mineral nutrient accumulation in grains of legumes. In South African cropping systems, there is hardly any literature on possible relationship between rhizosphere pH and nutrient availability in chickpea. A study on this matter would improve our knowledge on the subject.

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CHAPTER 3: MATERIALS AND METHODS

3.1 Study site

The study was conducted at the University of Venda's experimental farm in Thohoyandou and University of Limpopo, Syferkuil experiment farm. Thohoyandou is situated at a height of 595m above sea level, latitude of 22.97556ºS and longitude of 30.44444ºE (Tadross *et al*., 2006). The area receives an average of about 500 mm of rainfall per annum. The average minimum and maximum temperatures are 18ºC and 31ºC (Tadross *et al*., 2006). The site is characterized by deep, well-drained clay soils, classified as Rhodic Ferralsols with soil pH of 6.06 (Lusiba *et al*., 2016)

Syferkuil is situated at a height of 1230m above sea level, latitude of 23.8888ºS and longitude of 29.7386ºE (Thabang *et al*., 2012). The area receives an average of about 451mm of rainfall per annum. The average minimum and maximum temperatures are 10ºC and 25ºC, respectively (Thabang *et al*., 2012). The study site is characterized by sandy loam soil type) with a soil pH of 5.18 (Makonya *et al*., 2019).

3.2 Experimental design

Two field experiments were conducted during the 2017 and 2018 winter seasons (experiment 1 and 2, respectively). Treatments consisted of a factorial combination of two rates of phosphorus (P) fertilizer (0 and 90 kg P ha^{-1}), two desi chickpea genotypes (ACC1 and ACC5) and two rhizobial inoculation levels (with and without rhizobial strain). The treatments were laid out in a randomized complete block design (RCBD) and replicated three times. Each experimental unit was 2 m x 1.5 m each plot consisted of six rows. Seeds were soaked in a mixture of rhizobium powder and water and were planted immediately after inoculation. Uninoculated plots were sown before the inoculated ones in both experiments to prevent contamination of the uninoculated seed with the inoculum. Phosphorus fertilizers was band applied to all plots at the rate of 90 kg P ha^{-1} at planting as Single Super Phosphate. The plots were watered uniformly immediately after sowing to promote uniform germination, emergence and crop establishment. supplemental irrigation was also applied at different occasions in both experiments using sprinkler. The plots were kept weed-free throughout the growth duration of the crop using physical weed control.

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3.3 Measurements

3.3.1 Plant sampling and analysis

Plants were sampled from the outer rows from an area measuring 0.3 m^2 at vegetative stage, 50% flowering and at harvest maturity from each plot. On each occasion, the plants were cut at ground level. At the flowering stage the plants were separated into leaf and stem. During final sampling (harvest maturity), the plants were separated into leaf, stem and pods. All plant samples were oven-dried at 65^0C for 48 h (to constant weight), weighed and milled to a fine powder (0.85 mm sieve) and preserved for subsequent chemical analysis of macro and micronutrient elements.

3.3.2 Sampling and processing of soil and plant samples

Rhizosphere soil samples, defined as soil attached to and around roots of chickpea plants were collected by digging the whole root system along with its surrounding soil. The collected soil was placed into plastic bags (200 x 200 mm). Two samples were cored from each test plot. Non-rhizosphere bulk soil (soil away from roots of chickpea) was dug between experimental replicate blocks at a depth equivalent to that of the test chickpea roots.

Collected soil samples were individually sieved (2.0 mm sieve size), air-dried at room temperature and packaged for analysis of selected chemical properties. For each rhizosphere soil collected, the remaining plant stand was separated into root and shoot. Shoot material were placed into sample paper bags, oven-dried at 65 °C for 48 h (to constant weight). Each of these plant samples were weighed to determine shoot dry weights. Shoot plant samples were milled to fine powder (≤0.5 mm particle size) and packaged into 100x150 mm plastic bags and preserved for subsequent chemical analysis of macro- and micronutrient elements

3.3.3 Yield and yield components

Plant samples were oven dried for dry matter determination at the different growth stages. Vegetative dry matter was taken between 30 and 35 days after planting and flowering was determined visually when 50% of the plants from the experimental unit had exposed flowers. Physiological maturity was determined when the plants started experimenting browning of the pods and yellowing of plant leaves. Crop biomass and grain yield were determined at harvest maturity in both experiments. All the plants from 0.88 sq. meters of two guarded innermost rows of each experimental plot were cut at ground level. The pods were removed from all harvested plants and the shoots were dried at 65°C for 48 h to obtain total shoot dry weights. Pods were dried, threshed and seeds from threshed pods were weighed to obtain grain yield. Sub-samples of the seeds were used to determine 100 seed weight (100-SW). Harvest Index

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(%) was recorded as the ratio of dry seed yield per plot to the above ground dry biomass yield per plot taken at physiological maturity.

3.3.4 Determination of pH and mineral nutrients in rhizosphere soils

Rhizosphere and non-rhizosphere bulk soil pH were determined using 1:1.5 w/v of soil to $CaCl_2$. About 10 g of sieved soil was weighed into a volumetric flask and 15 ml of $CaCl_2$ solution added into the soil. The contents were mixed by shaking for 30 minutes. After 30 minutes, the mixture was allowed to settle for 30 minutes and pH was determined using a pH meter.

Macronutrients including P, K, Ca, and Mg were determined using the citric acid method (Dyer, 1894; Du Plessis and Burger, 1964). Acid digestion was done on samples followed by the measurement of P, K, Ca and Mg, performed through aspiration on an inductively coupled plasma–mass spectrometer (ICP-MS) (IRIS/AP HR DUO Thermo Electron Corporation, Franklin, Massachusetts, USA). Zn was extracted using a di-ammonium ethylene-diaminetetraacetic acid (EDTA) solution (Trierweiler and Lindsay, 1969).

3.3.5 Measurement of macro and micronutrient concentrations in shoot and grain samples

The shoots and grain were chemically analysed to determine their contents of N, P, K, Ca, Mg and Zn. The total N concentration was determined by the microKjeldahl method (Bremner, 1982). For the determination of the remaining elements, plant samples were subjected to wet digestion (Mehlich, 1984). From the digest, various elements were read using relevant procedures. P contents were determined colorimetrically using a spectrophotometer. The procedure involves the use of Vanadomolybdate yellow method (Murphy and Riley, 1962). A flame photometer was used for the determination of K in plant tissue (Heald, 1965). Aspiration on an inductively coupled plasma–mass spectrometer (ICP-MS) (IRIS/AP HR DUO Thermo Electron Corporation, Franklin, Massachusetts, USA) was used to determine Ca and Mg (Pratt, 1965).

3.4 Statistical analysis

Statistical analysis was carried out using STATISTICA software program, version 10. Analysis of variance was performed to compare the means of mineral nutrient concentrations in rhizosphere, shoots, and grain, also yield and yield components between the treatments and locations. Where means showed significant differences between treatments, Fisher's (LSD) Least Significant Difference test was used to separate the treatment means at $p \leq 0.05$.

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CHAPTER 4: VARIATION IN CONCENTRATION OF MACRO-AND MICRONUTRIENTS IN THE RHIZOSPHERE OF CHICKPEA AS AFFECTED BY RHIZOBIUM INOCULATION AND PHOSPHORUS FERTILIZER

ABSTRACT

Legumes increase the solubilisation and accumulation of mineral elements in the rhizosphere, but this depends on factors such as crop species and soil fertility, amongst others. There is a dearth of information on the effect of P with rhizobial inoculation in response of nutrients accumulation in the rhizosphere in the African continent. This study contributes knowledge on this crucial aspect which will likely lead to more other similar research reports in other settings. This study assessed the effect of fertiliser phosphorus and rhizobium inoculation on the concentration of nutrients in the rhizosphere of 2 chickpea genotypes. Field experiments were established at Thohoyandou and Syferkuil, South Africa in 2017 and 2018. At both locations, accession 5 accumulated higher concentrations of nitrogen, phosphorus, potassium and calcium compared to accession 1. Phosphorus plus rhizobium inoculation, P, rhizobium inoculation increased rhizosphere P in 2017 and rhizosphere N, P, K, Ca, Mg and Zn in 2018 at Syferkuil. Furthermore, application of phosphorus alone and in combination with rhizobium inoculation increased the concentration of most of the nutrients in the rhizosphere. Clearly, phosphorus fertilizer and rhizobium inoculation are important to enhance nutrients accumulation in the rhizosphere soil of chickpea.

Key words: Nutrients. Phosphorus. Rhizosphere. Rhizobial inoculation

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4.1 Introduction

Chickpea was probably introduced in South Africa by Asians and its consumption has increased and surpassed that of grain legumes that are native to Africa (Mpai and Maseko, 2018). Although South Africa imports chickpea, there are initiatives that encourage its cultivation and aimed at reducing its cost and boosting the country's economy and global competitiveness (http://www.univen.ac.za/news/farmers-encouraged-plant-chickpeas/). However, the cultivation of chickpea by small-scale farmers that have adopted are faced with a challenge of poor soil fertility. The most deficient mineral elements in smallholder cropping systems in southern Africa include N, P K, B, Zn and exchangeable cations (Mohale *et al*., 2014; Mtangadura *et al*., 2017; Mthembu et al., 2018). When grown in infertile soils and without the application of fertilizers, chickpea adopts a variety of mechanisms that enhance the solubility of nutrients (Li *et al*., 2004; Pang *et al*., 2018). The pulse has been shown to alter the rhizosphere pH which results in increased solubilisation of nutrients (Xue *et al*., 2016; Stagnari *et al*., 2017). However, these mechanisms hardly supply sufficient nutrients for sustainable growth and yield.

In order to improve the nutritional status of their soils, majority of smallholder farmers in South Africa apply organic fertilizers (Materechera, 2012; Scheepers and du Toit, 2016). However, challenges associated with the application of organic fertilizers is that often, large quantities are required to meet nutrient requirement of crops and that they have a low nutrient use efficiency. As a result of the low efficiency, organic fertilizer hardly sustain high yields and result in decreased yields in the long-term (Mtangadura *et al*., 2017). In contrast, the application of inorganic fertilizers improves crop yields by about 30 to 50% (Stewart *et al*., 2005). For example, research conducted in Thohoyandou, South Africa revealed that significantly higher growth, grain yields and water use efficiency of chickpea were achieved with the application of 90 kg P/ha compared with the zero control (Madzivhandila *et al*., 2012; Ogola *et al*., 2013). Unfortunately, given that smallholders have limited access to credit, they hardly afford the recommended rates of phosphate fertilizer due to its high cost (Mpai and Maseko, 2018). As a result, they apply significantly lower rates of P compared to recommended rates which leads to lower growth and grain yield because P fertilizer has a low efficiency and bioavailability in soils (Cordell *et al*., 2009).

Therefore, there is a need for alternative approaches that can improve the solubility and uptake of P especially in fields cultivated by the majority of low-income farmers. One such approach includes the application of biofertilizers. Biofertilizers are able to solubilize native soil nutrients which ultimately result in increased concentration of nutrients in the soil solution for better absorption by plants (Calvo *et al*., 2014; Thonar *et al*., 2017).

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Various biofertilizers including rhizobial inoculants are produced and readily available in South Africa. Rhizobial inoculants produce plant growth regulators that enhance the ability of plants to solubilise nutrients in soils (Tailor and Joshi, 2014). Through this mechanism, inoculants exert positive effects on growth and yield of crops grown as well as the nutritional status of soils especially that which contain ineffective rhizobia. Despite their availability and accessibility in South Africa, majority of smallholder farmers hardly use rhizobial inoculants. Through the use of rhizobial inoculants in the production of chickpea for example, smallholder farmers could reduce expenditure on synthetic fertilizers. When other grain legumes were inoculated with rhizobial inoculants alone or in combination with fertilizers, they increased the concentration of nutrients in soils (Nyoki and Ndakidemi, 2018).

In South African cropping systems, there is hardly any literature on possible relationship between application of P, rhizobium inoculation, and P in combination with rhizobium inoculation on the concentration of nutrients in the rhizosphere of chickpea grown in different locations having contrasting soil type. Generally, inoculation with rhizobial inoculants alone in the production of legumes could provide more than 50% of the Nitrogen fertilizer required for crop production in most marginal lands (Chianu *et al*., 2012). The inoculation of legumes with rhizobium has been shown to increase the concentration of nutrients in both the rhizosphere and in the grain (Nyoki and Ndakidemi, 2018). Mechanisms that are reported in rhizobia that enhance the solubility and therefore availability of nutrients include their ability to synthesize siderophores which sequester iron and supply it to host plants (Kumar *et al*., 2018). At the smallholder level, rhizobial inoculants supplemented with fertilizers enhanced legume production in East Africa (Mmbaga *et al*., 2014).

However, there is no published literature on the effect of P fertilization, the application of P plus rhizobium inoculation, and rhizobium inoculation alone on the concentration of mineral elements in the rhizosphere of chickpea established in South Africa. Results from such a study would improve our knowledge on the role of these inputs, especially that of the inorganic fertilizer plus the biofertilizer in enhancing the uptake and solubility of mineral nutrients. Better solubility and uptake are likely to promote increased accumulation in the grain which is the edible part by humans. In this study, two accessions of chickpea were grown at Thohoyandou (clay) and Syferkuil (sandy loam) farms with the application of P, P plus rhizobium, and rhizobium. The aim was to evaluate the effect of these inputs on the concentrations of selected macro-and micronutrient elements in the rhizosphere.

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4.2 Materials and methods

The detailed description of materials and methods is presented in chapter 3 but a brief summary is outlined here. The field experiment was conducted at two sites, Syferkuil farm and University of Venda experimental farm in 2017 and 2018 winter. Treatments consisted of a factorial combination of two chickpea genotypes (Accession 1 and Accession 5) and two phosphorus fertilizer levels (0 and 90 kg ha^{-1}) and two rhizobial inoculation levels (with and without rhizobial strain) arranged in a randomized complete block design and replicated three times. Rhizosphere soil samples were collected by digging the whole root system along with its surrounding soil. Collected soil samples were individually sieved, air-dried at room temperature and packaged and preserved for subsequent chemical analysis of macro and micronutrient elements. Nutrients elements which were determined include N, P, K, Mg, Ca and Zn. An analysis of variance (ANOVA) was used to compare the means of mineral nutrient concentrations between the accessions, the fertilizer treatments and the two study locations. Where means showed significant differences between treatments, Fisher's (LSD) was used to separate the mean at $p \leq 0.05$.

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4.3 Results

4.3.1 Physical and chemical properties of the soil

Soil collected from the study location in Thohoyandou contained 24% of sand, 16% of silt and 60% of clay while that from Syferkuil was made up of 87, 2, and 11% of sand, silt and clay, respectively (Table 4.1). The pH was moderately acidic (6.06) at Thohoyandou and slightly alkaline at Syferkuil (7.52) (Table 4.1). At Thohoyandou, organic C was 1.39%, ammonium-N was 23.5 mg/kg, and nitrate-N was 3.44 mg/kg, and at Syferkuil, the soil had 0.30% OC, 19.79 mg/kg ammonium-N, and 2.4 mg/kg nitrate-N. Extractable P was 35 mg/kg at Syferkuil and 10.10 mg/kg at Thohoyandou, respectively. Of the exchangeable cations at Syferkuil, the K, Na, Mg, Ca and S were 206; 80; 499; 863; and 9.3 mg/kg, respectively. At Thohoyandou however, the concentration of these were 80; 47; 438; 429; and 10.5 mg/kg (Table 4.1). The micronutrients included Cu, Fe, Mn, Zn, and B, and the soil at Syferkuil exhibited 1.1; 5.82; 31.85; 1.33; 0.11 and 9.3 while at Thohoyandou, there were 1.3; 3.77; 45.24; 1.8; 0.13, and 10.5 (Table 4.1). Lastly, the Cation exchange capacity was 9.3% at Syferkuil, and 10.5% at Thohoyandou (Table 4.1).

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Table 4.1 Physical and chemical properties in soil collected from the Syferkuil and Thohoyandou experimental sites in 2017.

Physical/chemical property	Location	
	Syferkuil	Thohoyandou
Sand (%)	87	24
Silt (%)	2	16
Clay (%)	11	60
Textural class	Sandy loam	Clay
Ph	7.52	6.06
Organic C (%)	0.30	1.39
NH_4^- (mg/kg)	19.79	23.5
NO_3^- (mg/kg)	2.4	3.44
P (mg/kg)	35	10.10
Exchangeable cations mg/kg		
K	132	80
Na	80	47
Mg	499	432
Ca	363	529
S	9.3	10.5
Cu	1.1	1.3
Fe	5.82	3.77
Mn	31.85	45.24
Zn	1.33	1.8
B	0.11	0.13
CEC	9.3	10.5

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4.3.2 Mineral accumulation in the rhizosphere of chickpea

When the test accessions were grown at the Syferkuil study location in 2017, they showed significant differences in the concentration of N, P and K while Ca, Mg and Zn were similar in the rhizosphere, However, in 2018 the concentrations of N, P, K, Mg and Zn were significantly greater in the rhizosphere of ACC5 compared to ACC1 while Ca was similar between the test accessions (Table 4.2). The concentrations of N, P and K were markedly higher in the rhizosphere of ACC5 compared to that of ACC1 (Table 4.2). In fact, the concentration of P was two-fold greater in the rhizosphere of ACC5 than ACC1. Similar to the 2018 season at Syferkuil, the concentrations of N, P, K and Mg were significantly greater in the rhizosphere of ACC5 compared to ACC1 while Ca and Zn was similar between the test accessions in 2018 (Table 4.2).

Inoculation of chickpea with *Bradyrhizobium japonicum*, the application of 90 kg ha^{-1} of P, and rhizobium inoculation plus the application of P resulted in similar concentrations of N, Ca, Mg and Zn in the rhizosphere of chickpea at Syferkuil in 2017 (Table 4.2). However, the application of P, and the supply of rhizobial inoculant plus P resulted in markedly enhanced concentration of rhizosphere P compared to the control (Table 4.2). In contrast, plants raised with rhizobial inoculant as well as the controls exhibited the least P level in their rhizosphere (Table 4.2). The concentrations of P and K were markedly highest in the rhizosphere of plants raised with P plus rhizobium inoculation, followed by that grown with the application of P, second lowest in rhizobial-inoculated plants, and least in controls in 2018 (Table 4.2). Plants grown with rhizobial bacteria plus P, and *Bradyrhizobium japonicum* exhibited greater concentration of Zn in the rhizosphere while controls had the lowest (Table 4.2).

There was significant interaction of genotype x fertilizer on P in both seasons in Syferkuil (Table 4.2). For both accessions, the application of P plus rhizobial inoculant promoted increased P in the rhizosphere, followed by that of plants supplied with P, second-least in rhizobial-inoculated treatments, and lowest controls that were established at Syferkuil in 2017 and in 2018 (Fig. 4.1 A & B).

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Table 4.2. Effect of Genotype, *Rhizobium* and P application on the concentration of macro-and micronutrients in rhizosphere of chickpea grown at Syferkuil farm in 2017 & 2018 (Mean ± SE in same column with dissimilar letter are significantly different at p ≤0.05.

