EFFECT OF BIOFERTILIZERS ON GRAIN YIELD AND BIOLOGICAL NITROGEN FIXATION OF TEPARY BEAN (*PHASEOLUS ACUTIFOLIUS*)

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K.P MNISI

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by

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DECLARATION

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

MNISI K.P (Ms) **03/04/2023**

Surname,Initials (title) **Date**

ABSTRACT

Tepary bean, a drought-tolerant bean, has become popular among poor small-scale farmers in semi-arid countries. Field experiments were conducted on the effect of biofertilizers (Bradyrhizobium *japonicum* (B. *japanicum*) inoculation, versicular arbuscular mycorrhizae, and seaweed extract) on grain yield and biological nitrogen fixation of tepary bean in two different locations, namely Syferkuil and Ga-Molepo farm. One-way, two-way, and three-way analysis of variance (ANOVA) were used to compare bradyrhizobium inoculation, versicular arbuscular mycorrhizae (VAM), and seaweed extract application performance on plant growth and yield parameters (50% emergence, 50% flowering, plant height, chlorophyll content, number of branches per plant, number of pods per plant, pod length, 90% maturity, number of seeds per pod, 100 seed weight, pod weight and grain yield). Amongst these plant growth and yield parameters, a significant difference was observed in emergence, plant height, chlorophyll content, number of branches per plant, number of pods, pod length and number of seeds per plant in response to location, VAM, and seaweed extract. The location had significant differences in 50% emergence, plant height, chlorophyll content, number of branches per plant and number of pods per plant. VAM showed a significant difference in plant height, chlorophyll content, pod length and the number of seeds per pod. Seaweed extract had a significant effect on plant height and pod length. The interaction effect of VAM and seaweed extract levels at Syferkuil showed no significant impact on the chlorophyll content of tepary beans. A significant difference was observed in chlorophyll content in response to the interaction effect of VAM, seaweed extracts and location. The interaction of location, VAM and seaweed extract on chlorophyll content also observed a significant difference.

This study also determined the treatment effect on the tepary bean's biological nitrogen fixation (BNF). The ^{15}N natural abundance approach was used to evaluate nitrogen fixation. Shoot dry matter, %Ndfa and N-fixed of tepary bean grown at Ga-Molepo increased significantly than at Syferkuil. Versicular arbuscular mycorrhizae, bradyrhizobium inoculation and seaweed extract had no significant difference in dry matter, %Ndfa and N-fixed. However, the results showed that treatments influenced these parameters. VAM (inoculated), seaweed extract (application) and bradyrhizobium (un-inoculated) fixed the most N at Ga-Molepo (164.96; 183. 81 and 180. 25 kg/ha, respectively) and therefore showed more significant dry matter accumulation. At Syferkuil, VAM (un-inoculated), bradyrhizobium (inoculated) and seaweed (no application) contributed the most symbiotic N (56. 1; 43. 48 and 42.97 kg/ha, respectively).

Tepary beans planted at Ga-Molepo significantly obtained greater mean dry matter (32. 70) than Syferkuil (16. 47). Tepary beans grown at Ga-Molepo significantly received a greater mean %Ndfa (27. 19) at Syferkuil (12. 57). The percent N derived from fixation was 35% at Syferkuil and 22% at Ga-Molepo. These outcomes confirmed the view of this study that production and biological nitrogen fixation of tepary beans (and other grain legumes) can be enhanced using biofertilizers.

Keywords: Tepary bean, parameters, treatments,%Ndfa and N-fixed.

DEDICATION

I dedicate this study to my two younger brothers, Makoela Thakgalo and Makoela Tetelo, and above all, to the baby I am carrying. I hope you all follow in my academic footsteps and succeed more than I did.

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ACRONYMS

ABA	:	Abscisic acid
AM	:	Arbuscular mycorrhizal
AMF	:	Arbuscular mycorrhizae fungi
ANOVA	:	Analysis of variance
BCMV	:	Bean common mosaic virus
BNF	:	Biological Nitrogen Fixation
CBB	:	Common bacterial blight
KCl	:	Potassium chloride
Leg	:	Legume
Ndfa	:	%N derived from atmospheric fixation
Ref	:	Reference
SWE	:	Seaweed extracts
VAM	:	Versicular Arbuscular Mycorrhizae

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CHAPTER 1: INTRODUCTION

1.1 Introduction

Tepary bean (*Phaseolus acutifolius L.*) is one of the most versatile legume crops, adapting well to various environmental conditions (Muñoz *et al*., 2004). Its production is mainly carried out by small-holder African farmers (Shisanya, 2003). This plant is grown for its seeds, which contain essential minerals, a high protein content of up to 24% oil, and 33% saturated fats (Bhardwaj and Hamama, 2004; 2005; Porch *et al*., 2017).

It has a relatively high concentration of essential amino acids, similar to the common bean (Porch *et al*., 2017). The plant is also cultivated for its leafy vegetation, and its haulms can feed livestock (Molosiwa *et al*., 2014). On average, African bean yields are very low compared to other bean-growing continents. Edaphic and climatic limits and biotic restrictions are responsible for this restriction. Improved cultivars better suited to low soil fertility can be employed, as well as managing soil fertility and optimizing root symbioses to encourage plant nutrient uptake to increase output. Legumes such as tepary beans create two different kinds of root symbioses, a legume-rhizobial symbiosis between the host plant and its microsymbiont and arbuscular mycorrhizal (AM) symbiosis. These symbioses affect plant nutrient uptake and are essential as they permit the extraction of nutrients from the soil and their delivery to the interior of the host root (Jansa *et al*., 2011). For example, arbuscular mycorrhizae fungi acquire carbon molecules from photosynthesis in plants, which are necessary to their metabolism, and obligate symbionts, which must interact with the plant to complete their life cycle (Bago and Bécard, 2002). The symbiotic effect also includes the ability of tepary beans to fix atmospheric N through a symbiotic N fixation with rhizobia bacteria (Shisanya, 2002; Mohrmann *et al*., 2017). Additionally, the availability of inoculants, which are sufficient to meet the N requirements of plants, facilitates the biological nitrogen fixation (BNF) process of diverse legume species (Bhattacharyya and Jha, 2012; Malusá and Vassilev, 2014; Fukami *et al*., 2017). The agricultural practices aligned with sustainable production (e.g. BNF, the application of inoculants and biostimulants) are encouraged as they are safe for the environment and improve yield (Bulgari *et al*., 2015).

Vesicular-arbuscular mycorrhizae (VAM) is also aligned with sustainable practices as beneficial micro-organisms that contribute significantly to soil nutrients by enhancing the physical, chemical, and biological characteristics of the soil (Parewa *et al*., 2014). Numerous studies have shown that VAM can affect how phosphorus from the soil is mobilized to benefit the host plants (Lalitha *et al*., 2017). Due to its low solubility, low mobility, and fixation in the soil, phosphorus is one of the elements that plants find the most challenging to absorb. Along with the advantages of VAM, using seaweed extract as a biostimulant for plants has been another effective, sustainable agriculture approach in recent years. Seaweed extracts derived from macroalgae have several economic applications in agriculture, including soil fertilization and growth stimulants (Khan *et al*., 2009). Auxins, cytokinins, polyamines, gibberellins, abscisic acid, and brassinosteroids are a few plant growth regulators found in seaweed extract that stimulate the growth of several crops (Stirk *et al*., 2014; Papenfus *et al*., 2013). Seaweed extract contains amino acids, antibiotics, vitamins, and trace minerals. The benefits of seaweed extract include enhancing yield and acting as a biofertilizer (Du Jardin, 2015). According to Khan *et al*. (2009), Mattner *et al*. (2013) and Hernández-Herrera *et al*. (2014), seaweed extract improves seed germination, plant growth, root development, and increases the production and quality of vegetables such as tomato, and bean, and extended post-harvest shelf life.

1.2 Problem statement

Although various agricultural sustainable practices have been mentioned above, the production of tepary beans in South Africa is still neglected. The low production is attributed to the low fertilizer use, lack of established varieties, the unreliability of landraces and low economic returns. Other limitations to tepary production include low soil fertility, which is a significant limitation (Bationo *et al*., 2006). Additionally, P has low solubility, mobility, and fixation, making it challenging for most plants to absorb. Due to its transformation into inaccessible forms that plants cannot absorb, P fertilizer recovery is limited (Osborne and Rengel, 2002; Wang *et al*., 2005).

Furthermore, soil nitrogen alone may be insufficient for sustaining tepary bean needs throughout the growing season. Most soils lack efficient rhizobia essential to increase nodulation and seed yield (Kellman *et al*., 2005). There is a need to evaluate the potential of Rhizobium inoculants, VAM, and seaweed extracts in addressing the insolubility of P and low N fixation by tepary bean.

1.3 Rationale

Tepary beans can fix atmospheric nitrogen (N), which lowers the need for inorganic nitrogen fertilizer and lowers the risk of water contamination from runoff into streams, lakes, rivers, and tributaries (Knight-Mason and Bhardwaj, 2016). It has a high level of bean weevil resistance (Kusolwa and Myers, 2011). Natural antioxidants in the seeds reduce the risk of certain cardiovascular diseases, including coronary heart disease and colon cancer (Jiri and Mafongoya, 2016). Its low glycaemic index makes it suitable for people with diabetes (Weil, 2015). The inoculation of seeds with the appropriate rhizobia influences root nodulation and increases biological nitrogen-fixing (Tang *et al*., 2001). The vesicular-arbuscular mycorrhizae microorganism is crucial to the dynamics of soil nutrients.

Additionally, it helps with nutrient absorption and enhances the soil's chemical, biological, and physical qualities (Parewa *et al*., 2014; Prakash and Verma, 2016). Seaweed extract enhances crop yield and improves plants' resistance to frost and diseases. It also increases nutrient uptake, promotes vigorous growth, and helps prevent flower pests and diseases (Lawson, 2018). Using biofertilizers can potentially enhance biological nitrogen fixation and the yield of tepary beans.

1.4 Aim

This study aims to evaluate bradyrhizobium inoculation, VAM and seaweed extract effects on grain yield and biological N-fixation of tepary bean.

1.5 Objectives

i. To determine the morphological, physiological parameters and grain yield of tepary bean in response to bradyrhizobium inoculation, seaweed extract and VAM application.

ii. To determine the effect of bradyrhizobium inoculation, seaweed extract and VAM on biological N-fixation of tepary bean.

1.6 Hypotheses

i. Bradyrhizobium inoculation, seaweed and VAM application do not affect tepary bean's morphological, physiological parameters and grain yield.

ii. Bradyrhizobium inoculation, VAM and Seaweed extract do not affect the biological N-fixation of tepary beans.

CHAPTER 2

LITERATURE REVIEW

2.1 Origin, domestication, and distribution of tepary bean

Tepary beans (*Phaseolus acutifolius*) are indigenous to the Sonoran Desert in northwestern Mexico and the southwestern United States. The wild tepary bean is grown in Arizona, New Mexico, Texas, and all over Mexico. Tepary beans are cultivated in dry areas worldwide, including North and Central America, Europe, South Asia, and Africa (ILDIS, 2018). Four plant varieties (var.) are recognized for tepary bean, including *latifolius*, *var. acutifolius* (domesticated and wild) and *var. tenuifolius* (Blair *et al*., 2012). Around 5,000 years ago, tepary beans were cultivated in several African nations, including South Africa, Malawi, Botswana, Uganda, Swaziland, Lesotho, Morocco and Algeria. In South Africa, they are grown mainly by smallholder farmers in drier areas, particularly in the Limpopo province (Shisanya, 2003).

2.2 Description of tepary bean

Tepary beans are a short-season leguminous crop that can be grown yearly in semi-arid climates. Tepary bean plants begin to grow between 27 and 40 days after germination and mature in 60 to 80 days (Jury and Vaux, 2007). Varieties of short-lived wild tepary beans reach maturity in two months in tropical areas. In milder climates, such as along Algeria's coast, the growing period could last up to 120 days (Jury and Vaux, 2007). Unlike ordinary beans, tepary beans are vine-like with a taproot system, more foliage and smaller leaves, and a more branching, bushy architecture. The seed size and coat colour vary by landrace or variety. In the wild, tepary bean vines are indeterminate, twining, or weakly trailing climbers of trees and bushes. Domesticated plants are bushier, can reach 0.30 m and a diameter of 0.50

m, have three narrow, sharp leaflets, and have either white or light-coloured blooms. Its fruit is a tiny pod that contains two to seven seeds and is about 0.03175 to 0.3302 meters long.

Wild pods are significantly more dehiscent than domesticated types (which do not distribute their seed when mature). Wild tepary bean seeds are smaller, darker, and mottled than domestic seeds, which are about 8.50 mm long and appear in various colours. Tepary roots create a symbiotic relationship with microbes that fix nitrogen (Felger and Rutman, 2015; FAO, 2010). R3254, a strain of *Rhizobia sp*., increases nodulation by fixing up to 260 kg N ha^{-1} (Shisanya, 2002a). Its protein-rich grains lessen starvation and improve soil fertility (Prasanna *et al.,* 2001).

2.3 Biotic stress tolerance of tepary bean

Tepary beans are a significant legume crop that can be used to introduce novel genes into other Phaseolus species or ordinary beans (Souter *et al*., 2017). Both common and tepary beans experience considerable output losses due to viral, fungal, and seed-transmitted bacterial diseases such as the common bacterial blight (CBB) (Vargas *et al*., 2014). Accessions of tepary beans are resistant to a wide range of ailments, such as bean golden mosaic virus, ashy stem blight, powdery mildew, and CBB. These genotypes are a vital genetic resource for developing disease resistance in tepary beans. Tepary bean breeding lines have been reported to include CBB and bean common mosaic virus (BCMV) resistance (Vargas *et al*., 2014). Tepary bean production might be increased by introducing enhanced tepary bean types with diverse disease resistance.

