THE RESPONSE OF SYMBIOTIC PERFORMANCE, GROWTH AND YIELD OF CHICKPEA (*Cicer arietinum* L.) GENOTYPES TO PHOSPHORUS FERTILIZER RATES AND RHIZOBIAL INOCULATION

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DECLARATION

I, Anza Muthabi hereby declare that this research dissertation for my MSc degree at the University of Venda is my original work and has not been submitted for any degree at this or any other university or institution. The dissertation does not contain other persons writing unless specifically acknowledged and referenced accordingly.

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Dedication

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ABSTRACT

Chickpea (*Cicer arietinum* L) is adapted to cool-seasons and its organs are of high nutritive value and serve as cheap sources of protein, especially in developing countries. Chickpea crop is mainly grown for human consumption, animal feed and for medicinal purposes. The introduction and promotion of chickpea to especially small-scale South African crop farmers has multiple objectives including the improvement of soil fertility. Small-scale farmer's flounder to afford Nfertilizers, coupled with the challenges faced by programmes aimed at assisting them about soil fertility in their cropping fields that are still without enough N concentration to meet N demand. It is therefore important that other alternatives that can help improve the N status of soils be explored. The shoot $\delta^{13}C$ is an indicator of WUE in C3 plants. However, shoot-WUE is affected by a variety of factors including genotypes, phosphorus fertilizer application and availability of native or introduced rhizobial bacteria. However, not much is known on whether application of phosphate fertilizer, seed inoculation with rhizobial strain affect the shoot C/N ratio of chickpea genotypes in South Africa. Therefore, field experiments were established at Thohoyandou and Syferkuil in Limpopo to assess the role of phosphorus fertilization and rhizobial inoculation on C assimilation, C/N ratio and shoot-WUE of chickpea genotypes. Field experiments were conducted during winter season in 2016 and 2017 (April to August). Treatments consisted of a factorial combination of two rates of phosphorus fertilizer (0 and 90 kg P ha^{-1}), four desi chickpea genotypes (ACC#1, ACC#2, ACC#3 and ACC#5) and two rhizobial inoculation levels (*bradyrhizobium* strain and without rhizobial strain. In Thohoyandou, ACC#1 showed greater grain yield in 2016 and 2017. Which was associated with more branches and greater plant height. Furthermore, the interaction between genotypes, phosphorus fertilizer and rhizobial inoculation had significant effect on grain yield in 2016. ACC#1, 3 and 5 of chickpea genotypes fixed the most N compared to that of ACC#2. In addition, ACC#5 had the highest soil N-uptake in both seasons followed by ACC#3, while ACC#1 had the least value of soil N-uptake in both seasons.

Phosphorus fertilizer application increased the fixation of N by 36.8% ($P \leq 0.01$), and similarly in soil N-uptake by difference of 59.9% compared to control in 2016. Furthermore, rhizobial inoculation increased N-fixed in 2016 and soil N-uptake in both seasons. ACC#5 had the highest N fixed at phosphorus-fertilized with *bradyrhizobuim* across two locations in both seasons. ACC#5 depended more on soil N-uptake than fixing its own N as compared to ACC#1. N fixation differed across seasons; however, ACC#3 had greater N-fixed in both locations. Moreover, chickpea genotype that fixed more N had least $\delta^{15}N$. This finding indicates that N fixation is exhibited by the genotypes that depend less on $\delta^{15}N$, because N_2 fixation is inhibited by high soil N concentration or $\delta^{15}N$. Furthermore, ACC#2 and ACC#3 had greater $\delta^{13}C$ at Thohoyandou in 2017; chickpea genotypes had significant effect on $\delta^{13}C$ at $P \leq 0.05$ at Thohoyandou, 2016. The results showed that ACC#1 with phosphorus fertilizer application and no *bradyrhizobium* strain showed greater $\delta^{13}C$. Also, $\delta^{13}C$ increased with a decrease in N-fixed (r=.1000), this indicates that there was a functional relationship between plant WUE and N fixation in chickpea, probably because improved water use in legumes supports N fixation.

Key words: Chickpea genotypes, Phosphorus fertilizer, Rhizobial inoculation, yield components, grain yield, symbiotic performance, N fixation, C accumulation, $\delta^{13}C$ and WUE

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List of abbreviations

ACC	Accessions
ANOVA	Analysis of variance
ARC	Agricultural research council
BNF	Biological nitrogen fixation
C	Carbon

C/N	Carbon/Nitrogen ratio
DAFF	Department of agriculture, fisheries and forestry
DAE	Days after emergence
DM	Dry matter
FAO	Food and agriculture organisation
FAOSTAT	The Food and Agriculture Organization Corporate Statistical Database
HI	Harvest index
LAI	Leaf area index
LSD	Least significant difference
N	Nitrogen
Ndfa	Nitrogen derived from the atmosphere
P	Phosphorus
RHS	Royal horticultural society
RUBISCO	Ribulose-1,5-bisphosphate carboxylase/oxygenase
SWT	Seed weight
USA	United State of America
WUE	Water use efficiency

CHAPTER 1

1.1 INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an ancient crop that originated in South-Eastern Turkey and belongs to the genus *Cicer*, tribe *Cicereae*, and family Fabaceae (Corp *et al*., 2004). The name *Cicer* is of Latin origin, derived from the Greek word 'kikus' meaning force or strength. The word *arietinum* is also Latin, translated from the Greek 'krios', another name for both ram and chickpea, an allusion to the shape of the seed, which resembles the head of a ram (van der Maesen, 1989).

The global demand of chickpea has increased for the past three decades, for example; the number of countries importing chickpea increased from 30 to 150 between the years 1981 and 2011. Moreover, the crop reached a global record of 13.3 million ha under cultivation and production figures 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 but production remained at 13.1 million tons (FAOSTAT, 2016). In Africa, Ethiopia is the largest producer of chickpea and accounts for about 46% of the continent‘s production (Kassie *et al*., 2009). It is also the seventh largest producer worldwide and contributes about 2% to the total world chickpea production (Kassie *et al*., 2009).

Given the expansion of land under chickpea production, it makes the crop one of the most important pulse crops. In human diet, it serves as an important source of protein for millions of people particularly those in developing countries. Importantly, the grain of chickpea is a good source of micronutrients (Thavarajah and Thavarajah , 2012), is high in carbohydrates, protein (20-22%), and rich in fiber and minerals such as phosphorus, calcium, magnesium, iron and zinc (Gaur *et al*., 2010). The biomass of chickpea is used as animal feed and being a winter crop, it makes its feed a good source of protein and other nutrients during the dry season (Bampidis & Christodoulou, 2011).

Along with water, Nitrogen (N), phosphorus (P) and Carbon (C) are the most important mineral nutrients limiting the growth and development of crops in both natural and managed ecosystems (Graham and Vance, 2003; Sinclair and Rufty, 2012). Their availability and acquisition is therefore crucial for plant development and functioning. Most grain legumes such as chickpea source their N from both the soil and through symbiotic N fixation (Siddiqi and Mahmood, 2001), and contributes significant quantity of N to cropping systems. Moreover, through root proliferation and its deep tap root system, chickpeas improve soil properties and soil biological health as well as soil nitrogen enrichment through biomass addition and N_2 fixation (Singh and Shivakumar, 2010).

For enhanced phosphorus availability in soils, a variety of mechanisms including acidification of the rhizosphere (Gunes *et al*., 2007), secretion of greater extracellular acid phosphatase activity (Li *et al*., 2004) and increase in concentration of rhizosphere carboxylates (Veneklaas *et al*., 2003; Wouterlood *et al*. 2004) have been shown in chickpeas. However, the phosphorus contributed through these various mechanisms is hardly enough to meet the phosphorus-demand of the crop. As a symbiotic legume, the biological N_2 fixation process is phosphorus demanding; shortage of phosphorus in the soil has the biggest impact on grain N_2 fixation. Crop response to phosphorus fertilization depends on soil available phosphorus as well as the genotype. In general, phosphorus fertilization promotes root growth, which in turn improves the growth efficiency of crops, enhance WUE and increase grain yield (Schwember *et al*., 2019).

For optimum growth, development and fecundity, plants need C among other mineral nutrients, and of all mineral nutrients, it ranks second to N as the most important mineral nutrient to plants. C is acquired by C3 plants through carbon dioxide fixation during the photosynthetic process; indeed, C accumulation represents a direct measure of photosynthetic activity and therefore the growth and development of such plants (Raines and Paul 2006; Smith and Stitt, 2007). Therefore, the C concentration in shoots of C3 plants is closely related to their growth. It is clear from the foregoing that any practice(s) that enhance acquisition of C, N and P would lead to increased growth and yield of chickpea and other legumes. A number of studies have documented the effect of phosphorus fertilizer application and rhizobial inoculation. This would in future lead to less reliance on importation of the crop, which makes it costly and therefore affordable to few. One such experiment was through assessing the use of external phosphorus fertilization along with rhizobium inoculation or phosphorus and inoculation alone which were hypothesized to enhance the acquisition of C for optimum growth and therefore biomass production and as well as N contributed through N fixation would be enhanced. The objective of this study was to assess the effect of phosphorus fertilizer rates, rhizobial inoculation and chickpea genotypes on grain yield and yield components, symbiotic performance, N fixation, C accumulation and water-use efficiency in Limpopo, South Africa. The hypotheses tested was that phosphorus fertilizer application, rhizobial inoculation and chickpea genotypes affects grain yield and yield components, symbiotic performance, N fixation, C accumulation and water-use efficiency.

CHAPTER 2 LITERATURE REVIEW

2.1 Background on chickpea in South Africa

Chickpea (*Cicer arietinum* L.) is native to South-East Turkey but has since been introduced to many other countries almost across the world. It is classified as belonging to the genus *Cicer*, tribe Cicereae, and family Fabaceae (Corp *et al*., 2014). Chickpeas in South Africa are commonly referred to as "Garbanzo beans" (DAFF, 2012).

Chickpea is not grown widely in South Africa, but a few stands of unimproved accessions and landraces of grown in some parts of Mpumalanga and Limpopo provinces (Thangwana and Ogola, 2012). The legume grain is either sold raw or as a canned product. Moreover, chickpea seeds are sold in markets either dry or canned for industrial use (ARC-Institute, 2008). South Africa does not produce chickpeas commercially to meet its demand. Therefore, the country relies on imported chickpeas to meet the local demand, and imports are mainly from India and Italy. Over the years, local demand for chickpea has increased and its importation figures increased as well. For example, the USA has since increased its export of chickpea to South Africa since the year 2011 (Rawal and Navarro, 2019). There is also a high global demand for chickpeas, which in the past three decades increased from 30 to 150 countries. Moreover, cultivation and production of the crop increased substantially from 11.75 million ha in 2011 to 13.5 m ha in 2013 (FAOSTAT, 2016). Figures recorded in the year 2016 indicated that Asian countries contributed the most tons of chickpea with 74% of global production (FAO, 2016). In the African continent however, Ethiopia was the largest producer and accounted for about 46% of the continent's production (Kassie *et al*., 2009).

Moreover, there are a number of scientific publications that have been produced over the years in South Africa. These include the crop's response in terms of growth, grain and other yield components to phosphorus fertilization when established in the summer and winter seasons (Madzivhandila *et al*., 2012), and also the effect of rhizobial inoculation on chickpea nodulation (Ogola, 2015). However, no interactive effect of phosphorus fertilizer and rhizobial inoculation has been evaluated on chickpea productivity in South Africa prior this study.

2.2 Uses and nutritional value of the chickpea

In South Africa chickpea grain is consumed as dahl (a thick soup used like gravy) or a dry pulse where it is roasted or boiled whilst the green pods are eaten as vegetables, and the remains of chickpea (such as the leaves, Husk and stems) are beneficial to livestock farmers for animal feed (DAFF, 2012). In addition to being beneficial to livestock feed, chickpea residues can be used to fertilize the soil and supply soil with free nitrogen (N) and that is beneficial to the ecosystem. Furthermore, the grain of chickpea is rich in micronutrients including selenium, iron and zinc and macronutrients including calcium, magnesium, potassium, copper and phosphorus (Thavarajah and Thavarajah, 2012). Chickpea seeds contain enhanced levels of unsaturated fatty acids (Jukanti *et al*., 2012), high in protein content (23%), carbohydrates and fiber: low in fat and cholesterol. They are an important human food with a high protein digestibility and are rich in phosphorus and calcium (Thavarajah and Thavarajah, 2012).

2.3 The ^{15}N and ^{13}C Natural abundance and their role in crops

Of the two most stable isotopes of N, soils are more enriched in ^{15}N than ^{14}N, an imbalance caused by isotopic fractionation during N cycling (Högberg *et al*., 1999). The significantly higher ^{15}N value of soil N compared to that in the atmosphere allows for determination of the amount of ^{15}N that a legume can acquire from the soil. When C3 plants such as chickpea photosynthesise, there is natural discrimination in terms of uptake between the heavy isotope of carbon, ($^{13}CO_2$) and the lighter isotope of carbon ($^{12}CO_2$); the discrimination favours the lighter isotope (Condon et al., 2004). Factors including the poor reactivity of the heavier isotope of C with RUBISCO (ribulose1, 5-bisphosphate carboxylase) as well as its slow diffusion through stomatal apertures contribute to its discrimination (Mapope *et al*., 2016). The discrimination against the heavy ^{13}C isotope is used as a measure of water-use efficiency in C3 plants; where shoot $\delta^{13}C$ values are less negative; they indicate that the crop is more efficient in water-use (Mohale *et al*., 2014).

Therefore, it is clear that these two stable isotopes of C and N are indicators of eco-physiological processes in plants. For example, shoot $\delta^{15}N$ and $\delta^{13}C$ in leguminous plants indicate symbiotic N nutrition and greater water-use efficiency (Schulze *et al*., 2006). Several experiments have validated that shoot $\delta^{15}N$ and $\delta^{13}C$ of chickpea are surrogates of N nutrition and water-use efficiency, respectively (Dalal *et al*., 2013).

2.4 The role of P fertilization in the growth, C accumulation, N_2 fixation and grain yield of chickpea

South African soils are largely acidic, with high Al and Fe concentrations and great phosphorus absorptive capacity, all which results in low plant-available phosphorus in the soil solution (Weil and Brady, 2017). As is the case with production of most grain legumes across the African continent, successful production of chickpea is severely limited by inherently low soil phosphorus levels. Therefore, there should be a comprehensive programme to ensure better phosphorus supply and availability because as a legume, it is sensitive to phosphorus deficiency. Through the N_2 fixation process, chickpeas have a high phosphorus demand because the process consumes large amounts of energy (Schulze *et al*., 2006) and energy-generating metabolism strongly depends upon the availability of phosphorus (Israel, 1987; Plaxton, 2004). Once applied to cropping fields, phosphate fertilizers react in the soil solution and accumulates as sparingly available phosphorus.

Various chickpea genotypes have specialized phosphorus-uptake mechanisms including production of carboxylates (Kabir *et al*., 2015) which enables better absorption. Chickpea grown with the application of phosphorus increased shoot dry weight, and formed more nodules, had higher nodule dry weight, enhanced N_2 fixation and grain yield (Yahiya *et al*., 1995; Madzivhandila *et al*., 2012). The enhanced growth, N_2 fixation and grain yield from phosphorus fertilization is often caused by its role in N_2 fixation and photosynthesis, which lead to enhanced metabolism and development of aboveground and belowground plant organs: such as early root formation, development of lateral and fibrous roots and better root proliferation for increased access and uptake of water. However, there are reports that have indicated that 40 kg P ha^{-1} which was applied in clay loam soil did not affect N_2 fixation by desi and kabuli chickpeas in Saskatchewan, Canada (Walley *et al*., 2005). Hence, N_2 fixation may require high rate of phosphorus fertilizer for it to be effective in the fixation process. Moreover, different genotypes of chickpea are likely to respond differently to phosphorus fertilization partly due to a variation in the rooting pattern between genotypes. In general, a nutrient-efficient chickpea genotype would be one that produces higher yields per unit phosphorus applied compared with other genotypes under similar agro ecological conditions.

Furthermore, carbon isotope discrimination is affected by soil phosphorus levels, for example, low phosphorus availability in soils result in more negative shoot $\delta^{13}C$ values which is caused by reduced photosynthetic assimilation of intercellular CO_2 in leaves (Beardall *et al*., 2004). An increase in external phosphorus supply stimulates net photosynthesis through changing CO_2 assimilation capacity, which in turn enhances carbon fixation and shoot dry matter. In addition, *bradyrhizobium*-inoculated plants alleviate water stress through the production of lumichrome, which decreases leaf stomatal conductance and reduces water loss via transpiration in the leaves (Phillips *et al*., 1999; Dakora, 2003; Mehboob *et al*., 2009). However, in South Africa region there is absence of documented information concerning the effect of phosphorus fertilizer on symbiotic performance in respect of chickpea genotypes.

2.5 Effect of Rhizobium inoculation on the growth, C accumulation, N_2 fixation and grain yield of chickpea

The most commonly used bio-fertilizer by researchers and commercial grain legume farmers for enhanced N_2 fixation by grain legumes in South Africa is rhizobial inoculant (Pule-Meulenberg *et al*., 2011; Mapope and Dakora, 2016). Although majority of studies have shown a significant increase in the growth, grain yield and N contribution by legumes as a result of rhizobial inoculation (Kyei-Boahen *et al*., 2017), some studies have not shown a significant different and also an opposite effect (Ogola, 2015; Chibeba *et al*., 2017). Currently, the ineffectiveness of some commercial rhizobial strains has been attributed to the competitiveness of native rhizobial bacteria (Chibeba *et al*., 2017). In addition, poor soil fertility, especially low-phosphorus in soils where rhizobial inoculation is carried out does reduce its effectiveness (Schutz *et al*., 2018). Currently, there is one published article on the response of nodulation, growth and yield-related parameters of chickpea to rhizobial inoculation and it showed that nodulation was markedly enhanced by rhizobial inoculation (Ogola, 2015).

Studies on the role of rhizobium inoculation on various parameters of chickpea reported that rhizobial inoculation promotes large leaf area index (LAI) and greater dry matter (Ali *et al*., 2004). Moreover, number and dry weight of nodules were greater with inoculants when compared with the control (Ogola, 2015). However, there is a need to investigate the impact of commercial *bradyrhizobial* inoculants on symbiotic performance and ^{13}C discrimination by chickpea genotypes in South Africa. In addition, the study found a similar response in shoot dry weight, grain yield, harvest index, intercepted total radiation and radiation use efficiency between rhizobial-inoculated chickpea and un-inoculated controls. Without a doubt, one study cannot have answered all questions on the potential of a trusted bio-fertilizer in grain legumes. There is therefore a need to have more studies on the effect of some of these factors on yield-related factors and N contribution by chickpea grown in the North-East part of South Africa, especially where the chickpea is inoculated.

2.6 The importance of P fertilization and rhizobium inoculation in the growth and symbiotic performance of chickpea

The concentration of native soil phosphorus or population of compatible native rhizobial strains in cropping systems are crucial in determining the successful production of chickpeas. Cropping systems low in soil phosphorus and with soil rhizobial, bacteria that is non-compatible to chickpea hardly produce great yields. The use of a combination of commercial inoculant strains and P fertilization results in better performance of the crop as these nodulation, nodule dry weight, seed and biological yields as well as nitrogen fixation (Ali *et al*., 2004; Saini *et al*., 2004; Elkoca *et al*., 2008). The better results obtained from seed inoculation with commercial rhizobia along with phosphorus fertilization could be partly because the rhizobia have an ability to produce plant growth regulators and solubilize nutrients, particularly phosphatase in soils (Peix *et al*., 2001).

Research in enhanced grain legume production requires that objectives focus on reducing phosphorus-fertilizer inputs without compromising yield or quality, and for that, agronomic strategies should seek to address this using inoculants and phosphorus but at reasonably low quantities to avoid the reported wasteful and inefficient phosphorus fertilizer use (White *et al*., 2013). However, there is no literature concerning the effect of phosphorus fertilizer and rhizobial inoculation on N_2 fixation, C concentration and water-use efficiency of chickpea crop in South Africa. Therefore, it was of great importance to investigate the effect, in order to determine how phosphorus fertilizer rates affect chickpea genotypes biological N fixation, water-usage and discrimination of ^{13}C in dry regions of South Africa.

CHAPTER 3

MATERIALS AND METHOD

3.1 Experimental site

The study was conducted in two locations which were University of Venda's experimental farm at Thohoyandou (22° 58' S, 30° 26' E; 595 m above sea level), and University of Limpopo in Syferkuil experiment farm (23° 50' S, 39° 40' E; 1230 m above sea level) Limpopo, South Africa. The daily temperatures at Thohoyandou vary from about 10˚C to 24˚C in winter, with an annual rainfall of 500 mm, which is highly seasonal with 95% occurring between October and March (Mzezewa and Van Rensburg, 2011). The daily temperature at Syferkuil vary from 4˚C to 20˚C in winter, average rainfall is about 451 mm but varies temporarily. Both Thohoyandou and Syferkuil are classified as semi-arid areas. Soils at Thohoyandou are classified as deep, well-drained clay soil soil with the pH of 5.10 (Lusiba *et al*., 2017), and at Syferkuil are deep loamy sand soil with the pH of 5.18 (Makonya *et al*., 2019).

3.2 Experimental design

Field experiments were conducted in North-Eastern South Africa at two sites during winter season in 2016 and 2017 (April to August). Seeds were sown between 19th and 24th April (season 1), and second experiment in 2017 seeds were sown between the 14th and 19th April (season 2). Treatments consisted of a factorial combination of two rates of phosphorus fertilizer (0 and 90 kg P ha^{-1}), four desi chickpea genotypes (ACC#1, ACC#2, ACC#3 and ACC#5) and two rhizobial inoculation levels (*bradyrhizobium* strain and without rhizobial strain). In each site, treatments were arranged in randomized complete block design and replicated three times.

3.3 Soil sampling and analysis

Soil samples were collected at both locations before planting. Sample points were distributed randomly in both experimental site to warrant representatively. Jarret style shallow sampling auger was used to extract soil from 15-20 cm depth. Six samples were taken from each experimental site and air dried for 10 days. Soil samples collected prior planting were analyzed for phosphorus and nitrogen. As mentioned above, soil samples were air dried at room temperature. Dried soil was sieved using 2 mm sieve. The soil that was sampled before planting was mixed to make a composite sample in each location for analysis respectively for available P and total N. Available P was extracted using bray P-1 (Bray and Kurtz, 1945) method. Total nitrogen was measured using the Kjeldahl N method (Bremmer and Mulvaney, 1982). The physical and chemical properties of the soil at the two sites before sowing and applying phosphorus fertilizer have been indicated in Table 3.1.

3.4 Cultural practices

Superphosphate fertilizer (16.5% P_2O_5) was applied before planting to plots according to the treatments. Inoculated seeds were soaked in a mixture of *bradyrhizobium* strain powder and water at a rate recommended by manufacture and dried before planting. Field sowing was done manually at a spacing of 0.4 m inter-row and 0.1m intra-row spacing. The area of each plot was 2 m^2 with 5 rows per plot. All the plots were irrigated uniformly after planting to promote uniform germination and crop establishment using sprinklers. Supplemental irrigation was applied in all the experiments when necessary. Weeds were eliminated in both experimental areas throughout the seasons using hands and hand hoes.

3.5 Measurements

3.5.1 Crop phenology

Days to flowering was recorded as the number of days from the date of planting to the date on which 50% of the plants in a plot had produced at least one flower. Days to physiological maturity was recorded as the number of days from planting to the period when 90% of the stems and pods of the plants in a plot had lost their green colour and turned to light golden yellow.

3.5.2 Grain yield and yield components

Grain yield and yield components was determined from 15 plants from the middle rows at harvest maturity. The above ground dry biomass yield (kg ha^{-1}) was determined by air drying the above ground biomass (including the seed yield). Thereafter, dried samples were weighed for total dry matter and pods dry weight using weighing balance. Pods were manually removed from all the harvested plants and number of pods per plant were manually counted. Total number of pods per harvested plot were weighed for pods dry weight (g). All the pods were threshed by hand, and number of seeds per pod and 100 seed weight (SWT) was determined. Harvest Index (%) was recorded as the ratio of dry seed yield per plot to the above ground dry biomass yield per plot taken at physiological maturity. Harvest index was calculated using the equation below (Sinclairs *et al*., 1995):

$$\text{Harvest index (\%)} = \frac{\text{Seed yield}}{\text{Total above dry biomass yield}} \times 100$$

3.5.3 Sampling, processing and measurement of N_2 fixation

Chickpea plants were sampled at pod-filling stage at 68 days after emergence (DAE) in each experimental site. Three plants were randomly dug up per plot, and oven-dried at 60°C for 48 hrs. The dry biomass was weighed and ground into fine powder (0.85 mm) for ^{15}N and ^{13}C isotope analysis. Reference plants (non-N_2-fixing plants), which included *Amarunthus viridis*, *Biden pilosa*, *Emex spinose* and *Eleusine indica* in Thohoyandou; *Sonchus asper*, *Malva neglecta*, and *Euphorbia hirta* in Syferkuil, were sampled from each field for determining N uptake by chickpea. Reference plants were also processed the same way as the legume, for ^{15}N analysis. All ground plant material was kept in zipper plastic sample bags prior to analysis (Mapope and Dakora, 2016).

N_2 fixation by the chickpea genotypes was determined through the ^{15}N natural abundance technique. About 2.0 mg of ground chickpea plant material was weighed into Al tin capsules to determine %N and $^{15}N/^{14}N$ ratio values using a Carlo Erba NA1500 elemental analyzer (Fisons Instruments SpA, Strada, Rivoltana, Italy) coupled to a Finan MAT252 mass spectrometer (Finnigan, MAT CombH, Bremen, Germeny) via Conflo II Open-Split Device (Junk and Svec, 1958; Mariotti, 1983; Mapope and Dakora, 2016).

Samples were run against an in-house reference material and the results normalized against, and reported relative to atmospheric N air as an international standard. The ^{15}N natural abundance, expressed as δ (delta) notation, the % deviation of the ^{15}N natural abundance of the sample from atmospheric N (0.36637 atom % ^{15}N), was calculated according to the following relationship in equation below (Mariotti *et al*., 1981; Unkovich *et al*., 2008):

$$\square^{15}N = \frac{(^{15}N/^{14}N)_{sample} - (^{15}N/^{14}N)_{atm}}{(^{15}N/^{14}N)_{atm}} \square 100$$

The proportion of N derived from atmosphere (%Ndfa) was calculated using the equation below (Shearer and Kohl, 1986):

$$\%Ndfa = \frac{\delta^{15}N_{ref} - \delta^{15}N_{leg}}{\delta^{15}N_{ref} - Bvalue} \times 100$$

Where ^{15}Nref is the ^{15}N natural abundance of reference plants, ^{15}N leg is the ^{15}N natural abundance of legume, and the B value, the ^{15}N natural abundance of each chickpea genotype grown wholly dependent on N_2 fixation for its N nutrition. The B value used to calculate the %Ndfa of chickpea was -2.10‰ which was determined under glasshouse conditions (Shah *et al*., 1997).

Amount of N-fixed was determined as shown in the equation below (Belane and Dakora, 2010):

$$\text{N fixed per plant} = \%\text{Ndfa} \times \text{legume biomass}$$

N-fixed per hectare estimated using plant density).

N content was calculated as a product of %N and plant dry matter.