Years	Treatments	N	P	K	Ca	Mg	Zn
2017				mg/kg			
	Genotype						
	Accession 1	25±2.00b	38±1.85b	142±11.90b	413±15.09	640±18.10	2.35±0.12
	Accession 5	29±1.00a	76±1.80a	171±15.19a	430±21.08	631±16.2	2.55±0.18
	Fertilizer Treatments (FT)						
	Control	25±1.00	32±1.40cd	188±13.97b	408±23.16	631±9.1	1.54±0.2
	Rhizobium (R)	26±3.00	36±.1.2c	202±21.74a	419±14.13	638±18.1	2.09±0.14
	Phosphorus (P)	26±2.01	74±3.64b	206±13.57a	434±21.24	646±20	2.13±0.11
	R+P	27±1.01	90±2.50a	213±16.16a	467±13.6	661±15.13	3.21±1.09
	F-statistics						
	Genotype (G)	23.09^{***}	8.47^{*}	10.22^{*}	1.65^{ns}	0.44^{ns}	0.88^{ns}
	Fertilizer Treatments (FT)	0.41^{ns}	16.69^{***}	0.28^{ns}	1.26^{ns}	0.59^{ns}	0.84^{ns}
	G x FT	0.57^{ns}	14.30^{***}	1.59^{ns}	1.22^{ns}	0.00^{ns}	0.12^{ns}
2018	**Genotypes**						
	Accession 1	23±1.20b	26.25±3.28b	100.33±7.73b	665±0.27	420±0.14b	1.13±0.08b
	Accession 5	27±1.1a	74.83±4.35a	129.83±5.61a	673±0.26	475±0.12a	1.34±0.04a
	Fertilizer Treatments (FT)						
	Control	23±1.0b	18.16±1.22d	80.83±3.84d	552±0.11c	395±0.17b	0.74±0.15c
	Rhizobium (R)	26±2.00	35.16±2.05c	101.50±14.14c	685±0.29b	462±0.17b	1.13±0.12b
	Phosphorus (P)	27±3.00	70.50±4.19b	131.16±12.95b	698±0.33b	463±0.93a	1.36±0.06ab
	R+P	28±2.00	82.66±4.30a	146.83±13.22a	741±0.21a	471±0.07a	1.41±0.05a
	F-statistics						
	Genotype (G)	15.57***	11.40***	9.52***	0.04^{ns}	8.63***	4.9*
	Fertilizer Treatments (FT)	6.97^{ns}	6.41***	8.25***	10.31^{ns}	3.52^{ns}	8.09^{ns}
	G x FT	2.54^{ns}	4.97*	1.61^{ns}	4.80^{ns}	0.39^{ns}	1.05^{ns}

Values (Means ± S.E) with dissimilar letters in a column are significantly different at **p*≤0.05. ** *p*≤0.01. ****p*≤0.001 and *ns* = not significant

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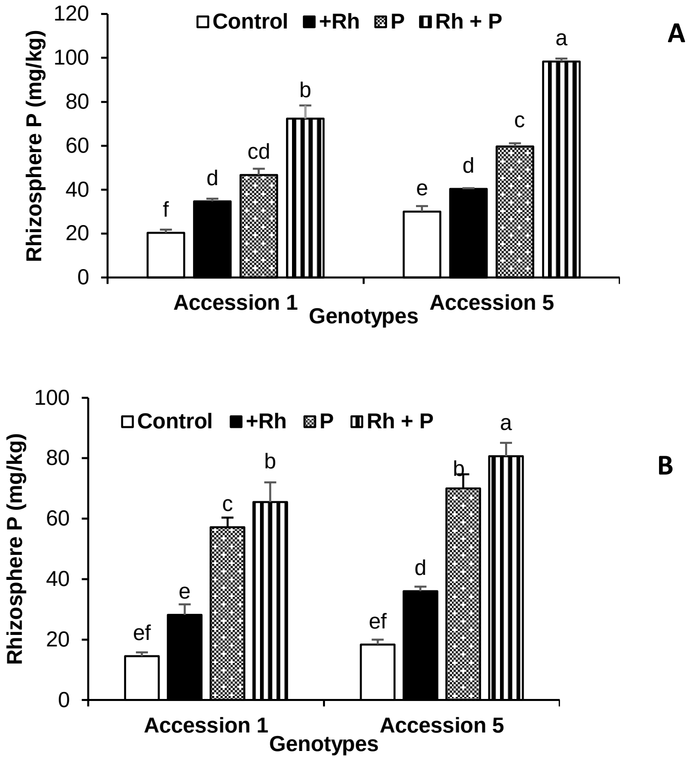

Fig. 4.1 Interactive effect of genotype x fertilizer treatment on P concentration in the rhizosphere at Syferkuil in A) 2017 and B) 2018.

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Results from the Thohoyandou study location on chickpea grown in 2017 showed that the concentrations of N, P, and K were significant while that of Ca, Mg, and Zn were similar between the test genotypes (Table 4.3). The concentrations of N, P, and K were markedly greater in the rhizosphere of ACC5 compared to that of ACC1. However, in 2018 cropping season, chickpea was significant greater in the concentration of N, P, K, Ca, Mg and Zn in the rhizosphere of the test accessions (Table 4.3). Interestingly, all these essential nutrient elements exhibited significantly higher concentrations in the rhizosphere of ACC5 compare to ACC1 (Table 4.3). Phosphorus was more than four-fold greater in the rhizosphere of ACC5 than ACC1. Phosphorus was more than four-fold greater in the rhizosphere of Acc. 5 than Acc. 1. At Thohoyandou in 2017, the application of P plus rhizobium inoculation as well as the supply of P, resulted in greater concentration of P, K, Ca, Mg and Zn, the rhizosphere relative to inoculation, and controls (Table 4.3). Rhizosphere P, K, Ca, Mg and Zn was significantly enhanced by the application of P plus rhizobial inoculant, and by the P application, while rhizobium inoculation resulted in second lowest, and controls showed the least at Thohoyandou in 2018.

There was significant genotype x treatment fertilizer interaction for the other nutrients in the rhizosphere exception of N, Ca and Mg in 2017, and Mg in 2018 the (Table 4.3). Application of P in combination with rhizobial inoculant, gave markedly higher concentration of P, K and Zn in the rhizosphere of both accessions of chickpea grown in 2017 and in rhizosphere N, P, K, Ca and Zn in 2018 (Fig. 4.2 & 4.3). The concentration of N, P, K, Ca and Zn in the rhizosphere was increased by P plus *Bradyrhizobium japonicum*, followed by that raised with the application of P, second-least in rhizobial inoculation and lowest in control (Fig. 4.2 & 4.3). ACC5 was high where P plus rhizobial inoculation was applied, second highest in the supply of P, followed by inoculation, and controls compare to ACC1 in 2017 (Fig. 4.2).

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Table 4.3. Effect of Genotype, *Rhizobium* and P application on the concentration of macro-and micronutrients in rhizosphere of chickpea grown at Thohoyandou farm in 2017 & 2018. Mean ± SE in same column with dissimilar letter are significantly different at p ≤0.05.

Years	Treatments	N	P	K	Ca	Mg	Zn
2017		mg/kg					
	Genotype						
	Accession 1	22±0.00b	20±2.83b	87±5.19b	779±34.32	498±45.12	5.15±0.38
	Accession 5	37±0.00a	98±.05a	138±14.58a	725±43.38	492±63.13	4.96±0.23
	Fertilizer Treatments (FT)						
	Control	20±0.00	57±12.95b	82±2.93cd	703±37.37c	346±52.16c	4.06±0.32b
	Rhizobium (R)	21±0.01	84±9.09b	92±5.44c	761±25.60b	466±38.22b	4.71±0.46b
	Phosphorus (P)	23±0.00	103±4.00ab	105±4.76b	813±57.35ab	504±46.15ab	9.1±0.46a
	R+P	23±0.02	126±18.4a	144±10.11a	836±24.55a	525±53.16a	6.84±0.54a
	F-statistics						
	Genotype (G)	6.64*	18.33***	12.10***	1.19^{ns}	0.52^{ns}	0.17^{ns}
	Fertilizer Treatments (FT)	0.04^{ns}	22.17***	4.65*	1.47^{ns}	2.62*	0.09^{ns}
	G x FT	2.55^{ns}	6.69**	15.45***	0.11^{ns}	5.00^{ns}	4.34*
2018							
	Genotypes						
	Accession 1	33.1±0.01b	20.33±2.6b	84.02±7.20b	562±21.36b	430±21.19b	2.51±0.30b
	Accession 5	45.2±0.05a	56.75±3.28a	126.55±4.80a	811±31.35a	512±24.11a	3.92±0.41a
	Fertilizer Treatments (FT)						
	Control	27.4±0.01c	14.66±1.72c	76.00±4.86b	503±18.61c	415±31.26b	1.50±0.27c
	Rhizobium (R)	31.3±0.01b	30.33±3.84b	79.20±5.58b	625±26.29bc	424±32.21b	3.00±0.15b
	Phosphorus (P)	34.1±0.01b	56.66±3.45a	112.20±4.5ab	743±23.42ab	521±33.12a	3.80±0.53a
	R+P	51.2±0.05a	59.25±4.22a	187.50±6.96a	876±26.46a	541±28.09a	b
	F-statistics						4.56±0.38a
	Genotype (G)	6.69**	15.88***	5.06*	3.52**	5.79**	7.52**
	Fertilizer Treatments (FT)	10.75***	11.18***	11.65***	11.77***	12.08**	12.81***
	G x T	0.96*	1.96**	3.81*	0.61*	4.09ns	1.59*

Values (Means ± S.E) with dissimilar letters in a column are significantly different at *p≤0.05. ** p≤0.01. ***p≤0.001 and *ns* = not significant

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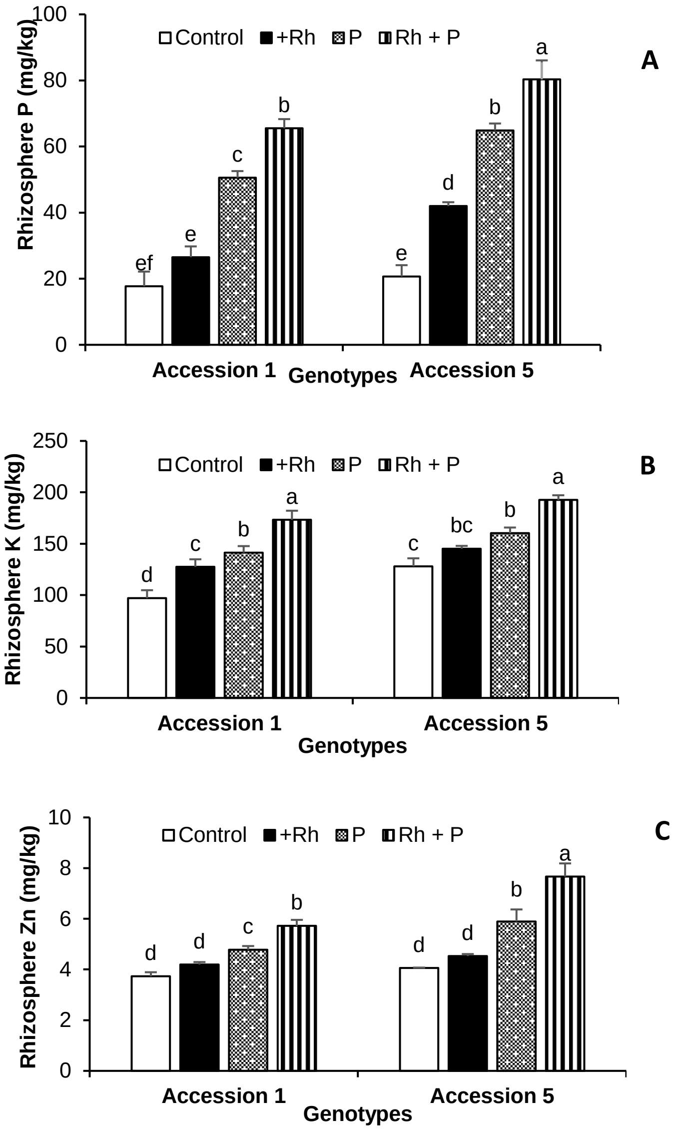

Figure 4.2 The interactive effect of genotype x fertilizer treatment in the rhizosphere A) Phosphorus B) Potassium, C) Zinc at Thohoyandou in 2017.

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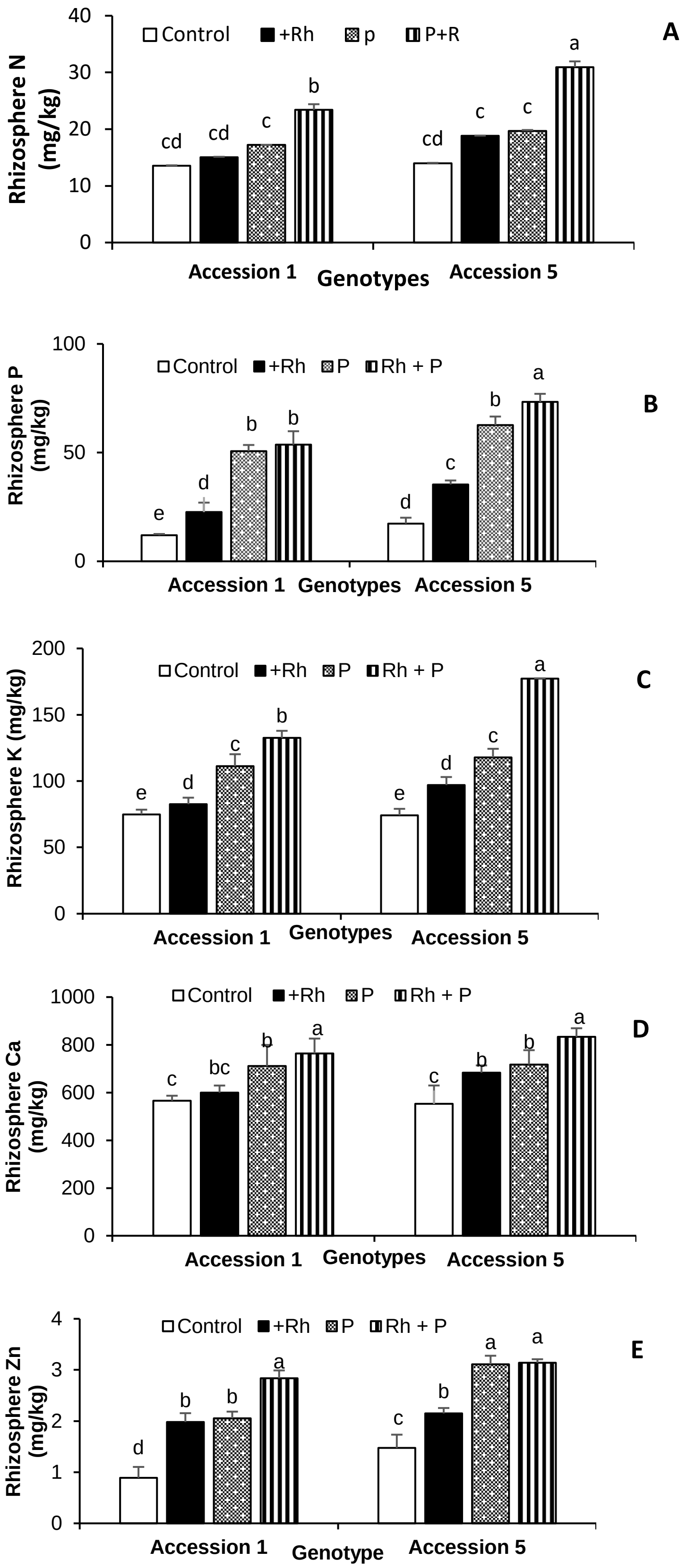

Figure 4.3 The interactive effect of genotype x fertilizer treatment in the rhizosphere A) Nitrogen, B) Phosphorus C) Potassium, D) Calcium and E) Zinc at Thohoyandou in 2018

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4.3.3 Comparison of rhizosphere mineral elements across the locations in 2017 and 2018

Between the study locations in 2017, accessions showed significant differences in the concentration of the selected mineral nutrients (Table 4.4). For example, N, P, Ca and Zn were markedly higher in the rhizosphere of plants grown at Thohoyandou than at Syferkuil. However, the levels of K and Mg were greater in the rhizosphere of chickpea that was established at Syferkuil (Table 4.4). Across the locations in 2017, the study showed significant interactions including that of genotype x fertilizer treatments on N, P, K and Zn; genotype x location on N, P and K; Fertilizer treatment x location on P, K and Zn; and genotype x fertilizer treatments x location on N, P, K, Ca and Zn (Table 4.4).

Concentration of macro-and micronutrients in the rhizosphere of chickpea grown at Syferkuil and Thohoyandou during the 2018 cropping season revealed significant differences (Table 4.4). N, P, Ca, and Zn were markedly greater in the rhizosphere of plants grown at Thohoyandou while K, and Mg were significantly greater at in plants established at the Syferkuil experimental farm. There were significant interactions for genotype x fertilizer treatments on all the test nutrients except Mg; genotype x location on all selected nutrients except for Ca and Mg; fertilizer treatments x location on all test macro-and micronutrients; and genotype x fertilizer treatments x location on N, P, K, Ca, and Zn (Table 4.4).

There was significant interaction for genotype x location interaction on N and P in 2017, ACC5 accumulated the highest concentrations of majority of nutrients when grown at Thohoyandou than at Syferkuil, Furthermore, the interaction for genotype x location shows that the rhizosphere K was greater at Syferkuil compared to Thohoyandou (Fig. 4.4 B). However, rhizosphere N of ACC1 in Thohoyandou was similar to that of ACC5 in Syferkuil. For fertilizer treatment x location for P, K, and Zn in 2017, the rhizosphere P and Zn was markedly increased at Thohoyandou by the application of P plus rhizobial inoculant while controls exhibited the least (Fig. 4.5 A & C). For genotype x fertilizer treatment x location for N, P, K, Ca, and Zn in 2017, ACC1 without fertilizer addition in Syferkuil had the lowest rhizosphere N and the highest was recorded in ACC5 supplied with P+R at both sites (Fig. 4.6 A, B, C and E). Lowest P was recorded in ACC1 without fertilizer application in Syferkuil and the highest P was in both accessions supplied with P + R at both sites (Fig 4.6 B). No difference between rhizosphere Ca on accessions 1 supplied with P+R in both sites (Fig. 4.6 D).

For the genotype x location interaction on N, P, K and Zn in 2018, the rhizosphere N was highest in all genotypes at Thohoyandou relative to Syferkuil (Fig.4.7A). In contrast, rhizosphere P was the highest on ACC1 and ACC5 at Syferkuil compared to Thohoyandou Fig. 4.7 B). Rhizosphere K, and Zn was greater at Thohoyandou on both accessions (Fig.4.7

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C & D). There was fertilizer treatment x location interaction in 2018, the application of P plus rhizobial inoculation increased the rhizospheric concentration of N, P, K, Ca and Mg in both location (Fig.4.8 A, B, C, D, E & F). For genotype x fertilizer treatment x location in 2018, the application of P plus rhizobial inoculant promoted the highest accumulation of N, P, K, Ca and Zn while the supply of P resulted in highest rhizospheric P; Ca; and Zn at Syferkuil (Fig. 4.9 A, B, C, D & E). Overall, controls accumulated the least N, P, K, Mg, Ca, and Zn.

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Table 4.4. Effect of Genotypes, *Rhizobium* and P application on the concentration of macro-and micronutrients in rhizosphere of chickpea grown at Syferkuil and Thohoyandou in 2017 and 2018 (Mean ± SE in same column with dissimilar letter are significantly different at p ≤0.05).