2.4 Tolerance of tepary bean to abiotic stress

Tepary bean is a legume that is tolerant of heat and drought, requires little water for growth, and resembles soybeans in terms of forage quality (Baath *et al.,* 2020). Tepary beans are drought and heat-adapted legumes that use little water to support growth and feed rates similar to soybeans (Baath *et al*., 2018). Tepary bean is widely employed to make common beans more resilient to abiotic conditions like drought and high heat (Beebe *et al*., 2013; Moghaddam *et al*., 2021b). It is tolerant to unfavourable agronomic circumstances such as excessive salt concentrations, lack of water, pests, and bacteria that kills normal beans. However, the ability to tolerate stress from extreme heat and drought did not transfer to the ability to tolerate flooding. In reaction to flooding, most forage legumes experience a >40% drop in N content in the shoots and leaves (Striker and Colmer, 2017). In addition, a study on cool-season grain legumes revealed that the white lupin (*Lupinus albus L*.) and flood-vulnerable pea (*Pisum sativum L.*) species had N contents that had decreased by more than 40% (Pampana *et al*., 2016). Waterlogging can decrease root conductivity, which lowers N absorption and limits N transport and distribution in plants. As a result, biomass buildup is reduced (Kaur *et al*., 2020). The swift restoration of root nodulation following flooding may be related to the slight drop in the nitrogen content of tepary beans (Dron *et al.*, 2002). Despite the high cost of flooding, legumes like the common bean and soybean have different genetic variants that make them more tolerant. These legume species have a similar conserved genetic mechanism for flooding resistance (Soltani *et al.,* 2017, 2018; Wu *et al*., 2017). Phaseolus genus and tepary beans may have a similar genetic history. Due to their underutilization as food crops with strong heat and drought resistance, tepary beans have not gotten much attention in flood studies (Beebe *et al*., 2013; Burbano-Erazo *et al*., 2021; Moghaddam *et al*., 2021b). Tepary bean's ability to survive heat and drought due to stomatal control may allow them to tolerate the abiotic stress of floods (Baath *et al*., 2020). According to Moghaddam *et al*. (2021b), tepary beans generate a variety of chemicals that lessen the effects of abiotic stress on plants (2021). However, drought and flooding can cause stomata to close and reduce CO_2 uptake. Climate warming will make the multibillion-dollar issue of crop loss from floods more significant. Researchers and farmers may find tepary beans and other crops that can withstand various biotic and abiotic stresses to be useful resources. According to Morton (2007), rainfed agriculture is impacted by the drought problem worldwide. Rainfed agriculture accounts for 84% of all arable land, substantially impacting global trade and the economy. Smallholder and "subsistence" farmers in developing nations will be severely impacted by global climate change, including related droughts and heat stress. Blum (2009) has identified constitutive and adaptive cultivars that promote effective water use and consequent avoidance of drought as important traits for improving yield in drought-prone environments. This conceptual framework emphasizes the significance of identifying plant characteristics and mechanisms influencing superior drought stress tolerance. *Phaseolus vulgaris* L., sometimes known as kidney bean, is a fantastic source of protein (around 22%), minerals (Ca, Cu, Fe, Mg, Mn, and Zn), and vitamins (folic acid). In poorer countries, it is the most important cereal legume for human nutrition (Beebe, 2012). Additionally, beans are less adaptable to harsh situations like very little rainfall, high temperatures, or low soil fertility than cereal legumes like cowpea (Beebe *et al*., 2011). Drought impacts more than 60% of dry bean production worldwide (Beebe *et al*., 2008; Rao, 2001). The endemic arid regions of bean cultivation include the highlands of much of eastern and southern Africa, north-eastern Brazil, and Central America (Beebe, 2012). The severity, nature, and length of stress determine how drought affects kidney beans (Beebe *et al*., 2013). In bean-growing regions susceptible to climate change, developing drought-tolerant bean cultivars is crucial for reducing crop failure and enhancing food security (Beebe *et al*., 2011). Since kidney beans are grown in diverse habitats where seasonal droughts and large interannual variations in soil water availability can occur, the rationale for maintaining normal metabolic function under drought stress has developed multiple mechanisms to maintain plant water conditions within reasonable ranges (Beebe *et al*., 2013). Beans under moderate to severe drought stress had decreased canopy biomass, yield index, seed number, weight, and days to maturity (Nunez-Barrios *et al*., 2005; Beebe *et al*., 2013). Early roots, deep rooting, and increased photosynthetic diffusivity have all been recognized as key factors in the common soybean's ability to withstand drought (Rao, 2001; Rao *et al*., 2009; Beebe *et al*., 2013). Beebe *et al*. (2008) found that selection for drought tolerance improved yield potential and increased plant performance in different environments (non-stress, low phosphorus stress, etc.) and that selection under drought stress increased plant performance in the wild. It has been proposed that the key to overall soybean improvement lies in the refined genes that have inherited inefficiencies (excessive vegetative growth) from them. However, additional development is possible through an intraspecific cross with a sister species in the genus Phaseolus. Small leaves use less water, whereas stomal control is not osmotic control (Beebe *et al*., 2013; Mohamed *et al*., 2005). Tepary bean possesses unique alleles that can be transferred into other beans to help them better adapt to abiotic stress (Souter *et al*., 2017). Despite successful breeding efforts, the number of tepary bean gene pools that show potential for increased adaptation to drought stress and yield is still limited. Within the genus Phaseolus, many cultivars exhibit variable levels of yield index. For instance, interspecific lines created by crossing kidney bean seeds with those from its secondary gene pool (*P. dumosus*, *P. coccineus*) frequently display excessive vegetative growth and poor grain yields.

Conversely, drought-tolerant legume lines have lower shoot biomass but produce more grain. This has been attributed to the increased recruitment of photosynthetic factors for crop development by Beebe *et al*. (2008) and Klaedtke *et al*. (2012), who found that differences in crop biomass distribution may affect the drought resilience of crops. Many late-summer storms produce intense, high-volume rainfall in short periods, which may result in significant runoff and soil erosion (Daniel *et al*., 2006). Such storms can be a significant obstacle to effective land management. This is because conventional tillage is commonly applied to agricultural lands in this region (Hossain *et al*., 2004).

2.5 Medicinal uses of tepary bean

In particular, the Tepary bean (*Phaseolus acutifolius*) lectins have drawn attention because they have lower toxicity than other bean lectins (Ferriz-Martínez *et al.,* 2015). Lectin fraction of tepary bean is produced using molecular weight exclusion chromatography, demonstrating in vitro differential lethal effects on cancer cell types, with colon cancer cells being the most sensible ones (García-Gasca *et al.,* 2012). According to Moreno-Celis *et al.* (2020), tepary bean lectin fraction affects colon cancer because it showed early premalignant lesion inhibition in rats that had previously received treatment with dimethylhydrazine or azoxymethane, which indicated that it might have a protective effect against colon cancer (Moreno-Celis *et al.,* 2017). According to Alatorre *et al.* (2018), tepary bean lectin fraction may have contributed to the immune system's in vivo activation by changing the lymphocyte-granulocyte ratio. Additionally, tepary bean lectin fraction can alter the distribution of occludin and have a deleterious impact on intestinal permeability, protein digestibility, and other factors (Pita-López *et al.,* 2020). Natural antioxidants in the seeds help lower the risk of various cardiovascular ailments, including type 2 diabetes, colon cancer, and coronary heart disease (Jiri and Mafongoya, 2016). Studies suggest that tepary beans may be effective in treating cancer and may be ten times more so than chemotherapy (Hart, 2012). The lectins in tepary beans inhibited non-transformed cells and some cancer cells from increasing (Bogler, 2014).

2.6 Nutritional importance of tepary bean

Tepary beans are primarily grown for human use and for feeding cattle. Tepary bean grains are abundant in vitamins, fibre, carbs, and protein (24%) (Bhardwaj, 2013). Like all crops, tepary beans' nutritional makeup can be impacted by environmental factors and the time of sowing (Ghadimian *et al*., 2020; Bhardwaj *et al*., 2002). Tepary bean seeds contain all the necessary minerals Ca, Mg, Cu, Fe, K, Mn, S, Zn, and Na, as well as roughly 33% saturated fat, 67% unsaturated fat, 24% monounsaturated fat, and 42% polyunsaturated fat (Bhardwaj and Hamama, 2005). Tepary beans' fat profile differs slightly from common beans in their saturated, unsaturated, and monounsaturated fatty acid compositions (Bhardwaj and Hamama, 2005). In another study, iron values for tepary beans were 10.7 mg 100 g^{-1} in seeds, while reported values for common beans ranged from 5.9 to 6.7 mg 100 g^{-1} (Bhardwaj and Hamama, 2004). According to research conducted in the United States and Mexico, chemicals such as lectin toxins in tepary beans play a more substantial role in chemotherapy by slowing cancer growth (García-Gasca *et al*., 2012). According to Weil (2015), tepary bean contains more calcium, iron, zinc, magnesium, phosphorus, and potassium than other beans, reducing digestive discomfort and gassiness. Tepary is also less digestible than other beans because of its lower polyunsaturated lipid content and anti-enzymatic chemicals.

2.7 The biological fixing of nitrogen in tepary beans

The gap between fertilizer inputs and nitrogen removal is high in Africa's semi-arid regions, where fertilizer input is less preferred for rainfed systems. Dryland agriculture is more likely to have negative nutrient budgets than irrigated systems (Adu-Gyamfi *et al*., 2007). Symbiotic N fixation is essential for agricultural sustainability since nitrogen (N) fertilizer is difficult to obtain in underdeveloped nations (Adgo and Schulze, 2002). Rhizobia, a type of soil bacterium, and tepary bean work in symbiosis to provide biological nitrogen (N_2) fixation (Mohrman *et al*., 2007). According to Herridge *et al*. (2008), the legume-rhizobia symbiosis, which results in the growth of nodules on the host plant's stems or roots, accounts for 60% of all biological nitrogen fixation. Rhizobial bacteria convert ambient N_2 to NH_3 using the nitrogenase enzyme and then trade this nitrogenous solute for photosynthates from the host plant (Peoples *et al*., 2002). Several biological processes occur concurrently inside the nodules once the symbiotic nitrogen fixation process has been established in mature nodules. In this biological nitrogen fixation process, host plants and bacteroids will trade carbon-nitrogen metabolism, and metabolites will be transported across cell membranes (Clarke *et al*., 2014; Udvardi and Poole, 2013). Tepary bean cultivation could be a valuable resource for poor small-holder farmers because it can fix significant amounts of nitrogen (Shisanya, 2005). According to Shisanya (2002b), tepary bean fixes up to 260 kg of nitrogen per hectare using *Rhizobium spp*. Strain R3254. Using nitrogen fixed by the tepary bean may remove or reduce the need for inorganic nitrogen fertilizer, reducing the risk of water pollution from run-off into streams, lakes, rivers, and tributaries. Tepary bean, if successful, can be used as a sustainable crop for environmentally friendly agriculture by reducing growers' reliance on synthetic nitrogen fertilizers (Knight-Mason and Bhardwaj, 2016).

2.8 Effect of inoculation on growth, yield and nutrient composition of tepary bean

Nitrogen is typically the primary nutrient limiting plant growth in natural ecosystems (Graham and Vance, 2003). Nitrogen fertilizer application to a crop entails a high financial and environmental cost. Bacteria that influence plant growth are used as inoculants. They improve the substance, leading to better root development, mineral absorption, and water uptake rate in general (Dobbelaere *et al*., 2001). Inoculation increases nitrogen levels in seeds and crop wastes, increases yields, and reduces input costs for inorganic and chemical fertilizers. According to Giller (2001), rhizobium has been shown to increase plant growth and biomass production by increasing soil roots' nutrient intake. According to Sharma *et al*. (2000), the seed inoculation of crops with this bacterium significantly impacts plant height and biomass dry matter. Numerous researchers have advocated rhizobium bacteria in promoting legumes' growth (Rudresh *et al*., 2005; Malik *et al*., 2006). Rhizobium inoculation on beans has been shown to provide N to the crop (Togay *et al*., 2008). According to the findings of Zhang *et al*. (2002), seed inoculation with appropriate Rhizobium bacteria increases the number of seeds and pods per plant as well as grain yield (2000 kg/ha) in beans, implying that using rhizobia in inoculating legumes can significantly increase growth and yield productivity as well as soil fertility. There is no doubt that specificity exists between the rhizobia strain and the legume variety, and compatibility between the two is essential for successful nodulation and nitrogen fixation (Emam and Rady, 2014; Allito *et al*., 2015).

2.9 Beneficial effect of vesicular-arbuscular mycorrhizae (vam)

Vesicular-Arbuscular Mycorrhizae (VAM) is a helpful fungus that improves soil's physical, chemical, and biological qualities and is crucial to the dynamics of soil nutrients (Parewa *et al*., 2014; Jaiswal *et al*., 2016; Jha and Subramanian, 2016). The mycorrhizal symbiotic relationship is a mutually beneficial link between fungi and plants that aids in the uptake of phosphorus and other nutrients from the soil. As soil phosphorus is insoluble in most circumstances and has low mobility and fixation, it is a challenging nutrient for plants to obtain. Mycorrhizal plants get additional phosphorus by absorbing and translocating P from far-flung locations that would otherwise be inaccessible to plant roots. It has been claimed that inoculating plants with mycorrhizal fungi will replace the 30 kg/ha of phosphorus in plant soil (Lalitha *et al*., 2017; Almagrabi and Abdelmoneim, 2012). Excessive phosphorus treatment can alter root colonization by limiting arbuscule growth and lowering mycorrhizal fungus biomass per plant (Smith and Read, 2008).