3.5.4 Measurement of $^{13}C/^{12}C$ and C concentration

Shoot $^{13}C/^{12}C$ ratio and %C in the selected chickpea genotypes was determined using mass spectrophotometer that was used for the $^{15}N/^{14}N$. The isotopic ratios of C were referenced and reported in the standard notation relative to Pee Dee Belemnite (Fry, 2006), as showed in the equation below: $\delta^{13}C = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$

Where: R sample is the isotopic ratio ($^{13}C/^{12}C$) of the sample and R standard = 0.06747; which is the isotopic ratio of PBD, a universally accepted standard from Belemnite Pee Dee limestone (Craig, 1957).

3.6 Statistical analysis

Statistica-10 was used to analyze data. Analysis of variance (ANOVA) was used to evaluate the effect of P fertilizer rates and rhizobial inoculation and chickpea genotypes on grain yield and yield components, symbiotic performance, N-fixation, C accumulation and WUE in Limpopo, South Africa. Where significant differences were found, the means were separated using Fisher's least significance difference (LSD) test at p≤ 0.05. Correlation analyses were performed to compare C/N ration and shoot dry matter (during flowering), $\delta^{13}C$ and N-fixed, C concentration and C content, well as C concentration and shoot dry matter. Where significant differences were found, the Duncan's multiple range test was used to separate treatment means at p≤ 0.05.

Table 3.1 Physio-chemical properties of the soil prior planting (2016)

Physical/chemical property	Location	
	Syferkuil	Thohoyandou
Sand (%)	87	24
Silt (%)	2	16
Clay (%)	11	60
Textural class	Sandy loam	clay
Ph	7.5	6.1
Organic C (%)	0.3	1.4
NH_4^- (mg/kg)	19.8	23.5
NO_3^- (mg/kg)	2.4	3.4
P (mg/kg)	35	10.1
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	5.8	3.8
Mn	31.9	45.2
Zn	1.3	1.8
B	0.1	0.1
CEC	9.3	10.5

CHAPTER 4 EFFECT OF PHOSPHORUS APPLICATION AND RHIZOBIAL INOCULATION ON GRAIN

YIELD AND YIELD COMPONENTS OF CHICKPEA GENOTYPES IN THOHOYANDOU AND SYFERKUIL FARM.

ABSTRACT

Chickpea (*Cicer arietinum* L) is adapted to cool season/areas and its organs are of high nutritive value and serve as cheap sources of protein, especially in developing countries. The crop is mainly grown for human consumption, animal feed and for medicinal purposes. One of the major limiting factors to possible high chickpea grain yields is the deficiency of mineral nutrients in cropping fields, especially phosphorus. The field study was established to evaluate the effect of phosphorus fertilizer rates, rhizobial inoculation and chickpea genotypes on grain yield and yield components Limpopo, South Africa. The hypotheses tested was that phosphorus fertilizer application, rhizobial inoculation and chickpea genotypes increase grain yield and yield components across two location. The experiment was conducted in winter in 2016 and 2018. Treatments consisted of a factorial combination of two rates of phosphorus fertilizer (0 and 90 kg ha^{-1}), 4 chickpea genotypes (ACC#1, ACC#2, ACC#3 and ACC#5) and two rhizobial inoculation levels (with and without rhizobial strain). Grain yield and yield components were determined at harvest maturity. In Thohoyandou, ACC#1 showed greater grain yield in 2016 and 2017. Application of phosphorus enhanced grain yield in Thohoyandou in both seasons and Syferkuil in 2017. In addition, significant increase of yield, pods dry weight and number of pods was obtained at Syferkuil in 2017 as compared to Thohoyandou. Moreover, phosphorus fertilizer application increased grain yield and yield components. Furthermore, the interaction between genotypes, phosphorus fertilizer and rhizobial inoculation had significant effect on grain yield in 2016. In addition, ACC#1 proved to be the best performing accessions at 90 kg ha^{-1} when inoculated with *bradyrhizobium* strain.

Key words: chickpea genotypes, rhizobial inoculation, phosphorus fertilier, yield and yield componets

4.1 Introduction

Chickpea (*Cicer arietinum* L) is better adapted in cool seasons/areas and its organs are of high nutritive value and serve as cheap sources of protein, especially in developing countries (FAO, 2015). The crop is mainly grown for human consumption, animal feed and for medicinal purposes. Although chickpea is currently the second-most cultivated legume crop in the world, after common beans (FAOSTAT, 2016), in South Africa, there are no records of its commercial cultivation despite its high domestic demand in the country. The largest producer of chickpea in the African continent is Ethiopia but the country produces grain yields that are below that reported in experimental fields (Fikre, 2016).

One of the major limiting factors to possible high grain yields is the deficiency of mineral nutrients in cropping fields, especially phosphorus (Wolde-meskel *et al*., 2018). In the African continent, the deficiency of phosphorus is not unique to Ethiopia but has been widely reported in smallholder cropping fields in South Africa (Mohale *et al*., 2014; Sosibo *et al*., 2017). Indeed, the deficiency of phosphorus in South African soils is reportedly the main reason why field experiments conducted in the country on the growth and grain yield of chickpea have included phosphorus application as a main factor (Mpai *et al*., 2018; Madzivhandila *et al*., 2012; Ogola *et al*., 2013; Lusiba *et al*., 2017).

In addition to the application of phosphate fertilizers, the phosphorus nutrition of soils as well growth and yield-related parameters of crops can be improved through the application of biofertilizers (Kyei-Boahen *et al*., 2017; Schutz *et al*., 2018). For example, Togay *et al*. (2008) reported that rhizobium inoculation increased plant height, number of branches, number of pods and seeds, as well as grain yield of chickpea. In general, these inputs improve the solubilization of the organic pool of most nutrients and the cleavage of mineral-bound nutrients (Alori *et al*., 2017). This is partly because bio-fertilizers contain microorganisms, which enhance nutrient uptake and efficiency, thereby improving crop quality.

When the efficiency of phosphate fertilisers is low, crops can absorb 20-40% during the season they are applied (Beegle and Durst, 2002). Therefore, there is a need to increase the efficiency of phosphorus fertilizers. Research has shown that the co-application of phosphorus and rhizobial inoculant increased number of pods per plant and seed yield on chickpea plant (Tahir *et al*., 2009). Moreover, the study conducted by Fatima *et al*. (2006) indicated that the combination of phosphorus application with rhizobium inoculation increased growth and yield. Therefore, combination of rhizobial inoculation with phosphorus is needed in chickpea production to increase number of pods per plant with an increase in seed yield (Tahir *et al*., 2009). However, only a handful of studies have reported on the effect of phosphorus fertilizer rates and rhizobial inoculation on yield and yield components of chickpea in Limpopo Province of South Africa.

The objective of this study was to assess the effect of phosphorus fertilizer rates and rhizobial inoculation on shoot biomass, grain yield and yield components of four chickpea genotypes in two locations that are ecologically representative of the dry environments of Limpopo Province, South Africa. The hypotheses tested was that phosphorus fertilizer application, rhizobial inoculation *(bradyrhizobium*) and chickpea genotypes affects grain yield and yield genotypes in the study locations.

4.2 Materials and methods

A detailed description of materials and methods has been presented in chapter 3, but a brief summery is outlined here. Field experiment was conducted at two sites, University of Venda's experimental farm at Thohoyandou (22° 58' S, 30° 26' E; 595 m above sea level), and University of Limpopo, Syferkuil experiment farm (23° 50' S, 39° 40' E; 1230 m above sea level) Limpopo, South Africa. The experiment was conducted in winter (April to August) in 2016 and 2017. Treatments consisted of a factorial combination of two rates of phosphorus fertilizer (0 and 90 kg P ha^{-1}), four desi chickpea genotypes (ACC#1, ACC#2, ACC#3 and ACC#5) and two rhizobial inoculation levels (with and without rhizobial strain) arranged in randomized complete block design (RCBD) and replicated three times. Each experimental unit was 2 m x 1.5 m with an area of 3 m^2.

Grain yield and yield components (shoot biomass, number of pods, pod weight, 100 seed weight, and harvest index) were measured from 15 sampled plants at harvest maturity. Ten plants were selected randomly in the middle of the two rows at an area of 0.88 m^2 in each plot considering that reflects the characteristics of the entire population. Statistica-10 was used to analyse the data. Analysis of variance (ANOVA) was used to evaluate the measured effects of phosphorus fertilizer rates and rhizobial inoculation on grain yield and yield components of chickpea genotypes.

4.3 Results

Chickpea genotype affected shoot biomass (P≤0.01), pods dry weight (P≤.001), number of pods per plant (P≤0.001), seed per pod (P≤0.05), grain yield (P≤0.001) and harvest index (P≤0.05) at Thohoyandou in 2016 (Table 4.1), and grain yield (P≤0.05) and all yield components in 2017, (Table 4.2). ACC#1 had greater grain yield in both seasons, which ranged from 2321 kg ha^{-1} in 2017 to 3497 kg ha^{-1} in 2016. At Syferkuil, characterized by sandy soils, genotype affected shoot biomass, pods dry weight, number of pods plant, grain yield (P≤0.001) and harvest index in 2016 (Table 4.3), and number of pods per plant and grain yield (P≤0.05) in 2017 (Table 4.4). In contrast to Thohoyandou, ACC#2 genotype consistently had greater yield and yield components at Syferkuil in both seasons.

Application of phosphorus fertilizer increased grain yield and yield components in both seasons, except for seed per pod, 100 SWT, and harvest index in 2017, at Thohoyandou (Table 4.1 and Table 4.2). Greater grain yield was achieved at 90 kg ha^{-1} in 2016 and 2017 (ranging from 2375 kg ha^{-1} to 2979 kg ha^{-1}) respectively. In contrast, phosphorus fertilizer application increased shoot biomass, 100 SWT but decreased harvest index in 2016 for both locations (Table 4.1 and Table 4.3). In Syferkuil, phosphorus fertilizer increased shoot biomass, 100 SWT and harvest index in 2016 (Table 4.3), and in 2017 it increased shoot biomass, pods dry weight, pods per plant and grain yield (Table 4.4).

Rhizobial inoculation increased grain yield at Thohoyandou for both seasons (Table 4.1 and Table 4.2), and had a positive effect on yield components (shoot biomass, pod dry weight, seed per pod) only in 2016 (Table 4.1). In contrast, rhizobial inoculation increased 100 SWT in 2017 (Table 4.4) and shoot biomass (P≤0.05), pods dry weight (P≤0.05), number of pods per plant (P≤0.001), seed per pod (P≤0.01), 100 SWT (P≤0.01) and grain yield (P≤0.01) in 2016 in Syferkuil (Table 4.3).

The results showed interaction between genotypes and phosphorus fertilizer pods per plant (P≤0.001) and 100 SWT (P≤0.05) in 2016 (Table 4.1), and pods dry weight and grain yield in 2017 (Table 4.2), while seed per pod was affected in both seasons (Table 4.1 and Table 4.2) at Thohoyandou. However, the figures only showed the interaction on grain yield; hence, it was the focal point of this study. In contrast, genotypes X phosphorus fertilizer interaction affected pods dry weight in 2016 (Table 4.3) and 100 SWT in 2017 (Table 4.4) at Syferkuil. Moreover, the interaction between genotypes and rhizobial inoculation, it affected grain yield (ACC#5 with rhizobial inoculation) (figure 4.1 b) at Thohoyandou in both seasons (Table 4.1 and Table 4.2), and shoot biomass in 2016 (Table 4.1) and pods dry weight in 2017 (Table 4.2).

The interaction between phosphorus fertilizer and rhizobial inoculation increased shoot biomass and seed per pod at Thohoyandou in both seasons (Table 4.1 and Table 4.2), and pods dry weight, 100 SWT and grain yield (rhizobial inoculation at 90 kg ha^{-1}) in 2016 (figure 4.7 b). In contrast to Thohoyandou, phosphorus fertilizer and rhizobial inoculation increased grain yield and yield components in 2016, and it only affected harvest index ($P \leq 0.05$) in 2017 (Table 4.4).

There was significant difference when genotypes interacted with phosphorus fertilizer and rhizobial inoculation on grain yield and yield components, except harvest index in 2016 (Table 4.1), and only pods per plant ($P \leq 0.05$) in 2017 at Thohoyandou (Table 4.2). In Syferkuil, chickpea genotypes with phosphorus fertilizer and rhizobial affected, shoot biomass and pods per plant in 2017 (Table 4.4), and pods dry weight and 100 SWT in both seasons (Table 4.3 and Table 4.4).

4.4 Discussion

Grain yield was associated with chickpea yield components such as shoot dry matter, pods dry weight, pod $plant^{-1}$, seeds per pod and harvest index. In 2016, chickpea genotype affected shoot dry matter in both locations. However, in 2017 there was a significant variation in Thohoyandou but not Syferkuil. The highest shoot biomass in both locations was recorded in ACC#2 at Thohoyandou, which indicates that it is the best performing genotype.

Moreover, application of phosphorus fertilizer increased shoot biomass at both locations in 2016/2017. The role of phosphorus fertilizer, which enhances the metabolism, development of below and aboveground plant organs such as early root formation, development of lateral and fibrous roots and better root proliferation for increased access and uptake of water could attribute partly to the enhanced growth by phosphorus fertilizer application. Similar results were found by Madzivhandila *et at*. (2012) who indicated that growth was greater at 90 kg ha^{-1} in Thohoyandou location in winter.

Rhizobial inoculation also showed significant effect on shoot biomass in 2016. Wondwosen *et al*. (2016) reported that inoculated chickpea plants improved yield components as compared to control treatments. Hence, rhizobial inoculation has been shown to Increase chlorophyll content in legume plants, and as a results it increases photosynthesis, growth and productivity (Nyoki and Ndakidemi, 2014; Mmbaga *et al*., 2015).

Genotypes that had higher shoot biomass, also recorded higher pods dry weight, and grain yield in both location and seasons. In Thohoyandou, ACC#1 showed greater grain yield in 2016 and 2017, which was associated with more branches and greater plant height in order to accommodate more number of pods. In addition, genotypes significantly affected grain yield in both seasons at Syferkuil. Similarly, association between shoot biomass, pod dry matter and pod per plant to grain yield was found in Syferkuil in both seasons. In 2016, grain yield was very low as compared to 2017 at Syferkuil. Frost occurred early May 2016, as a result it caused low growth in chickpea plants that were developing hence frost damages plant cells that result to low growth rate (www.rhs.org)

Phosphorus fertilizer application increased grain yield and yield components. Legume plants require phosphorus for obtaining good quality yield. Phosphorus fertilizer application enhanced grain yield in Thohoyandou in both seasons, and Syferkuil in 2017. However, grain yield for 2016 in Syferkuil may be misleading due to the frost damage the experiment in that year. Significant results are supported by the study done by Zafar *et al*. (2003) which shows that the application of phosphorus fertilizer increases 100-seed weight (g) and number of pods plant^{-1} because phosphorus fertilizer application encourages flowering and fruiting which result to an increase in grain yield. Moreover, rhizobial inoculation also showed positive effect on grain yield and yield components. In Thohoyandou, inoculated plants had higher grain yield in both seasons. However, Syferkuil greater obtained yield in 2017 as compared to Thohoyandou; hence, it also had greater pod dry weight and pod per plant. Which means that pod dry weight and pod per plants can be used to indication/estimate grain yield in chickpea plants because there was always an association between pod dry weight and pod per plant in this study. Studies have proven that the success of rhizobial inoculation depends on prevailing environmental conditions, soil richness, application method of effective rhizobial cells, presence of high population of competing strains of rhizobia and plant genotype (Afzal and Bano, 2014). This means that Syferkuil provided favorable conditions to the bacterial strain for chickpea growth as compare to Thohoyandou; hence, Makonya *et al*. (2019) reported that Polokwane (Syferkuil) showed temperature range that is conducive to chickpea production.

Furthermore, the interaction between genotypes, phosphorus fertilizer and rhizobial inoculation had significant effect on grain yield in 2016. In addition, ACC#1 proved to be the best performing accessions at 90 kg ha^{-1} when inoculated with *bradyrhizobium* strain. Inoculation of composite strains of rhizobium with phosphorus, gave a better yield than inoculation of a single strain of rhizobium (Tahir *et al*., 2009).

4.5 Conclusion

The results showed that phosphorus fertilizer application and rhizobial inoculation (*bradyrhizobium* strain) had significant effect on grain yield and yield components of chickpea genotypes. Best performing accessions differed in two locations. In Thohoyandou, ACC#1 and ACC#5 proved to be the best performing ones. While in Syferkuil, ACC#2 consistently performed better in 2016 and 2017. Hence, those genotypes can be classified as nutrient-efficient genotypes because they produced higher yields per unit phosphorus fertilizer applied compared with other genotypes that were planted under similar agro ecological conditions. Moreover, yield components were associated with grain yield of chickpea in both locations. Therefore, the results were consistent with the expectation.

Table 4.1 the effect of phosphorus fertilizer, rhizobial inoculation and chickpea genotypes on yield and yield components at Thohoyandou in 2016

Treatment	Shoot DM (kg ha^{-1})	Pod DW (kg ha^{-1})	Number of Pod per plant	Seed per pod	100 SW (g)	Grain yield (kg ha^{-1})	HI %
Accessions #1	6847±163a	380±36.92a	165.5±15.89a	1.45±0.13ab	24.30±0.60	3407±316.29a	41.85±2.72a
#2	5043±426a	261±12.14b	107.1±8.01b	1.23±0.20b	23.24±0.47	2204±145.24b	38.49±2.92ab
#3	4767±721b	268±21.03b	57.6±4.56d	2.06±0.35a	25.70±1.63	2225±195.47b	38.74±2.66ab
#5	4995±580ab	262±17.40b	80.7±3.75c	1.25±0.10b	24.64±0.86	1811±140.25b	33.66±2.58b
P-fertilizer (kg ha^{-1})							
0	4412±560b	226±11.20b	76.1±4.47b	1.32±0.69b	23.08±0.37b	1608±84.22b	38.83±1.70a
90	6415±481a	292±15.37a	102.3±9.10a	1.63±0.22a	25.86±0.86a	2575±153.17a	32.78±1.58b
Rhizobium							
Inoculated	6125±487a	287±12.61a	86.1±3.65a	1.56±0.17a	25.28±0.89	2392±148.25a	35.55±1.54
Uninoculated	4702±593b	233±13.22b	99.6±7.09a	1.38±0.14b	23.65±0.44	1716±95.93b	36.95±2.00
F-Statistics							
Accession (A)	5.33 **	6.63***	19.14***	3.08*	3.92 NS	5.07***	2.64 *
Phosphorus (P)	23.05 ***	12.24***	7.01**	4.47 *	8.85**	18.97***	1.98*
Rhizobium (R)	11.64 **	8.82**	2.87 NS	3.67 *	2.68 NS	11.44***	0.22 NS
A*P	0.16 NS	0.40 NS	18.17***	106.93***	3.29*	0.25 NS	0.71 NS
A*R	3.49 *	2.71 NS	10.73***	66.02***	5.73 NS	5.06**	0.12 NS
P*R	4.89 *	4.16*	0.05 NS	11.58***	18.42*	4.83*	0.43 NS
A*P*R	22.88 ***	19.79***	3.79**	59.66***	9.22***	17.94***	1.03 NS

NS= not significant. *, **, ***= significant at P<0.05, P<0.01, P<0.001 respectively.

Table 4.2 the effect of phosphorus fertilizer, rhizobial inoculation and chickpea genotypes on yield and yield components at Thohoyandou in 2017

Treatment	Shoot DM (kg ha^{-1})	Pod DW (kg ha^{-1})	Pod per plant	Seed per pod	100 SW (g)	Grain yield (kg ha^{-1})	HI %
Accessions #1	4742±312c	253±18.14b	45.83±4.20b	1.5±0.1a	21.47±0.59ab	2321±150a	23.25±1.38a
#2	6907±573a	207±17.47b	61.84±6.40a	1.3±0.0b	20.18±0.57b	1782±151b	20.68±0.71ab
#3	5219±376bc	262±16.46ab	49.99±2.98b	1.3±0.1b	19.73±0.50b	2144±121ab	20.94±1.06a
#5	6191±484ab	348±37.87a	51.82±5.97b	1.2±0.0b	22.68±1.23a	2598±257a	17.57±0.71b
P-fertilizer (kg ha^{-1})							
0	4567±244b	197±10.37b	46.69±2.88b	1.3±0.1	20.84±0.69	1751±84b	22.08±1.14
90	5934±347a	329±18.50a	56.86±3.69a	1.3±0.0	21.18±0.46	2979±162a	21.72±0.74
Rhizobium							
Inoculated	5678±338	230±12.70	52.56±3.25	1.3±0.0	21.33±0.61	2695±187a	21.41±0.77
Uninoculated	5010±332	225±12.97	51.45±3.88	1.3±0.1	20.70±0.56	2042±117b	21.43±1.07
F-Statistics							
Accession (A)	4.8 **	6.53 **	3.61 *	6.06 **	2.93 *	3.78 *	3.95 **
Phosphorus (P)	9.81 ***	40.99 ***	8.99 **	3.41 NS	0.54 NS	48.35 ***	0.07 NS
Rhizobium (R)	1.99 NS	0.08 NS	0.10 NS	3.89 NS	0.16 NS	8.81 **	0.00 NS
A*P	1.11 NS	4.08**	1.47 NS	5.86 **	0.65 NS	3.31 *	0.33 NS
A*R	0.24 NS	6.04 **	3.55 *	3.07 *	0.33 NS	4.84 **	0.51 NS
P*R	4.37 *	1.51 NS	0.35 NS	4.31 *	0.01 NS	0.00 NS	0.04 NS
A*P*R	2.68 NS	1.65 NS	3.54 *	1.81 NS	0.21 NS	2.55 NS	1.64 NS

NS= not significant. *, **, ***= significant at P<0.05, P<0.01, P<0.01 respectively

Table 4.3 the effect of phosphorus fertilizer, rhizobial inoculation on yield and yield components of chickpea genotypes at Syferkuil in 2016

Treatment	Shoot DM (kg ha^{-1})	Pod DW (kg ha^{-1})	Pod per plant	Seed per pod	100 SW (g)	Grain yield (kg ha^{-1})	HI %
Accessions #1	519±111b	20.83±2.19b	7.40±0.93b	1.27±0.11	17.38±1.73	1450±124.57b	22.87±1.28ab
#2	852±172a	36.75±5.53a	11.86±1.04a	1.15±0.10	23.61±1.03	1740±129.31a	18.58±1.75b
#3	817±133a	33.97±3.16a	14.54±2.39a	1.00±0.08	20.71±0.57	1760±1147.56a	24.13±1.55a
#5	731±154ab	27.12±3.51ab	11.24±0.95a	1.05±0.08	19.66±1.18	1492±137.37b	27.25±2.09a
P-fertilizer (kg ha^{-1})							
0	536±70b	22.18±0.25	10.06±0.49a	1.11±0.06	19.07±0.77b	1799±181.19	33.13±2.33a
90	926±115a	30.30±3.11	10.85±1.04a	1.12±0.08	21.86±0.71a	1611±153.26	22.05±1.31b
Rhizobium							
Inoculated	779±100a	27.59±1.91a	13.61±0.81a	0.90±0.06b	20.42±0.71	1661±137.01a	24.35±0.89
Uninoculated	677±104b	20.57±1.62b	8.03±0.78b	1.16±0.06a	22.54±0.85	1616±145.78b	26.91±2.37
F-Statistics							
Accession (A)	1.28 *	3.98 *	5.67 **	1.09 NS	0.11 NS	11.87 ***	2.32 *
Phosphorus (P)	4.18 **	0.17 NS	1.09 NS	0.56 NS	3.65 *	1.03 NS	13.14 ***
Rhizobium (R)	2.77 *	4.37 *	9.12 ***	7.12 **	7.66 **	5.02 **	0.54 NS
A*P	1.20 NS	11.28 ***	0.76 NS	2.04 NS	0.65 NS	1.11 NS	1.10 NS
A*R	0.34 NS	0.94 NS	0.13 NS	1.87 NS	1.44 NS	0.54 NS	0.12 NS
P*R	6.46 **	30.99 ***	6.0 **	3.98 *	16 87 ***	4.12 **	2.54*
A*P*R	1.50 NS	9.84 ***	1.02 NS	1.55 NS	4.01 *	1.11 NS	1.23 NS

NS= not significant. *, **, ***= significant at P<0.05, P<0.01, P<0.01 respectively

Table 4.4 the effect of phosphorus fertilizer, rhizobial inoculation on yield and yield components of chickpea genotypes at Syferkuil in 2017

Treatment	Shoot DM (kg ha^{-1})	Pod DW (kg ha^{-1})	Pod per plant	Seed per pod	100 SW (g)	Grain yield (kg ha^{-1})	HI %
Accessions							
#1	5074±340	366.36±27.03	72.99±3.06b	1.32±0.06	17.27±0.28	3146±234a	15.58±1.13
#2	5249±391	379.54±32.55	94.77±6.19a	1.31±0.07	17.83±0.44	3093±263a	16.52±1.44
#3	4768±444	338.12±23.84	64.99±4.89b	1.24±0.04	18.13±0.39	2267±166b	15.72±1.23
#5	5038±301	374.47±24.80	90.97±6.63a	1.30±0.07	17.79±0.27	3066±205a	14.62±0.51
P-fertilizer (kg ha^{-1})							
0	4588±212b	336.23±18.46b	70.48±3.33b	1.30±0.04	17.63±0.19	2684±182b	15.72±0.96
90	5598±307a	397.94±23.89a	98.21±4.77a	1.29±0.04	17.88±0.30	3355±171a	15.50±0.59
Rhizobium							
Inoculated	5240±318	376.58±20.69	85.67±4.91	1.33±0.04	18.11±0.20a	3023±159	15.73±0.67
Uninoculated	4931±243	361.41±18.48	89.50±4.69	1.26±0.04	17.41±0.28b	2860±196	15.49±0.90
F-Statistics							
Accession (A)	0.26 NS	1.66 NS	7.35 ***	0.40 NS	1.61 NS	3.40 *	0.45 NS
Phosphorus (P)	10.58 **	4.92 *	24.14 ***	1.68 NS	0.77 NS	6.92 **	0.04 NS
Rhizobium (R)	0.60 NS	0.30 NS	0.33 NS	0.01 NS	6.13 **	0.36 NS	0.04 NS
A*P	1.69 NS	0.87 NS	2.11 NS	2.05 NS	4.60 **	0.49 NS	0.23 NS
	0.58 NS	0.83 NS	7.62 ***	2.76 NS	1.84 NS	1.51 NS	0.31 NS
	1.97 NS	0.87 NS	0.10 NS	0.06 NS	0.67 NS	0.00 NS	5.56 *
	4.66 **	3.56 *	6.19 **	1.17 NS	3.29 *	2.32 NS	0.83 NS

A*R

P*R

A*P*R

NS= not significant. *, **, ***= significant at P<0.05, P<0.01, P<0.01 respectively

Table 4.5 the effect of phosphorus fertilizer and rhizobial inoculation on yield and yield components of chickpea genotypes at two locations, Thohoyandou and Syferkuil in 2016.

