CULTIVATION OF AFRICAN POTATO (*Hypoxis hemerocallidea* Fisch., C.A.Mey. & Avé-Lall.) USING A NUTRIENT SOLUTION

by

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DECLARATION

I, **Patience Seyram Akakpo** with student number do hereby declare that this research project submitted to the Central University of Technology, Free State for the degree **Master of Agriculture**, is my own independent work; complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology, Free State; and has not been submitted before to any institution by myself or any other person in fulfilment (or partial fulfilment) of the requirements for the attainment of any qualification.

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ABSTRACT

The aim of this study was to investigate the cultivation of African potato (*Hypoxis hemerocallidea* Fisch., C.A.Mey. & Avé-Lall.) using nutrient solutions. The specific objectives were to determine the effects of different concentrations of potassium (K) on the agronomic attributes, mineral and the phytochemical (i.e. primary metabolites) contents as well as to evaluate the relationship between K levels, agronomic attributes, mineral and primary metabolite accumulation in African potato. The study was conducted in the greenhouse, Central University of Technology, Free State, Bloemfontein Campus. The effects of four K levels were studied for 9 months (October, 2018 – August, 2019). Culture plantlets of African potato were obtained and grown. The four levels of K used were 4.00, 6.00, 8.00 and 10.00 meq L^{-1}, arranged in a randomised complete block design with six replications. Agronomic attribute data were collected in three stages; 18 weeks, 32 weeks and 40 weeks after transplanting respectively for the first, second and third data collection. The results of this study agree with the hypothesis of the study. Significant effects of K levels on agronomic attributes were observed only after the first measurement. Optimization of agronomic attributes started at 8.00 meq L^{-1} and heightened at 10.00 meq L^{-1} in the second measurement. Results revealed that K level of 4.00 meq L^{-1} showed a positive effect on yield parameters. Root fresh mass and root dry mass produced during the third measurent showed no significant differences at 4.00 and 10.00 meq L^{-1} K levels. There was no significant mineral accumulation in the leaf due to K levels. Calcium and B were significantly accumulated in the corm at 4.00 meq L^{-1} K level. Whilst alanine was optimized at K level of 10.00 meq L^{-1}, malic acid decreased with increase in the levels of K used until the level of 8.00 meq L^{-1}. Multivariate analysis (PCA) revealed the strong synergistic and antagonistic relationship between K levels and all minerals used in the nutrient solution. Potassium showed positive relationship with S, Mg, Zn, Mn and Cu in the leaf, whilst K association with S, Mg and N was negative in the corm. More minerals were accumulated in the corm at K level of 4.00 meq L^{-1}.

Two principal components, PC1 and PC2 accounted for most of the variations in the PC analysis. Most of the parameters loaded positively on PC1, which accounted for most of the variations. Generally, the study revealed a low K level of 4.00 meq L^{-1} with increased Ca:Mg ratio could be recommended for growing African potato. It is therefore possible to cultivate African potato in a controlled environment using nutrient solutions.

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CHAPTER ONE

1.1 Background and motivation to the study

About 80% of world population depends on medicinal plants to treat illnesses (Okigbo *et al*., 2008; Shinwari, 2010; Chandra and Rawat, 2015). Recently, medicinal plants have been recognized globally, leading to an exponential growth on their usage not only for health care, but also for socioeconomic support benefits such as income generation, better education and improved livelihoods (Pandey and Das, 2013; Rasethe *et al*., 2019). Dzoyem *et al*. (2013) reported that global and national markets are growing for herbal medicine, and significantly increased economic gains through sales of medicinal plant products. Annually, large volumes of medicinal plant materials are increasingly traded globally. The medicinal plant trade in 2015 was estimated to be $25.6 billion and expected to reach $35.4 billion in 2020 with an annual growth rate of 6.6% (Roosta *et al*., 2017). Due to the demand for natural products, medicinal plant usage is gaining popularity among communities around the world.

Africa has a strong tradition on the use of plant for medicininal purpose (Dzoyem *et al.*, 2013). African countries that are rich in plant biodiversity in the world include South Africa, a home to diverse indigenous plant species, consisting about 30 000 germplasm, approximately 10% of the global higher plant species (Street and Prinsloo, 2013; Xego *et al*., 2016). About 3 000 of these indigenous plant species are used in various applications as food, medicine, cosmetic applications as well as fuel (Vermaak *et al*., 2011; Xego *et al*., 2016) however, nearly 350 species are mostly used and traded medicinal plants (Xego *et al*., 2016). About 80% of the population in South Africa, mostly rural dwellers and the urban poor use medicinal plants for primary health care, socio-cultural and socio-economic gain, putting enormous pressure on plant population, especially the wild sources, mostly due to indiscriminate harvesting, leading to extinction of most of these plants (Xego *et al*., 2016; Nsibande and Zhu, 2017).

Although, the importance of medicinal plant preservation has been realized, many challenges face the availability of these plants. These challenges include poor seedling establishment, environmental factors, the intensive care required and the

assumption of some conservative African traditional healers that cultivated medicinal plants have a lesser healing ability compared to wild plants (Fennell *et al.,* 2004; Moyo *et al*., 2015). Different measures are therefore needed to ensure the sustainability of these important plant species to meet the growing demand (Moyo *et al*., 2015). Ex-situ and In-situ conservation techniques always preserve plant species, nonetheless increased cultivation is a long-term strategy to increase yield and prevent indiscriminate harvesting of medicinal plants (Moyo *et al*., 2015). In recent years, the use of nutrient solutions in growing crops has increased and has proven to be successful (Khetsha, 2013; Sedibe and Allemann, 2013).

African potato (*Hypoxis hemerocallidea* Fisch., C.A.Mey. & Avé-Lall.) is one of the widely used medicinal plants in South Africa (Bassey and Gous, 2018; Kumar *et al.,* 2019). Traditional healers of Southern Africa use African potato and other *Hypoxis* species for curing various diseases in humans and animals such as cardiac diseases, infertility, diabetes, internal parasites, burns, ulcers, immune boosting, testicular cancer and prostate hypertrophy (Pereus *et al.*, 2018; Otunola and Afolayan, 2019). Though not well documented in veterinary medicine, they are used to treat gall-sickness, heart-water, sores and cracked hooves of animals (Appleton *et al*., 2012).

1.2. Justification for the study

The demand for African potato is increasing (Kumar *et al.,* 2019; Mofokeng *et al.,* 2020). For sustainable use and commercial production of African potato, improved cultivation methods are necessary (Nsibande and Zhu, 2017; Kumar *et al.,* 2019; Mofokeng *et al.,* 2020). Cultivation with nutrient solution has proven to increase growth rate and quality of plants (Sedibe and Allemann, 2012; Nyakane *et al*., 2019).

Therefore, cultivation of African potato in a controlled environment could improve the growth rate and phytochemical content. It has been reported that cultivation of African potato requires high levels of nitrogen, phosphorus and potassium at the early stages of growth to produce good biomass (McAlister and van Staden, 1995). However, the cultivation using combinations of major cations like potassium, calcium and magnesium in nutrient solutions using organic media requires further studies. Among these major elements, potassium is essential in plant metabolism, cation-anion

balance and stress resistance which promote yield and quality of plants (Chrysargyris *et al*., 2017; Hasanuzzaman *et al.,* 2018). It is against this background that the current study seeks to investigate the cultivation of African potato using nutrient solutions containing different levels of potassium to establish ideal standards for mass production of African potato.

1.3. Problem statement

There is a growing demand for African potato derived medicine, which is mostly derived from plants harvested in the wild. However, the wild populations are said to be declining rapidly and locally extinct in some areas due to indiscriminate harvesting, habitat loss and inadequate efforts to conserve these plants. Cultivation is seen as an alternative in preserving these species for sustainable use, but little is known about the cultivation of African potato using a balanced nutrient solution.

1.4. Hypothesis

Cultivation in a controlled environment using nutrient solutions may positively affect growth and phytochemical content of African potato.

1.5. Purpose of the study

Aim

The aim of the study was to investigate the cultivation of African potato fertigated using nutrient solutions in a greenhouse.

Objectives

Specific objectives of this study were to determine the effects of different levels of potassium in nutrient solutions on:

- The agronomic attributes, mineral and phytochemical (i.e. primary metabolites) contents of African potato.

- The relationship between potassium levels and the measured agronomic attributes as well as the mineral and primary metabolite accumulation in African potato.

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CHAPTER TWO

Literature Review

2.1 Introduction

Medicinal plants are globally treasured resources (Chen *et al*., 2016) not only for promoting health and general well-being of humans and animals but, also for social and economic benefits all over the world (Street and Prinsloo, 2013). Medicinal plant usage has therefore developed and increased steadily with civilization over the years in various communities as part of their rich culture, tradition and scientific heritage gaining popularity now all over the world (Dzoyem, *et al.*, 2013; Srivastava, 2018).

Globally, developing communities depend largely on medicinal plants for their livelihood (Shinwari and Qaiser, 2011). Many people around the world, about 80%, still depend largely on medicinal plants to treat diseases and for their health care needs especially in developing countries (Chandra and Rawat, 2015; Chen *et al*., 2016). The World Health Organization (WHO) acknowledges the use of traditional and alternate systems of medicine to support the health system of developing nations (Rao and Rajput, 2010; Mahomoodally, 2013). WHO believes that both wild and cultivated plant materials could be used for medicine (Rao and Rajput, 2010).

The increasing demand for medicinal plant products has renewed pharmaceutical industry interest in the production of herbal health care formulations, herbal-based cosmetic products, and herbal nutritional supplements (Dzoyem *et al*., 2013; Chen *et al.,* 2016). Therefore, the demand for medicinal plants keeps increasing at a fast rate putting enormous pressure on these valuable resources especially the wild sources. Growing demand for medicinal plants worldwide calls for effective use and conservation measures of these valuable resources. To achieve sustainable use, focus must be on conservation.

Cultivation is seen as a measure to meet the growing demand and to combat the effects of indiscriminate wild harvesting coupled with other anthropogenic activities on medicinal plants. With decreasing plant materials from natural sources and increasing global demand, the medicinal and aromatic plants need cultivation to ensure their

steady supply and conservation (Rao *et al*., 2004). One of the ways of increasing production during cultivation is through the use of nutrient solutions. The use of nutrient solution in crop production has significantly increased globally as it offers opportunity for ideal growth and better performance (Trejo-Téllez and Gómez-Merino, 2012).

African potato (*Hypoxis hemerocallidea* Fisch., C.A.Mey. & Avé-Lall.) is one of the important medicinal plants of South Africa that is used widely and carefully researched for its efficacy against several diseases (Van Wyk, 2015; Otunola and Afolayan, 2019). Much research has been done on propagation of African potato using modern technologies (Nsibande *et al*., 2015; Kumar *et al*., 2016; Nsibande and Zhu, 2017). However, little has been done on cultivation of African potato to meet the growing demands. According to Appleton *et al*. (2012), *Hypoxis* plants are usually not cultivated with the thinking that they are readily available in nature. This is untrue because some areas may experience local extinction due to high demand and destructive harvesting (Appleton *et al*., 2012; Nsibande and Zhu, 2017).

2.2 Medicinal plants

2.2.1 Medicinal plants of South Africa

Africa has a wide variety of plant species that are endemic, with South Africa being one of the biodiversity hotspots. South Africa has almost 30 000 plant species of which about 10% is used medicinally mainly in the country (Xego *et al*., 2016; Van Wyk and Prinsloo, 2018). In South Africa, the use of indigenous medicinal plants as medicine significantly exceeds the use of allopathic medicines (Nirmal *et al*., 2013). There are over 200 000 native traditional healers in South Africa providing health services to about 80% of the population who generally use herbs together with contemporary medical services (Xego *et al*., 2016). Over 700 medicinal plant species are actively used in traditional medicine throughout the whole of South Africa (Dold and Cocks, 2002) with an estimated 63 000 commercial harvesters involved in the medicinal plant trade (Van Wyk and Prinsloo, 2018). Indiscriminate harvesting of wild plant populations is seen to have negative impact on biodiversity making some to face the risk of dying out completely from their natural sources (Street and Prinsloo, 2013; Xego

et al., 2016).

About 40 South African plant species are on the International Union for Conservation of Nature (IUCN) Red List as endangered species due to global trade. The rich plant diversity is shockingly declining at a fast rate due to over-harvesting. Currently, the rate of harvesting and habitat destruction have led to the extinction of more species while other species are under pressure of going extinct. There are 62 southern African plant taxa thought to be extinct (Xego et *al*., 2016).

Between 1997 and 2009, there has been 254% rise in the number of threatened taxa recorded owing to the fact that 1 092 threatened taxa have been listed for the first time whilst 909 taxa moved from unthreatened lists to threatened lists (Raimondo, 2011). A total of 2 577 (13%) of South Africa's plant taxa are threatened with extinction whilst 2 232 (11%) are recorded under other categories of conservation concern. The total population of South African plants of crucial conservation concern has therefore risen to about 24% (Raimondo, 2011). Most of these plants of conservation concern are medicinal plants.

2.2.2 South Africa's medicinal plant trade

The domestic trade in herbal medicines in South Africa is valued at about R2.9 billion yearly constituting 5.6% of the National Health budget (Rasethe *et al*., 2019). At least 166 medicinal plant species were traded in the Eastern Cape Province, amounting to 525 metric tons of plant material at approximate cost of R27 million yearly. Also, KwaZulu-Natal recorded an estimated 4 500 metric tons of plant materials sold each year and nearly 1 200 metric tons of plants are traded in Durban street markets per year (Dzoyem *et al*., 2013). There are more than 30 million consumers of the medicinal plant materials in South Africa, hence, the trade is active and spreading rapidly. More than 500 000 South Africans are directly involved in this trade of which rural women form a greater proportion of this number (Dzoyem *et al*., 2013; Xego *et al*., 2016).

South Africa's export value of medicinal plant increased between 2006 and 2015 with Europe, Americas and some parts of Africa as the main markets. An estimated average of 6 036 tons of medicinal plant materials were exported during the same period (DAFF, 2016). From 2011 to 2015, the volume of export increased by 8%. In

2015, South Africa's exports exceeded the imports. A total of 8 632 569 tons of medicinal plant materials valued at approximately $7.5 million were exported against an overall import volume of 3 209 223 tons prized around $3.5 million, thus making South Africa a net exporter of medicinal plant materials (DAFF, 2016).

2.2.3 Conservation of medicinal plants

Habitat destruction mainly due to anthropogenic activities, over harvesting, global warming and climate change are seriously affecting medicinal plant populations. About 15 000 medicinal plant species are threatened with extinction (Rao and Rajput, 2010; Chen *et al.*, 2016). There are many conservation measures to ensure the dwindling medicinal plant resources are protected and saved for continuous sustainable use. According to Okigbo *et al.* (2008), effective preservation measures should be in these areas of in-situ and ex-situ conservation, education and research as well as intensive management to prevent extinction of these plants especially in Africa.

Africa is known to have about 216 million hectares of forest, but the African continent is also known to have one of the highest global rates of deforestation, with an expected loss of 1% each year through deforestation (Mahomoodally, 2013). Interestingly, Africa also has the highest rate of endemism with the Republic of Madagascar leading by 82% (Mahomoodally, 2013; Catarino and Romeiras, 2020). The continent of Africa thus contributes about 25% of the global trade in biodiversity (Mahomoodally, 2013). Nonetheless, the contradiction is that despite this huge potential and diversity, the African continent has only few drugs commercialized globally (Mahomoodally, 2013).

A huge percentage of about 80 percent of medicinal plants in the world market is obtained from the wild (Dzoyem *et al*., 2013; Roosta *et al.*, 2017). The growth in demand for medicinal plants has led to unsustainable harvesting mostly from natural habitats. About 20% of the wild medicinal plant resources are almost exhausted as a result of the growing human population coupled with habitat loss and higher plant consumption rate (Chen *et al.*, 2016).

