# RESPONSE OF TEPARY BEAN (*Phaseolus acutifolius*) TO VESICULAR-ARBUSCULAR MYCORRHIZA AND PHOSPHORUS APPLICATION RATES

by

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# ABSTRACT

Tepary bean (*Phaseolus acutifolius*) is a legume crop cultivated for its high nutritional status and is tolerant to biotic and abiotic stress. It can potentially fix atmospheric nitrogen (N), reducing the need for chemical fertilizers. The current study aimed to evaluate how vesicular-arbuscular mycorrhiza and phosphorus application rates influence tepary beans' growth, yield components, and biological nitrogen fixation. The experiments were carried out simultaneously at Syferkuil and Ga-Molepo during the 2020/2021 summer growing season. The field experiment was laid out in a split-plot design with four replications. Treatment factors were phosphorus (P) and vesicular-arbuscular mycorrhiza (VAM). Phosphorus was the main factor with five treatment levels (0, 20, 40, 60, and 80 kg/ha P), whereas VAM was the sub-factor with two levels (inoculated with 50 kg/ha and uninoculated).

Growth and yield parameters were measured, and the amount of nitrogen fixed was determined. Soil samples were collected before planting and after harvest to determine the chemical composition of the soil. The collected data were subjected to ANOVA at a 5% significance level. Selected parameters such as the number of days to 50% emergence and plant height were significantly affected by P application. Pod length was influenced considerably by the VAM application. A significant impact on plant height, chlorophyll content, pod weight and grain yield was observed between the two locations.

The 60 kg/ha P application resulted in the highest plant height as compared to the application of 80 kg/ha P. The reason could be that the P requirement was already met. The interactive effects of 60 kg/ha and VAM inoculation increased the pod length. This could be due to the fact that organic fertilizers contain plant nutrients, growth-promoting substances, and beneficial microflora when combined with inorganic fertilizers, which provide favorable soil conditions to improve nutrient use efficiency. Dry matter, Percent N derived from the atmosphere (%Ndfa) and N fixed increased with an increase in P application, except for 0 kg/ha and 20 kg/ha P for %Ndfa and N fixed, respectively. The $\delta^{15}N$ value decreased with an increase in P application. Application of 80 kg/ha P fixed the highest amount of N at both locations. The findings of this study demonstrated that the application of P and VAM was effective on the growth, yield components and N fixation of tepary bean.

**Key Words:** Tepary bean, phosphorus, vesicular-arbuscular mycorrhiza, biological nitrogen fixation

# DECLARATION

I declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Agronomy has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

Malatji J.M. (Ms) March, 2023

# DEDICATION

A special dedication to my parents (Peter and Welhemina), my siblings (Weaver, Evans, Paul and Martha), my son (Blessing) and the Malatji family at large, who have always desired the best for me and supported me in all aspects of life.

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# ACRONYMS

Al : Aluminium

ANOVA : Analysis of variance

BNF : Biological nitrogen fixation

$CO_2$ : Carbon dioxide

$N_2$ : Dinitrogen

VAM : Vesicular arbuscular mycorrhiza

AM : Arbuscular mycorrhizal

ATP : Adenosine triphosphate

%Ndfa : Percent N derived from the atmosphere

Tn : Minimum temperature

Tx : Maximum temperature

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# CHAPTER 1: INTRODUCTION

## 1.1 Introduction

Tepary bean (*Phaseolus acutifolius*) is a dry-land crop species that is believed to have originated in the Mexican states of Jalisco and the Sonoran Desert region of Sinaloa around 5000 years ago, where wild tepary accessions with characteristics similar to those of the domesticated tepary bean were discovered (Blair *et al.,* 2012). It is an excellent crop for cultivation in arid and semi-arid areas due to its distinctive genetic characteristics, which include tolerance to drought, heat, and salt stress (Porch *et al*., 2013: Rao *et al.,* 2013). The crop can fix atmospheric nitrogen through symbiotic interaction with brady-rhizobium bacteria (Mohrmann *et al.,* 2017).

Tepary beans are primarily produced for their dry and highly nutritious grains. It has a protein level of about 24%, vital minerals such as iron (Fe), calcium (Ca), magnesium (Mg), copper (Cu), potassium (K), manganese (Mn), sulfur (S), zinc (Zn), and sodium (Na); oil and 67% unsaturated fatty acids, 33% saturated fatty acids, 42% polyunsaturated fatty acids and 24% monounsaturated fatty acids (Bhardwaj and Hamama, 2004; Porch *et al.,* 2017). Tepary bean is also high in fibre necessary for a healthy digestive system, and it stabilizes glucose and cholesterol levels, making it suitable for consumption by diabetic people. Additionally, tepary bean has a reasonably high quantity of essential amino acids equivalent to common bean (Porch *et al.,* 2017). The crop is cultivated as a leafy vegetable in certain instances, and the haulms are utilized as animal feed (Molosiwa e*t al.,* 2014).

The mineral phosphorus (P) is crucial for the growth and development of plants (Malhotra *et al*., 2018). Phosphorus fertilizers are essential for preventing deficiencies and increasing crop production. However, due to their limited availability and high cost, Smallholder farmers utilise a minimal amount of fertilizers (about 0.4 kg/ha) when available (Sanginga and Woomer, 2009). According to Sinyolo and Mudhara (2018), smallholder farmers in developing countries have low uptake of improved farm inputs such as fertilizers due to high transaction costs incurred as a result of their remote location, insufficient information, and a lack of credit markets. Organic fertilization adoption is low among farmers in rural areas, affecting yields and overall food security (Muluneh *et al*., 2022). Vesicular-arbuscular mycorrhiza (VAM) is established by the symbiotic association between certain phycomycetous fungi and angiosperm roots (Subodh and Dipita, 2020). According to Subodh and Dipita (2020), the fungus colonizes the root cortex creating a mycelial network, characteristic vesicles (bladder-like structures) and arbuscules (branched finger-like hyphae). Vesicular-arbuscular mycorrhiza increases the host plants’ capacity to withstand stress through a process known as induced systemic tolerance (Verma *et al*., 2020). Due to hyphae's ability to extend outside of the nutrient depletion zone, mycorrhizae aid in increasing the surface area of plant roots (Schnepf *et al*., 2008). The process involves the external mycelium of the fungi absorbing P from the soil, translocation towards the surface of the roots, and transfer into the cortical root cells (Schnepf *et al*., 2008). Following inoculation, these fungi give plants access to insoluble P, such as rock phosphate (Kumar *et al*., 2020). The inoculated plant's use of nitrogen (N) is significantly improved by arbuscular mycorrhizal fungi (El-Sherbeny *et al*., 2022). Vesicular-arbuscular mycorrhiza accelerates plant development and produces high yields by promoting biological nitrogen fixation (Bona *et al*., 2015).

## 1.2 Problem statement

Tepary bean is a domesticated crop considered to be under-researched and under-utilized. This is demonstrated by the lack of breeding initiatives to improve the yield (Gujaria-Verma *et al.*, 2016). The crop is mainly planted using unimproved wild accessions or landraces (Mhlaba *et al.,* 2018). Novel genes for stress tolerance have been discovered in tepary beans. Though tepary bean has a high probability of surviving in an arid and semi-arid climate, the unavailability of nutrients can reduce crop production. All growth traits in a plant become limited when P concentration is low in the soil because of its low mobility, solubility, and fixation in the soil (Al-Karaki *et al.,* 2008). According to Wissuwa *et al*. (2005), leaf area and total root growth become lower when P is low and increase with an increase in the amount of P available in the soil. Barbagelata *et al*. (2002), recommends applying 87 kg/ha to 217 kg/ha P for soybean production. Since tepary bean and soybean are from the same family, the recommendation can be used to estimate P requirement of tepary bean. Phosphorus is one of the most difficult nutrients for plants to obtain. This is because P is fixed in the soil, resulting in insufficient amounts for plants to grow and develop to their optimum potential (Malhotra *et al*., 2018). Phosphorus is one of the most difficult nutrients for plants to obtain. It is essential to comprehend how P fertilizer inputs and outputs interact (Bindraban *et al.*, 2020). This is necessary for increasing the productivity of tepary beans while reducing the cost of production. According to Sharma *et al.* (2013), lower phosphorus application rates will lead to abnormal growth and poor yields. In contrast, an excessive application can lead to toxicities (Lambers, 2022). Mogano (2021) reported no significance in grain yield and yield components under 30 and 60 Pkg/ha. Therefore, correct rates are needed to enhance plant growth and yield.

Vesicular arbuscular mycorrhizae are useful in agriculture, horticulture afforestation, and rehabilitation of deserts. In recent years, it has been presented that the VAM fungi provide host plants with several other advantages besides phosphate uptake (Juwarkar *et al.,* 2004). Among these advantages are biological control of root diseases, formation of nodules and fixation of nitrogen in legumes, hormone production, drought resistance, and improved nutrient absorption such as Zn, N, Mg, K, S and Ca (Diagne *et al.*, 2020). Inoculation of plants suppresses soil-borne pathogens, especially those concerning wilting and root rot pathogens (Souza *et al*., 2015). Little to no information has been reported on the interactive effect of VAM and P and therefore, there is a need to assess the response of tepary beans to phosphorus and VAM for improved productivity.

### 1.3 Rationale

Beans represent the most significant potential for hunger and malnutrition reduction (ASI, 2013). Tepary beans can maximize the availability of food, especially protein (Chakravorty, 2020). According to Porch *et al.* (2017), the nutritional profile of the tepary bean, such as mineral elements and flavour, is the same as that of the common bean, making it a desirable crop for consumption by a human. Moreover, it is also considered a good crop for production by resource-strapped farmers (Bhardwaj *et al.,* 2002). This is associated with its increased protein content and adaptability or resistance to abiotic and biotic stresses, making it a food security crop in the future (Chakravorty, 2020).

One of the essential nutritional components in a plant is phosphorus. Phosphorus is responsible for photosynthesis, the transfer and storage of energy, respiration, cell enlargement and division, and other processes in plants (Sandhya *et al*., 2013). Its acquisition occurs through root cells, tips, and root hairs. Phosphorus-based fertilizers have been shown to provide better crop responses by increasing root production during the early stages of plant growth, have a smaller negative impact on the environment, improve biological nitrogen fixation, aid in seed production, and consequently increase grain yield and bean quality, thereby increasing crop producer returns (Bildirici and Yilmaz, 2005; Bhattacharya, 2019). The symbiotic relationship between the mycorrhizal fungi and the roots of the crops is one of the elements that promote absorption (Dighton, 2009). Using VAM increases nodule formation in the roots, thereby increasing nitrogen fixation, plant growth, and yield (Diagne *et al.*, 2020). The application of phosphorus and VAM has the potential to improve nitrogen fixation, thereby increasing the yield of tepary beans.

### 1.4 Aim

To evaluate grain yield and nitrogen fixation of tepary bean in response to vesicular-arbuscular mycorrhiza and phosphorus application rates.

### 1.5 Objectives

i. To determine the effect of phosphorus application rates and vesicular-arbuscular mycorrhiza on the growth and yield of tepary beans.
ii. To determine the effect of phosphorus application rates and vesicular-arbuscular mycorrhiza on the symbiotic performance of tepary bean.

### 1.6 Hypotheses

i. Phosphorus application rates and vesicular-arbuscular mycorrhiza application do not affect tepary bean yield.
ii. Phosphorus application rates and vesicular-arbuscular mycorrhiza application do not affect the tepary bean's symbiotic performance.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Introduction

Legumes are beneficial as human and animal food and soil-improving components of agriculture and agroforestry. Phaseolus beans are important legumes for food consumption, especially in Latin America, Africa, and Asia (Zambre *et al*., 2006). The tepary bean (*Phaseolus acutifolius* L. Gray) is one of the five cultivated species of the Phaseolus. It has the potential for agricultural production in many parts of the world (Zambre *et al*., 2006). Tepary bean is desired for its adaptability to biotic and abiotic factors. Including legumes in the diet is essential in controlling and preventing various diseases and is the cheapest source of supplementary proteins (Bukya, 2014).

Legumes such as tepary beans can fix atmospheric nitrogen. Biological nitrogen fixation is one of the most important sources of nitrogen in the production of leguminous crops (Mohammadi *et al*., 2012). A symbiotic relationship between rhizobia and legume plants can provide large quantities of nitrogen to the plant. The factors influencing the growth, survival, and metabolic activity of nitrogen-fixing bacteria and plants and their ability to forge efficient symbiotic interactions include low pH, nutrient availability, temperature, and moisture status (Werner and Newton, 2005). Nitrogen fixation under phosphorus (P) deficiency is often reduced because a low P supply induces changes in the relative growth of nodules and shoots (Hogh-Jensen *et al*., 2002).

Phosphorus plays an essential role in biological nitrogen fixation, for the symbiotic fixation of nitrogen to occur, the roots have to interact with compatible rhizobia in the soil and factors that affect root growth or the activity of the host plant would affect nodulation (Freire, 2006). Phosphorus is the basis for forming valuable energy, essential for sugar formation and translocation (Aulakh *et al*., 2003). Low soil phosphorus and poor phosphorus utilisation efficiency are significant constraints limiting most grain legumes' productivity.

## 2.2 Plant description and characteristics of Tepary bean.

### 2.2.1 Plant description and growth habit

*Phaseolus acutifolius*, or the tepary bean, is a legume native to the southwestern United States and Mexico and has been grown there by the native peoples since pre-Columbian times (Gray, 2018). Tepary bean (*Phaseolus acutifolius* L. Gray) is one of the five cultivated species of the genus Phaseolus (Gray, 2018). It is more drought-resistant than the common bean (*Phaseolus vulgaris*) and is grown in desert and semi-desert conditions from Arizona through Mexico to Costa Rica. Tepary bean has the potential for agricultural production in many parts of the world (Bhardwaj, 2013). It is a small nitrogen-fixing plant which can form associations with nitrogen-fixing bacteria (Felger and Rutman, 2015).

Tepary beans exhibit an annual growth habit, as do other members of the genus. The tepary bean is an annual crop and can be climbing, trailing, or erect with stems up to 4 m long (Gray, 2018). Wild forms of tepary bean are twining or weakly trailing indeterminate vines that can climb shrubs and trees (FAO, 2010). The domesticated plants are bushier, up to 33.02 cm in height and 50.8 cm in diameter. The specific epithet, acutifolius, is derived from the Latin acutus (pointed, acute) and folius (leaved) (Gray, 2018). Leaves are trifoliate with narrow, pointed leaflets and have white or light-coloured flowers (Wolf, 2018). A narrow-leafed variety, tenuifolius, and a broader-leafed variety, latifolius, are known. Fruit are small pods, about 3.18 cm to 7.67 cm long, containing 2 to 7 seeds (Gray, 2018). The wild pods are strongly dehiscent (scattering their seed when mature), while the domesticated forms are less so (ASA, 2020). According to Stephens (2018), the seeds of this species are smaller than those of common beans and vary in seed coat colour among races (commonly white or brown). Domesticated tepary bean seeds are about 0.85 cm long and can be brown, beige, black, mottled, or white, while wild seeds are smaller, dark, and mottled (FAO, 2010). They mature in about 60 to 120 days after planting.

### 2.2.2 Adaptation to climatic conditions

The ecosystems around the world are rapidly impacted by the effects of climate changes (Chmura *et al*., 2018), which causes changes in the levels of carbon dioxide (Pourkheirandish *et al*., 2020), a global increase in temperature and a change in rainfall patterns. Such changes result in reduced water availability which puts pressure on agriculture as it limits the usage of agricultural land for production. It has been estimated that agriculture will need to produce about 60% to 100% more food than it is currently producing by the year 2050 (Chmura *et al*., 2018). As a result, food security is becoming a significant challenge to address in the coming decade (Raggi *et al*., 2019) as the changes in climate give rise to predictions of yield reduction in crops, especially for those with greater sensitivity to abiotic stress like a common bean (Porch and Hall, 2013).