Treatment	Shoot DM (kg ha^{-1})	Pod DW (kg ha^{-1})	Pod per plant	Seed per pod	100 SW (g)	Grain yield (kg ha^{-1})	HI %
Location							
Thohoyandou	3693±270a	220±133a	87.05±6.44a	1.52±0.12a	24.47±0.50a	2139±112a	27.43±0.74

Syferkuil	1727±72b	38±24b	10.08±0.87b	1.12±0.05b	12.00±2.17b	584±47b	29.81±2.62
F-Statistics Location (L)	1187 ***	39.79 ***	534.61 ***	77.47 ***	782.44 ***	417.80 ***	1.27 NS
L*A	5.28 *	5.35 **	15.25 ***	24.01 ***	41.30 ***	3.76 NS	4.51 **
L*P	4.68 **	15.75 ***	33.12 ***	0.13 NS	190.23 ***	5.79 **	9.87 **
L*R	8.33 **	10.63 ***	0.03 NS	53.64 ***	14.11 ***	15.08 ***	0.53 NS
L*A*P	0.38 NS	0.37 NS	14.80 ***	33.13 ***	1.01 NS	0.56 NS	0.45 NS
L*A*R	3.26 *	1.92 NS	9.12 ***	19.96 ***	23.37 ***	2.08 NS	1.95 NS
L*P*R	5.67 *	8.85 **	0.16 NS	9.51 **	150.78 ***	13.03 ***	18.50 ***
L*A*P*R	12.39 ***	14.08 ***	2.22 NS	24.82 ***	93.46 ***	11.42 ***	4.16 **

NS= not significant. *, **, ***= significant at P<0.05, P<0.01, P<0.01 respectively

Table 4.6 the effect of phosphorus fertilizer and rhizobial inoculation on yield and yield components of chickpea genotypes at two locations, Thohoyandou and Syferkuil in 2017.

Treatment	Shoot DM ($kg\ ha^{-1}$)	Pod DW ($kg\ ha^{-1}$)	Pod per plant	Seed per pod	100 SW (g)	Grain yield ($kg\ ha^{-1}$)	HI %
Location							
Thohoyandou	5344±239	253.95±15.45b	52.03±2.48b	1.30±0.03	21.01±0.41a	2221±147b	24.21±1.48a
Syferkuil	5082±198	364.93±15.43a	79.28±3.42a	1.29±0.03	17.76±0.18b	2918±198a	15.84±0.56b
F-Statistics							
Location (L)	0.18 NS	37.39***	85.58 ***	0.25 NS	51.99***	11.81 ***	67.98 ***
L*A	0.12 NS	0.93 NS	0.81 NS	2.76*	2.91*	1.00 NS	5.07 **
L*P	0.28 NS	5.81 NS	0.71 NS	1.37 NS	0.01 NS	1.12 NS	1.47 NS
L*R	0.01 NS	0.15**	0.00 NS	2.72 NS	0.01 NS	0.62 NS	1.43 NS
L*A*P	0.28 NS	1.32 NS	1.17 NS	6.22***	0.66 NS	0.78 NS	3.08 *
L*A*R	0.80 NS	0.77 NS	2.66 NS	3.09*	0.40 NS	0.81 NS	6.90 ***
L*P*R	0.57 NS	0.00 NS	0.38 NS	1.50 NS	0.13 NS	0.95 NS	0.80 NS
L*A*P*R	4.07 **	1.40 NS	2.29	2.60 NS	0.20 NS	0.80 NS	7.65 ***

NS= not significant.
*, **, ***= significant at P<0.05, P<0.01, P<0.01 respectively

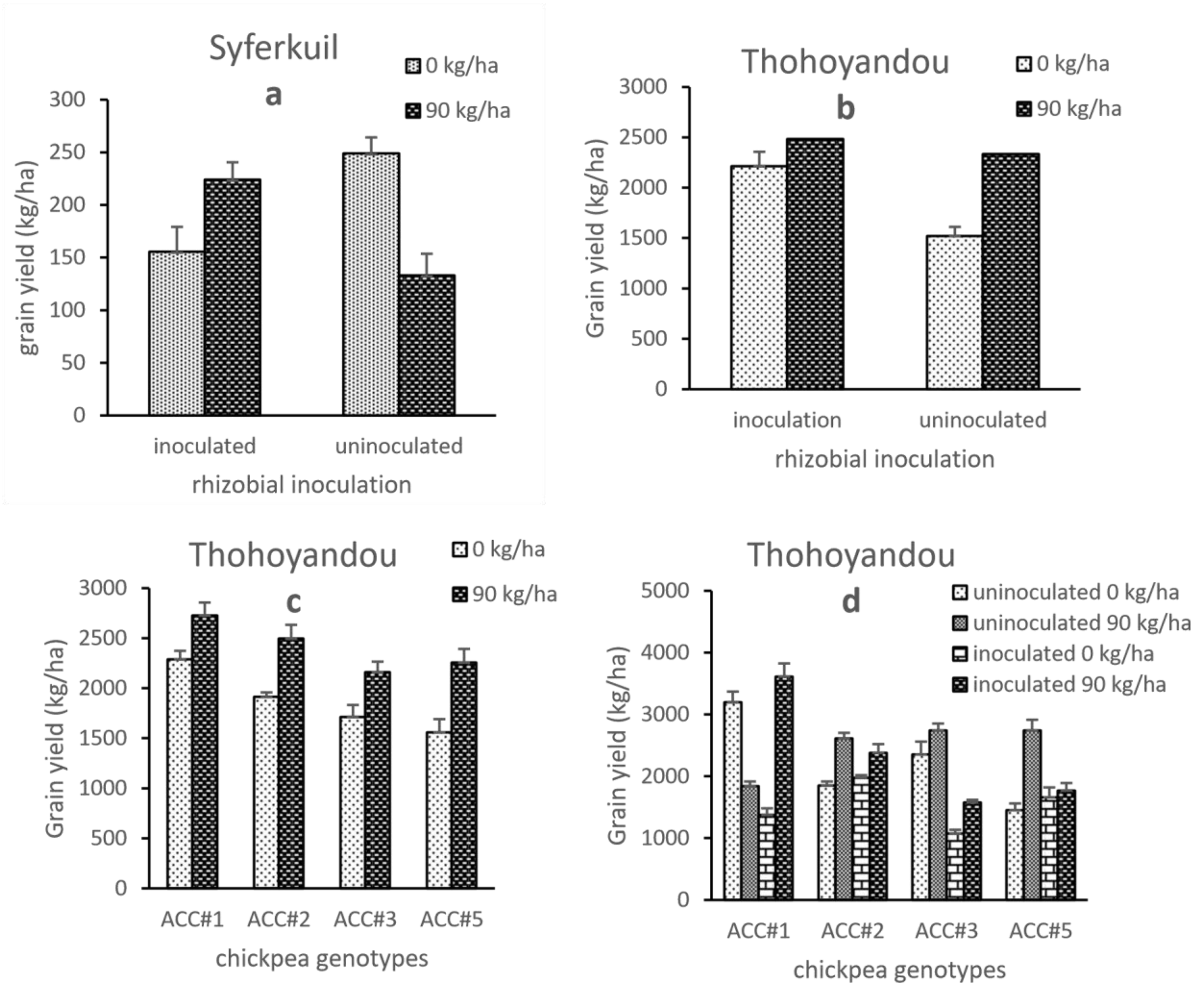

Figure 4.1: The interactive effect of phosphorus fertilizer application and rhizobial inoculation (a) and (b); phosphorus fertilizer and chickpea genotypes (c); phosphorus fertilizer, rhizobial inoculation and chickpea genotypes (d) on grain yield (kg ha^{-1}) in Thohoyandou and Syferkuil location, 2016.

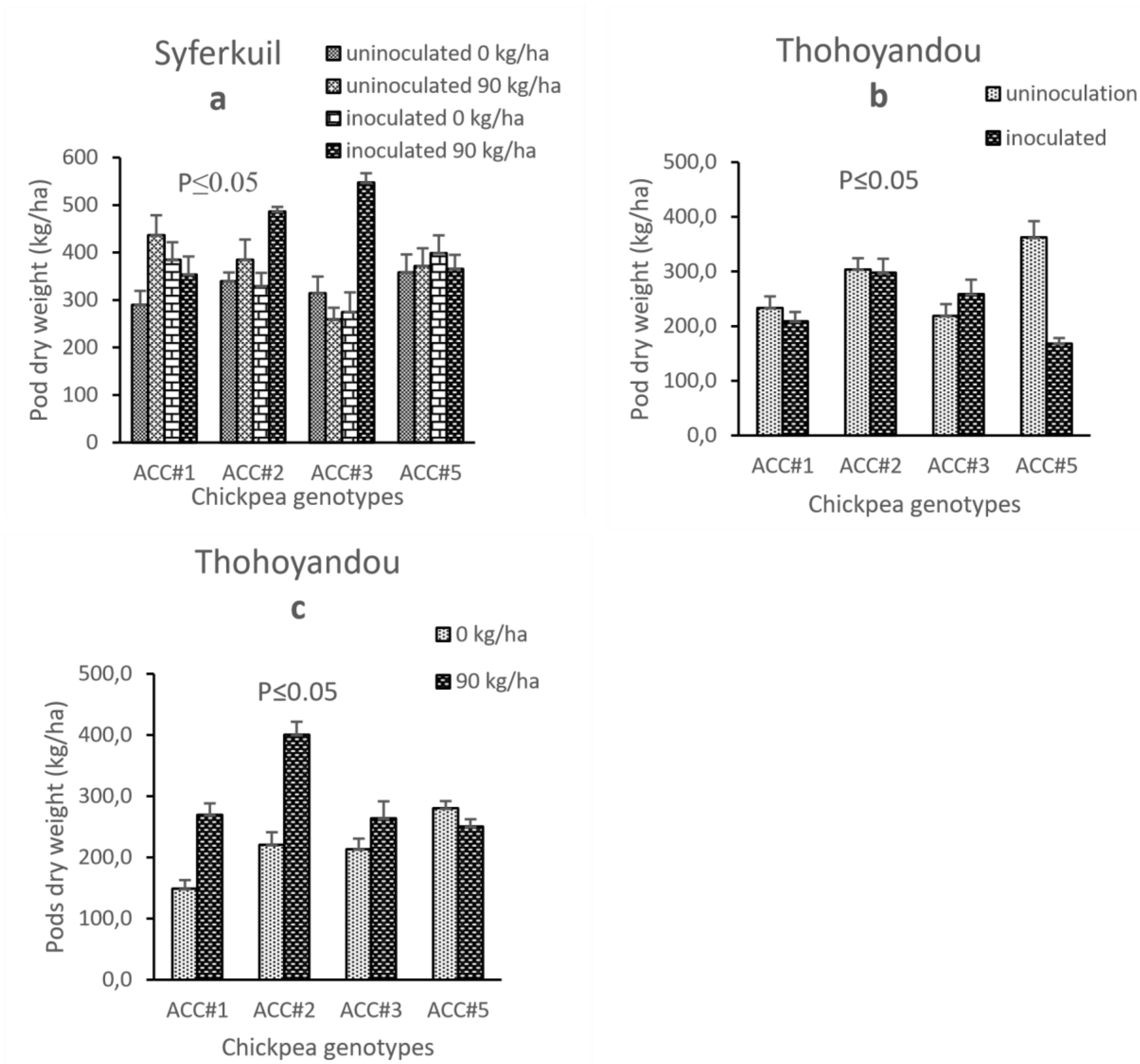

Figure 4.2: The interactive effect of phosphorus fertilizer application, rhizobial inoculation and chickpea genotypes (a); rhizobial inoculation and chickpea genotypes (b); phosphorus fertilizer and chickpea genotypes (c) on pods dry weight (kg ha^{-1}) in Thohoyandou and Syferkuil location, 2017.

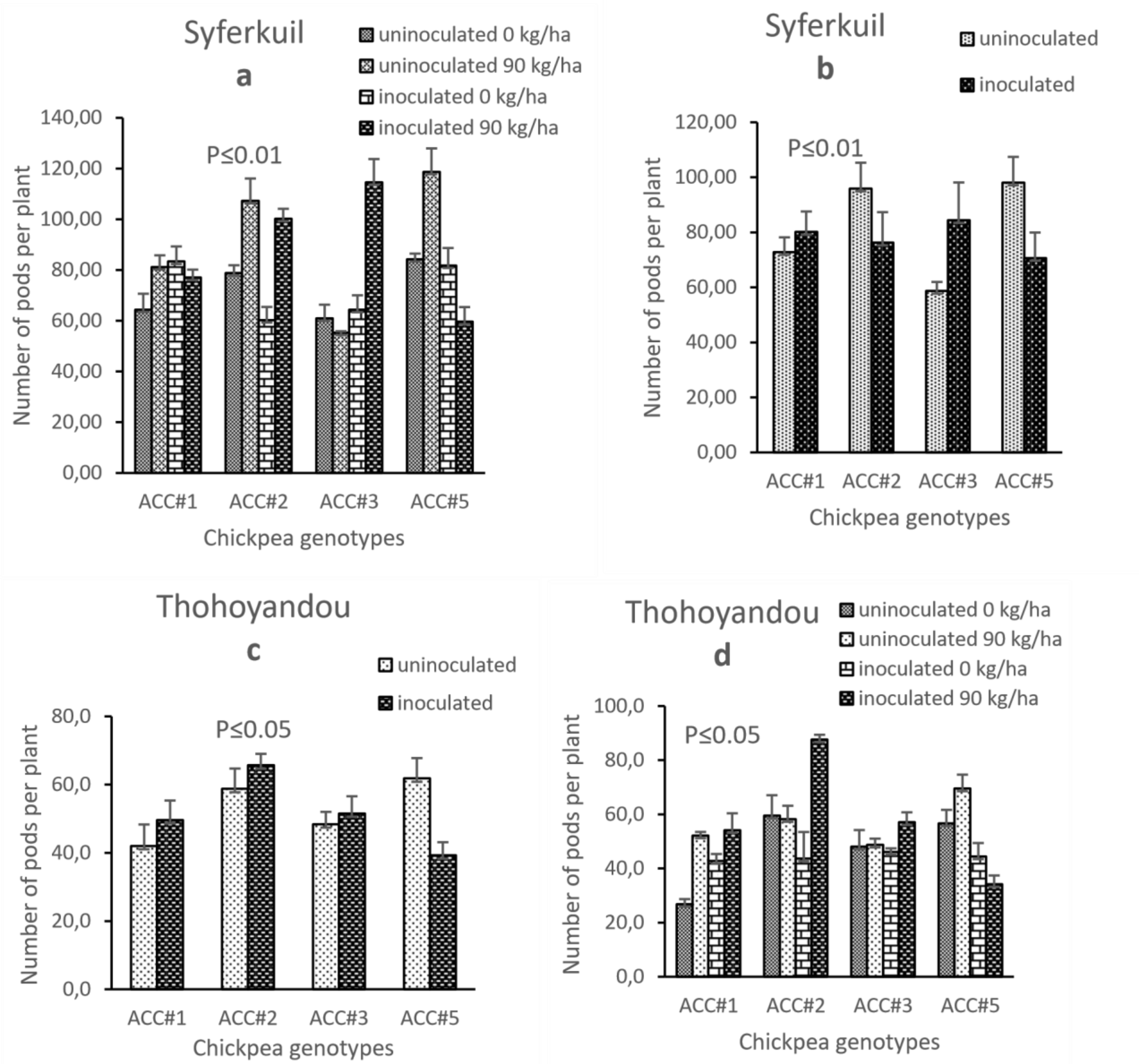

Figure 4.3: The interactive effect of phosphorus fertilizer application, rhizobial inoculation and chickpea genotypes (a) and (d); rhizobial inoculation and chickpea genotypes (b) and (c) on number of pods per plant on chickpea genotypes in Thohoyandou and Syferkuil location, 2017.

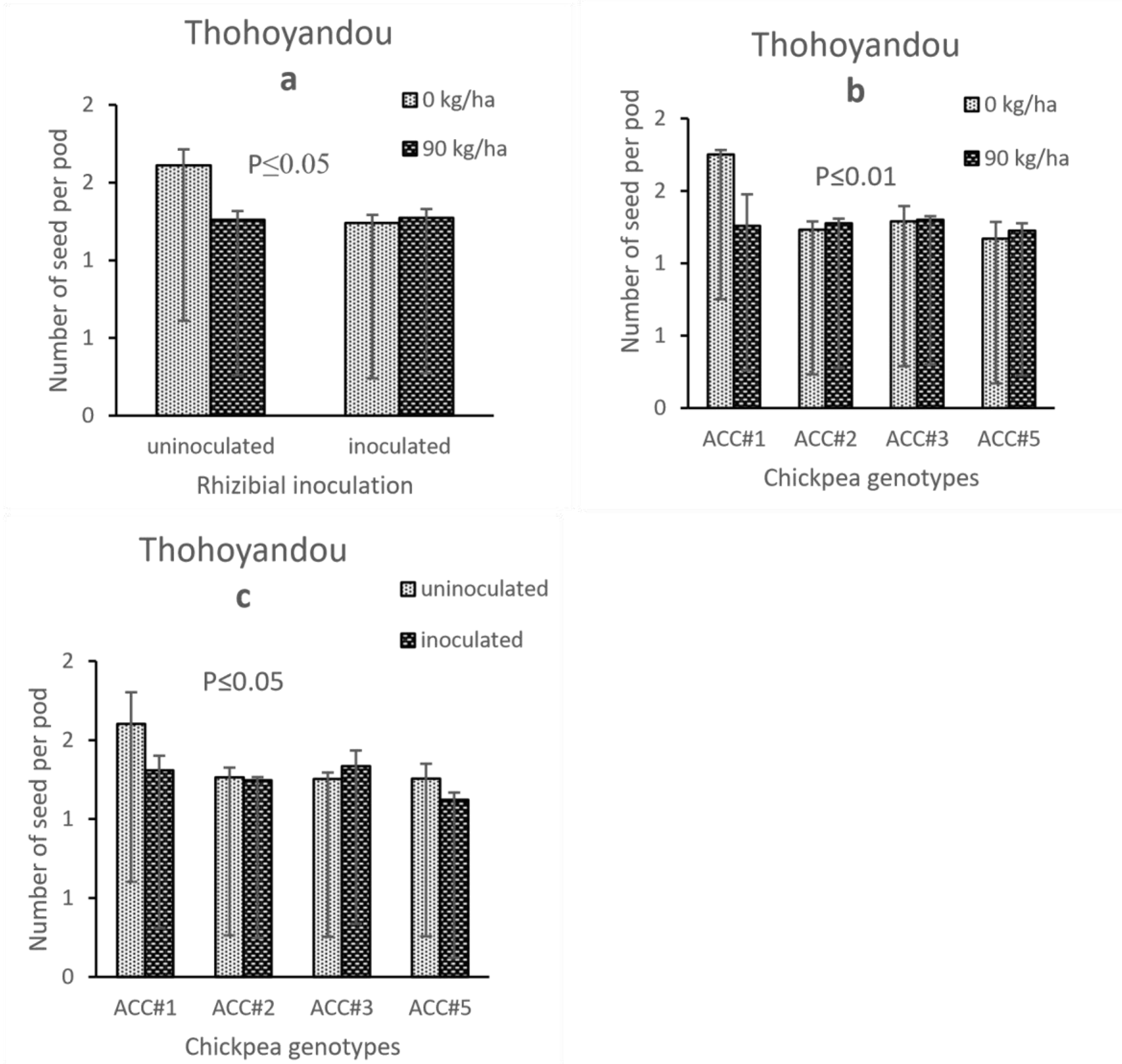

Figure 4.4: The interactive effect of phosphorus fertilizer application and rhizobial inoculation (a); phosphorus fertilizer and chickpea genotypes (b); rhizobial inoculation and chickpea genotypes (c) on number of seeds per pod in Thohoyandou location, 2017.

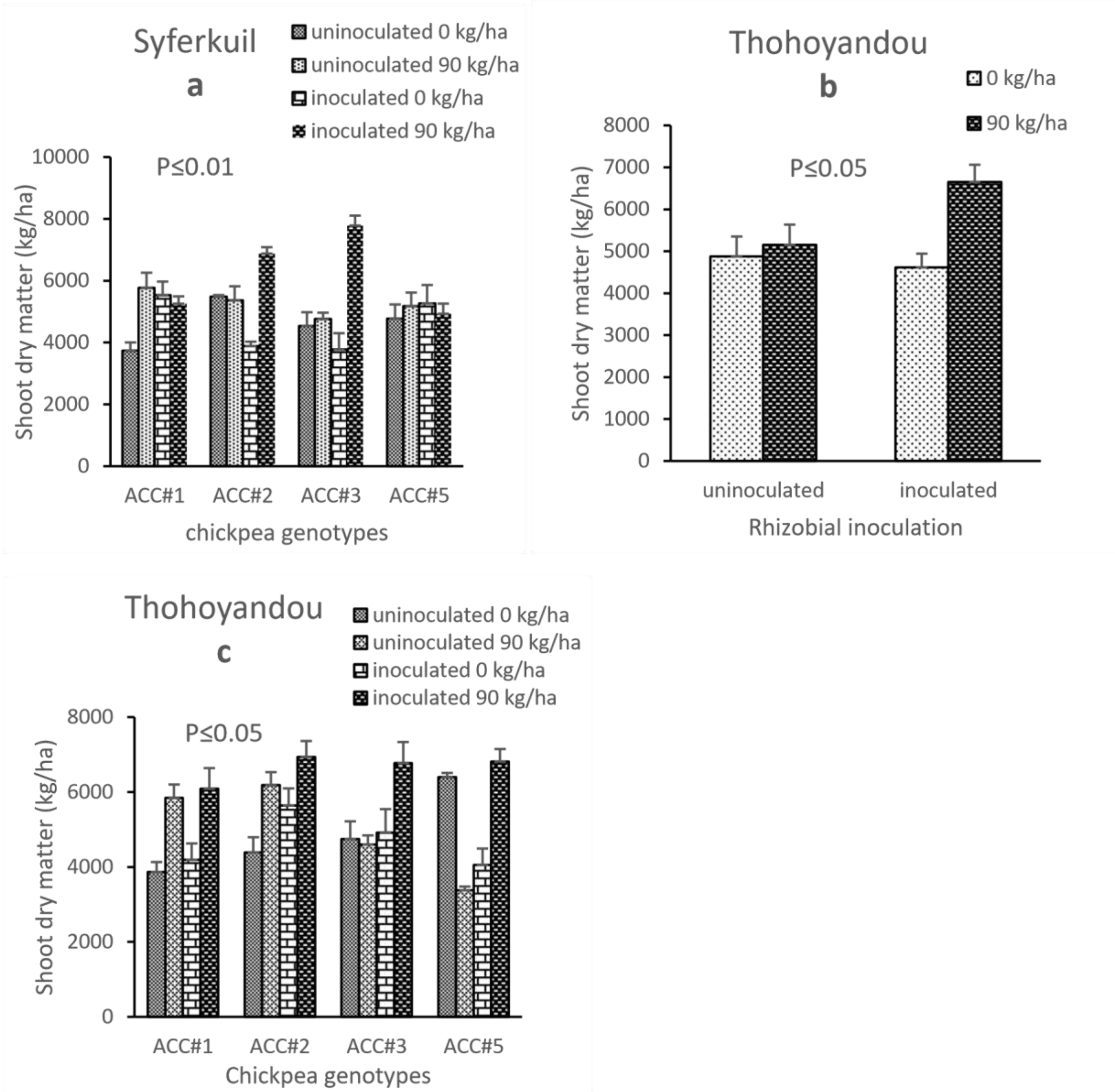

Figure 4.5: The interactive effect of phosphorus fertilizer, rhizobial inoculation and chickpea genotypes (a) and (c) phosphorus fertilizer application and rhizobial inoculation (b) on shoot dry weight (kg ha^{-1}) in Thohoyandou and Syferkuil location, 2017.

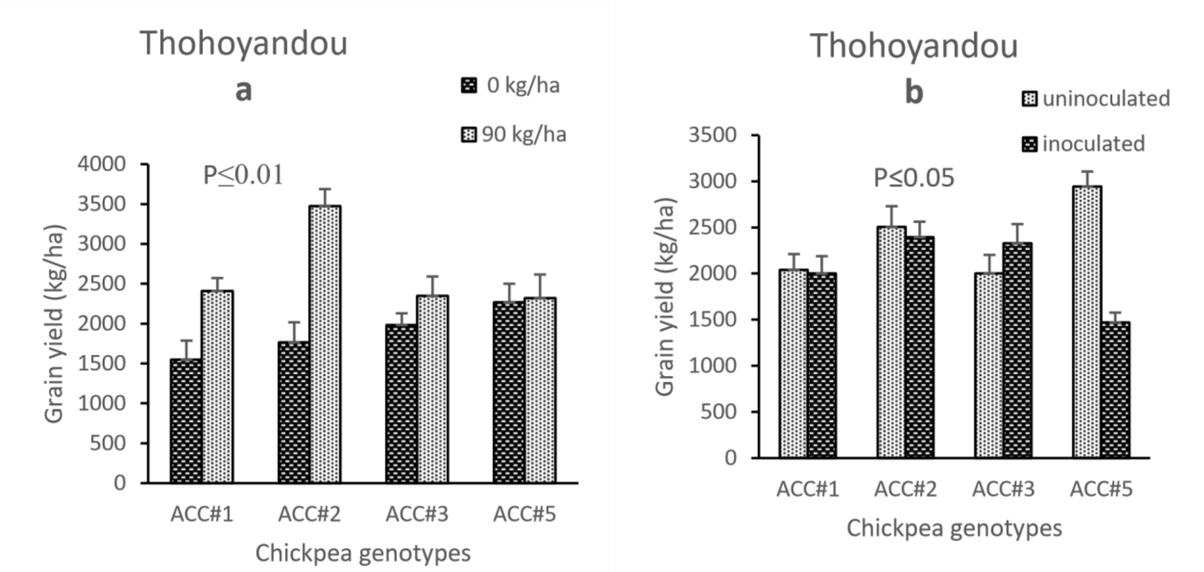

Figure 4.6: The interactive effect of phosphorus fertilizer application and chickpea genotypes (a); rhizobial inoculation and chickpea genotypes (b) on grain yield (kg ha^{-1}) in Thohoyandou location, 2017.

CHAPTER 5

EFFECT OF PHOSPHORUS FERTILIZER AND RHIZOBIAL INOCULATION ON N_2 FIXATION ON CHICKPEA GENOTYPES IN LIMPOPO PROVINCE OF SOUTH AFRICA

ABSTRACT

The introduction and promotion of chickpea to especially small-scale South African crop farmers has multiple objectives including the improvement of soil fertility. Small-scale crop farmers cannot afford N-fertilizers; it is coupled with the challenges faced by programmes aimed at assisting them address soil fertility means that their cropping fields are still without enough N level to meet N demand. It is therefore important that other alternatives that can help improve the N status of soils be explored. This study evaluated the effect of phosphorus fertilizer, rhizobia inoculation and chickpea genotypes on symbiotic performance and N_2 fixation at Thohoyandou and Syferkuil in two seasons. The hypotheses tested was that phosphorus fertilizer application, rhizobial inoculation and chickpea genotypes affects symbiotic performance and N_2 fixation. Chickpea plants were sampled at pod-filling stage at (68 days after emergence) DAE in each experimental site. The dry biomass was weighed and ground into fine powder (0.85 mm) for ^{15}N and ^{13}C isotope analysis. ACC#1, 3 and 5 fixed greater N compared to ACC#2. In addition, ACC#5 had the highest soil N-uptake in both seasons followed by ACC#3, while ACC#1 had the least value of soil Nuptake in both seasons. Phosphorus fertilizer application increased the fixation of N by (36.77%), and similarly in soil N-uptake (59.78%) compared to control in 2016. Furthermore, rhizobial inoculation increased N-fixed in 2016 and soil N-uptake in both seasons. ACC#5 had the highest N fixed at phosphorus-fertilized with *bradyrhizobuim* across two locations in both seasons. ACC# 5 had greater N-fixed at 90 kg ha^{-1}, in contrast to ACC#1 (which had performed better in control), while ACC#3 had greater soil N-uptake in the presence of phosphorus and *bradyrhizobium*. Although N_2 fixation differed across seasons. ACC#3 had greater N-fixed in both locations but in different seasons. Chickpea genotype that fixed more N had least $\delta^{15}N$ suggesting that N_2 fixation is exhibited by the genotypes that depend less on $\delta^{15}N$ because N_2 fixation is inhibited by high soil N concentration or $\delta^{15}N$. Hence chickpea genotype that depend more on atmospheric N, it minimize fixation, hence its growth will be depended the atmospheric N than N_2 from the soil. Therefore, to achieve maximum fixation of N_2 in similar environmental condition to North-east of South Africa, is best to inoculate ACC#3 and 5 at 90 kg P ha^{-1}.