In South Africa and neighbouring countries, the bulk of the harvested plant materials (about 85%) such as bulbs, rhizomes and bark are non-renewable (Fennell *et al.,* 2004). In some cases, the whole plant is harvested. Sustainable approaches to

harvesting in the wild are not prioritized by most collectors since the short-term economic gain for plants traded outweighs the long-term benefits to all in conserving wild populations of plants (Fennell *et al.,* 2004; Van Wyk and Prinsloo, 2018). Many species are therefore becoming extremely scarce, mainly outside protected areas. The exploitation of *Warburgia salutaris* (Bertol.f.) Chiov. (Canellaceae), *Cassine transvaalensis* (Burtt Davy) Codd (Celastraceae), *Alepidea amatymbica* Eckl. & Zeyh. (Apiaceae) and *Erythrophleum lasianthum Corbishley* (Leguminosae), for instance, were documented as early as 1938 (Fennell *et al.,* 2004).

There has been much research on the protection and sustainable utilization of medicinal plants over the years with a lot of different suggestions among which are the formation of systems for species inventorying and status monitoring, and the need for conservation management practices based on both in situ and ex situ strategies (Chen *et al*, 2016). It has been observed that for medicinal plants with increasingly inadequate materials, sustainable harvesting from the wild can be an effective conservation measure. However, medicinal plant harvesting from the wild is seriously affecting plant populations especially in China and South Africa where large medicinal plant populations are demanded (Chen *et al*, 2016).

Cultivation is generally accepted in some countries however, some conservative traditional healers from South Africa and Botswana for example think that cultivation with fertilizers and modern techniques will result in less effective medicinal plants compared with the wild harvested plants (Fennell *et al.,* 2004; Moyo *et al*., 2015). Most exporting countries involved in medicinal plant cultivation for years know the importance of good agricultural practices to ensure the safety and quality of the plants produced to guarantee the acceptance of the cultivated plant materials by the companies dealing with herbs. The future is promising for cultivated medicinal plants as worldwide commodity of trade (Fennell *et al.,* 2004; Rao and Rajput, 2010).

2.2.4 Cultivation of medicinal plants

Despite the numerous conservation strategies studied, cultivation to grow medicinal plants as crops is seen as the only real solution to the diminishing medicinal plant resources (Fennell *et al.,* 2004). The world has about 422 000 of the plant species

documented globally of which about 72 000 are used for medicine accounting for nearly 17.1% of the total plant species on earth (Chandra and Rawat, 2015). However, only few species are being cultivated (Rao *et al*., 2004). About 3 000 medicinal plants are traded globally but only 900 are under cultivation (Rao and Rajput, 2010).

Currently in this modern time, various techniques are being used in cultivation of specific plants to meet the needs of the growing global population. Medicinal plants are not left out in the process of cultivation. Rao and Rajput (2010) are of the view that the practical way to conserve the world's rich biodiversity of medicinal plants and also gain profitable economic return is to cultivate them. Cultivation with modern processing techniques in a scientific way has an outstanding ability to bring out the economic power in these medicinal plants (Rao and Rajput, 2010). Accordiding to Heuberger *et al.* (2010), field cultivation under controlled conditions helps to improve drug safety and pharmaceutical quality characterized by its identity, purity and active constituents.

Likewise, large-scale cultivation of medicinal plants will help alleviate the increased pressure on wild populations and bring the prices of plants to a lower, more acceptable range that can be stable for a long period without frequent fluctuations. Good agricultural practices (GAP) for medicinal plant cultivation have been developed over the years to control the production, safeguard quality and help the standardization of herbal medicines (Chen *et al*., 2016).

2.2.5 General cultivation of medicinal plants

Germany and some Asian countries such as China, India, Indonesia, and Nepal produce medicinal plants on commercial scale. In Russia, about 50 000 tons of medicinal plants are consumed yearly out of which about 50% are cultivated (Shinwari, 2010). Genetic as well as cultural improvements enhances the successful commercial cultivation of medicinal plants. Numerous attempts have been made to improve the cultivation of medicinal plants. These includes the use of good agro-technological practices, integrated cropping systems like crop rotation and intercropping, selection of suitable sites among others (Chapman and Chomchalow, 2005; Heuberger *et al*., 2010; Li et *al*., 2015).

Not much has been done on the cultivation of medicinal plants in Southern Africa. A

few plants, such as buchu (*Agathosma betulina* Willd.), rooibos tea (*Aspalathus linearis* (Burm.f.) R.Dahlgren.) and devil's claw (*Harpagophytum procumbens* (Burch.) DC. ex Meisn.) are under cultivation as export commodities in South Africa (Fennell *et al.,* 2004). Also, large-scale farming of *Sutherlandia frutescens* (L.) R.Br. is now being undertaken by Phyto Nova (Pty) Ltd, though small-scale cultivation and commercialization of *Sutherlandia* began since 1990 in the Cape Province (Drewes, 2012).

Few trials on the domestication of commonly used medicinal plants and aromatic plants have been carried out at several places in South Africa. These trials are geared towards small-scale cultivation first to gather important data for large-scale commercial cultivation (Jäger and van Staden, 2000; Tanga *et al*., 2018). Despite some challenges faced in medicinal plant cultivation, knowledge of the cultivation practices of specific herbs, adequate data on medicinal plant cultivation, necessary facilities and industries, coupled with favorable environmental conditions are key to achieving higher production of quality medicinal plants through cultivation (Jäger and van Staden, 2000).

It is believed that the effectiveness of medicinal plants is affected by genetic and environmental factors such as the biochemistry of species, climate, geographical location, season and other conditions necessary for growth and development. These factors can cause changes in plant growth, development and phytochemical content of medicinal plants. Africa is relatively far behind in the growth and control of its medicinal plant industry, research is therefore on going to explore the numerous aspects essential for the development of the medicinal plant trade in Africa especially in the area of pharmacology and toxicology (Fennell *et al.,* 2004).

2.3 Plant metabolites

Plants synthesize huge amounts of metabolites of varied structures and abundance that are important in plant growth, development and reaction to the environmental pressures. These metabolites are the biochemicals responsible for plant yield and quality as well as treasured wells of nutrients, energy and medicine for humans and animals. Metabolites are organic substances, which can be divided into two basic

classes namely, primary and secondary metabolites (Hong *et al.,* 2016; Shakya, 2016; Indrajeet and Rajesh, 2018).

2.3.1 Primary metabolites

Primary metabolites are the organic substances produced by all plants and other living organisms through photosynthesis and are crucial for the existence, growth and development of the species. They include the general building blocks of carbohydrates, lipids and proteins (Hong *et al*., 2016; Shakya, 2016). Primary metabolites are also directly responsible for cell component production (Ramawat, 2007). The metabolic pathways of primary metabolites and the actual compounds synthesized through these basic methods are very similar, though not equal in some cases amongst animals, bacteria, fungi, plants, and other organisms (Aharoni and Galili, 2011).

Furthermore, primary metabolites are vastly preserved in their structures and are freely existing in great quantities (Ramawat, 2007; Hong *et al*., 2016). Normally primary metabolites from higher plants for commercial use are bulky chemicals with low value, primarily used in industrial raw materials, foods or food additives and are easily obtainable. Examples include vegetable oils, fatty acids (for soap and detergent production) and carbohydrates (sucrose, starch, pectin and cellulose). However some primary metabolites such as myoinositol and *β*-carotene are expensive due to difficult extraction, isolation and purification processes involved (Ramawat, 2007).

2.3.2 Secondary metabolites

Secondary metabolites are complex plant products biosynthetically produced from primary metabolites and are not usually included in metabolic activities (Verma and Shukla, 2015; Indrajeet and Rajesh, 2018; Thakur *et al*., 2018). Over 200 000 of these secondary metabolites have been discovered so far (Aharoni and Galili, 2011). They include volatile oils, flavonoids, alkaloids, glycosides, tannins, anthocyanins, quinones, lignans, steroids, terpenoids and resins among others (Ramawat, 2007; Indrajeet and Rajesh, 2018; Thakur *et al*., 2018). Secondary metabolites in plants are produced in much smaller quantities (mostly below 1% dry weight) than primary metabolites (Ramawat, 2007; Thakur *et al*., 2018).

The availability and the amount of these compounds are influenced by the hereditary factors and their expression, the biochemical and physiological processes of the plant as well as biotic and abiotic environmental differences. Thus, production of secondary metabolites, and the amount produced may be restricted to some plant families, genus, species or even individual plants at specific physiological stages of development (ie, seasonally or daily) (Ramawat, 2007; Ncube *et al*., 2012; Thakur *et al.*, 2018). Studies show that though production of secondary metabolites is controlled by genes, their biosynthesis and accumulation is amazingly due to environmental effects (Ncube *et al*., 2012; Mohiuddin, 2019).

Secondary metabolites are critical for plants to cope with environmental pressures through the maintenance of homeostasis and to act as compounds of defence to protect plants against insects, pests, herbivores, phytopathogens besides acclimatization to the environment (Hong *et al*., 2016; Thakur *et al*., 2018; Mohiuddin, 2019). The role of secondary metabolites in the strong interaction of plants with the environment for their existence and fitness makes these metabolites as vital as primary metabolites (Verma and Shukla, 2015).

These accumulated secondary metabolites are useful to humans by finding use as agrochemicals, food additives, therapeutics, pharmaceuticals, nutraceuticals, dyes, fragrances, stimulants, hallucinogenes, as well as vertebrate and human poisons. Secondary metabolites are active principles that make plants useful medicinally (Chomel *et al.,* 2016; Jamwal *et al*., 2018; Thakur *et al*., 2018).

The various metabolites and their complex metabolic pathways call for extensive research into their biochemical nature so as to maximise the production of secondary metabolites of interest. Moreover, there need to be careful engineering of the whole metabolic process to redirect primary metabolites into secondary metabolites without any negative effect on the plant such as the plant fitness (Aharoni and Galili, 2011).

2.3.3 Production of plant metabolites

Plant metabolism is a complex system compared with the metabolism of other living organisms (Aharoni and Galili, 2011). Various metabolic pathways exist for the synthesis of both primary and secondary metabolites which comprise glycolysis, the

citric acid cycle (CAC) also called tricarboxylic acid cycle (TCA) or the Krebs cycle, aliphatic amino acids, pentose phosphate pathway, shikimate pathway and conspicuously the aromatic amino acids (AAAs) essential for protein biosynthesis in all living organisms (Aharoni and Galili, 2011; Dias *et al.,* 2016; Mohiuddin, 2019). Whilst primary metabolites are produced from photosynthesis (Ramawat, 2007) the numerous secondary metabolites are synthesized from primary metabolites through the various metabolic pathways and are classified based on the biosynthetic pathways. Generally, three main classes of secondary metabolites exist namely phenolic compounds (phenylpropanoids and flavonoids) required for lignin synthesis and are common to all higher plants, followed by terpenoids or isoprenoids and steroids, and finally, nitrogen or sulphur compounds such as the alkaloids, glucosinolates and cyanogenic glycosides respectively (Verma and Shukla, 2015; Chomel *et al.,* 2016). The biosynthesis routes produce both primary and secondary metabolites as shown in (Figures 2.1 and 2.2) (Verma and Shukla, 2015; Bhatla and Lal, 2018).

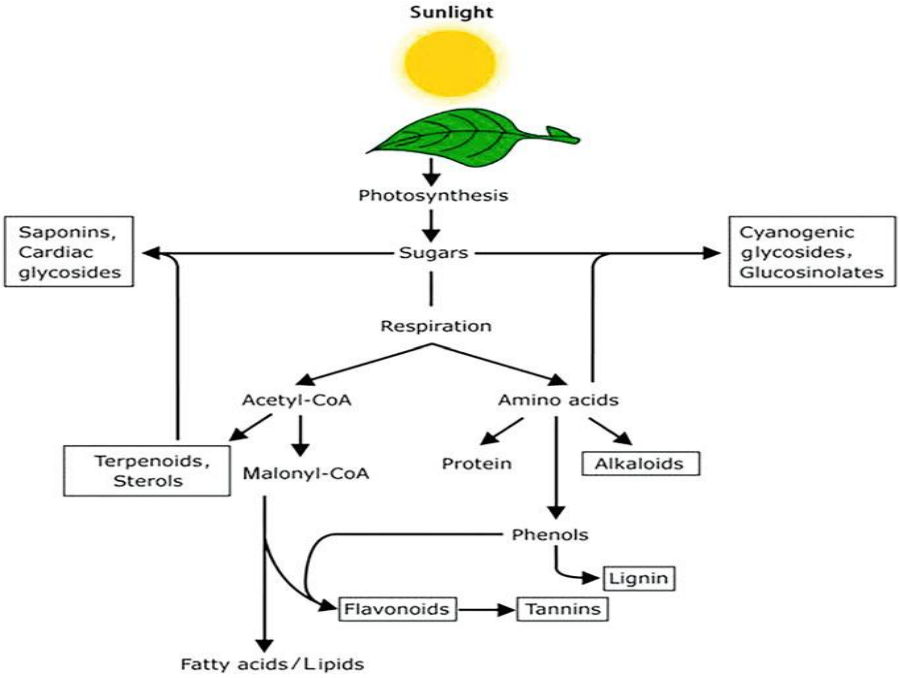

Figure 2.1 A simplified depiction of the interrelationship between the major primary and secondary metabolic pathways. Secondary metabolites are shown in boxes (Bhatla and Lal, 2018).

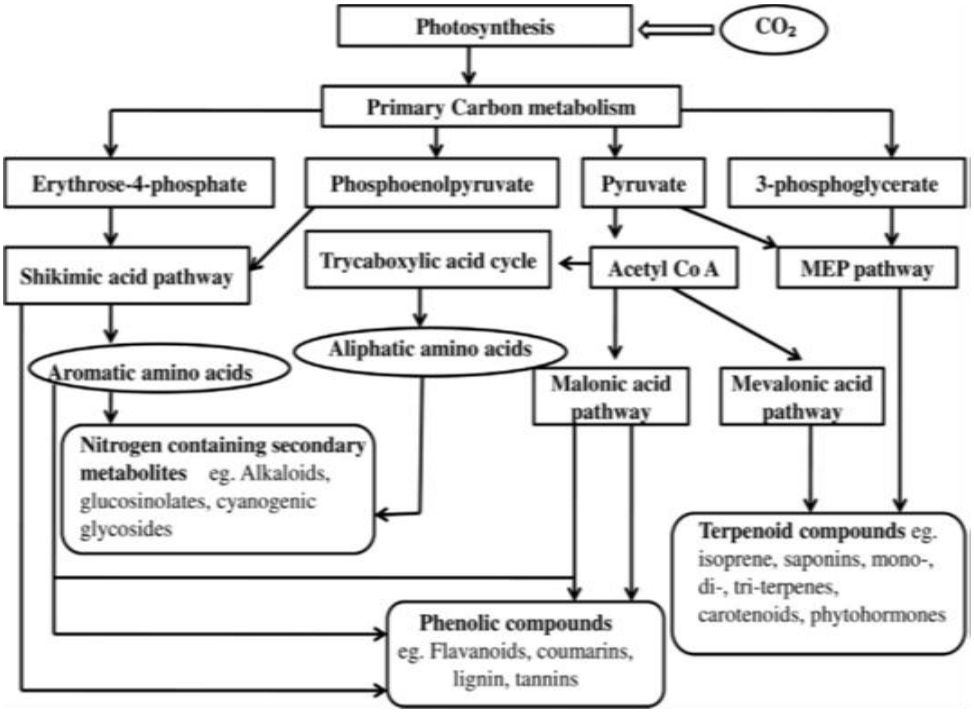

Figure 2.2 A general outline of biosynthetic pathways of secondary metabolites in plants with examples (Verma and Shukla, 2015).