2.10 Effect of seaweed extract biostimulant on tepary beans

An effective biostimulator, seaweed extract boosts plant resistance to adverse conditions and stimulates life processes. It improves the yield and quality of the product. Auxins and cytokinins, as well as gibberellins, amino acids, and alginates, are among its constituents (Basak, 2008; Kavipriya *et al*., 2011; Rathore *et al*., 2009). Kelpak, made from the brown seaweed *Ecklonia maxima*, has a higher concentration of auxin than cytokinin (11.0 mg/L auxin vs 0.031 mg/L cytokinins). Seaweed extract is essential for raising chlorophyll levels in leaves, according to Selvam and Sivakumar (2013). It encourages the elongation of cells and the growth of plants and their root systems (Basak, 2008; Basak and Mikos-Bielak, 2008; Matysiak and Kaczmarek, 2008; Russel, 2002). Furthermore, seaweed extract increases plant hormones, which help to boost crop quality and quantity as well as plant health (Matysiak and Adamczewski, 2006; Matysiak and Kaczmarek, 2008; Oyoo *et al*., 2010; Russel, 2002). Seaweed liquid fertilizers have been shown to boost the root system (Slavik, 2005), increase total yield (Ashour *et al.,* 2020b), and increase chlorophyll content. Kelpak promotes root system growth by improving nutrient uptake from the soil solution.

Since the beginning of plant breeding, seaweed extracts have been used in agricultural activities as biostimulants (Hassan *et al.,* 2017). Based on their nutritional worth, algal cells (either microalgae or seaweeds) are a treasure trove of sources for colours, proteins, lipids, polysaccharides, minerals, and antioxidants, as well as a variety of biological components (Ashour *et al.,* 2019; Heneash *et al.,* 2015; Zaki *et al.,* 2021; El-Shenody *et al.,* 2019; Sharawy *et al.,* 2020). Seaweeds are also one of the most vital elements of marine ecosystems, serving critical environmental roles (Abo-Taleb *et al.,* 2020). However, seaweed extracts can be employed in various industrial biological processes (Ashour, 2019). Seaweeds are suitable for biofertilizers due to biological influence and biocompatibility since they share biological ingredients with plants. Due to this considerable advantage, seaweeds are currently at the top of the list of plant biostimulants, which has accelerated several plant treatment processes, primarily to benefit and support organic and sustainable agriculture (Tarakhovskaya *et al*., 2007). According to reports, some of the most popular extracts are *Pterocladia capillacea* (Ashour *et al.,* 2020a), *Ascophyllum nodosum* (Xu *et al.,* 2015), *Ecklonia maxima*, *Sargassum spp*. (Bhattacharyya *et al.,* 2015), *Ulva lactuca, Laminaria spp., Pterocladia gymnosperm, Durvillaea potatumum, Caulerpa sertularioides, Senecio johnstonii,* and *Sargassum liebmannii*

(Hernández-Herrera *et al.,* 2014) *Padina gymnospora*, and *S. johnstonii* (Drobek *et al.,* 2019).

Although numerous seaweed extract supplements exist for plants, foliar spray administration has been sufficiently and widely employed in modern agriculture to boost the production of many commercial crops, with highly encouraging results (Rouphael *et al*., 2017). The applicability of seaweed extract foliar spraying has been investigated (Ashour, 2020b), along with its quick and simple handling procedure, with an emphasis on promoting growth and raising productivity (Murugalakshmikumari *et al.,* 2020; Ahmed and Shalaby, 2012; Valencia *et al.,* 2018).

Seaweed extracts bolster plants' natural defences against environmental hazards, nourish crops, and encourage the establishment of better biomass. Since seaweed extracts have highly stable, promising stages, they are frequently advocated for use in supporting organic agriculture (Ashour *et al*., 2020b). The production is rising quickly, and they compete for market share with chemical pesticides and fertilizers due to the easily accessible of recently created seaweed extract products.

CHAPTER 3

MORPHOLOGICAL, PHYSIOLOGICAL PARAMETERS AND GRAIN YIELD RESPONSE OF TEPARY BEAN TO INOCULATION, SEAWEED EXTRACT AND VAM APPLICATION.

3.1. Introduction

Tepary bean (*Phaseolus acutifolius A. Gray*) is described as more tolerant to numerous biotic and abiotic stresses (high temperature, drought, and diseases) than common beans (Muñoz *et al*., 2021). Tepary bean is still abandoned and underutilized with no scientific backing despite its capacity to contain critical mineral elements (Bhardwaj and Hamama, 2005; Mhlaba *et al*., 2018). The crop is mainly grown by smallholder farmers in sub-Saharan Africa with poor soil and minimal farm input systems (Jiri and Mafongoya, 2016). Nitrogen and phosphorus are two essential nutrients for crop growth and development. Most soils have low levels of these nutrients in developing countries, including South Africa (Hikosaka, 2004).

According to Ghany *et al*. (2013), as artificial fertilizers release nutrients more quickly than conventional manures, they were used excessively by Indian farmers during the Green Revolution because they were under pressure to feed billions of people. Inorganic fertilizer releases residual toxic substances into the soil, altering its health and leaving behind hazardous residues that affect the soils fertility. Also, beneficial soil microorganisms die because of excessive fertilizer use (Mishra *et al*., 2012).

Utilizing inorganic fertilizers have good benefits and enhanced agricultural productivity. Microbial inoculants improve soil productivity by mobilising nutrients from the soil and making them available for plant uptake (Selvakumar *et al*., 2009). As a result of the symbiotic relationship between Rhizobium and legume root systems, nitrogen is fixed. Rhizobium bacteria applied with the appropriate strain increase nodulation and yield (Giri *et al*., 2010).

Vesicular Arbuscular Mycorrhiza (VAM) develops a mutualistic symbiosis with the host plant and has a favourable impact on nutrient uptake, plant health, and soil fertility, which leads to a favourable influence on plant growth (Ramasamy *et al*., 2011). VAM fungus assists plants in absorbing nutrients from the soil, including phosphate and micronutrients. Because the knowledge applies to human endeavours to manage, restore, and sustain ecosystems, and symbiosis is vital for plant nutrient uptake in agroecosystems, mycorrhizal technology becomes essential in low-input systems (Brundrett, 2004). Another alternative to chemical fertilizers is seaweed extracts which are biodegradable and environmentally friendly (Ganapathy Selvam and Sivakumar, 2013). It is used widely as a foliar spray to increase yields (Eman *et al*., 2008). Seaweed contains macro- and micronutrients, amino acids, vitamins, cytokinins, auxins, and abscisic acids (ABA)-like growth substances that influence cellular metabolism in treated plants, resulting in increased growth and crop yield (Durand *et al*., 2003; Stirk *et al*., 2003). This study aimed to determine the morphological, physiological parameters and grain yield of tepary beans in response to inoculation, seaweed extract and VAM application.

3.2. Materials and Methods

3.2.1. Study area

The experiment was carried out in the summer of 2021 at the University of Limpopo Experimental farm (Syferkuil), which is situated at (23°59'35" S, 29°33'46" E), and Ga-Molepo community (24° 01' 52.0" S, 29 44' 16.0" E). The Experimental farm's climate is semi-arid, with annual rainfall ranging from 300 to 1000 mm (Mpandeli *et* *al*., 2019). Syferkuil's yearly average temperature range, according to Mokoka *et al*. (2018), is 13 °C to 30 °C. Ga-Molepo has an average annual temperature of 33.2 °C and receives 400 to 600 mm of precipitation (Maree, 2016). Sandy loam is the type of soil found at both sites (Sebetha *et al*., 2009; Maree, 2016). Ga-Mothapo represents the weather distribution of Syferkuil because it follows the same global positioning system (GPS).

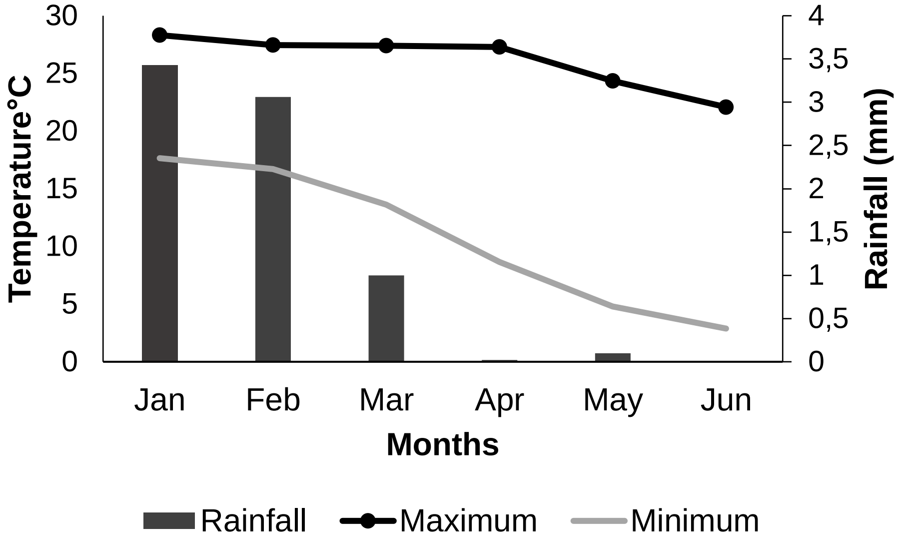

Figure 3.1: Average rainfall and temperature from the Capricorn district weather station for the summer growing season of 2020–2021.

3.2.2. Experimental design and treatments

A split-split plot design was used to layout the experiment. The experiment consisted of 3 treatment factors where factor A was bradyrhizobium *japonicum* inoculation (Application and No application), factor B was vesicular-arbuscular mycorrhizae (Application and No application), and factor C was seaweed extract (application on the leaves and with no application), and they were replicated four times. Each replication consisted of eight treatment combinations.

3.2.3. Procedure and management of the field

Each replication consisted of eight plots with a total area of 3 m × 3 m per plot (9 m^2) in size, with 1 m spacing between replications. Each plot had five rows with an inter-row spacing of 60 cm and an intra-row spacing of 15 cm. All plots received superphosphate granule fertilizer for management at 60 kg/ha. Bradyrhizobium inoculant was applied at the rate of 250 g per 25 kg of tepary seeds under the shade and mixed with a small amount of water and sugar to ensure that the seeds were coated with the inoculant and the effectiveness of the bacteria. After mixing, the seeds were allowed to dry under the shade before planting. To prevent exposure to direct sunlight, the treated seeds were planted into moist soil and quickly covered with soil. VAM was applied in the soil, per row, as layering following recommended rates outlined by Erman *et al*. (2011). Seaweed extract (Kelpak) was sprayed on the foliage following the label instructions. It was administered early in the morning, as directed on the label. Supplementary irrigation was applied when the crops showed signs of drought at Syferkuil.

In comparison, the study at Ga-Molepo was conducted under rainfed conditions. Weeding was done when it was necessary as part of management techniques. The weeds were pulled out manually and mechanically using a hand hoe. Since the harmfulness thresholds of the pests were not exceeded, no insecticides were applied.

3.3. Data collection

3.3.1. Soil sampling and analysis

Soil samples were collected randomly before planting and at harvest at a depth of 0-15 cm and 15-30 cm using a soil auger. Three samples were collected per plot and mixed as a composite sample. Collected soil samples were air-dried, ground to a fine powder and sieved using a 25 mm mass sieve. Soil samples were analyzed 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 were analyzed using automated Dumas dry combustion method using a LECO TruSpec CN (LECO Corporation, Michigan, USA; Matejovic, 1996). Clay percentage. P, K, Zn, Mn, and Cu were measured using the Ambic-2 extraction method. Concentrations of exchangeable Ca and Mg, pH and exchangeable acidity were determined with a KCl solution. Nitrogen (N) and organic carbon were analyzed using the automated Dumas dry combustion method using a LECO TruSpec CN (LECO Corporation, Michigan, USA; Matejovic, 1996).

3.3.2. Growth, grain yield components, plant physiology and morphology measurements

Days to reach 50% emergence and 50% flowering were observed and recorded when 50% of plants had emerged and developed flowers. All growth and yield components were measured from five representative plants per plot. A chlorophyll meter was used to measure the amount of chlorophyll content on fully developed intact leaves. The number of leaf branches was at pod development. A 100 cm measuring stick was used to measure the height of the plants. The number of pods per plant and seeds in each pod was counted at harvest. Pod length was measured using a 30 cm ruler at harvest maturity from five representative plants in each plot. Days to reach 90% physiological maturity were recorded when the leaves turned yellow and the pods turned brown. The pods and seeds weights were determined by weighing harvested pods and seeds per plot on a scale. The weight of a hundred seeds was determined by weighing two samples of a hundred seeds per plot. Grain yield was calculated from seed yield per plot.

3.3.3 Data analysis

Data collected were subjected to analysis of variance using Statistica software version 10 at a significance threshold of 5%, and the means were separated using Tukey. Three-way ANOVA was used to compare inoculation, VAM, and seaweed extract application performance.

3.4 Results

Tables 3.1 and 3.2 showed significant differences in bradyrhizobium inoculation, VAM and seaweed extract and their interactions on 50% flowering and 50% emergence. Tables 3.3 and 3.4 demonstrate appreciable variations between bradyrhizobium inoculation and its interaction on plant height and chlorophyll content. Location, the interaction of location and VAM, and the interaction of VAM and seaweed showed a significant difference in plant height (Table 3.3). Location, the interaction of VAM and seaweed, and interaction of location, VAM and seaweed showed a significant difference in chlorophyll content (Table 3.4). The number of branches and pods per plant was not influenced by VAM inoculation, seaweed extract and the interactions (data not shown). However, location significantly affected the number of branches and pods per plant. Pod length was significantly affected by location, VAM, location, and seaweed extract interactions (data not shown). A single factor of location, VAM, seaweed extract, bradyrhizobium inoculation, and other interactions did not affect pod length. Location, VAM, seaweed extract, bradyrhizobium inoculation and their interactions did not significantly influence 90% maturity (Table 3.5). Inoculation, VAM, seaweed extract, location, and their interactions did not significantly affect the grain yield of tepary bean (Table 3.6).

It has been observed that the levels of phosphorus, potassium, calcium and magnesium, total cation, and pH (KLC) zinc, copper and clay% at Syferkuil were higher than that of Ga-Molepo before planting and at harvest. The Level of manganese, organic carbon and nitrogen was higher at Ga-Molepo than at Syferkuil, both at planting at harvest (Tables 3.7 and 3.8).