Key words: chickpea genotypes, phosphorus fertilizer, rhizobial inoculation, symbiotic performance and N_2 fixation

5.1 Introduction

The introduction and promotion of chickpea to especially small-scale South African crop farmers has multiple objectives including the improvement of soil fertility. As a legume, chickpea has the potential to contribute and therefore increase the concentration of essential mineral nutrients. Across smallholder cropping fields in South Africa, it is rare that majority contain optimal N content, in fact, the reverse is true (Mohale *et al*., 2014). N is the most deficient of essential mineral nutrients needed to maintain the growth and yield of crops. Reasons for the inherently low N in small-scale farmer's cropping fields in South Africa are many and include the high cost of N-fertilizers. It should be noted that of late, the South African government subsidize farmers with inorganic fertilizers including LAN (Shabangu, 2015); however, the amount of fertilizer given to farmers is insufficient to meet the N need of crops. In addition, the programme has challenges of poor management (Moloto *et al.,* 2018). That small-scale crop farmers do not afford N-fertilizers coupled with the challenges faced by programmes aimed at assisting them address soil fertility means that their cropping fields are still without adequate N level to meet crops' N demand. It is therefore important that other alternatives that can help improve the N status of soils be explored.

In Africa, much tonnage chickpea grain is contributed by Ethiopia with a national productivity of 1.9 tons ha^{-1} (FAOSTAT, 2016). South Africa imports chickpea grain, which is becoming one of most consumed with an increasing demand (Abate *et al*., 2012; Mpai and Maseko, 2018). In South Africa, there have been field experiments aimed at encouraging local production of chickpea (Madzivhandila *et al*., 2012; Thangwana and Ogola, 2012; Ogola *et al*., 2013; Dakora *et al*., 2015; Ogola 2015; Lusiba *et al*., 2017, Mpai and Maseko, 2018). Some of these field experiments were established using seeds of both desi and kabuli types of chickpea (Madzivhandila *et al*., 2012; Thangwana and Ogola, 2012; Ogola *et al*., 2013).

Of the few published studies on N_2 fixation by chickpea in South African cropping fields Dakora *et al*. (2015) reported that when grown in three agro-ecologies of the Mpumalanga Province, both types of chickpea were dependent on atmospheric N_2 fixation for their N nutrition and also fixed N into the selected cropping fields. For example, the study recorded that the dependence of the kabuli type of chickpea on fixation of atmospheric N_2 ranged between 3 and 92%. The desi-type chickpea, on the other hand, showed dependence on N_2 fixation, which ranged from 21 to 82%.

Furthermore, Dakora *et al*. (2015) showed that the kabuli-type chickpea contributed 0.1-34 kg N ha^{-1} while their desi counterparts fixed 1-20 kg N ha^{-1}. Although Dakora *et al*. (2015) were among the first to publish on N_2 fixation by chickpea in South Africa, they showed that the grain legume has potential to contribute N into especially low-N cropping fields. However, this varied between the types of chickpea, genotypes within each type and different agro-ecosystems.

Along with water, Nitrogen (N), phosphorus (P) and Carbon (C) are the most important mineral nutrients limiting the growth and development of crops in both natural and managed ecosystems (Graham and Vance, 2003; Sinclair and Rufty, 2012). Their availability and acquisition is therefore crucial for plant development and functioning. Most grain legumes, such as chickpea source their N from both the soil and through symbiotic N_2 fixation (Siddiqi and Mahmood, 2001; Abera, 2015), and contributes significant quantity of N to cropping systems. Moreover, through root proliferation and its deep tap root system, chickpeas improve soil properties and soil biological health as well as soil N enrichment through biomass addition and N_2 fixation (Singh and Shivakumar, 2010).

There is a need to have more studies on the dependence of chickpea on symbiotic N_2 fixation as well as its ability to contribute N into cropping fields. Knowledge on this is crucial because assessing the symbiotic grain legume under cropping fields for its N_2 fixation and N contribution could provide biological solutions that overcome N limitation. This could be more important in the North-East of South Africa which is characterized by poor soils. Indeed, studies conducted at Limpopo in South Africa have shown that the growth, yield, water-use efficiency of chickpea as well as the physio-chemical properties of soils where chickpea is established are affected by the application of bio-fertilizers (Lusiba *et al*., 2017; Lusiba *et al,.* 2018).

Ronga *et al*. (2019) defined bio-fertilizers as products that contain living microorganisms or natural substances and improve the physio-chemical and biological properties of soils, stimulate plant growth, and enhance soil fertility. The most commonly used bio-fertilizer by researchers and commercial grain legume farmers for enhanced N_2 fixation in South Africa is rhizobial inoculant (Pule-Meulenberg *et al*., 2011; Mapope and Dakora, 2016). Although majority of studies have shown a significant increase in the growth, grain yield and N contribution by legumes as a result of rhizobial inoculation (Kyei-Boahen *et al*., 2017), some contributing results have been reported (Ogola, 2015; Chibeba et al., 2017). Currently, the ineffectiveness of some commercial rhizobial strains has been attributed to the competitiveness of native rhizobial bacteria (Chibeba et al., 2017). In addition, poor soil fertility, especially low-phosphorus in soils where rhizobial inoculation is carried out, does reduce its effectiveness (Schutz *et al*., 2018). Currently, there is one published article on the response of nodulation, growth and yield-related parameters of chickpea to rhizobial inoculation in South Africa and it showed that nodulation was markedly enhanced by rhizobial inoculation, but in shoot dry weight, grain yield, harvest index, intercepted total radiation and radiation use efficiency were not affected (Ogola, 2015).

Without a doubt, one study cannot have answered all questions on the potential bio-fertilizer in grain legumes. There is therefore a need to have more studies on the effect of some of these factors on yield-related factors and N contribution grown in the North-East part of South Africa by chickpea, especially where the chickpea is inoculated. South African soils are largely acidic, with high Al and Fe concentrations and greater phosphorus absorptive capacity, all which results in low plant-available phosphorus in the soil solution (BÜhmann *et al*., 2006). As is the case with production of most grain legumes across the African continent, successful production of chickpea should be severely limited by inherently low soil phosphorus levels. Therefore, adequate supply of phosphorus at early stages of plant growth is important for the development of roots as well as for seed formation and yields up soil fertility by fixing large amount of atmospheric nitrogen through root nodules, growth and yields of chickpea (Meena *et al*., 2005; Jat and Ahlawat, 2006; Singh *et al*., 2010). There are however, reports that have indicated that phosphorus (40 kg ha^{-1}) did not affect N fixation by desi and kabuli chickpeas (Walley *et al*., 2005). Hence, N fixation may require high rate of phosphorus fertilizer for it to be effective in the fixation process. However, the high cost of phosphorus fertilizer limits its use among smallholder farmers. Moreover, using Nfixing legume for its production will reduce the cost of fertilizer. Legumes play a significant role in sustainable agriculture through their ability to improve soil fertility and health. Legumes, with a mutual symbiotic relationship with some bacteria in soil, can improve nitrogen amount through biological nitrogen fixation (BNF). Nevertheless, to maximize such functions, legumes need more phosphorus as it is required for energy transformation in nodules

Apart from low N, small-scale farmer's cropping fields in South Africa are also deficient in phosphorus (Mpai and Maseko, 2018). In fact, the low soil phosphorus levels in smallholder cropping fields including in the north-east of the country, is one of major reasons why researchers include phosphorus application as a factor in their research experiments (Madzivhandila *et al*., 2012; Ogola *et al*., 2013; Lusiba *et al*., 2017). Overall, studies conducted on grain legumes in the African continent have shown that the application of phosphorus fertilizer increases the symbiotic performance (%Ndfa and N-fixed) of grain legume relative to un-fertilized treatments (Samago *et al*., 2018; Maseko *et al*., 2020). However, there is no recently published literature on the effect of phosphorus application on the symbiotic performance of chickpea in South African conditions.

Surely, there is a need for such studies, especially given that some smallholder farmers are adopting chickpea and are likely to cultivate it in low-phosphorus fields.

Besides, phosphorus also plays a significant role in root development, nutrient uptake, and growth of legume crops. However, most of the agricultural soils have inadequate amounts of phosphorus to support efficient BNF as it exists in stable chemical compounds that are least available to plants. The deficiency of phosphorus causes significant yield reduction in leguminous crops. Therefore, there is a need to enhance phosphorus use efficiency (PUE) for better legume productivity and soil sustainability. Improving the PUE of applied fertilizer requires enhanced phosphorus acquisition from the soils by crops for growth and development. It is necessary to better exploit soil phosphorus resources through increasing labile soil phosphorus using leguminous crops in a rotation cycle (Mitran *et al*., 2018). Therefore, this study assessed the effect of phosphorus fertilizer, rhizobia inoculation and chickpea genotypes on symbiotic performance and N_2 fixation in Limpopo, South Africa.

5.2 Material and methods

A detailed description of materials and methods has been presented in chapter 3, but a brief summery is outlined here. Field experiment was conducted at Thohoyandou (22° 58' S, 30° 26' E; 595 m above sea level), and Syferkuil (23° 50' S, 39° 40' E; 1230 m above sea level) Limpopo, South Africa. The experiment was conducted in winter in 2017 and in 2018. Treatments consisted of a factorial combination of two rates of phosphorus fertilizer (0 and 90 kg ha^{-1}), four chickpea genotypes (ACC#1, ACC#2, ACC#3 and ACC#5) and two rhizobial inoculation levels (with and without rhizobial strain). Superphosphate fertilizer (16.5% P2O5) was applied before planting to plots according to the treatments. Inoculated seeds were soaked in a mixture of *bradyrhizobium* strain powder and water at a rate recommended by manufacture and dried before planting.

Chickpea plants were sampled at pod-filling stage at (68 days after emergence) DAE in each experimental site, wherein five plants were randomly sampled from middle rows in each plot. The dry biomass was weighed and ground into fine powder (0.85 mm) for ^{15}N and ^{13}C isotope analysis. Reference plants (non-N_2-fixing plants), which included herbaceous annual weeds, were sampled from each field for determining N uptake by chickpea. Reference plants were also processed the same way as the legume, for ^{15}N analysis (Mapope and Dakora, 2016).

N fixation by the chickpea genotypes was determined through the ^{15}N natural abundance technique. About 2.0 mg of ground chickpea plant material was weighed into Al tin capsules to determine %N and $^{15}N/^{14}N$ ratio values using a Carlo Erba NA1500 elemental analyzer (Fisons Instruments SpA, Strada, Rivoltana, Italy) coupled to a Finan MAT252 mass spectrometer (Finnigan, MAT CombH, Bremen, Germeny) via Conflo II Open-Split Device (Junk and Svec, 1958; Mariotti, 1983; Mapope and Dakora, 2016).

Samples were run against an in-house reference material and the results normalized against and reported relative to atmospheric N air as an international standard. The ^{15}N natural abundance, expressed as δ (delta) notation, the % deviation of the ^{15}N natural abundance of the sample from atmospheric N (0.36637 atom % ^{15}N), was calculated according to the following relationship in equation 5.1 (Mariotti *et al*., 1981; Unkovich *et al*., 2008):

$$\delta^{15}N = \frac{(^{15}N/^{14}N)_{sample} - (^{15}N/^{14}N)_{atm}}{(^{15}N/^{14}N)_{atm}} \times 100$$

The proportion of N derived from atmosphere (%Ndfa) was calculated using equation 5.2 (Shearer & Kohl, 1986):

$$\%Ndfa = \frac{\delta^{15}N_{ref} - \delta^{15}N_{leg}}{\delta^{15}N_{ref} - B\,value} \times 100$$

Where ^{15}Nref is the ^{15}N natural abundance of reference plants, ^{15}N leg is the ^{15}N natural abundance of legume, and the B value, the ^{15}N natural abundance of each chickpea genotype grown wholly dependent on N_2 fixation for its N nutrition. The B value of the selected chickpea genotypes was determined under glasshouse conditions.

Amount of N-fixed was determined as shown in equation 5.3 (Peoples *et al*., 2008):

$$\text{N fixed per plant} = \%\text{Ndfa} \times \text{legume biomass}$$

N-fixed per hectare estimated using plant density).

N content was calculated as a product of %N and plant dry matter.

5.3 Results

5.3.1 Symbiotic performance of chickpea genotypes grown at Thohoyandou in response to phosphorus fertilizer and rhizobial inoculation.

The shoot biomass showed varietal differences and ACC#2 and 5 with the highest compared to that of ACC#1 in 2016: and ACC#1 and 5 in 2017 (Table 5.1). N content varied with genotypes in both seasons at Thohoyandou. However, it ranged from (1.08 g plant^{-1}) to (2.14 g plant^{-1}) in 2016 and from (1.69 g plant^{-1}) to (2.33 g plant^{-1}) in 2017. Shoot $\delta^{15}N$ and Ndfa similarly varied between the selected genotypes at Thohoyandou in both seasons. ACC#5 and 1 markedly exhibited highest $\delta^{15}N$ in both seasons and ACC#2 with the lowest $\delta^{15}N$ across two seasons (Table 5.1). In addition, ACC#2 and 3 had the highest value of Ndfa in 2016/17 and ACC#1 and 5 with the lowest ranged from (35.06-45.96%) in 2016 and from (18.76-33.30%) in 2017. ACC#2 fixed the most N_2 in 2016; however, in 2017 ACC#1, 3 and 5 fixed the most N_2 compared to that of ACC#2. Lastly, ACC#5 had the highest soil N-uptake in both seasons followed by ACC#3, while ACC#1 had the least value of soil N-uptake in both seasons (Table 5.1)

The application of phosphorus fertilizer had a significant effect only on the growth and N content of the selected chickpea genotypes. For example, chickpea established with the application of phosphorus fertilizer showed greater shoot biomass and N content compared to control at Thohoyandou location in the 2016 (Table 5.1). There was no variation among genotypes on N concentration in both seasons. Phosphorus fertilizer application significantly increased N content in both seasons compared to control. Furthermore, $\delta^{15}N$ varied between phosphorus-fertilized and un-fertilized (control) in 2016. Phosphorus application decreased shoot $\delta^{15}N$ by 7.5% in 2016 as compared to control. Similarly, Ndfa percentage was greater in control in 2016. In addition, phosphorus fertilizer application increased the fixation of N by 36.77% ($P \leq 0.01$), and similarly in soil N-uptake by difference of 59.78% compared to control in 2016 (Table 5.1).

Similar to the trend shown by the application of phosphorus, rhizobial inoculation had a significant effect on the shoot biomass and the N concentration in 2016 (Table 5.1). Rhizobial-inoculated chickpea showed significantly greater shoot biomass and N level compared to the uninoculated control. *Bradyrhizobium* inoculant increased shoot biomass (45 g plant^{-1}) by 40.48% compared to control in 2016. N concentration was higher (7.29%) in inoculated chickpea compared to control chickpea in 2016. Furthermore, rhizobial inoculation increased N-fixed in 2016 and soil N-uptake in both seasons. In 2016, soil N-uptake ranged from 0.74 kg ha^{-1} to 1.84 kg ha^{-1}, while it ranged from 3.46 kg ha^{-1} to 4.46 kg ha^{-1} in 2017. $\delta^{15}N$ and Ndfa were not affected by rhizobial inoculation, however, greater $\delta^{15}N$ was found at uninoculated plants in both seasons (Table 5.2).

The interaction between phosphorus fertilizer application and chickpea genotypes had significant effect on shoot biomass (P≤0.05), N content (P≤0.01), soil N-uptake (P≤0.01) in 2016, and Nfixed (P≤0.05) in 2017 (Figure 5.1a, Figure 5.3a, Figure 5.7a and Figure 5.6b) respectively. Phosphorus fertilizer application increased shoot biomass, N content, N-fixed and soil N-uptake in ACC# 2, 3 and 5. Wherein ACC#2 and 5 had greater N-fixed at 90 kg ha^{-1}, in contrast to ACC#1, which performed better in control (Figure 5.6b). Similarly to N-fixed, soil N-uptake was increased by the application of phosphorus fertilizer in ACC#3 and 5 (Figure 5.7a).

Moreover, the interaction between phosphorus fertilizer and chickpea genotypes significantly affected shoot biomass (Figure 5.1b) in 2016, N content (Figure 5.3c), N-fixed (Figure 5.6c) and soil N-uptake (Figure 5.7d) in 2017. ACC#2 and 3 had greater shoot biomass, N content and Soil N-uptake in control treatment as compared to ACC#1 and 5 that performed best in the presence of rhizobial inoculant (Figure 5.7b). Similarly, the interaction between phosphorus fertilizer application and rhizobial inoculation increased N content (Figure 5.3d) and soil N-uptake (Figure 5.7c) in 2017.

The interaction between phosphorus fertilizer, rhizobial inoculation and chickpea genotypes significantly affected N-fixed and soil N-uptake in 2016. ACC#3 had the highest N fixed at phosphorus-fertilized with *bradyrhizobuim* (Figure 5.6d). N-fixed was increased by the application of phosphorus and rhizobial inoculant in ACC#1, 3 and 5: with the highest N-fixed by ACC#5. ACC#2, however, fixed more N in the absence of phosphorus and rhizobial strain (Figure 5.6a). Similarly, to N-fixed, ACC#3 had greater soil N-uptake in the presence of phosphorus and *bradyrhizobium*. While ACC#1 performed well with rhizobial inoculant and no application of phosphorus (Figure 5.7b)

5.3.2 Symbiotic performance of chickpea genotypes grown at Syferkuil in response to phosphorus fertilizer and rhizobial inoculation

The shoot dry weight showed varietal differences and ACC#5 had greater shoot biomass both seasons compared to that of ACC#1 as shown in Table 5.2. Similarly, N concentration exhibited differences; ACC#1, 2 and 5 accumulated the most relative to ACC#3 and it ranged from 1.04 % to 1.56% in 2016. The N content, shoot $\delta^{15}N$ and soil N-uptake were similar between the selected genotypes established at Syferkuil in 2016. The percentage Ndfa ranged from 18.59 to 29.37%, and markedly greater in ACC#1, 2 and 3 while ACC#5 had the lowest. ACC#1 and 5 fixed the most N compared to that by ACC#2 ($P \leq 0.05$).

The application of phosphorus had a significant effect only on the growth and N content of the selected chickpea genotypes. For example, chickpea established with the application of phosphorus showed increased shoot dry weight and N content at Syferkuil in the 2016 cropping season (Table 5.2). The N concentration was similar between phosphorus-fertilized and unfertilized controls while the shoot $\delta^{15}N$, %Ndfa, N-fixed and soil N-uptake also revealed no differences. Moreover, genotypes had no significant effect on N content and $\delta^{15}N$ in both seasons. However, ACC#1 and ACC#3 showed higher N content in both seasons, ranging from 1.11 g $plant^{-1}$ to 2.14 g $plant^{-1}$ in 2016 and 1.48 g $plant^{-1}$ to 2.53 g $plant^{-1}$ in 2017 (Table 5.2). ACC#5 exhibited the lowest N content with greater $\delta^{15}N$ (4.51‰) in 2016. N-fixed varied among test genotypes (Table 5.1 and 5.2). ACC#3 had high N-fixed in both seasons (ranging from 0.048 kg ha^{-1} in 2016 to 0.74 kg ha^{-1}) in 2017. Furthermore, genotypes had no significant variation on soil N-uptake in 2016. However, genotypes varied in soil N-uptake in 2017 ($P \leq 0.01$). ACC#1 (5.08 kg ha^{-1}) and ACC#5 (4.15 kg ha^{-1}) had greater soil N-uptake (Table 5.2).

Results from the 2017-growing season revealed significant differences for the shoot dry weight, N-fixed and soil N-uptake at Syferkuil (Table 5.2). ACC#5 revealed markedly enhanced shoot dry weight compared to its counterparts, which recorded similar and least mean values. The highest N-fixers at Syferkuil in the 2017 cropping season were ACC#2 and ACC#3 while ACC#1 and ACC#5 ranked as the lowest N-fixers. In this study, chickpea established at Syferkuil in 2017 revealed that ACC#1 had the highest soil N-uptake, followed by ACC#5 and the least was recorded in ACC#2 and ACC#3.

Phosphorus fertilizer had significant effect on shoot biomass and N content in both seasons, Ndfa, N-fixed and soil N-uptake in 2017 (Table 5.2). Shoot biomass ($P \leq 0.01$), N content ($P \leq 0.01$), Nfixed (0.01) and soil N-uptake ($P \leq 0.001$) were increased by phosphorus fertilizer application at 90 kg ha-1. In contrast, the un-fertilized control plants had the largest shoot %Ndfa. The shoot N concentration and $\delta^{15}N$ had similar mean values at Syferkuil in 2017 in 2017 (Table 5.2). Soil Nuptake was significantly higher in 2017 as compared to 2016. Hence, 90 kg ha^{-1} increased soil Nuptake by 49.19% as compared to control.

Similar to the trend shown by the application of phosphorus, inoculation with rhizobial inoculant also had a significant effect on the growth and the N content (Table 5.2). Rhizobial-inoculated chickpea showed significantly increased shoot biomass and N content in 2016 (Table 5.2).

Bradyrhizobium inoculant increased shoot biomass (P≤0.001) by 20.38%-24.24% in 2016 and 2017, respectively. N content was significantly higher in 2017 (P≤0.01) as compared to 2016 (P≤0.001). For example, rhizobial-inoculated planted plants recorded increased shoot dry weight, exhibited the highest N-fixed and depended the most on soil N. Interestingly, the N concentration and content, shoot $\delta^{15}N$ and percentage Ndfa showed no differences between rhizobialinoculated chickpea and their un-inoculated counterparts. Rhizobial inoculation increased N content by 6.94% in 2017 when compared with 2016. Furthermore, soil N-uptake was higher in 2017 also than in 2016. In 2016, soil N-uptake ranged from 0.19 kg ha^{-1} to 0.23 kg ha^{-1}, while it ranged from 3.14 kg ha^{-1} to 4.81 kg ha^{-1} in 2017. $\delta^{15}N$ was not significantly affected by rhizobial inoculation, however, greater $\delta^{15}N$ was found at uninoculated plants in both seasons (Table 5.2).

The interaction between phosphorus fertilizer application and rhizobial inoculation had significant effect on N content (P≤0.01), soil N-uptake (P≤0.01) in 2016, and shoot biomass (P≤0.05) in 2017 (Figure 5.3d, Figure 5.7e and Figure 5.1c) respectively. Moreover, the interaction between phosphorus fertilizer, rhizobial inoculation and chickpea genotypes significantly affected N concentration (P≤0.05), $\delta^{15}N$ (P≤0.05), soil N-uptake (P≤0.05) in 2016, N-fixed (P≤0.05) in 2017, N content and Ndfa in both seasons. In N concentration, ACC#1 exhibit greater N concentration at 90 kg ha^{-1} with no *bradyrhizobium* strain, while ACC#3 performed best in the presence of rhizobial inoculant at 90 kg ha^{-1} (Figure 5.2a).

5.4 Discussion

In this study, ACC#1, 2 and 5 at the Syferkuil in 2016 as well as ACC#2 and 3 in 2017 at Thohoyandou exhibited greater N concentration. A number of published literature has shown that in general, the shoot N concentration is a parameter that does not vary between plant species, genotypes and locations (Beyan *et al*., 2018; Samago et al., 2018; Yahaya *et al*., 2019). Across the growing locations where significant variation shoot N concentration was realized, ACC#2 ranked as that which accumulated the most compared to its counterparts. It is interesting to note that ACC#2 also accumulated the highest shoot C concentration at both study locations (Chapter 6). Furthermore, it revealed the highest shoot dry weight. The uptake and accumulation of N in aboveground organs of plants is determined by the ability of plants to absorb N from the soil (Mohammed and Abdalla, 2013). This varies with the rooting depth and root biomass of plants, which is different between cultivars. The markedly higher shoot N by ACC#2 could suggest that it had larger root biomass that was effective in the interception and uptake of water and N. The increased absorption and accumulation of N supported the growth as well as photosynthetic fixation of C as shown by enhanced C accumulation.

Given that N content is calculated as a product of shoot dry weight and N concentration, in general, genotypes that show high shoot dry weight, also exhibit greater N content (Mokgehle et al., 2014; Mapope and Dakora, 2016). Although the N content was similar between the genotypes evaluated at the Syferkuil study site, at the Thohoyandou, the relationship between shoot dry weight and N content was observed for ACC#5.

There was no variation among chickpea genotypes on $\delta^{15}N$ in Thohoyandou; however, phosphorus fertilizer, rhizobial inoculation and chickpea genotypes had significant effect on $\delta^{15}N$ at Syferkuil location. According to published literature, there exist an inverse relationship between shoot $\delta^{15}N$ and shoot %Ndfa, that is, where genotypes, plant species or study locations show increased shoot $\delta^{15}N$, they reveal decreased %Ndfa (Nyemba and Dakora, 2010; Mohale *et al*., 2014). This suggesting that, when legumes depend more on the uptake of soil N, then there is less reliance on fixation of atmospheric N_2, which is denoted as Ndfa. This was certainly the case in this study, especially with ACC#5, which recorded the highest shoot $\delta^{15}N$ in both the 2016 and 2017 seasons, and the lowest shoot %Ndfa (Table 5.1). In addition, ACC#2 exhibited the least shoot $\delta^{15}N$ but the highest %Ndfa, during both cropping seasons at Thohoyandou. The N-fixed and soil N-uptake between the test genotypes showed an inverse relationship. For example, ACC#5, which fixed the least N, showed the highest soil N-uptake at Thohoyandou and at Syferkuil in 2017. Suggesting that ACC#5 obtained much of its N from the soil as opposed to through symbiosis. However at Thohoyandou it had greater N-fixed and low soil N-uptake.

Largely, chickpea raised with the application of phosphorus showed similar shoot N concentration except for that grown at Thohoyandou in the 2017 growing season. The increased accumulation of N in shoots of chickpea established with the supply of phosphorus could have been as a result of the fact that the application of adequate rates of phosphorus increases the uptake of N (Sharma *et al*., 2017). Furthermore, the application of phosphorus is associated with markedly improved root architecture including increased length, width, and diameter of root, including improved proliferation of their roots (Razaq *et al*., 2017). Other studies that has corroborated this on grain legumes include that by Samago *et al*. (2018) who showed it on common bean established in Ethiopia. In addition, shrub legumes of the Iberian Peninsula showed the same response when grown under controlled conditions (Miguez-Montero *et al*., 2020). In both experimental sites and over the two cropping seasons, the application of phosphorus increased the N content.

The enhanced plant N accumulation is a direct effect of phosphorus having led to increased shoot biomass of the chickpea grown at Syferkuil and Thohoyandou. The greater biomass could have been supported by the application of phosphorus. Hence, increased fertility associated with phosphorus application (for example increased uptake of Mg, Ca, S and N) supports formation of a larger and extensive root system which absorbed and intercepted nutrients, particularly N for the non-fertilized controls, as shown by the higher plant N content (Razaq *et al*., 2017). Chickpea grown at Thohoyandou in 2016 and at Syferkuil in 2017 with the application of phosphorus revealed increased N-fixed as well as soil N-uptake. This response was also shown in other legumes including dry bean (Samago *et al*., 2018).