Years	**Location**	N	P	K	Ca	Mg	Zn
2017		mg/kg					
	Syferkuil	22±0.01b	55.39±5.60b	203.95±10.31a	437±16.12b	644±17.07a	2.49±0.28b
	Thohoyandou	31±0.00a	164.22±42.46a	122.07±10.43b	799±17.34a	489±24.10b	5.11±0.28a
	F-statistics						
	Location	8.40***	7.35**	27.17***	10.5***	14.4***	4.85***
	G x FT	6.89**	7.70***	9.68***	11.31***	0.01ns	13.29***
	G x L	5.08*	6.4**	3.5*	2.22ns	1.04ns	0.01ns
	FT x L	0.03ns	5.38*	0.74*	2.38ns	0.00ns	7.98***
	G x FT x L	2.58*	6.36***	4.44*	4.41*	0.81ns	5.45***
2018	**Location**						
	Syferkuil	22.3±0.00b	44.95±4.88b	192.08±15.59a	626±21.18b	491±41.10a	1.23±0.05b
	Thohoyandou	36.7±0.02a	54.39±4.40a	115.62±12.93b	730±23.33a	432±18.13b	3.53±0.26a
	F-statistics						
	Location	3.41***	3.08***	7.04**	0.33ns	5.32**	5.0**
	G x FT	13.46***	7.68***	14.97**	7.81**	0.00ns	7.60***
	G x L	5.71***	3.19***	6.36**	0.91ns	0.04ns	7.79**
	FT x L	7.95***	3.48*	4.54**	3.73**	4.13**	10.18**
	G x FT x L	1.02*	0.48*	3.24*	1.19*	0.76ns	1.70*

Values (Means ± S.E) with dissimilar letters in a column are significantly different at **p*≤0.05. ** *p*≤0.01. ****p*≤0.001 and *ns* = not significant

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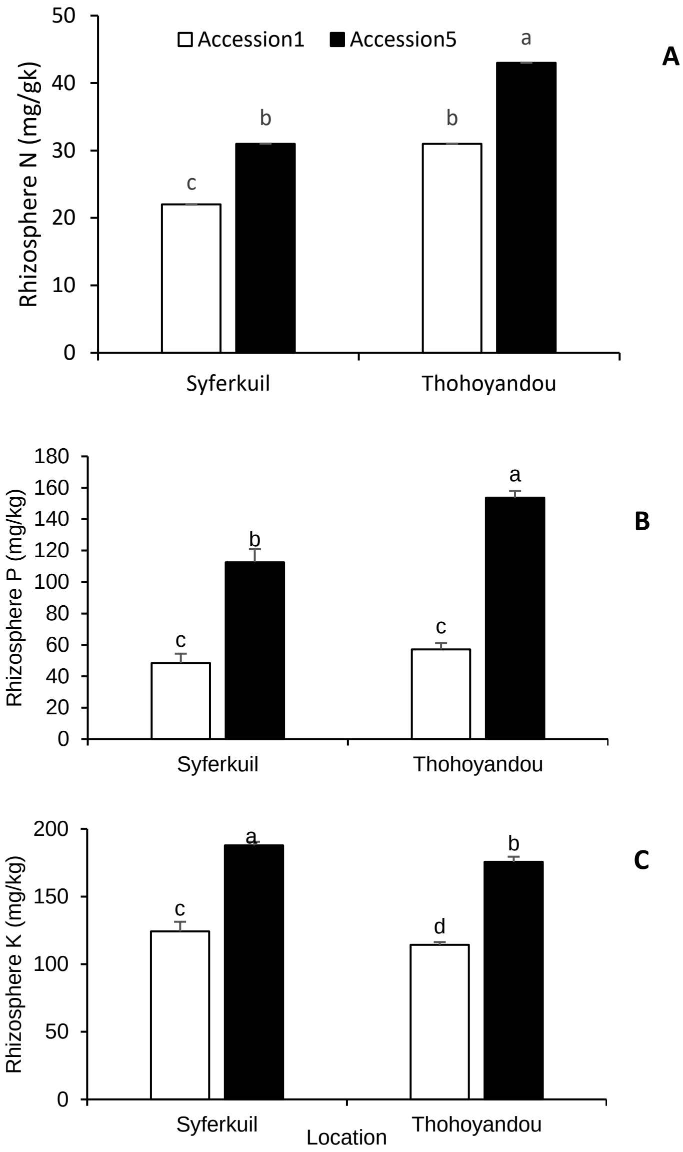

Figure 4.4 The interactive effect of genotype x location in the rhizosphere A) Nitrogen, B) Phosphorus, C) Potassium at Syferkuil in 2017.

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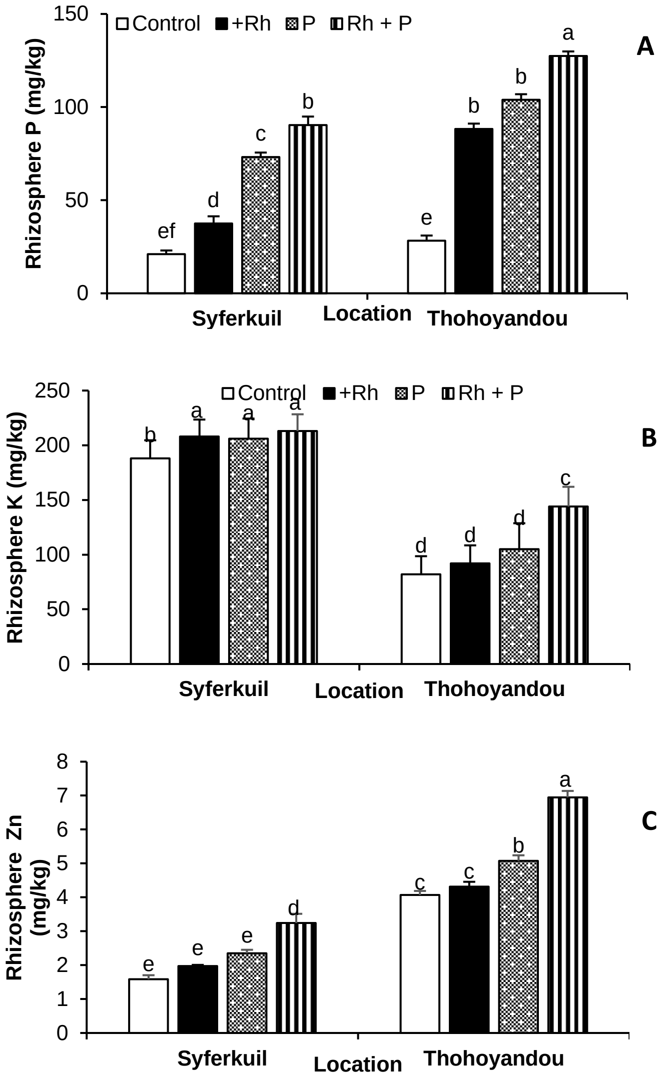

Figure 4.5 The interactive effect of fertilizer treatment x location in the rhizosphere A) Phosphorus, B) Potassium, C) Zinc at Syferkuil and Thohoyandou in 2017.

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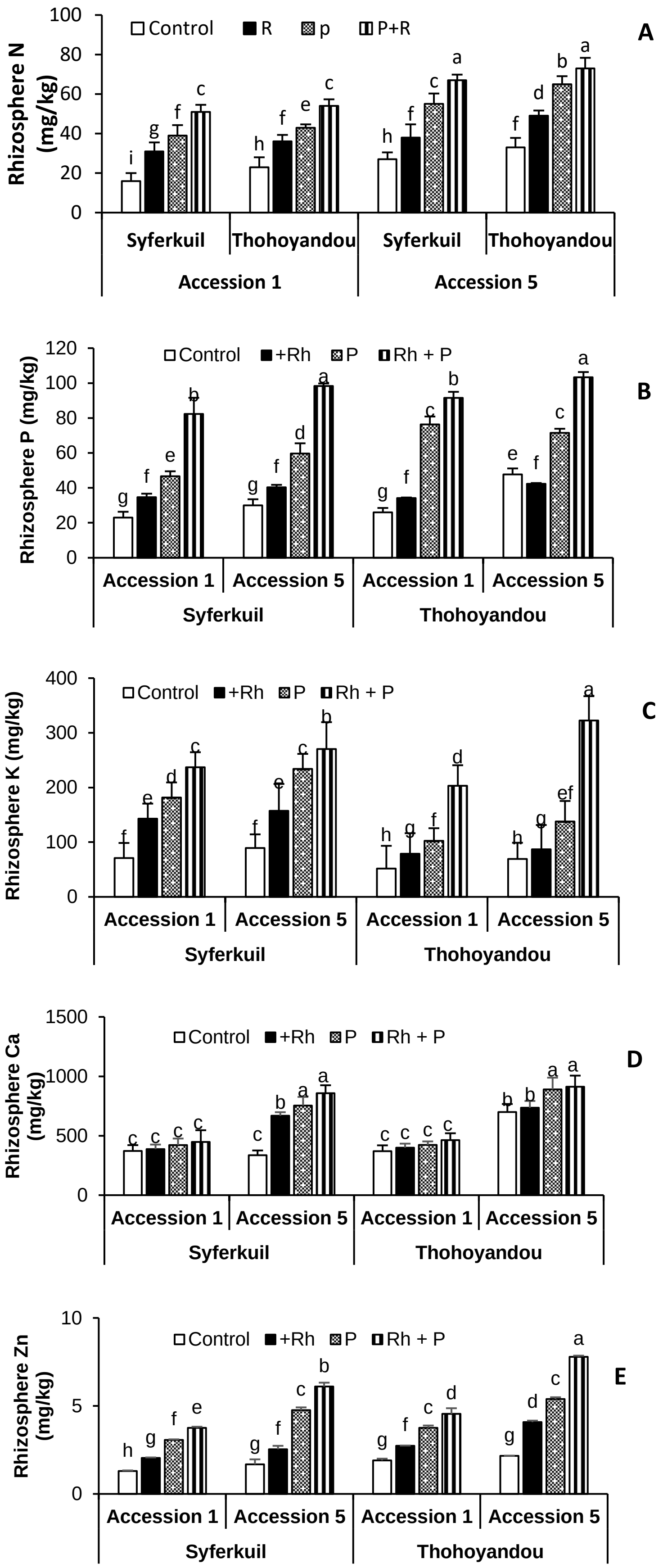

Figure 4.6 The interactive effect of genotype x fertiliser treatment x location in the rhizosphere A) Nitrogen, B) Phosphorus, C) Potassium, D) Calcium, E) at Syferkuil and Thohoyandou in 2017.

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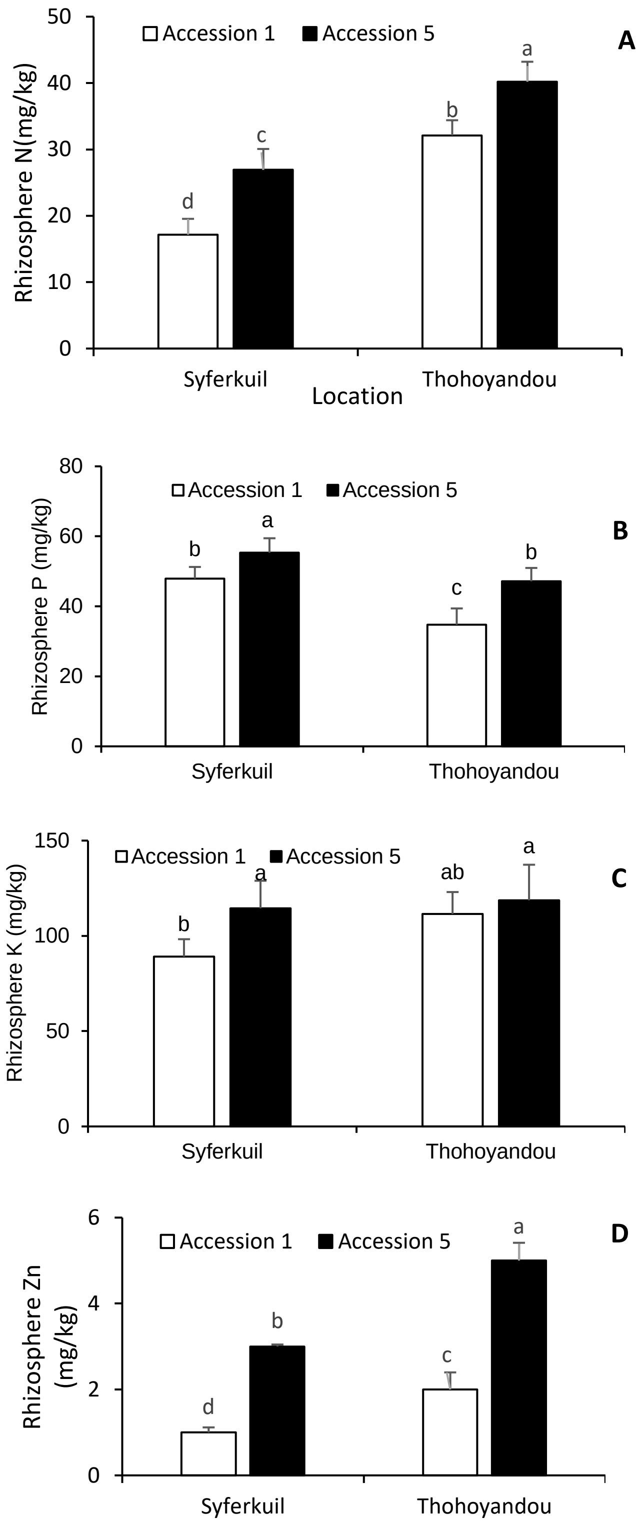

Figure 4.7 The interactive effect of genotype x location in the rhizosphere A) Nitrogen, B) Phosphorus, C) Potassium and D) Zinc at Syferkuil and Thohoyandou in 2018.

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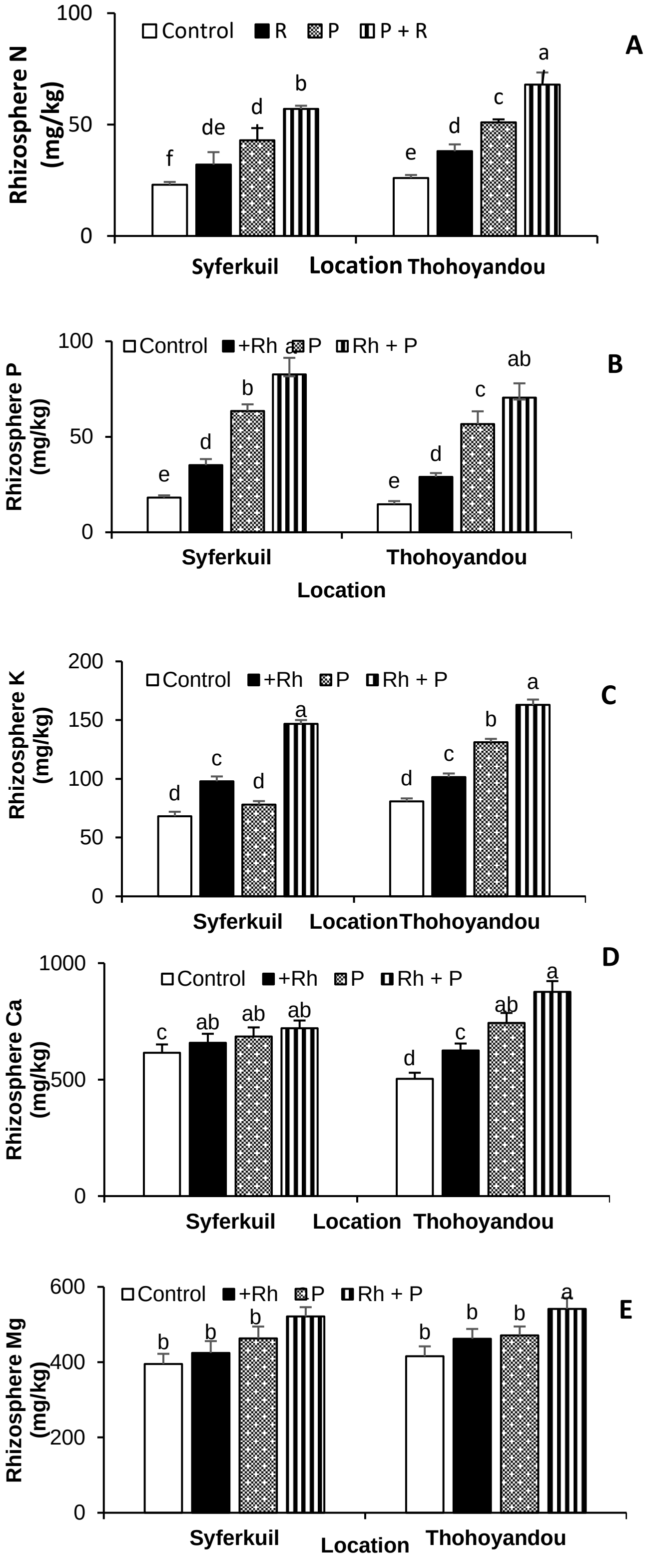

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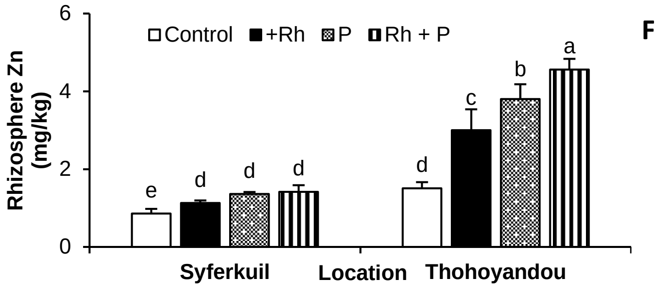

Figure 4.8 Interactive effect of fertilizer treatment x location in the rhizosphere A) Nitrogen, B) Phosphorus, C) Potassium, D) Calcium, E) Magnesium and F) Zinc at Syferkuil and Thohoyandou in 2018.