2.3.4 Factors that influence plant metabolite production

Several factors both biotic and abiotic, contribute to growth, development and metabolite production in plants. Changes in these factors cause alterations in substrate availability and several disturbances in the plant environment thereby affecting biochemical and physiological activities such as photosynthesis and the means of defense signalization (Gouvea *et al.,* 2012). The various responses by plants to these factors usually lead to secondary metabolite accumulation in plants as plants try to adjust to the stress in the changing environments. Those factors responsible for the maintenance of plant homeostasis include temperature, hydric availability, ultraviolet radiation intensity, presence of atmospheric gas, type of soil and nutrients, the interaction of plant–microorganism and herbivores coupled with other mechanical stimuli (Gouvea *et al.,* 2012; Kapoor *et al.*, 2018; Mohiuddin, 2019). Many approaches are being used by scientists to help plants accumulate the necessary bioactive compounds in a short period of time. The use of soil nutrients is one of the approaches.

2.3.4.1. Effects of soil nutrients on metabolite production

Soil and soil nutrients play an important role in the production of primary metabolites for growth and development of plants, which consequently affects the secondary metabolism of plants (Gouvea *et al.,* 2012). Soil contains the necessary nutrients to promote secondary metabolite accumulation and enhance nutraceutical plant quality (Ávila-Juárez *et al*., 2017). Nutrient-deficient soils produce plants with decreased growth rate (reduced primary metabolite levels) and increased secondary metabolites, chiefly phenolic compounds (Gouvea *et al.,* 2012). The synthesis of secondary metabolites is often low in normal conditions when plants do not have any biotic or abiotic stress. However, its production increases with exposure to various stresses in which synthesis of phenylpropenoids and phenolic compounds were found higher during stress situations in plants (Indrajeet and Rajesh, 2018). However, it was stressed that any method which alter metabolism in plants can reduce plant yield. Therefore, appropriate techniques need to be developed to increase yield and quality of plants (Ávila-Juárez *et al.*, 2017; Indrajeet and Rajesh, 2018).

Studies on *Cistus monspeliensis* L. by Rivoal *et al*. (2010) revealed that the type of soil impacted the production of terpenes in that the siliceous soil with less nutrient content produced seven times more terpenes than the calcareous soils with relatively higher nutrient levels. This led to the conclusion that in the presence of all factors favorable to growth, the growth processes prevail (i.e. favourable synthesis of primary metabolites) upon the synthesis of secondary metabolites. However, lack of nutrients like nitrogen (N) causes plants to use relative supplies to produce secondary metabolites like terpenes (Gouvea *et al.,* 2012).

Generally, secondary metabolite synthesis increases with increasing carbon–nutrient balance in plants except with N compounds (Gouvea *et al.*, 2012). Thus, soils with low nutrient status might produce slower growing plants that synthesize higher secondary metabolites particularly, phenolic derivatives (Gouvea *et al.*, 2012). Increased N in the growth media mostly promotes the synthesis of N metabolites like alkaloids, glycosides cyanogenics and glucosinolates. However, it must be noted that, the available N absorbed in plant tissues has a great effect on secondary metabolite production therefore synthesis does not depend only on soil N (Gouvea *et al.*, 2012).

Acid soils mostly reduce the conversion of ammonia to nitrate (Gouvea *et al.,* 2012) which prevent better N absorption hence plants grown in acid soils tend to yield higher secondary metabolites particularly phenolic compounds (Gouvea *et al.,* 2012). Also, the synthesis of secondary metabolites is affected by changing N and K ratios in the growing media (Gouvea *et al.,* 2012). Potassium is an essential mineral in plant metabolism, encouraging the synthesis of carbohydrates, fats and proteins, increasing crop yield and quality (Ibrahim *et al*., 2012; Samet *et al.,* 2015; Chrysargyris *et al*., 2017).

In an experiment with *Labisia pumila* (Blume) Fern.-Vill., Ibrahim *et al*. (2012) found that increased K fertilization promoted the synthesis of soluble protein, the invertase activity and phenylalanine ammonia-lyase activity increased steadily. At the highest K level, higher net photosynthesis, stomatal, conductance, intercellular CO_2, apparent quantum yield and lower dark respiration rates were observed. Increased production of total phenolics, flavonoids and ascorbic acid were also seen at higher K concentration but there was a decreased antioxidant enzyme activity with high K fertilization (Ibrahim *et al*., 2012). Also, low levels of N, P, S and K can increase the synthesis of the derivatives of ordinary chiquimic acid, hydrolyzable and condensed tannins (Gouvea *et al.,* 2012).

Nitrogen and P fertilizers are very important nutrient factors in plant growth and development as well as biosynthesis of secondary metabolites. Nitrogen and P influence the flavonoid content in St. John's Wort (*Hypericum perforatum L*.). Also, N fertilizers help to control some gene expressions of certain genes of some plant species like *Arabidopsis* (Verma and Shukla, 2015). Phosphorus increases the leaf biomass, total phenolic and rosmarinic acid levels. However, P has no effect on quality and quantity of volatile oils in *Salvia officinalis* L. (garden sage) (Verma and Shukla, 2015).

The application of micronutrients boosted primary metabolite production which may improve the secondary metabolites in *Cassia angustifolia* M.Vahl. Chlorophyll, protein and phenol contents were affected by $FeSO_4$, $ZnSO_4$ and $CuSO_4$ (Verma and Shukla, 2015). The production of secondary metabolites of diffent concentrations in micro propagated *Hypoxis hemerocallidea* was greatly improved by the addition of zinc (Zn)

in different concentrations. Plant biomass and the build-up of most phenolic acids was increased with Zn addition but, the levels of hypoxoside and antioxidant activity decreased (Kumar *et al.,* 2019).

Sprinkling of the leaves of *Digitalis grandiflora* Mill. with manganese (Mn) and molybdenum (Mo) solution increased the concentration of cardioactive heterosides to more than double whilst a study on boron (B) deficiency in palms revealed a reduced synthesis of phenolic compounds (Gouvea *et al*., 2012). In another research on *Eugenia uniflora* L., the spathulenol and the caryophyllene oxide in the essential oils of the leaves corelated strongly with S, Ca, Fe nutrients' balance and phenolic compounds present in the leaves whereas the selenin epoxide-1,3,7 (11)trien-8-one was linked to K, Cu, and manganese (Gouvea *et al*., 2012). Increasing buildup of Cr, Fe, Zn and Mn improved oil production to about 35% in *Brassica juncea* L. plant whilst betalains production in *Beta vulgaris* L. plant was encouraged by the accumulation of Cu^{2+} (Indrajeet and Rajesh, 2018).

Some heavy metals like Ni, Ag, Fe and Co are known to alter the metabolic processes of plants affecting the production of photosynthetic pigments, sugars, proteins and nonproteinthiols which also affect secondary metabolite synthesis in several plants (Thakur *et al.*, 2018). Plant nutrients therefore play a vital role in the synthesis of both primary and secondary metabolites for plant growth, yield, nutritional and medicinal quality.

2.4 Nutrient solutions

Organic and/or conventional farming methods cannot always meet the demands of the growing population. Many techniques are now employed in agriculture to maximise crop production in terms of yield and secondary metabolite content of the plants. These techniques include the use of nutrient solutions with different nutrient proportions applied to growth media or directly on the plants (Ávila-Juárez *et al.,* 2017). In support, Verma and Shukla (2015) stated that different chemical effects on secondary metabolites undoubtedly show that chemicals needed by the plants for growth and development also influence the accumulation of secondary metabolites.

To increase secondary metabolites in plants, ionic ratios need to be varied in the nutrient solutions wisely to avoid nutrient toxicity or deficiency due to synergistic or antagonistic behaviour of nutrients (Ávila-Juárez *et al.,* 2017). In addition, it has been recorded that plants respond differently to nutrient availability in order to adapt to the changing nutrient concentrations, therefore chemical imbalances lead to chemical stress, which yields various secondary metabolites accordingly (Verma and Shukla, 2015).

Recently, crop production using nutrient solutions has significantly increased globally. Growing plants with nutrient solutions offer another method to enhance growth and yield of plants. The use of nutrient solution allows for an ideal growing environment for maximum plant performance which results in higher competitiveness and better incomes (Trejo-Téllez and Gómez-Merino, 2012). Additionally, the use of nutrient solutions for production helps to study the effects of high and low concentrations of nutrients in solution because plant nutrition does not only affect plants but other animals and humans that depend on plants. The availability of all essential minerals in plants impacts on our diets and therefore the supply of mineral fertilizers has economic and environmental effects (Maathuis, 2009).

The use of nutrient solution in growing plants in hydroponic systems is a flexible technology, which can be used for both village or backyard production systems to high-tech space stations. Hydroponic technology can be used effectively for the production of food as well as other plants of economic importance such as medicinal plants under adverse environmental conditions and highly populated areas (Trejo-Téllez and Gómez-Merino, 2012). Mugundhan *et al.* (2011) emphasised that domestic cultivation of medicinal plants using hydroponic techniques is a practical substitute that helps to overcome the problems inherent in plant extracts, misidentification, genetic and phenotypic inconsistency, extract variability and instability, lethal constituents and contaminants. In addition, controlled environments help lessen cultivation challenges and to engineer changes in plant secondary metabolites.

2.4.1 The role of nutrients

There are about 14 important nutrients needed by plants for growth and development. Out of these, six are needed in large amounts and are called macronutrients whilst the remaining are needed in small amounts and are called micronutrients. These nutrients are normally absorbed by plants in ionic forms from the soil solution through the roots (Maathius, 2009). These mineral nutrients needed by plants are N, P, K, Mg, Ca, S, Fe, Mn, Zn, Cu, B, Mo, Cl and Ni (Maathius, 2009; Bindraban *et al*., 2015). In addition, C, H and O are also needed, making a total of about 17 elements for the desired growth and development of plants grown with nutrient solutions (Mugundhan *et al.*, 2011; Bindraban *et al*., 2015; Njira and Nabwami, 2015). These essential elements are important that without them, plants cannot complete their life cycles (Ávila-Juárez *et al*., 2017).

Macronutrients consist of the major cations, which are K, Ca and magnesium (Jones, 2012). Potassium helps in enzyme activity. It helps to maintain water status of plants and the turgor pressure of plant cells. It is involved in closing and opening of stomata necessary for the accumulation and translocation of newly formed carbohydrates. Potassium also regulates other biochemical processes that affect the growth and metabolism of plants. Likewise, K helps plants to survive the exposure to the numerous biotic and abiotic pressures (Jones, 2012; Wang *et al.*, 2013; Hasanuzzaman *et al.,* 2018). Calcium maintains cell integrity and membrane permeability, improves pollen germination and growth as well as cell growth. It activates enzymes for cell division and elongation and forms part of cell walls. It is also important for protein synthesis and carbohydrate transfer and also helps to detoxify heavy metals in plants (Mugundhan *et al.*, 2011; Jones, 2012). Magnesium on the other hand is a component of chlorophyll. It serves as a co-factor in most enzymes that activate phosphorylation processes as a bridge between pyrophosphate structure of adenosine tri-phosphate (ATP) or adenosine di-phosphate (ADP) and the enzyme molecule. It also stabilizes the ribosome particles in the configuration for protein synthesis (Mugundhan *et al.*, 2011; Jones, 2012).

Other macronutrients are C for the synthesis of organic compounds, O for the producing energy from sugar molecules, H responsible for water formation, N is

important for chlorophyll, amino acid and protein production, P necessary for photosynthesis and growth, forms part of plasma membranes, it is also needed for and nucleic acid build up as well as in phosphorylation processes and S is responsible for the synthesis of amino acids and proteins (Mugundhan *et al.*, 2011; Neocleous and Savvas, 2019).

Micronutrients include Mn, which is a constituent of chlorophyll. Mn helps in the metabolism of nearly 35 enzymes and acts as a metallic catalyst and protein activator. Mn activates enzymes for nitrogen metabolism (i.e. glutamine synthase and arginase), gibberellic acid, ribonucleic acid (RNA) fatty acids biosynthesis as well as polymerase activation (Mugundhan *et al.*, 2011; Ávila-Juárez *et al*., 2017). Zinc is a constituent of enzymes and auxins, a co-factor responsible for respiration, photosynthesis and hormone synthesis in plants (Mugundhan *et al.*, 2011; Indrajeet and Rajesh, 2018). Boron helps in the transport of sugar, building of cell wall and lignification. It also helps in carbohydrate, RNA, indoleacetic acid and phenolic metabolism. It is included in the cellular membrane, important in stomatal control and reproduction (Ávila-Juárez *et al*., 2017; Jehangir *et al*., 2017). Molybdenum is needed in biochemical and physiological processes and as a key constituent of mononuclear enzymes, metabolic processes as well as cycles of C, N and sulphur (Mugundhan *et al.*, 2011; Ávila-Juárez *et al*., 2017). Iron helps in photosynthesis, Cl is important in root growth, Ni is involved in nitrogen release whilst Cu is for enzyme activation (Mugundhan *et al.*, 2011).

Other elements like Sodium (Na) which is essential for water movement, maximum biomass accumulation and performing functions of K as it replaces it in most cases and Silicon (Si) responsible for cell wall hardiness can also be used in nutrient solutions (Mugundhan *et al.*, 2011; Bindraban *et al*., 2015).

2.4.2 Formulation and the use of nutrient solutions

Since 1939, there has been various preparations of essential elements in nutrient solutions for plant growth. Hence, instructions for universal nutrient solutions (UNSs) was introduced with varying concentrations of the essential elements. However, these modern UNSs do not differ much from the original ones apart from the introduction of chelated iron (Ávila-Juárez *et al*., 2017; Combrink, 2019).

Many herbs flourish well with basic nutrient solutions, which are readily available on the market as ready made products however, care needs to be taken to avoid nutrient deficiencies. Single nutrient solutions consist of nutrients N, P, K, Ca, Mg, S, Fe, Mn, B, Cu, Zn and Mo in various ratios with the electrical conductivity (EC) of this solution being nearly 2.5 whilst the pH adjusted to 5.5 - 6.5. With day length less than 11 hours, the EC should be increased to 3.0-3.6. However, the concentration of nitrogen needs to be maintained at 210ppm. With these settings, a smaller root system grows, and extra energy is offered for shoot (vegetative) development. The higher EC guarantees satisfactory nutrition even with a smaller root structure (Mugundhan *et al*., 2011).

Due to the complex nature of plant nutrition, differences in nutrient solutions could bring about ionic imbalances of the elements in solution thereby affecting plant growth and the production of useful metabolites (Ávila-Juárez *et al*., 2017). This is supported by Pii *et al*. (2015) findings that, plants consequently face nutrient deficiencies and/or toxicities that change their physiology. Therefore, these differences in nutrient solutions makes it difficult selecting the most suitable UNS for research. The importance of effective management of fertilizer programmes is to make sure that all nutrients are well balanced throughout the plants' life cycle to enhance quality and yield of the plants (Hochmuth and Hochmuth, 2015; Ávila-Juárez *et al*., 2017).

2.4.3 Behaviour of nutrients: Nutrient-nutrient interactions

Combining fertilizers in different ratios appropriately according to the needs of plants aims at providing the correct nutrients that end up in the specific plants of interest. However, in actuality, nutrients in fertilizers do not all end up in the target plant. About 20–80% of nutrients in fertilisers may not be available to the plants. This may be environmental loss, temporal accumulation in the soil owing to numerous complex soil interactions and nutrient-nutrient interactions during uptake that prevent their instant availability to the plant (Bindraban *et al*., 2015). The availability and subsequent absorption, translocation and allocation of plant nutrients are influenced by a lot of factors ranging from physical, chemical and biological factors but not only the type of nutrient source (Pii *et al*., 2015). Some of these factors include pH, redox potential and microbial activity. Nutrients are usually absorbed by plants in ionic forms. Owing to the similarities in the uptake forms of some elements, antagonistic and synergistic

interactions exist preventing the plants from taking in the right amount of nutrients. Understanding these interactions well is important for the application of balanced nutrient solutions for efficient cultivation of plants (Fageria, 2001; Bindraban *et al*., 2015).