The N concentration and content were enhanced in rhizobial-inoculated chickpea established at Thohoyandou in 2016 (N concentration) and 2017 (both N concentration and content) and at Syferkuil in 2016 (N content). Rhizobial inoculation outperformed control in both location, suggesting that N fixation in chickpea plants is maximized by inoculating them with bacterial strain. Pule-Meulenberg *et al*. (2011); Mapope and Dakora (2016) stated that the most commonly used bio-fertilizer by researchers and commercial grain legume farmers for enhanced N_2 fixation by grain legumes in South Africa is rhizobial inoculant. Given that the inoculation of legumes with competitive commercial rhizobial bacteria result in a marked increase in the population of bacteria (Zilli *et al*., 2019), apart from N_2 fixation, their other role is the solubilization of N in the soil.

The interaction between phosphorus fertilizer application and rhizobial inoculation had significant effect on N content, soil N-uptake in 2016, and shoot biomass in 2017. In addition, between phosphorus fertilizer interacting with rhizobial inoculation and chickpea genotypes significantly affected N concentration and soil N-uptake in 2016, N-fixed in 2017, N content and Ndfa in both seasons. Wherein ACC#2 and 5 had greater N-fixed at 90 kg ha^{-1}, in contrast to ACC#1, while ACC#3 had greater soil N-uptake in the presence of phosphorus and *bradyrhizobium*. While ACC#1 performed well with rhizobial inoculant and no application of phosphorus (Figure 5.7b) in both locations. This indicate that phosphorus fertilizer together with rhizobial inoculant enhances N fixation and other symbiotic performance. In this case, ACC#3 and 5 performed best in the presence of phosphorus and *bradyrhizobium* inoculant across two location; hence it had greater N-fixed and low Ndfa. These results may suggest that inoculated ACC#3 and 5 had the ability to solubilize nutrients in the soil more than ACC#1 and 2 (Peix *et al*., 2001). Moreover, the use of a combination of commercial inoculant strains and phosphorus fertilization has been reported to increase the performance of the crop as these reportedly enhanced nodulation and nitrogen fixation (Ali *et al*., 2004; Saini *et al*., 2004; Elkoca *et al*., 2008).

5.5 Conclusion

The results showed that chickpea genotypes grown in ACC#5 performed better in soil N-uptake at both locations when inoculated with *bradyrhizobuim* and with the application of phosphorus in Thohoyandou than in Syferkuil. The results in line with the hypothesis that stated phosphorus fertilizer and rhizobial inoculation will increase N_2 fixation. However, ACC#2 performed better 0 kg ha^{-1} of phosphorus fertilizer. Therefore, to achieve maximum fixation of N in similar environmental condition to North-East of South Africa, is best to inoculate ACC#3 and 5 at 90 kg P ha^{-1}.

Table 5.1 the effect of phosphorus fertilizer and rhizobial inoculation on symbiotic performance of chickpea genotypes at Thohoyandou, 2016/2017

Treatment	Shoot DM (g plant^{-1})	N concentration (%)	N Content (g plant^{-1})	$\delta^{15}N$ (‰)	Ndfa (%)	N-Fixed (kg ha^{-1})	Soil N-Uptake (kg ha^{-1})
				2016			
Accessions							
#1	27.07±2.34c	4.28±0.21	2.14±0.44a	1.59±0.08b	35.06±2.17b	1.65±0.08c	1.29±0.12c
#2	46.16±3.25a	4.11±0.12	1.47±0.22ab	1.39±0.11b	45.96±4.13a	1.87±0.13a	1.69±0.13bc
#3	38.51±2.76b	4.18±0.08	1.08±0.15b	1.86±0.13a	44.94±4.20ab	1.17±0.11b	2.20±0.17b
#5	43.66±3.42a	4.06±0.16	1.94±0.13a	1.90±0.17a	35.66±2.66b	1.18±0.17b	3.32±0.35a
P-fertilizer (kg ha^{-1})							
0	31±3.53b	4.19±0.13	1.27±0.15b	1.60±0.13a	42.65±3.06	0.98±0.03b	0.74±0.05b
90	43±3.34a	4.10±0.13	1.79±0.16a	1.48±0.10b	37.71±1.82	1.55±0.13a	1.84±0.11a
Rhizobium							
Inoculated	42±2.29a	3.94±0.10a	1.48±0.12	1.56±0.11	38.07±1.89	1.57±0.12a	1.57±0.12a
Uninoculated	25±2.13b	4.25±0.15b	1.65±0.22	1.64±0.10	42.52±3.21	1.18±0.11b	1.19±0.12b
F-Statistics							
Accession (A)	6.45 **	0.12 NS	7.83 **	4.07 *	4.78 *	10.12 ***	8.03 ***
Phosphorus (P)	12.86 ***	2.01 NS	17.57 ***	1.54 NS	2.87 NS	17.03 ***	7.99 ***
Rhizobium (R)	9.02 ***	4.56 *	1.75 NS	2.85 NS	1.96 NS	4.32 *	4.92 *
A*P	5.30 **	2.36 NS	3.81 *	1.96 NS	3.00 NS	2.78 NS	3.22 *
A*R	3.82 *	0.25 NS	2.08 NS	2.85 NS	2.53 NS	1.89 NS	2.93 NS
P*R	2.09 NS	1.02 NS	0.23 NS	3.01 NS	0.89 NS	3.13 NS	0.00 NS
A*P*R	1.19 NS	0.87 NS	0.70 NS	1.89 NS	2.41 NS	9.11 ***	3.58 *
				2017			
Accessions							
#1	88.24±8.93a	2.45±0.17	1.69±0.01b	5.99±0.21ab	22.81±2.92b	2.27±0.55a	2.99±0.28b
#2	64.74±6.21b	2.60±0.13	2.16±0.28ab	5.25±0.25b	33.31±2.43a	1.08±0.35b	3.54±0.22b
#3	65.96±4.36b	2.56±0.11	2.35±0.15a	5.25±0.23b	30.18±2.56a	2.39±0.42a	3.91±0.37a
#5	97.50±8.72a	2.25±0.12	2.33±0.28a	6.21±0.24a	18.76±2.07b	2.47±1.01a	5.50±0.59a
P-fertilizer (kg ha^{-1})							
0	79.14±5.59	2.40±0.10	1.69±0.01b	5.75±0.18	29.00±1.49a	1.85±0.19	4.25±0.41
90	76.03±5.17	2.64±0.09	2.46±0.02a	5.60±0.19	18.73±1.97b	2.14±0.20	3.68±0.34
Rhizobium							
Inoculated	77.34±5.07	2.50±0.08	2.93±0.28a	5.61±0.17	27.69±1.58	2.65±0.19	4.45±0.47a
Uninoculated	77.59±6.01	2.55±0.11	2.08±0.27b	5.74±0.20	25.16±1.69	2.31±0.61	3.46±0.37b

F-Statistics							
Accession (A)	4.68 **	1.80 NS	4.20 *	3.89 *	9.60 **	14.01 ***	6.87 **
Phosphorus (P)	0.20 NS	0.05 NS	9.19 ***	0.33 NS	28.39 ***	0.03 NS	1.72 NS
Rhizobium (R)	0.15 NS	0.15 NS	8.23 ***	0.28 NS	0.60 NS	1.28 NS	5.62 *
A*P	0.75 NS	0.30 NS	1.27 NS	0.35 NS	0.25 NS	3.61 *	2.89 NS
A*R	0.48 NS	1.96 NS	12.66 **	0.22 NS	1.74 NS	5.34 **	6.52 **
P*R	0.01 NS	0.33 NS	14.98 **	0.54 NS	1.64 NS	0.84 NS	4.76 *
A*P*R	1.94 NS	0.57 NS	1.86 NS	0.80 NS	2.05 NS	2.47 NS	0.13 NS

NS= not significant *, **, ***= significant at P<0.05, P<0.01, P<0.01 respectively

Table 5.2 the effect of phosphorus fertilizer rates and rhizobial inoculation on symbiotic performance of chickpea genotypes at Syferkuil, 2016/2017

Treatment	Shoot DM (g plant^{-1})	N (%)	N content (g plant^{-1})	δ_{15}N (‰)	Ndfa (%)	N-Fixed (kg ha^{-1})	Soil N-Uptake (Kg ha^{-1})
				2016			
Accessions #1	19.57±0.98b	1.32±0.09a	2.14±0.05	4.23±0.58	24.69±1.79a	0.054±0.00a	0.22±0.02
#2	21.41±0.85ab	1.56±0.17a	1.21±0.18	4.14±0.55b	25.67±2.90a	0.038±0.00b	0.21±0.01
#3	24.11±1.17a	1.04±0.02b	1.80±0.15	4.46±0.87	29.37±1.63a	0.048±0.00ab	0.18±0.01
#5	26.65±1.41a	1.20±0.11a	1.11±0.21	4.51±0.58	18.59±1.22b	0.049±0.00a	0.23±0.01
P-fertilizer (kg ha^{-1})							
0	20.91±0.71b	1.34±0.13	1.70±0.19b	4.36±0.15	23.69±1.32	0.049±0.00	0.21±0.01
90	28.29±1.65a	1.30±0.09	2.31±0.11a	4.34±0.15	24.32±1.54	0.048±0.00	0.24±0.02
Rhizobium Inoculated	25.86±1.18a	1.39±0.10	2.28±0.10a	4.47±0.19	23.41±1.61	0.048±0.00	0.23±0.02
Uninoculated	20.59±0.85b	1.31±0.07	1.01±0.13b	4.27±0.12	22.80±1.21	0.049±0.00	0.19±0.01
F-Statistics							
Accession (A)	4.13 *	6.10 **	1.55 NS	0.14 NS	6.23 **	3.45 *	0.58 NS
Phosphorus (P)	8.98 ***	0.90 NS	9.21 ***	0.77 NS	0.86 NS	1.56 NS	0.96 NS
Rhizobium (R)	11.23 ***	1.73 NS	5.03 **	1.25 NS	2.89 NS	0.67 NS	1.02 NS
A*P	3.01 NS	0.63 NS	0.36 NS	0.77 NS	1.95 NS	0.21 NS	0.78 NS
A*R	1.96 NS	0.95 NS	2.08 NS	1.45 NS	0.65 NS	1.89 NS	1.96 NS
P*R	0.98 NS	1.69 NS	4.96 **	1.25 NS	1.13 NS	0.18 NS	6.58 **
A*P*R	3.22 NS	3.96 *	5.56 **	4.00 *	11.87 ***	0.09 NS	3.22 *
				2017			
Accessions #1	84.13±6.31b	0.024±0.002	2.53±0.24	6.27±0.18	20.28±2.06	0.58±0.05b	5.08±0.54a

#2	78.27±7.34b	0.026±0.001	1.48±0.18	6.23±0.20	17.84±2.36	0.89±0.14a	3.25±0.30b
#3	75.87±5.72b	0.026±0.001	1.90±0.22	6.35±0.15	15.84±1.39	0.74±0.13a	3.27±0.30b
#5	128.90±9.24a	0.025±0.001	1.78±0.19	6.58±0.17	17.15±1.59	0.53±0.10b	4.15±0.45ab
P-fertilizer (kg ha^{-1})							
0	62.85±4.41b	0.026±0.001	1.34±0.12b	6.23±0.13	19.86±1.36a	0.57±0.07b	2.84±0.19b
90	86.25±7.20a	0.025±0.001	2.57±0.22a	6.48±0.12	15.38±1.08b	0.81±0.09a	5.59±0.42a
Rhizobium Inoculated	86.04±7.81a	0.026±0.001	2.45±0.10	6.33±0.13	16.87±1.48	0.94±0.09a	4.81±0.33a
Uninoculated	65.17±4.46b	0.024±0.001	1.96±0.19	6.39±0.12	18.45±1.18	0.61±0.04b	3.14±0.22b
F-Statistics							
Accession (A)	7.25 **	0.22 NS	0.90 NS	0.66 NS	2.48 NS	4.52 **	4.50 **
Phosphorus (P)	9.33 **	2.93 NS	11.84**	0.08 NS	11.74 **	8.47 **	35.60 ***
Rhizobium (R)	13.74 ***	0.15 NS	26.76 ***	1.75 NS	1.46 NS	11.40 **	17.59 ***
A*P	0.56 NS	0.60 NS	0.18 NS	0.46 NS	2.11 NS	1.00 NS	2.01 NS
A*R	1.35 NS	1.62 NS	0.65 NS	0.15 NS	1.89 NS	2.96 NS	0.23 NS
P*R	4.67 *	0.34 NS	1.22 NS	0.03 NS	2.82 NS	0.51 NS	0.21 NS
A*P*R	0.61 NS	0.11 NS	9.21***	0.37 NS	3.37 *	3.45 *	2.22 NS

NS= not significant. *, **, ***= significant at P<0.05, P<0.01, P<0.01 respectively

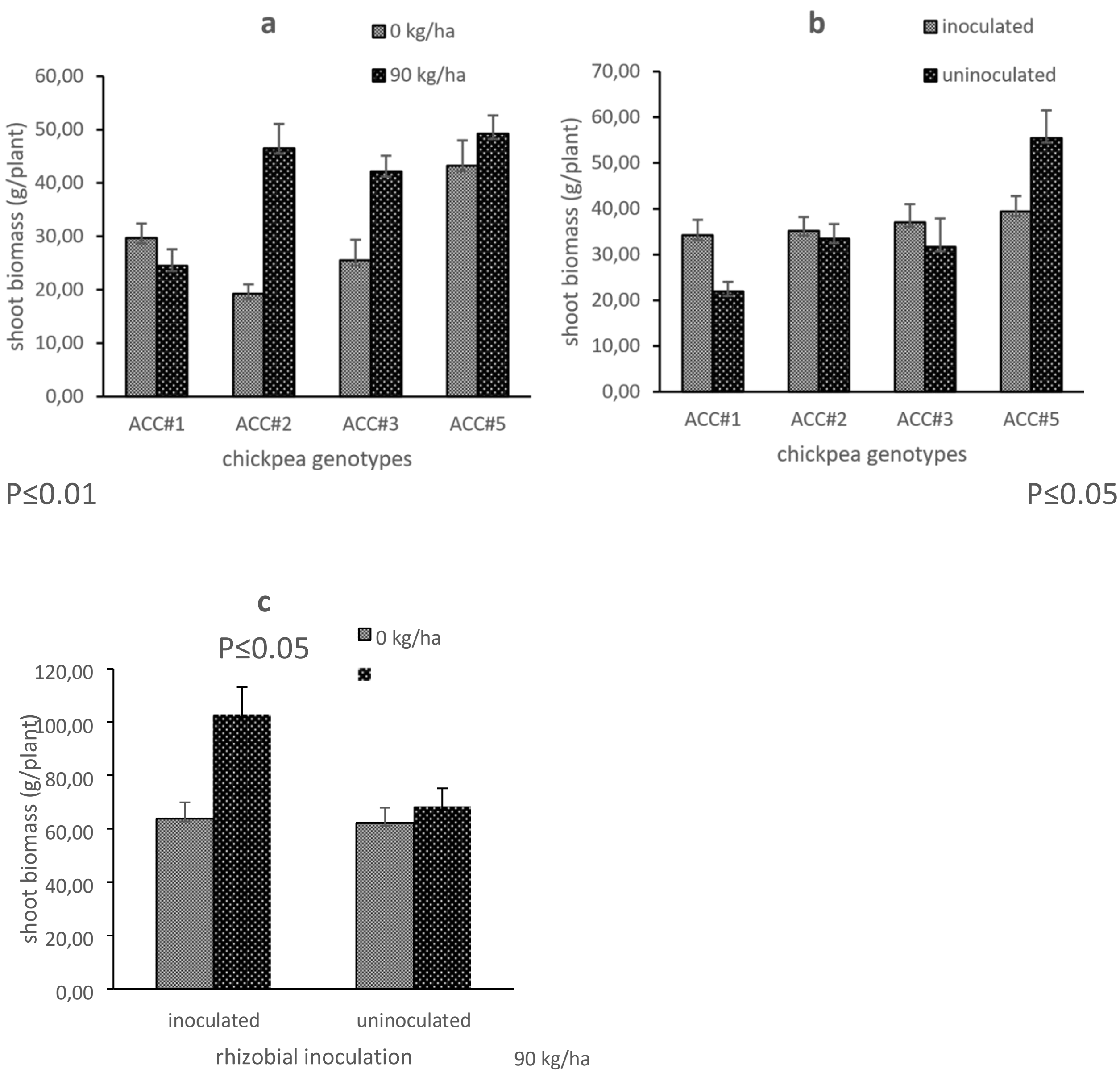

Figure 5.1: The interactive effect of phosphorus fertilizer application and chickpea genotype (a); rhizobial inoculation and chickpea genotypes (b) in 2016 at Thohoyandou; rhizobial inoculation and phosphorus fertilizer application (c) in 2017 at Syferkuil on shoot biomass (g plant^{-1}), respectively.

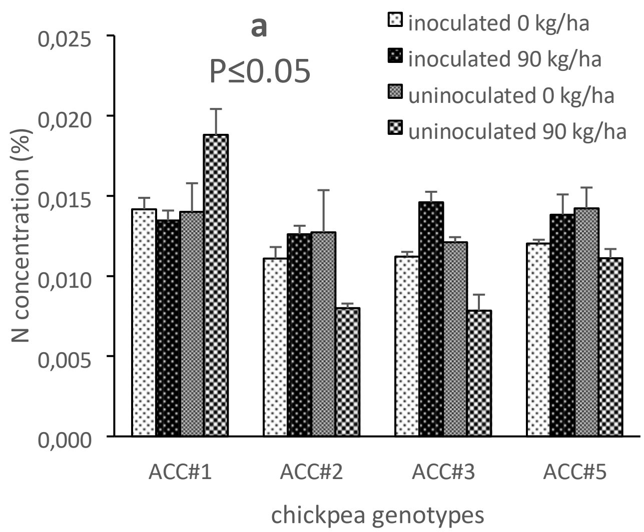

Figure 5.2: The interactive effect of phosphorus fertilizer application, rhizobial inoculation and chickpea genotype (a) on N concentration (%) at Syferkuil location in 2016.

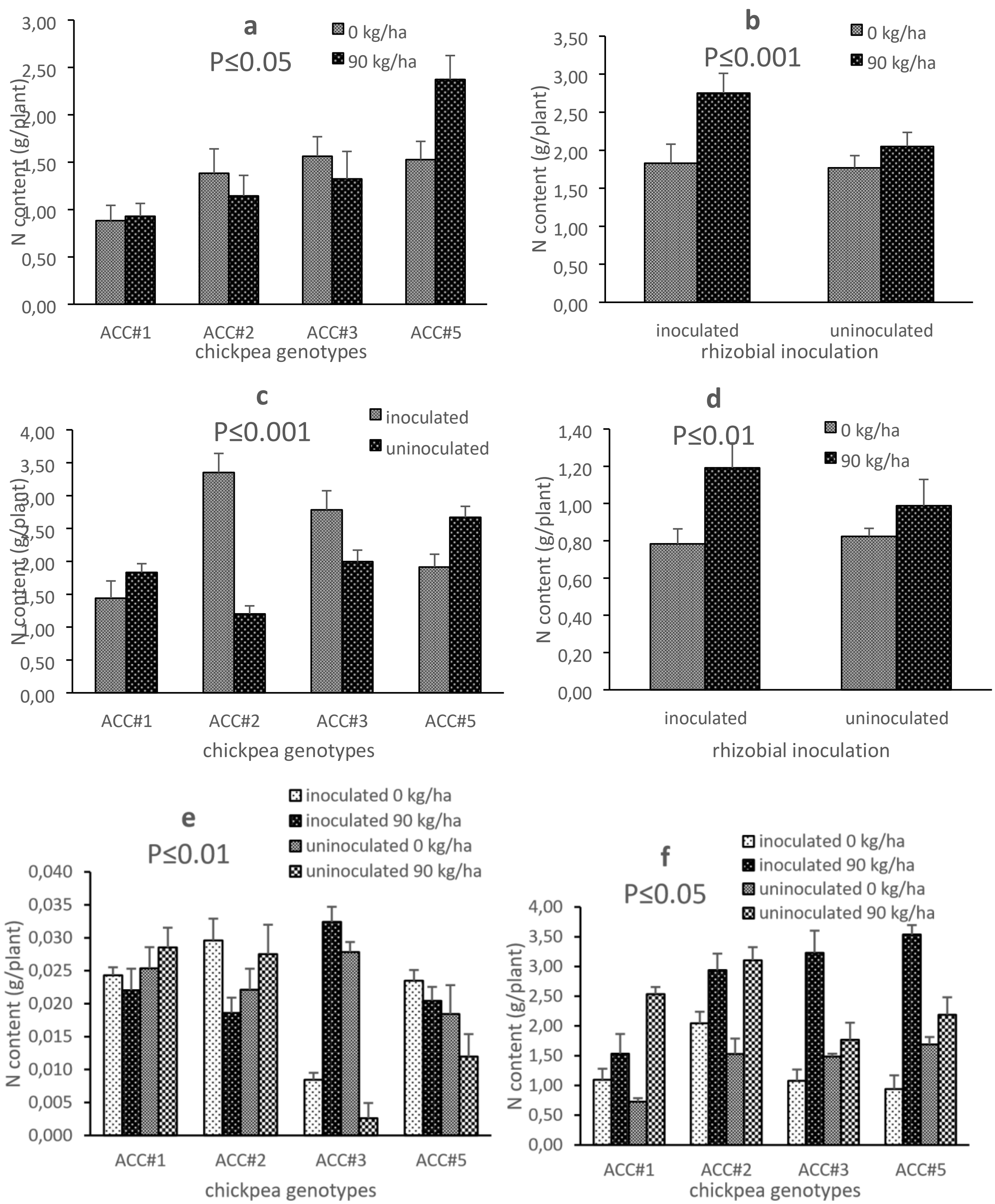

Figure 5.3: The interactive effect of phosphorus fertilizer application and chickpea genotype (a) in 2016; phosphorus fertilizer application and rhizobial inoculation (b); rhizobial inoculation and chickpea genotypes (c) in 2017 at Thohoyandou; phosphorus fertilizer and rhizobial inoculation (d); phosphorus fertilizer, rhizobial inoculation and chickpea genotypes (e) in 2016 and (f) in 2017 at Syferkuil; on N content (g plant^{-1}), respectively.

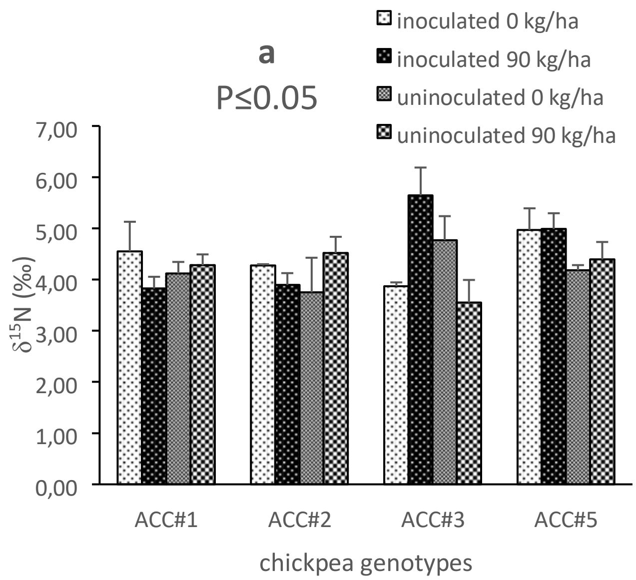

Figure 5.4: The interactive effect of phosphorus fertilizer application, rhizobial inoculation and chickpea genotype (a) on $\delta^{15}N$ at Syferkuil location in 2016.

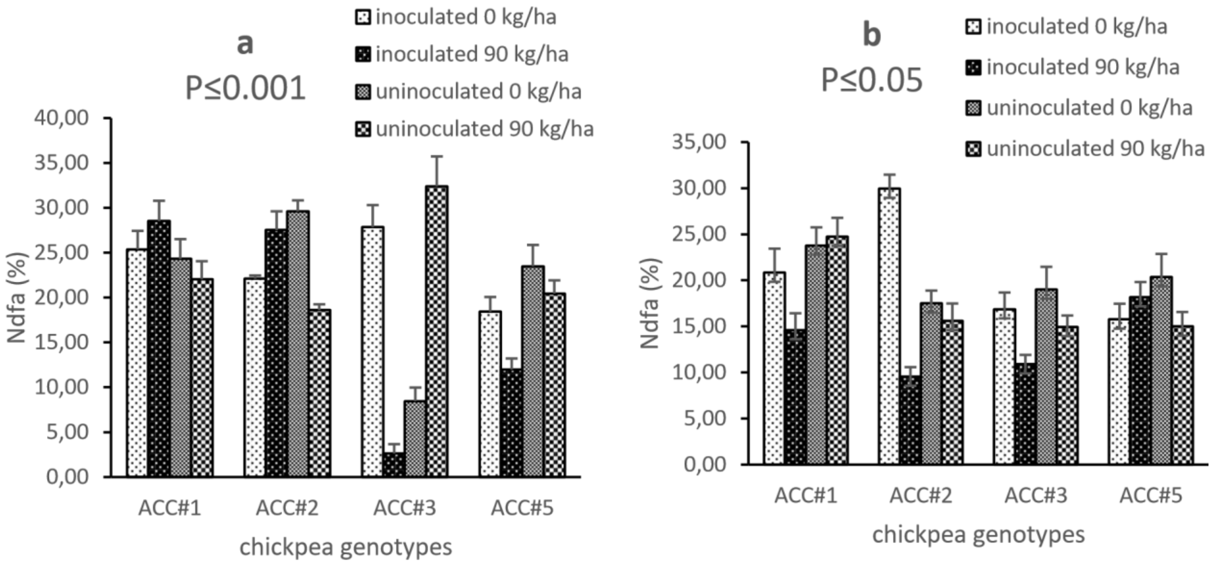

Figure 5.5: The interactive effect of phosphorus fertilizer application, rhizobial inoculation and chickpea genotype (a) and (b) on Ndfa (%) at Syferjuil in 2016 and 2017, respectively.