After planting, magnesium levels for Ga-Molepo decreased while they rose for Syferkuil. Exchange acidity decreased for Ga-Molepo while remaining constant for Syferkuil. For both locations, it was discovered that the acid saturation was lower at harvest than it was before planting. After planting at Syferkuil, zinc declined while copper increased. After planting, potassium and manganese levels rose in both areas, although calcium levels decreased in both locations (Tables 3.7 and 3.8).

Table 3.1: Three-way ANOVA for 50% emergence parameters of tepary bean in response to VAM, bradyrhizobium inoculation and seaweed extract application in Syferkuil and Ga-Molepo.

Source of Variation	**Degree of Freedom**	**Sum of square**	**Mean square**	**F-test**	**P Value**
50% emergence					
Location	1	219.45	219.45	6.96	*ns*
VAM	1	3.00	3.00	0.10	*ns*
Inoculation	1	56.95	56.95	1.81	*ns*
Seaweed	1	0,03	0.03	0.00	*
Location*VAM	1	4.28	4.28	0.14	*ns*
Location*Inoculation	1	11.63	11.63	0.37	*ns*
VAM*Inoculation	1	3.40	3.40	0.11	*ns*
Location*Seaweed	1	0.03	0.03	0.00	*
VAM*Seaweed	1	1.65	1.65	0.05	*
Inoculation*Seaweed	1	0.00	0.00	0.00	*
Location*VAM*Inoculation	1	3.83	3.83	0.12	*ns*
Location*VAM*Seaweed	1	1.95	1.95	0.06	*ns*
Location*Inoculation*Seaweed	1	0.00	0.00	0.00	*
VAM*Inoculation*Seaweed	1	5.25	5.25	0.17	*ns*
Location*VAM*Inoculation*Seaweed	1	6.90	6.90	0.22	*ns*
Error	304	9578.75	31.50		
Total	319	9897.12			

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

Table 3.2: Three-way ANOVA for 50% flowering parameters of tepary bean in response to VAM, bradyrhizobium inoculation and seaweed extract application in Syferkuil and Ga-Molepo.

Source of Variation	**Degree of Freedom**	**Sum of square**	**Mean square**	**F-test**	**P Value**
50% Flowering					
Location	1	0.11	0.11	0.00	*
VAM	1	12.80	12.80	0.05	*
Inoculation	1	0.45	0.45	0.00	*
Seaweed	1	30.01	30.01	0.11	*ns*
Location*VAM	1	12.80	12.80	0.05	*
Location*Inoculation	1	0.45	0.45	0.00	*
VAM*Inoculation	1	19.01	19.01	0.07	*ns*
Location*Seaweed	1	30.01	30.01	0.11	*ns*
VAM*Seaweed	1	3.20	3.20	0.01	*
Inoculation*Seaweed	1	0.80	0.80	0.00	*
Location*VAM*Inoculation	1	19.01	19.01	0.07	*ns*
Location*VAM*Seaweed	1	3.20	3.20	0.01	*
Location*Inoculation*Seaweed	1	0.80	0.80	0.00	*
VAM*Inoculation*Seaweed	1	0.31	0.31	0.00	*
Location*VAM*Inoculation*Seaweed	1	0.31	0.31	0.00	*
Error	304	84674.60	278.53		
Total	319	84807.89			

ns= non-significant

Table 3.3: Three-way ANOVA for tepary bean for plant height of tepary bean in response to VAM, bradyrhizobium inoculation and seaweed extract application in Syferkuil and Ga-Molepo.

Source of Variation	Degree of Freedom	Sum of square	Mean square	F-test	P Value
Plant Height					
Location	1	1643.48	1643.48	27.02	*ns*
VAM	1	10.95	10.95	0.18	*ns*
Inoculation	1	125.00	125.00	2.06	*ns*
Seaweed	1	0.15	0.15	0.00	*
Location*VAM	1	248.51	248.51	4.09	*ns*
Location*Inoculation	1	112.81	112.81	1.85	*ns*
VAM*Inoculation	1	4.70	4.70	0.08	*ns*
Location*Seaweed	1	508.54	508.54	8.36	*ns*
VAM*Seaweed	1	21.74	21.74	0.36	*ns*
Inoculation*Seaweed	1	53.96	53.96	0.89	*ns*
Location*VAM*Inoculation	1	4.23	4.23	0.07	*ns*
Location*VAM*Seaweed	1	13.04	13.04	0.21	*ns*
Location*Inoculation*Seaweed	1	5.15	5.15	0.08	*ns*
VAM*Inoculation*Seaweed	1	184.53	184.53	3.03	*ns*
Location*VAM*Inoculation*Seaweed	1	110.22	110.22	1.81	*ns*
Error	304	18491.2	60.8		
Total	319	21538.2			

*ns= non-significant, * $p \leq 0.05$ and** $p \leq 0.001$.*

Table 3.4: Three-way ANOVA for chlorophyll content of tepary bean in response to VAM, bradyrhizobium inoculation and seaweed extract application in Syferkuil and Ga-Molepo.

Source of Variation	Degree of Freedom	Sum of square	Mean square	F-test	P Value
Chlorophyll content					
Location	1	125903.06	125903.06	450.83	*ns*
VAM	1	51.97	51.97	0.19	*ns*
Inoculation	1	12.62	12.62	0.05	*
Seaweed	1	14.35	14.35	0.05	*
Location*VAM	1	73.38	73.38	0.26	*ns*
Location*Inoculation	1	13.38	13.38	0.05	*
VAM*Inoculation	1	183.38	183.38	0.66	*ns*
Location*Seaweed	1	49.02	49.02	0.18	*ns*
VAM*Seaweed	1	2119.95	2119.95	7.59	*ns*
Inoculation*Seaweed	1	5.16	5.16	0.02	*
Location*VAM*Inoculation	1	830.63	830.63	2.97	*ns*
Location*VAM*Seaweed	1	4128.21	4128.21	14.78	*ns*
Location*Inoculation*Seaweed	1	254.97	254.97	0.91	*ns*
VAM*Inoculation*Seaweed	1	171.76	171.76	0.62	*ns*
Location*VAM*Inoculation*Seaweed	1	463.11	463.11	1.66	*ns*
Error	304	84898.2	279.3		
Total	319	219173.2			

*ns= non-significant, * $p \leq 0.05$ and** $p \leq 0.001$.*

Table 3.5: Three-way ANOVA for 90% maturity of tepary bean in response to VAM, bradyrhizobium inoculation and seaweed extract application in Syferkuil and Ga-Molepo.

Source of Variation	**Degree of Freedom**	**Sum of square**	**Mean square**	**F-test**	**P Value**
90% Maturity					
Location	1	0.45	0.45	0.00	*ns*
VAM	1	6.05	6.05	0.01	*ns*
Inoculation	1	2.81	2.81	0.00	*ns*
Seaweed	1	7.20	7.20	0.01	*ns*
Location*VAM	1	6.05	6.05	0.01	*ns*
Location*Inoculation	1	2.81	2.81	0.00	*ns*
VAM*Inoculation	1	27.61	27.61	0.03	*ns*
Location*Seaweed	1	7.20	7.20	0.01	*ns*
VAM*Seaweed	1	0.80	0.80	0.00	*ns*
Inoculation*Seaweed	1	2.81	2.81	0.00	*ns*
Location*VAM*Inoculation	1	27.61	27.61	0.03	*ns*
Location*VAM*Seaweed	1	0.80	0.80	0.00	*ns*
Location*Inoculation*Seaweed	1	2.81	2.81	0.00	*ns*
VAM*Inoculation*Seaweed	1	2.81	2.81	0.00	*ns*
Location*VAM*Inoculation*Seaweed	1	2.81	2.81	0.00	*ns*
Error	304	316339.3	1040.59		
Total	319	316440.0			

*ns= non-significant, * $p \leq 0.05$ and** $p \leq 0.001$.*

Table 3.6: A three-way ANOVA for grain yield of tepary bean in response to VAM, bradyrhizobium inoculation and seaweed extract application in Syferkuil and Ga-Molepo.

Source of Variation	**Degree of Freedom**	**Sum of square**	**Mean square**	**F-test**	**P Value**
Grain yield (kg/ha)					
Location	1	23802.76	23802.76	0.87	*ns*
VAM	1	1734.54	1734.54	0.06	*ns*
Inoculation	1	11381.53	11381.53	0.41	*ns*
Seaweed	1	1411.74	1411.74	0.05	*ns*
Location*VAM	1	1.69	1.69	0.00	*ns*
Location*Inoculation	1	7545.35	7545.35	0.27	*ns*
VAM*Inoculation	1	401.55	401.55	0.01	*ns*
Location*Seaweed	1	0.00	0.00	0.00	*ns*
VAM*Seaweed	1	736.71	736.71	0.03	*ns*
Inoculation*Seaweed	1	603.63	603.63	0.02	*ns*
Location*VAM*Inoculation	1	5.97	5.97	0.00	*ns*
Location*VAM*Seaweed	1	100.02	100.02	0.00	*ns*
Location*Inoculation*Seaweed	1	194.99	194.99	0.01	*ns*
VAM*Inoculation*Seaweed	1	3182.32	3182.32	0.12	*ns*
Location*VAM*Inoculation*Seaweed	1	2868.35	2868.35	010	*ns*
Error	304	8346690	27456		
Total	319	8400662			

*ns= non-significant, * p ≤ 0.05 and** p ≤ 0.001.*

Table 3.7: Analysis of soil chemicals before planting

Location	P	K	Ca	Mg	Exch. Acidity	Total cation	Acid	pH	Zn	Org. C	N	Clay
			g/mL			cmol/L	Sat. %	KCL		%	%	%
Syferkuil	40.81	214.69	787.63	474.63	0.09	8.48	1.06	7.53	1.51	< 0.05	< 0.05	18,.06
Ga-Molepo	4.06	196.94	583.25	173.94	0.11	4.96	2.25	4.71	0.78	0.80	0.07	17.88

P=Phosphurus, K=Potassium, Ca=Calcium, mg=Magnesium, Exch.Acidity= Exchangeable Acidity, Acid Sat.%=Acid saturation, pH= Potential of Hydrogen, KLC. chloride, Zn=Zinc, Org.C= Organic carbon, N%= Nitrogen percent and Clay %= Clay percent.

Table 3.8: Soil chemical results at harvest

Location	P	K	Ca	Mg	Exch. Acidity	Total cation	Acid	pH	Zn	Mn	Cu	Org. C	N	Clay
			g/mL			cmol/L	Sat. %	(KCL)		g/mL		(%)	(%)	(%)
Syferkuil	46.63	259.69	833.56	561.63	0.09	9.54	1.00	7.75	2.04	21.88	2.97	0.50	<0.05	17.81
Ga-Molepo	4.75	228.81	609.25	167.81	0.09	5.09	1.63	4.65	0.75	35.56	2.38	0.78	0.07	19.94

P=Phosphurus, K=Potassium, Ca= Calcium, mg= Magnesium, Exch. Acidity= Exchangeable Acidity, Acid Sat.%=Acid saturation, pH= Potential of Hydrogen, KLC= Potassium chloride,Zn=Zinc, Org.C= Organic carbon, N%= Nitrogen percent and Clay %= Clay percent.

Location showed significant (p≤0.05) differences on days to reach 50% emergence (Figure 3.2). Syferkuil took fewer days to get 50% emergence at an average mean of 1.46, and Ga-Molepo took more days with 3.11. The location also had significant (p≤0.001) differences in plant height (Figure 3.3). In comparison to Ga-Molepo, which obtained shorter plants of 29.44 cm, Syferkuil obtained a substantially longer plant with a length of 33.97 cm.

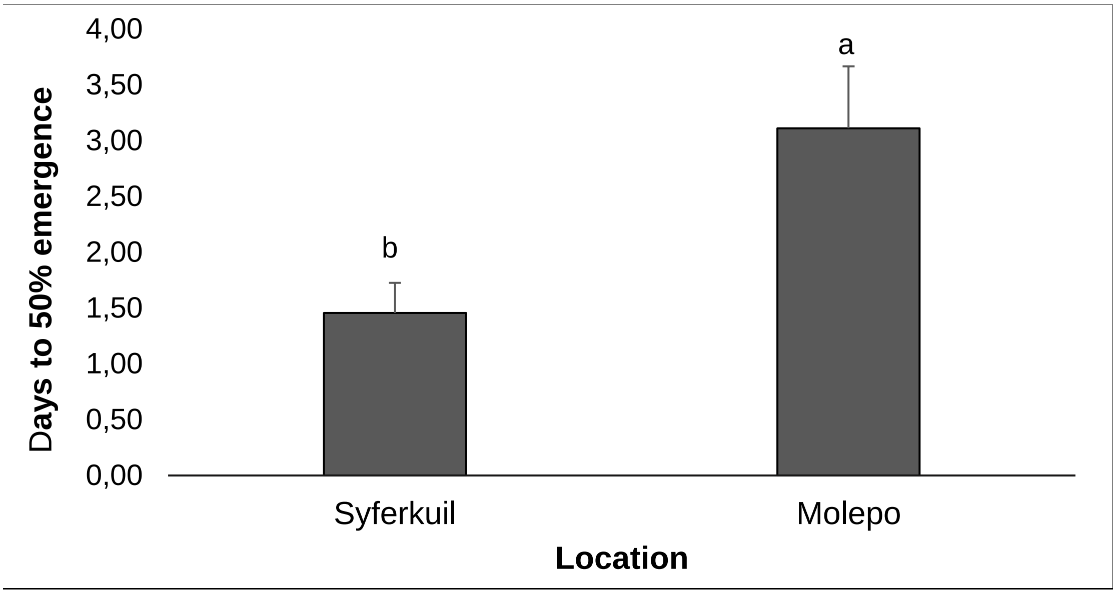

Figure 3.2: A one-way mean value for days to 50% emergence in response to different locations (Syferkuil and Ga-Molepo) and error bars indicate standard error.