The effects of climate change on agricultural production can be managed effectively by identifying and characterizing new sources tolerant to abiotic stress (Buitrago-Bitar *et al*., 2021). The common bean *(Phaseolus vulgaris* L) is one of the food crops considered necessary due to its contribution to the human diet, as it has higher nutritional value/status (Leal-Delgado *et al*., 2019). However, a limitation may be presented in its adaptation and yield due to its sensitivity to abiotic stresses (Polania *et al*., 2016). For this reason, related species such as tepary beans need more attention as they can withstand different abiotic stresses (Moghaddam *et al*., 2021). The crop can also be used for breeding purposes to enhance stress tolerance in common beans.

According to Jiri and Mafongoya (2016), the tepary bean can be able to survive drought climates, therefore, it is a resilient crop. Gary (2010) reported that the tepary bean is resistant to disease, heat and drought and has high nutritional value. It also offers a quick harvest, meaning that it can be harvested within a short period. Tepary beans provide hope to smallholder bean farmers experiencing climate change in southern Africa as they have naturally evolved with resistance to drought and high-temperature conditions (Andrews, 2014). Tepary beans can produce a significant yield with an annual rainfall of less than 400 mm (Andrews, 2014). Compared to common beans, tepary beans are the most yielding, which is attributed to tepary beans being one of the drought-resistant legume species in the world (Weil, 2015). Based on these findings, tepary beans can be expected to have significant potential for introduction into arid and semi-arid areas.

### 2.2.3 Utilization of tepary bean

Worldwide, legumes are valued as an inexpensive and sustainable meat alternative and are considered the second most important food source after cereals (Maphosa and Jideani, 2017). They are valued for their nutrition as they provide about 20-45% protein with essential amino acids, 5-37% dietary fibre and ±60% complex carbohydrates (Maphosa and Jideani, 2017). They provide essential vitamins and minerals and are low in fat with less cholesterol. Additionally, legumes can be used in physiological, cultural, economic, and medicinal roles due to the beneficial bioactive compounds found in the crops. The bioactive compounds contain antioxidant properties, which help prevent some heart diseases, cancers, osteoporosis, and other degenerative diseases. This can be beneficial in patients with diabetes and celiac disease.

Tepary bean is produced for its mature, dry seeds consumed after boiling, steaming, baking, or frying. They can be used in soups, whole-maize, or stews (Jiri and Mafongoya, 2016). It can also be consumed as bean sprouts or as a green bean. In some parts of the world, like Malawi, the leaves are edible, although they are tougher than the common bean. Phaseolus beans have been extensively used as animal feed. In developing and underdeveloped countries, the green foliage, deseeded pods, and roots and shoots of bean plants are used as natural fertilizers, especially after composting (Sathe, 2016). Because the legume roots fix nitrogen, they help conserve soil quality. For this reason, many developing countries extensively use legumes for soil quality conservation.

## 2.3 Biological nitrogen fixation (BNF)

Biological nitrogen fixation (BNF) can be described as the reduction of dinitrogen ($N_2$) to ammonia carried out by prokaryotes (Bottomley and Myrold, 2015), and it is a process that maintains the soil nitrogen status under normal conditions. The *Rhizobium* and *Bradyrhizobium* bacteria colonize the host plant’s root system, forming nodules to house the bacteria (Wagner, 2011). The bacteria then initiate the fixation of nitrogen required by the plant. Access to fixed nitrogen permits the plant to produce leaves fortified with nitrogen that can be recycled throughout the plant (Wagner, 2011). This causes an increase in the photosynthetic capacity of the plant leaves, which in turn yields nitrogen-rich seed. The availability of nitrogen and oxygen concentration regulates nitrogen fixation. Under nitrogen-deficient conditions, plants inoculated with the correct type of bacteria produce greater shoot mass and nitrogen content than plants which have not been inoculated.

Microorganisms that perform biological nitrogen fixation are important because this element is an essential component of nucleic acids, proteins, and other nitrogen compounds (Dakora, 2003). Therefore, nitrogen is an integral component of life for all living beings. The process of BNF performed by symbiotic nitrogen fixing bacteria with legume species can help promote plant growth by supplying nitrogen and several other mechanisms, such as the production of siderophores, exopolysaccharides, and phytohormones; protection against phytopathogenic fungus and phosphate solubilization (Dakora, 2003).

The two most essential symbioses are $N_2$ fixation and mycorrhizae acquisition of phosphorus (P) and other nutrients (Bonfante and Anca, 2009). For the cultivation of legumes, the relationship between rhizobia and mycorrhiza is of great importance because these bacteria influence the infection rate and mineral nutrition as well as the chemical and physical conditions of the soil by adding organic waste and increasing the growth of these plants (Parniske, 2008).

The extent or amount of nitrogen fixed varies with legume species and variety (Table 2.1). According to Dabessa *et al*. (2018), seasonal African grain legumes fix about 15-210 kg N/ha. The selection of suitable legume genotypes that can form symbiotic relationships with rhizobia is essential for significant legumes (Dabessa *et al*., 2018). The amount of nitrogen fixed depends on all the factors that influence BNF, as they can enhance or inhibit biological fixation. It is, therefore, essential to keep other factors in control to increase the chances of the symbiotic relationship between the bacteria and the plant.

**Table 2.1:** Estimates of amounts of N fixed annually by crop legumes in southern Africa

| Crop | $N_2$ fixed (kg/ha) | Source |
|---|---|---|
| Bambara nut | 52 | Rowe and Giller (2003) |
| Cowpea | 47 | Rowe and Giller (2003) |
| Groundnut | 33 | Rowe and Giller (2003) |
| Groundnut | 58-188 | Mokgehle *et al*. (2014) |
| Pigeonpea | 39 | Rowe and Giller (2003) |
| Pigeonpea | 3-82 | Mapfumo *et al.* (2000) |
| Pigeonpea | 96 | Chikowo *et al*. (2004) |
| Cowpea | 28 | Chikowo *et al*. (2004) |

## 2.4 Factors affecting the response of legumes to inoculation and BNF

### 2.4.1 Soil temperature

Temperature plays an essential role in the success of biological nitrogen fixation due to its effect on microorganisms' survival and growth rate. According to Kabahuma (2013), temperature affects nodulation, survival, and persistence of microorganisms in the soil. The planting date can be adjusted to conditions where the temperature is minimal or will not significantly affect the organisms responsible for BNF. Low temperatures delay the onset of nodulation and reduce the nodule growth rate, resulting in small nodule sizes (De Lira Juniora *et al*., 2005). Nitrogenase activities are high at around 12–35 °C and reach a maximum between 20–25 °C in most legumes (Liu *et al*., 2010). Irrigation can be used to control the soil temperature, although temporary. According to Aranjuelo *et al*. (2015), the high temperature decreased the growth of plants due to its adverse effects on nodule growth and development, resulting in reduced nodule biomass and depletion of total soluble protein content. $N_2$ fixation was more sensitive to high temperatures than photosynthesis, especially when plants are exposed to temperatures higher than 25 °C (Aranjuelo *et al*., 2015). For effective symbiosis to occur, the bacteria should have the ability to survive in arid and hot conditions.

### 2.4.2 Soil moisture

The amount of water available in the soil influence BNF and inoculation. Less or excessive moisture levels drastically affect BNF, as moisture stress can affect the potential of the nodules to function. Drought stress is one of the main factors affecting the legume-rhizobium symbiosis's ability to fix nitrogen. Moisture stress significantly affects nodulation, nitrogen fixation and yield. This may be because moisture stress causes biochemical and physiological changes, affecting plant growth and development (Boutraa, 2010). It causes suppression in plant photosynthetic effectiveness (Lawlor and Cornic, 2002). The physiological response of symbiotic nitrogen fixation to drought stress has been associated with several mechanisms, including carbon deficiency and nodule carbon metabolism, oxygen restriction, and feedback regulation by the accumulation of N fixation products (Schwember *et al*., 2019). In a study by Serraj (2003), the combined effects of carbon dioxide ($CO_2$) enrichment and water deficiencies on nodulation and $N_2$ fixation in soybean were examined to test the carbon shortage hypothesis. A drought-tolerant $N_2$ fixation allocated around four times as much $^{14}C$ to nodules under drought compared to a genotype with drought-sensitive $N_2$ fixation. The augmentation of photo assimilation and an improved partitioning of carbon to nodules, whose significant effects were to sustain nodule growth and help sustain $N_2$ rates under soil water shortages, was reported to be the essential effect of $CO_2$ enrichment on soybean during drought.

The relationship between nodule permeability to oxygen ($O_2$) and drought stress with $N_2$ fixation was examined in soybean nodules (Serraj, 2003). The results concluded that $O_2$ limitation appears to be only partially responsible for decreasing nodule activity during the early phases of water deficit stresses. An increased ureide content in shoots and nodules during drought resulted in an adverse feedback reaction between ureides and nodule activity. It led to the initial suspicion that ureides were involved in the drought response of $N_2$ fixation (Serraj *et al.,* 1999). This hypothesis was supported by concrete evidence that the ureides and amides produced by nitrogenase inhibit its activity. Understanding the regulation of $N_2$ fixation and how it responds to soil drying requires understanding all three physiological mechanisms.

According to Kabahuma (2013), nodulation is inhibited by low soil water levels. Singh and Kataria (2012) found that water stress decreased leghaemoglobin, nitrate and nitrite contents and the activity of enzymes of nitrogen assimilation, such as nitrate reductase and nitrite reductase, in two chickpea genotypes. Water holding capacity, influenced by the soil type, can also affect BNF. Excessive moisture may result in waterlogging, which prevents the development of root hairs and sites of nodulation and interferes with the normal diffusion of oxygen in the root system of plants (Worku, 2016). Streeter (2003) reported depression of 30–40% of nitrogen content in leaves and pods of soybean plants when irrigation was withheld for four weeks during the reproductive stage. Youn *et al*. (2008) reported that waterlogging reduced the nodules and nodule dry weight of super nodulating soybean mutants.

### 2.4.3 Soil pH

Soil pH affects the host plant and the bacteria involved in the symbiotic relationship. Most leguminous plants require a neutral or only a slightly acid soil pH for growth, with nodulation problems expected once the pH falls below 5.5 (Ferguson *et al*., 2013).

According to Rohyadi *et al.* (2004), vesicular-arbuscular mycorrhiza (VAM) increases with soil pH, implying that VAM can survive in alkaline but deteriorate in acidic conditions. Aluminium (Al) and manganese ions may cause injury to plant roots in soils with pH levels below 5.0, thus leading to aluminium toxicity and limiting the availability of essential nutrients (Rout *et al*., 2001). These effects can also reduce water and nutrient uptake by the plants, thereby reducing the survival of microorganisms in the soil. According to Ferguson *et al*. (2013), low soil pH of 4.0-4.5 highly reduced the number of nodules, dry nodule weight, fresh nodule weight and nitrogenase activity of common beans, cowpea, and soybean.

On the other hand, Sulieman and Hago (2009) discovered that nodulation failure in common beans was due to high calcareous soil, which prevailed at a pH of 8.1 and 8.5 in the topsoil and subsoil, respectively. Therefore, maintaining the soil pH promotes optimal plant growth and BNF in many forage crops. Limestone may be added to raise the pH of the soil and thus raise BNF.

### 2.4.4 Mineral soil nutrition

The reduction of BNF due to soil fertility will often be related to either an excess of soil nitrates or a deficiency of some essential nutrient limiting plant growth and development (Chalk *et al*., 2010). Phosphorus is one of several elements which affect nitrogen fixation, along with nitrogen, it is a principal yield-limiting nutrient in many regions of the world (Weisany *et al*., 2013). Excess soil nitrate levels will depress BNF, thus, the application of fertilizer nitrogen (N) to legume crops that can utilize BNF is not advisable. According to Wang *et al*. (2020), low phosphorus levels in acidic soils, for example, delay nodulation and infection of primary roots. Available nitrogen in the soil is another important factor for BNF (Dabessa *et al*., 2018). High nitrogen levels inhibit infection and nitrogen fixation (Weisany *et al*., 2013). Aluminium and manganese toxicity and low calcium levels inhibit mycorrhiza growth and nodulation at a soil pH of less than 5 (Drew *et al*., 2012).

## 2.5 Effect of phosphorus availability on the development and productivity of legumes

Phosphorus is the most crucial component for sufficient grain yield (Brady and Weil, 2002). For instance, the amount of P absorbed is a key factor in the high seed production of legumes (Khan *et al*., 2003). The application of P enhances haricot bean production (Kassa *et al*., 2014). According to Yuan *et al.* (2010), one of the many factors influencing the crop's region low grain production of beans is the inability to apply fertilizer at the optimum rate. Using P fertilizers from outside sources at the acceptance rate for the crop could be one way to solve the issue.

An adequate P supply is critical early in a plant's reproduction stage for the development of its reproductive organs. Additionally, a sufficient supply of P is linked to faster root development and earlier maturation of crops, especially grain crops. In plants lacking in P, fruit and seed production and quality are negatively impacted (Silva and Uchida, 2000). Beans cultivated in P-deficient soils exhibit poor nodulation and weak plant vigour (Mweetwa *et al*., 2016). Due to erosion and high fixation losses, most soils do not always have enough P for crop growth (Brady and Weil, 2002). This problem is most severe in highly weathered acid soils that dominate the highlands of the tropics (Mweetwa *et al*., 2016). Phosphorus availability can also be affected by water stress which delays solubility and absorption by the plant.

## 2.6 Agronomic traits associated with drought tolerance and yield gains

Agronomic parameters such as biomass accumulation, harvest index, and partitioning to seed and deep root systems are known to have a significant contribution to yield improvement and identifying genotypes that are drought tolerant in *Phaseolus species* (Mukeshimana *et al*., 2014). Similarly, selection has focused on photosynthate-mobilizing features, including harvest index, pod partitioning index and pod harvest index, to increase yield gains under drought stress conditions (Assefa *et al*., 2013). The number of pods per plant, root length, canopy biomass, number of seeds per pod, pod partitioning index, hundred-seed weight, and harvest index are all positively correlated with common bean seed yield under drought stress (Asfaw *et al*., 2017).

In a study conducted on common bean, traits like harvest index, pod partitioning index, hundred-seed weight, and the number of pods per plant have high broad-sense heritability and genetic advance values of more than 50% (Asfaw *et al*., 2017; Assefa *et al*., 2013). High broad-sense heritability and genetic advance indicate the presence of additive genes in the trait and suggest reliable crop improvement by selecting such characteristics (Rana *et al*., 2015). Under drought stress conditions, the pod harvest index, the partitioning of biomass from pods to seeds, exhibits a strong association with seed yield (Ambachew *et al*., 2015). The pod harvest index is lowered in drought-susceptible common bean genotypes, making this characteristic a helpful selection criterion for assessing drought tolerance (Assefa *et al*., 2013). The seed yield of tepary bean is positively correlated with the number of pods per plant and hundred-seed weight under water-limited conditions (Kuruvadi and Valdez, 1993) and harvest index in non-stressed conditions (Mhlaba *et al*., 2018a). High broad-sense heritability is present in the number of pods per plant, hundred-seed weight, and seeds per pod under drought-stress conditions (Kuruvadi and Valdez, 1993). This suggests that using these traits to elect drought-tolerant tepary bean genotypes may be possible. To maximize yield gains of tepary bean under water-limited situations, other important agronomic features, such as pod harvest index and pod partitioning index, which have been demonstrated to be effective for breeding in common bean, could also be targeted for selection.