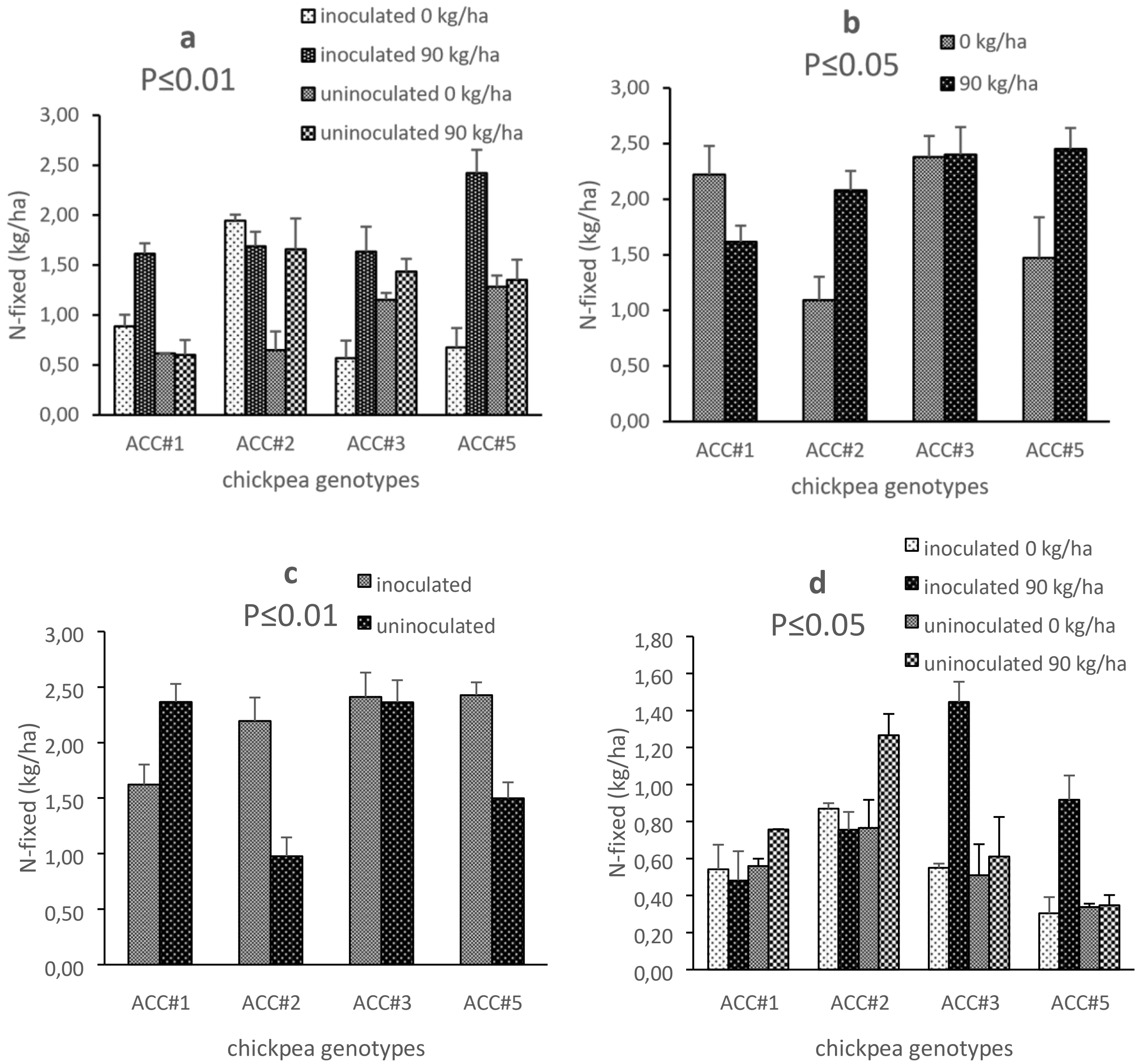

Figure 5.6: The interactive effect of phosphorus fertilizer application, rhizobial inoculation and chickpea genotype (a) in 2016; phosphorus fertilizer application and chickpea genotypes (b); rhizobial inoculation and chickpea genotypes (c) in 2017 at Thohoyandou; phosphorus fertilizer application, rhizobial inoculation and chickpea genotypes (d) in 2016 at Syferkuil location on Nfixed (kg ha^{-1}), respectively.

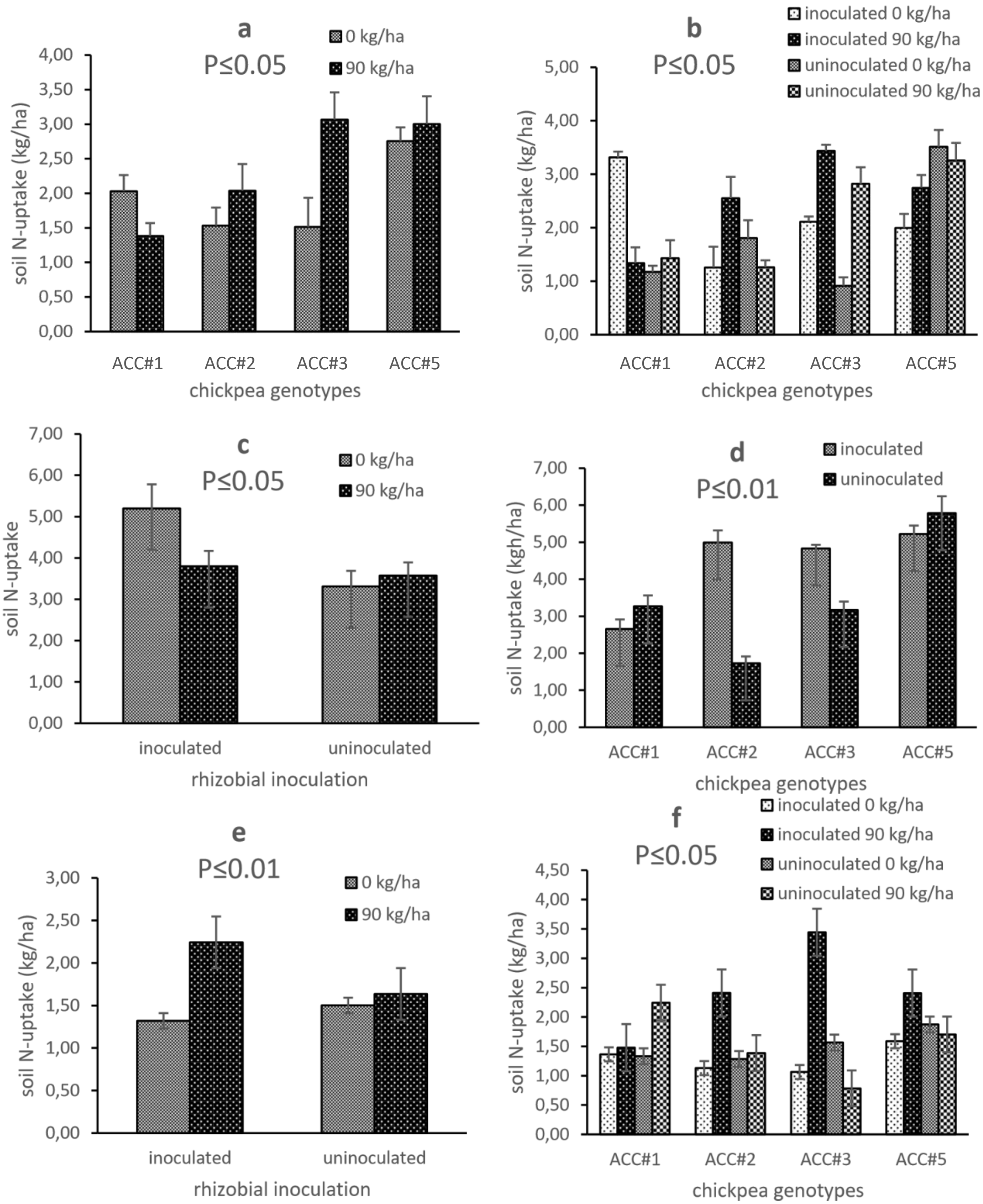

Figure 5.7: The interactive effect of phosphorus fertilizer application and chickpea genotype (a); phosphorus fertilizer, rhizobial inoculation and chickpea genotypes (b) in 2016; phosphorus fertilizer application and rhizobial inoculation (c); rhizobial inoculation and chickpea genotypes (d) in 2017 at Thohoyandou; phosphorus fertilizer application and rhizobial inoculation; phosphorus

fertilizer, rhizobial inoculation and chickpea genotypes (f) in 2016 at Syferkuil on soil N-uptake (kg ha^{-1}), respectively.

CHAPTER 6

EFFECT OF PHOSPHORUS AND RHIZOBIUM INOCULATION ON THE C ASSIMILATION AND WATER-USE EFFICIENCY OF CHICKPEA IN LIMPOPO PROVINCE OF SOUTH AFRICA.

ABSTRACT

Chickpea growing countries in Asia largely cultivate the pulse in regions that are characteristically cooler temperature. Chickpea production was greater at winter season as compared to summer season in Thohoyandou. According to literature, the shoot $\delta^{13}C$ is an indicator of WUE in C3 plants. However, shoot-WUE is affected by a variety of factors including genotypes, phosphorus fertilizer application and availability of native or introduced rhizobial bacteria. There is not much known on whether application of phosphate fertilizer, seed inoculation with rhizobial strain affect the shoot C/N ratio of chickpea genotypes in South Africa. Therefore, field experiments were established at Thohoyandou and Syferkuil to assess the role of phosphorus fertilization and rhizobial inoculation on C assimilation, C/N ratio and shoot-WUE of chickpea genotypes. The hypotheses tested was that phosphorus fertilizer application, rhizobial inoculation and chickpea genotypes increase shoot C accumulation and water-use efficiency. In this study, ACC#2 and ACC#3 had greater C concentration (ranging from 39.63% to 40.48%) respectively at Thohoyandou (2017); chickpea genotypes had significant effect on $\delta^{13}C$ at $P \leq 0.05$ at Thohoyandou, 2016. The results showed that ACC#1 with phosphorus fertilizer application and no *bradyrhizobium* strain showed greater $\delta^{13}C$. Interestingly. Also, $\delta^{13}C$ showed to increases with a decrease in N-fixed (r=.1000*), this indicates that there was a functional relationship between plant WUE and N fixation in C3 plants, and that is because improved water use in legumes supports N fixation. ACC#1 and ACC#5 have the potential to become significant drought tolerant genotypes in dry regions of Limpopo province in South Africa for poor-resourced farmers. Furthermore, it is important to screen chickpea genotypes with superior symbiosis and C accumulation and improved WUE for increased food security in dry areas such as South Africa.

Key words: Chickpea genotypes, Phosphorus fertilizer, Rhizobial inoculation, C accumulation, $\delta^{13}C$ and WUE

6.1 Introduction

Chickpea-growing countries in Asia largely cultivate the pulse in regions that are characteristically cooler and of late include traditionally warmer areas as well (Pokhrel, 2019). This legume food crop grows well in areas with temperatures ranging from 15 to 30°C but hardly above 32°C (Agriculture Victoria, 2018). Given this background, research on chickpea in South Africa has assessed its adaptation to cultivation in both the summer and winter cropping seasons (Madzivhandila *et al*., 2012; Lusiba *et al*., 2016). Of these studies, only one has shown increased water-use efficiency in chickpea grown during the summer relative to the winter season with 90 kg P ha^{-1} (Ogola *et al*., 2013). There is, however, not enough literature on possible effect of planting material, inputs and environmental factors on water-use efficiency of the crop in South African conditions. It is therefore necessary for farmers to have broader and better knowledge through other studies that address these questions in order.

The carbon isotope discrimination for shoot ($\delta^{13}C$) is an indicator of water-use efficiency (WUE) in C3 plants (Mohale *et al*., 2014; Mapope and Dakora, 2016). However, shoot-WUE is affected by a variety of factors including genotypes or cultivar, phosphorus fertilizer application (Hatfield *et al.*, 2011) and availability of native or introduced rhizobial bacteria (Ahmad *et al*., 2011). For example, application of phosphate can enhance shoot-WUE through increased photosynthetic activity, root size and production of organic acids (Vance *et al*., 2003). Environmental factors that affect shoot-WUE include temperature through its effect on the photosynthetic rate and therefore the carbon fixation by plants (Kirkham, 2005). Photosynthetic performance is inhibited in plants at locations with higher temperatures because of lower Rubisco activity (Singh *et al*., 2014). Another factor that vary between locations is relative humidity and where different, C3 plants show differences in photosynthetic C fixation, which has a direct influence to carbon accumulation and shoot-WUE (Farquhar *et al*., 1989; Araus *et al*., 2002). Lastly, often, different locations vary in soil fertility and this affect photosynthetic carbon fixation by plants (Reyes, 2015).

As a legume, where the residues of chickpea are incorporated into soils, their rate of N transformation into organic matter can be predicted through the shoot C/N ratio (Hobbie, 1992). A C3 plant with relatively higher quality residues is one, which the aboveground litter show a C/N ratio that is < 24 g g^{-1} (Abera *et al*., 2013). Where the C/N ratio is above 24 g/g, it indicates that the particular plant litter has a slow decomposition and N mineralization. There is little literature on the rate of chickpea's litter decomposition and N mineralization (Gan *et al*., 2011). The few literature, however, indicate that the crop has a relatively lower shoot C/N ratio which mean it has potential to increase organic carbon where incorporated into agricultural soils (Mpai and Maseko, 2018). There is not much known on whether application of phosphate fertilizer, seed inoculation with rhizobium and environmental factors affect the shoot C/N ratio of legumes.

In South Africa, chickpea is fast becoming one of main grain legume crops given its increased demand. In order to maintain supply, there is need for government and local academic institutions to promote local cultivation of the crop. Factors that need to be addressed in promoting its local production include the effect that phosphate application, rhizobium inoculation and different locations have on the crop's C assimilation, water-use efficiency and C/N ratio. In order to contribute knowledge on this, field experiments were established at Thohoyandou and Syferkuil to assess the role of phosphorus fertilization and rhizobial inoculation on the growth, C assimilation, C/N ratio and shoot-WUE of chickpea genotypes. The hypotheses tested was that phosphorus fertilizer, rhizobial inoculation and test genotypes affects C accumulation and WUE all study locations.

6.2 Materials and Methods

A detailed description of materials and methods has been presented in chapter 3, but a brief summery is outlined here. Field experiment was conducted at Thohoyandou (22° 58' S, 30° 26' E; 595 m above sea level), and Syferkuil (23° 50' S, 39° 40' E; 1230 m above sea level) Limpopo, South Africa. The experiment was conducted in winter in 2016 and in 2017. Treatments consisted of a factorial combination of two rates of phosphorus fertilizer (0 and 90 kg ha^{-1}), four chickpea genotypes (ACC#1, ACC#2, ACC#3 and ACC#5) and two rhizobial inoculation levels (with and without rhizobial strain).

Chickpea plants were sampled at pod-filling stage at (68 days after emergence, DAE) in each experimental site. Three plants were randomly dug up per plot, and oven-dried at 60°C for 48 hrs. The dry biomass was weighed and ground into fine powder (0.85 mm) for ^{13}C isotope analysis. All ground plant material was kept in zipper plastic sample bags prior to analysis (Mapope and Dakora, 2016).

C fixation by the chickpea genotypes was determined through the ^{13}C natural abundance technique. About 2.0 mg of ground chickpea plant material was weighed into Al tin capsules to determine %N and $^{15}N/^{14}N$ ratio values using a Carlo Erba NA1500 elemental analyzer (Fisons

Instruments SpA, Strada, Rivoltana, Italy) coupled to a Finan MAT252 mass spectrometer (Finnigan, MAT CombH, Bremen, Germeny) via Conflo II Open-Split Device (Junk and Svec, 1958; Mariotti, 1983; Mapope and Dakora, 2016). The isotopic ratios of C were referenced and reported in the standard notation relative to Pee Dee Belemnite (Fry, 2006), as showed in equation 6.1:

$$\delta^{13}C = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \ldots\ldots\ldots\ldots\ldots\ldots\ldots \textbf{6.1}$$

Where: R sample is the isotopic ratio ($^{13}C/^{12}C$) of the sample and R standard = 0.06747; which is the isotopic ratio of PBD, a universally accepted standard from Belemnite Pee Dee limestone (Craig, 1957).

Statsoft software 10th edition was used to analyze data. Analysis of variance (ANOVA) was used to evaluate the effect of P fertilizer rates and rhizobial inoculation (*bradyrhizobium*) on chickpea genotypes. Fisher's least significant difference (LSD) test was used to separate means that were significantly different (P<0.05). Correlation analysis was conducted to assess the relationship between the various parameters.

6.3 Results

6.3.1 C accumulation, C/N ratio and water-use efficiency of chickpea genotypes grown at Thohoyandou

Results on shoot C concentration of the selected chickpea genotypes that were grown at Thohoyandou study location in 2016 showed no significant differences (Table 6.1). In 2017, ACC#3 had greater shoot C concentration (40.48%) compared to ACC#1 and ACC#5 (Table 6.1). At the Thohoyandou experimental location, C content was markedly different between the test genotypes (Table 6.1). For example, ACC#2, ACC#3 and ACC#5 exhibited greater C content compared to ACC#1. When grown during the 2017 cropping season, ACC#5 had higher C content and ACC#1 and ACC#3 the lowest. The shoot C/N ratio of chickpea established at Thohoyandou in 2016 was statistically similar between the four genotypes. However, in 2017, ACC#1 exhibited the highest compared to ACC#2 and ACC#3. Interestingly, the shoot $\delta^{13}C$ was not significantly different between the selected accessions at Thohoyandou, in both cropping seasons (Table 6.1).

The application of phosphorus fertilizer to the selected accessions of chickpea grown at Thohoyandou had no significant effect on shoot C concentration and C/N ratio (Table 6.1). By contrast, phosphorus fertilization increased the C content of chickpea planted at Thohoyandou in both growing seasons. Chickpea that was planted without application of phosphorus at Thohoyandou had 8.82 g plant^{-1} C content compared to 18.50 g plant^{-1} in their phosphorusfertilized counterparts (Table 6.1). This represented a more than 2-fold increase in C content with the application of phosphorus. Control plants showed significantly less negative shoot $\delta^{13}C$ values (-29.74‰) compared to the most negative (-30.03‰) exhibited by chickpea supplied with phosphorus at Thohoyandou in 2016. This indicates that the control plants were more water-use efficient compared to that supplied with phosphorus at Thohoyandou. In 2017, the study revealed no significant difference in shoot $\delta^{13}C$ between plants raised with phosphorus application and controls.

Rhizobial inoculation had no significant effect on C accumulation, C/N ratio and the water-use efficiency of the selected chickpea accessions that were planted at Thohoyandou in 2016 (Table 6.1). C concentration values ranged from 45.69% to 45.98% whilst C content ranged from 18.36 g plant^{-1} to 20.39 g plant^{-1} for the control and rhizobium-inoculated plants, respectively. Mean values for the shoot C/N ratio and $\delta^{13}C$ ranged from 13.59 g g^{-1} to 12.60 g g^{-1} and from -29.93‰ to -29.34‰, respectively (Table 6.1). For plants established in 2017, the C content was increased in rhizobial-inoculated chickpea while the other parameters were similar (Table 6.1).

In 2016, the interaction between genotypes, phosphorus fertilizer and rhizobial inoculation affected C concentration (Figure 6.1a), wherein ACC#1 had greater C concentration at 90 kg ha^{1} in the absence of *bradyrhizobium* strain. In 2017, the interactive effect between genotypes with phosphorus fertilizer: and phosphorus fertilizer with rhizobial inoculation was presented in Figure 6.1b and Figure 6.1c. Furthermore, the interaction between genotypes and rhizobial inoculation significantly affected C content (Figure 6.2a). ACC#5 had the highest C content (22.5 g plant^{-1}) in the absence of *bradyrhizobium* strain, while other test genotypes had high C content when inoculated with rhizobium. Similarly, genotypes X phosphorus fertilizer interaction affected C content (Figure 6.2b), greater C content was obtained at 90 kg ha^{-1} in ACC#2 and ACC#5. Similarly, to 2016, the interaction between genotypes and phosphorus fertilizer increased C content at 90 kg ha^{-1} in 2017 at ACC#5.

Moreover, the interaction between genotypes, phosphorus fertilizer and rhizobial inoculation affected $\delta^{13}C$ (Figure 6.3a) in 2016. ACC#2 exhibited less negative values (29.40‰) when interacting with rhizobial inoculation in absence of phosphorus fertilizer, and ACC#1 showed more negative values of $\delta^{13}C$ (-30.58‰), which indicate that ACC#2 was more water-use efficient as compared to ACC#1 (Figure 6.3a)

Though 2016 results showed no significance in C/N ratio, C/N ratio was significantly affected by the interaction between genotypes and phosphorus fertilizer in 2017 (Figure 6.4a); genotypes and rhizobial inoculation (Figure 6.4b); and three was interaction of genotypes, phosphorus fertilizer and rhizobial inoculation (Figure 6.4c). The application of phosphorus fertilizer reduced C/N ratio in test genotypes except ACC#5 which performed best at 90 kg ha^{-1}, ACC#1 however had the greatest C/N ratio at control (0 kg ha^{-1}) (Figure 6.4a). Furthermore, ACC#5 obtained high C/N ratio when inoculated with *bradyrhizobium* strain, whereas ACC#2 performed better in the absence of rhizobium. Lastly, the interaction between genotypes, phosphorus fertilizer and rhizobial inoculation increased C/N ratio (28.56 g g^{-1}) at ACC#5, and it had a least C/N ratio of 16.10 at 90 kg ha-1 and with the presence of rhizobial strain (Figure 6.4c).

ACC# 1 however showed greater C content of 13.51 g plant^{-1}. Furthermore, C/N ratio showed significant variation in genotypes (ranging from 33.48 g g^{-1} to 45.67 g g^{-1}). Highest C/N ration was obtained at ACC#2 and ACC#3 while ACC#1 had the least C/N ratio of 33.48 g g^{-1}.

C concentration showed no significant variation again in 2017, however greater C concentration was obtained in ACC#3 (40.88%) followed by ACC#1 (39.01%) (Table 6.1). C content varied significantly in C content by P≤0.05 (Table 6.1) in 2017. ACC#2 had the greatest C content (32.96 g plant^{-1}) followed by ACC#5 (26.95 g plant^{-1}). Phosphorus fertilizer showed significant difference (P≤0.001) in C content in 2017. 90 kg ha^{-1} increased C content by 63% difference as compared to control (Table 6.2). Table 6.2 also shows the significant effect on phosphorus fertilizer on C/N ratio. The results show that C/N ratio was greater at control by 19.50 g g^{-1}, while 90 kg ha^{-1} had the least value of C/N ratio of 17.37 g g^{-1}.

6.3.2 Effect of P application and rhizobium inoculation on the C assimilation, C/N ratio and water-use efficiency of chickpea grown at Syferkuil farm

There was no significant variation in C accumulation, C/N ratio and $\delta^{13}C$ between the test genotypes in both 2016 and 2017 (Table 6.2). The C/N ratio was greater in ACC#2 compared to ACC#1 and ACC#5 in 2016 while the genotypes revealed no differences in C/N ratio in 2017. The application of phosphorus had no effect on the C concentration, C/N ratio and shoot $\delta^{13}C$ in both cropping seasons at the Syferkuil location (Table 6.2). In contrast, the C content was highest in phosphorus-fertilized plants grown in 2017 but had similar C content in 2016. Interestingly, rhizobial inoculation showed no significant effect on shoot C concentration and content, C/N ratio and the shoot $\delta^{13}C$ of desi chickpea established at Syferkuil in 2016 and 2017.

6.3.3 Comparison of C concentration and content, C/N ratio and shoot $\delta^{13}C$ across the locations

When grown at Thohoyandou in the 2016 growing season, chickpea accumulated significantly higher shoot C concentration relative to that at Syferkuil (Table 6.3). In 2017, plants established at Thohoyandou had the highest C content and shoot $\delta^{13}C$ while the C/N ratio and shoot $\delta^{13}C$ were enhanced in chickpea at Syferkuil in 2016.

6.4 Discussion

The genotypes did not show significant differences in C concentration in the shoots between the growing locations and the cropping seasons and it ranged between 38.42% at Syferkuil in 2016 to 45.32% at Thohoyandou in 2016. In fact, the only marked difference in %C between the selected genotypes was revealed at the Thohoyandou site in 2017 where ACC#3 accumulated the most %C in shoot. Similarly, previous studies show that in most cases, different genotypes of legumes exhibit no significant differences in %C values (Pule-Meulenberg *et al*., 2011; Yahaya *et al*., 2019). Whether this is related to their genetic make-up, is yet to be determined. In this study, the only marked difference was shown where ACC#3 exhibited superior %C. Although the only significant difference in shoot %C in the study, it could have been as a result of genotypic differences because plants do vary in the control of their C accumulation (Maseko and Dakora, 2015). The high accumulation of C by ACC#3 in this study is, however, contrary to the performance of the genotype as it ranked among those with the least growth and yield-related parameters (see Tables 6.1 - 6.2). Indeed a direct relationship between shoot %C and shoot dry weight has been shown in other legumes (Makoi *et al*., 2010). Similarly, direct relationship between growth and shoot %C has been shown in soybean genotypes (Mapope and Dakora, 2016). Therefore, the performance by ACC#3 was not unexpected.

In this study, the values of shoot %C varied between 38.42% and 45.32% for the genotypes, 38.38 and 45.95% for the phosphorus treatment, and between 38.95 and 45.98% for the rhizobium inoculation treatment (Tables 6.1 to 6.2). Overall, the shoot %C in this study ranged from 38.38 to 45.98%. According to literature, C concentration determined in legume has exhibited values of up to 30% (Sprent *et al*., 1996). Interestingly, there has been literature on legumes that has revealed shoot %C values that were greater than 30% (Post *et al*., 2007; Makoi *et al*., 2010; Pule-Meulenberg *et al*., 2011; Mohale *et al*., 2014; Maseko and Dakora 2015; Mapope and Dakora, 2016; Yahaya *et al*., 2019). In these instances, Post *et al*. (2007) suggested that where %C values of legumes are above 35%, such high values could be indicative of high lipid distribution within the particular plant organ. Although the lipid distribution in shoot of chickpea was not determined, the mean values of %C were similar to other grain legumes.

The C content shown in this study was largely greater in ACC#2, except at Thohoyandou in 2017 where ACC#5 revealed the highest. That ACC#2 had the highest C content is not surprising because the genotype recorded the highest shoot dry weight and given that C content is a product of shoot dry weight and %C, it had to rank higher. The application of phosphorus increased the C content of plants established at Thohoyandou during both cropping seasons and only for chickpea grown in 2016 at Syferkuil. Similar to the argument made for C content between genotypes, that C content is a product of %C and shoot dry weight, it was expected that the supply of phosphorus would reveal markedly higher C content in this locations because it also resulted in shoot dry weight (see Tables 4.1, 4.2 and 4.3). In addition, the supply of phosphorus could have increased photosynthetic performance of the plants and therefore their capacity to assimilate CO_2, which could have increased carbon fixation and therefore shoot dry matter and C content.

Furthermore, the application of phosphorus and its improved uptake by plants could have also increased the availability and uptake of especially Ca, Mg and S (Fageria, 2009), all which enhance photosynthesis, plant growth and therefore C concentration and content. Except for the shoot $\delta^{13}C$ of plants established at Thohoyandou in 2016, largely, the application of phosphorus had no significant effect on the WUE of chickpea. The markedly increased WUE in chickpea grown at Thohoyandou in 2016 with phosphorus application could have been caused by the enhanced efficiency of carbon fixation, which is increased by the supply of phosphorus and increases the efficiency of light harvesting or carboxylation processes (Zhang *et al*., 2013; Wang *et al*., 2018).

Rhizobial inoculation showed no significant effect on the parameters at Syferkuil in both cropping seasons. Although this was the case at Thohoyandou, it was only in the 2016 season. Rhizobial inoculation increased C content in chickpea grown in 2017 at Thohoyandou as compared to 2016. In most cases where legumes are inoculated with rhizobium inoculant, there is report of increased of the population of bacteria (Zilli *et al*., 2019). However, the effectiveness of the bacteria that is introduced through inoculation depends on the competitiveness of the native bacteria (Chibeba *et al*., 2017). Where effective and competitive, introduced bacteria have had positive effects on the shoot dry weight, C concentration and content and C/N ratio (Pule-Meulenberg *et al*., 2011).

However, even with effective rhizobial strains, like this study, other studies have shown no effect on the shoot $\delta^{13}C$ or WUE of legumes (Pule-Meulenberg *et al*., 2011). Whether the lack of significant differences in the parameters was because of inefficiency of the introduced rhizobial inoculant or effectiveness of the indigenous rhizobia as shown by other studies (Chibeba *et al*., 2017), remains unknown. Whatever the case, the was markedly improved C content in chickpea grown in 2017 at Thohoyandou. Perhaps this was because of a build-up of the introduced rhizobial bacteria since these have been supplied in 2016 as well. In that case, the commercial bacteria could have improved the uptake and solubilization of especially N, P, Fe and K (Biswas *et al*., 1999) and hence the increase C content as these nutrients are involved in photosynthesis and plant growth.