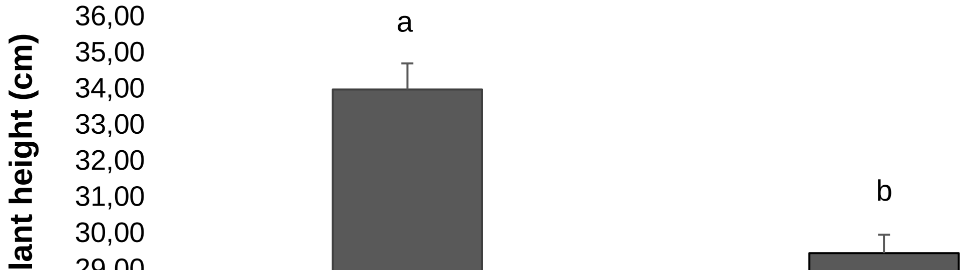

Figure 3.3: A one-way mean value for plant height in response to a different location (Syferkuil and Ga-Molepo) and error bars indicating a standard error.

Vesicular arbuscular mycorrhizae and location significantly influenced plant height (Figure 3.4). The interaction effect of VAM (no application) × Syferkuil significantly increased plant height by 35.04 cm, while the interaction effect of VAM (application) × Syferkuil especially obtained less plant height (32.91 cm).

Significant longer plants (30.14 cm) were observed under the interaction of VAM (application) × Ga-Molepo. Interaction effect of VAM (no application) × Ga-Molepo significantly obtained shorter plants (28.74 cm). The interactive effect of VAM (no application and application) and Syferkuil greatly influenced plant height more than that of VAM (no application and application) and Ga-Molepo.

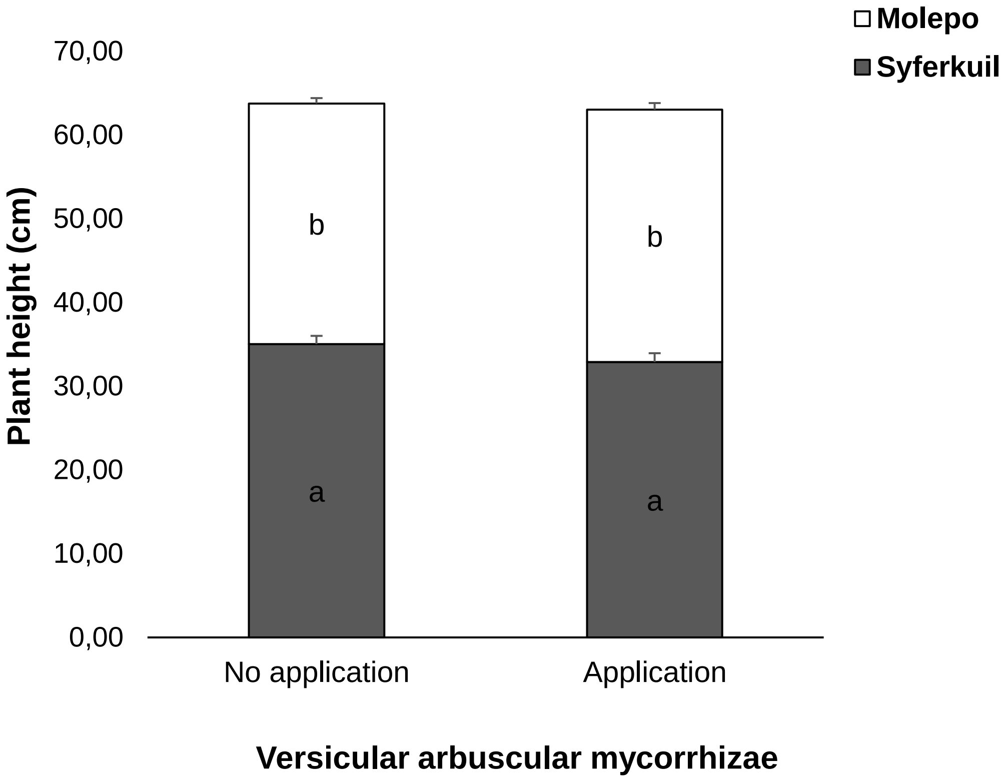

Figure 3.4: A two-way mean value for plant height in response to the interaction effect of VAM and location and error bars indicating a standard error.

Figure 3.5 shows the interactive effect of seaweed extract and location on plant height. Interaction effect of seaweed extract (no application) × Syferkuil significantly increased plant height (35.21 cm), while application of seaweed extract × Syferkuil interaction significantly obtained less plant height (32.73 cm). When seaweed extract is applied, Ga-Molepo's plants grow to a much higher height (30.72 cm), however, when seaweed extract is not applied, Ga-Molepo's plants grow to a significantly lower height (28.16 cm).

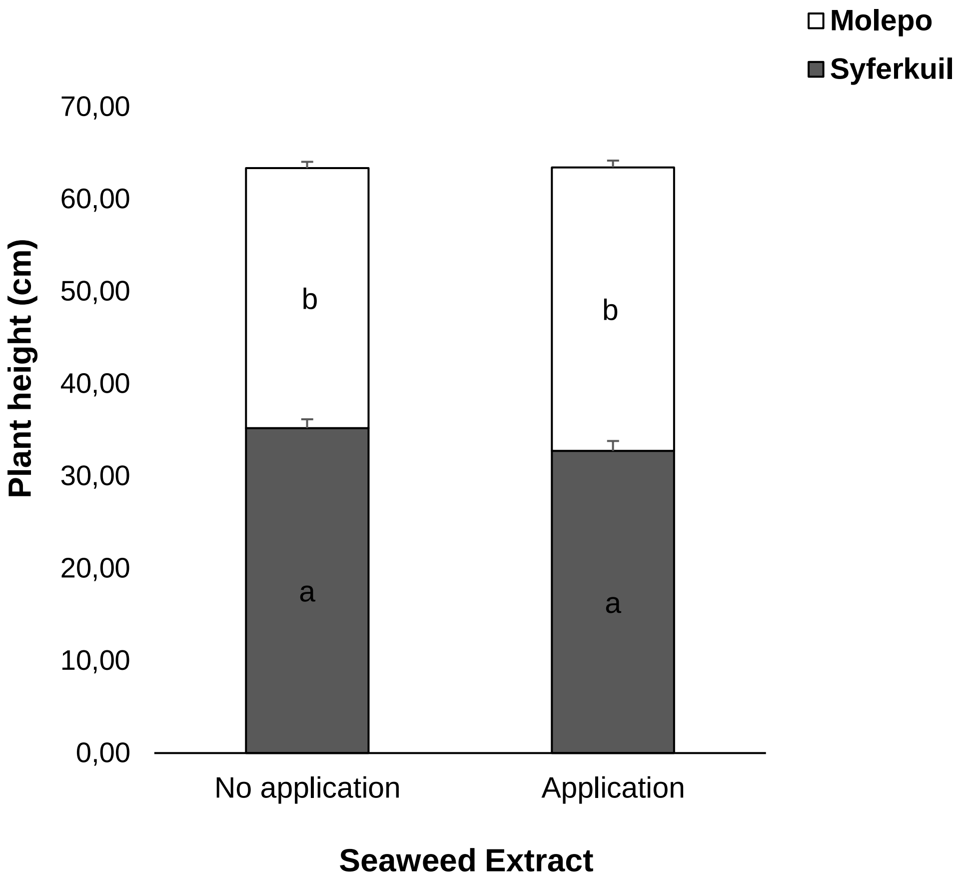

Figure 3.5: A two-way mean value for plant height in response to the interaction effect of seaweed extract levels and location and error bars indicating a standard error.

Figures 3.6 shows that location had a significant difference in chlorophyll content of tepary bean (p≤0.001). The highest chlorophyll concentration was substantially found in Syferkuil (66.40 CCI) and the lowest in Ga-Molepo (26.73 CCI).

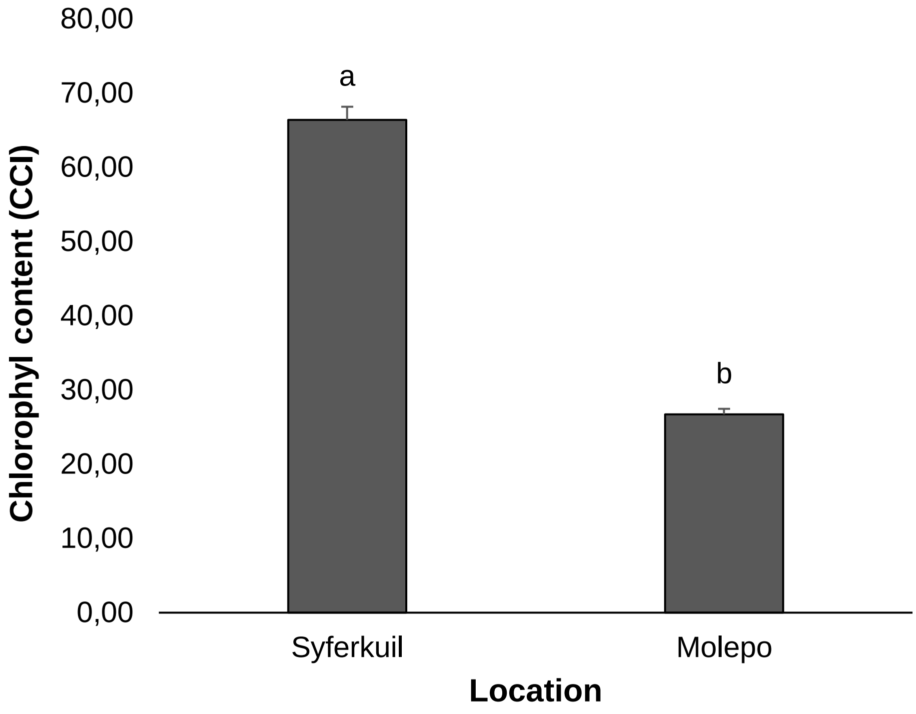

Figure 3.6: A one-way mean value for chlorophyll content in response to location and error bars indicate standard error.

Chlorophyll content was significantly influenced by vesicular-arbuscular mycorrhizae and seaweed extract (Figure 3.7). The interactive effect of VAM (no application and application) and seaweed extract (no application and application) significantly did not affect the chlorophyll content (43.80 CCI) of tepary beans.

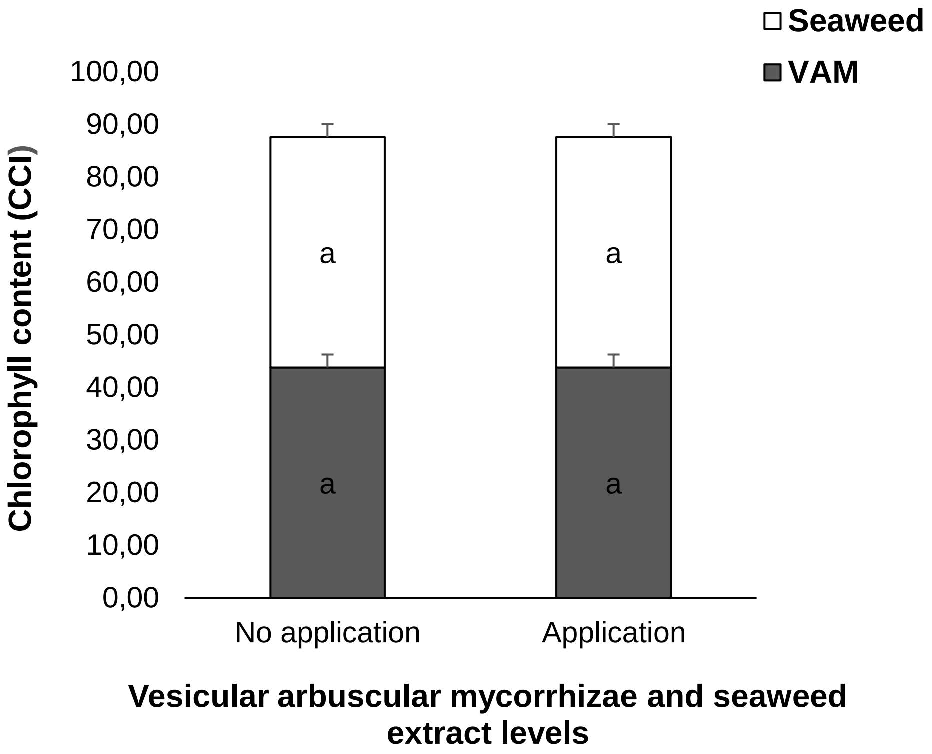

Figure 3.7: A graph indicating two-way mean values for chlorophyll content in response to the interaction effect of VAM and seaweed extract levels at Syferkuil and error bars indicating a standard error.

Location showed significant (p≤0.001) differences in the number of branches and number of pods per plant (Figures 3.8 and 3.9). A significantly high number of branches (5.64) was observed at Ga-Molepo. In contrast, Syferkuil obtained less number of branches per plant (3.61). On the contrary, a significantly high number of pods, with 26.13, was observed in Syferkuil, while Ga-Molepo obtained fewer pods per plant (17.81).

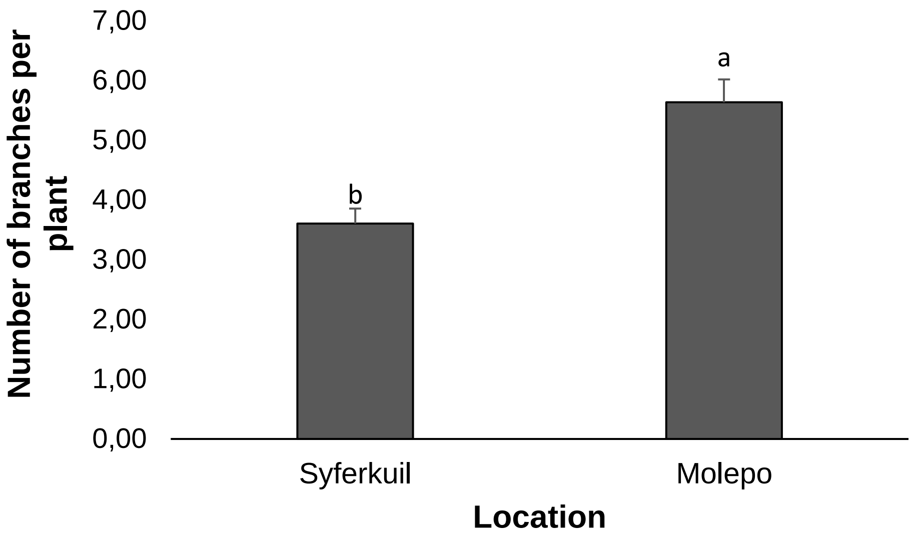

Figure 3.8: A one-way mean value for the number of branches in response to location and error bars indicating standard error.