Days to flowering and maturity were phenological characteristics that showed poor and negative associations with seed yield but substantial and negative correlations with plant height, number of pods per plant, and biomass accumulation (Mhlaba *et al*., 2018b). These features help identify and choose genotypes with mechanisms for surviving drought, but they may also reduce other yield components, which could reduce yield.

## 2.7 Physiological and biochemical traits of seeds in response to drought tolerance

Under drought stress conditions, physiological characteristics like stomatal conductance are positively linked with the number of pods per plant, yield, total biomass production and the number of seeds per pod in *Phaseolus species* like a common bean (Langat *et al*., 2019). Under drought stress conditions, stomatal conductance shows moderate to high associations with yield per plant, biomass above ground, seed per pod, hundred-seed weight, and harvest index in common beans (Langat *et al*., 2019). According to Langat *et al*. (2019), the common bean exhibits stomatal conductance under drought stress, which has high broad-sense heritability and approximately 20% genetic advance as a mean percentage, suggesting that selection using stomatal conductance would increase genetic gains in water-limited environments.

In common beans, there is a moderate association between chlorophyll content, plant height, and the number of pods per plant (Darkwa *et al*., 2016). According to Asfaw *et al*. (2017), chlorophyll content has a high broad-sense heritability (0.84%) and high genetic advance (30%), indicating that an indirect selection for this feature will increase yield gains in situations with drought stress. Under drought stress conditions, carbon isotope discrimination is a significant yield-determinant trait that can be used to measure transpiration efficiency (Polania *et al*., 2016). High broad-sense heritability (between 71% and 90%) for carbon isotope discrimination has been observed in wheat, peanut, and common bean (Beebe *et al*., 2013). Additionally, seed yield and carbon isotope discrimination are positively correlated (Hall, 2004), indicating that selecting this trait will probably increase yield under drought stress. Compared to common beans, tepary beans have higher net photosynthetic rates because their stomata close more quickly due to a more significant soil water deficit, increasing their water use and carboxylation efficiencies (Mwale *et al*., 2020). These characteristics are emphasized as being the main reasons why *P. acutifolius* can tolerate drought and use water effectively in drought-stressed areas (Mohamed *et al*., 2005).

Numerous alterations in the concentrations of primary metabolites, such as sugars, amino acids, and organic acids, occur in plants during drought stress (Lemoine *et al*., 2013). As a result, quick screening of tepary bean genotypes for drought resistance now includes the analysis of biochemical characteristics (Silvente *et al*., 2012). Under drought stress conditions, starch content, amino acids (such as proline), and soluble sugars show favourable correlations with seed yield and pod harvest index in common beans (Andrade *et al*., 2016). In common beans under drought stress, the number of soluble sugars, including glucose, stachyose, and maltose, is positively correlated with seed yield, making these features suitable biochemical markers for choosing drought-tolerant genotypes for breeding.

The presence of pleiotropic genetic effects or genetic links is suggested by the strong and positive correlations between seed yield and agronomic, physiological, and biochemical variables (Tar'an *et al*., 2002). Minor and significant alleles influence this, allowing simultaneous improvement of seed production and other desirable features. Therefore, highly heritable qualities and a favourable correlation with seed yield should be the focus of selection for drought tolerance in tepary beans.

## 2.8 Effect of drought stress on photosynthesis

Lack of water is a common problem that hinders plant growth in field conditions. It significantly affects plants' growth, morphology, physiology, and biochemistry (Jill *et al*., 2012). In response to drought stress, plants typically regulate their stomata, change their osmotic balance, and mount an anti-oxidative defence to mitigate harm (Jianhui *et al*., 2017). However, prolonged high-intensity drought stress could slow plant growth, alter the morphological structure and biomass distribution pattern, or even result in plant mortality (Wegener and Jansen, 2013). The response of crops to drought stress and drought resistance mechanisms have therefore attracted attention. According to research (Efeoglu *et al*., 2009), drought significantly inhibits plant growth and reduces photosynthesis. Water stress impacts a plant’s productivity by decreasing photosynthesis (Traub *et al*., 2017). Drought may interfere with $CO_2$ uptake by leaves, affect how $CO_2$ is absorbed by the carboxylation centre, and lower the net photosynthetic rate (Zhang, 1999). Closely linked to photosynthesis is stomatal conductance, with effects on the influx of carbon dioxide and efflux of water vapour (Traub *et al*., 2017). Plants must balance their carbon and water resources. Under drought stress, plants close their stomata to conserve water, reducing the availability of carbon dioxide for photosynthesis. Stomatal closure is the primary mechanism limiting photosynthesis under drought stress (Flexas *et al*., 2004). In the study on beans, stomatal conductance decreased photosynthetic activity under drought stress (Zlatev and Yordanov, 2004).

## 2.9 Effect of drought stress on yield

Drought stress reduces common bean yields yearly in over 60% of the production zones (McClean *et al*., 2011). To maintain the sustainability of the global bean crop, it is essential to breed bean cultivars with increased drought tolerance (Beebe *et al*., 2012). This improves the food security and financial stability of subsistence bean producers. The physiology of drought tolerance in common beans and related plants should be further researched to help breeders create more resilient cultivars (Beebe *et al*., 2013). While research on other crop's mechanisms for drought tolerance aids efforts in beans (Passioura, 2012), it is ultimately necessary to investigate the existence and scope of these mechanisms in common bean germplasm before they can be exploited in a breeding program.

Additionally, studies on drought incorporating various metabolic and physiological parameters are necessary (Pinheiro and Chaves, 2011). Drought stress causes the abortion of developing reproductive tissues (Traub *et al*., 2017). However, in agronomic conditions, tolerant genotypes have lower fruit abortion rates, frequently via mobilizing photosynthate reserves from other plant sections (Yang and Zhang, 2006).

When the amount of water in the soil is reduced, and transpiration continues to lose water without being supplemented by rain or irrigation, drought stress occurs. Reduced water content decreased leaf water potential, turgor loss, stomata closure, and reduced cell development and enlargement are all signs of drought stress (Jaleel *et al*., 2009). Stress from drought is a concern because it restricts plant growth, resulting in decreased yields, which raises or lowers food prices. Water use efficiency is crucial for a plant's ability to respond to drought (Edwards *et al*., 2012). The ratio of dry matter production to water use is referred to as water use efficiency (Hatfield and Dold, 2019). Two common bean cultivars (D81083 and Sayyad) experienced a decrease in leaf area, chlorophyll content, dry matter, and yield due to the introduction of drought stress (Emam *et al*., 2010). In small, red-seeded common beans, post-flowering drought stress decreased seed yield, pods per plant, and 100 seed weight (Rezene *et al*., 2013).

### 2.10 Role of mycorrhizae

Fungi represent a significant portion of soil rhizosphere microflora and influence plant growth. The symbiotic association generated by fungi with plant roots (mycorrhizae) increases the root surface area, enabling the plant to absorb water and nutrients more efficiently from a large soil volume (Khanday *et al.*, 2016). The mycorrhizal association increases nutrient and water availability and protects plants from abiotic stresses (Miransari, 2010). Mycorrhizae play a significant role in enhancing plant growth through different mechanisms. These mechanisms include mycorrhizal interaction with the rhizosphere in the contribution of soil organic carbon and impact microbial community structures that ultimately affect the N cycle. Another mechanism involves the impact of mycorrhizae on the photosynthetic activity of plants and its subsequent effect on plant growth and resistance to environmental stress (Palacios and Winfrey, 2020).

# CHAPTER 3

# EFFECT OF VESICULAR-ARBUSCULAR MYCORRHIZA AND PHOSPHORUS APPLICATION RATES ON VEGETATIVE PARAMETERS AND YIELD COMPONENTS OF TEPARY BEAN *(PHASEOLUS ACUTIFOLIUS)*

## 3.1 Introduction

Grain legumes are essential to sub-Saharan African smallholder farmers' livelihoods as an economical source of nutritional protein (Vanlauwe *et al.,* 2019; Odendo *et al.,* 2011). Despite their importance, smallholder farmers' yields of legumes are still much lower than they could be (Baijukya *et al.*, 2021). This is partly due to the crops grown on infertile soils without fertilizer application. Jiri and Mafongoya (2016) assert that climate change and variability significantly impact smallholder farmers in southern Africa. It necessitates the adoption of various agronomic techniques and crop selections by farmers. With integrated soil fertility management techniques, such as using phosphorus (P) fertilizers, inoculants, animal manure, seeds from superior cultivars, and composts, grain legume yield can be boosted (Giller *et al.*, 2013). Legumes require phosphorus fertilizers and rhizobia that fix nitrogen for the highest yield (Baijukya *et al.*, 2021).

*Phaseolus acutifolius,* a leguminous crop high in protein that can withstand drought, has been overlooked and disregarded by studies for a long time (Jiri and Mafongonya, 2016). It can make people more resilient to the effects of climate change. All stages of plant development require proper nutrition, which can dramatically lower the crop's economic worth. One of the primary nutrients that prevent plants from growing is phosphorus. Phosphorus reserves are abundant in most agricultural soils, and artificial fertilizers are primarily to blame for their accumulation (Sandhya *et al.*, 2013). However, a sizable portion of the soluble inorganic phosphate added to the soil quickly transforms into insoluble forms, rendering it unavailable to plants for fixation (Sandhya *et al.*, 2013). Cell signal transduction, sugar breakdown, energy and nutrient movement within plants, cell division and growth, photosynthesis, and cell division and growth are just a few of the processes in that phosphorus is involved (Sandhya *et al.*, 2013). This shows that P can promote plant growth, improving yield. A key element in the high seed production of legumes, according to Khan *et al.* (2003), is the amount of P absorbed. Haricot bean production increases with P application (Zebire and Gelgelo, 2019). Low grain yields result from failing to supply fertilizers at the optimum rate (Zebire and Gelgelo, 2019).

Tepary beans are an example of a legume that uses symbiotic relationships with bacteria to fix atmospheric nitrogen. Tepary beans and other mycorrhiza-associated plants can survive in situations with limited access to nutrients (Nurhikmah *et al.*, 2018). This is connected to the ability of mycorrhizae to draw phosphorus out of the soil. Mycorrhizae might potentially accumulate hazardous substances in addition to boosting nutrient intake, which raises the possibility of using this biofertilizer to reclaim land (Simanjuntak *et al.*, 2014).

Additionally, plants with mycorrhizae are more resistant to heavy metals due to their higher total P levels than plants without them (Nurhikmah *et al.*, 2018). Through the action of the enzyme phosphatase, Vesicular Arbuscular Mycorrhiza (VAM) activity increases the amount of P available to plants (Nurhikmah *et al.,* 2018). With organic acids, VAM can solubilize P in the soil (Rakshit and Bhadoria, 2010). This will reduce the likelihood of P fixation and ensure that the plants use the administered P. The application of VAM can promote field crop growth (Manjulata, 2015). This context makes it necessary to research the impact of VAM and phosphorus levels on the development and yield components of tepary beans. The study's objective was to determine the effect of phosphorus application rates and vesicular-arbuscular mycorrhiza on tepary beans' growth and yield.

## 3.2 Materials and methods

### 3.2.1 Description of the study site

During the 2020/21 summer planting season, the research was carried out at the University of Limpopo experimental farm, also known as Syferkuil (23° 59' 35" S, 29° 33' 46" E), and Ga-Molepo settlement (24° 01' 52.0" S, 29° 44' 16.0" E). The crop was planted and harvested in January and April respectively. The University of Limpopo experimental farm's environment is classified as semi-arid, with daily average temperatures ranging from 13 to 30 °C during planting season and mean annual precipitation varying from 300 to 1000 mm per year (Mpandeli *et al.*, 2019; Mokoka *et al.*, 2018). The mean yearly rainfall of Ga-Molepo varies from 400 mm to 600 mm, and the minimum and maximum temperature range from 0.6 ˚C to 33.2 ˚C (Maree, 2016). Both locations have sandy loam-like soil (Sebetha *et al.*, 2009; Maree, 2016).

Figure 3.1 shows the climatic conditions data collected from the weather station in the Capricorn district. The Syferkuil and Ga-Molepo sites are located in the Capricorn district in Limpopo. The rainfall pattern from the two study sites ranged from 0 to 0.9 mm from January to July. The minimum temperature (Tn) ranged from 1.4 to 17.53 °C, while the maximum temperature (Tx) ranged from 20.9 to 29.3 from January to July (figure 3.1).

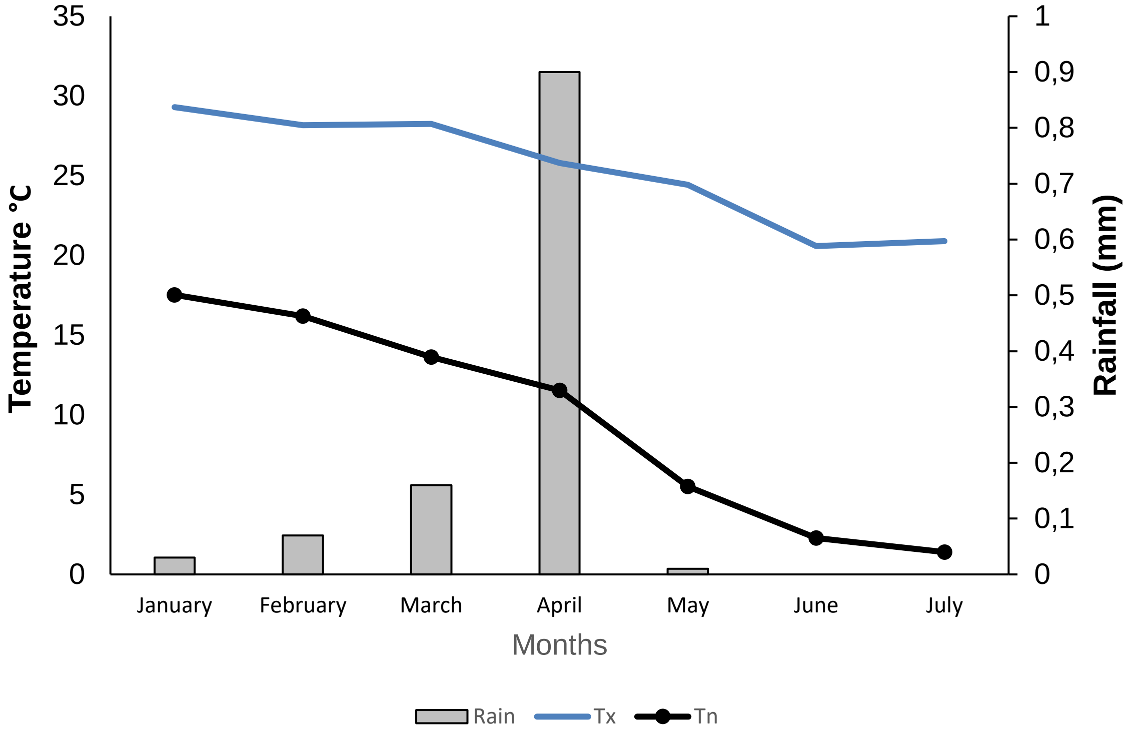

**Figure 3.1:** Average temperature and rainfall from weather station in the Capricorn district.

### 3.2.2 Experimental design

The field experiment was laid out in a split-plot design with four replications. There were ten different treatment combinations as a result of the main treatment factor, phosphorus fertilizer rates using a single superphosphate containing 10.5% P (0, 20, 40, 60, and 80 kg/ha), and the subfactor, VAM inoculation levels (VAM inoculation at a recommended rate of 50 kg/ha and without inoculation). The seeds were planted 5 cm deep. Planting was done at a 9 $m^2$ area per plot with a 60 cm and 15 cm inter- and intra-row spacing, respectively. Each plot consisted of 5 rows, with 20 plants per row. The net plot was 15 m X 30m, making a total plot area of 450 $m^2$. During planting, phosphorus and VAM were both directly applied to the soil. Management procedures, including thinning and weeding, were carried out when necessary. To help the more established seedlings grow appropriately, weak, dead, or extra seedlings were manually removed. A hand hoe was used to manage the weeds physically.