2.4.3.1 Antagonism

Antagonism arises due to competitions among nutrient ions during uptake. Jointly, nutrients are absorbed through numerous transporters, many of which transport more than one kind of nutrient. For instance, the iron regulated transporter is induced mainly by Fe deficiency but, it transports Fe, Mn, Cu, Zn and perhaps additional divalent cations into the plant (Bindraban *et al*., 2015). Studies done by Dimkpa *et al*. (2014) reported that Zn decreased the absorption of Fe and Mn in bean (*Phaseolus vulgaris* L.) whereas increased Cu decreased Zn, Fe, Mn and Ca levels in the shoots of bean. Likewise, it has been reported that, exposure of barley to Fe decreased the absorption of Mn, Zn and Cu in the xylem sap of barley. Markedly, such antagonism is not only with soil-root uptake routes because, a foliar application of Fe too reduced Mn, Zn and Cu absorption in wheat (Bindraban *et al*., 2015). Similarly, in a study on barley, cucumber and tomato, iron-deficiency encouraged a major rise in Mg and Mn in the dicots whilst P and S reduced in cucumber, remained unchanged in barley and tomato compared with the control. In the same investigation, increased Fe raised the level of Zn in all plants used for the experiment (Pii *et al*., 2015).

Antagonism between nutrients usually happens with unbalanced ratios of nutrients available in solution. An example is the absorption of Mn over Fe in Arabidopsis and Zn over Fe and Mn in the shoots of bean (Dimkpa *et al*., 2014; Bindraban *et al*., 2015). Also, it has been reported that, high levels of K can hinder the uptake of Ca and P and Mg in solution (Fageria, 2001). Hochmuth and Hochmuth (2015) recorded Mg deficiency in tomato plants grown with extra K in solution.

2.4.3.2 Synergism

Comparable to antagonism, synergism exists between nutrients in solution as the combined presence of two nutrients improves crop yield compared with the individual availability of the nutrients in question. However, it must be noted that synergism

usually occurs between N and K as well as N and P which affects not only yield but also helps to elucidate their joint effect on root growth and the significance for corresponding applications which eventually lead to increased nutrient use efficiency (Rietra *et al.,* 2017). Some micronutrients show synergistic relationships with macronutrients. An example is seen with Zn application causing an increased yield of wheat on a calcareous soil, with the highest increase as a result of increased macronutrient application (Rietra *et al.,* 2017).

There existed synergistic relationship between P and Mn, K and Mn as well as Mg and Ca, in barley, when P was increased. It was said that, increasing N use raised Ca and Mn build up in maize with Mn increasing throughout the plants life whilst Ca accumulation occurred at tasselling (Bindraban *et al*., 2015). The authors further recorded that the addition of NH_4^+-N and P improved Zn and Fe absorption by maize in calcareous soil through processes that change root characteristics and acidifies the rhizosphere. Reports revealed that, in rice (*Oryza sativa* L.), barley (*Hordeum vulgare* L.), corn (*Zea mays* L.), cucumber (*Cucumis sativus* L.) and tomato (*Solanum lycopersicum* L.), Ca concentration was higher when (NO_3-N) was applied to the nutrient solution compared to application of NH_4-N (Fageria, 2001).

2.5 Cultivation of African potato (*Hypoxis hemerocallidea*)

2.5.1 African potato

Hypoxis hemerocallidea formerly called *Hypoxis rooperi* T.Moore (Katerere, 2013) is generally identified as African potato (the latest name coined for commercial purposes) (Van Wyk 2008). However, the plant does not produce tubers as the name African potato suggests but rather, corms/rhizomes are produced. This name therefore leads to confusion with tuberous *Plectranthus esculentus* N.E.Br. (Nair and Kanfer, 2008b; Katerere, 2013). Other common names include Yellow star, Star lily, starflowers (English); sterretjie, Afrika-patat (Afrikaans); inkomfe, ilabatheka (isiZulu); inongwe (isiXhosa); moli kharatsa, lotsane (Sesotho); ilabatheka, zifozonke (isiSwati) (DAFF, 2012; Street and Prinsloo, 2013; Rungqu *et al.,* 2019).

African potato is one of the species in the family Hypoxidaceae. This family is well recognized and widely used in Africa for its medicinal value in treating various diseases due to phenolic glycosides contained in their tissues especially in the corm. Hypoxoside, a norlignan diglucoside, (E)-1,5-bis (3'-hydroxy-4'-0-*β* -D-glucopyranosylphenyl)pent-1-en-4-yne, known for its medicinal value is one of the significant phytochemicals in the corms of *Hypoxis* species. The worth and importance of *Hypoxis* species in medicine is because hypoxoside cannot be easily produced synthetically hence making *Hypoxis* species the key sources of this phytochemical (Street and Prinsloo, 2013; Nsibande, 2017; Nsibande *et al.,* 2018). Kumar *et al.* (2019) added that pharmacological efficiency of *H. hemerocallidea* is partly due to phenolic composites and hypoxoside.

Some other commonly used *Hypoxi*s species are *H. acuminata* Baker, *H. colchicifolia* Baker, *H. argentea* Harv. ex Baker*, H. filiformis* Baker*, H. gerrardii* Baker, *H. parvifolia* Baker*, H. obtusa* Burch. ex Ker Gawl.*, H. rigidula* Baker and *H. galpinii* Baker among others. About twelve *Hypoxis* species were stated to be used in medicine in Southern Africa (Nsibande *et al.,* 2018; Rungqu *et al.,* 2019). However, *H. hemerocallidea* (African potato) is one of the species widely used and carefully researched (Van Wyk, 2015; Nsibande *et al.,* 2018; Otunola and Afolayan, 2019). This plant has become famous as a result of millions of rands spent on research and development since 1967, when *Hypoxis* phytosterols were first developed into a branded product (Harzol®) by an entrepreneur from Johannesburg (R.W. Liebenberg), who successfully marketed and sold it in Germany for the treatment of benign prostate hypertrophy (Van Wyk, 2011). Ncube *et al.* (2013) supported saying, herbal preparations from *H. hemerocallidea* became widespread as far back as 1967.

The genus *Hypoxis* has gained important commercial position due to the use in traditional African medicine because of the sterols/phytochemicals produced and the claims that it has anti-human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) activity (Katerere and Eloff, 2008). According to Van Wyk (2015) African potato happens to be among the leading plant species of research interest in recent years having about 80% of research publications in the last decade. It has become the number one most commonly marketed species among 60 other medicinal plant species in the Eastern Cape Province (Drewes and Khan, 2004).

Likewise, Appleton *et al*. (2012) as well as Ncube *et al.* (2013) recorded that studies on the most popular medicinal plants sold in South Africa revealed *H. hemerocallidea* and *H. colchicifolia*, as regular species traded at medicinal plant markets in eastern KwaZulu-Natal or through mail order from herb sellers in the Gauteng Province. The authors further stated that, research done in the Eastern Cape Province showed *H. hemerocallidea* is not only the most often sold species but along with *H. colchicifolia*, they rank the top four medicinal plants mostly used in traditional medicine to cure diabetes.

Street and Prinsloo (2013) confirmed that the corms of *H. hemerocallidea* could be used to manage type 2 diabetes mellitus in adults and was found to be one of the best 10 most regularly traded medicinal plant species. Potgieter *et al.* (2017) and Pereus *et al*. (2018) also reported that *H. hemerocallidea* and *H. colchicifolia* are the two most wanted *Hypoxis* species for traditional medicine such as herbal teas and tinctures in South Africa. In addition, Bassey and Gous (2018) reported that among the *Hypoxis* species, *H. hemerocallidea* find uses in traditional health care system of more than 85% of South Africans and it has become one of the mainly used and widely traded herbal species in South Africa.

2.5.2 Classification, origin and distribution

African potato is in the family of monocotyledonous perennial geophytes Hypoxidaceae. It belongs to the genus *Hypoxis* and the species *hemerocallidea*. Species in the family Hypoxidaceae are widely distributed all over the world. However, they largely spread across the Southern Hemisphere and the Torrid Zone with limited distribution of just some few species in the Northern Hemisphere (Kocyan *et al.,* 2011; Nsibande *et al*., 2018; Pereus *et al.*, 2018). Hypoxidaceae plants are herbaceous producing corms or rhizomes and are usually 20 cm or less tall, though a few reach a meter or more. Flowers of this family are yellow, white, pink, or hardly orange and are typically less than 2 cm in diameter (Kocyan *et al.,* 2011). There are nine genera and about 155 species of the family Hypoxidaceae, with *Hypoxis* being the leading genus with about 90 species and are well known for their medicinal value (Kocyan *et al.,* 2011; Bassey and Gous, 2018; Rungqu *et al.,* 2019).

The genus *Hypoxis* in Africa is widespread in the continent, but southern Africa is regarded the home of diversity and endemism (Pereus *et al.*, 2018; Rungqu *et al.,* 2019). *Hypoxis* has about 76 species in Africa (Bassey and Gous, 2018; Rungqu *et al.,* 2019). Nearly 41 species are reported to be indigenous to countries in the Southern African Development Community (SADC), such as South Africa, Malawi, Mozambique, Namibia, Botswana, Lesotho, Swaziland, Zimbabwe, and the Democratic Republic of Congo (Bassey and Gous, 2018; Otunola and Afolayan, 2019). However, about 29 species are clearly noted to be endemic to South Africa (Bassey *et al.*, 2014).

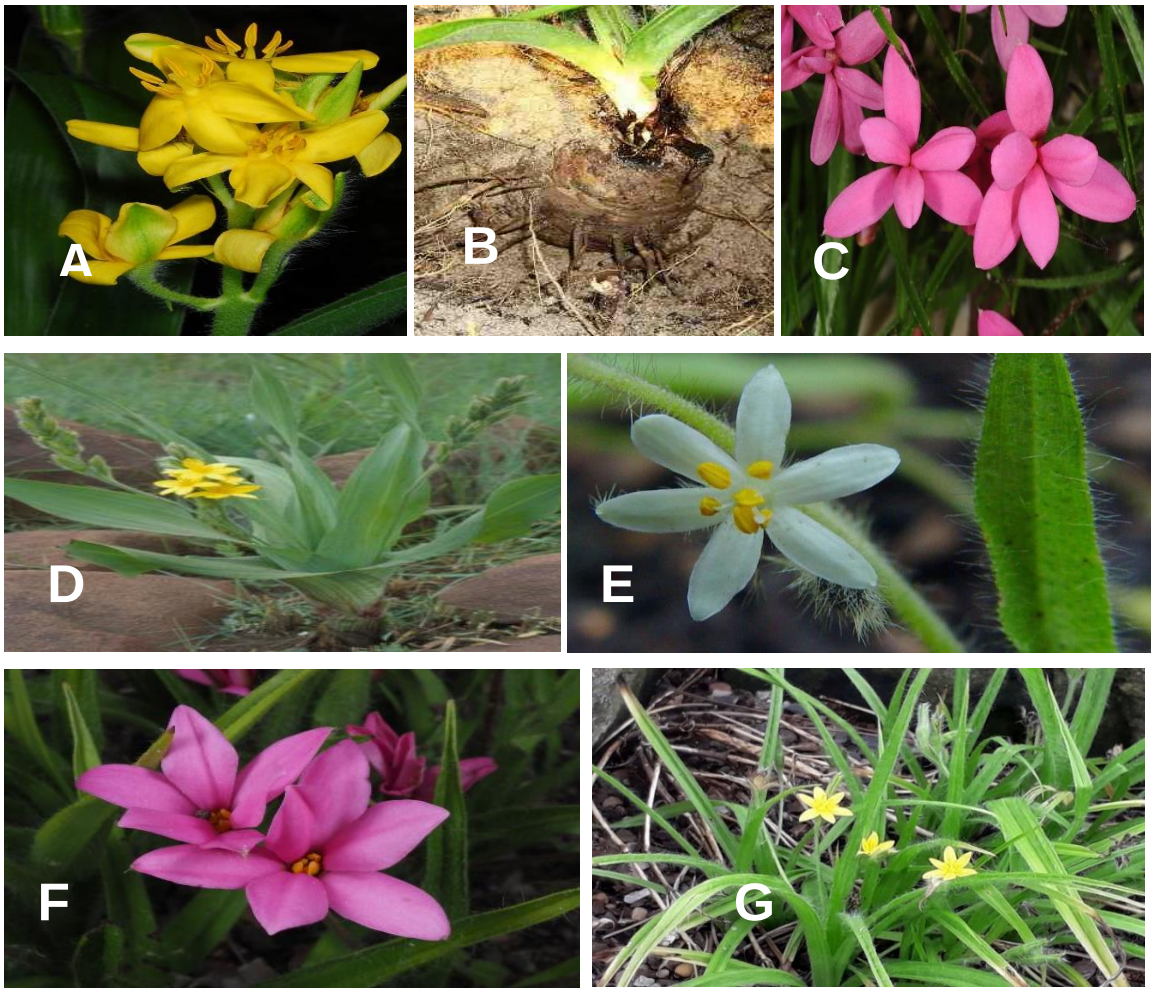

Figure 2.3 Pictures of some *Hypoxis* species with the various plant parts. A = *Hypoxis hemerocallidea*, B = *Hypoxis hemerocallidea* roots, corm and leaves, C = *Hypoxis milloides* Baker, D = *Hypoxis colchicifolia*, E = *Hypoxis membranacea* Baker, F = *Hypoxis baurii* Baker, G = *Hypoxis decumbens* L. (Sources: POWO, 2019; SANBI, 2020).

In South Africa, altitudes higher than 1 000 m above sea level are favourable for *Hypoxis* plants where they grow in open natural grasslands. These plants do well in semi shade and/or full sun; and prefer average amounts of water. Predominantly these species are found in the Eastern Cape, Western Cape, KwaZulu-Natal provinces, spreading to the interior Mpumalanga, Gauteng, Limpopo and the Free State Provinces (Bassey and Gous, 2018; Otunola and Afolayan, 2019; Rungqu *et al.,* 2019).

The genus is distinguished by having yellow hairy flowers with six stamens, trilocular ovaries without a beak and free perianth segments with ovaries pointed at the base (Zimudzi, 2014; Oguntibeju *et al*., 2016; Nsibande, 2017). African potato is identified by long strap-like leaves held on dense green hairy stems that are unbranched. The leaves are green during spring, summer and autumm, are arranged usually into three ranks and maybe somewhat hairy. The stems hold stalks supporting 2-12 bright yellow, star shaped flowers which appear between October and January (Katerere, 2013; Oguntibeju *et al.*, 2016; Otunola and Afolayan, 2019).

The stemless perennial geophyte has plenty adventitious roots on a large black-brown fibrous corm which are bright yellow when freshly cut open (Sathekge, 2010; Katerere, 2013), but turns dark brown due to oxidation upon exposure to air and secretes gummy yellowish juice (Sathekge, 2010). The corms may range 10-15 cm diameter (Drewes *et al.*, 2008). The roots allow these plants to persist under unfavourable circumstances (Sathekge, 2010). The availability of numerous *Hypoxis* species throughout South Africa under the common name ‘African potato’ justifies its extensive use in the South African traditional medicine systems (Boukes *et al.,* 2008; Bassey and Gous, 2018).

2.5.3 Uses of African potato

Traditional healers of Southern Africa and other African countries as well as the populace use African potato as well as other *Hypoxis* species for curing and/or managing various diseases (Pereus *et al.*, 2018; Otunola and Afolayan, 2019). These diseases include infertility (barrenness and impotency), cardiac diseases, testicular cancer, diabetes, arthritis, endometriosis and premenstrual syndrome. *Hypoxis* species are also used for the treatment of common colds, flu, asthma, tuberculosis,

skin problems, burns, gastric and duodenal ulcers, dysentery, internal parasites, vomiting, prostate hypertrophy and urinary tract infections. Furthermore, these plants are used to cure headaches, dizziness, high blood pressure, obesity, nervous disorders (including epilepsy and childhood convulsions), depression, insanity and sexually transmitted diseases. In addition, they are also used as laxative, vermifuge, for immune boosting especially for HIV/AIDS patients and healing of septic wounds (Pereus *et al.*, 2018; Kumar *et al.*, 2019; Otunola and Afolayan, 2019). These plants are also used as food especially during famine (DAFF, 2012; Pereus *et al.*, 2018).