The results indicated that C concentration in chickpea legume was enhanced by phosphorus fertilizer at 90 kg ha^{-1} interacting with *bradyrhizobium* on selected chickpea genotypes in 2016 as shown in Figure 6.1a, and between genotypes and phosphorus fertilizer; and phosphorus fertilizer with rhizobial inoculation as presented in Figure 6.1b and Figure 6.1c. Literature report the effect on phosphorus fertilizer rates on C accumulation and of rhizobium bacteria solely, but there is dearth literature on the interactive effect of phosphorus fertilizer and rhizobial inoculation on chickpea genotypes.

Furthermore, the interaction between chickpea genotypes and phosphorus fertilizer increased C content in 2016 and 2017. ACC#2 and ACC#5 had greater C content at 90 kg ha^{-1}; also, ACC#2 and ACC#5 had higher C content with rhizobial inoculation compared to uninoculated control. Which again indicates that C content is directly proportional to shoot biomass (r=.9112***). Taiz and Zeiger (2002) reported on Bambara groundnut plants, that greater C content was higher in accumulated dry matter, and Mohale *et al*. (2014) stated that photosynthetic C accumulation accounts for a substantial amount of plant total biomass. Thus, the findings are comparable to those of Mohale *et al*., (2014) that Bambara groundnut plants that exhibited greater C content, always accumulated higher DM. Taiz and Zeiger (2002); Mohale *et al*. (2014) also stated that photosynthetic C accumulation accounts for a substantial amount of plant total biomass.

The interactive effect of phosphorus fertilizer rates and rhizobial inoculation on chickpea genotypes had significant effect on $\delta^{13}C$ at $P \leq 0.05$ (Figure 6.3a). Results showed ACC#1 with phosphorus fertilizer application in the absence of *bradyrhizobium* had greater $\delta^{13}C$. The results (Table 6.1) showed that $\delta^{13}C$ increases with a decrease in N fixed (r=1.000). This indicates that there is a functional relationship between plant WUE and N fixation in C3 plants, and that is because improved water use in nodulated legumes supports nodule functioning. Mohale *et al*. (2014) reported that there is a functional relationship between symbiotic N nutrition and plant WUE, between N fixation and photosynthesis, and between WUE and plant growth in Bambara groundnut.

6.5 Conclusion

The ^{15}N and ^{13}C natural abundance were used to assess symbiotic functioning and plant water use. Taken together, these data show a close relationship between $\delta^{15}C$ values, symbiotic N nutrition, and WUE efficiency on chickpea legumes with an influence of phosphorus fertilizer rates and rhizobial inoculation IN ACC#3 and 5 from different experimental sites. With proper management of soil fertility such as P fertilizer and *bradyrhizobuim*, this legume has the potential to become a significant food security crop and a bio-fertilizer in cropping systems of resourcepoor farmers in Africa. Furthermore, it is important to screen chickpea genotypes with superior symbiosis and carbon accumulation and improved WUE for increased food security in dry areas such as South Africa.

Table 6.1 Effect of P application and rhizobial inoculation on the C assimilation, C/N ratio and $\delta^{15}C$ of chickpea grown at Thohoyandou in 2016/2017.

Treatment	C concentration (%)	C content (g/plant)	C/N ratio (g g^{-1})	$\delta^{13}C$ (‰)
		2016		
Accessions #1	45.32±0.52	10.53±1.01c	13.10±0.62	-29.94±0.09
#2	44.48±0.36	20.49±1.50a	13.19±0.41	-29.93±0.13
#3	45.08±0.47	17.39±1.22b	12.85±0.44	-29.76±0.11
#5	44.43±0.48	19.19±1.75ab	13.12±0.52	-29.92±0.16
P-fertilizer (kg ha^{-1})				
0	45.95±0.32	8.82±0.50b	13.41±0.41	-29.74±0.08a
90	45.71±0.34	18.50±0.97a	12.74±0.28	-30.03±0.07b
Rhizobium				
Inoculated	45.69±0.31	18.36±1.04	13.59±0.30	-29.93±0.08
Uninoculated	45.98±0.35	20.39±1.61	12.60±0.37	-29.34±0.11
F-Statistics				
Accession (A)	0.45 NS	15.00 ***	1.87 NS	0.87 NS
Phosphorus (P)	2.01 NS	22 97 ***	0.87 NS	6.12 **
Rhizobium (R)	1.00 NS	2.68 NS	2.01 NS	1.85 NS
A*P	1.76 NS	8.09 **	1.74 NS	1.65 NS
A*R	0.87 NS	18.04 ***	1.77 NS	0.89 NS
P*R	1.45 NS	0.98 NS	0.98 NS	1.08 NS
A*P*R	4.98 *	1.87 NS	2.84 NS	3.89 *
		2017		
Accessions #1	38.98±0.32b	90±4.30bc	22.28±1.29a	-28.51±0.18
#2	39.63±0.38ab	102±9.38b	16.58±1.25bc	-28.52±0.29
#3	40.48±0.28a	79±6.33c	15.32±0.40c	-29.01±0.23
#5	38.90±0.30b	150±10.82a	19.29±1.86ab	-28.52±0.21
P-fertilizer (kg ha-1)				
0	39.38±0.35	78±6.84b	18.73±0.91	-28.62±0.17
90	39.62±0.25	105±7.77a	17.24±0.88	-28.64±0.16
Rhizobium				
Inoculated	39.64±0.27	120±8.01a	17.22±0.83	-28.40±0.16
Uninoculated	39.36±0.33	79±4.88b	16.22±0.63	-28.86±0.15
F-Statistics				
Accession (A)	4.80 **	14.61 ***	9.69 ***	0.94 NS
Phosphorus (P)	0.49 NS	6.74 **	1.38 NS	0.00 NS
Rhizobium (R)	0.70 NS	17.27 ***	2.16 NS	3.82 NS
A*P	6.48 ***	3.49 *	3.43 *	0.58 NS
A*R	0.98 NS	2.31 NS	3.92 *	2.01 NS
P*R	3.89 *	2.90 NS	1.89 NS	0.01 NS
A*P*R	1.29 NS	0.69 NS	3.27 *	0.15 NS

NS= not significant. *, **, ***= significant at P<0.05, P<0

Table 6.2 Effect of P application and rhizobium inoculation on the C assimilation, C/N ratio and $\delta^{15}C$ of chickpea genotypes at Syferkuil in 2016/2017.

Treatment	C Concentration (%)	C content (g/plant)	C/N ratio (g g^{-1})	$\delta^{13}C$ (‰)
		2016		
Accessions #1	39.62±0.59	13.51±1.03	33.48±1.56b	-27.73±0.16
#2	39.54±0.47	10.95±0.31	45.67±4.27a	-27.89±0.21
#3	38.92±0.61	11.98±0.83	39.73±2.93ab	-27.66±0.16
#5	38.42±0.61	13.30±0.84	36.90±2.57b	-27.91±0.12
P-fertilizer (kg ha^{-1})				
0	38.95±0.40	14.30±0.91	37.18±1.29	-27.87±0.10
90	39.41±0.41	15.07±1.26	42.81±3.58	-27.69±0.12
Rhizobium				
Inoculated	38.95±0.40	14.69±1.12	40.10±2.42	-27.72±0.10
Uninoculated	39.45±0.45	13.86±0.92	39.08±2.33	-27.82±0.11
F-Statistics				
Accession (A)	0.25 NS	0.25 NS	5.12 *	0.98 NS
Phosphorus (P)	1.42 NS	1.0 NS	1.02 NS	2.09 NS
Rhizobium (R)	0.98 NS	0.56 NS	1.84 NS	1.50 NS
A*P	1.47 NS	1.89 NS	0.75 NS	1.78 NS
A*R	0.89 NS	1.10 NS	1.58 NS	0.14 NS
P*R	1.56 NS	2.04 NS	1.09 NS	1.03 NS
A*P*R	1.01 NS	0.09 NS	0.98 NS	2.20 NS
		2017		
Accessions #1	39.03±0.42	25.69±3.88b	18.57±1.33	-28.32±0.19
#2	38.90±0.34	32.96±3.65a	18.01±0.87	-28.23±0.13
#3	40.88±1.26	24.87±2.77b	18.91±0.88	-28.38±0.19
#5	38.43±0.55	26.95±2.58b	18.35±0.70	-28.34±0.15
P-fertilizer (kg ha-1)				
0	38.81±0.30	21.58±1.41b	19.50±0.75a	-28.30±0.12
90	39.80±0.70	34.25±2.23a	17.37±0.45b	-28.34±0.11
Rhizobium				
Inoculated	39.32±0.73	28.43±2.44	18.70±0.53	-28.30±0.12
Uninoculated	39.30±0.26	26.84±2.22	18.21±0.78	-28.34±0.11
F-Statistics				
Accession (A)	2.13 NS	3.62 *	0.15 NS	0.12 NS
Phosphorus (P)	1.79 NS	24.12 ***	5.07 *	0.05 NS
Rhizobium (R)	0.00 NS	3.54 NS	0.36 NS	0.04 NS
A*P	0.95 NS	0.27 NS	0.37 NS	0.31 NS

A*R	1.86 NS	0.22 NS	0.67 NS	0.89 NS
P*R	0.45 NS	0.42 NS	2.05 NS	0.03 NS
A*P*R	0.47 NS	1.96 NS	0.25 NS	0.28 NS

NS= not significant. *, **, ***= significant at P<0.05, P<0.01, P<0.01 respectively

Table 6.3 Comparison of C concentration and content, photosynthetic N use efficiency, and $\delta^{13}C$ on chickpea genotypes affected by of phosphorus application and rhizobial inoculation at Thohoyandou and Syferkuil, South Africa in 2016/2017.

Treatment	C concentration (%)	C content (g/plant)	C/N ratio (g g^{-1})	$\delta^{13}C$ (‰)
		2016		
Location				
Thohoyandou	0.45±0.00a	16.53±1.14	13.07±0.24b	-29.89±0.06b
Syferkuil	0.39±0.00b	13.37±0.67	40.27±2.06a	-27.80±0.08a
F-Statistics				
Location (L)	247.18 ***	2.44 NS	199.26 ***	470.04 ***
L*A	1.39 NS	0.29 NS	1.05 NS	0.33 NS
L*P	0.02 NS	0.62 NS	3.05 NS	4.92 *
L*R	1.21 NS	0.23 NS	1.84 NS	3.15 NS
L*A*P	1.90 NS	0.28 NS	0.51 NS	0.32 NS
L*A*R	0.60 NS	0.69 NS	0.14 NS	0.80 NS
L*P*R	0.07 NS	0.07 NS	5.20 *	1.17 NS
L*A*P*R	1.15 NS	0.22 NS	0.37 NS	5.13 **
		2017		
Location				
Thohoyandou	0.40±0.00	94.24±5.75a	18.28±0.77	-28.63±0.12b
Syferkuil	0.39±0.00	27.92±1.64b	18.46±0.46	-28.32±0.08a
F-Statistics				
Location (L)	0.22 NS	97.29 ***	0.10 NS	4.5 *
L*A	0.39 NS	0.22 NS	5.95 **	0.4 NS
L*P	0.86 NS	0.01 NS	0.12 NS	0.0 NS
L*R	0.10 NS	1.38 NS	0.00 NS	2.0 NS
L*A*P	3.00 *	0.35 NS	1.02 NS	0.8 NS
L*A*R	2.64 NS	0.41 NS	2.02 NS	1.8 NS
L*P*R	2.03 NS	0.80 NS	0.01 NS	0.0 NS

NS significant. *, **, ***= significant at P<0.05, P<0.01, P<0.01 respectively

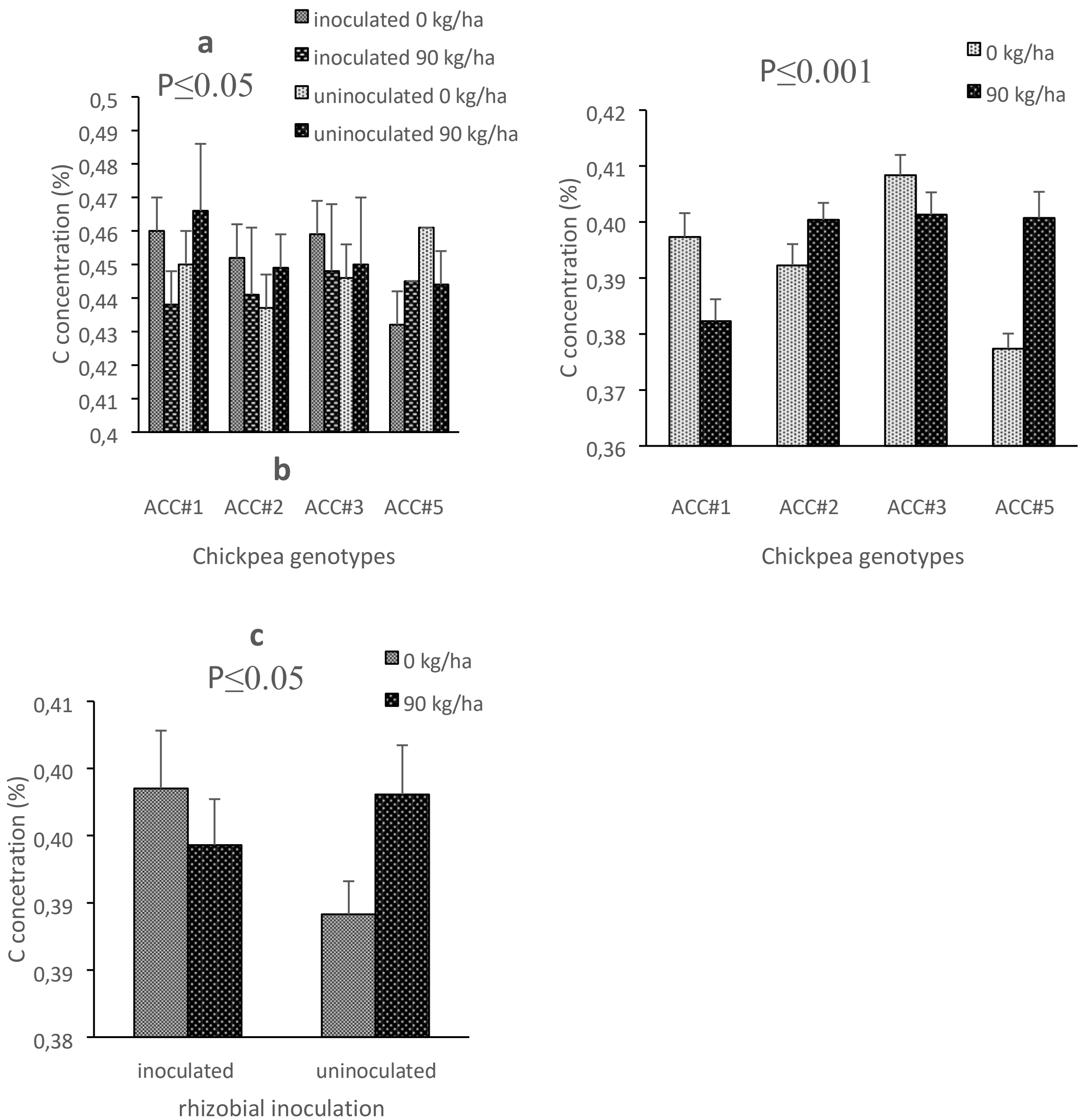

Figure 6.1: The interactive effect of phosphorus fertilizer application, rhizobial inoculation and chickpea genotype (a) in 2016; rhizobial inoculation and chickpea genotypes (b); phosphorus fertilizer application and chickpea genotypes (c) on C concentration (%) at Thohoyandou location in 2016

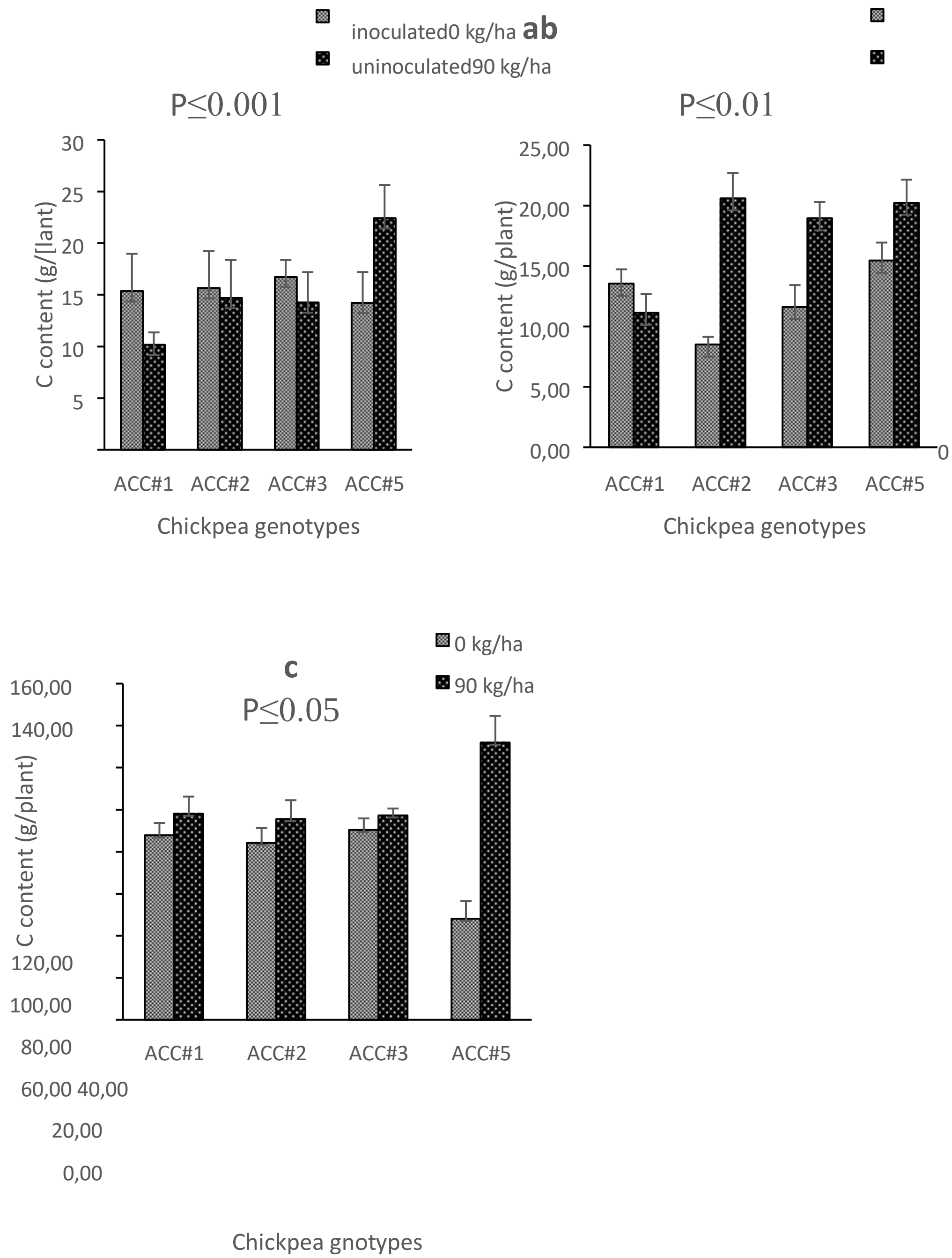

Figure 6.2: The interactive effect of rhizobial inoculation and chickpea genotypes (a), phosphorus fertilizer application and chickpea genotypes in 2016; and phosphorus fertilizer application and chickpea genotypes on C content (g/plant) at Thohoyandou in 2017.

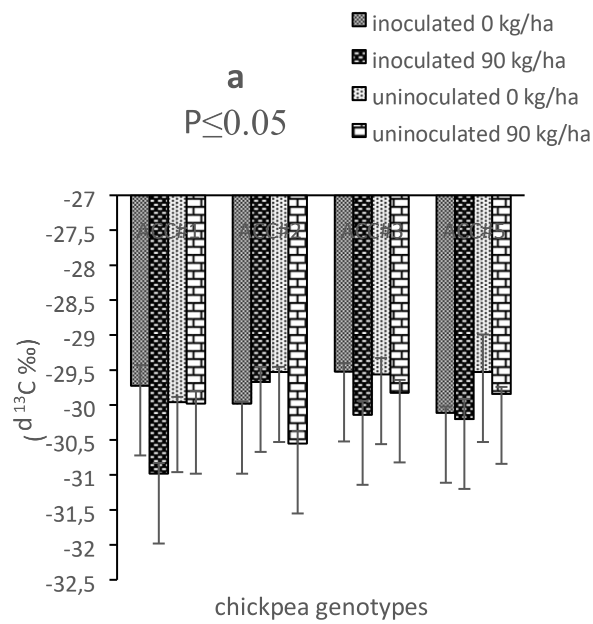

Figure 6.3: The interactive effect of phosphorus fertilizer application, rhizobial inoculation and chickpea genotypes on δ^{13}C (‰) at Thohoyandou location in 2016.

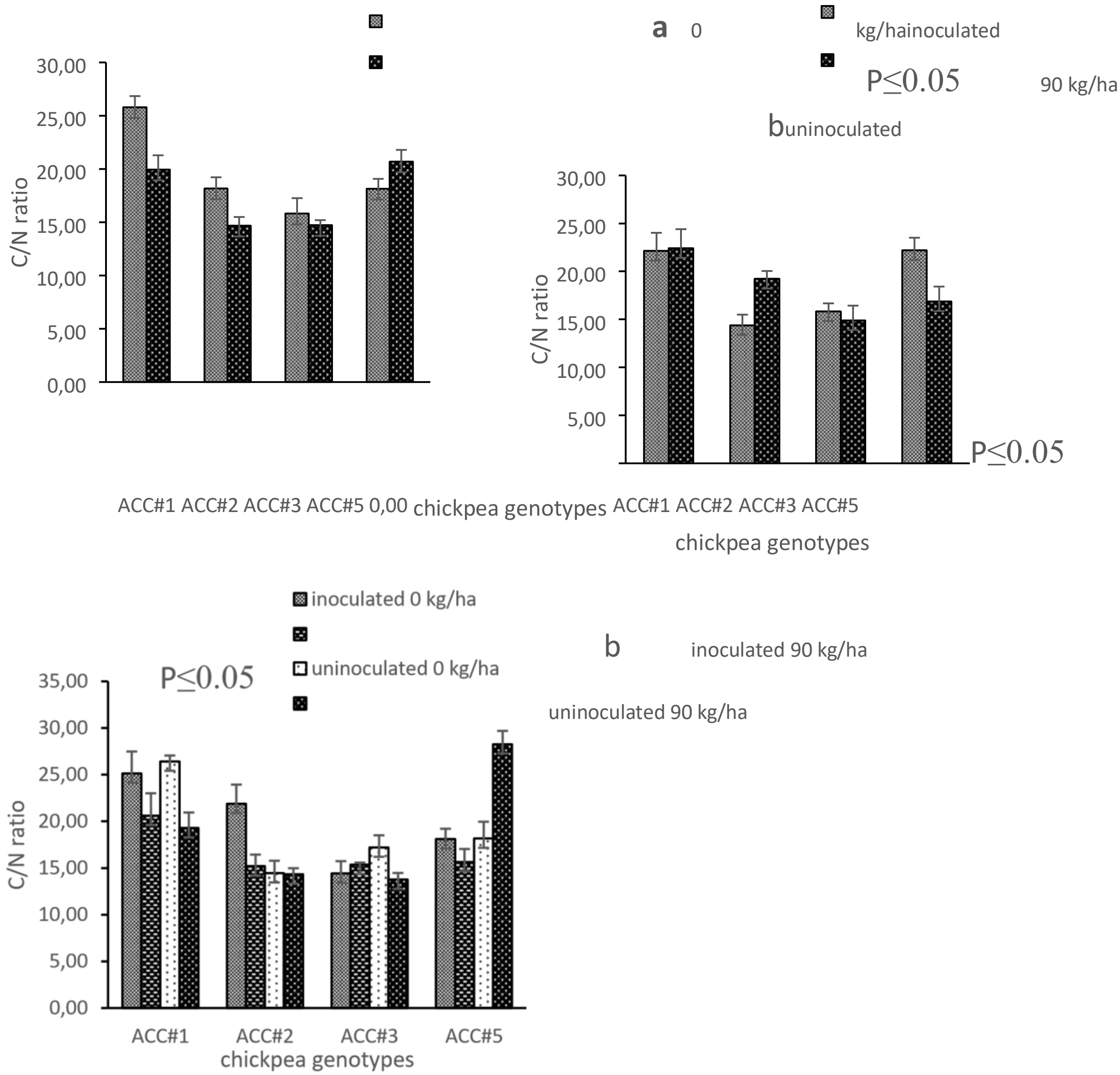

Figure 6.4: The interactive effect of phosphorus fertilizer application and chickpea genotype (a) and rhizobial inoculation and chickpea genotypes (b); phosphorus fertilizer application rhizobial inoculation and chickpea genotypes (c) on C/N ratio at Thohoyandou location in 2017.

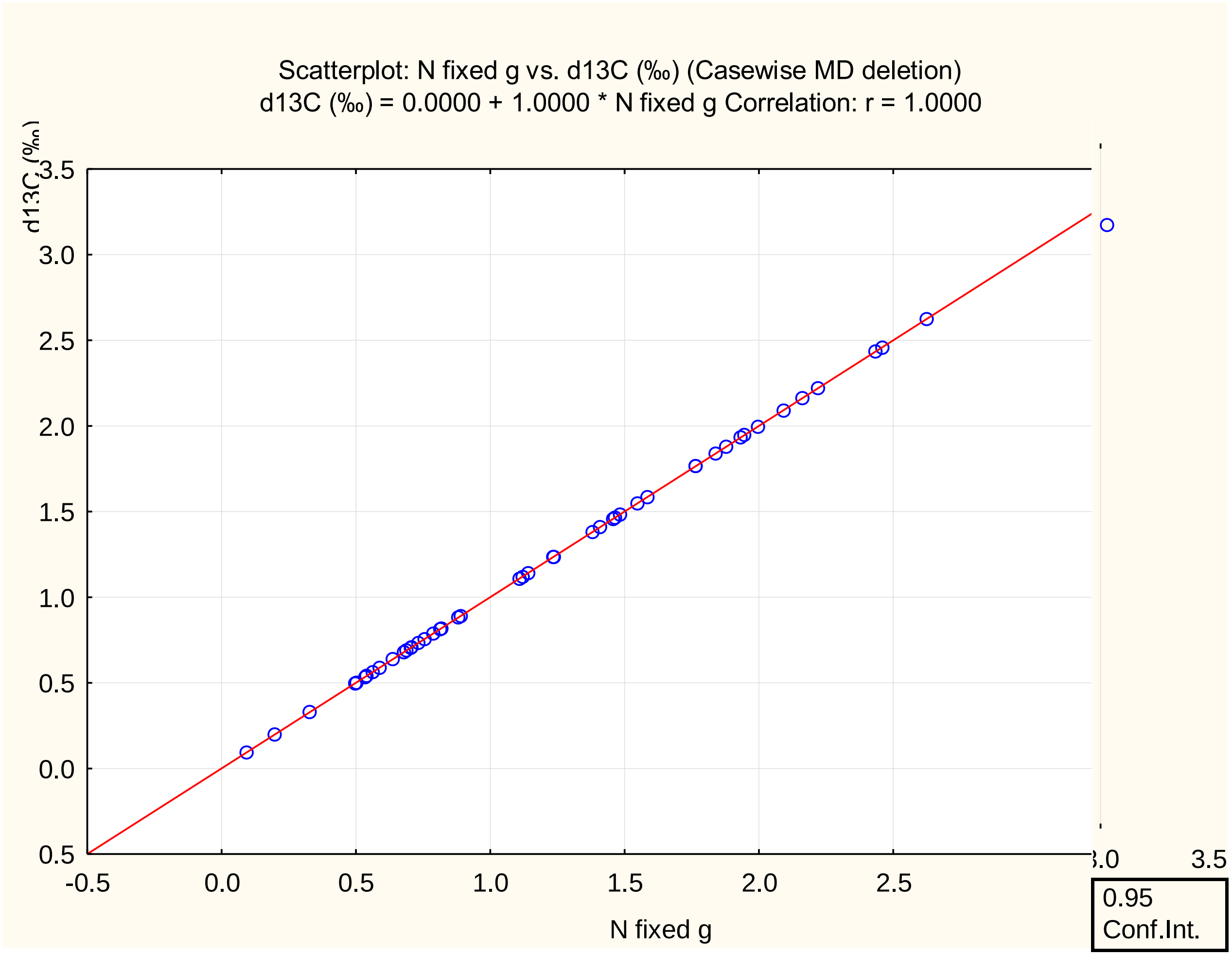

Figure 6.5: The correlation of $\delta^{13}C$ (‰) and N-Fixed ($kgha^{-1}$) on chickpea at Thohoyandou location, 2016.