### 3.2.3 Soil sampling and analysis

Soil samples were collected randomly before planting and at harvest at 0-30 cm depth. A composite sample was obtained by mixing three samples per plot. Both pre-planting and post-harvest soil samples were analysed for phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), exchangeable acidity, total cations, acid saturation, zinc (Zn), manganese (Mn), copper (Cu), pH, nitrogen (N), organic carbon, and clay percentage. The ambic-2 extraction method measured the extractable P, K, Zn, Mn and Cu. Concentrations of exchangeable Ca and Mg, pH and exchangeable acidity were determined with a KCl solution.

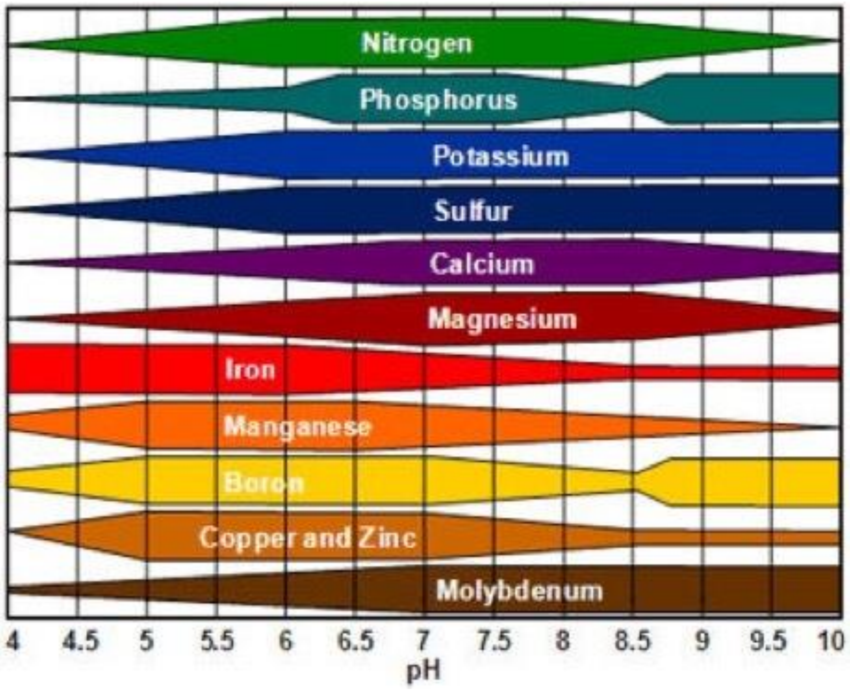

**Figure 3.2:** Plant nutrients' relative availability as influenced by soil pH. Sourced from Foth (1991).

### 3.2.4 Vegetative parameters, grain yield and yield components measurements

The representative data were collected from the three middle rows of each treatment. The vegetative parameters were measured from planting to physiological maturity, and data were collected from 5 randomly selected plants. The days to 50% emergence and flowering were counted and recorded. The number of plants per plot was counted after emergence and during harvest to determine the population. Plant height was measured using a measuring tape during the reproductive stage. Plant height was measured by keeping the measuring tape close to the stem of the crop. Without straightening the crop, the measurement was taken from the ground level to the tallest stem. Two leaves per plant were measured using a chlorophyll meter to determine the chlorophyll content. The leaves chosen for sampling were from the middle part of the plant and were fully intact, clean, dry, green, and free of disease or damage. The number of branches per plant was counted and recorded at physiological maturity. The number of pods on each plant was counted throughout harvest maturity, and after harvesting, the length of the pods was measured with a ruler. The number of seeds in each pod was counted. Pod weight, seed weight, and 100 seed weight were measured using a measuring scale.

### 3.2.5 Statistical analysis

The data were analyzed using Statistica software (StatSoft, TIBCO Software Inc., Dell, Tulsa, Oklahoma), the obtained data were subjected to an analysis of variance, and mean separation was carried out using Duncan Multiple Range Test at a 5% level of significance.

## 3.3. Results

### 3.3.1 Soil chemical analyses collected at Syferkuil and Ga-Molepo.

The chemical properties of soil samples taken at Syferkuil and Ga-Molepo for pre-planting and harvest are shown in Tables 3.1 and 3.2. Soil analysis at harvest revealed an increase in P, K, exchangeable acidity, acid saturation, Zn, Mn, and Cu compared to the samples collected at planting for both locations. Syferkuil exhibited higher levels of Ca, Mg, total cation, and organic carbon, however, Ga-Molepo had higher levels of Ca, Mg, total cation, and organic carbon at harvest. Soil samples collected at planting and harvest showed slightly alkaline soil at Syferkuil (7.73), whereas Ga-Molepo had acidic soil (4.62 and 4.78) for both pre-planting and at harvest. The percentage of N remained constant at Syferkuil, while Ga-Molepo showed an increase at harvest. Clay percentage was lower at harvest for both locations than pre-planting.

**Table 3.1:** Chemical properties of soil collected from Syferkuil and Ga-Molepo. The data reflects the average of soil samples collected.

| **Location** | **P** | **K** | **Ca** | **Mg** | **Exchangeable acidity** | **Total Cation** | **Acid saturation** |
|---|---|---|---|---|---|---|---|
| | | | **mg/L** | | **cmol/L** | | **%** |
| | | | **Pre-planting** | | | | |
| Syferkuil | 53.70 | 249.50 | 826.95 | 534.50 | 0.08 | 9.25 | 1.00 |
| Ga-Molepo | 5.65 | 207.65 | 467.55 | 132.35 | 0.09 | 4.04 | 2.20 |
| | | | **Harvest** | | | | |
| Syferkuil | 61.25 | 238.20 | 810.00 | 522.55 | 0.10 | 9.05 | 1.10 |
| Ga-Molepo | 8.35 | 259.15 | 548.05 | 132.60 | 0.15 | 4.64 | 3.10 |

**Table 3.2:** Chemical characteristics of soil collected at Syferkuil and Ga-Molepo. The data represents a mean of the collected soil samples.

| **Location** | **pH** | **Zn** | **Mn** | **Cu** | **Org. C** | **N** | **Clay** |
|---|---|---|---|---|---|---|---|
|  | **KCl** |  | **mg/L** |  |  | **%** |  |
|  |  |  | **Pre-planting** |  |  |  |  |
| Syferkuil | 7.73 | 2.16 | 16.00 | 2.76 | 0.51 | 0.05 | 19.95 |
| Ga-Molepo | 4.62 | 0.76 | 18.15 | 1.60 | 0.67 | 0.06 | 19.30 |
|  |  |  | **Harvest** |  |  |  |  |
| Syferkuil | 7.73 | 3.15 | 22.25 | 3.01 | 0.19 | 0.05 | 17.45 |
| Ga-Molepo | 4.78 | 1.01 | 29.40 | 1.91 | 0.73 | 0.07 | 18.80 |

### 3.3.2 Growth and yield components of tepary bean in response to VAM and P application at Syferkuil and Ga-Molepo

Tepary bean growth and yield components in response to VAM and P treatment at Syferkuil and Ga-Molepo are presented in Tables 3.3, 3.4, 3.5, and 3.6 using three-way ANOVA. The results for parameters 50% emergence, plant height, chlorophyll content, pod length, pod weight, and grain yield displayed significant differences in response to VAM and P application at Syferkuil and Ga-Molepo. Significant difference was observed on the number of days to 50% emergence and plant height due to the application of different P-rates (Table 3.3). Tables 3.3 and 3.4 showed a significant difference between the locations of plant height and chlorophyll content, respectively. VAM inoculation levels and the interaction of VAM and P rates significantly differed in pod length (Table 3.5). A significant difference in pod weight and grain yield was observed between the locations (Table 3.6). The analysis of variance confirmed the significance of the parameters and parameters with non-significant difference are not presented.

**Table 3.3:** Three-Way ANOVA analysis for growth and yield components of tepary bean in response to VAM and P application at Syferkuil and Ga-Molepo.

| **Source of Variation** | **Degree of freedom** | **Sum of square** | **Mean square** | **Variance ratio** | ***p* value** |
|---|---|---|---|---|---|
| **50% emergence** | | | | | |
| VAM | 1 | 0.49 | 0.49 | 0.01 | 0.92$^{ns}$ |
| Phosphorus | 4 | 609.49 | 152.37 | 3.06 | 0.02* |
| Location | 1 | 4.16 | 4.16 | 0.08 | 0.77$^{ns}$ |
| VAM*Phosphorus | 4 | 3.20 | 0.80 | 0.02 | 1.00$^{ns}$ |
| VAM*Location | 1 | 0.49 | 0.49 | 0.01 | 0.92$^{ns}$ |
| Phosphorus*location | 4 | 31.09 | 7.77 | 0.16 | 0.96$^{ns}$ |
| VAM*Phosphorus*Location | 4 | 3.20 | 0.80 | 0.02 | 1.00$^{ns}$ |
| Residual | 380 | 18919.36 | 49.79 | | |
| Total | 399 | 19574.31 | | | |
| **50% flowering** | | | | | |
| VAM | 1 | 0.06 | 0.06 | 0.00 | 0.99$^{ns}$ |
| Phosphorus | 4 | 11.48 | 2.87 | 0.01 | 0.99$^{ns}$ |
| Location | 1 | 14.70 | 14.70 | 0.05 | 0.83$^{ns}$ |
| VAM*Phosphorus | 4 | 2.50 | 0.62 | 0.00 | 1.00$^{ns}$ |
| VAM*Location | 1 | 1.38 | 1.38 | 0.00 | 0.95$^{ns}$ |
| Phosphorus*location | 4 | 8.93 | 2.23 | 0.01 | 1.00$^{ns}$ |
| VAM*Phosphorus*Location | 4 | 1.33 | 0.33 | 0.00 | 1.00$^{ns}$ |
| Residual | 380 | 115405.33 | 303.70 | | |
| Total | 399 | 115441.10 | | | |
| **Plant height** | | | | | |
| VAM | 1 | 26.55 | 26.55 | 0.76 | 0.38$^{ns}$ |
| Phosphorus | 4 | 323.27 | 80.82 | 2.31 | 0.05* |
| Location | 1 | 3155.35 | 3155.35 | 90.19 | 0.00** |
| VAM*Phosphorus | 4 | 193.09 | 48.27 | 1.38 | 0.24$^{ns}$ |
| VAM*Location | 1 | 12.80 | 12.80 | 0.37 | 0.55$^{ns}$ |
| Phosphorus*location | 4 | 171.35 | 42.84 | 1.22 | 0.23$^{ns}$ |
| VAM*Phosphorus*Location | 4 | 82.20 | 20.55 | 0.59 | 0.67$^{ns}$ |
| Residual | 380 | 13294.82 | 34.99 | | |
| Total | 399 | 17426.29 | | | |

**Table 3.4:** Three-Way ANOVA analysis for growth and yield components of tepary bean in response to VAM and P application at Syferkuil and Ga-Molepo.

| **Source of Variation** | **Degree of Freedom** | **Sum of Square** | **Mean Square** | **Variance ratio** | ***p* value** |
|---|---|---|---|---|---|
| **Chlorophyll content** | | | | | |
| VAM | 1 | 82.39 | 82.39 | 1.07 | 0.30 $^{ns}$ |
| Phosphorus | 4 | 538.74 | 134.68 | 1.75 | 0.14 $^{ns}$ |
| Location | 1 | 3080.66 | 3080.66 | 40.13 | 0.00** |
| VAM*Phosphorus | 4 | 234.67 | 58.67 | 0.76 | 0.55 $^{ns}$ |
| VAM*Location | 1 | 74.62 | 74.62 | 0.97 | 0.32 $^{ns}$ |
| Phosphorus*location | 4 | 544.19 | 136.05 | 1.77 | 0.13 $^{ns}$ |
| VAM*Phosphorus*Location | 4 | 164.91 | 41.23 | 0.54 | 0.71 $^{ns}$ |
| Residual | 380 | 29172.24 | 76.77 | | |
| Total | 399 | 33736.09 | | | |

**Table 3.5:** Three-Way ANOVA analysis for morphological and physiological parameters of tepary bean in response to VAM and P application at Syferkuil and Ga-Molepo

| Source of Variation | Degree of freedom | Sum of square | Mean square | Variance ratio | *p* value |
|---|---|---|---|---|---|
| **Number of branches per plant** | | | | | |
| VAM | 1 | 6.08 | 6.08 | 0.37 | 0.54$^{ns}$ |
| Phosphorus | 4 | 44.26 | 11.06 | 0.67 | 0.61$^{ns}$ |
| Location | 1 | 0.37 | 0.37 | 0.02 | 0.88$^{ns}$ |
| VAM*Phosphorus | 4 | 6.54 | 1.63 | 0.10 | 0.92$^{ns}$ |
| VAM*Location | 1 | 2.44 | 2.44 | 0.15 | 0.70$^{ns}$ |
| Phosphorus*location | 4 | 5.03 | 1.26 | 0.08 | 0.99$^{ns}$ |
| VAM*Phosphorus*Location | 4 | 5.23 | 1.31 | 0.08 | 0.99$^{ns}$ |
| Residual | 380 | 6288.96 | 16.55 | | |
| Total | 399 | 6363.78 | | | |
| **Pod length** | | | | | |
| VAM | 1 | 1.18 | 1.18 | 3.71 | 0.05* |
| Phosphorus | 4 | 1.23 | 0.31 | 0.96 | 0.43$^{ns}$ |
| Location | 1 | 0.14 | 0.14 | 0.42 | 0.52$^{ns}$ |
| VAM*Phosphorus | 4 | 3.67 | 0.92 | 2.87 | 0.02* |
| VAM*Location | 1 | 0.39 | 0.39 | 1.22 | 0.27$^{ns}$ |
| Phosphorus*location | 4 | 1.78 | 0.44 | 1.39 | 0.23$^{ns}$ |
| VAM*Phosphorus*Location | 4 | 2.14 | 0.53 | 1.68 | 0.15$^{ns}$ |
| Residual | 380 | 121.27 | 0.32 | | |
| Total | 399 | 131.45 | | | |
| **Number of seeds per pod** | | | | | |
| VAM | 1 | 0.86 | 0.86 | 1.22 | 0.23$^{ns}$ |
| Phosphorus | 4 | 1.36 | 0.34 | 0.48 | 0.75$^{ns}$ |
| Location | 1 | 2.17 | 2.17 | 3.07 | 0.08$^{ns}$ |
| VAM*Phosphorus | 4 | 3.59 | 0.90 | 1.27 | 0.28$^{ns}$ |
| VAM*Location | 1 | 0.05 | 0.05 | 0.08 | 0.78$^{ns}$ |
| Phosphorus*location | 4 | 2.56 | 0.64 | 0.91 | 0.46$^{ns}$ |
| VAM*Phosphorus*Location | 4 | 1.85 | 0.46 | 0.66 | 0.62$^{ns}$ |
| Residual | 380 | 268.21 | 0.71 | | |
| Total | 399 | 280.79 | | | |

**Table 3.6:** Three-Way ANOVA analysis in response to VAM and P application of tepary bean growth and yield components at Syferkuil and Ga-Molepo.