Though not well documented, African potato as well as other *Hypoxis* species are used in traditional veterinary medicine in the treatment of gall-sickness, infertility, prevent abortion in cattle, heartwater (ehrlichiosis), redwater (bovine babesiosis), sores and cracked hooves of farm animals (Appleton *et al*., 2012; Katerere, 2013; Bassey *et al*, 2014). Extracts of African potato are also recognized for anti-inflammatory, anti-neoplastic, antioxidant, and anti-infective properties. African potato is processed by the pharmaceutical industry to produce health powders, tablets, capsules and creams in treating the diseases (Owira and Ojewole, 2009; Nsibande et *al.,* 2018).

African potato is used for other non-medicinal purposes such as its use by the Basotho people as a charm against lightning and thunder storms. It is used as an emetic against dreadful dreams. Together with other species of *Hypoxis* the leaves are used to make ropes to build reed enclosures and to decorate huts especially among the Zulus. The ropes are also used for the sewing of grain baskets. The corms and leaves are used in preparing black dyes to polish floors (Singh, 2009; Street and Prinsloo, 2013; Pereus *et al.*, 2018). African potato is also used in horticulture along with other *Hypoxis* species as suitable garden plants due to the hardiness of the rhizomes and how the plants flower easily in favourable environmental conditions such as lack of fires (Singh, 2009; Pereus *et al.*, 2018). Flowers in several species are formed successively throughout the growing period maintaining colourful garden beds for longer periods (Singh, 2009). Kocyan *et al.* (2011) added that in the temperate regions of southern Africa, species of Hypoxidaceae are significant spring and summer flowering bulb plants.

2.5.4 Production, marketing and exports

The *Hypoxis* genus has great capacity as a source of new drugs for immune system regulation and it is of extreme economic importance (Nsibande, 2012; Pereus *et al.*, 2018). *Hypoxis hemerocallidea* is regarded by businesspersons as a 'wonder herb' and 'miracle cure', and leads the South African herbal trade based on this genus (Potgieter *et al.*, 2017). South Africa is involved in local, regional and international trade of African potato (Street and Prinsloo, 2013; Van Wyk, 2015).

Drewes *et al.* (2008) further reported that a study conducted in a local retail pharmacy showed that *Hypoxis* extract, in various forms, solely or as a mixture with aloe and *Sutherlandia frutescens* extract, assorted amino acids and a collection of vitamins allures the ever-growing market. Some use local names like ‘Vuselela’, ‘Vikelela’ whilst others depend on attractive names like ‘Down to Earth’, ‘Smart *Hypoxis*’ ‘Nutriherb’ or ‘Hypo-Plus’ among others to sell their products. Mostly, the formulations are in capsule form (holding between 60 and 600 mg herbal extract per capsule) with prices alternating between R47 for 30 capsules to R130 for 60 capsules. All the formulations are branded as ‘immunoactive’ but most are used for various conditions such as asthma, allergies, high blood pressure, diabetes, impotence and enlarged prostate. Other uses are for immunity in cancer and HIV/AIDS patients, treating haemorrhoids, *Herpes simplex* and varicose ulcers.

Recently, *Hypoxis* extracts are being used in the formulation of skin creams. A large percentage of these skin products have *Hypoxis* (sometimes precisely referring to ‘rooperol’) as a main constituent in addition to tea tree oil, aloe, comfrey, evening primrose oil or Vitamin E. These mixtures are used precisely to remove skin blemishes but are also believed to have antiviral and antifungal properties (Drewes *et al.*, 2008). Some of examples of the *Hypoxis* extracts in use in South Africa are presented in Table 2.1 below. *Hypoxis* plants usually *Hypoxis hemerocallidea* are therefore versatile and need to be conserved.

Table 2.1 Herbal formulations using *Hypoxis* corm extract available in South Africa.

Trade name	Form	Therapeutic claims	Obtainable from	Composition
Moducare®	Capsule	Immune system booster serves as a cure for flu and infections as well as treats allergies and painful autoimmune disorders eg. rheumatoid arthritis	Health stores and Pharmacies	20 mg of β-sitosterol and 0.2 mg of its glucoside, this ratio is considered 'critical' for the product efficiency
Hypo-Plus	Capsule	As a supplement, energy booster and regulates the immune system	Health stores and Pharmacies	Have a mixture of assorted amino acids, selected vitamins and *Hypoxis* extract
African potato/ *Hypoxis hemerocallidea*	Capsule	As immune booster and acts agaist HIV/AIDS symptoms	Health stores	300 mg dry extract of *Hypoxis hemerocallidea* in a capsule
Immuniser	Capsule	As immune booster	Pharmacies	Unrevealed quantities of *Hypoxis hemerocallidea* and *Aloe vera*
Herbal immune booster	Tincture	As immune booster	Pep stores	500 mg of *Hypoxis* and 55 mg plant sterols per 20 ml
Medico herbs	Capsule	As immune modulator and lessen allergy symptoms	Health stores	Sterols and sterolins
African Potato (*Hypoxis hemerocallidea*)	Capsule	Control immune system and reduce allergy symptoms	Health stores	Dry extract of *Hypoxis hemerocallidea* 60 x 350 mg capsules
Immuno active	Capsule	Immune booster	Pharmacies	500 mg of *Hypoxis hemerocallidea. Aloe ferox* is also added.
Down to earth	Cream	Works against skin blemishes, rheumatoid arthritis, acne, and acts as antibacterial and antifungal agent	Health stores	Sterols
Afrigetics™ *Hypoxis*	Capsule	Herbal HIV/AIDS cure, immune booster	Pharmacies and Health stores	Dry extract of *Hypoxis hemerocallidea* 60 x 300 mg in a capsule
African Potato Capsules *(Hypoxis)*– Phyto Green	Capsule	Immune enhancer, heals enlargements of prostate gland, urinary tract infections, cancer and tumour growth and rheumatoid arthritis	Pharmacies and Health stores	Dry extract of *Hypoxis hemerocallidea* 60 x 450 mg per capsule
African potato extract – South Africa's miracle herb	Tonic	For immune boosting and as a remedy for HIV/AIDS symptoms	Health stores	Sterols and sterolins
Stameta	Tonic	For treating nervous disorders, skin disorders, improves sexual	Health stores	*Hypoxis rooperi* extracts, *Mentha piperital*, *Pimpinella*

		performance and poor blood quality, treats hypertension, chest, lung and kidney infections. It is also used for fever and flu, heart conditions, back pain, persistent tiredness, menstrual pain, it washes out bile, remedies bleeding gums and body sores in addition to harden bones and serves as immune system enhancer		*anisum*, Aloe (unspecified). Strengthened with multivitamins (unspecified), calcium, magnesium, potassium, phosphorus and iron
African potato	Tonic	Used to heal rheumatism, high blood pressure, gout and arthritis, cancer, tuberculosis, yuppi flu, psoriasis, eczema, varicose veins bad blood circulation and prostate complications	Health stores	An indefinite amount of *Hypoxis rooperi* extracts. Comprises sterols and sterolins

Source: Ncube *et al.* (2013)

2.5.5 Chemical composition of African potato

Though, the roots and the leaves contain some amount of hypoxoside, studies show that the corms are the site of highest levels followed by the roots with the leaves as the least accumulators. It has also been recorded that, a clear difference existed between the chemicals in the leaf and the corms with the leave compounds being more complex. The corms however had phytochemicals that were not seen in the leaves. Therefore, corms are apparently the only plant parts that can be used for medicine (Katerere, and Eloff, 2008; Ncube *et al.*, 2013; Nsibande *et al.,* 2018). However, a recent study on *H. hemerocallidea* by Bassey and Gous (2018) recommended that the roots of *Hypoxis* plants should also be used in preparing the herbal medicines from these plants. Also, Otunola and Afolayan (2019) suggested that all plant parts of African potato may be used for making animal feed, tea or dietary supplements due to the occurrence of nutrients and essential trace elements in the corms, peels, leaves and roots of this plant.

African potato contains phytosterol glucosides (*β*-sitosterol) and its glucoside, a nontoxic diglucoside hypoxoside, its aglycone rooperol and sterolins (Nair and Kanfer, 2008b; Bassey *et al.*, 2014). Unknown lectin-like compounds have also been found in the extracts of African potato (Katerere and Eloff, 2008). Nsibande *et al.* (2018) also detected some unnamed biochemical composites that were alike in all the species studied although in variable quantities.

Chemical analysis of the corms of African potato revealed the presence of various groups of secondary metabolites, *viz*., glycosides, polyphenols, saponnins, steroids, and tannins (Street and Prinsloo, 2013). Nsibande *et al.* (2018) identified total phenolic compounds in *Hypoxis* species. Three cytokinins were also found in the rootstock of *H. hemerocallidea* namely, zeatin, zeatin riboside, zeatin glucoside (Street and Prinsloo, 2013).

A more recent study on *H*. *hemerocallidea* by Kumar *et al*. (2019) showed the presence of phenolic acids, comprising hydroxybenzoic acid derivatives like gallic acid, *p*-hydroxybenzoic acid, protocatechuic acid, salicylic acid, syringic acid and vanillic acid as well as hydroxycinnamic acid derivatives like caffeic and *p*coumaric acids. In addition, chlorogenic, sinapic and ferulic acids were also found.

Furthermore, a first-time report on essential oil composition of *H. hemerocallidea* was given by Rungqu *et al.* (2019). These authors detected in variable quantities and composition some essential oils from the leaves (both fresh, dry) and the corms of *H. hemerocallidea*. The most abounding constituents seen in the essential oils were sabinene, linalool, *α*-terpineol and *β*-caryophyllene. Monoterpenes, monoterpenoids and sesquiterpenes were also found to be the main chemical classes of compounds (Rungqu *et al.,* 2019). In a recent study conducted by (Otunola and Afolayan, 2019), crude protein as well as minerals such as Ca, Zn, Cu, Mn, Mg, K, Fe, Na and P were detected in various quantities in the corms, peels, roots and leaves of *H. hemerocallidea.*

Hypoxoside is the key glycoside obtained from African potato (Boukes and van de Venter, 2012; Bassey *et al.*, 2014). Hypoxoside and the sterol *β*-sitosterol, are thought to be the most significant phytochemicals obtained from *H. hemerocallidea* that are offered in the market (Nair and Kanfer, 2008a; Nsibande *et al.,* 2018). Boukes and van de Venter (2012), Street and Prinsloo (2013) and Bassey *et al*. (2014) also reported that the worth of this plant may be due to some chemical constituents present in the plants among which hypoxoside, and its aglycone derivative rooperol as well as sitosterol are possibly the most famous compounds.

The high levels of phytosterols like *β*-sitosterols has shown effectiveness against benign prostatic hyperplasia (Street and Prinsloo, 2013). Singh (2009) added that the hydrolysed form of hypoxoside, (rooperol) reduced the growth of cancerous cells. Singh further reported that *Hypoxis* species have significant potential as anti-cancer drug. Boukes and van de Venter (2012) strengthened that rooperol has numerous *in vitro* results including anticancer, anti-inflammatory, antioxidant and antibacterial properties. In support, Pereus *et al.* (2018) reiterated that the therapeutic uses of *Hypoxis* species are due to the occurrence of hypoxoside, which is transformed to the active constituent rooperol with medicinal properties linked to cancer, HIV/AIDS and inflammations.

These authors further stated that *Hypoxis* also contains sitosterol, used for boosting the immune system. Nevertheless, a combination of active constituents rather than individual compounds offers a vital simultaneous result on several pharmacological targets (Bassey *et al.*, 2014; Zimudzi, 2014). The biologically active rooperol may be gotten by hydrolysis of hypoxoside with *β*-glucosidase an enzyme found mainly in the alimentary canal. Hypoxoside has two glucose molecules which are removed by hydrolysis with the *β*-glucosidase enzyme to produce the aglycone rooperol (Boukes and van de Venter, 2012; Street and Prinsloo, 2013).

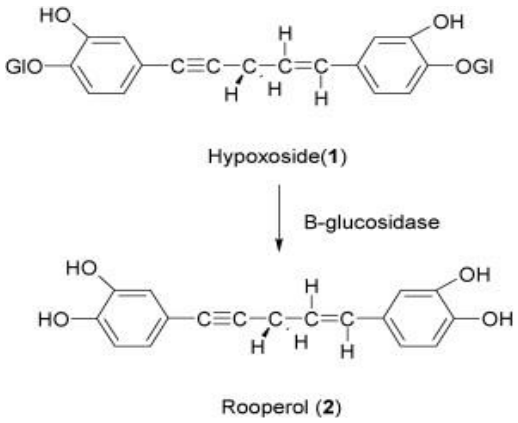

Figure 2.4 Structural representation of hypoxoside (1) and rooperol (2) and the production of (2) from (1) in the presence of the enzyme *β*-glucosidase (Nsibande, 2017).

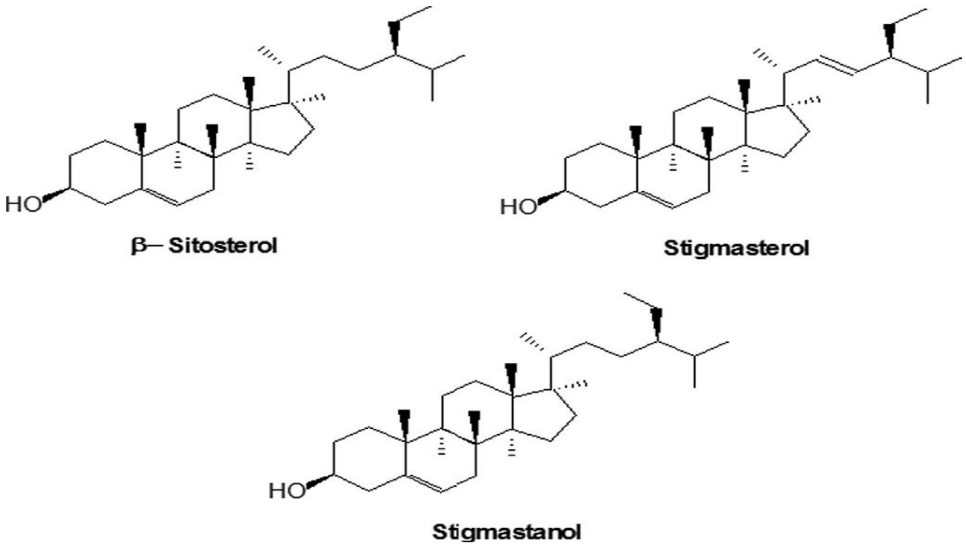

Figure 2.5 Structural representation of *β*-sitosterol, stigmasterol and stigmastanol obtained from *Hypoxis* species (Ncube *et al*., 2013).

2.5.6 Safety and toxicity

The toxicity of *Hypoxis* species have been studied extensively. Many of these studies revealed that *Hypoxis* species are not toxic even after a long period of use (5 years) of high doses of 2 400 mg per day of plant extract (i.e. 4 capsules 3 times per day) (Ncube *et al.*, 2013). Street and Prinsloo (2013) also reported that *Hypoxis* extracts containing 45% hypoxoside lack toxicity. The authors continued that the extracts and their active constituent hypoxoside have not shown toxicity in numerous medical or toxicity studies (Street and Prinsloo, 2013; Zimudzi, 2014). It was further reported that there was neither cytotoxicity nor genotoxicity in the *in vitro* studies of water extracts from four *Hypoxis* species *viz*., *H. acuminata, H. colchicifolia, H. hemerocallidea and H. rigidula* and a commercial formulation using the neutral red uptake assay, the alkaline comet assay and the cytome assay in human hepatoma (HepG2) cells (Ncube *et al.*, 2013). The oral consumption of hypoxoside presented no toxicity and there was no lethality in the brine shrimp assays nor cytotoxicity in Vero monkey cells (Katerere, 2013). The long practice of African potato use in traditional medicine is a form of a ‘clinical trial’ because any sign of toxicity would have stopped the users and traditional healers from using the plants (Drewes and Khan, 2004).