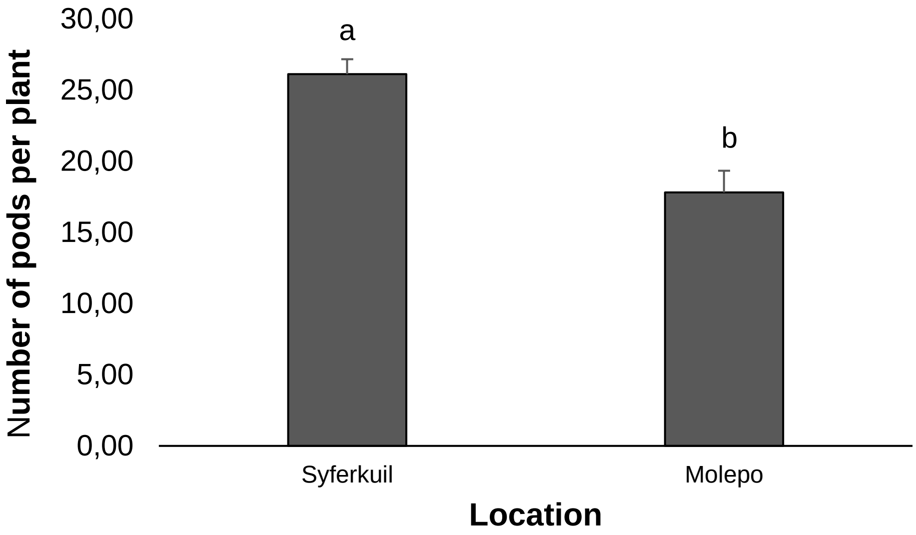

Figure 3.9: A one-way mean value for the number of pods per plant in response to location and error bars indicating a standard error.

Location and vesicular-arbuscular mycorrhizae significantly affected pod length (Figure 3.10). No application of VAM at Syferkuil resulted in much longer pods (6.42 cm), whereas VAM at Syferkuil resulted in shorter pods (6.40 cm). When VAM was used, Ga-Molepo exhibited significantly increase in pod length (6.31 cm), whereas when VAM is not applied, Ga-Molepo showed decreased pod length (6.16 cm).

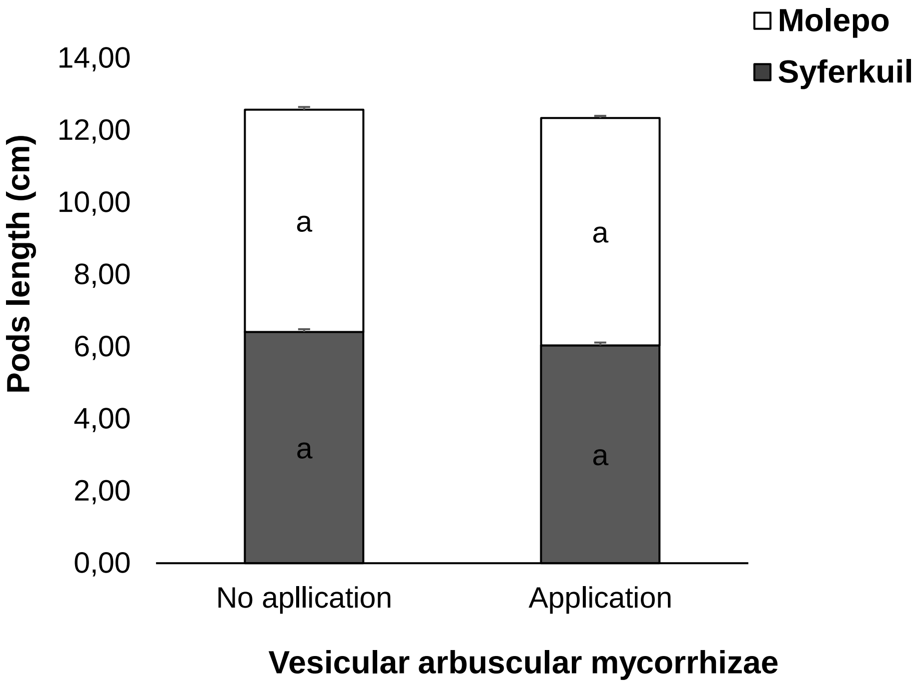

Figure 3.10: A two-way mean value for pod length in response to the interaction effect of VAM levels and location and error bars indicate standard error.

Pod length was significantly ($p \leq 0.05$) influenced by the interaction of seaweed extract and location (Figure 3.11). No application of seaweed extract at Syferkuil significantly had shorter pods (6.18 cm). Compared to previous treatments, applying seaweed extract at Syferkuil dramatically lengthened the pod (6.27 cm). The relationship between seaweed extracts (no application) and Ga-Molepo exhibits increased pods (6.35 cm). Interactions that seaweed extract has (application) Ga-Molepo significantly reduced the length of the pod (6.12 cm) compared to other treatments.

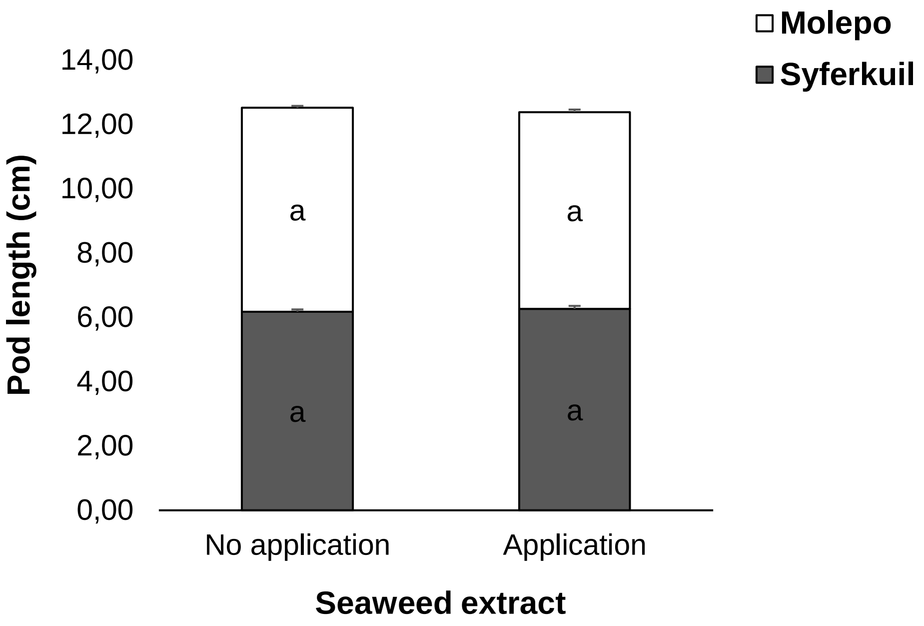

Figure 3.1: A two-way mean value for pod length in response to the interaction effect of seaweed levels and location and error bars indicating the standard error.

The most significant average number of seeds per pod (4.74) was seen in Syferkuil when VAM was not applied (Figure 3.12). Contrary, When VAM was not applied, a significantly lower number of seeds per pod (4.34) were seen at Ga-Molepo.

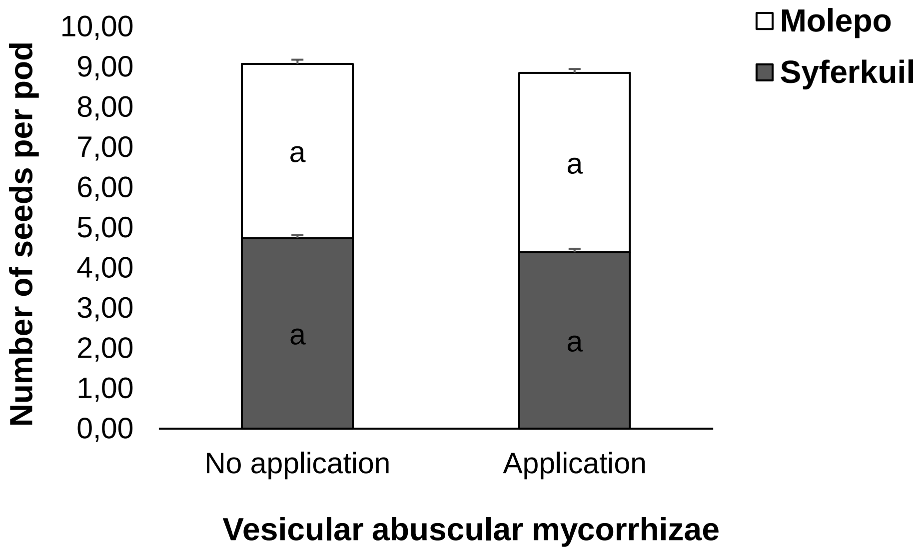

Figure 3.12: A two-way mean value for the number of seeds per pod in response to the interaction effect of VAM levels application and location and error bars indicating a standard error.

3.5 Discussion

3.5.1 Effect of location on growth parameters

There was a significant difference among locations on the growth parameters (Ga-Molepo and Syferkuil). Syferkuil greatly influenced 50% of emergence, plant height, chlorophyll content and the number of pods per plant. Although grain yield at Syferkuil did not show any significant difference, there was an increase in yield due to the performance of growth parameters. This might be due to the availability of adequate moisture for production because Syferkuil received supplementary irrigation. This result is comparable to that of Saleh *et al*. (2018), who found that increasing water application boosted most plant growth parameters and pod yield.

Ga-Molepo performed poorly regarding growth parameters (50% emergence, plant height, chlorophyll content and the number of pods per plant. This performance resulted in a yield decrease at Ga-Molepo. An enormous amount of rain after planting at Ga-Molepo peaked in February, which is when emergence was supposed to occur. The rainfall dropped drastically during the vegetative stage. This result implies that the heavy rains that fell during the emerging period prevented growth and caused moisture stress at the vegetative stage. The outcomes are in line with those of Lamichhane *et al*. (2019), who found that heavy rainfall events following sowing resulted in soil crusts that made it difficult to distinguish between soil clods and soil surface crusts, which caused 12% losses in seedling emergence and about 11% of seedling deaths. The findings by Abayomi and Abidoye (2009) also observed that as soil moisture stress increased, a water deficit during the vegetative phase decreased plant height, the number of leaves per plant, and the number of flowers per plant.

Ga-Molepo obtained a significantly increased number of branches at an average of 5,64, while Syferkuil obtained a smaller number per plant (3.61). This result is supported by Green-Tracewicz *et al*. (2010), who claim slow branch growth is influenced by unfavourable growing conditions, including too much soil moisture and agricultural methods like late planting, a decrease in the ratio of red to far-red light, and high plant density.

3.5.2 The interaction effect of seaweed extract and location on growth and yield parameters was studied.

The tepary bean's plant height, chlorophyll content, and pod length were significantly affected by seaweed extract and its interactions with the location. The application of seaweed extract had an appreciable impact on the quantity of chlorophyll and plant height, while no application of seaweed increased plant height at Syferkuil. This finding suggests that the crop survived because the site had high soil nutrients such as Mg, P, K and Ca had supplementary irrigation, which increased this plant height. Similar findings were reported by Kovácik *et al*. (2009), who observed an inhibitory effect on plant development after applying seaweed to chamomile plants. This result is also consistent with that of Latique *et al*. (2013), who found that foliar application of seaweed extract had no appreciable impact on the quantity of chlorophyll in plant leaves.

Interaction of seaweed extract application at Molepo shows a significant increase in plant height (30.72 cm), while seaweed extract application at Syferkuil significantly increased the pod length of tepary bean (6.27 cm). This may be because seaweed contains highly adequate nutrition, which encourages crop development and yield as well as biotic and abiotic resistance (Zewail, 2014). According to Badar *et al*. (2015) and Alves *et al*. (2016), a boost of growth and development in crops by seaweed extract is most likely due to the generation of hormones and the presence of macro- and micronutrients that enhanced amino acid transport, cell division, cell enlargement, and nutrient uptake (N, P, and K). Elansary *et al.* (2016) and Khan *et al.* (2009) observed an increase in leaf area, number, and dry weight after treatment with seaweed extracts. Ramesh *et al*. (2013) also found that seaweed extract (NAA) at 40 ppm boosted soybean plant height. In comparison, Vasantharaja *et al*. (2019) discovered that seaweed extract increased *Vigna unguiculata's* shoot length, leaf number, and yield.

3.5.3 Interaction effect of VAM and location on plant height and number of seeds per pod

The highest chlorophyll content, plant height, and the number of seeds per pod were significantly increased by no application of VAM at Syferkuil. This is because soil chemical analysis (Figures 3.7 and 3.8) showed that soil phosphorus before planting was enough for plant growth, hence no application of VAM did not impact plant height and the number of seeds per pod of tepary bean. This study's findings are consistent with those of AL-zalzaleh and AL-zalzaleh, (2007), who stated that among all treatments, the highest plant height of 154 cm was recorded in VAM un-inoculated *Parkinsonia aculeata* growing in agricultural soil and VAM-inoculated *Nerium oleander* grew in agricultural soil, the lowest height was 49 cm.