| **Source of Variation** | **Degree of freedom** | **Sum of square** | **Mean square** | **Variance ratio** | ***p* value** |
|---|---|---|---|---|---|
| **100 Seed weight** | | | | | |
| VAM | 1 | 0.00 | 0.00 | 0.00 | 0.99$^{ns}$ |
| Phosphorus | 4 | 2.61 | 0.65 | 0.04 | 1.00$^{ns}$ |
| Location | 1 | 1.74 | 1.74 | 0.11 | 0.74$^{ns}$ |
| VAM*Phosphorus | 4 | 0.26 | 0.07 | 0.00 | 1.00$^{ns}$ |
| VAM*Location | 1 | 0.05 | 0.05 | 0.00 | 0.95$^{ns}$ |
| Phosphorus*location | 4 | 0.09 | 0.02 | 0.00 | 1.00$^{ns}$ |
| VAM*Phosphorus*Location | 4 | 0.44 | 0.11 | 0.01 | 1.00$^{ns}$ |
| Residual | 380 | 5967.48 | 15.70 | | |
| Total | 399 | 5973.00 | | | |
| **Pod weight** | | | | | |
| VAM | 1 | 3915.73 | 3915.73 | 0.08 | 0.77$^{ns}$ |
| Phosphorus | 4 | 90161.69 | 22540.42 | 0.48 | 0.75$^{ns}$ |
| Location | 1 | 504987.69 | 504987.69 | 10.75 | 0.00** |
| VAM*Phosphorus | 4 | 17176.68 | 4294.17 | 0.09 | 0.99$^{ns}$ |
| VAM*Location | 1 | 1353.98 | 1353.98 | 0.03 | 0.87$^{ns}$ |
| Phosphorus*location | 4 | 24042.35 | 6010.59 | 0.13 | 0.97$^{ns}$ |
| VAM*Phosphorus*Location | 4 | 7541.20 | 1885.30 | 0.04 | 1.00$^{ns}$ |
| Residual | 380 | 17857782.06 | 46994.16 | | |
| Total | 399 | 18542296.73 | | | |
| **Grain yield** | | | | | |
| VAM | 1 | 2899.76 | 2899.76 | 0.14 | 0.71$^{ns}$ |
| Phosphorus | 4 | 36174.77 | 9043.69 | 0.43 | 0.79$^{ns}$ |
| Location | 1 | 231714.70 | 231714.70 | 10.96 | 0.00$^{**}$ |
| VAM*Phosphorus | 4 | 10011.46 | 2502.87 | 0.12 | 0.98$^{ns}$ |
| VAM*Location | 1 | 657.72 | 657.72 | 0.03 | 0.86$^{ns}$ |
| Phosphorus*location | 4 | 9007.67 | 2251.92 | 0.11 | 0.98$^{ns}$ |
| VAM*Phosphorus*Location | 4 | 4224.99 | 1056.25 | 0.05 | 1.00$^{ns}$ |
| Residual | 380 | 8032698.19 | 21138.68 | | |
| Total | 399 | 8343509.26 | | | |

The number of days to 50% emergence significantly differed as a result of phosphorus application (Figure 3.3). While not significantly different, 60 and 80 kg/ha P applications differed considerably from the other treatments. The difference between treatments 20 and 40 kg/ha P was not statistically significant from one another, although they were statistically different from the other treatments. Treatment 0 kg/ha P had the lowest number of days to 50% emergence, while treatment 80 kg/ha P took longer to reach 50% emergence.

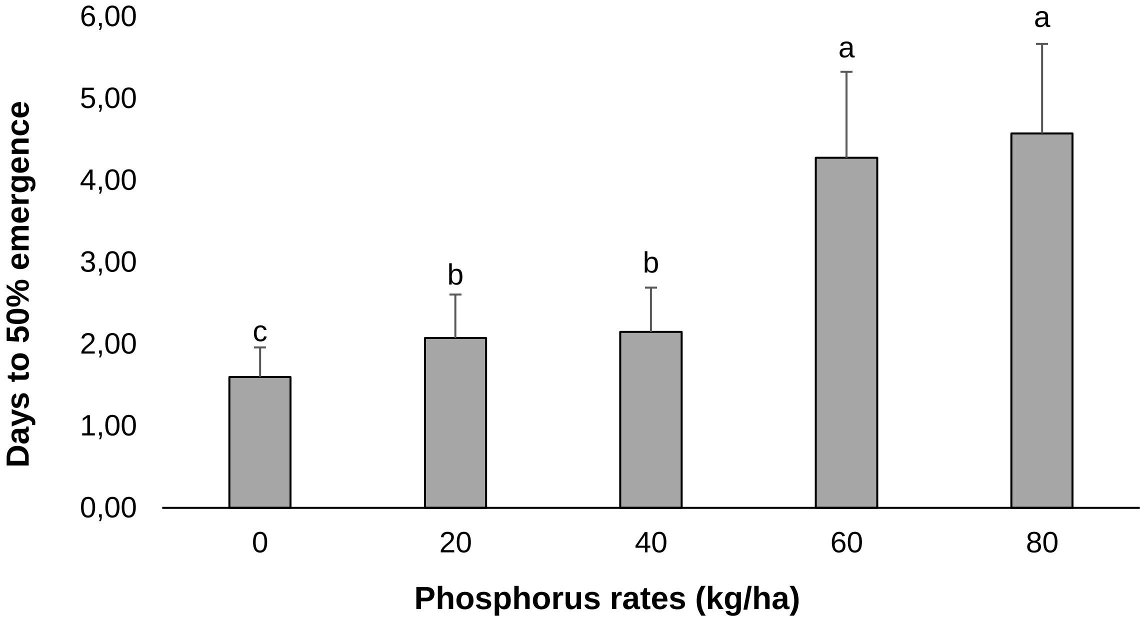

**Figure 3.3:** P rates on the number of days to 50% emergence. The error bars represent the standard error. Bars followed by the same letters are non-significant at p≤0.05.

The levels of VAM inoculation resulted in a significant difference in the length of the pods. VAM inoculated treatment was significantly higher than uninoculated treatment (Figure 3.4). VAM application resulted in a higher pod length of 6.43 cm, while no application had a lower pod length of 6.30 cm.

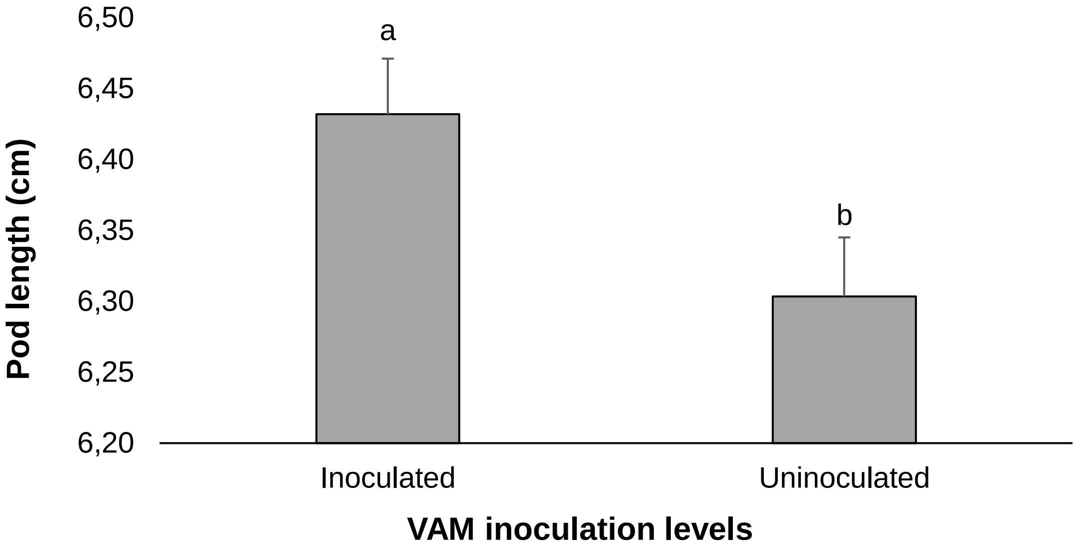

**Figure 3.4:** Pod length in response to VAM inoculation levels. The standard error is indicated by the error bars. Bars with identical letters are not significant at $p \leq 0.05$.

The interactive effect of VAM and P significantly differed in pod length. The interaction of P and VAM inoculated plants varied with the interaction of P and uninoculated plants. When compared to the other treatments, the application of 60 kg/ha of P inoculated resulted in longer pods, while 40 kg/ha of P uninoculated produced shorter pods (Figure 3.5).

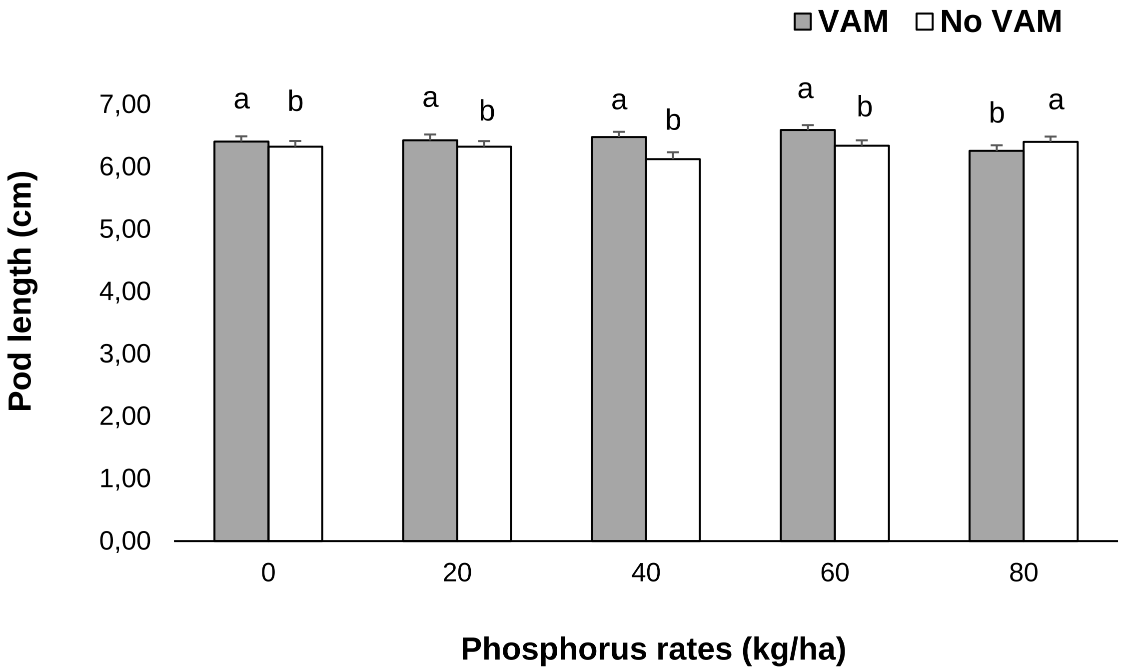

**Figure 3.5:** Two-way interactive effect of VAM inoculation levels and P rates on pod length. The error bars indicate the standard error. Bars with a common letter(s) in a column do not differ significantly at p≤0.05.

There was a significant difference between the means of applied P fertilizer rates on plant height. Treatments 20, 40, and 80 kg/ha P did not differ significantly, although they differed from the other treatments (Figure 3.6). The application of 60 kg/ha of P differed substantially from the application of 0 kg/ha of P. Application of 60 kg/ha P resulted in longer plants (34.42 cm). In contrast, the application of 0 kg/ha P had shorter plants (28.38 cm).

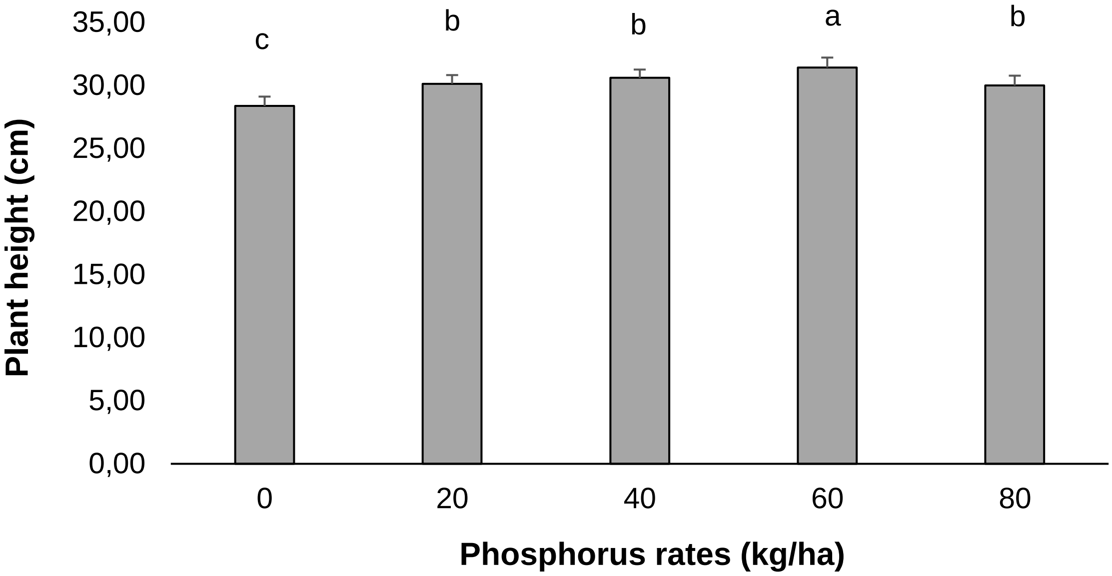

**Figure 3.6:** Plant height in response to P rates. The error bars represent the standard error. Bars followed by the same letters are non-significant at $p \leq 0.05$.

A highly significant difference was observed between the locations of plant height. The tepary bean plants in Syferkuil were taller than those surveyed in Ga-Molepo (Figure 3.7).

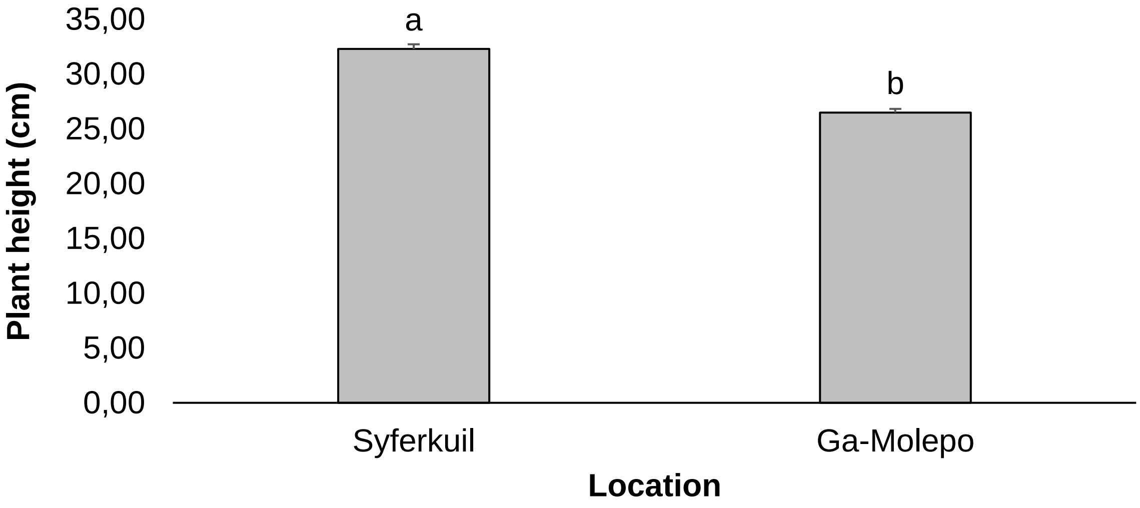

**Figure 3.7:** Plant height of tepary bean at Syferkuil and Ga-Molepo. The standard error is indicated by the error bars. Bars with identical letters are not significant at p≤0.05.

The difference in chlorophyll content was significant at p≤0.05. Figure 3.8 shows that the chlorophyll content at Ga-Molepo was higher than that at Syferkuil.