Nevertheless, a study reported that lasting consumption of African potato extracts may well result in the reduction of the rate of glomerular filtration and can raise plasma creatinine levels in rats, signifying weakening of kidney function. Furthermore, experiments with animals have shown that water extracts of African potato may result in bradycardia and brief hypotension in guinea-pigs and rats *in vitro* and *in vivo*, respectively; while rooperol reportedly raised myocardial contractility *in vivo* in baboons, perhaps owing to its catechol structure (Owira and Ojewole, 2009). Care is therefore needed in the consumption of African potato and its products.

2.5.7 Conservation status

Due to the huge demand for African potato, populations are quickly reducing in South Africa (Singh, 2009; Kumar *et al*., 2019; Mofokeng *et al.,* 2020). Williams *et al*. (2013) also recorded that the species is declining. The species is endangered in South Africa mainly by overharvesting and habitant destruction (Street and Prinsloo, 2013; Pereus *et al.*, 2018). According to Appleton *et al.* (2012), *Hypoxis* plants are usually not cultivated with the reason that they are readily available in nature however, this reasoning is false because in some areas, the plant is locally extinct due to high demand and destructive harvesting. The massive and unsustainable harvesting from the wild is risking the survival of these species (Nsibande and Zhu, 2017). However, only KwaZulu-Natal, South Africa has started preservation work by keeping 250 hectares of land to protect of *H. hemerocallidea* (Mofokeng *et al.,* 2020).

Approximately 31 300 corms of *H. hemerocallidea* were sold yearly from 54 outlets in Durban and 11 000 kg/year of the corms were sold in the Eastern Cape Province at the cost of R322 500 (Appleton *et al.,* 2012; Street and Prinsloo, 2013). Pressure on wild populations of *Hypoxis* species is very high in that in an ecological market studies, all available *Hypoxis* species (*H. hemerocallidea and H. colchicifolia*) had been totally removed from the study area monitored (Appleton *et al.,* 2012). Fennell and van Staden (2003) observed that geophytes form a large proportion of plants sold at medicinal plant markets. An estimated 20 000 tonnes of plant materials are sold nationally at the value of R270 million which is able to improve livelihoods of rural communities involved in the growing of indigenous geophytes or bulbs.

A recent survey by the South African National Biodiversity Institute (SANBI) justified that the extensive commercial misuse of *H. hemerocallidea* since 1997 led to the declines in some sub-populations, especially in Gauteng, South Africa, where it is further endangered by habitat loss and degradation. Though the species is decreasing in populations, it is however widely distributed in natural habitats and hence the species is not regarded at this time to be vulnerable or near threatened. The species is probably not abundant outside of nature reserves in Gauteng (Williams *et al.*, 2016). Due to the rapid decline of African potato populations as a result of uncontrollable harvesting from the wild, cultivation to meet demand with sustainable good quality supply as well as conserve the species is the best option (Kumar *et al*., 2016; Kumar *et al*., 2019; Mofokeng *et al.,* 2020).

2.5.8 Cultivation of African potato

The demand for the widely esteemed African potato is rising therefore, needs to be cultivated safely to meet the growing demands (Kumar *et al*., 2019). Kumar *et al*. (2016) further stated that the current status and over exploitation shows there should be conservation strategies to reduce pressure on wild plants and to produce large numbers of plants for commercial use and providing the opportunity for the analysis of bioactive compounds. African potato cultivation will not only ease pressure on natural resources, but will also provide practical means to enhance secondary metabolite production, uniformity, and safety by reducing inconsistency in quality and composition of plants, thereby decreasing the risk of contamination and increasing yield through agricultural practices (Mofokeng *et al.,* 2020).

Cultivation of these plants is seen as a means to supply the informal, formal and industrial markets as well as a conservation measure to reduce the pressure of harvesting on natural populations (Appleton *et al*., 2012). Enormous work has been done on tissue culture propagation and seed sprouting using modern technologies (Nsibande *et al*., 2015; Kumar *et al*, 2016; Nsibande and Zhu, 2017); however, there is still scanty information on the cultivation of African potato and there are no decisive techniques which can adequately supply the market with the required plant materials at an affordable price (Street and Prinsloo, 2013). There is therefore the demand to target the effects of external factors on physiological growth and physiological and

genomic manipulation of bulb and flower initiation (Fennell and van Staden, 2003).

Sequentially, Fennell and van Staden (2003) reported studies that were conducted to explore the possibility of producing *Hypoxis* secondary metabolites *in vitro* on low nitrogen substrate in the dark. Root-type cultures of *Hypoxis rooperi* were induced from flower buds and corms. Secondary metabolite concentrations increased gradually on the media with low nitrogen levels and with the cultures in the dark but were too small for feasible production *in vitro* (Fennell and van Staden, 2003).

In another experiment, McAlister and van Staden (1995) investigated some areas of cultivation (namely herbicide and fertilizer use) of *H. hemerocallidea.* It was reported that 2,4-D amine and glyphosate killed the plants whilst low levels of paraquat encouraged good growth and produced a good hypoxoside content. With N, P and K fertilization, results show that low concentrations of N did not significantly decrease the biomass buildup in corms but hugely raised hypoxoside accumulation. Reduced levels of N and P or low N and K produced the highest amount of hypoxoside. Moreover, addition of N, P and K at increased levels gradually caused the best biomass production. It was then concluded that the initial increased biomass production may be necessary in cultivation as it may offer a good corm volume in which secondary metabolites are stored at a later stage (McAlister and van Staden, 1995). The control experimental plants still showed the best biomass production and accumulated the highest hypoxoside content. *H. hemerocallidea* seemingly does well in poor soils with low fertility. High levels of N, P and K are therefore required in the early stages of growth to build up a good biomass and could be stopped when the plants fully establish (McAlister and van Staden, 1995).

In a study carried out by Kumar *et al*. (2019) to determine the effects of different concentrations of Zn on the production of secondary metabolites in micro propagated *H. hemerocallidea*, addition of Zn greatly improved the concentrations of hydroxybenzoic acid derivatives. These derivatives are gallic acid, p-hydroxybenzoic acid, protocatechuic acid, salicylic acid, syringic acid and vanillic acid. The peak concentration of cafeic acid and p-coumaric acid was recorded in plants grown on standard Murashige and Skoog (MS) medium comprising the normal 30 µM-Zn concentration. Augmentation with Zn (200 µM) encouraged the maximum levels of

chlorogenic acid and sinapic acid while 100 µM of Zn considerably raised the concentration of ferulic acid. Noticeable high amounts of hypoxoside was reported in the control and plantlets grown with Zn (800 µM) whilst there was a marked reduction in the other concentrations used. Basically, it was seen that Zn heightened plant biomass and the build-up of most phenolic acids, but notably decreased the levels of hypoxoside and antioxidant activity (Kumar *et al.,* 2019).

2.5.8 Climatic requirements of African potato

There is limited information on the cultivation and cultivation practices of African potato however, the Department of Agriculture, Forestry and Fisheries (DAFF) published the following guidelines for its cultivation.

Rainfall and temperature

African potato does well in full sunlight in warm and cold subtropical zones however, few species can be seen on cliff faces or in forests. The plant is exceptionally hardy and drought resistant, so does not need much water for survival (DAFF, 2012). The species mainly grows in regions having between 600 and 1000 mm annual rainfall, which includes the semi-arid and dry-sub humid zones of South Africa (Mofokeng *et al.,* 2020).

2.5.9 Cultivation

Propagation

African potato is propagated using seed, tissue culture and corms (DAFF, 2012). However, seed germination and conventional vegetative methods are very problematic though corm division is a much more rapid and successful technique of multiplying the plants (Kumar *et al.*, 2016; Nsibande and Zhu, 2017). Seeds must be collected just before the capsules open and could be treated with fungicide and boiling water prior to planting into a well composted soil. Seeds may take approximately a year to sprout and needs an extra year for seedling transplanting (DAFF, 2012). To obtain better germination rate, seeds must be cold treated (mixed with vermiculite and stored in the refrigerator) for nearly six to eight weeks prior to sowing in well prepared seed boxes (DAFF, 2012).

Soil requirements

African potato is grown in different soil types though well-drained soils are required (DAFF, 2012). Soil preparation is done based on the method of harvesting, irrigation practices and the contour of the land. The field needs to be worked into long rows of mounds (DAFF, 2012).

Planting

Seeds should be sown in early spring and must be planted 1 mm deep and covered with fine grass compost. For a year-old corm, planting should be done 10 cm apart in rows and 20 cm between rows whilst corms that need to be grown for longer periods say three years should be spaced 20 cm apart in rows and 50 cm between rows to favour the large corm size (DAFF, 2012). There are no known chemicals (chemical additives, chemical fertilisers or insecticides) that are used to grow African potato (DAFF, 2012).

Irrigation

The soil used must be moist, and not damp. The soil must have low humidity and be well ventilated. The plants should be watered weekly with 25 mm of water per week for the first three months. In cool temperatures, the plants should be kept dry throughout winter (DAFF, 2012).

Pest and disease control

American bollworm, termites, spotted maize beetle, stink bug and grasshopper feed on African potato. Whilst porcupines uproot the corms, centipedes devour the external cover. Registered pesticides are available in South Africa for most of these insect pests. Athough these chemicals are available, the best control method is to have a good sanitization program (DAFF, 2012).

Harvesting method

Harvesting of African potato occurs all year round using the handpick system once the corms attain a weight of 250g. It could be picked during summer when the plants are vigorously growing (DAFF, 2012).

2.6 Conclusion

From the literature reviewed, it is evident that African potato is under pressure due to indiscriminate harvesting (for both domestic and commercial use) and habitat loss. Production of these plants in large quantities with high medicinal value is necessary for conservation and to meet the ever-growing demand. The use of nutrient solutions with different ratios of potassium in a controlled environment to cultivate African potato may increase plant growth and yield as well as the production of phytochemicals responsible for the plant's medicinal properties.

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CHAPTER 3

Cultivation of African potato (*Hypoxis hemerocallidea* Fisch., C.A.Mey. & Avé-Lall.) using a nutrient solution containing potassium

Abstract

African potato (*Hypoxis hemerocallidea* Fisch., C.A.Mey. & Avé-Lall.) is a valuable medicinal plant that needs to be cultivated. The objective of this study was to determine the effect of nutrient solution containing potassium (K) on the yield attributes, mineral content and synthesis of primary metabolites of African potato plants grown in a greenhouse. Four K levels, 4.00, 6.00, 8.00 and 10.00 meq L^{-1} of K were evaluated in the cultivation of African potato plants for 9 months (October, 2018 - August, 2019). Plantlets of African potato were grown in the greenhouse, Central University of Technology, Free State, Bloemfontein Campus. The experiment was set up in a randomised complete block design with six replications. Agronomic attribute data were collected at three stages; 18 weeks, 32 weeks and 40 weeks after transplanting respectively for the first, second and third data collection. The results of this study agree with the study hypothesis. Significant K effects were observed only after the first measurement. During the second and the third data collection, chlorophyll content, leaf area, fresh corm mass, root dry and root fresh mass were significantly affected by the K levels. Mineral analysis data were collected at 18 weeks whilst primary metabolite data were collected at 32 weeks. Mineral accumulation in the leaf was not significant with variation in K levels; however, significantly higher Ca and B were accumulated in the corm at 4.00 meq L^{-1} K levels. Alanine was significantly affected at 10.00 meq L^{-1} K level while malic acid decreased with increased K levels until 8.00 meq L^{-1}. Low K level of 4.00 meq L^{-1} could be adequate for cultivating African potato plants of good medicinal value. African potato could therefore be cultivated using nutrient solutions in a controlled environment.

3.1 Introduction

Increasing global demand and subsequent overexploitation of medicinal plant resources in the wild call for the cultivation of medicinal plants to meet the growing demands (Chen *et al.,* 2016; Kumar *et al*., 2016; Xego *et al*., 2016). African potato is one of the most popular medicinal plants in South Africa known for curing many diseases and widely harvested from the wild without any serious commercial cultivation (Kumar *et al.*, 2016; Mofokeng *et al.,* 2020). However, only KwaZulu-Natal, South Africa, has kept 250 hectares of land to protect *H. hemerocallidea* (Mofokeng *et al.,* 2020).

Application of fertilizers is seen as one of the suitable cultivation practices that could enhance growth and development as well as useful metabolite accumulation of plants Major elements such as N, P, K, Ca, Mg, O, H, P and S are needed by plants in large quantities for various enzymatic, physiological processes and structural development. Physiological and structural development processes these elements are needed for include organic compound build up, energy production, chlorophyll, amino acid, protein synthesis, photosynthesis, growth and build-up of plasma membranes (Mugundhan *et al.,* 2011; Jones, 2012; Wang *et al.,* 2013; Neocleous and Savvas, 2019).

Trace elements such as Fe, Mn, Zn, B, Cu and Mo are used by plants for metabolic activities, enzyme activation, transport of molecules, synthesis of hormones, structural build up and physiological processes (Mugundhan *et al.,* 2011; Ávila-Juárez *et al*., 2017; Indrajeet and Rajesh, 2018). All these elements can be supplied for plants uptake through application of fertilizers. However, fertilizers need to be managed properly to achieve maximum plant performance and the production of useful metabolites as imbalances of elements could affect plant growth and metabolite synthesis due to chemical stress (Verma and Shukla 2015; Ávila-Juárez *et al.,* 2017). Fertilizer application could be through broadcasting, banding, foliar application and nutrient soultions.

The use of a well-balanced nutrient solution is ideal to ensure maximum plant production (Mugundhan *et al.*, 2011; Trejo-Téllez and Gómez-Merino, 2012). To optimize growth, nutrient solutions are specifically formulated to suit a specific crop nutrient requirements (Ávila-Juárez *et al*., 2017; Combrink, 2019). There is scanty information on cultivation of African potato and the use of nutrient solution. Potassium is essential and the most abundant cation in plants (Wang *et al.,* 2013). Potassium plays a vital role in plant metabolism, cation-anion balance and stress resistance in plants which increases plant yield and quality through the synthesis of useful metabolites (Samet *et al.*, 2015; Chrysargyris *et al*., 2017; Hasanuzzaman *et al.,* 2018).Therefore, the objective of this study was to determine the effects of potassium containing nutrient solution on the yield attributes, mineral content and synthesis of primary metabolites of African potato plants grown in a greenhouse.

3.2 Materials and Methods

3.2.1 Description of the experimental site

The study was conducted in the greenhouse of the Department of Agriculture, Central University of Technology, Free State, Bloemfontein Campus. The study area is situated at 29°07’16.78”S 26°12’45.95”E, and altitude of 1 395 m above sea level.

Figure 3.1 A section of the greenhouse, gowing units and young African potato plants. Source: (Central University of Technology, Free State, South Africa).

The total area of the greenhouse is 72 m^2 (i.e. 12 m x 6 m in length and width, respectively). The greenhouse structure was of a transparent polyethylene sheet fitted on metal frames. At the beginning of the study, the temperature in the greenhouse was maintained at 26°C during the day and 12°C at night. The temperature was continuously regulated throughout the study period to mimick the weather conditions of each month. The temperature sensor in the greenhouse was connected in such a way that it triggers and activates an axial fan fitted at one end of the greenhouse. The ambient weather condition in Bloemfontein during the experimentation period are shown in Table 3.1.

Table 3.1 Average monthly weather conditions for the period of the experiment (October 2018 - August 2019) (South African Weather Service, 2019).