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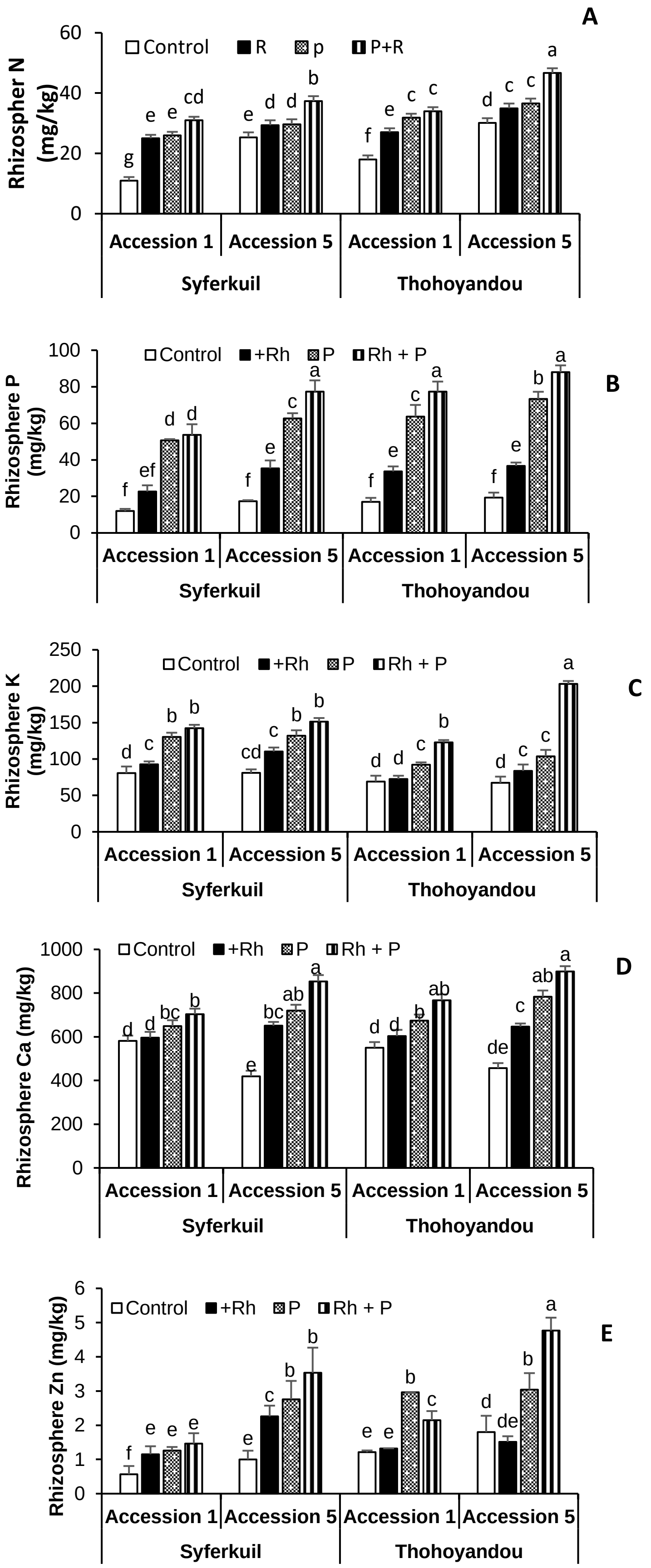

Figure 4.9 The interactive effect of genotype x fertilizer treatment x location in the rhizosphere A) Nitrogen, B) Phosphorus, C) Potassium, D) Calcium, E) Zinc at Syferkuil and Thohoyandou in 2018.

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4.4 DISCUSSION

Genotypes exhibited differences in concentration of nutrients in the rhizosphere. For example, accession 5 revealed a markedly higher concentration of most of the test nutrients (N, P, and K in 2017, and N, P, K, Mg and Zn in 2018 at Syferkuil, as well as N, P, and K in 2017, and N, P, K, Ca, Mg and Zn in 2018 at the Thohoyandou site) (Tables 4.2 and 4.3) compared to accession 1. The enhanced accumulation of most of the macro-and micronutrients in the rhizosphere of accession 5 at both sites was despite the differences in the pH, texture, and fertility of the soil in the 2 locations (Table 4.1). Although the study did not determine possible causes of the variation, it has been reported that genotypes may differ in their ability to access and solubilise nutrients in the rhizosphere and this is controlled largely by genetic make-up, composition of root exudates, and root architecture (Rengel and Marschner, 2005).

Therefore, it is highly that accession 5 could have a rooting system composed of different but superior chemistry and biology. These include fine roots with small diameter that allowed it access to a wider surface area; exhibiting of greater capacity to solubilise more nutrients; and ability to secret exudates that are capable of solubilisation of the test mineral nutrients (Darra, 1993; Dakora and Phillips, 2002). The results are in accordance with the findings of Matseo (2019) who reported differences in concentration of nutrients in the rhizosphere of desi-type chickpea established in the Mpumalanga and Gauteng Provinces of South Africa.

The application of P, and P plus rhizobium inoculation resulted in greater accumulation of P in the rhizosphere compared to rhizobium inoculation, and the control at both study locations in 2017 and 2018 (Table 4.2 and 4.3). It is only when chickpea was established at Syferkuil in 2018 that the application of P plus rhizobium showed a significantly higher concentration of P in the rhizosphere relative to that of plants growth with the supply of P (Table 4.2). Although rhizosphere soil pH was not determined in this study, the application of phosphate fertilizer has been widely reported to alter (largely decrease) the soil pH in the rhizosphere (Bolan *et al*., 1991; Nyoki and Ndakidemi, 2018). Lower pH in the rhizosphere enhances the solubility and desorption from various particles (Penn and Camberato, 2019) and thus the concentration and accumulation of P would likely increase in rhizosphere of soil supplied with P fertilizer. Moreover, P fertilization enhances the exudation of organic acids by roots and increases the capacity of the exudates to mobilize P, including improving the activity of microorganisms in the soil (Nuruzzaman *et al*., 2005). However, it is not clear in literature as to the particular rate of P fertilizer that make these mechanisms possible. Also, the application of 90 kg P ha^{-1} could have resulted in a marked increase in extractable soil P, and therefore enhanced the P concentration in the rhizosphere of the selected chickpea (Li *et al*., 2004).

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These factors, individually or in combination, could explain the increased accumulation of P in the rhizosphere with the application of P at these test study locations with contrasting soil types.

Rhizobium inoculant not only enhances a legume's N_2-fixing potential, it also increases the concentration of nutrients in the rhizosphere (Nyoki and Ndakidemi, 2018) perhaps due to enhanced solubility of the nutrients. For example, rhizobia is able to synthesize siderophores that chelate oxide-bound and particle-bound P and is capable of synthesizing other phytohormones such as auxins, cytokinins and gibberelins, lumichrome, riboflavin which increase the availability of P in soils (Schutz *et al*., 2018). Therefore, increases in rhizosphere P with application of P plus rhizobium inoculation was not unexpected at both sites despite the contrasting soil types.

In general, the P plus rhizobium inoculation treatment increased the accumulation of Ca and Mg in the rhizosphere of chickpea grown in 2018 at both sites (Table 4.2 & 4.3), and that of Mg in 2017 at Syferkuil (Table 4.2). Through increased synthesis of indole acetic acid, biofertilisers including rhizobium, are able to enhance the solubilisation and uptake of Mg and Ca, along with other cations (Bambara and Ndakidemi, 2010; Menendez and Garcia-Fraile, 2017).

In contrast to the micronutrients, fertiliser application affected the concentration of Zn in the rhizosphere of plants grown at both locations during the second cropping year where rhizosphere Zn was greater with the application of P, Rhizobium inoculation, and P plus rhizobium inoculation compared to the control, while at Thohoyandou, P plus rhizobium exhibited the largest . Significantly higher Zn in the rhizosphere of the rhizobium-inoculated common bean has been reported in other studies (Bambara and Ndakidemi, 2010; Nyoki and Ndakidemi, 2018). Inoculation of chickpea enhanced the symbiotic biological nitrogen fixation process through which legumes alter the balance in cation/anion in the rhizosphere, leading to acidification of the rhizosphere, and therefore increased dissolution of Zn among other mineral nutrients (Marschner, 1995).

The greater concentration of K in the rhizosphere with the application of P plus rhizobium inoculant compare to other treatments could be attributed partly to the ability of certain bacteria including that which is native in soils and commercial solubilise the mineral form of K in soils through a range of mechanisms including the production of organic and inorganic acids, chelation and exchange reactions (Hassan *et al*., 2017; Rashid *et al*., 2016). Although there is scanty literature that shows that the application of *Bradyrhizobium japonicum* increases K in the rhizosphere, it is likely that it produces organic acids by rhizobium application which enables it to solubilise K in soils, as shown in this study.

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The markedly higher concentration of K in the rhizosphere with the application of P in the current study is comparable to previous studies that the application of P fertilizer markedly enhanced the concentration of K in the rhizosphere of a soybean (Nyoki and Ndakidemi, 2018).

In this study, the concentration of N, P, Ca, and Zn was higher in the rhizosphere of chickpea established at the clay soil of Thohoyandou and K and Mg were markedly greater at Syferkuil (sandy soil) in 2017. In contrast N, P, Ca and Zn concentration were greater at Thohoyandou (clay soil) in 2018 (Table 4.7). Overall, majority of the test nutrients exhibited greater concentrations in the rhizosphere of chickpea grown in the clay soil of Thohoyandou probably due to greater efficacy of the biofertilizer because of the greater capacity of fine textured soil to retain soil organic matter and mineral nutrients and exhibit higher microbial activity of fine-textured soils, relative to the largely sandy soils at Syferkuil (Hamarashid *et al*., 2010). Also, the generally higher fertility (higher OC, N, Ca, S, Mn, and Zn) of the clay soil in Thohoyandou could have increased the efficacy of the rhizobium as biofertilizers showed improved efficiency when applied in relatively fertile soils (Kyei-Boahen *et al*., 2017; Schutz *et al*., 2018).

4.5 CONCLUSION

The combination of P+R enhances nutrients accumulation, therefore there is a root interaction and nutritional interrelationship between nutrients accumulation in the rhizosphere soil which varies with genotype. Accession 5 accumulated higher nutrients compare to accession 1, these may be that accession 5 would have a rooting system composed of different but superior chemistry and biology. However, studying the root biology and chemistry of the accessions is needed before a definite conclusion can be made.

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CHAPTER 5. EFFECT OF P APPLICATION AND RHIZOBIUM INOCULATION ON CONCENTRATION OF MACRO-AND MICRONUTRIENTS ON SHOOTS AND GRAIN OF CHICKPEA GENOTYPES

ABSTRACT

The growth, development, and mineral nutrition of cultivated plants, especially in low-input cropping systems, is dependent on nutrients contributed through weathering of the parent material. Naturally, parent materials contain different concentrations of nutrients, and therefore soils derived from these parent materials also contain varying concentrations. when food crops are cultivated on such nutrient-poor soils without the application of sufficient quantities of nutrients, they become deficient in mineral nutrients. Although plants that are grown in poor soils adopt mechanisms for enhancing nutrient uptake, such mechanisms hardly contribute nutrients that are sufficient for the need of plants. Therefore, this study assessed the effect of fertiliser phosphorus and rhizobium inoculation on the concentration of nutrients in the shoots and grain of two chickpea genotypes. Field experiment were established at Thohoyandou and Syferkuil, South Africa in 2017 and 2018. At both locations, accession 5 accumulated higher concentration of Nitrogen, Phosphorus, Potassium, Magnesium, Calcium and Zinc in the shoots and grain. The Rhizobium inoculation, application of P, and P plus rhizobia inoculant increased nutrients accumulation on shoots and grain at Syferkuil. Similarly, the application of P and P plus rhizobial inoculant increased concentrations of N, P, K and Zn on chickpea shoots at flowering stage. Application of phosphorus, and rhizobium inoculant plus phosphorus resulted in greater accumulation of nutrients at both sites and season. At both locations, accession 5 exhibited greater ability for the uptake and accumulation of nutrients compared to accession 1. Clearly, fertiliser phosphorus and rhizobium inoculation are important for enhance nutrients uptake and accumulation by chickpea, but this may vary with genotypes.

Key words: Chickpea, Grain, Nutrients, Phosphorus, Rhizobium inoculation, Shoots

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5.1 Introduction

In South Africa, chickpea is fast becoming one of the most consumed grain legumes after common bean, soybean, cowpea, groundnut and Bambara groundnut (Mpai and Maseko, 2018). Although the grain of chickpea is the most common and widely consumed plant part by humans, in other parts of the world, the shoot is also widely utilised as food and feed for humans and livestock, respectively (Bampidis and Christodoulou, 2011). Furthermore, the green leaves and twigs of chickpea are regularly harvested, cooked and eaten as a vegetable and serve as sources of mineral nutrients and protein (Ibrikci *et al*., 2003). Unfortunately, reports indicate that malnutrition, which is caused largely by the deficiency of mineral nutrients in aboveground parts of chickpea, is prevalent in many regions where chickpea is part of the staple food (Marles, 2017).

The growth, development, and mineral accumulation of plants especially those cultivated in low-input cropping systems, is dependent on nutrients contributed through weathering of the parent material. Naturally, parent materials contain different concentrations of nutrients, and therefore soils derived from these parent materials also contain varying concentrations. For example, soils that originate from ancient weathered sandstones and shales contain high levels of boron but very low concentrations of P (Wisheu *et al*., 2000; Maqshoof *et al*., 2012). Additionally, the weathering of a granitic parent material results in low concentrations of P (Porder and Ramachandran, 2012). Thus, when food crops are cultivated on such nutrient-poor soils without the application of sufficient quantities of nutrients, they become deficient in mineral nutrients. Although plants that are grown in nutrient-poor soils adopt mechanisms for enhancing nutrient uptake, such mechanisms hardly contribute nutrients that are sufficient for the need of plants.

Majority of cropping fields in inland South Africa are derived from granitic parent material and therefore contain low concentrations of P (Buhmann *et al*., 1996; Mpai and Maseko, 2018). Although, all essential nutrients are needed for the growth and yield of plants (Reid, 2001), plants need major macronutrients including P in higher concentrations and their deficiency has the most negative effect on the physiological, biochemical and metabolic functioning of plants (O'Hara, 2002). Therefore, the deficiency of P, especially in cropping fields in inland South Africa, should be a concern to farmers that intend to adopt and cultivate crops such as chickpea. The application of P increased the nodulation, growth, yield-related parameters, water-use efficiency and overall performance of chickpea in areas located in the Northeast of South Africa (Madzivhandila *et al*., 2012; Ogola *et al*., 2013, Ogola, 2015; Lusiba *et al*., 2017). However, the studies did not assess the accumulation of mineral nutrients by the chickpea crop.

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The inoculation of grain legumes with rhizobium has shown significant and non-significant increases of nutrients in various organs. For example, the concentration of N in shoots of rhizobium-inoculated cowpea was similar to that in un-inoculated controls (Ferreira et al., 2013). In contrast, the inoculation of cowpea with *Bradyrhizobium japonicum* significantly improved the accumulation of Zn, Fe, Cu and Mn in aboveground and belowground organs (Nyoki and Ndakidemi, 2014).

The accumulation of mineral nutrients in aboveground plant organs is affected by factors including cultivar, soil fertility status, rhizobium inoculation, and the application of P fertilizer (Tian *et al*., 2012; Xu *et al*., 2016). Most assimilates, including mobile nutrient elements are translocation from the shoot to the pods or grain during grain filling (Wien *et al.,* 1976) but this varies with cultivars (Nascente and Stone, 2018). Furthermore, variation in translocation and accumulation of nutrients in grain legumes have been reported (Araujo and Teixera, 2003).

The concentration of macro-and micronutrients in plant tissue pulse varies between leaves and grains (Dakora and Belane, 2019; Edelman and Colt, 2016). For example, apart from significant differences in the concentration of selected macro-and micronutrients between genotypes, Dakora and Belane, (2019) showed markedly greater levels of Fe, Cu, Zn, Mn and B in leaves compared to grain of cowpea that was grown without inoculation nor application of fertilizers. Similarly, grain legumes exhibited variation in the accumulation of nutrients between leaves, pods, and grain where organic fertiliser was applied (Gulmezoglu and Kayan, 2011).

Although the effect of genotypes, rhizobial inoculation and P application on growth and yield of chickpea has been well documented is South Africa and elsewhere, there is a dearth of information in literature on the effect of the application of P, rhizobium inoculation, and the co-application of P plus rhizobium on the concentration of nutrients in shoot and grain of chickpea. Knowledge on possible variation in the accumulation of nutrient between genotypes of chickpea at different developmental stages, and between leaves and grain for chickpea is needed. Also, the role of rhizobium inoculation, P application, and P plus rhizobium on nutrient accumulation at flowering, maturity, and grain filling stage is lacking. Such studies are likely to generate useful information on practices that can lead to the cultivation of nutritious shoot and grain material of chickpea and in improving nutritional security. Therefore, this study evaluated the response of nutrients accumulation and partitioning in chickpea to genotype, application of P at two sites with contrasting soil types.

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5.2 Materials and methods

The detailed description of materials and methods has been presented in chapter 3 but a brief summary is outlined here. The field experiment was conducted at two sites, Syferkuil farm and University of Venda experimental farm in 2017 and 2018 winter. Treatments consisted of a factorial combination of two chickpea genotypes (Accession 1 and Accession 5) and two phosphorus fertilizer levels (0 and 90 kg ha^{-1}) arranged in a randomized complete block design and replicated three times. Shoots were collected at flowering and harvest maturity and oven-dried at 65 °C for 48 hours. Plant samples and grain were ground to fine powder and packaged into plastic bags and preserved for subsequent chemical analysis of macro and micronutrient elements. The shoots and grain were chemically analysed to determine their contents of N, P, K, Ca, Mg and Zn. The total N concentration was determined by the microKjeldahl method. For the determination of the remaining elements, samples were subjected to wet digestion (Mehlich, 1984). Data on nutrients element on chickpea shoots and grain were subjected to an analysis of variance using a STATISTICA software version 10.1

5.3 Results

5.3.1 Mineral accumulation in the shoots at flowering

Genotype affected the concentration of N, P, K, Ca, Mg and Zn in plant shoots at flowering stage in Syfekuil in 2018 but had no effect on Mg and Zn accumulation in 2017 (Table 5.1). In 2018 all the macro and micronutrients were greater in the shoots of ACC5 compared to ACC1 (Table 5.1). Similarly, ACC5 accumulated higher levels of nutrients compared to ACC1 in 2017. Similarly, N, P, K, Ca, Mg and Zn were higher in the shoots of ACC5 compared to ACC 1 in Thohoyandou (clay soil) in 2018 (Table 5.2). Fertilizer treatment had significant effect on the accumulated P, K, Ca, Mg and Zn at flowering stage at Syferkuil 2017. However, in 2018 fertilizer treatment had a significant effect on all the nutrients elements in both sites and seasons, while Thohoyandou the effect of fertilizer on accumulation of N, P, K, Ca and Zn was significant in 2017 (Table 5.2). For both accessions, the application of P plus rhizobium inoculation promoted nutrients accumulation on the shoots, followed by that of plants supplied with P, second-least in rhizobial-inoculated treatments, and lowest controls that were established at Syferkuil in 2017 and in 2018 (Figs. 5.1 A, B, C, D, E and 5.2 A, B, C, D, E).

When established in 2017, the study revealed significant interaction of genotype x fertilizer treatments on P, K, Ca and Zn while that of the other N and Mg was similar, while in 2018 the interaction between genotype and fertilizer treatment was on N, K, Ca and Zn at Syferkuil (Table 5.1).

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In Thohoyandou 2017 and 2018 there was significant interaction of G x FT on Zn only (Fig. 5.3 A & B). For both accessions, the application of P plus rhizobium inoculation increased Zn on the shoots, followed by that of plants supplied with P, second-least in rhizobial-inoculated treatments, and lowest controls.

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Table 5.1 The effect of rhizobial inoculation and phosphorus application on the concentrations of macro-and micronutrient concentrations in the shoots of chickpea accessions at flowering at the Syferkuil farm in 2017 and 2018. (Mean ± SE in same column with dissimilar letter are significantly different at p ≤0.05).