Molepo significantly decreased in plant height and number of seeds per pod when VAM was not applied, (28.74 cm and 4.34 cm respectively. This could result from low soil phosphorus before planting (Table 3.7), and most plants find it difficult to obtain phosphorus due to its low mobility and solubility in the soil (Osborne and Rengel, 2002). Phosphorus is essential to plants' growth and productivity (Malhotra *et al*., 2018). The findings of this study corroborate what Nasim, 2005 reported. The overall phosphorus concentration in soil is relatively low, primarily transferred to roots by diffusion. However, P has a very low diffusion coefficient, so it is readily depleted from the root zone. VAM association can significantly improve a plant's access to P sources in the soil (Nasim, 2005).

VAM application at Molepo resulted in considerably long plants (30.14 cm). Ga-Molepo's soil phosphorus increased following VAM application. The increase in soil phosphorus implies that due to VAM application, arbuscular mycorrhizae fungi managed to mobilize phosphorus in the soil and make it available for plant uptake hence there was an increase in height. This study's findings are similar to those of AL-zalzaleh and AL-zalzaleh, 2007, who reported that the mean plant height was higher in the VAM inoculated treatment compared to the VAM un-inoculated treatment. VAM-inoculated *Prosopis chilensis* grew 5.54% taller than un-inoculated plants. VAM inoculation increased plant height in *Ficus infectoria* by 2% over un-inoculated plants. The results are further supported by Grant *et al*. (2005), who reported that when VAM is added to the soil, the amount of phosphorus available to plants increases, as does the amount of phosphorus in the plant's tissues.

3.5.4 Effect of Bradyrhizobium inoculation on growth and yield parameters studied

Bradyrhizobium inoculation did not show any significant difference in the growth, yield and yield parameters studied in both locations. These could be brought on by local rhizobia that occasionally competes with newly introduced strains in the field (di-Cenzo *et al*., 2018). Indigenous rhizobia are reported to be more competitive than the inoculant strain and thus inhibits its effect (Rodriguez *et al*., 2010; Yates *et al*., 2011). Environmental circumstances, such as moisture stress, do not support the growth of Rhizobium bacteria (Yang *et al*., 2019). At Ga-Molepo, the study was carried out under rainfed conditions, and between March and May 2021, the amount of rain at Ga-Molepo rapidly decreased, leaving the soil with insufficient moisture, which may have had an adverse influence on the efficacy of recently introduced strains (Aldesuquy *et al*.,2013). The deficient phosphorus at Ga-Molepo and nitrogen level at Syferkuil observed may potentially be to blame for this non-response (Table 3.7 and 3.8). This is by the study of Malhotra *et al*. (2018), who reported that rhizobium needs phosphorus to drive energy for atmospheric nitrogen fixation by fostering development. This is further supported by Hikosaka (2004), who reported that phosphorus (P) and nitrogen (N) are two essential nutrients for crop growth and development, and their levels are naturally low in most developing country soils, including South Africa. The absence of water in leaves affects chlorophyll synthesis, encourages its breakdown, and hastens the yellowing of leaves, thus affecting crop yield.

3.6 Conclusion

Based on the results, Syferkuil performed better on days to reach 50% emergence, plant height, chlorophyll, and the number of pods per plant than Ga-Molepo, which had a poor performance generally because of its soil pH, low nutrient status and dependence on rainfall. This resulted in an increase in grain yield at Syferkuil and a decrease at Ga-Molepo. The soil in Ga-Molepo was highly acidic, which makes it not favourable for bradyrhizobium inoculation. However, despite the environmental conditions of Ga-Molepo, it obtained more branches than Syferkuil. Seaweed extracts and VAM at Ga-Molepo increased plant height, pod length, and the number of seeds per pod. Bradyrhizobium inoculation did not show any influence on the parameters studied. This indicates that these biofertilizers can increase tepary bean productivity even in less favourable conditions. More studies are recommended to quantify the results obtained from this study.

CHAPTER 4

EFFECT OF BRADYRHIZOBIUM INOCULATION, SEAWEED EXTRACT AND VAM ON BIOLOGICAL NITROGEN FIXATION OF TEPARY BEAN.

4.1. Introduction

Tepary bean is a legume known for its high protein content and ability to form symbiotic relationships with Rhizobia bacteria, allowing them to fix atmospheric nitrogen and replenish it in the soil for future crops (Mapp, 2008; Moghaddam *et al*., 2021a). Nitrogen fixation is a critical process involving the reduction of molecular nitrogen to form ammonia, which is the form of nitrogen used by living systems to synthesise many bioorganic compounds. Biological nitrogen fixation has the added benefit of being environmentally friendly, making it ideal for sustainable agriculture. Nitrogen biologically fixed could be absorbed directly by plants, leaving the environment unaffected. Inoculation with Rhizobial strains is one strategy for increasing symbiotic N fixation by legumes and thus yield in crop production systems (Amba *et al*., 2013). Inoculation of legumes with Rhizobium causes more nitrogen fixation and makes it available to the plants; therefore, it is used as an alternative to urea to reduce production costs (Karim *et al*., 2001). The mutual interactions of soil fungus and root tissues of the host plant form the Vesicular Arbuscular Mycorrhiza (VAM) structure in plant roots. The primary function of VAM is to increase soil P availability and, thus, P uptake by macrosymbionts (Toljander, 2006). Several decades of research on various aspects of root symbionts have revealed that the dual interaction of AM fungi and Rhizobium has improved legume growth, nodulation, and yield (Gill and Singh, 2002; Talaat and Abdallah, 2008), as well as nutrient status (Talaat and Abdallah, 2008; Chakrabarty *et al*., 2007). According to Clark and Zeto (2000), Arbuscular Mycorrhiza fungi (AM fungi) help legumes fix nitrogen by supplying phosphorus and other immobile nutrients. According to Tavasolee *et al.* (2011), effective AM fungi can improve rhizobial infection performance. Arbuscular Mycorrhiza fungi help plants absorb phosphates more efficiently in legume plants, phosphate stimulates nodule production, increasing the rate of atmospheric nitrogen fixation Schmidt (2005). Seaweed extract (SWE) is a natural organic fertilizer that contains highly effective nutrients and promotes faster seed germination as well as increased yield and resistance in many crops (Zewail, 2014).

In contrast to chemical fertilizers, SWE extracts are biodegradable, non-toxic, non-polluting, and non-hazardous to plants. Exogenous SWE application has already been shown to improve plant growth, yield, and quality, as Abdel Mawgoud et al. (2010) reported on the celeriac plant and Abou El-Yazied *et al*. (2012) on Snap Bean. Higher concentrations of seaweed extract increase grain N, P, and K uptake (Rathore *et al*., 2009). Biofertilizers are an alternative to improve the conditions of global fields because they do not pollute the soil or atmosphere and help to produce healthy foods (Andrew *et al*., 2007). This study aimed to determine the effect of rhizobium inoculation, seaweed extract and VAM on the biological N-fixation of tepary bean.

4.2. Materials and Methods

4.2.1. Study area, experimental design and treatments

The study area and experimental designs are explained in detail in Chapter 3.

4.2.2 Plant Sampling and processing

Three representative plants were dug up at flowering, and roots and shoots were separated. Fresh weight for the shoot and roots were recorded then after the samples were oven-dried at 60 °C until they reached a constant weight, and the dry weight was also recorded. Plants were ground to a particle size of 0.25 mm, placed in plastic bags, and labelled. The samples were packaged and transported to the University of Pretoria's Stable Isotope Laboratory to analyse the ^{15}N isotopic composition.

4.2.3 Measurements of N_2 fixation

The ^{15}N natural abundance approach was used to evaluate nitrogen-fixing. Three plants from each treatment plot were carefully dug up for each location. The shoots and pods were dried in an oven at 60 °C until they reached a constant weight, processed through a 0.451 mm sieve, weighed and stored in vials before ^{15}N analysis. Three non-legume plants (weeds) from each location were sampled as reference plants for measuring tepary bean N uptake from soil. The reference plants were handled in the same way as the tepary bean.

4.2.4 $^{15}N/^{14}N$ analyses

Ground reference plant samples of 2.5 mg and legume samples of 2.0 mg were weighed into tin capsules, placed onto a Carlo Erba NA1500 Elemental Analyzer via Conflo II Open-Split Device and run to measure the ratio of $^{15}N/^{14}N$ and N concentration (percent N) in the plant material.

4.2.5 Nitrogen content

Isotope composition ($\delta^{15}N$) was measured according to Unkovich *et al*. (2008) as follows:

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

The result of %N and sample weight was used to calculate the N content of the plant samples.

%N derived from atmospheric fixation (%Ndfa)

The following is how the amount of nitrogen produced by N-fixation was calculated (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 $\delta^{15}N_{ref}$ denotes the ^{15}N natural abundance of non-fixing reference plants (weeds), $\delta^{15}N_{leg}$ denotes N-fixing legume (tepary bean), and B represents the ^{15}N natural abundance of tepary bean plants that rely only on N_2 fixation of their N nutrition.

Where $\delta^{15}N_{leg}$ is the ^{15}N natural abundance of the legume, $\delta^{15}N_{ref}$ is the ^{15}N natural abundance of the reference plant, and B is the natural abundance of ^{15}N in tepary bean plants which solely obtain their N through N_2 fixation. The percentage % of Ndfa was calculated using the B value (-1.80) (Balboa and Ciampiti, 2020). The quantity of fixed N was calculated as shown below (Peoples *et al*., 2002)

N-fixed = %Ndfa × legume biomass N

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

4.2.5 Data Analysis

Data collected were subjected to analysis of variance using Statistica software version 10 at a significance threshold of 5%, and the means were separated using Tukey. Three-way ANOVA was used to compare symbotic parameters in response to bradyrhizobium inoculation, VAM, and seaweed extract application.

4.3 Results

4.3.1 δ^{15}N and %Ndfa values

Dry matter, δ^{15}N, %Ndfa and N-fixed did not show a significant difference in response to the application of VAM, bradyrhizobium inoculation and seaweed extract at Syferkuil. According to the data in Table 4.1, the δ^{15}N values of the treatments used on tepary beans grown in Syferkuil ranged from 6.87‰ to 7.42‰. The treatments with the highest δ^{15}N values are VAM and seaweed extract. High N-fixed was observed under no application of VAM, while low N-fixed was obtained under VAM application (Table 4.1). The treatment with the highest δ^{15}N values (VAM) derived the most N from atmospheric N_2 fixation, while the one which obtained average δ^{15}N values (Bradyrhizobium inoculation) showed the lowest %Ndfa values.

Table 4.1: Analysis of dry matter, the symbiotic performance of tepary bean samples collected at Syferkuil during the 2020/2021 summer growing season.

VAM	Dry matter g	$\delta^{15}N$ ‰	Ndfa %	N-fixed mg
Inoculated	13.28	7.41	10.01	26.60
Un-inoculated	19.66	6.88	15.12	56.10
F-statistics	1.97 ^{ns}	1.24 ^{ns}	1.24 ^{ns}	3.60 ^{ns}
Bradyrhizobium inoculation				
Inoculated	18.27	7.39	10.14	43.48
Un-inoculated	14.67	6.90	14.99	39.22
F-statistics	0.57 ^{ns}	1.11 ^{ns}	1.11 ^{ns}	0.06 ^{ns}
Seaweed extract				
Application	13.63	6.87	15.27	39.73
No application	19.31	7.42	9.87	42.97
F-statistics	1.52 ^{ns}	0.26 ^{ns}	1.40 ^{ns}	0.03 ^{ns}

ns= non-significant

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

Tepary bean dry matter and symbiotic performance were not affected by VAM, bradyrhizobium inoculation, and seaweed extract at Ga- Molepo. Dry matter ranged from 28.54 g under no seaweed extract to 36.87 g under the application of seaweed extract (Table 4.2). The treatment with the highest $\delta^{15}N$ values is bradyrhizobium inoculation, and the lowest was observed under no inoculation of bradyrhizobium. Un-inoculated bradyrhizobium derived the most N from atmospheric N_2 fixation (180.5 mg) and %Ndfa values (31.23%), while inoculated bradyrhizobium derived less (144.18 mg) and %Ndfa of (23.14%). Amongst all treatments, Seaweed derived the highest N-fixation (183.81 mg).

Table 4.2: Dry matter and symbiotic performance of tepary bean samples collected at Ga-Molepo during the summer growing season of 2020/2021.

VAM	**Dry matter G**	**$\delta^{15}N$ ‰**	**Ndfa %**	**N-fixed mg**
Inoculated	32.80	2.31	29.07	164.96
Uninoculated	32.61	2.52	25.31	159.47
F-statistics	0.001 ^{ns}	0.328 ^{ns}	0.328 ^{ns}	0,0160 ^{ns}
Bradyrhizobium inoculation				
Inoculated	34.51	2.65	23.14	144.80
Un-inoculated	30.89	2.18	31.23	180.50
F-statistics	0.589 ^{ns}	1.663 ^{ns}	1.663 ^{ns}	0287 ^{ns}
Seaweed extract				
Application	367	2.42	27.14	183.81
No application	284	2.41	27.24	140.62
F-statistics	3.817 ^{ns}	0.0002 ^{ns}	0.0002 ^{ns}	1.069 ^{ns}

ns= non-significant

4.3.3 $\delta^{15}N$ values of reference plants

The shoots of three non-legume species were sampled at Syferkuil and Ga-Molepo as reference plants for estimating the %Ndfa of tepary bean. For Syferkuil, the values of those three weed species ranged from 7.85‰ to 9.30‰, with a combined $\delta^{15}N$ mean value of 8.43‰ (Table 4.3). At Ga-Molepo, The $\delta^{15}N$ values at that site ranged from

2.94‰ to 4.89‰. The combined mean $\delta^{15}N$ value of reference plants used for estimating %Ndfa was 3.99‰ (Table 4.3).

Table 4.3: $\delta^{15}N$ of non-legume plant species used as reference plants to estimate soil N uptake by tepary bean. For every sample, three runs were conducted.