Months	Ave. Min. Temp. (°C)	Ave. Max. Temp. (°C)	Total Rainfall (mm)	Ave Humidity (%)	Ave. Wind Speed (m/s)	Ave. Pressure (hpa)
Oct 2018	10.5	28.5	22.8	32.2	2.1	864.3
Nov 2018	13.2	31.6	15.6	25.7	2.7	861.3
Dec 2018	16.0	34.5	23.2	27.9	2.9	859.6
Jan 2019	17.0	34.4	48.6	31.3	2.6	859.4
Feb 2019	15.5	30.4	163.6	49.8	2.3	861.1
Mar 2019	14.9	30.6	55.2	50.5	1.8	862.1
Apr 2019	10.2	24.4	122.0	69.1	1.5	863.6
May 2019	6.6	23.7	17.2	63.1	0.9	866.2
Jun 2019	1.0	20.5	0.0	55.5	1.2	868.1
Jul 2019	0.8	20.2	0.0	49.4	1.5	866.8
Aug 2019	3.8	25.3	0.0	27.7	1.7	874.8

3.2.2 Planting, irrigation and general management of plants

African potato plantlets generated from tissue culture were obtained from the Agricultural Research Council of South Africa-Vegetable and Ornamental Plant Institute. The experiment was established on the 29th of October 2018. A black 9 L potting bag filled with organic potting media consisting of a mixture of compost and palm peat in a ratio of 2:1 respectively was used to grow the African potato plants. Initially, two plantlets were grown in a single pot, after a month of establishment one plantlet was thinned per pot.

Plants were fertigated using a K- nutrient solution prepared using Mangaung Metropolitan municipality water. The water chemical analysis was determined prior to the preparation of the experimental nutrient solution (Appendix 1). The nutrient solutions were adjusted to a pH of 5.5 (± 0.1) by adding 47 ml 60% nitric acid per 1 000 L of water to lower the HCO_3^- to 0.40 meq L^{-1} and the electrical conductivity of the solution was kept at 1.62 ± 0.01 mS cm^{-1} (Combrink, 2019).

Custom-made growing units consisting of recirculating nutrient solutions were used. These growing units were 110 L rectangular plastic containers obtained from a local plastic made items supplier. Plant containing potting bags were placed on top of the lid that separate the growing plants from the nutrient solution reservoir underneath. Each growing unit had an irrigation pump of 700 L hr^{-1} flow rate capacity fitted to a 10 mm transparent hose. The hose was connected by a 4 mm polythene tubing connected to the individual dripper which supply water to the pot.

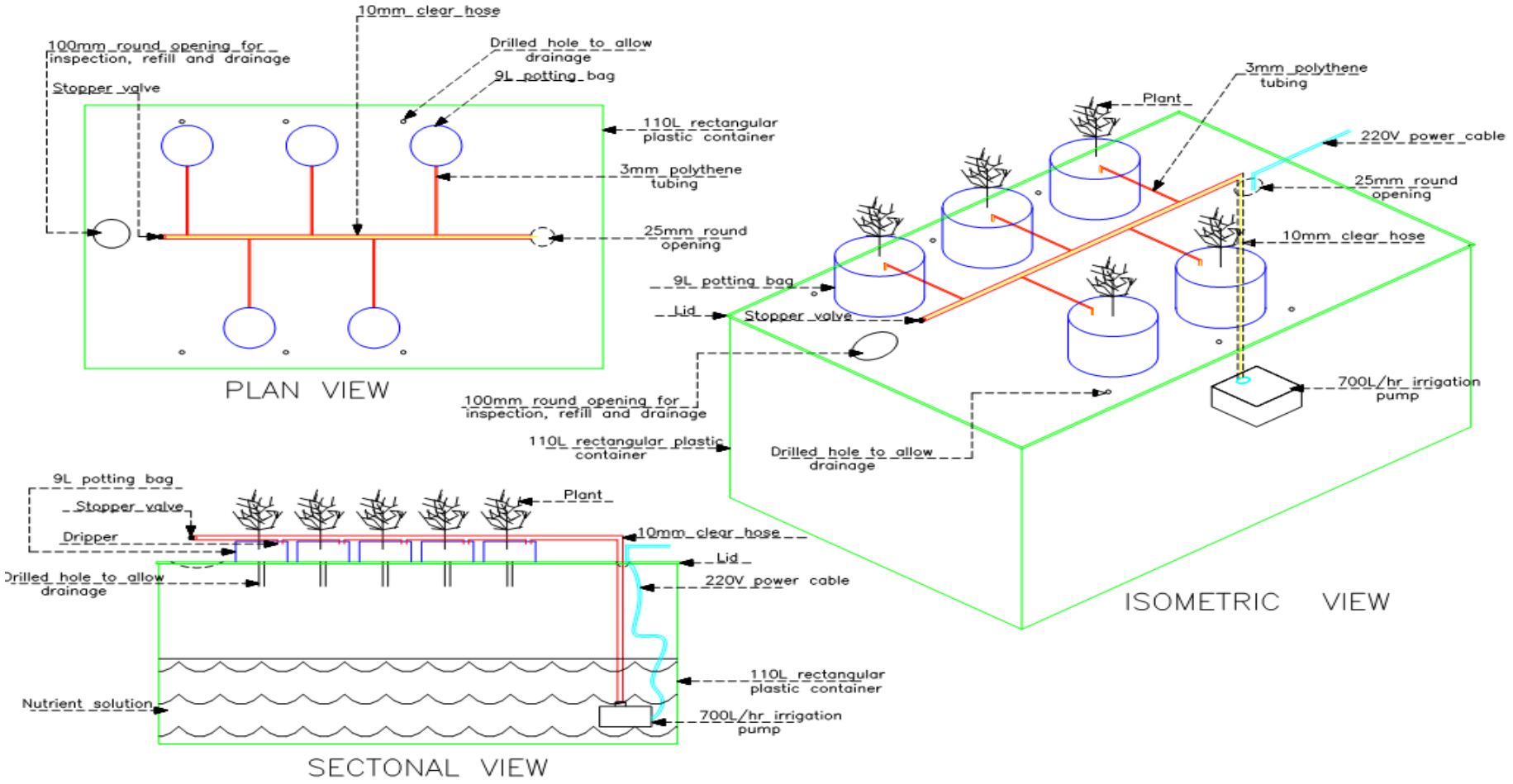

Figure 3.2 A schematic representation of the growing unit and irrigation system used to grow African potato plants.

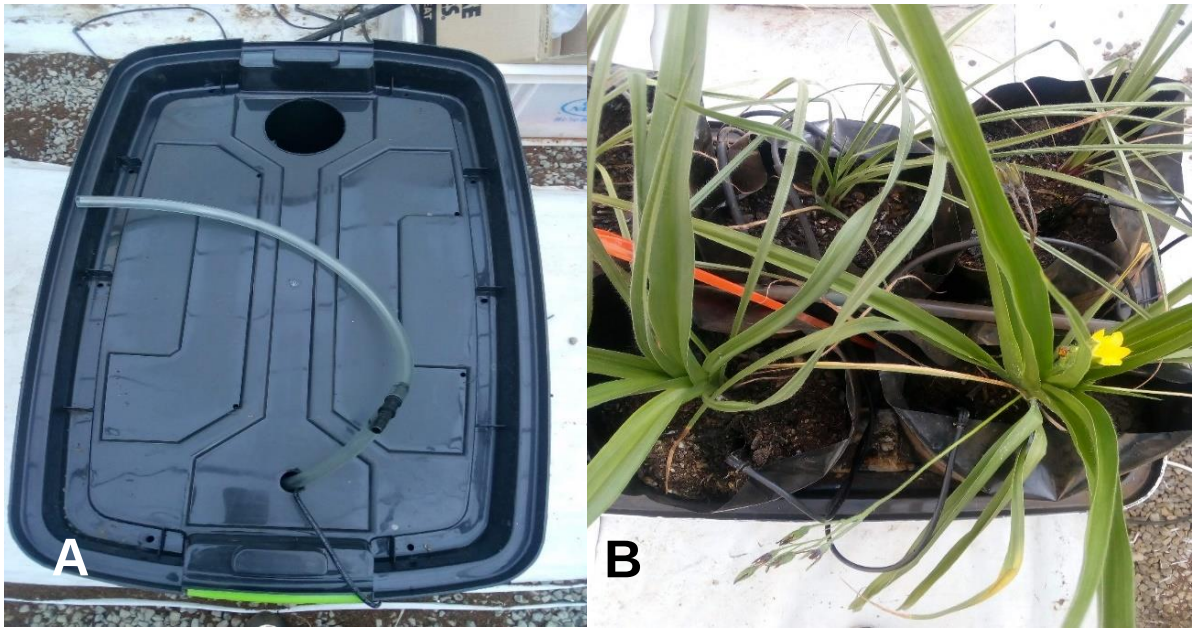

Figure 3.3 The growing unit: A = the custom-made growing unit being built. B = the completed growing unit with African potato plants. (Source: Central University of Technology, Free State, South Africa).

Table 3.2 Micronutrients used in the nutrient solution to grow African potato.

Mineral	Fertilizer	Application (g 1000 L^{-1} of water)
Iron (Fe)	Libfer, (Fe-EDTA 13%)	6.54
Manganese (Mn)	Manganese Sulphate	2.19
Zinc (Zn)	Zinc Sulphate	1.24
Boron (B)	Boric Acid	1.94
Copper (Cu)	Copper Sulphate	0.27
Molybdenum (Mo)	Ammonium Molybdate	0.09

Source: (Combrink and Kempen, 2011; Combrink, 2019).

Irrigation was scheduled and often adjusted according to the age of plants, season and the moisture content of the growing media (DAFF, 2012). Accumulation of salts in the growth media was minimized by applying large volumes of nutrient solution (3 hours irrigation) in each irrigation cycle to ensure 10% to 15% leaching (Nyakane *et al*., 2019). Nutrient solution used was replaced with fresh solution once a month to avoid nutrient imbalances (Sedibe and Allemann, 2013; Nyakane *et al*., 2019).

Micronutrients used in all experimental treatments are shown in Table 3.2 (Combrink and Kempen, 2011; Combrink, 2019).

3.2 3 Experimental design

Four levels of K were evaluated at 4.00, 6.00, 8.00 and 10.00 meq L^{-1} of K and the experiment was arranged in a randomised complete block design consisting of six blocks used as replications. Each experimental unit had five potted plants consisting of one plant in each potting bag. The experimental units were placed 60 cm apart between blocks and 40 cm apart between units within a block. Randomization and the arrangement of blocks (field plan) is shown in Table 3.3. The sources of the major minerals were potassium nitrate (KNO_3), calcium nitrate crystal [$Ca(NO_3)_2$] with NH_4, mono potassium phosphate (MKP) KH_2PO_4, mono ammonium phosphate (MAP) ($NH_4H_2PO_4$), magnesium sulphate ($MgSO_4$) and potassium sulphate (K_2SO_4) (Combrink and Kempen, 2011; Combrink, 2019). Treatment levels of K and the rest of anions and cations were applied as shown in Table 3.4. Fertilizer rate for the nutrient solutions of K levels is shown in Table 3.5.

Table 3.3 The experimental lay out.

Blocks (replications)	**Treatments**			
1	K3R1	K4R1	K1R1	K2R1
2	K1R2	K2R2	K4R2	K3R2
3	K2R1	K1R3	K3R3	K4R3
4	K1R4	K4R4	K2R4	K3R4
5	K3R5	K2R5	K1R5	K4R5
6	K1R6	K3R6	K4R6	K2R6

Table 3.4 Mineral composition of the nutrient solution used to evaluate the effect of potassium on cultivated African potato.

K levels (Treatments)	Ions (meq L^{-1})						
	NH_4^+	K^+	Ca^{++}	Mg^{++}	NO_3^-	$H_2PO_4^-$	$SO_4^=$
4.00	1.00	4.00	8.55	2.45	12.00	1.10	2.39
6.00	1.00	6.00	7.00	2.00	12.00	1.10	2.39
8.00	1.00	8.00	5.44	1.56	12.00	1.10	2.39
10.00	1.00	10.00	3.89	1.11	12.00	1.10	2.39

Note: Prescribed nurient solutions; Low in potassium: K1 (4.00 meq L^{-1}); Normal, control: K2 (6.00 meq L^{-1}); High in potassium: K3 (8.00 meq L^{-1}); Very high in potassium: K4 (10.00 meq L^{-1}).

Table 3.5 Fertilizers and their quantities used to formulate the nutrient solutions of the potassium levels.

Fertilizer	K levels (Treatments) (meq L^{-1})			
	4.00	**6.00**	**8.00**	**10.00**
	Rate of application (g 1000 L^{-1} of water)			
KNO_3	296.94	470.00	662.00	815.00
$[Ca(NO_3)_2]$ with NH_4	910.01	726.00	541.00	356.00
KH_2PO_4	118.32	97.00	76.00	54.00
$NH_4H_2PO_4$	26.45	45.00	62.00	81.00
$MgSO_4$	254.61	199.00	145.00	90.00
K_2SO_4	11.31	50.00	72.00	128.00

Source: (Combrink and Kempen, 2011; Combrink, 2019).

3.2.4 Parameters

Agronomic attribute data were collected in three stages occurring after 18 weeks, 32 weeks and 40 weeks for first, second and third data collection respectively. The experiment lasted for 9 months (October, 2018 – August, 2019). Mineral analysis data were collected at 18 weeks whilst primary metabolite data were collected at 32 weeks. Mineral as well as phytochemical analysis (primary metabolites) were done using the specific analytical procedures, which are described separately below.

Agronomic attributes

Chlorophyll content, leaf area, plant height, number of leaves and roots were determined at each harvest. In addition, both fresh and dry mass of leaf, corm and root were measured at harvest.

The leaf area was determined using a portable leaf area meter (CI-202 portable Laser Leaf Area Meter CID Bio-Science, USA). The chlorophyll content was measured at harvest using a portable non-destructive chlorophyll meter (Optisciences CCM-200, USA).

The number of roots and leaves were observed and counted whereas the masses of fresh and dry roots, leaves and corms were measured using a laboratory scale (Denver Instrument APX – 602, USA). The roots and corms were washed immediately under running tap water while still attached to the mother plants. For each plant, the roots were counted and then detached from the corm for weighing. The corm was also detached from the plant afterwards and weighed. All measurements for each plant took approximately five minutes. Harvested materials were oven dried prior to dry mass and the mineral contents measurements (Sedibe and Allemann, 2013).

Disease and pest control

There was leafspot disease occurrence during the experimental period which was controlled using VIRIKOP, a broad-spectrum fungicide and bactericide. A 50 g/10 L of water of VIRIKOP was used as recommended for flowers and ornamentals in a full cover spray since there is no fungicide or bactericide recommended for African potato. A repeat application was done after a week.

Thrips invasion was also noticed and controlled using CYPERMETHRIN 200EC as prescribed for flowers and ornamentals using the dosage 1 ml/10 L water since there is no dosage prescribed for African potato. A full cover spray was used and repeated after one week.

Mineral analysis

Plant materials (corms and leaves) for mineral analysis were oven dried at 60°C for 72 hours. (Singh and Praharaj, 2017). The dried plant materials were milled using micro hammer mill following the procedure used by Sedibe and Allemann (2013). Minerals determined were N, P, K, Ca, Mg, S, Fe, Zn, Cu, Mn, B and Mo. The total concentration of K, Ca, P, Mg, Fe, Zn, Mn, B, and Cu in milled leaf and corm tissues were determined using atomic emission spectrometry with Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES) (PerkinElmer Inc. - Optima 4300 DV ICP-OES, USA).

The Dumas combustion method was used for total nitrogen determination in a Leco FP-528 combustion nitrogen analyzer (LecoCorp, St.Joseph, MI, USA) (Sedibe and Allemann, 2013). Plant sulphur was also determined by ICP-OES using an extract solution, however, another instrument (JY Horiba Ultima, USA) was used in its determination (Sedibe and Allemann, 2012). Molybdenum in plant samples was determined by calorimetric method after digesting samples with HNO_3 and $HCLO_4$ (Singh and Praharaj, 2017).