Years	Treatments	N	P	K	Ca	Mg	Zn
2017	**Genotypes**	mg/g					
	Accession 1	30.4±0.09b	3.7±0.01b	24.4±0.12b	21.1±0.16b	15.6±0.09	0.025±0.0
	Accession 5	33.6±0.07a	4.2±0.01a	28.8±0.09a	27.7±0.15a	17.4±0.16	0.026±0.0
	Fertilizer treatments (FT)						
	Control	33.2±0.14	3.7±0.01ab	21.9±0.14c	21.8±0.27b	12.4±0.21b	0.022±0.0b
	Rhizobium (R)	33.5±0.15	3.8±0.02a	26.4±0.22b	22.1±0.19b	14.1±0.15ab	0.025±0.0b
	Phosphorus (P)	33.6±0.18	3.9±0.02a	28.2±0.80b	26.2±0.35a	17.8±0.25a	0.028±0.ab
	P+R	34.6±0.07	4.0±0.02a	30.8±0.09a	27.1±0.17a	19.9±0.09a	0.030±0.0a
	F-statistics						
	Genotype (G)	5.23*	11.67***	6.32*	8.29***	0.82ns	2.64*
	Fertilizer Treatments (FT)	2.17**	2.41**	4.54*	1.00*	0.50^{ns}	3.76*
	G x FT	1.04^{ns}	6.4***	1.53*	3.65*	0.69^{ns}	2.19**
2018							
	Genotype						
	Accession 1	22.1±0.12b	2.6±0.04b	23.8±0.18b	19.1±0.18b	14.1±0.08b	0.22±1.51b
	Accession 5	33.8±009a	4.8±0.06a	40.0±0.12a	26.4±0.15a	16.9±0.18a	0.28±2.12a
	Fertilizer treatments (FT)						
	Control	28.9±0.03c	2.5±0.00c	18.0±002c	17.9±0.24b	14.5±0.23c	0.20±0.70b
	Rhizobium (R)	31.4±0.03b	3.3±0.00b	24.4±0.18b	24.5±0.25a	15.4±0.29b	0.23±0.30b
	Phosphorus (P)	34.0±0.03a	4.7±0.02ab	27.7±0.05b	25.1±0.36a	15.6±0.13b	0.28±0.89b
	P + R	37.6±0.05a	7.2±0.05a	31.4±0.10a	26.4±0.20a	18.7±0.07a	0.35±2.12a
	F-statistics						
	Genotype (G)	5.04*	2.72***	4.30***	10.60**	1.08*	9.47***
	Fertilizer Treatments (FT)	4.22***	13.57***	8.68**	2.20*	0.63^{ns}	4.37**
	G x FT	1.42*	7.57^{ns}	5.99***	1.22*	0.64^{ns}	1.36*

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Table 5.2 The effect of rhizobium inoculation and phosphorus application on the concentrations of macro-and micronutrient concentrations in shoots of chickpea accessions at flowering grown at the Thohoyandou in 2017 and 2018(Mean ± SE in same column with dissimilar letter are significantly different at p ≤0.05).

Years	Treatments	N	P	K	Ca	Mg	Zn
2017	**Genotypes**			mg/g			
	Accession 1	40.2±0.18b	0.1±0.01b	25.4±0.12b	23.5±0.10b	4.9±0.00	0.039±0.0
	Accession 5	49.7±0.22a	3.6±0.00a	67.2±0.62a	28.7±0.18a	5.0±0.00	0.042±0.0
	Fertilizer treatments (FT)						
	Control	40.4±0.32b	3.5±0.02b	25.7±0.13c	23.8±0.14c	4.8±0.00	0.032±0.0b
	Rhizobium (R)	42.9±0.27b	4.2±0.02a	42.6±1.18bc	31.9±0.66b	4.9±0.01	0.036±0.0b
	Phosphorus (P)	44.4±0.42b	4.4±0.03a	63.0±0.64ab	33.5±0.51b	6.0±0.02	0.039±0.0b
	P+R	51.2±0.31a	4.5±0.01a	70.3±0.70a	50.3±0.19a	6.06±0.11	0.049±0.0a
	F-statistics						
	Genotype (G)	10.67**	8.44*	8.25***	7.48**	0.16ns	0.39**
	Treatments (FT)	1.33**	3.45*	7.92***	1.15**	0.24ns	5.01*
	G x FT	2.88ns	0.30ns	2.81ns	0.26ns	1.06ns	4.15**
2018	**Genotypes**						
	Accession 1	32.1±0.12b	3.0±0.04b	23.8±0.18b	19.1±0.18b	12.9±0.08b	0.025±0.0b
	Accession 5	33.8±0.09a	4.8±0.06a	31.0±0.12a	26.4±0.15a	16.1±0.18a	0.028±0.0a
	Fertilizer treatments (FT)						
	Control	28.9±0.15c	2.5±0.00d	18.0±0.18c	17.9±0.24b	14.5±0.23c	0.020±0.0b
	Rhizobium (R)	31.4±0.03bc	3.3±0.00c	24.4±0.02b	21.3±0.25a	15.4±0.29b	0.023±0.0b
	Phosphorus (P)	34.0±0.03b	4.7±0.02ab	27.7±0.05b	23.5±0.36a	15.6±0.13b	0.028±0.0ab
	P + R	37.6±0.05a	7.2±0.05a	31.4±0.10a	24.5±0.20a	18.7±0.07a	0.035±0.0a
	F-statistics						
	Genotype (G)	3.60*	3.56**	2.61*	8.35**	6.6**	12.94**
	Fertilizer Treatments (FT)	6.69**	9.98**	7.42**	12.43*	10.66**	19.39**
	G x FT	1.98ns	5.40ns	0.52ns	1.52ns	1.05ns	1.95*

Values (Means ± S.E) with dissimilar letters in a column are significantly different at *p≤0.05. ** p≤0.01. ***p≤0.001 and *ns* = not significant

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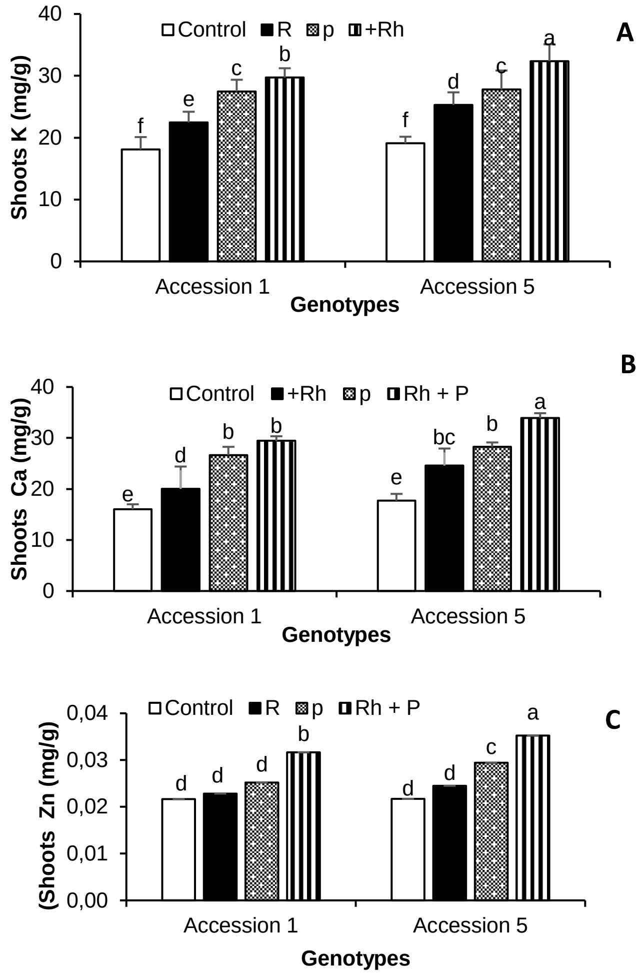

Fig 5.1 The interactive effect of genotype x fertilizer treatments on shoots A) Phosphorus B) Potassium C) Calcium and D) Zinc at flowering, Syferkuil in 2017

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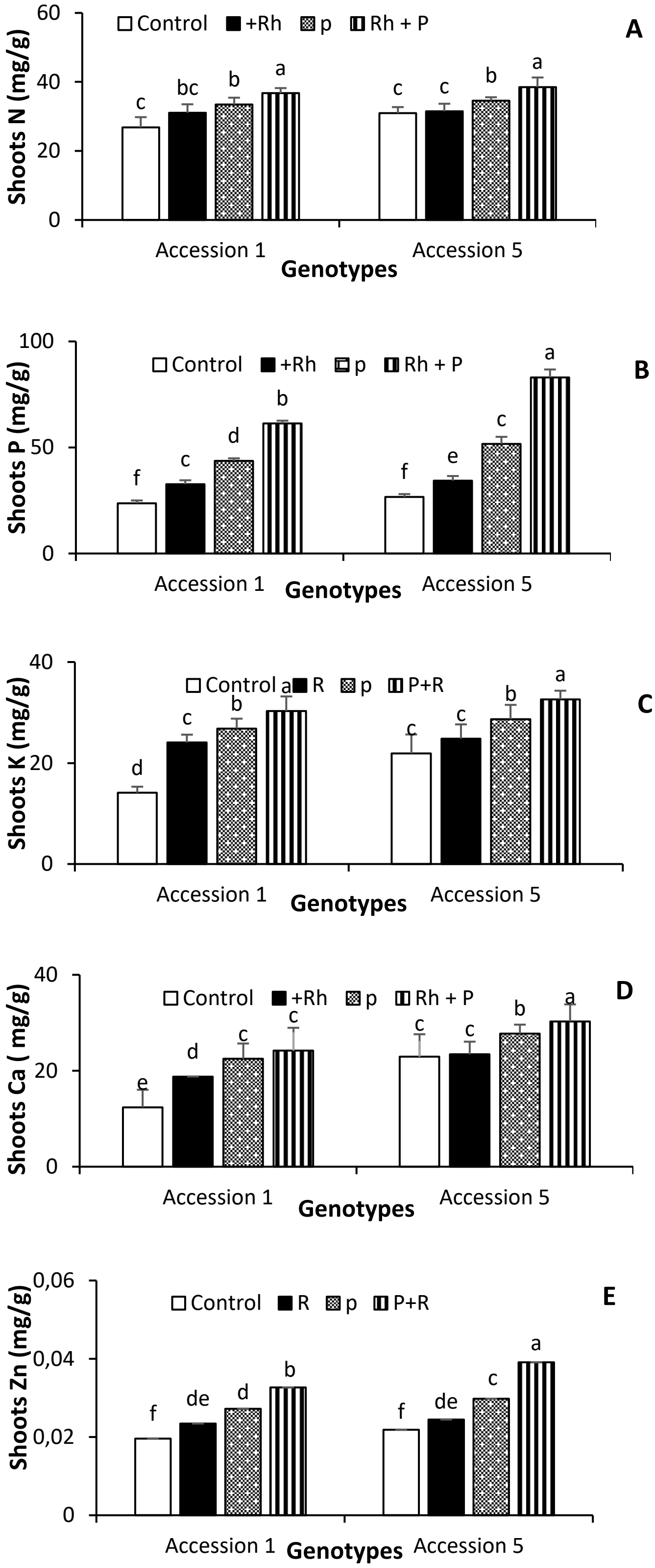

Fig 5.2 The interactive effect of genotype x fertilizer treatment on A) Nitrogen, B) Phosphorus C) Potassium D) Calcium and E) Zinc at flowering, Syferkuil in 2018

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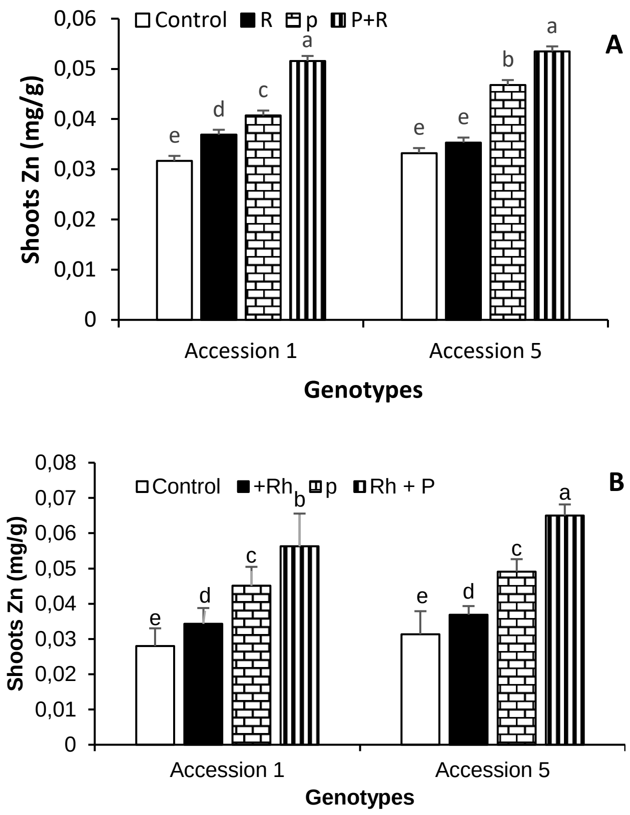

Fig 5.3 The interactive effect of genotype x fertilizer treatments on shoots Zinc concentration chickpeas at flowering, Thohoyandou in A) 2017 A) 2018.

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5.3.2 Mineral accumulation in the shoots at harvest maturity

The concentrations of N, P, K, Ca, Mg, and Zn were greater in shoots of ACC5 compared to that in ACC1; In fact, the N and P concentrations were 2-fold higher while Zn was 4-fold increase in ACC5 than ACC1 at Syferkuil in 2017 (Table 5.3). In 2018 cropping season at Syferkuil farm revealed significant differences between the two accessions (Table 5.3). Similar to the results shown in 2017, all the selected essential nutrient elements accumulated in significantly higher concentrations in shoots of ACC5 compared to that of ACC1 (Table 5.3). At Thohoyandou in 2017, there was a significant difference in the concentration of N, P, K, Ca, Mg and Zn (Table 5.4). The concentrations of nutrients were higher in shoots of accession 5 compared to that of ACC1 (Table 5.4). Shoot levels of N, P, Ca, Mg and Zn revealed significantly differences, while K was similar between the test accessions established at Thohoyandou 2018 (Table 5.4). ACC5 exhibited markedly greater shoot concentrations of N, P, Mg, Ca and Zn compared to its ACC1(Table 5.4).

Similarly, fertilizer treatments affected nutrients accumulation in chickpea shoots at harvest maturity at both sites and season (Table 5.3 & 5.4). The application of P plus rhizobial inoculation resulted in markedly higher accumulation of N, P, K, Ca, Mg and Zn during the 2017 at Syferkuil. The was a significant interaction for genotype x fertilizer treatments for the N P, K, Ca and Zn for both the 2017 and 2018 cropping seasons at Syferkuil (Table 5.3), while at Thohoyandou there was significant interaction for genotype x treatments for the P and Ca for both the 2017 and 2018 cropping season. The concentration of N, P, Ca on harvested shoots was greater on P plus rhizobial inoculant, P, followed by R and the least concentration of N, P, Ca and Zn was on control (Fig 5.4 A, B & D). Furthermore, ACC5 was greater on concentration of shoots N, P, Ca and Zn. However, K was higher on P plus rhizobial inoculant followed by P application and equal concentration of K was observed on rhizobial inoculant and on control (Fig 5.4 C). Concentration of N on harvested shoots was greater on P plus rhizobial inoculant, P, R and the least concentration was on controls, also ACC5 had greater N compared to ACC1 (Fig 5.5 A). Shoots P, K, Ca and Zn concentration was greater on P plus rhizobial inoculant and P, R and control were similar in ACC5 and ACC1(Fig 5.5 B, C, D & E). The concentration of harvested shoots on P and Ca was greater on P plus rhizobial inoculant, secondly P, followed by R and the least concentration was on controls, also ACC5 had greater P and Ca concentration compared to ACC1 (Fig 5.6 A & B).

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Table 5.3 The effect of rhizobium inoculation and phosphorus application on the concentrations of macro-and micronutrient concentrations in the shoots at harvest of chickpea accessions grown at the Syferkuil farm in 2017 and 2018. (Mean ± SE in same column with dissimilar letter are significantly different at p ≤0.05).

Years	Treatments	N	P	K	Ca	Mg	Zn
2017		mg/g					
	Genotypes						
	Accession 1	2.0±0.02b	1.2±0.03b	11.2±0.30b	3.4±0.02b	3.5±0.03b	0.005±0.0b
	Accession 5	4.8±0.05a	2.5±0.00a	18.5±0.32a	4.8±0.05a	4.8±0.03a	0.020±0.0a
	Fertilizer treatments (FT)						
	Control	3.2±0.02b	1.2±0.04b	11.7±0.5b	2.9±0.04b	3.8±0.03b	0.003±0.0d
	Rhizobium (R)	3.5±0.05b	2.0±0.02ab	11.9±0.5b	3.3±0.04ab	4.1±0.06bc	0.007±0.0c
	Phosphorus (P)	3.7±0.06a	2.4±0.02a	12.2±0.0a	4.1±0.04ab	4.2±0.04ab	0.009±0.0b
	P+R	4.1±0.04a	2.4±0.06a	12.8±0.03a	5.1±0.12a	4.8±0.09a	0.024±0.0a
	F-statistics						
	Genotype (G)	6.38*	9.73*	4.51**	5.64*	3.16*	2.53*
	Fertilizer Treatments (T)	4.37*	1.90**	1.23***	2.3*	4.35**	1.05**
	G x FT	5.07**	0.61*	8.54**	2.48*	0.13ns	7.75**
	Genotype						
2018	Accession 1	3.1±3.26b	2.4±0.01b	10.2±0.06b	13.1±0.13b	2.2±0.06b	0.19±0.86b
	Accession 5	9.7±1.30a	3.7±0.01a	12.8±0.06a	23.8±0.09a	5.6±0.06a	0.29±1.30a
	Fertilizer treatments (FT)						
	Control	3.2±5.23c	2.0±0.00b	9.2±005c	18.1±0.10b	1.9±0.05c	0.20±0.71b
	Rhizobium (R)	5.1±2.59b	2.7±0.00b	10.6±0.13b	21.1±0.21a	4.4±0.02b	0.26±2.59a
	Phosphorus (P)	7.9±1.42ab	3.0±0.00a	12.9±0.06b	22.2±0.09a	5.8±0.03b	0.28±1.42a
	P + R	8.4±1.23a	3.5±0.01a	14.9±0.03a	23.2±0.15a	7.6±0.04a	0.29±1.23a
	F-statistics						
	Genotype (G)	9.28***	5.64***	0.25ns	1.14ns	3.09***	13.43**
	Fertilizer Treatments (FT)	6.44***	17.21***	3.49*	3.89*	9.10***	4.50**
	G x FT	3.72***	3.76*	4.50**	2.83*	0.44ns	8.70***

Values (Means ± S.E) with dissimilar letters in a column are significantly different at *p≤0.05. ** p≤0.01. ***p≤0.001 and *ns* = not significant

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Table 5.4 The effect of rhizobium inoculation and phosphorus application on the concentrations of macro-and micronutrient concentrations in shoots of chickpea accessions at harvest stage grown at the Thohoyandou in 2017 and 2018(Mean ± SE in same column with dissimilar letter are significantly different at p ≤0.05).