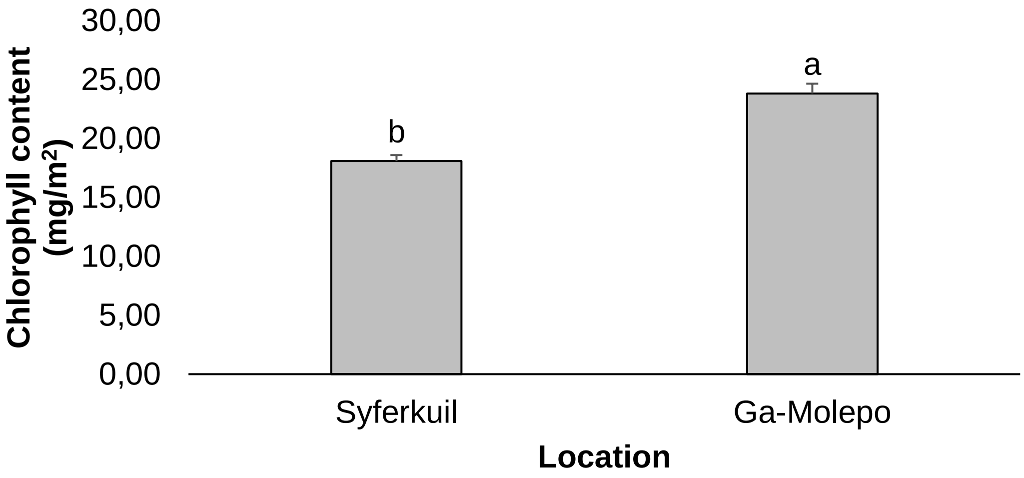

**Figure 3.8:** Chlorophyll content of tepary bean at Syferkuil and Ga-Molepo. The error bars indicate the standard error. Different letters over error bars indicate significant differences between the locations at the p≤0.05 level.

Figure 3.9 showed significant differences between the locations on pod weight. The tepary bean plants at Syferkuil produced a greater pod weight than that observed at Ga-Molepo.

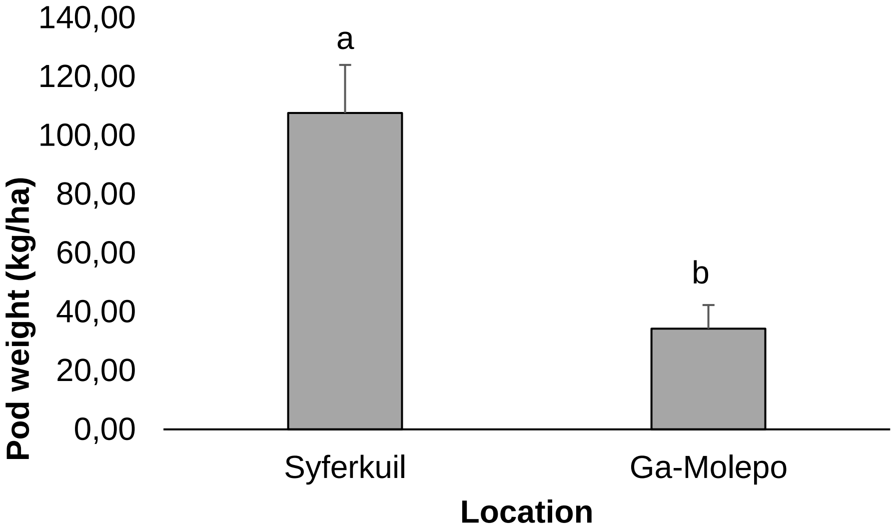

**Figure 3.9:** Pod weight of tepary bean at Syferkuil and Ga-Molepo. The error bars indicate the standard error. Bars with dissimilar letters are significant at a 5% level of significance.

There was a significant variation between the locations of grain yield (Figure 3.10). Syferkuil produced a significantly higher yield than Ga-Molepo.

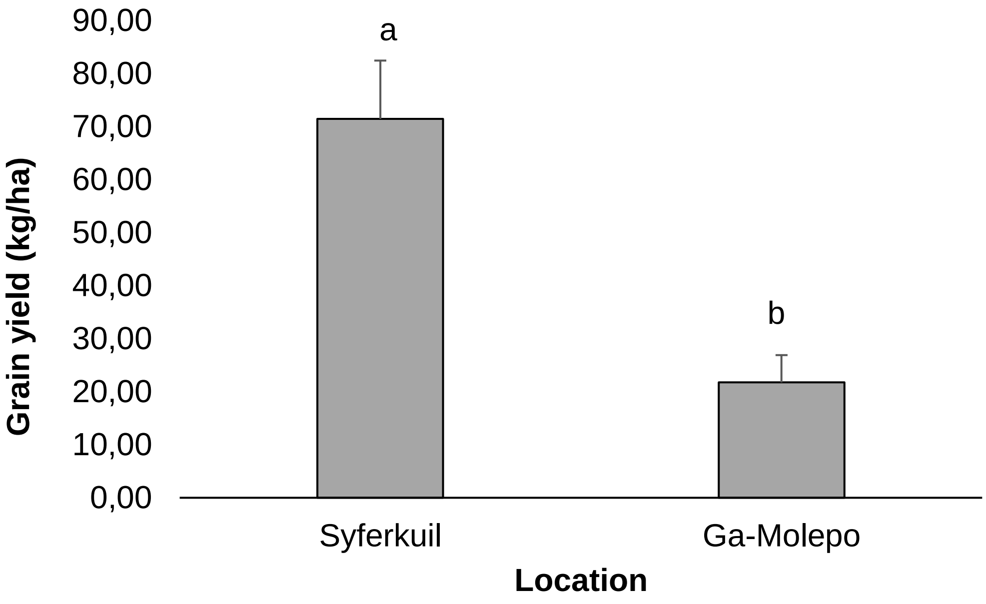

**Figure 3.10:** Grain yield ANOVA of tepary bean at Syferkuil and Ga-Molepo. The error bars represent the standard error. Bars followed by different letters are significant at p≤0.05.

## 3.4. Discussion

### 3.4.1 Effect of P on the number of days to 50% emergence

In the present study, phosphorus application significantly affected the days to 50% of emergence. The days to 50% emergence under 80 kg/ha P was substantially higher than in the other treatments. The number of days to reach 50% emergence increased with an increase in P application. The study shows that adding P fertilizer resulted in the poor emergence of tepary beans. The negative impacts of excessive mineral P supply are likely what delayed seedling emergence in treatments with P supply. This occurs because fertilizers contain salts, increasing the osmotic pressure and thereby affecting the capacity of the seeds/seedlings to absorb water (Mike, 2012). Due to a high concentration in the soil that exceeds the acceptable limit, fertilizer application adjacent to seeds may have a negative impact on germination (Mike, 2012). Phosphorus appears to directly impact germination mechanisms, as evidenced by the reduced germination in the treatments with increased P levels (Johnson, 2004). Due to the ion balance of other required ions of physiological significance, such as Zn inside the seed, which is a crucial nutrient for the seed's germination process, phosphorus exposure exhibits an antagonistic effect on germination (Ajouri *et al*., 2004). Absorption of micronutrients and N is reduced by high P (Ova *et al.*, 2015). Similar observations were made by Chmelíková and Hejcman (2012), who discovered that *Trifolium arvense* L. field emergence was significantly affected by P fertilizer treatment. Mao *et al.* (2021) reported that adding P considerably reduced seedlings' survival rate, and the negative impacts of P application may be attributed to a higher carbon cost due to the tissue maintenance of plants with higher P concentration (Mao *et al.*, 2021).

### 3.4.2 Effect of VAM on pod length

Significant differences in pod length were found due to the effect of VAM inoculation levels. Treatment with VAM was much more effective than treatment without it. The increased pod length of inoculated plants may be related to the mycorrhizae’s capacity to promote nutrient uptake, particularly highly immobile elements such as P and micronutrients like Zn and Cu (Dash and Gupta, 2011). Zinc deficiency may result in the loss of flowers, leaves, and fruits, reducing the yield (Ahmad *et al.*, 2012). Plant production is increased using fertilizers like Zn and Cu, which have an efficient crop response, such as expanding the pod length (Davarpanah *et al.*, 2016). The findings of this study are supported by those of Yaseen *et al.* (2021) and Devhade *et al.* (2018), who discovered that pod length was significantly impacted by VAM inoculation levels and that inoculated treatments on the green gram (*Vigna radiata*) and French bean (*Phaseolus vulgaris*) had longer pod length as compared to uninoculated treatments.

### 3.4.3 Interactive effect of VAM inoculation levels and P rates on pod length

Significantly higher pods were measured in treatment with 60 kg/ha P inoculated. This may be because organic fertilizers contain plant nutrients, growth-promoting substances, and beneficial microflora combined with inorganic fertilizers, which provide favourable soil conditions to enhance nutrient use efficiency (Bam *et al.*, 2022). Kalaiyarasan *et al.* (2017) stated that mycorrhizal inoculation and phosphorus application increase photosynthetic activity, which leads to maximum crop growth (Kirschbaum, 2011), and as a result, increases pod length. Nouri *et al.* (2014) reported that plant growth was increased in mycorrhizal plants, and there was an improvement in mineral nutrition. Similar results of significant differences in pod length have been reported by Yaseen *et al.* (2013), mentioning that VAM inoculation results in increased pod length. The highest P application, inoculated, resulted in a lower pod length. The excess P supply reduces VAM's ability to colonize the roots, decreasing membrane cell permeability (Indriani *et al.*, 2016). According to Curtis and Swift (2004), high soil phosphorus levels greatly minimize VAM infection.

### 3.4.4 Plant height in response to P rates.

The effects of different P fertilizer application rates on plant height varied significantly. Compared to the highest P rate of 80 kg/ha, the application of 60 kg/ha P resulted in maximum plant height. This could be because high phosphorus levels frequently lead to nutrient interactions and may affect the availability of other nutrients required for bean growth (Zebire and Gelgelo, 2019). Another reason may be the fact that the P requirement was already fulfilled. The P application of all the treatments in this study resulted in significantly higher plant height than the application of 0 kg/ha P. This is in contrast with the study reported by Zebire and Gelgelo (2019), where a non-significant response in plant height following P application on haricot beans was noted, and the maximum rate of P application (69 kg/ha) had no impact on plant height as compared to the application of 46 kg/ha P. Furthermore, according to Turuko and Mohammed (2014), the use of P fertilizer had no discernible impact on the common bean plant's height. Zafar *et al.* (2011) found that plant height increased when phosphorus fertilizer was applied at 60 kg/ha.

### 3.4.5 Response of tepary bean height to location

The locations highly influenced plant height. The effect of soil pH recorded in this study might have caused this. Ga-Molepo's pH was substantially lower than Syferkuil, which had an alkaline pH (Table 3.2), resulting in significantly shorter plants. According to Minasny *et al.* (2016), soil pH is classified as the "master soil variable" because it affects a wide range of soil chemical, physical and biological properties and processes that impact plant growth and yield. The microbial activity requires a pH ranging from 5.5-8.8 to function appropriately (Pietri and Brookes, 2008). This implies that mycorrhiza at Ga-Molepo was ineffective since the pH was lower than that required by microorganisms to survive. Similar results were observed by Correa *et al.* (2006), who reported that low pH had a significant impact on *Medicago sativa* plant development. Correa *et al.* (2006) also indicated that plants grew significantly more when the pH was higher than 5.0, whereas they grew poorly when the pH was lower.

Another reason for a shorter plant height at Ga-Molepo might be related to moisture deficiency. Water plays a crucial role in the growth of beans (Ahmed *et al.*, 2018). Rainfall was supplemented by irrigation at Syferkuil, whereas Ga-Molepo depended solely on rainfall. The study sites received less rainfall during the crop growing period and since Syferkuil was supplemented with irrigation, it can be concluded that moisture increased plant height at Syferkuil, resulting in a greater plant height than Ga-Molepo.

### 3.4.6 Chlorophyll content of tepary bean in at Syferkuil and Ga-Molepo

In comparison to Syferkuil, Ga-Molepo had a greater chlorophyll concentration. This may be because the N percentage at Ga-Molepo was more significant than at Syferkuil (Table 3.2). Chlorophyll is a crucial photosynthetic pigment for plants, and it plays an essential role in determining photosynthetic capacity and, consequently, plant growth (Li *et al.*, 2018). Nitrogen and P are some of the essential elements necessary for chlorophyll synthesis. The chlorophyll synthesis is directly impacted by N, Mg, and iron (Fe) deficiencies (Kalaji *et al.*, 2018). According to Hamann *et al.* (2020), the photosynthetic pigment increased with an increase in N application. Despite Syferkuil soil being higher in P and Mg than Ga-Molepo's, chlorophyll was still low. Based on Figure 3.2, it can be concluded that Fe was higher at Ga-Molepo as compared to Syferkuil, which might have affected the chlorophyll content. Buapet *et al.* (2008) showed that chlorophyll concentration was not significantly impacted by P supplementation but increased after applying nitrogen. As a result of the positive correlation between the amount of N in the plant and the quantity of the photosynthetic pigment, the chlorophyll content in the leaf is used to estimate the nutritional level of N in plants (Jia *et al.*, 2021).

Several other factors, like temperature, affect the chlorophyll content of a crop. According to Li *et al.* (2018), chlorophyll is produced through a series of enzymatic reactions; however, if the temperature is too high or low, the enzyme reaction is inhibited, and the original chlorophyll may even be destroyed. For Mungbean, 28-30 °C is the ideal temperature for chlorophyll synthesis, with a decline in the photosynthetic rate at 36 °C (Islam *et al.*, 2022). Since the two study sites fall under the same district, the temperature for both locations did not vary (Figure 3.1). Therefore, the temperature did not lead to a significant difference between the locations. The maximum temperature for both locations ranged between 29.3 °C and 25.79 °C during the growing period (figure 3.1), and it can be considered an ideal temperature for chlorophyll synthesis. Zhao *et al.* (2020) found that chlorophyll biosynthesis was suppressed at low temperatures, decreasing chlorophyll accumulation.

### 3.4.7 Response of tepary bean pod weight and grain yield to location

The study showed a significant difference between the two locations on pod weight and grain yield. In comparison to samples obtained in Ga-Molepo, Syferkuil had much higher pod weight and grain yield. The observed variations in pod weight and grain yield could be attributed to the difference in climatic conditions between the locations, which affect the availability and functions of both P and VAM. Less than 300 mm of rain was recorded in the Ga-Molepo area during the summer growing season of 2020/2021. If the plants do not receive sufficient moisture during the early developmental stages, germination and emergence can be negatively affected, leading to poor crop stand. This is supported by Staton (2020), who indicated that inadequate soil moisture could cause uneven and spotty emergence during germination. Crop stand establishment is essential for the crop’s life cycle because it can result in well-established planting, rapid development, and high crop yields (Dong *et al.*, 2008).

At Syferkuil, irrigation was used to supplement the rainfall, leading to a higher pod weight and grain yield than Ga-Molepo. The reproductive stage experienced less than 1mm of rainfall at Ga-Molepo. This negatively affects crop yield (Ndimbo *et al.*, 2015) as moisture deficiency leads to flower abortion (Staton, 2020), thereby affecting the number and weight of the pods and grains. Similar findings showed that the soil's water deficiency significantly impacted corn's vegetative and yield properties (Çakir, 2004).