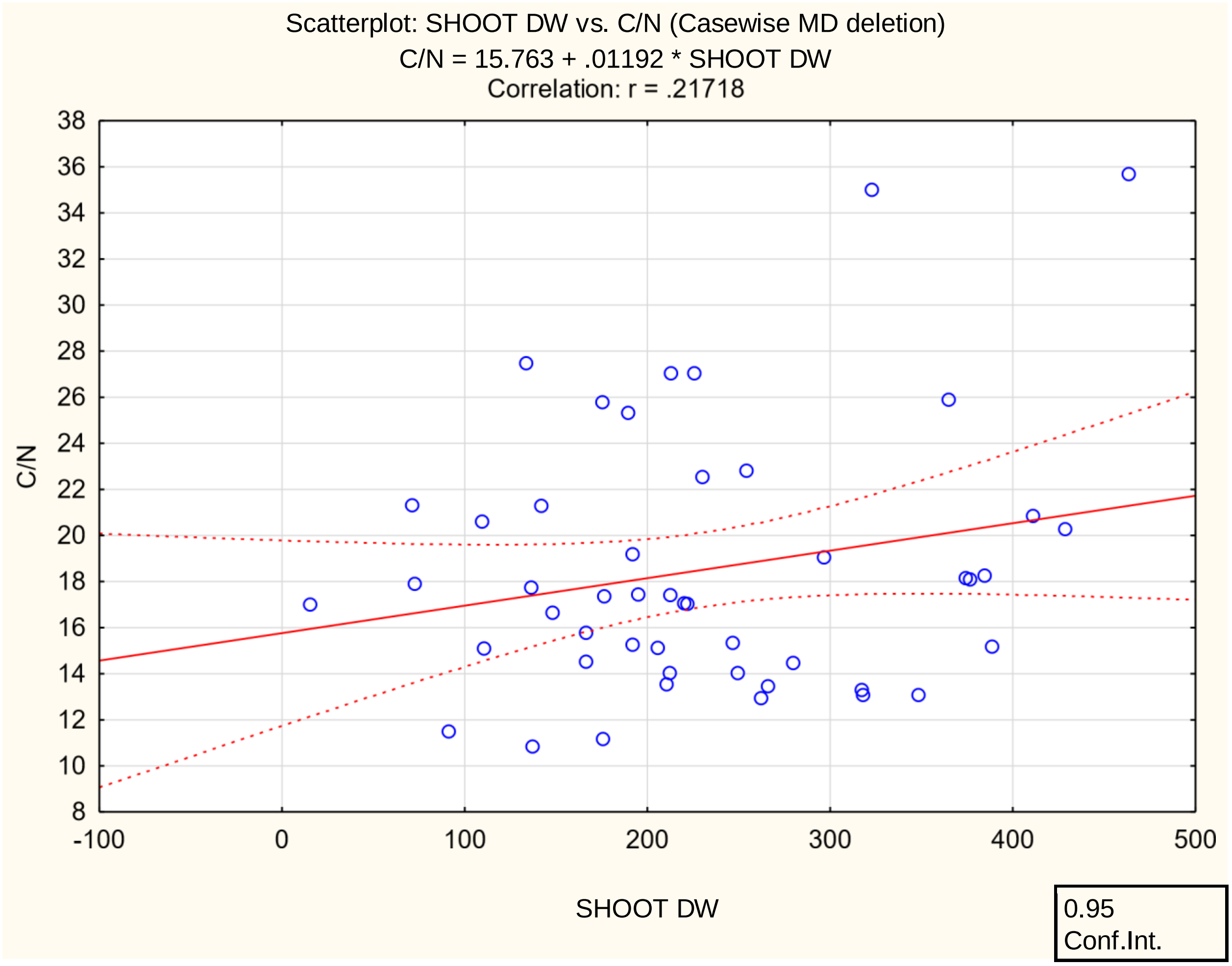

Figure 6.6: The correlation of shoot dry matter and C/N ratio on chickpea at Thohoyandou location, 2017.

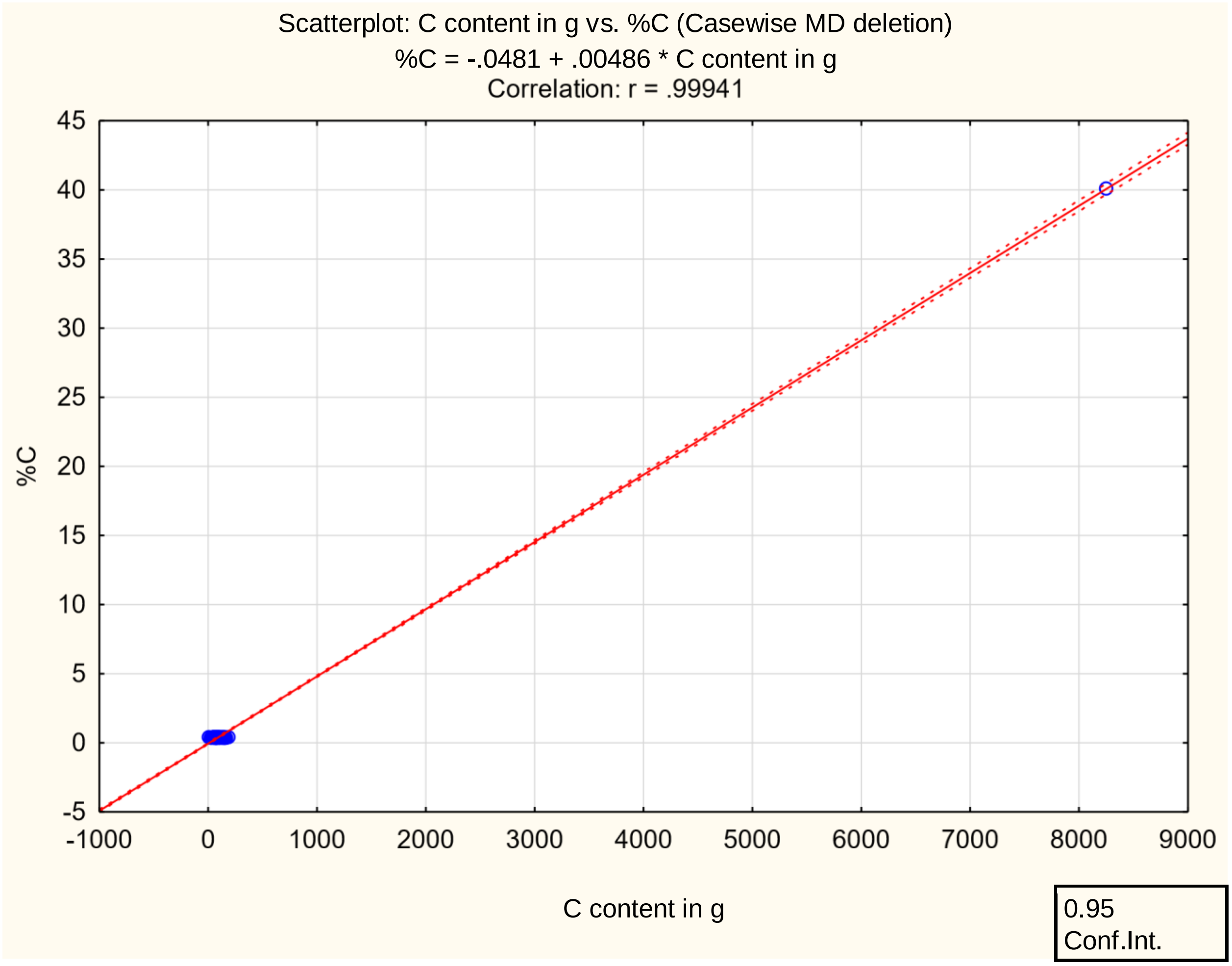

Figure 6.7: The correlation of C concentration and C content on chickpea at Thohoyandou location, 2017.

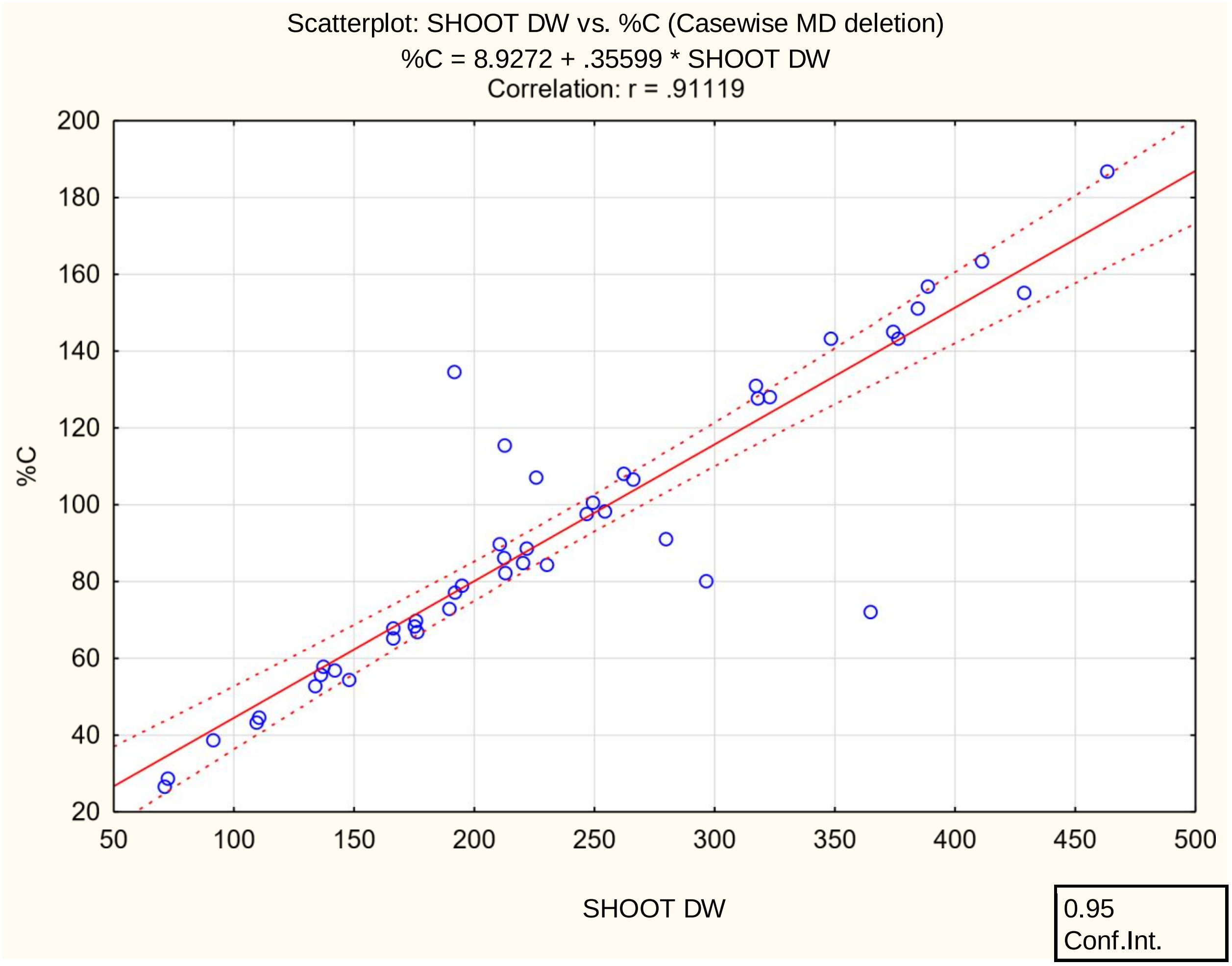

Figure 6.8: The correlation of C concentration and shoot biomass on chickpea at Thohoyandou location, 2017.

CHAPTER 7: GENERAL DICUSSION

As a legume, chickpea has the potential to contribute and increase the concentration of essential mineral nutrients. Nitrogen (N) is the most deficient of essential mineral nutrients needed to maintain the growth and yield of crops. Reasons for the inherently low N in small-scale farming fields in South Africa are many and include the high cost of N-fertilizers, hence it is important that other alternatives that can help improve the N status of soils be explored. Along with water, nitrogen, phosphorus and carbon are the most important mineral nutrients limiting the growth and development of crops in both natural and managed ecosystems (Graham and Vance, 2003; Sinclair and Rufty, 2012). Most grain legumes such as chickpea source their N from both the soil and through symbiotic N fixation (Siddiqi and Mahmood, 2001; Abera, 2015), and contributes significant quantity of N to cropping systems. Moreover, chickpeas improve soil properties and soil biological health as well as soil nitrogen enrichment through biomass addition and N_2 fixation (Singh and Shivakumar, 2010).

For enhanced phosphorus availability in soils, a variety of mechanisms including acidification of the rhizosphere (Gunes *et al*., 2007), secretion of greater extracellular acid phosphatase activity (Li *et al*., 2004) and increase in concentration of rhizosphere carboxylates (Veneklaas *et al*., 2003; Wouterlood *et al*. 2004) have been shown in chickpeas. However, the phosphorus contributed through these various mechanisms is hardly enough to meet the phosphorus demand of the crop. As a symbiotic legume, the N_2 fixation process is phosphorus demanding; shortage of phosphorus has the biggest impact on grain legumes, which rely on N_2 fixation for their N. Although crop response to phosphorus fertilization depends on soil available phosphorus as well as genotype, in general, phosphorus fertilization promotes root growth, which in turn improves the growth efficiency of crops, enhance nutrient and WUE and increase yields nutrition (Schwember *et al*., 2019).

For optimum growth, development and fecundity, plants need C among other mineral nutrients, and of all mineral nutrients, it ranks second to N as the most important mineral nutrient to plants. C is acquired by C3 plants through carbon dioxide fixation during the photosynthetic process; indeed, C accumulation represents a direct measure of photosynthetic activity and therefore the growth and development of such plants (Raines and Paul 2006; Smith and Stitt, 2007). Therefore, the C concentration in shoots of C3 plants is closely related to their growth. It is clear from the foregoing that any practice(s) that enhance acquisition of C, N and P would lead to increased growth and yield of chickpea and other legumes. A number of studies have documented the effect of phosphorus fertilizer application and rhizobial inoculation. This would in future lead to less reliance on importation of the crop, which makes it costly and therefore affordable to few. One such experiment was through assessing the use of external phosphorus fertilization along with rhizobium inoculation or phosphorus and inoculation alone which was hypothesized to enhance the acquisition of C for optimum growth and therefore biomass production and as well as N contributed through N_2 fixation would be enhanced. The results supported the hypotheses of this study entirely.

High grain yield was associated with high yield components such as shoot dry matter, pods dry weight, pod/plant, seed per pod and harvest index. In 2016, chickpea genotype affected shoot dry matter in both locations. However, in 2017 there was a significant variation in Thohoyandou but not Syferkuil. Moreover, phosphorus fertilizer and rhizobial inoculation (*bradyrhizobium* bacteria) solely had significant effect on shoot dry matter in both locations. Phosphorus fertilizer application increased shoot dry biomass in 2016 and 2017.

The enhanced growth by phosphorus fertilizer could have been caused by the role of phosphorus fertilizer in photosynthesis, which enhances the metabolism and development of aboveground plant organs such as early root formation, development of lateral and fibrous roots and better root proliferation for increased access and uptake of water. Similar results were found by Madzivhandila *et at*. (2012) who indicated that growth is greater at 90 kg ha^{-1} in Thohoyandou location in winter. Rhizobial inoculation increased shoot biomass in 2016. Wondwosen *et al*. (2016) reported that inoculated chickpea plants improves yield components as compared to control treatments. However, inoculated plants with *Bradyrhizobium* strain proved to perform greater as compared to non-inoculated plants. Hence, rhizobual inoculation has been proven to Increase chlorophyll content in legume plants, and as a results in increases photosynthesis, growth and productivity (Nyoki and Ndakidemi, 2014; Mmbaga *et al*., 2015).

Phosphorus fertilizer application also increased grain yield at 90 kg ha^{-1}. Hence, legume plants require phosphorus for obtaining good quality yield at Thohoyandou in both seasons, and Syferkuil in 2017. However, in 2016, Syferkuil results may be misleading due to the damage done by frost in 2016. However, significant results are supported by the study done by Zafar *et al*. (2003) which shows that the application of phosphorus fertilizer increases 100-SWT (g) and number of pods $plant^{-1}$ because phosphorus fertilizer application encourages flowering and fruiting which result to an increase in grain yield. Moreover, rhizobial inoculation also showed positive effect on grain yield. In Thohoyandou, inoculated plants had higher grain yield in both seasons. Furthermore, the interaction between genotypes, phosphorus fertilizer and rhizobial inoculation had significant effect on grain yield in 2016. In addition, ACC#1 proved to be the best performing accessions at 90 kg ha^{-1} when inoculated with *bradyrhizobium* strain. Inoculation of composite strains of rhizobium with phosphorus, gave a better yield than inoculation of a single strain of rhizobium (Tahir *et al*., 2009).

Furthermore, the results in chapter 5 showed that there was variation among genotype on shoot biomass. ACC#5 was recorded greater shoot biomass, N-fixed and soil N-uptake across two location in both seasons. There was no variation among chickpea genotypes on $\delta^{15}N$ in Thohoyandou; however, phosphorus fertilizer, rhizobial inoculation and chickpea genotypes showed significant variation in $\delta^{15}N$ at Syferkuil location. Soil N uptake by chickpea genotypes was proportional to $\delta^{15}N$, thus where plant dependent more on N from the soil also absorbed greater amount of N as compared to those that dependent more on Ndfa. Whenever shoot $\delta^{15}N$ was greater, Ndfa low, soil N uptake by chickpea was markedly greater, and vice-versa. Therefore the results indicates that soil-N inhibits N_2 fixation in the legume, and It is likely possible that the genotypes that fixed larger amounts of N and took up less amount of N in the presence of high soil N, formed symbioses that were relatively tolerant of mineral N (Dakora *et al*., 1992; Dakora, 1998; Ayisi *et al*., 2000).

The application of phosphorus fertilizer had a significant effect only on the growth and N content of the selected chickpea genotypes in both locations. However, phosphorus application decreased shoot $\delta^{15}N$ by 7.5% in 2016. Similarly, Ndfa percentage was greater in control in both seasons. Soil N-uptake and N-fixed was significantly higher in 2017 as compared to 2016. Application of 90 kg P ha^{-1} increased soil N-uptake by 49.19% as compared to control across two location in both seasons in line with previous studies that N fixation is encouraged by the availability of optimum phosphorus fertilizer in the soil in other countries (Veneklaas *et al*., 2003; Wouterlood *et al*., 2004).

Rhizobial-inoculated chickpea showed significantly increased shoot biomass and N concentration; wherein *bradyrhizobium* inoculant increased shoot biomass at Thohoyandou in 2016 and at Syferkuil, in both seasons. Furthermore, rhizobial inoculation increased N-fixed in 2016 and soil N-uptake in both seasons. Results showed that rhizobial-inoculated plants with greater shoot biomass exhibited the highest N-fixed and depended the most on soil N across two locations in 2016 and 2017. Therefore rhizobial inoculation outperformed control in both location, and that means N fixation by chickpea plants is maximized by inoculating them with bacterial strain. (Pule-Meulenberg *et al*., 2011; Mapope and Dakora 2016).

The interaction between phosphorus fertilizer application and rhizobial inoculation had significant effect on N content, soil N-uptake in 2016, and shoot biomass in 2017. In addition, between phosphorus fertilizer interacting with rhizobial inoculation and chickpea genotypes significantly affected N concentration and soil N-uptake in 2016, and N-fixed in 2017, N content and Ndfa in both seasons. Wherein ACC#2 and 5 had greater N-fixed at 90 kg ha^{-1}, in contrast to ACC#1 (which had performed better in control). This indicate that phosphorus fertilizer together with rhizobial inoculant enhances N fixation and other symbiotic performance. In this case, ACC#3 and 5 proved to perform best in the presence of phosphorus and *bradyrhizobium* inoculant across two location; hence it had greater N-fixed and low Ndfa. This indicate that inoculated ACC#3 and 5 had the ability to solubilize nutrients in the soil more than ACC#1 and 2 hence rhizobia with phosphorus have an ability to produce plant growth regulators and solubilize nutrients (Peix et al., 2001). Moreover, the use of a combination of commercial inoculant strains and phosphorus fertilization has been reported to increase the performance of the crop as these reportedly enhanced nodulation and nitrogen fixation (Ali *et al*., 2004; Saini *et al*., 2004; Elkoca *et al*., 2008).

Moreover, genotypes did not show significant differences in C concentration in shoot between the growing locations and the cropping seasons and it ranged between 38.42% at Syferkuil in 2016 to 45.32% at Thohoyandou in 2016. In fact, the only marked difference in %C between the selected genotypes was revealed at the Thohoyandou site in 2017 where ACC#3 accumulated the most %C in shoot. Interestingly, literature has shown that in most cases, different genotypes of legumes exhibit no significant differences in %C values has been shown in a number of studies (Pule-Meulenberg *et al*., 2011; Yahaya *et al*., 2019). Whether this is related to their genetic makeup, is yet to be determined.

The C content shown in this study was largely greater in ACC#2, except at Thohoyandou in 2017 where ACC#5 revealed the highest. That ACC#2 had the highest C content is not surprising because the genotype recorded the highest shoot dry weight and given that C content is a product of shoot dry weight and %C, it had to rank higher. The application of phosphorus increased the C content of plant established at Thohoyandou during both cropping seasons and only for chickpea grown in 2016 at Syferkuil. Similar to the argument made for C content between genotypes, that C content is a product of %C and shoot dry weight, it was expected that the supply of phosphorus would reveal markedly higher C content in this locations because it also resulted in increased shoot dry weight. In addition, the supply of phosphorus could have increased enhanced the photosynthetic performance of the plants and therefore their capacity to assimilate CO_2, which could have increased carbon fixation and therefore shoot dry matter and C content. Furthermore, the application of phosphorus and its improved uptake by plants could have also increased the availability and uptake of especially Ca, Mg and S (Fageria, 2009), all which enhance photosynthesis, plant growth and therefore C concentration and content.

Except for the shoot $\delta^{13}C$ of plants established at Thohoyandou in 2016, largely, the application of phosphorus had no significant effect of the WUE of chickpea. The markedly increased WUE in chickpea grown at Thohoyandou in 2016 with phosphorus application could have been caused by the enhanced efficiency of carbon fixation, which is increased by the supply of phosphorus and increases the efficiency of light harvesting or carboxylation processes (Wang *et al*., 2018).

Rhizobial inoculation showed no significant effect on the parameters at Syferkuil in both cropping seasons. Although this was the case at Thohoyandou, it was only in the 2016 season. Rhizobial inoculation increased C content in chickpea grown in 2017 at Thohoyandou. In most cases where legumes are inoculated with rhizobium inoculant, there is report of increased of the population of bacteria (Zilli *et al*., 2019). However, even with effective rhizobial strains, like this study, other studies have shown no effect on the shoot $\delta^{13}C$ or WUE of legumes (Pule-Meulenberg *et al*., 2011). Whether the lack of significant differences in the parameters was because of inefficiency of the introduced rhizobial inoculant or effectiveness of the indigenous rhizobia as shown by other studies (Chibeba *et al*., 2017), remains unknown. However, the markedly improved C content in chickpea grown in 2017 at Thohoyandou was perhaps due to a build-up of the introduced rhizobial bacteria since these have been supplied in 2016 as well. In that case, the commercial bacteria could have improved the uptake and solubilization of especially N, P, Fe and K (Biswas *et al*., 1999). Hence, the increase C content as these nutrients are involved in photosynthesis and plant growth.

The results indicated that C concentration in chickpea legume was enhanced by appliocation phosphorus fertilizer at 90 kg ha^{-1} interacting with *bradyrhizobium* on selected chickpea genotypes in 2016, and between genotypes and phosphorus fertilizer; and phosphorus fertilizer with rhizobial inoculation. Although the effect of phosphorus fertilizer rates on C accumulation and of rhizobium bacteria has been documented, there was dearth literature on the interactive effect of phosphorus fertilizer and rhizobial inoculation on chickpea genotypes. Furthermore, the results from this study proved that the interaction between rhizobial inoculation and phosphorus fertilizer increased C content in 2016-2017, and C concentration. ACC#2 and ACC#5 had greater C content at 90 kg ha^{-1} and with rhizobial inoculation. Taiz and Zeiger (2002) reported on Bambara groundnut plants, that greater C content was higher in accumulated dry matter, and Mohale *et al*. (2014) stated that photosynthetic C accumulation accounts for a substantial amount of plant total biomass. Thus the findings resemble Mohale *et al*., (2014) findings who reported that Bambara groundnut plants that exhibited greater C content, always accumulated higher dry matter. Taiz and Zeiger (2002); Mohale *et al*. (2014) also stated that photosynthetic C accumulation accounts for a substantial amount of plant total biomass.

In conclusion

The results showed that phosphorus fertilizer application and rhizobial inoculation (*bradyrhizobium* strain) had significant effect on grain yield and yield components, symbiotic performance, N fixation, C accumulation and WUE of chickpea genotypes. Therefore, is concluded that yield components, N fixation, C accumulation and WUE were associated with phosphorus fertilizer application and rhizobial inoculation in ACC#3 and ACC#5 throughout the experiments at both locations. Moreover, grain yield of chickpea in both locations was enhanced by an increase in yield components. In addition, to achieve maximum N_2 fixation in similar environmental condition to Northeast of South Africa, is best to inoculate ACC#3 and 5 with *bradyrhizobium* inoculant at 90 kg P ha^{-1}. Furthermore, we have used the ^{13}C isotope to assess plant water use. Results showed a close positive relationship between $\delta^{15}C$ values, symbiotic N nutrition, and WUE efficiency on chickpea legumes. Hence, the results were aligned with the

expectation.

However, it is recommended that, before definite conclusion can be drawn, further studies using different rhizobial strains be inoculated in chickpea genotypes, and varying phosphorus fertilizer levels be used with rhizobial strains in future studies. Nonetheless, with proper management of soil fertility such as phosphorus fertilizer and *bradyrhizobium*, this legume has the potential to become a significant food security crop and a bio-fertilizer in cropping systems of resource-poor farmers in Africa. Furthermore, it is important to screen chickpea genotypes with superior symbiosis and carbon accumulation and improved WUE for increased food security in dry areas such as South Africa.

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