Years	Treatments	N	P	K	Ca	Mg	Zn
2017	**Genotypes**	mg/g					
	Accession 1	11.4±008b	1.3±0.00b	10.2±0.07b	28.0±0.38b	2.0±0.03b	0.011±0.0b
	Accession 5	18.1±0.26a	2.7±0.01a	12.4±0.05a	38.6±0.43a	5.5±0.05a	0.013±0.0a
	Fertilizer treatments (FT)						
	Control	11.0±010b	1.3±0.01b	10.0±0.08	21.8±020b	4.6±0.04b	0.009±0.0b
	Rhizobium (R)	11.9±0.07b	1.4±0.01b	10.8±0.09	23.1±0.59b	4.8±0.02b	0.013±0.0ab
	Phosphorus (P)	15.8±0.40a	1.7±0.02b	11.3±0.21	24.4±0.68b	5.0±0.04ab	0.013±0.0ab
	P+R	18.1±0.39a	1.9±0,02a	12.7±0.14	29.0±0.41a	5.3±0.08a	0.017±0.0a
	F-statistics						
	Genotype (G)	6.75**	3.46*	12.43***	2.61**	0.59**	0.65*
	Fertilizer Treatments (FT)	1.20*	1.50*	0.35*	4.28*	2.52*	1.74*
	G x FT	0.51ns	3.32*	0.45ns	2.99*	1.67ns	1.38ns
2018	**Genotypes**						
	Accession 1	18.1±0.12b	1.2±0.01b	22.0±0.04	15.6±0.09b	2.9±0.02b	0.04±0.0b
	Accession 5	20.1±0.12a	1.8±0.00a	22.4±0.05	17.6±0.09a	3.7±0.0a	0.05±0.0a
	Fertilizer treatments (FT)						
	Control	14.0±0.08b	0.9±0.00c	9.1±0.09b	15.6±0.12b	3.3±0.01b	0.003±0.0b
	Rhizobium (R)	17.6±0.15ab	1.2±0.00b	9.4±0.06b	16.0±0.15b	3.4±0.04b	0.004±0.0b
	Phosphorus (P)	21.8±0.08a	1.4±0.00b	8.5±0.06b	16.4±0.13b	3.7±0.03ab	0.005±0.0a
	P + R	22.9±0.11a	1.7±0.00a	9.7±0.05a	18.5±0.13a	4.1±0.02a	0.006±0.0a
	F-statistics						
	Genotype (G)	4.56*	6.40**	0.43ns	1.99*	1.31*	16.03***
	Fertilizer Treatments (FT)	11.19***	8.41**	1.38**	3.87**	3.24*	5.41**
	G x FT	0.62ns	5.63*	0.14ns	4.70*	10.81ns	0.55ns

Values (Means ± S.E) with dissimilar letters in a column are significantly different at **p*≤0.05. ** *p*≤0.01. ****p*≤0.001 and *ns* = not significant

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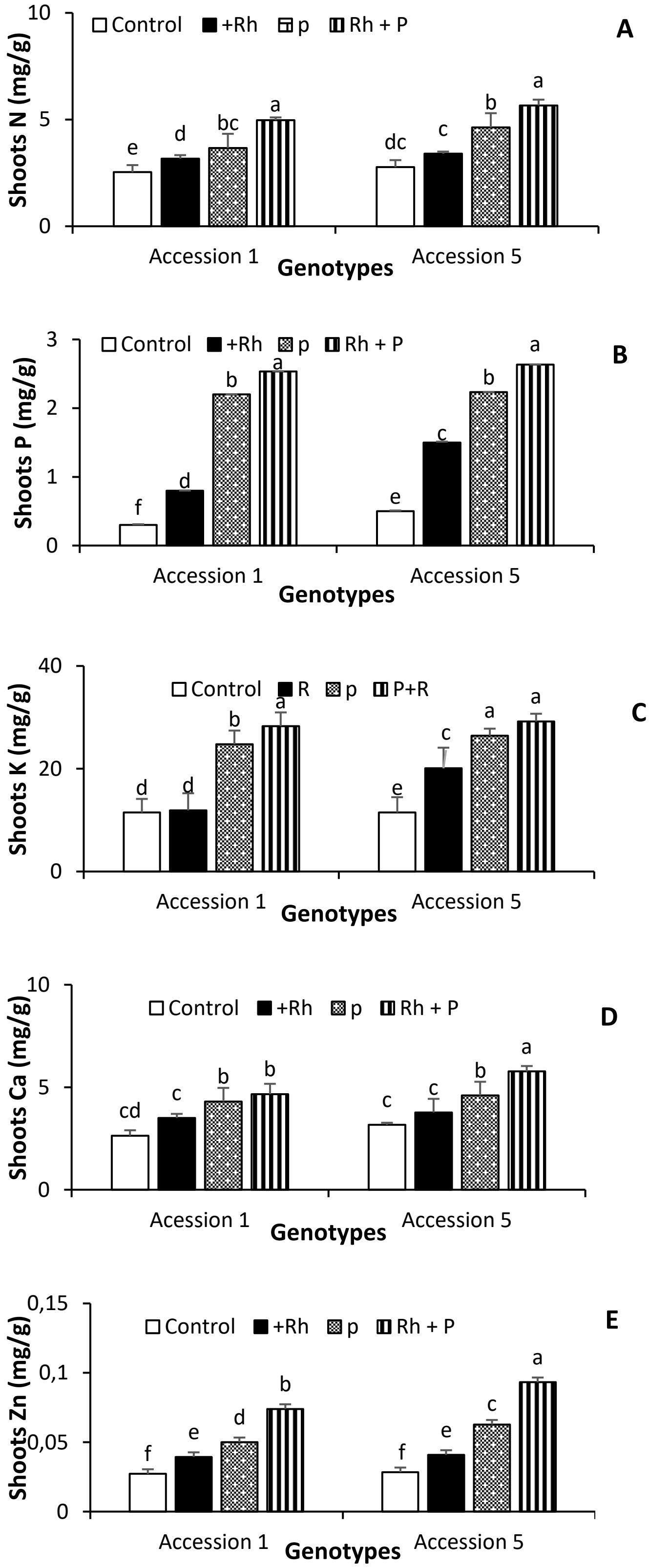

Fig 5.4 The interactive effect of genotype x fertilizer treatments on shoots A) Nitrogen, B) Phosphorus C) Potassium D) Calcium and E) at harvest, Syferkuil in 2017

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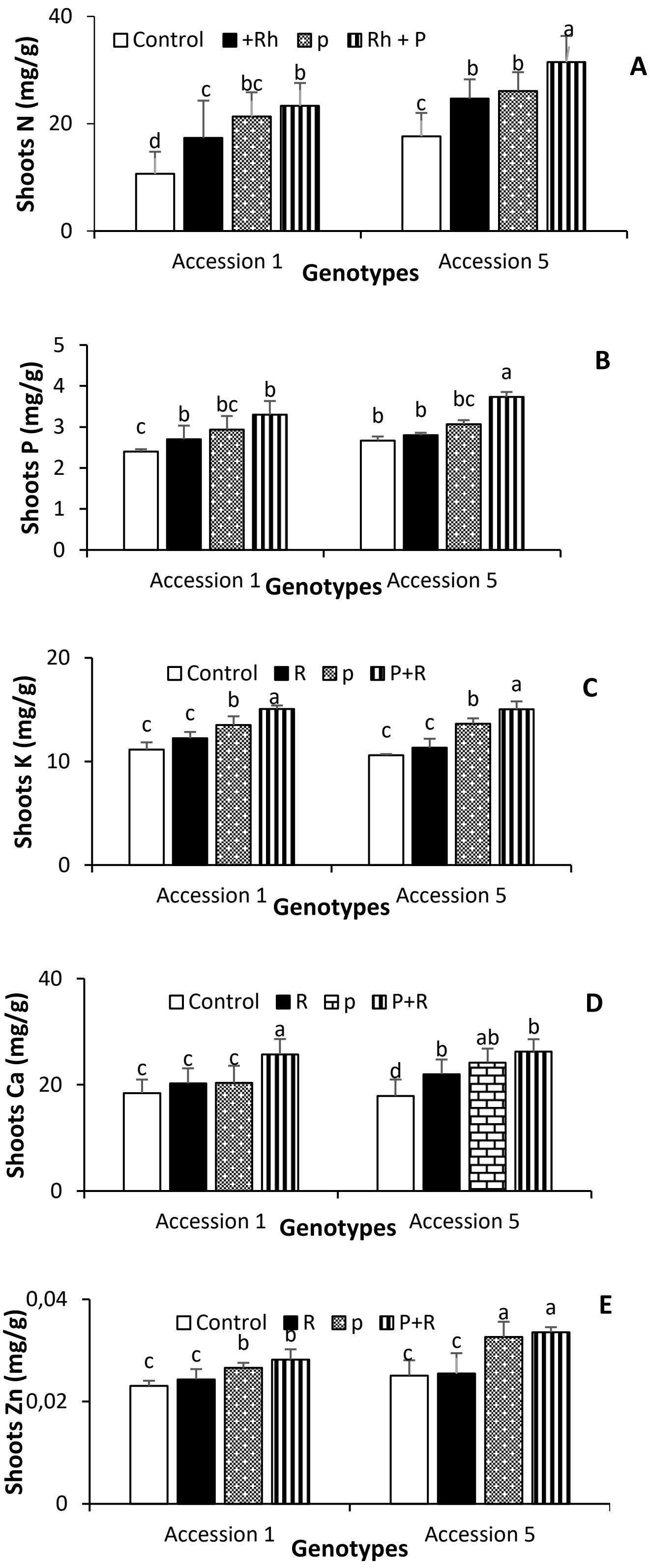

Fig 5.5 The interactive effect of genotype x fertilizer treatments on shoots A) Nitrogen, B) Phosphorus C) Potassium D) Calcium and E) at harvest, Syferkuil in 2018

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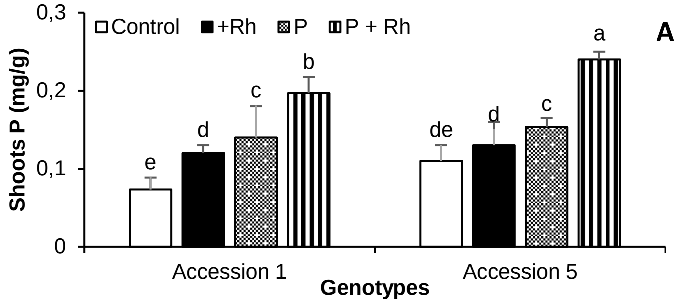

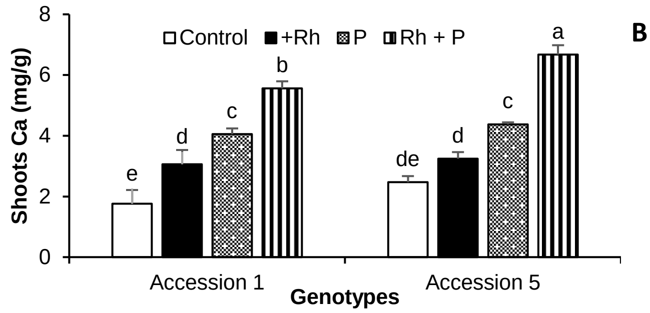

Fig 5.6 The interactive effect of genotype x fertilizer treatment on shoots A) Phosphorus B) Calcium at harvest, Thohoyandou in 2017.

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5.3.3 Mineral accumulation in the grain

Accession 5 accumulated greater amount of N, P, K, Ca, Mg and Zn in the grain at the sandy loam soil of Syferkuil in both seasons (Table 5.5). In Thohoyandou (clay soils) in contrast, ACC 5 accumulated greater N, P, K (2017) and N, P and Zn (2018) compared to ACC1(Table 5.6). Similarly, the effect of fertilizer treatment on accumulation of the studied macro and micronutrients was significant in both seasons at Syferkuil (Table 5.6). Phosphorus plus rhizobium inoculant followed by plants with P followed on plants with rhizobial inoculant the least nutrients concentration was on the control.

There was a significant interactions of genotype x fertilizer treatments for N, P, K and Ca in the grain in 2018 at Syferkuil, while at Thohoyandou the interaction between genotype ant fertilizer treatment was on N, P and Zn (Table 5.5 & 5.6). Interactions in 2017, highest grain N was recorded in ACC5 supplied with P+R and the second highest was ACC1 with P+R (Fig. 5.7 A). The control in ACC5 was similar to inoculated ACC1 on grain P (Fig 5.7 B).

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Table 5.5 The effect of rhizobium inoculation and phosphorus application on the concentrations of macro-and micronutrient concentrations in the grain of chickpea accessions at Syferkuil farm in 2017and 2018. (Mean ± SE in same column with dissimilar letter are significantly different at p ≤0.05).

Years	Treatments	N	P	K	Ca	Mg	Zn
2017		mg/g					
	Genotypes						
	Accession 1	19.2±0.06b	3.1±0.00b	16.2±0.03b	1.1±0.00b	1.24±0.00b	0.019±0.0b
	Accession 5	21.5±0.04a	4.2±0.00a	23.4±0.02a	1.5±0.00a	1.82±0.00a	0.028±0.0a
	Fertilizer treatments (FT)						
	Control	18.9±0.01b	4.06±0.00b	16.3±0.023b	1.2±0.15b	1.80±0.007b	0.028±0.0b
	Rhizobium (R)	20.4±0.02b	4.07±0.00b	21.4±0.022ab	1.4±0.00ab	1.82±0.004b	0.030±0.0ab
	Phosphorus (P)	21.2±0.04b	4.2±0.00a	24.7±0.024a	1.5±0.00a	1.84±0.002b	0.031±0.0a
	P+R	26.2±0.30a	4.3±0.00a	29.8±0.029a	1.6±0.00a	1.95±0.005a	0.033±0.0a
	F-statistics						
	Genotype (G)	9.41^{*}	13.42^{***}	6.48^{*}	3.10^{*}	2.24^{*}	4.21^{**}
	Fertilizer Treatments (FT)	4.69^{*}	8.38^{***}	9.64^{***}	5.11^{*}	8.19^{**}	2.32^{*}
	G x FT	3.59^{ns}	2.92^{ns}	1.04^{ns}	9.83^{ns}	0.59^{ns}	1.56^{ns}
2018	**Genotype**						
	Accession 1	13.4±0.07b	2.9±0.00b	11.9±0.01b	1.1±0.00b	1.1±0.00b	0.29±0.98b
	Accession 5	16.7±0.05a	3.9±0.00a	19.5±0.01a	1.4±0.00a	1.5±0.00a	0.35±1.51a
	Fertilizer treatments (FT)						
	Control	14.0±0.06c	2.1±0.00b	11.7±0.00b	1.1±0.00b	1.3±0.00b	0.19±0.50c
	Rhizobium (R)	23.7±0.00b	3.6±0.00a	12.1±0.02a	1.3±0.00ab	1.5±0.00a	0.31±0.27b
	Phosphorus (P)	25.2±0.04b	3.7±0.00a	12.2±0.09a	1.4±0.00a	1.6±0.00a	0.34±0.39a
	P + R	38.4±0.01a	3.7±0.00a	14.3±0.03a	1.5±0.00a	1.6±0.00a	0.39±1.74a
	F-statistics						
	Genotype (G)	21.59^{***}	7.69^{***}	3.02^{*}	4.04^{**}	8.17^{**}	10.42**
	Fertilizer Treatments (FT)	8.38^{***}	3.95^{***}	1.46^{*}	9.11^{**}	3.94^{**}	6.71^{***}
	G x FT	4.89^{**}	1.94^{*}	4.09^{**}	4.00^{**}	1.94^{ns}	2.16^{ns}

Values (Means ± S.E) with dissimilar letters in a column are significantly different at *$p \leq 0.05$. ** $p \leq 0.01$. ***$p \leq 0.001$ and *ns* = not significant

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Table 5.6 The effect of rhizobium inoculation and phosphorus application on the concentrations of macro-and micronutrient concentrations in grain of chickpea accessions grown at the Thohoyandou in 2017 and 2018(Mean ± SE in same column with dissimilar letter are significantly different at p ≤0.05).

Years		N	P	K	Ca	Mg	Zn
2017	**Treatments**	mg/g					
	Genotypes						
	Accession 1	24.5±0.11b	3.2±0.01b	17.8±0.01b	1.8±0.0	1.6±000	0.026±0.0
	Accession 5	31.1±0.21a	3.7±0.00a	22.7±0.02a	1.8±0.0	1.7±0.00	0.032±0.0
	Fertilizer treatments (FT)						
	Control	27.3±0.21b	3.1±0,01b	12.7±0.01c	1.7±0.00	1.6±0.00	0.028±0.01b
	Rhizobium (R)	27.4±0.10b	3.3±0.01b	18.9±0.02bc	1.8±0.0	1.6±0.00	0.029±0.0b
	Phosphorus (P)	36.0±0.55a	3.8±0.02b	21.7±0.05ab	1.8±0.0	1.7±0.00	0.033±0.0a
	P+R	41.3±0.29a	3.9±0.02a	23.0±0.02a	1.9±0.01	1.7±0.00	0.039±0.0a
	F-statistics						
	Genotype (G)	6.49^{*}	14.00^{**}	9.98^{***}	0.55^{ns}	0.21^{ns}	3.45^{ns}
	Fertilizer Treatments (FT)	9.32^{***}	3.35^{*}	5.22^{*}	0.43^{ns}	1.01^{ns}	7.43***
	G x FT	3.6^{*}	0.39^{ns}	0.37^{ns}	0.18^{ns}	0.60^{ns}	0.21^{ns}
2018	**Genotype**						
	Accession 1	25.0±0.0b	1.9±0.01b	9.2±0.01	1.4±0.00	1.3±0.00	0.045±0.00b
	Accession 5	37.2±0.13a	2.4±0.01a	9.3±0.02	1.5±0.0	1.4±0.00	0.066±0.01a
	Fertilizer treatments (FT)						
	Control	21.8±0.13b	1.8±0.00b	21.3±0.02b	1.2±0.00c	1.2±0.00	0.043±0.00c
	Rhizobium (R)	24.1±0.03b	2.1±0.00b	21.6±0.02b	1.3±0.00b	1.3±0.00	0.052±0.00bc
	Phosphorus (P)	26.9±0.02b	2.6±0.00a	22.6±0.01b	1.6±0.00b	1.4±0.00	0.060±0.01b
	P + R	31.7±0.14a	2.9±0.00a	33.1±0.04a	1.8±0.00ab	1.5±0.00	0.091±0.00a
	F-statistics						
	Genotype (G)	12.93^{*}	7.11^{*}	0.85^{ns}	18.01^{ns}	0.17^{ns}	12.93^{**}
	Fertilizer Treatments (FT)	5.24^{**}	9.77^{**}	0.65^{**}	23.50^{**}	1.93^{ns}	6.5**
	G x FT	2.55*	2.74^{*}	0.77^{ns}	1.00^{ns}	0.17^{ns}	2.24^{*}

(Means ± S.E) with dissimilar letters in a column are significantly different at *p≤0.05. ** p≤0.01. ***p≤0.001 and *ns* = not significant

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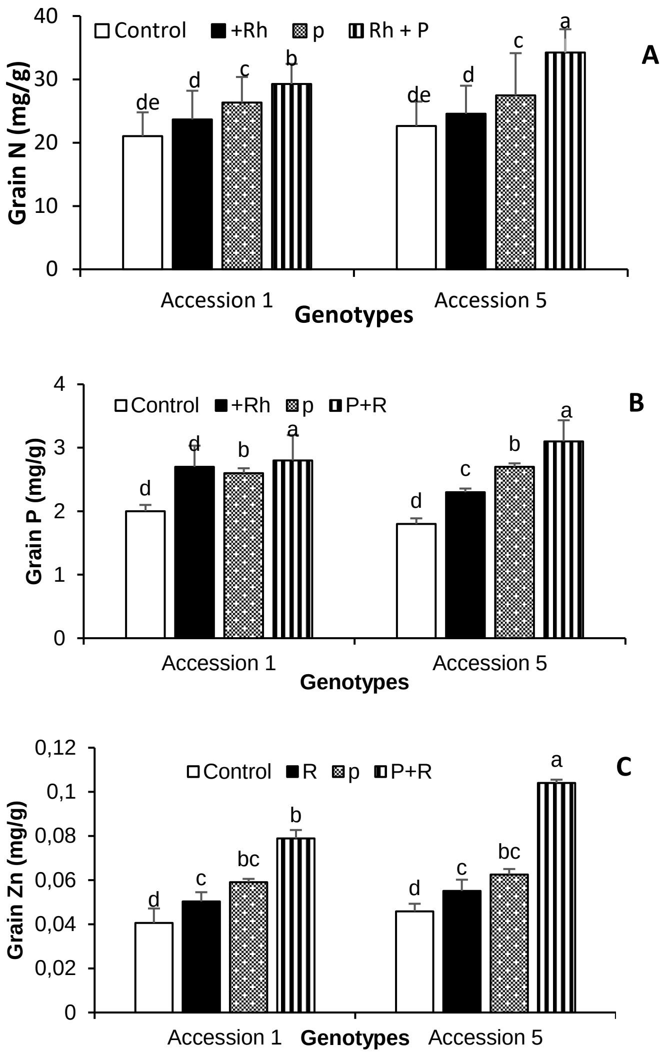

Fig 5.7 Interactive effect of genotype x fertilizer treatment on grain A) Nitrogen B) Phosphorus C) Zinc concentration at Thohoyandou 2018.