The environmental conditions at Syferkuil were more suitable, as seen by the increased pod weight and grain yield compared to Ga-Molepo. It is a result of the AM fungus creating external mycelium surrounding the roots, which may have served to increase the number of nutrients available to the roots' surface, increasing water and nutrient uptake, thus increasing pod weight and grain yield. Moisture stress has the potential to inhibit nodulation, and this renders the application of VAM ineffective at Ga-Molepo as the fungi are affected by low levels of water. Common beans' moisture stress strongly influenced nodulation, nitrogen fixation, and grain yields (Ndimbo *et al.*, 2015). Rhizobia have the most trouble growing in marginal lands with little rainfall. The alteration of rhizobial cells by water stress will eventually decrease infection and nodulation of legumes (Zahran, 1999). Tepary beans has the potential to produce a significant yield with an annual rainfall of less than 400 mm (Andrews, 2014). However, figure 3.1 showed average monthly rainfall of less than 1 mm during the life cycle of the crop. Although it is indicated that tepary has the potential to produce significant yield under low rainfall, the rainfall received was extremely low and this leads to the conclusion that there was water stress at Ga-Molepo. Kandel and Endres (2019) reported that dry, hot weather and short periods of water saturation cause nodules to slough off. Elhassan *et al.* (2010) found that the application of P and inoculation with arbuscular mycorrhizal fungus considerably increased the growth components and nodulation of faba beans, as a result, yield parameters increased.

Soil pH has a significant effect on biological nitrogen fixation. Tepary bean at Syferkuil performed better due to the symbiosis relation between the crop and VAM, which increases nitrogenase activity, thereby increasing N fixation. The soil pH at Ga-Molepo was acidic, while the pH at Syferkuil was alkaline (Table 3.2). Ferguson *et al.* (2013) reported that once the pH drops below 5.5, problems related to nodulation are expected, and this is because soil pH impacts chemical and biological activities (Devkota *et al.*, 2022). Microbial metabolism increases with an increase in soil pH (Anderson *et al.*, 2018), this implies that VAM deteriorates in acidic conditions. Aluminium toxicity occurs under acidic soils, limiting the availability of essential nutrients (Rout *et al.*, 2001). Even when soil testing reveals sufficient amounts of certain plant nutrients, these nutrients might become unavailable in acidic conditions. Ga-Molepo had a significantly lower nutritional status as compared to Syferkuil.

Figure 3.2 shows the availability of different nutrients at different pH levels, and elements such as N and P become unavailable at a pH less than 6.5. The soil pH at Ga-Molepo was acidic, affecting tepary bean production by decreasing yield. Phosphorus increased slightly after applying different P levels at Ga-Molepo (Table 3.2), and it can be related to P fixation. Phosphorus is usually unavailable for plants' optimum growth and development due to fixation (Malhotra *et al.*, 2018). The lack of phosphorus may also prevent nitrogen from being fixed through its impact on growth and nodule formation, nodule function, and host plant growth. . Since the P starting points at the different locations varied significantly, it can be concluded that this led to uneven levels of soil P after the application of experimental P rates, which then resulted in higher pod weight and grain yield at Syferkuil as compared to Ga-Molepo. Basic cations (Na, Ca, Mg, and K) are depleted as a result of leaching, and toxic metals (Al and Mn) become more soluble in acidic soil, which restricts the plant's access to water and nutrients (Yadav *et al.*, 2020). Calcium and sodium were higher at Ga-Molepo concerning Syferkuil. It can be concluded that Al toxicity occurred at Ga-Molepo as compared to Syferkuil, affecting the availability of nutrients and water for tepary beans. This results in severe root damage and decreased crop production (Yadav *et al.*, 2020).

### 3.5. Conclusion

The findings of this study conclusively reveal that VAM-inoculated plants outperformed uninoculated plants in terms of pod length. Plants cultivated with 60 kg/ha P had greater plant height. The interaction of VAM and P significantly increased the pod length of the tepary bean. Although plants planted at Syferkuil had less chlorophyll content than those grown at Ga-Molepo, they were taller, leading to increased pod weight and grain yield. The symbiotic interaction between VAM and tepary bean, which increased nutrient and water absorption and made P available for plant use, may have contributed to the grain yield. Additional research should be done to confirm the different combinations of VAM and P levels required to increase the growth and yield of tepary beans.

# CHAPTER 4

# SYMBIOTIC PERFORMANCE OF TEPARY BEAN IN RESPONSE TO PHOSPHORUS APPLICATION RATES AND VESICULAR-ARBUSCULAR MYCORRHIZA

## 4.1. Introduction

Legumes are crucial for sustainable farming due to their capacity to improve soil fertility (Reem, 2017). Leguminous plants can biologically fix nitrogen (N) from the atmosphere (Liu *et al.*, 2011). This occurs through a symbiotic interaction with certain soil bacteria (Jim, 2015). This can reduce or eliminate the need to apply N fertilizers because it can benefit legumes and any subsequent or intercropped crops (Liu *et al.*, 2011). According to Cheng (2008), the nitrogen fixation process occurs when molecular nitrogen is reduced to generate ammonia, which is the form of nitrogen utilized by living systems to create numerous bioorganic substances.

Due to soil nitrogen depletion and insufficient nitrogen fertilizer application, smallholder systems in Africa are especially challenged by low soil fertility (Rao *et al.*, 2016). Tepary beans can obtain some of the necessary nitrogen from the atmosphere through symbiotic nitrogen fixation for crop growth (Santachiara *et al.*, 2019; Fenta *et al.*, 2020). Numerous elements, such as the number of nodulating bacteria present and their density in the root zone during the emergence and development of root hairs, impact nitrogen fixation (Abdul Jabbar and Saud, 2012). Numerous studies have shown that legumes need more phosphorus as an energy source for nitrogen fixation to work at their best (Rotaru and Sinclair, 2009). Additionally, the phosphorus required to produce the mitochondrial and symbiosome membranes during nodule formation raises the N-fixing legume's requirement for phosphorus even further (Rotaru and Sinclair, 2009). Lack of P limits root development, photosynthesis, sugar transport, and other processes that either directly or indirectly affect legume plants' ability to fix nitrogen (Mitran *et al.*, 2018).

Vesicular arbuscular mycorrhizal (VAM) fungi play a significant role in soil-water relations and nutrient acquisition. The mycorrhizal fungus penetrates plant roots. Subsequently, their hyphae radiate into the surrounding soil, forming enormous networks of mycelium that can access volumes up to two orders of magnitude larger than those accessible to plants alone (Raven and Edwards, 2001; Makarov, 2019). This increase in root surface area and better nutrient absorption increases biological nitrogen fixation (Püschel *et al.*, 2017). Vesicular-arbuscular mycorrhizal has become a prominent part of low-input agricultural systems (Garg and Chandel, 2011; Follet and Hatfield, 2001). The AM symbiosis stimulates rhizobial activity (Chakrabarti *et al.*, 2010) and boosts BNF since AM fungi typically improve the P status of their host plants in low P availability (Smith and Read, 2008; Püschel *et al.*, 2017). When P availability is high, AM symbiosis rarely improves the host plant's P budget, making it less likely to increase BNF through improved P feeding (Smith and Read, 2008; Kleinert *et al.*, 2014).

Legumes have been reported to have improved nitrogen fixation and grain productivity due to increased phosphorus and other nutrients, better root nodulation, improved photosynthesis, beneficial interactions with rhizosphere microbes, and reduced environmental stressors by AM (arbuscular mycorrhizal) colonization (Javaid, 2010). The study aimed to determine tepary beans' symbiotic performance in response to phosphorus application rates and vesicular-arbuscular mycorrhiza**.**

## 4.2. Materials and methods

### 4.2.1. Site description, research design, treatment, and procedure.

The detailed methodology and the research design are outlined in Chapter three of the dissertation (sections 3.2.1, 3.2.2 and 3.2.3).

### 4.2.2. Determination of biomass

Three representative tepary bean plants were randomly selected at physiological maturity from each plot for biomass. With the aid of a fork, the plants were gently dug up, put in brown bags, and immediately taken to the lab for processing. Plants were separated into shoots and roots, weighed then dried in an oven at 60 °C to a constant weight, and the weight was recorded.

### 4.2.3. Measurements of $N_2$ fixation.

The $^{15}N$ natural abundance technique was used to evaluate nitrogen ($N_2$) fixation. Three tepary bean plants were carefully removed from each treatment and taken to the laboratory for processing. In the laboratory, the roots were separated from shoots and then shoots were oven-dried (at 60 °C) to a constant weight, weighed, ground (using a sieve with a mesh size of 0.45 mm), and stored before $^{15}N$ analysis. Tepary bean N uptake from the soil was determined using samples of three non-legume species growing as weeds in the experimental farm. The non-legume plants collected are referred to as reference plants, and they underwent a similar processing procedure to that of tepary beans.

### 4.2.4. $^{15}N/^{14}N$ isotopic analyses.

To measure the ratio of $^{15}N/^{14}N$ and N concentration (%N) in the plant material, about 2.0 mg of ground legume samples and 2.5 mg of reference plant samples were weighed into tin capsules, loaded on a Carlo Erba NA1500 Elemental Analyzer connected to a Finnigan MAT 252 Mass Spectrometer (Finnigan MAT GmbH, Bremen, Germany) via Conflo II Open-Split Device and ran. After every five runs of plant samples, an internal standard of Nasturtium spp. was added to account for isotopic fractionation machine faults.

The isotopic composition ($\delta^{15}$N) was measured by Unkovich *et al.* (2008):

$$\delta^{15}N(‰) = \frac{\left[{}^{15}\mathrm{N}/{}^{14}\mathrm{N}\right]_{\mathrm{sample}} - \left[{}^{15}\mathrm{N}/{}^{14}\mathrm{N}\right]_{\mathrm{atm}}}{\left[{}^{15}\mathrm{N}/{}^{14}\mathrm{N}\right]_{\mathrm{atm}}} \times 1000$$

The N content of the plant samples was determined as the product of %N and sample weight.

Percent N derived from atmospheric fixation (%Ndfa).

The formula used to determine the proportion of N derived by $N_2$ fixation is (Unkovich *et al.*, 2008):

$$\%\,\mathrm{Ndfa} = \frac{\delta^{15}\mathrm{N}_{\mathrm{ref}} - \delta^{15}\mathrm{N}_{\mathrm{leg}}}{\delta^{15}\mathrm{N}_{\mathrm{ref}} - \mathrm{B}} \times 100$$

Where B is the $^{15}$N natural abundance of tepary bean plants that rely only on $N_2$ fixation for their N nutrition, $^{15}N_{leg}$ is the $^{15}$N natural abundance of the legume, and $^{15}N_{ref}$ is the $^{15}$N natural abundance of the reference plant. The B value (−1.80 ‰) was used to calculate the %Ndfa (Balboa and Ciampitti, 2020).

The amount of N-fixed was calculated by Peoples *et al.* (2008):

N-fixed = %Ndfa × legume biomass N

Where legume biomass N was the N content of tepary bean shoots and pods.

## 4.3. Results

### 4.3.1. $\delta^{15}N$ values of reference plants.

To estimate the %Ndfa in tepary bean plants, the shoots of six non-legume species were collected at Syferkuil and Ga-Molepo as reference plants (Table 4.1). The $\delta^{15}N$ mean values at Syferkuil and Ga-Molepo are 8.22‰ and 6.40‰, respectively (Table 4.1). The $\delta^{15}N$ at Syferkuil ranged from 7.49‰ to 8.73‰, whereas Ga-Molepo ranged from 5.69‰ to 6.96‰ (Table 4.1).

**Table 4.1:** $\delta^{15}N$ of non-legume plant species used as reference plants. For every sample, three runs were conducted.

| **Location** | **Reference plants** | | **$\delta^{15}N$** |
|---|---|---|---|
| | Common names | Botanical names | ‰ |
| Syferkuil | Lesser Jack | *Rumex spinosus* | 8.73 |
| | Bull thistle | *Cirsium vulgare* | 7.49 |
| | Jungle rice | *Echinochloa colona* | 8.43 |
| | | **Mean** | **8.22 ±0.37** |
| Ga-Molepo | Carpetweed | *Mollugo verticillata* | 6.96 |
| | Horseweed | *Conyza canadensis* | 5.69 |
| | Bermuda grass | *Cynodon dactylon* | 6.54 |
| | | **Mean** | **6.40 ±0.23** |

### 4.3.2. Dry matter, $\delta^{15}N$, %Ndfa and N fixed values at Syferkuil.

Dry matter, $\delta^{15}N$, %Ndfa and N fixed was insignificant at Syferkuil for both P and VAM application (Table 4.2). Dry matter affected by P rates ranged from 8.52 g to 24.61 g. The $\delta^{15}N$ varied from 6.15‰ to 6.83‰, while Ndfa varied from 16.58% to 23.16%. The N fixed increased with an increase in the P application. VAM-inoculated plants had higher values of dry matter, $\delta^{15}N$, and N fixed than uninoculated plants, except for %Ndfa.

**Table 4.2:** Dry matter and symbiotic performance of tepary bean samples at Syferkuil during the 2020/2021 summer growing season.

| P rates (kg/ha) | Dry matter g | $\delta^{15}N$ ‰ | Ndfa % | N Fixed mg |
|---|---|---|---|---|
| 0 | $8.52^{c}$ | $6.35^{a}$ | $21.28^{a}$ | $36.19^{c}$ |
| 20 | $24.61^{a}$ | $6.83^{a}$ | $16.58^{b}$ | $69.64^{b}$ |
| 40 | $15.60^{b}$ | $6.70^{a}$ | $17.86^{b}$ | $57.83^{b}$ |
| 60 | $15.53^{b}$ | $6.36^{a}$ | $21.20^{a}$ | $68.85^{b}$ |
| 80 | $23.28^{a}$ | $6.15^{a}$ | $23.16^{a}$ | $101.47^{a}$ |
| F-statistics | $1.07^{ns}$ | $0.43^{ns}$ | $0.43^{ns}$ | $0.65^{ns}$ |
| VAM | | | | |
| Inoculated | $22.02^{a}$ | $6.52^{a}$ | $19.60^{a}$ | $80.79^{a}$ |
| Uninoculated | $13.00^{b}$ | $6.43^{a}$ | $20.44^{a}$ | $52.81^{b}$ |
| F-statistics | $2.73^{ns}$ | $0.06^{ns}$ | $0.06^{ns}$ | $1.25^{ns}$ |

*ns = non-significant*

### 4.3.3. Dry matter, $\delta^{15}N$, %Ndfa and N fixed values sampled at Ga-Molepo.

The dry matter and N fixed at Ga-Molepo increased with an increase in P application. A significant difference was observed in the $\delta^{15}N$, %Ndfa, and N fixed values resulting from the P application. The $\delta^{15}N$ values varied from 3.69‰ to 6.04‰, while Ndfa ranged from 4.40% to 33.05% (Table 4.3). Plants inoculated with VAM had higher values of dry matter and $\delta^{15}N$ than uninoculated plants, however, the values of %Ndfa and N fixed were lower.

**Table 4.3:** Analysis of Dry matter and symbiotic performance of tepary bean samples collected at Ga-Molepo during the 2020/2021 summer growing season.

| P rates (kg/ha) | Dry matter g | $\delta^{15}N$ ‰ | Ndfa % | N Fixed mg |
|---|---|---|---|---|
| 0 | $16.77^{c}$ | $6.04^{a}$ | $4.40^{d}$ | $10.98^{d}$ |
| 20 | $33.54^{b}$ | $5.62^{a}$ | $9.54^{c}$ | $61.73^{c}$ |
| 40 | $39.37^{b}$ | $5.24^{a}$ | $14.11^{b}$ | $103.86^{b}$ |
| 60 | $43.66^{a}$ | $5.12^{a}$ | $15.56^{b}$ | $155.84^{b}$ |
| 80 | $47.58^{a}$ | $3.69^{b}$ | $33.05^{a}$ | $343.83^{a}$ |
| F-statistics | $2.42^{ns}$ | $4.18^{*}$ | $4.81^{*}$ | $10.74^{*}$ |
| VAM | | | | |
| Inoculated | $41.91^{a}$ | $5.36^{a}$ | $12.66^{a}$ | $129.65^{b}$ |
| Uninoculated | $29.19^{b}$ | $4.88^{a}$ | $18.60^{a}$ | $142.08^{a}$ |
| F-statistics | $2.82^{ns}$ | $0.99^{ns}$ | $0.99^{ns}$ | $0.04^{ns}$ |

*ns = non-significant and * $p \leq 0.05$.*

There was a significant difference in the dry matter between the locations (Figure 4.1). Tepary bean plants from Ga-Molepo had significantly greater dry matter than tepary bean plants at Syferkuil.