Location	**Reference plants**		**$\delta^{15}N$**
	Common names	**Botanical names**	**%**
Syferkuil	Silverleaf nightshade	*Solanum elaeagnifolium*	7.85
	Barbeton daisy	*Gerbera jamesonii*	8.14
	Jungle rice	*Echinochloa colona*	9.30
		Mean	**8.43 ± 0.77**
Ga-Molepo	Sessile Joyweed	*Alternanthera sessilis*	4.89
	Bermuda grass	*Cynodon dactylon*	4.15
	Carpetweed	*Mollugo verticillata*	2.94
		Mean	**3.99 ±0.98**

Figure 4.1 shows the average mean for dry matter obtained at Syferkuil and Ga-Molepo. Location significantly showed a difference in dry weight ($p \leq 0.001$). Tepary beans planted at Ga-Molepo significantly obtained greater mean the dry matter of 32.70 g compared to the one grown at Syferkuil (16.47 g).

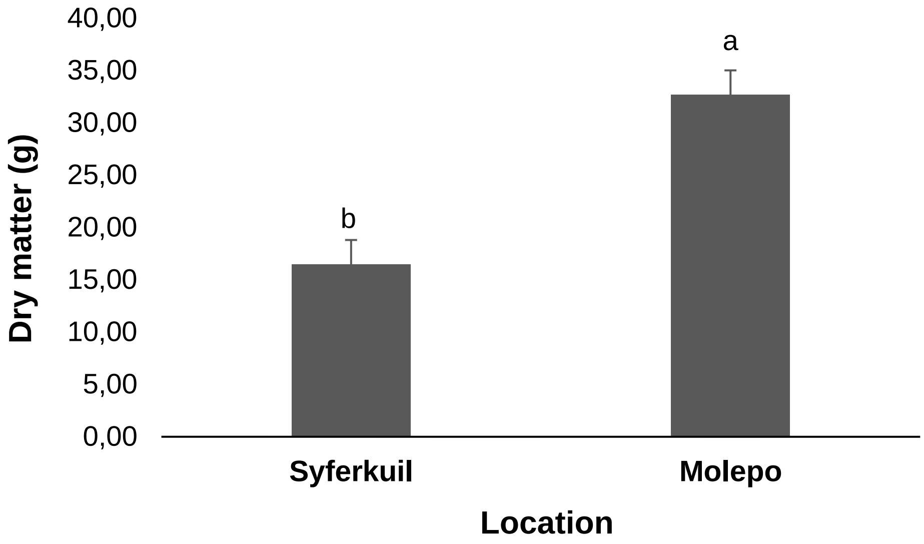

Figure 4.1: One-way mean value for dry matter in response to a different location (Syferkuil and Molepo) and bars indicating a standard error.

The %Ndfa differed significantly by location (Figure 4.2). Tepary beans planted in Ga-Molepo significantly outperformed Syferkuil in terms of mean %Ndfa. Ga-Molepo was greater at 27.19%, and Syferkuil was lower at 12.57%.

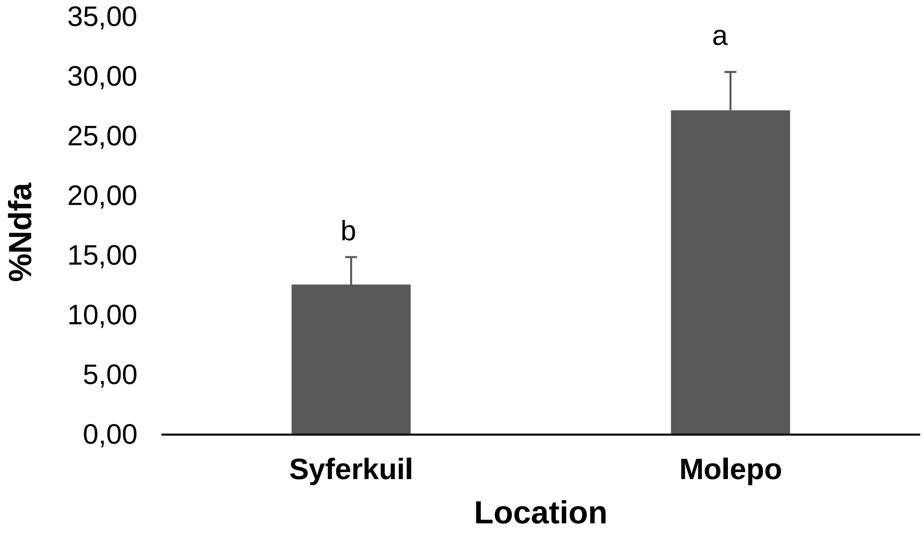

Figure 4.2: The one-way mean value for %Ndfa between two locations (Syferkuil and Ga-Molepo). Bars on top of the graphs show standard error.

The N-fixed showed substantial variations based on the location (Figure 4.3). Tepary beans grown at Ga-Molepo considerably increased the mean N-fixed. Ga-Molepo was greatest at 162.22 mg compared to Syferkuil, which obtained 41.35 mg.

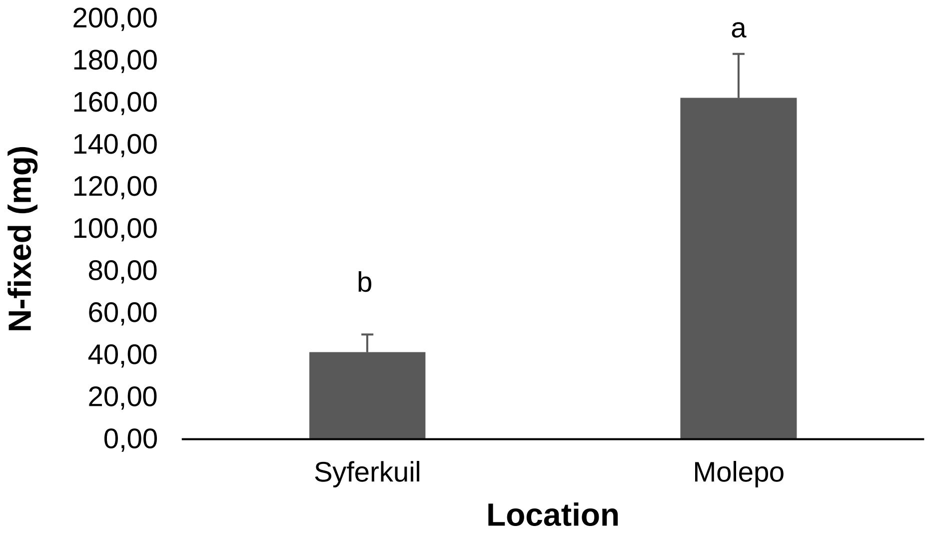

Figure 4.3: One-way ANOVA value for N-fixed in response to two locations (Syferkuil and Ga-Molepo). Bars showing the standard error.

4.4 Discussion

4.4.1 Location-specific symbiotic N contribution

The $\delta^{15}N$ natural abundance technique was used to evaluate N contribution by tepary beans grown at two locations in the Limpopo province of South Africa. To estimate soil N uptake by tepary bean, three different non-legume species were sampled and analyzed as reference plants for their $\delta^{15}N$ / $\delta^{14}N$ ratios (Table 4.3). The number of weeds, grasses and crop species analyzed included three from each location (Syferkuil and Ga-Molepo). The $\delta^{15}N$ values of these reference plants ranged from 7.85‰ to 9.3‰ at Syferkuil and 2.94‰ to 4.89‰ at Ga-Molepo. The difference between the combined mean $\delta^{15}N$ value of reference plants and the highest $\delta^{15}N$ value of tepary bean was 1.01‰ for Syferkuil and 1.34‰ for Ga-Molepo. Usually, the more significant this difference is between the $\delta^{15}N$ of the legume and the mean $\delta^{15}N$ of reference, the higher the precision of the $\delta^{15}N$ natural abundance technique (Unkovich *et al*., 1994). Therefore, the differences in this study indicate that the method has the potential for high-precision measurement of N_2 fixation in field-grown tepary beans. Although a mixture of herbaceous and graminaceous non-legume was collected for estimating soil N uptake by tepary bean, using combined mean $\delta^{15}N$ values eliminated any bias as some species from all two experimental sites showed markedly different $\delta^{15}N$ values. An evaluation of three treatments at two locations in South Africa revealed large differences in plant growth and symbiotic performance. At each site, the treatments exhibited marked variation in dry matter, pod, and grain yield, as well as in symbiotic N contribution. VAM (inoculated), seaweed extract (application) and bradyrhizobium (un-inoculated) fixed the most N at Ga-Molepo (164.96 mg; 183.81 mg and 180.25 mg) and therefore showed more significant dry matter accumulation (Table 4.2). The administration of these treatments is known to enhance nitrogen fixation and provide appropriate nutrients, thus, these results were expected. Amin *et al.* (2020), whose results clearly showed that various applied treatments increased measured growth characteristics (such as the number of leaves per plant, leaf area, and total chlorophyll (SPDS), the yield and components of the yield (such as the number of pods per plant, seed yield per plant, and seed yield), and chemical constituents (such as total protein, carbohydrates, nitrogen, and phosphorus). Bagyaraj, (2018) reported that VAM significantly enhances nodulation and nitrogen fixation by legume bacteria, primarily by providing the high phosphorus needed for the fixation process.

At Syferkuil, no inoculation of VAM, bradyrhizobium inoculation and no application of seaweed contributed the most symbiotic N (56.10; 43.48 and 42.97 mg/ha, respectively) (Table 4.1). This is because most of the nutrients in the soil were sufficient for production, and the location's environmental factors, including the availability of moisture, the local temperature, and the amount of rainfall, were favourable for bradyrhizobium activities that further increased. According to Irisarri *et al*. (2019), it is frequently believed that the inoculated strain determines how well symbioses and nitrogen fixation rates perform in crops that have been inoculated with commercial strains.

4.4.2 The effect of the location to dry matter, %Ndfa and N-fixed

Dry matter, $\delta^{15}N$, %Ndfa and N-fixed did not show a significant difference in response to the application of VAM, bradyrhizobium inoculation and seaweed extract at both locations. However, there was a significant difference in dry matter, %Ndfa and N-fixed in response to the location. Ga-Molepo performed better than Syferkuil. These parameters were directly affected by the soil nutrient status. Ga-Molepo displayed a greater soil N content before and after planting than Syferkuil. The results of this study are consistent with those of Diatta *et al.* (2020), who found that high soil nitrogen led to increases in dry shoot matter, shoot nitrogen content, and ^{15}N in their research. Syferkuil results may be due to having more soil P, which resulted in lowered mycorrhizal colonization levels, which also resulted in a barely less noticeable effect of VAM fungi on plants (Yadav and Aggarwal, 2014). According to Pharudi (2010), mycorrhizal associations tend to decline with rising soil phosphorus, which may account for this study's non-significant responses to nitrogen fixation.

4.5 Conclusion

In this study, no significant differences were found between the treatments tested; however, these treatments increased dry matter, $\delta^{15}N$ and %Ndfa. The results indicate that VAM inoculation, application of seaweed extract, and No inoculation of bradyrhizobium fixed the most N at Ga-Molepo. The results further revealed that No inoculation of VAM (un-inoculated), Inoculation of bradyrhizobium bacteria and no application of seaweed extract obtained the most symbiotic N at Syferkuil. A significant difference was observed on dry matter, $\delta^{15}N$ and %Ndfa in response to the location, where, Ga-Molepo performed better in all the parameters (dry weight, $\delta^{15}N$ and %Ndfa) than at Syferkuil. These results suggest that farmers at these sites, or in places with conditions comparable to them, need to improve the status of their soil nutrients to increase production. The tested treatment levels can be used to boost symbiotic N.

CHAPTER 5

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1. Summary

Tepary bean has been identified as a potential substitute crop capable of providing low-income families with adequate nutrition and protein found in red meat. Because of their ability to withstand drought and fix atmospheric nitrogen, they are well-suited to marginal areas with low rainfall and poor fertility. The effect of biofertilizers (VAM, bradyrhizobium inoculation, and seaweed extract) on 50% emergence, 50% flowering, plant height, chlorophyll content, number of branches per plant, number of pods per plant, pod length, 90% maturity, number of seeds per pod, 100 seed weight, pod weight, grain yield and nitrogen fixation of tepary bean was studied. Most measured parameters, including 50% emergence, plant height, chlorophyll content, number of branches per plant, number of pods, pod length, and number of seeds per plant, showed significant differences due to VAM, Seaweed extract, and location. Although there was no significant increase in grain yield, no application of seaweed extract at either location produced noticeably taller plants or longer pods. Applying seaweed extracts also resulted in a noticeable rise in tepary bean plant height in Ga-Molepo and pod length in Syferkuil. The study's null hypothesis was thus rejected because seaweed and VAM application affected tepary beans' morphological, physiological, and grain yield. However, bradyrhizobium inoculation did not affect the tepary bean's morphological, physiological, and grain yield. Experimental areas' environmental conditions (rainfall and soil nutrient status) influenced parameters such as 50% emergence, plant height, chlorophyll content, number of branches per plant, and number of pods per plant (Syferkuil and Ga-Molepo). The bradyrhizobium inoculation, VAM, and seaweed extract had no significant effect on dry matter, %Ndfa, or N-fixation. However, no significant dry weight, ^{15}N, and %Ndfa were all increased by VAM, seaweed extract and location. In this investigation, inoculation had no impact on the nitrogen fixation of tepary beans.

5.2. Conclusion and recommendations

To make recommendations to farmers, the study evaluated VAM, seaweed extract and bradyrhizobium inoculation on tepary beans. These biofertilizers were chosen as good stimulants because, unlike chemical fertilizers, they have the potential to increase production sustainably. The effects of VAM and seaweed extract on most growth parameters studied were highly significant. However, grain yield was not significantly affected by these treatments. VAM, bradyrhizobium inoculation, or seaweed extract also had no significant effect on dry matter, %Ndfa, or N-fixed. This study concluded that rhizobium inoculation is not a reliable biofertilizer that can be used to optimize growth, yield parameters, and N-fixation under adverse environmental conditions since it did not influence growth, grain yield and yield parameters of tepary bean. The study also concludes that VAM and seaweed extract can be recommended for use by farmers since they have improved some of the growth parameters. Since the study was conducted in one season. Future studies are recommended to assess this biofertilizer to validate the results from the study.

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