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5.4 DISCUSSION

5.4.1 Variation in concentration of macro-and micronutrients in shoots and grain of chickpea

Accession 5 showed greater nutrients accumulation at all growth stages, sites and growing season compared to accession 1. This was consistent with higher accumulation of nutrients in the rhizosphere and shoots of ACC5 compared to ACC1. These results may suggest that roots of the ACC5 were effective in the interception and absorption of nutrients which were sufficient to meet its nutrient demand for better growth. It is also possible that the genotype has the ability to produce organic acid for enhanced solubilisation of nutrient (Pang *et al*., 2018; Richardson *et al*., 2009). Through the growing season at both sites, all the genotypes accumulated high nutrients element at flowering which decreased at harvest maturity, which subsequently increased in the grain. This is due to the fact that the translocation of nutrients especially in grain legumes during the flowering, physiological maturity and grain-filling stages, varies between cultivars as some exhibit higher concentration compared to their counterparts (Nascente and Stone, 2018). The ability of ACC5 to take up more nutrients than ACC1 were reflected in the greater biomass and grain yield of ACC5 at all sites and season (Chapter 6).

The inoculation, application of P, and P plus rhizobia inoculant influenced nutrients accumulation in 2017 and 2018 at flowering stage in Syferkuil. When grown at Thohoyandou in 2017 and 2018, the application of P and P plus rhizobial inoculant increased the concentrations of N, P, K and Zn on chickpea shoots at flowering stage (Table 5.2). Alves *et al*. (1996) observed that P deficiency decreased total N concentrations in leaves of hybrid maize, indicating that low P levels reduced N uptake. Several studies have shown that the P nutritional status can affect the absorption of other nutrients and, consequently, influence crop nutrition and production. At harvest maturity chickpea that was raised with the supply of P revealed markedly higher N, P, K, and Mg while inoculation significantly enhanced the concentrations of P, K, and Ca (Table 5.3). This might be due to the fact that Phosphorus also interacts positively with other nutrient elements and plant growth (Fageria, 2006). It is clear from this results that a balanced P nutrition is essential for an appropriate development of Chickpea.

Application of P plus rhizobial inoculation resulted in markedly higher accumulation of N, P, K, Ca, Mg and Zn at flowering at Syferkuil and of N, P, K, Ca, and Zn in Thohoyandou compared with the control. High accumulation of N element is due to the fact that leguminous crops get into symbiotic associations with Rhizobium bacteria and transform elemental nitrogen in atmosphere into available forms for plant uptake. Chickpea being a leguminous crop, is able to form a symbiotic relationship with bacteria in soil to fix atmospheric nitrogen into ammonia.

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The application of P plus inoculation with *Bradyrhizobium japonicum* as well as the application of P alone resulted in high accumulation of all the selected nutrients in grain of chickpea planted in both sites in 2017. Chickpea shoots accumulated large amount of nutrients at flowering this decreased at harvest maturity but was high on grain. Gulmezoglu and Kayan, 2011 reported that grain legumes exhibit variation in the accumulation of nutrients between leaves, pods, and grain. These results suggest that when plants reach physiological maturity, most assimilates including mobile nutrients elements, are translocated from the shoot to the pods or grain. In general, research has shown that the concentration of macro-and micronutrients varies between leaves and grain of grain legumes (Dakora and Belane, 2019; Edelman and Colt, 2016).

5.6 CONCLUSSION

The results of this study revealed that the application of P plus inoculation with *Bradyrhizobium japonicum* as well as the application of P alone influenced the accumulation of mineral elements. Phosphorus levels in the nutrient solution increased nutrients accumulation. Better P and rhizobia availability in nutrient solution increased N, P, K, Ca, Mg and Zn accumulation on shoots and grain of two chickpea genotypes.

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CHAPTER 6. EFFECT OF *RHIZOBIUM* INOCULATION AND P APPLICATION ON YIELD-RELATED PARAMETERS OF CHICKPEA (*Cicer arietinum* L.) GENOTYPES

ABSTRACT

Low soil fertility is one of the major constraints to the productivity of chickpea and other legumes. Therefore, fertilizer (inorganic, organic, biofertilizer) may enhance growth, yield and overall productivity of legumes especially in marginal soils. However, there is a dearth of information in literature on the effect of phosphorus (P) plus rhizobium inoculation on yield and yield components of chickpea in contrasting soil types. Therefore, this study assessed the effects of P fertilizer application (0 and 90 kg P ha^{-1}) and rhizobium inoculation (with and without rhizobial strain) on grain yield and yield components of 2 Desi chickpea genotypes (ACC#1 and ACC#5) at two sites, Syferkuil and Thohoyandou, in NE South Africa that have contrasting soil types. Grain yield and yield components (shoot biomass, 100-SW, pod weight, number of pods per plant and harvest index) were assessed at harvest maturity. Grain yield was consistently greater in ACC#5 (2405 to 3965 kg ha^{-1}) compared to ACC#1 (2084 to 2623 kg ha^{-1}) at both sites and seasons. A similar trend was observed with the yield components. Also, highest grain yield (3308 to 5157 kg ha^{-1}) and yield components were recorded in chickpea plants that were supplied with a combination of P fertilizer and rhizobium inoculation and the lowest grain yield (1413 to 2397 kg ha^{-1}) and yield components produced in the zero control plots across sites and seasons. Moreover, the higher yielding ACC#5 appeared to be more responsive to fertilizer treatment compared to the lower yielding ACC#1 across both sites and seasons. These preliminary results indicate that chickpea productivity in this region may be enhanced by a combined application of fertilizer P and rhizobium inoculation irrespective of soil type but further studies, incorporating more chickpea genotypes, are recommended before definite conclusions can be drawn.

Key words: Chickpea, Phosphorus, Rhizobium inoculation, Yield

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6.1 Introduction

Soil-plant-microbe interaction has gained much importance in recent decades, partly because many types of microorganisms are known to inhabit soil, especially rhizosphere and play important role in plant growth and development. These microorganisms secrete different types of organic acids (Deubel and Merbach, 2005) thus lowering the pH in the rhizosphere. Use of these microorganisms as environment friendly biofertilizer helps to reduce the much expensive phosphatic fertilizers. Phosphorus fertilizers could help increase the availability of accumulated phosphate by solubilization, efficiency of biological nitrogen fixation and increase the availability of Fe, Zn through production of plant growth promoting substances (Kucey *et al*., 1989). *Rhizobium,* previously well known as a symbiotic N fixer, has been shown to be a symbiotic microorganism in recent years (Biswas *et al*., 2000). Most inoculation studies have focused on free living diazotrophs, although a few reports indicate r*hizobia* can act as plant growth promoting rhizobacteria, PGPR (Noel *et al*., 1996). The PGPR influences crop growth and development by releasing plant growth regulators (Glick and Bashan, 1997) and improving morphological characteristics of inoculated roots (Biswas, 1998), which favour nutrient uptake (Okon and Kapulnik, 1986).

Phosphorus is one of the essential mineral macronutrients, which is required for optimum yield of cultivated crops. Most of the essential plant nutrients, including phosphorus, are found in insoluble form in the soil (Abd-Alla,1994; Yadav and Dadarwal, 1997). Phosphorus in the soil has developmental activity in the plant's root growth. Depending on phosphorus applications, the contact area of the root expands with the growth of root which, in turn makes it easier for the plant to benefit from the other nutritional elements in higher proportions (Marschner, 1995). Phosphorus deficiency in legumes reduces leaf area, photosynthetic rate, and the number of nodes and branches (Qiu and Israel, 1992; Chaudhary *et al*., 2008). Number of pods, grain yield was increased with increased P levels up to 90 kg ha^{-1} over control in cowpea (Baboo and Mishia, 2001). Ram and Dixit (2001) also found that the application of P at 60 kg ha^{-1} significantly increased dry matter accumulation. Many researchers have reported improved performance of legumes when supplied with P (Ndakidemi *et al*., 2006; Madzivhandila *et al*., 2012; Ogola *et al*., 2013; Macil *et al*., 2017; Lusiba *et al*., 2017). Phosphorus significantly increased dry matter yield, yield components, leaf area, number of branches per plant and seed yield of cowpea (karikari *et al*., 2015). The individual effects of P fertilizer and rhizobium inoculation on yield and yield components of chickpea is well documented (Madzivhandila *et al*., 2012; Turuko and Mohammed 2014; Ogola, 2015). However, there is a dearth of information on the effect of a combination of rhizobium inoculation and P fertilizer application on the grain yield and yield components of chickpea grown in contrasting soil types. Therefore, the hypothesis tested in this study was that rhizobium inoculation and P fertilizer application

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would affect grain yield and yield components of 2 Desis chickpea types sown at two sites, Syferkuil and Thohoyandou, in NE South Africa that have contrasting soil types.

6.2 Materials and methods

The detailed description of materials and methods has been presented in chapter 3 but a brief summary is outlined here. The field experiment was conducted at Syferkuil (sandy loam soil) and Thohoyandou (clay soil) in two consecutive seasons, 2017 & 2018. Treatments consisted of a factorial combination of two rates of P fertilizer (0 and 90 kg P ha^{-1}), two desi chickpea genotypes (ACC1 and ACC5) and two rhizobial inoculation levels (with and without rhizobial strain). The treatments were laid out in a randomized complete block design (RCBD) and replicated three times. Each experimental unit was 2 m x 1.5 m. Yield and yield components such as shoot biomass, number of pods, pod weight, 100 seed weight, and grain yield and harvest index were measured from 0.88 sq. meters of two guarded innermost rows of each experimental plot at harvest maturity.

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6.3 RESULTS

The effects of genotypes and fertilizer treatments on grain yield and yield components were significant at both sites and seasons (Table 6.1 & 6.2). ACC5 exhibited higher shoot dry weight, number of pods per plant, pod weight, 100-seed weight, grain yield and harvest index compared to ACC1 at both sites and seasons (Table 6.1 & 6.2). The difference in grain yield between ACC5 and ACC1 was much greater at Syferkuil (48-60%; 1114-1485 kg ha^{-1}) compared to Thohoyandou (15-23%; 321-604 kg ha^{-1}) in both seasons (Table 6.1 & 6.2).

Grain yield was significantly greater with P fertilizer application plus rhizobium inoculation (4396 & 5157 kg ha^{-1}) compared with P (3694 & 4275 kg ha^{-1}), rhizobium (2760 & 3059 kg ha^{-1}) and the zero control (2079 & 2397 kg ha^{-1}), respectively in 2017 and 2018 seasons at the sandy loam site, Syferkuil (Table 6.1). Similar trend was observed was shoot biomass, number of pods per plant, pod weight, 100-SWT and HI (Table 6.1). In Thohoyandou, characterised by clay soils, P plus rhizobium application similarly produced the highest grain yield (4355 & 3308 kg ha^{-1}) followed by P (3072 & 2473 kg ha^{-1}), rhizobium inoculation (2464 & 1784 kg ha^{-1}) and the zero control (1812 & 1413 kg ha^{-1}) in 2017 and 2018, respectively (Table 6.2).

Only the interaction between genotype and fertilizer treatment on HI was significant at Syferkuil in both seasons (Table 6.1). In both seasons, ACC5 supplied with both P and rhizobium had the highest HI, while the lowest HI was realised in ACC1 with control (Fig. 6.1). In Thohoyandou (clay soil), in contrast, the interaction between genotype and fertilizer treatment affected 100-SWT, grain yield and harvest index in 2017, and shoot biomass, pod weight, grain yield and harvest index in 2018 (Table 6.2). In all cases, ACC5 supplied with a combination of P and rhizobium gave the best performance with ACC1 supplied with neither P nor rhizobium consistently performing poorest (Fig. 6.2 & 6.3).

Averaged across treatments and seasons, grain yield was 18% greater in Syferkuil (3052 kg ha^{-1}) compared to Thohoyandou (2585 kg ha^{-1}) (Table 6.1 & 6.2). Moreover, grain yield was greater in 2018 (3222 kg ha^{-1}) compared to 2017 (2882 kg ha^{-1}) in Syferkuil (Table 6.1) but in Thohoyandou greater yield was obtained in 2017 (2925 kg ha^{-1}) compared to 2018 (2245 kg ha^{-1}) (Table 6.2).

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Table 6.1. Effect of Genotype, *Rhizobium* and P application on gran yield and yield components of chickpea grown at Syferkuil farm in 2017 and 2018 (Mean ± SE in same column with dissimilar letters are significantly different at p ≤0.05).

years		Shoot biomass (kg ha^{-1})	No of pods per plant	Pod weight (kg ha^{-1})	100 seed weight (g)	Grain yield (kg ha^{-1})	HI (%)
2017							
	Genotypes						
	Accession 1	4355.8±383b	62.8±28b	4338.8±366.5b	14.6±0.3b	2325.4±259.6b	57.8±2.4b
	Accession 5	5897.8±429a	112.4±7.4a	5112.6±461.36a	18.2±0.4a	3439.3±279.7a	62.1±1.6a
	Fertilizer treatments (FT)						
	Control	3933±148d	71.9±18.1d	2947.2±133.2d	16.4±0.2d	2079.1±68.1d	50.1±2.2c
	Rhizobium (R)	4943.5±141c	81.8±1.5c	4104.7±166.31c	17.3±0.1c	2760.4±122.7c	58.9±1.1c
	Phosphorus (P)	6185.1±121b	108.8±1.3b	5326.2±147.6b	18.3±0.1b	3693.5±120.7b	62.9±0.1ab
	P+R	7445.7±265a	131.95±7.1a	5624.5±389a	19.6±0.3a	4396.4±130.4a	67.8±1.1a
	F-statistics						
	Genotype (G)	16.8***	14.76***	27.5***	8.9**	37.3***	26.16***
	Fertilizer Treatments (FT)	72.9***	8.44**	43.5***	42.7***	81.1***	28.4***
	G x FT	1.1^{ns}	1.02^{ns}	2.2^{ns}	0.8^{ns}	0.8^{ns}	4.7**
2018	**Genotypes**						
	Accession 1	4894.1±584.8b	77.5±7.9b	3832.6±363.2b	13.9±0.3b	2479.8±313.7b	60.6±6.6b
	Accession 5	5857.8±605.5a	89.6±7.2a	4509.6±368.3a	17.8±0.2a	3964.5±337.6a	78.6±11.8a
	Fertilizer treatments (FT)						
	Control	2974.9±388.2d	66.7±10.8d	2445.3±211.8d	17.6±0.5b	2397.3±81.2d	38.9±2.9d
	Rhizobium (R)	4366.3±137.5c	74.7±3.9c	3818.7±189.5c	16.7±0.4b	3058.8±81.2c	55.4±1.1c
	Phosphorus (P)	6022.9±218.1b	82.4±4.1b	4779.7±90.1b	17.9±0.2a	4275.2±129b	68.5±2.1b
	P+R	8139.7±344.4a	106.6±10.4a	5640.7±231.5a	18.4±0.2a	5157.3±160.6a	115.7±14.4a
	F-statistics						
	Genotype (G)	25.3***	14.5***	36.2***	5.6**	38.3***	11.8***
	Fertilizer Treatments (FT)	58.9***	3.8***	52.7***	54.6**	87.5***	19.4***
	G x FT	1.4^{ns}	1.9^{ns}	1.3^{ns}	0.3^{ns}	0.7^{ns}	4.4***

Values (Means ± S.E) with dissimilar letters in a column are significantly different at *p≤0.05. ** p≤0.01. ***p≤0.001 and *ns* = not significant

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Table 6.2 Effect of Genotype, *Rhizobium* and P application on grain yield and yield components of chickpea grown at Thohoyandou in 2017 and 2018. (Mean ± SE in same column with dissimilar letters are significantly different at p ≤0.05).

years		Shoot biomass (kg ha^{-1})	No of pods per plant	Pod weight (Kg ha^{-1})	100 seed weight (g)	Grain yield (Kg ha^{-1})	HI (%)
2017							
	Genotypes						
	Accession 1	6213.1±555.19b	55.2±3.3b	2286.7±291.7b	21.2±0.6b	2623±234.5b	34.9±0.6b
	Accession 5	7242.4±629.4a	62.3±4.3a	3810.1±346.6a	25.7±1.2a	3227.±354.6a	48.5±1.2a
	Fertilizer treatments (FT)						
	Control	4225.8±285.4d	44.1±1.8d	2191.8±112.4d	18.9±0.3d	1811.6±108.2d	30.5±0.3d
	Rhizobium (R)	5853.9±240.8c	52.3±0.8c	3083.9±111c	20.2±0.1c	2464.1±88c	41.5±0.1c
	Phosphorus (P)	7452.7±189.3b	61.3±2.1b	3939.7±89.4b	23.7±0.2b	3071.8±44.2b	47.2±0.2b
	P+R	9378.6±178.69a	77.4±3.6a	4978.2±272.6a	26.6±1.4a	4354.9±378.5a	60.2±1.4a
	F-statistics						
	Genotype (G)	21.1***	69.8**	21.8***	27.9**	27.9***	18.9***
	Fertilizer Treatments (FT)	96.8***	17.6**	113.2***	89.9***	89.9***	50.7**
	G x FT	0.7ns	1.3ns	1.6ns	6.4***	6.4***	2.7**
2018	**Genotypes**						
	Accession 1	5786.9±327.6b	54.1±1.6b	3803.4±265.8b	16.8±1.1b	2083.9±206.4b	34.7±2.2b
	Accession 5	6582.2±475.2a	66.3±1.1a	4273.8±338.2a	20.6±0.7a	2405.3±239.1a	37.7±2.2a
	Fertilizer treatments (FT)						
	Control	4555.7±81.2d	41.3±1.4c	2919.5±122.1d	18.5±0.6d	1413.3±78.7d	26.1±0.79d
	Rhizobium (R)	5582.8±211.4c	65.2±2.3b	3557.1±76.1c	19.1±1.8c	1784.4±70.3c	34.4±1.1c
	Phosphorus (P)	6473.3±90.9b	78.9±2.3b	4120.4±54.9b	20.5±0.5b	2473.3±82.5b	37.6±0.5b
	P+R	8124.7±399.1a	108.3±1.7a	5557.3±274.9a	24.7±0.3a	3307.8±157.5a	45.4±1.6a
	F-statistics						
	Genotype (G)	16.16***	1.28***	22.6***	0.019**	16.3*	22.4*
	Fertilizer Treatments (T)	33.11***	4.1**	129.8***	4.485**	11.3**	14.6**
	G x FT	20.36***	2.5ns	4.8**	0.194ns	5.8**	1.2*