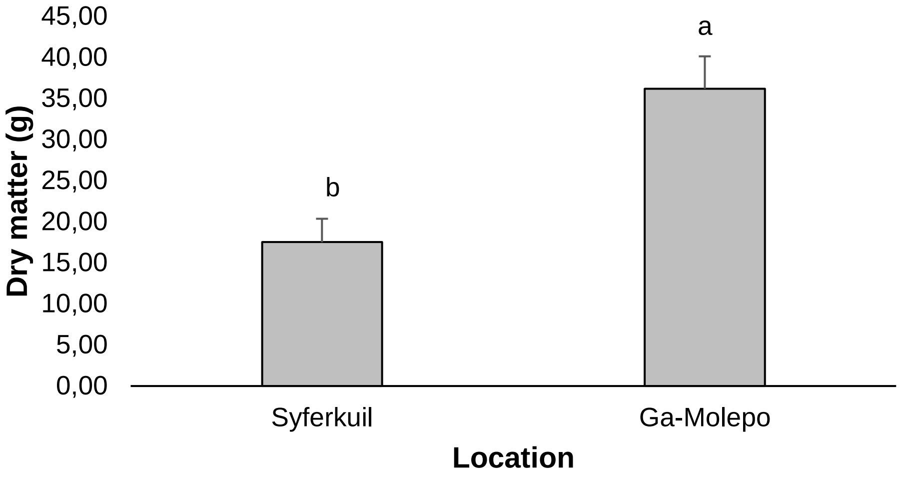

**Figure 4.1:** Dry matter of tepary bean sampled at Syferkuil and Ga-Molepo. Bars with dissimilar letters are significant at a 5% level of significance.

Figure 4.2 demonstrates a significant difference between Syferkuil and Ga-Molepo in the amount of N fixed. Ga-Molepo had a higher amount of N fixed at 135.25 mg, whereas N fixed at Syferkuil was 66.80 mg.

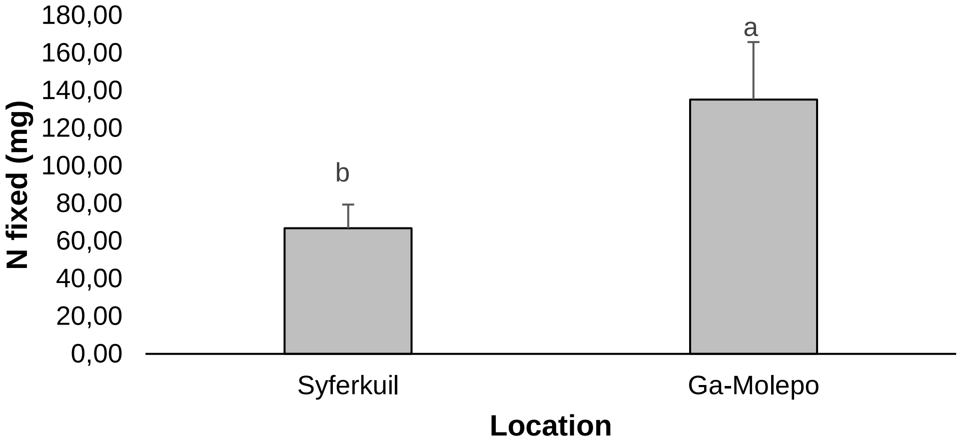

**Figure 4.2:** Effect of location on N fixed. Bars with dissimilar letters are significant at a 5% level of significance.

## 4.4. DISCUSSION

### 4.4.1. Location-specific symbiotic N contribution by tepary bean

Tepary bean growing at Syferkuil and Ga-Molepo was evaluated for its N contribution using the $^{15}N$ natural abundance approach. Three non-legume species were chosen as the reference plants, and their $^{15}N/^{14}N$ ratios were analyzed to determine the tepary bean plants (Table 4.1). The $\delta^{15}N$ readings at Syferkuil and Ga-Molepo ranged from 7.49‰ to 8.73‰ and 5.69‰ to 6.96‰, respectively (Table 4.1). Due to the P application, the difference between the highest tepary bean $\delta^{15}N$ value and the combined mean $\delta^{15}N$ value of reference plants was 1.39‰ for Syferkuil and 0.36‰ for Ga-Molepo. In contrast, for VAM levels, it was 1.70‰ for Syferkuil and 1.04‰ for Ga-Molepo. Typically, the $^{15}N$ natural abundance technique's precision increases with a more significant difference between the $\delta^{15}N$ value of the legume and the $\delta^{15}N$ mean value of the reference (Unkovich et al., 1994). This suggests that tepary bean $N_2$ fixation measurements were less precise. High fixation rates occur when a legume's $\delta^{15}N$ value approaches zero (Unkovich et al., 1994), and since the tepary bean has a high $\delta^{15}N$ value, the study shows that it is a poor fixing legume.

A non-significant difference was observed in dry matter, $\delta^{15}N$, %Ndfa and N fixed from samples collected at Syferkuil due to the application of both P and VAM. The insignificant difference in P levels can be associated with the very high concentration (53.70 mg/L) of P at planting (Table 3.1). The greatest N fixed (101.7 mg) was found in Syferkuil when 80 kg/ha P was applied, and this resulted in a greater %Ndfa (Table 4.1). Despite having the maximum dry matter and $\delta^{15}N$, the 20 kg/ha P application did not have the highest amount of N fixed. The application of 0 kg/ha P had the lowest dry matter, and the lowest amount of N fixed (36.19 mg). Inoculated plants produced more dry weight, $\delta^{15}N$, and N fixed than uninoculated tepary bean plants. This may be associated with the ability of VAM to extend the root surface area, thereby improving P acquisition (Makarov, 2019) which assisted in dry matter accumulation and nitrogen fixation.

Phosphorus application rates significantly differed on $\delta^{15}N$, %Ndfa and N fixed, whereas dry matter was insignificant at Ga-Molepo. The application of 80 kg/ha P and 60 kg/ha P had the highest amount of N fixed (155.84 and 343.83 mg, respectively), which stimulated an increase in dry matter and %Ndfa. Decreased dry weight, %Ndfa, and subsequently lower N fixed resulted from the treatment of 0 kg/ha P. The legume plants need P as adenosine triphosphate (ATP) during dinitrogen ($N_2$) fixation and the amount of N fixed increased as P application increased. Low P can limit bioenergetic processes impacting plant development and functions (Le Roux *et al.,* 2008). The soil analysis revealed that the soil at Ga-Molepo was moderately low in P (5.65 mg/L) at planting (Table 3.1). According to Aranjuelo *et al.* (2014), P is the primary component contributing to nodulation and BNF. This is shown by high N fixation in plants receiving 80 kg/ha P compared to low P-supplied plants in this study.

Although there was insignificant variation in the dry matter when plants were exposed to different P levels, dry weight increased with applying P. According to Dordas (2009), dry matter production is positively related to P availability, and when P supply is low, dry matter production becomes reduced, especially in leaves. The findings of this study are consistent with those of Magadlela *et al.* (2020), who indicated that legume plants grown in high P conditions had higher %Ndfa and BNF efficiency and that P-deficient plants had the lowest %Ndfa. It was also discovered that plants with low %Ndfa percentages altered their preferred nitrogen source to primarily soil N. (Magadlela *et al.,* 2020). Given that the P concentration was low during planting, the N percentage, which was 0.06%, may have contributed to the rise in N fixed at Ga-Molepo. These results align with previous research showing that *Mucuna pruriens* in nutrient-poor soils relied primarily on soil N to store the energy needed during BNF (Aranjuelo et al. 2014). VAM inoculation levels did not significantly impact dry matter, $\delta^{15}$N, %Ndfa and N fixed. Since soil acidity is known to reduce symbiotic nitrogen fixation in legumes (Mohammadi *et al.* 2012), which negatively affects the crops' growth (Bekere *et al.* 2013), this may be the source of the ineffectiveness of VAM. This would influence dry matter, $\delta^{15}$N, %Ndfa, and N fixed. Bordeleau and Prevost (1994) assert that soil pH can affect nodulation and nitrogen fixation in two situations: first, when the bacteria are sensitive to low pH, and second, when acidic conditions inhibit them following nodule establishment. Despite having more dry matter and $\delta^{15}$N, inoculated plants had less N fixed than non-inoculated plants. This is because the amount of N fixed decreases as the $\delta^{15}$N value increases (Unkovich *et al.*, 1994).

### 4.4.2. Comparison of geographical impacts on tepary bean's symbiotic N contribution

A comparison between the two locations showed that Ga-Molepo performed better than Syferkuil, it, therefore, produced greater dry matter and fixed more N (36.19 g and 135.25 mg, respectively). The chemical properties in Table 3.1 revealed that Ga-Molepo had considerably lower P (5.65 mg/L), while Syferkuil had a very high P amount (53.70 mg/L). The N percentage was lower at Syferkuil than Ga-Molepo (0.05 and 0.06 at planting and 0.05 and 0.07 at harvesting, respectively). To enhance P uptake and conservation, legumes have evolved physiological and morphological adaptations (Valdés-López and Hernández, 2008). This, as observed in this study, permits them to thrive in P-deficient soils. Nitrogen and P generally restrict plant growth because plants often grow better when both nutrients are provided to the soil (Elser *et al.,* 2007).

Interestingly, despite the soils having much lower P concentrations than Syferkuil, tepary beans cultivated in Ga-Molepo accumulated greater dry matter and increased N fixation. This might be explained by plants' different adaptations when there is a P shortage. The adaptations involve investing more energy below ground to increase the surface area of the roots for nutrient absorption (Keerthisinghe *et al.,* 1998).

While plants grown in environments with enough P increase their BNF and accumulate more N and P in their tissues, those grown with insufficient P maximize their utilization of N and P (Magadlela *et al.,* 2020). Phosphorus deficiency causes plants to produce more nodules, which increases the legume's capacity to fix atmospheric nitrogen (Magadlela *et al.,* 2020). This may have resulted in the tepary bean maintaining its dry matter. Phosphorus-deficient legumes form symbiotic relationships with soil microorganisms and AM fungi that fix nitrogen (Berruti *et al.,* 2016) due to the ability of endophytic microbes to transfer phosphate to plants and promote the uptake of N (Magadlela *et al*., 2020). The AM fungus symbiosis may enhance the effectiveness of soil P absorption and solubilization, leading to improved $N_2$ fixation (de Vries *et al.,* 2012). However, compared to VAM uninoculated plants, VAM inoculated plants showed lower N fixed levels (Table 4.3). This may be associated with soil acidity (pH-4.62 in Table 3.2) at Ga-Molepo, which reduces microorganisms' effectiveness. Irrespective of the fact that the soil at Syferkuil was more alkaline (pH-7.73) and the soil at Ga-Molepo was more acidic, the latter site accumulated more dry matter and had a greater level of enhanced N fixed. These findings concur with a previous work that indicated that *Calopogonium mucunoides Desv*. cultivated in acidic soils (pH 4) increased nodulation and biological N fixation (Ferreira *et al.,* 2016).

### 4.5. Conclusion

The application of P and VAM allowed tepary bean plants to grow in different locations with soils that varied in nutrition levels, resulting in distinct amounts of dry matter and N fixed. The results of this study revealed that P application enhanced dry weight and the amount of nitrogen that tepary beans could fix. Tepary bean plants developed a symbiotic relationship with VAM at Syferkuil, where inoculated plants were more effective at fixing nitrogen than non-inoculated plants. Plants inoculated with VAM at Ga-Molepo did not have a higher N fixation, however, Ga-Molepo performed better than Syferkuil. This suggests that tepary beans can withstand acidity and lack of soil moisture, and fix nitrogen under such conditions.

# CHAPTER 5

# SUMMARY, CONCLUSION AND RECOMMENDATIONS

## 5.1. Summary

This study aimed to investigate the effect of VAM and P on the growth, yield components and biological nitrogen fixation of tepary beans. Phosphorus treatments had no significant impact on the number of days to 50% flowering, chlorophyll content, number of branches per plant, pod length, number of seeds per pod, 100 seed weight, pod weight and grain yield. However, significant differences were recorded in the days to 50% emergence and plant height. An increase in P application increased plant height, with an application rate of 60 kg/ha having the greatest effect.

VAM inoculation levels influenced pod length but not the number of days to 50% emergence, 50% flowering, plant height, chlorophyll content, number of branches per plant, number of seeds per pod, 100 seed weight, pod weight and grain yield. There was a significant impact on plant height, chlorophyll content, pod weight and grain yield between the two locations. The combined effects of 60 kg/ha and VAM inoculation enhanced the pod length. In comparison to Ga-Molepo, which had an acidic soil condition and relatively low soil P, Syferkuil had an alkaline pH and a very high soil P content, which led to significantly larger pod weight and grain yield.

The findings of this study revealed that dry matter and the amount of nitrogen fixed were high at both locations, with an increase in P application. The phosphorus application rate of 80 kg/ha was the most effective treatment in fixing nitrogen, followed by the application of 60 kg/ha at Ga-Molepo and 20 kg/ha at Syferkuil, and then by 60 kg/ha. Although the yield was lower at Ga-Molepo, the nitrogen fixed was higher than at Syferkuil.

## 5.2. Conclusion and Recommendation

The present study focused mainly on the recommendation of optimum VAM inoculation and P concentration levels necessary to improve the performance of tepary bean crops in two different locations. The data serves as a foundation for helping farmers to select the ideal concentration level for enhancing growth and productivity in nutrient-deficient soils. It was found that plants treated with 80 kg/ha of phosphorus did not generate the highest yield, leading to the conclusion that 60 kg/ha of phosphorus was adequate to meet the crops' nutrient needs. Treatment 60 kg/ha P, inoculated, increased pod length and positively affected the crop yield. Therefore, it can be said that using VAM and P can increase tepary beans' production. The overall assessment of tepary beans in both locations revealed that the crop could be grown in acidic and alkaline conditions. Phosphorus can improve the efficacy of N fixation in tepary bean crops, as evidenced by phosphorus-promoting N fixation in both locations.

Due to the high accumulation of N brought on by P treatment at both locations, it is therefore recommended that farmers apply P to increase production. Using VAM could be a better alternative in areas where smallholder farmers have inadequate resources and little access to fertilizer inputs because it can increase yield. Farmers will benefit from the interactive effect of these treatment combinations and using VAM with P will ensure that the P applied is utilized effectively. Our results suggest that tepary beans can grow in alkaline and acidic conditions, increase yield and fix nitrogen. Smallholder farmers can have a high yield and all the possible benefits from the crop, including a source of protein, feed for livestock, and improved soil fertility if they have greater accessibility to VAM inoculants and P.

More research should be done to confirm the different combinations of VAM and P needed to improve tepary bean growth, yield and N fixation. VAM and P can be provisionally recommended for future tepary bean production by smallholder farmers to increase soil N whilst further research is conducted. The total above-ground biomass will help produce enough fodder for livestock to feed during dry winters, enhance soil fertility, and act as mulch, especially when mulch tillage is used to encourage conservation agriculture and protect the environment by reducing the need for inorganic fertilizers. Future studies should further examine the nutritional status of tepary bean seeds in response to P application rates and VAM.

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