Values (Means ± S.E) with dissimilar letters in a column are significantly different at *p≤0.05. ** p≤0.01. ***p≤0.001 and *ns* = not significant

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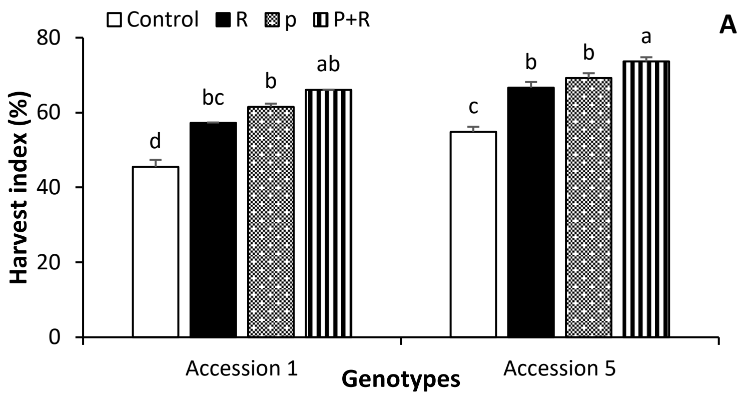

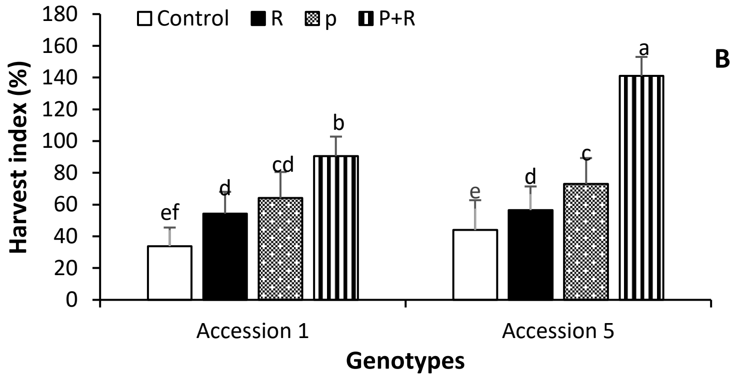

Fig 6.1 Interactive effect of genotype x fertilizer treatment on harvest index at Syferkuil in A) 2017 and B) 2018

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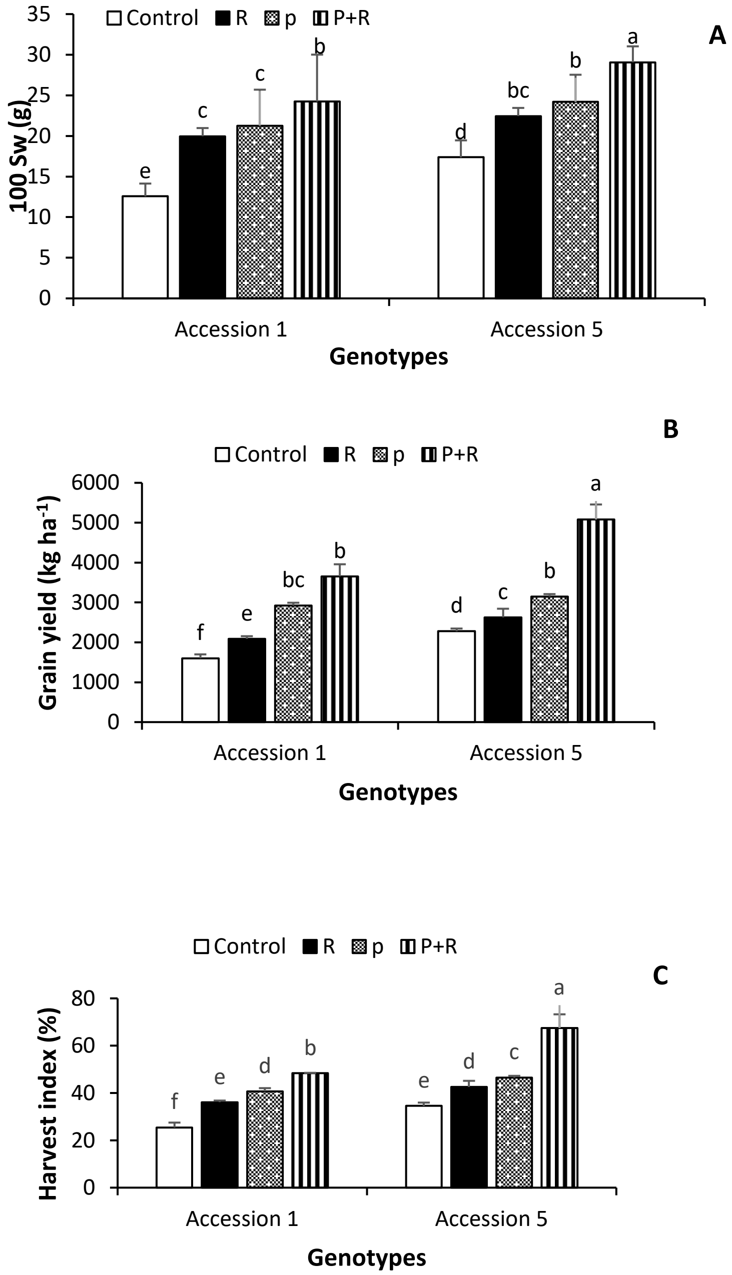

Fig 6.2 The interactive effect of genotype x fertilizer treatment on A) 100 SW, B) Grain yield and C) Harvest index at Thohoyandou in 2017

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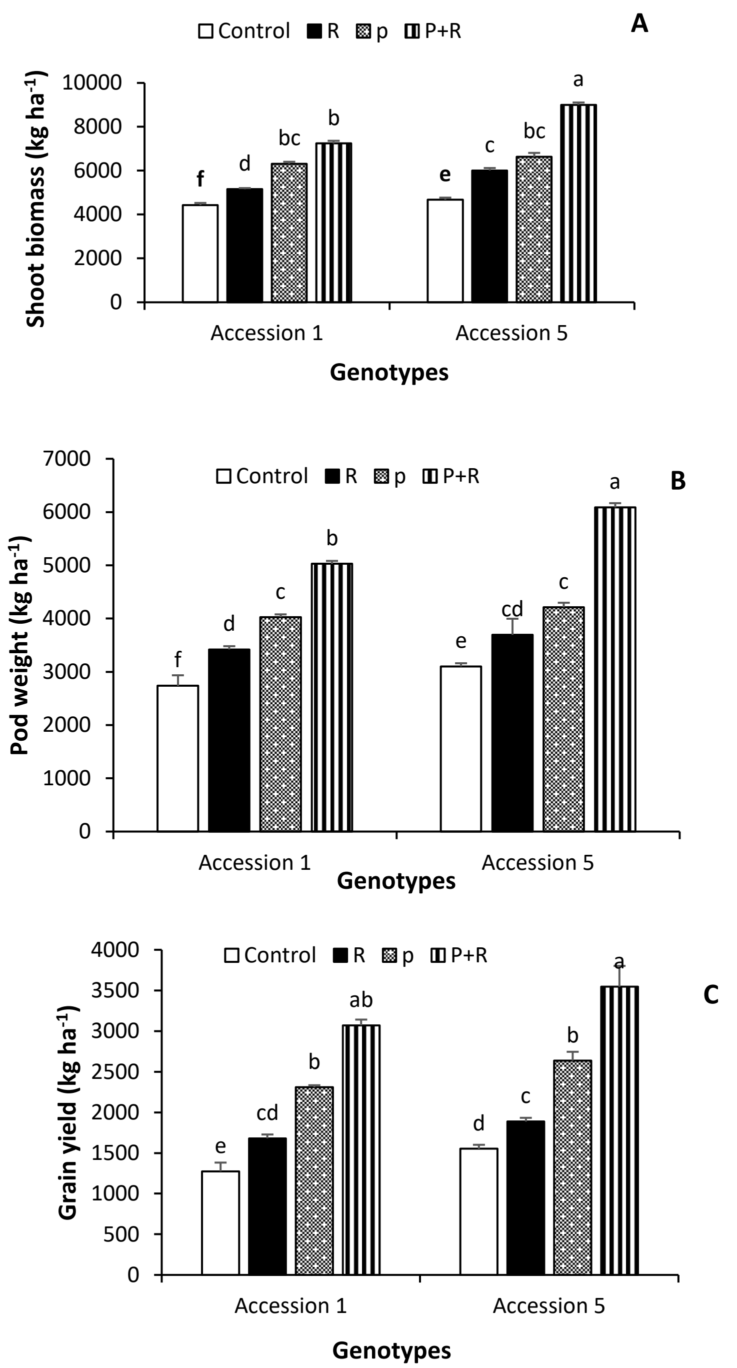

Fig 6.3 The interactive effect of genotype x fertilizer treatment on A) Shoot biomass, B) Pod weight and C) grain yield at Thohoyandou in 2018

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6.4. Discussion

The growth, development and yield of plants is affected by factors including genotype, environment and inputs. When established at both the Syferkuil and Thohoyandou areas during the 2017 and 2018 cropping seasons, ACC5 exhibited markedly higher yield-related parameters: shoot dry weight, number and dry weight of pods, 100-seed weight, grain yield and harvest index compared to ACC1. This finding is not a surprise as the performance of ACC5 was supported by the accumulation of markedly higher concentration of main and secondary macronutrients at Syferkuil and Thohoyandou, including Zn in the rhizosphere (see Tables 4.2 and 4.3). In fact, the growth and yield in this study was a reflection of the accumulation of nutrients in the rhizosphere of chickpea whereby ACC5 with the highest concentration of nutrients in the rhizosphere revealed greater growth and yield compared with ACC1.

Across the experimental sites and the cropping seasons involved in this study, in general, the co-application of P and *rhizobial* inoculation increased the growth-related parameters of chickpea compared to that of the other treatments. This could have been caused by various factors including that rhizobial inoculated plants largely show a significant decrease in stomatal conductance and enhanced water-use efficiency following partly through the production of phytohormones including abscisic acid and lumichrome as well as stomatal closure (Matiru and Dakora, 2005). Similarly, the application of P to plants stimulates increases in their water-use efficiency. Although not determined in this study, research has shown larger exploration and weight of roots of plants grown with the application of, especially optimum rates, P compared to control or that with the application of higher rates (Fageria *et al*., 2013). Taken together, increased root growth and improved water-use efficiency by plants promotes greater growth and yield. Also, P application helps in osmotic adjustment, stomatal regulation and enhances the photosynthetic rate and water retention in plants. In addition to increased water-use efficiency, the co-application of P specially to soil that contain low-P and the inoculation of legumes increases growth-related parameters (Razaq *et al*., 2017). Legume species, such as chickpea, have a greater demand for P which serves multiple functions in plants such as supporting their overall growth; production of adenosine triphosphate; and in meeting the P requirement of arbuscular mycorrhizal fungal communities (Maseko and Dakora, 2013). In fact, the combination of P and rhizobial inoculation ensures that the efficiency of P is improved (Schutz *et al*., 2018) through increased solubilisation of its organic pool and its cleavage from particles (Alori, 2015). This results in improved availability and therefore efficiency of an inorganic fertilizer.

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The markedly lower yield-related parameters by chickpea grown without rhizobial inoculation nor P application could have been as a result of the fact that these plants may have had to adopt P-solubilisation mechanisms including formation of mycorrhizas and exudation of carboxylates in order to acquire P. In general, this mechanism imposes a significant carbon cost to plants (Ryan *et al*., 2012), that which should have been utilized to improve their growth and yield.

There was a significant interaction between genotype and fertilizer treatment on grain yield (Thohoyandou) and some yield components (Thohoyandou and Syferkuil) in both seasons (Table 6.1 & 6.2). Although, ACC5 was generally higher yielding than ACC1 at both sites and seasons, it also appeared to have greater response to fertilizer treatment than ACC1. Indeed, the highest grain yield and yield components was recorded in ACC5 that was supplied with a combination of P and rhizobium and the lowest grain yield and yield components in ACC1 with no fertilizer additions. From these preliminary findings it is clear that ACC1 was not able to adapt to the low fertility status of the soils at both sites. In contrast, ACC5 was able to produce appreciable yield levels even without fertilizer additions. Therefore, further studies to assess the mechanism-(s) that ACC5 used to adapt to such soils is proposed.

6.5. Conclusion

Grain yield increased with fertilizer application and Rhizobium inoculation. The greater grain yield at high P levels was associated with greater shoot biomass, seed weight, pod per plant and 100- SW. Thus, application of P fertilizer and Rhizobium inoculation improved grain yield of chickpea at the sites of the current. However, there is need to carry out further field trials (involving a wider range of fertilizer rates plus rhizobium inoculation) before any agronomically and economically justifiable fertilizer recommendations for this area can be formulated for chickpea.

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CHAPTER 7. GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATION

7.1 General discussion

Chickpea is gaining prominence in South Africa with the domestic demand for the grain legume currently met through importation. Compared to grain legume food crops that are grown in South Africa, in general, imported food crops are costly and are of lower nutritional value. Therefore, there is a need to promote cultivation of the crop in South Africa which would likely reduce its current cost, improve its nutritional value, be of financial reward to local farmers, and boost South Africa ‘s economy and global competitiveness. In order to encourage and ensure its sustainable cultivation, research needs to focus on the role of different types and textures of soil, suitable cultivars as well as inputs on the growth and nutritional quality. Conventionally, crops are cultivated in soil that is made up of different textures. Although farmers, especially smallholder farmers, hardly select potential agricultural cropping fields based on the texture of the soil, typically within a cropping field, they know the fertility status of soils based on texture and/or colour.

Rhizosphere governs the chemistry of plant nutrient and affects growth of the plants. Nutrient requirement in agriculture has been rising and is likely to increase further to enhance the agriculture productivity across the globe in order to keep pace with growing food demand. In most of the cases, farmers apply fertilizers without knowing the rhizosphere role in a particular nutrient chemistry in relation to its availability to plants. Sometimes it creates unavailability of a nutrient to the plant showing deficiency symptoms, which result in yield decline. Mineral nutrients are usually obtained from the soil through plant roots, but many factors can affect the efficiency of nutrient acquisition. First, the chemistry and composition of certain soils can make it harder for plants to absorb nutrients. The nutrients may not be available in certain soils or may be present in forms that the plants cannot use. Soil properties like water content, pH, and compaction may exacerbate these problems.

This study evaluated the effect of P fertilizer and rhizobium inoculation on the accumulation of nutrients on the soil rhizosphere, shoots, grain, yield and yield components of two desi-type chickpea genotypes grown in soil with texture that clay and sandy-loam. The hypothesis tested was that the rhizobium inoculation, P, P plus rhizobium inoculation affect soil rhizosphere, shoots and grain nutrients accumulation and yield.

Genotypes exhibited differences in concentration of nutrients in the rhizosphere. For example, ACC5 revealed a markedly higher concentration of most of the test nutrients (N, P, and K in 2017, and N, P, K, Mg and Zn in 2018 at Syferkuil, as well as N, P, and K in 2017, and N, P, K, Ca, Mg and Zn in 2018 at the Thohoyandou site) (Tables 4.2, 4.3, 4.4 and 4.5) compared to ACC1. The enhanced accumulation of most of the macro-and micronutrients in the

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rhizosphere of ACC5 at both sites was despite the differences in the pH, texture, and fertility of the soil in the 2 locations (Table 4.1). Although the study did not determine possible causes of the variation, it has been reported that genotypes may differ in their ability to access and solubilise nutrients in the rhizosphere and this is controlled largely by genetic make-up, composition of root exudates, and root architecture.

Therefore, it is highly likely that ACC5 could have a rooting system composed of different but superior chemistry and biology which enhance the accumulation of nutrients in the rhizosphere, plant parts and grain yield compared to ACC1. These include fine roots with small diameter that allowed it access to a wider surface area; could exhibit greater capacity to solubilise more nutrients; and secret exudates that are capable of solubilisation of the test mineral nutrients. ACC5 showed high nutrients accumulation in the rhizosphere, shoots, grain in all sites and growing season compared to ACC1. Also is an indication that roots of the ACC5 were effective in the interception and absorption of nutrients which were sufficient to meet its nutrient demand for better growth. It is also possible that the genotype has the ability to produce organic acid for enhanced solubilisation of nutrient. When established at both the Syferkuil and Thohoyandou areas during the 2017 and 2018 cropping seasons, Accession 5 exhibited markedly higher yield-related parameters: shoot dry weight, number and dry weight of pods, 100-seed weight, grain yield and harvest index compared to Accession 1. This finding is not a surprise as the performance of Accession 5 was supported by the accumulation of markedly higher concentration of main and secondary macronutrients at Syferkuil and at the Thohoyandou site, including Zn in the rhizosphere.

Majority of the test nutrients exhibited greater concentrations in the rhizosphere of chickpea grown in the clay soil of Thohoyandou compared to sandy loam soil of Syferkuil, probably due to greater efficacy of the biofertilizer because of the greater capacity of fine textured soil to retain soil organic matter and mineral nutrients and exhibit higher microbial activity of fine-textured soils, relative to the largely sandy soils at Syferkuil. Also, the generally higher fertility (higher OC, N, Ca, S, Mn, and Zn) of the clay soil in Thohoyandou (Table 4.1) could have increased the efficacy of the rhizobium as biofertilizers showed improved efficiency when applied in relatively fertile soils.

The application of P plus inoculation as well as the application of P alone resulted in high accumulation of all the selected nutrients in grain of chickpea planted in both sites in 2017. Chickpea shoots accumulated large amount of nutrients at flowering this decreased at harvest maturity but was high on grain. These results suggest that when plants reach physiological maturity, most assimilates, including mobile nutrients elements, are translocated from the shoot to the pods or grain.

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In general, concentration of macro-and micronutrients varies between leaves and grain of grain legumes. ACC5 exhibited greater growth, yield and nutrients accumulation across agroecologies with different physical and chemical properties and perhaps biological properties. This suggest that accession can adapt under conditions in inland South Africa, when grown during winter season. It should be recommended for further evaluation in other localities and conditions. This study shows that the application of P plus Rhizobium inoculation increased growth, yield and nutrients accumulation on soil, plant shoots and grain in chickpea. These findings are the first on chickpea under South Africa (Limpopo) conditions.

7.2 General conclusion

Rhizobium inoculation and P application has a positive effect on root interactions and nutritional interrelationship between nutrients accumulation on the rhizosphere soil possibly due to the ability to enhance solubilisation and uptake of nutrients from the soil. P plus rhizobium inoculation as well as the application of P alone influenced the accumulation of mineral elements. Phosphorus levels in the nutrient solution increased nutrients accumulation. Better P and rhizobia availability in nutrient solution increased N, P, K, Ca, Mg and Zn and accumulation on chickpea and that Accession 5 performed better compare to Accession 1. Grain yield increased with fertilizer application and Rhizobium inoculation. Application of P fertilizer and Rhizobium inoculation improved grain yield of chickpea at the current sites. P plus rhizobium inoculation increased the accumulation of nutrients in the rhizosphere which lead to the increase in nutrients accumulation on plant parts and the grain yield.

7.3 Recommendations

- Studying the root chemistry and biology of the two accessions.
- This study may provide useful information for future study on relationships between rhizobium inoculation plus P on rhizosphere and under various field conditions.
- Studying the production of organic acid of two accessions.
- P fertilizer and Rhizobium inoculation may improve grain yield of chickpea at the current study.

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