Phytochemical analysis

Primary metabolite analysis

Primary metabolites were determined only in the corms of African potato. The corm samples were prepared for primary metabolite analysis using a method described by Nsibande *et al.* (2018) with slight modification. Subsequent to harvesting, corms were snap frozen using liquid nitrogen (-196°C), crushed and grounded immediately using a pestle and mortar. Milled materials were kept in airtight tubes and stored in liquid nitrogen then later transferred and stored in a freezer (-80°C) prior to analysis. About 50 mg of milled corms were extracted using 15 ml methanol-water. The mixture was sonicated for 20 min at room temperature and centrifuged at 1300 rpm for 15 min. The mixture was filtered through 0.45 µm syringe filters and the supernatant (1 ml) was transferred into 2 ml amber glass vials for Liquid chromatography–mass spectrometry (LC–MS) analysis.

Primary metabolites were determined using Ultra-High Performance Liquid Chromatography-Mass Spectrometry (UHPLC-MS) analysis method described by Mncwangi *et al*. (2014) with minor modification. The UHPLC analysis was performed on a Waters Acquity ultra-high-performance liquid chromatographic system with PDA detector (Waters, Milford, MA, USA). UHPLC separation was achieved on a UHPLC ultra C18 column (100 mm × 2.1 mm, i.d., 5-µm particle size, Restek) maintained at 35°C. The mobile phase consisted of 0.1% formic acid in water (solvent A) and LC-MS Grade Methanol (solvent B) at a flow rate of 0.3 ml min^{-1} and gradient elution was applied as follows: 85% A: 15% B to 65% A: 35% B in 4 min, changed to 50% A: 50% B in 2 min, to 20% A: 80% B in 1 min, maintaining for 1 min and back to initial ratio in 0.5 min. The analysis time was 9 min. Samples were introduced into the mobile phase with an injection volume of 1.0 µl (full-loop injection) for samples and 2.0 µl for reference standards. The UHPLC system was interfaced with a Xevo G2QTof MS (Waters, USA). The following mass spectrometry operating conditions were applied: source – ESI negative mode; capillary voltage – 3 kV; cone voltage 30 V; calibration – sodium formate; lock spray – leucine enkaphalin and scan mass range – 200 –1500 m/z.

3.2.5. Statistical analysis

Analysis of variance (ANOVA) was conducted using Statistical Analysis Systems software program (SAS inst., 2017) with General Linear Model (GLM) procedure. The Tukey's student range test was used to separate means that were significantly different at p value of 0.05 as described by Steel and Tourie (1980).

3.3 Results

3.3.1 Agronomic attributes

The results of the agronomic attributes are shown in Tables 3.6 and 3.7. The different levels of K had significant effect on agronomic attributes only after the first measurement. There were no significant treatment effects on the plant height, corm dry mass, number of roots, leaf fresh mass, leaf dry mass and number of leaves per plant irrespective of the time of measurements. Chlorophyll content was significantly affected by the different K levels. Chlorophyll content was significantly ($p<0.05$) higher in plants grown at 10.00 meq L^{-1} K concentration than plants grown at 4.00 and 6.00 meq L^{-1} but not significantly higher than plants grown in the nutrient solution of 8.00 meq L^{-1} K concentration. In the second measurement, increased chlorophyll increased leaf area. However, there was reduction in leaf area over time when compared with leaf area of the first measurement except for plants grown with 10.00 meq L^{-1} K concentration.

Leaf area of the 10.00 meq L^{-1} K treatment increased over time whilst leaf area reduced overtime with K treatments of 4.00, 6.00 and 8.00 meq L^{-1}. The leaf area of African potato was significantly ($p<0.05$) greater in plants fertigated with the highest K level of 10.00 meq L^{-1} than plants grown using 6.00 meq L^{-1} K, but not significantly higher than plants grown using a nutrient solution containing 4.00 and 8.00 meq L^{-1} K concentration during the second measurement. Likewise, the fresh corm mass obtained at 10.00 meq L^{-1} K was not significantly ($p<0.05$) different from plants produced at nutrient solution containing 4.00 and 8.00 meq L^{-1} K but different from fresh corm mass obtained from 6.00 meq L^{-1} K treatment.

Table 3.6 Summary of ANOVA of agronomic attributes of African potato affected by levels of potassium.

	Agronomic attributes													
Source	**DF**	**CHL_1**	**CHL_2**	**LA_1**	**LA_2**	**PH_1**	**PH_2**	**FCM_1**	**FCM_2**	**FCM_3**	**DCM_1**	**NR_1**	**NR_2**	**NR_3**
Replication	**5**													
K levels	**3**	ns	*	ns	*	ns	ns	ns	*	ns	ns	ns	ns	ns

	Agronomic attributes												
Source	**DF**	**RFM_1**	**RFM_2**	**RFM_3**	**RDM_1**	**RDM_2**	**RDM_3**	**NL_1**	**NL_2**	**LFM_1**	**LFM_2**	**LDM_1**	**LDM_2**
Replication	**5**												
K levels	**3**	ns	ns	*	ns	ns	*	ns	ns	ns	ns	ns	ns

Chlorophyll content is denoted by CHL; Leaf area (cm^2) is denoted by LA; Plant height (cm) is demoted by PH; Fresh mass of corms (g) denoted by FCM; drymass of the corms (g) denoted DCM; number of roots per plant denoted by NR; root fresh mass (g) denoted by RFM; root dry mass (g) denoted by RDM; number of leaves per plant denoted by NL; Leaf fresh mass (g) denoted by LFM; Leaf dry mass (g) denoted by LDM. Subscript 1, 2 and 3 represent first, second and third data collection respectively. **Note:** Agronomic attributes in bold are statistically significant at 0.05 p value. *** = significant at p<0.0001, ** = significant at p < 0.001, * = significant at p <0.05, ns = not significant at p<0.05, DF = degrees of freedom.

Table 3.7 Effects of potassium concentration in the nutrient solution on agronomic attributes of African potato.

K levels (meq L^{-1})	CHL_2	LA_1	LA_2	FCM_2	RFM_3	RDM_3
4.00	16.21±11.33^{b}	83.65±65.00^{a}	59.02±30.10^{ab}	54.13±19.24^{a}	56.12±25.04^{ab}	3.31±1.59^{ab}
6.00	13.10±12.18^{b}	53.90±59.21^{a}	39.15±27.79^{b}	34.91±10.53^{b}	43.63±25.83^{b}	2.59±1.63^{b}
8.00	20.48±10.71^{ab}	67.64±46.74^{a}	63.91±29.01^{ab}	42.14±16.07^{ab}	52.83±22.14^{b}	3.18±1.41^{ab}
10.00	28.51±7.10^{a}	51.76±43.52^{a}	87.59±19.21^{a}	57.13±14.52^{a}	74.82±24.12^{a}	4.59±1.78^{a}
$LSD_{T\ 0.05}$	10.72*	57.52^{ns}	29.47*	17.49*	18.72*	1.41*

Note: Statistical significance was at 0.05 P value. *** = p<0.0001, ** p < 0.001, * p <0.05, ns = not significant at p<0.05, ± = standard deviation, $LSD_{T\ 0.05}$ = least significant difference at 5% level of significance. The same letter within a column denotes non significance (P>0.05). Means with the same letter as superscripts within a column are not significantly different.

A significant effect of K occurred on the root dry and root fresh mass measured in the third measurement (Table 3.7). For this measurement, root fresh mass was highest at 10.00 meq L^{-1} K levels, where this mass obtained was not significantly higher for plants grown using a nutrient solution containing 4.00 meq L^{-1} of potassium. Again, there were no significant differences between 4.00, 6.00 and 8.00 meq L^{-1} for this parameter. Root dry mass of the 10.00 meq L^{-1} K treated plants produced the highest but was not significantly different from the root dry mass of the plants obtained at 4.00 and 8.00 meq L^{-1} K levels. The root dry mass was only significantly lower (p<0.05) at 6.00 meq L^{-1} compared with 10.00 meq L^{-1} K level.

3.3.2 Primary metabolite

Sixty-one types of primary metabolites were detected in the corms by the Liquid Chromatography–Mass Spectrometry (LC–MS) procedure and are listed in Table 3.8. Only 22 metabolites (in bold) were further analyzed statistically since some metabolites were not present in all the replications of the treatments. Analysis of variance revealed that K had no significant effect on most of the primary metabolites analysed. However, only alanine and malic acid were significantly (p<0.05) affected by the K levels of the nutrient solution as shown in Table 3.9 and Figures 3.4 - 3. 5. Alanine was highest at 10.00 meq L^{-1} of K, however, malic acid decreased with increase in the levels of K used until the level of 8.00 meq L^{-1}.

Table 3.8 Primary metabolites of African potato corms obtainined from LC-MS analysis as affected by potassium levels of the nutrient solution.

Acetylcarnitine	Choline	Fumaric acid	**Malic acid**	**Threonine**
Acetylcholine	**Creatine**	**Glutamic acid**	**Methionine sulfoxide**	Thymidine
Adenine	**Creatinine**	**Glycine**	Niacinamide	Thymidine monophosphate
Adenosine	Cystathionine	**Guanosine**	Nicotinic acid	Thymine
Adenosine monophosphate	Cystine	**Histamine**	**Norepinephrine**	Tryptophan
Alanine	Cytidine	Hypoxanthine	Ophthalmic acid	Tyrosine
Allantoin	**Cytidine monophosphate**	Inosine	Ornitine	**Uracil**
Argininosuccinic acid	**Cytosine**	**Isocitric acid**	Orotic acid	Valine
Asparagine	**Dimethylglycine**	Isoleucine	**Phenylalanine**	Xanthine
Aspartic acid	Dopa	Kynurenine	**Pyruvic acid**	2-Ketoglutaric acid
Asymmetric dimethylarginine	Dopamine	Lactic acid	**Serine**	4-Aminobutyric acid
Carnitine	**Epinephrine**	Leucine	Symmetric dimethylarginine	4-Hydroxyproline
Carnosine				

Note: Primary metabolites in bold were analysed statistically.

Table 3.9 Summary of ANOVA of primary metabolites of African potato detected using LC-MS as affected by levels of potassium.

		Primary metabolites							
Source	**DF**	**Alanine**	Aspartic acid	Carnitine	Creatine	Creatinine	Cytidine mono phosphate	Cytosine	Dimethylglycine
Replication	**5**								
K levels	**3**	*	ns	ns	ns	ns	ns	ns	ns
		Primary metabolites							
Source	**DF**	Epinephrine	Glutamic acid	Glycine	Guanosine	Histamine	Isocitric acid	**Malic acid**	Methionine sulfoxide
Replication	**5**								
K levels	**3**	ns	ns	ns	ns	ns	ns	*	ns
		Primary metabolites							
Source	**DF**	Norepinephrine	Phenylalanine	Pyruvic acid	Serine	Threonine	Uracil		
Replication	**5**								
K levels	**3**	ns	ns	ns	ns	ns	ns		

Note: Primary metabolites in bold are statistically significant at 0.05 p value, *** = significant at p<0.0001, ** = significant at p < 0.001, * = significant at p <0.05, ns = not significant at p<0.05, DF = degrees of freedom.

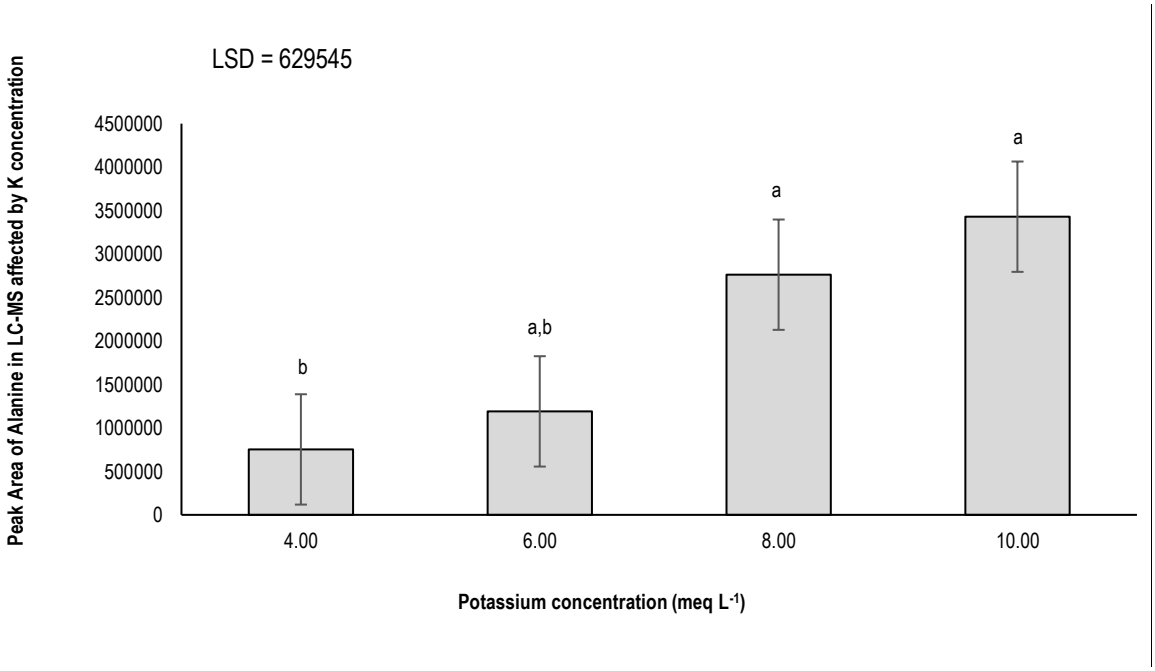

Figure 3.4 The peak area of alanine in LC-MS of plants grown with potassium levels.

Standard error (SE) is shown as vertical bars on the bar chart, the same letter on the bar charts denotes non significance ($p<0.05$), number of K treatments = 4, replications per treatment (n) = 6.

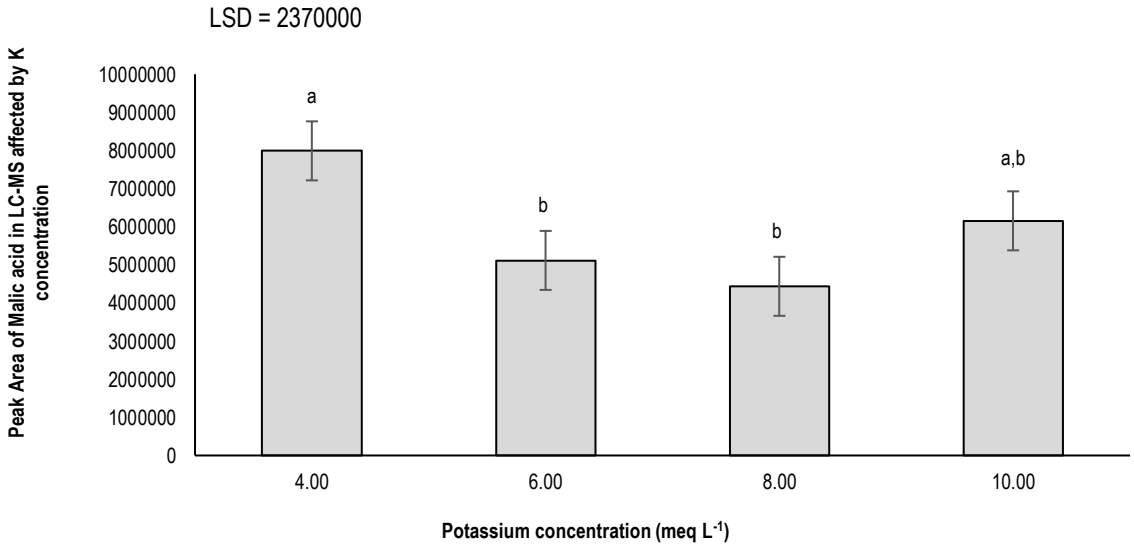

Figure 3.5 The peak area of malic acid in LC-MS of plants grown with potassium levels.

Standard error (SE) is shown as vertical bars on the bar chart, the same letter on the bar charts denotes non significance ($p<0.05$), number of K treatments = 4, replications per treatment (n) = 6.

Table 3.10 Effects of potassium levels on mineral accumulation in the leaf of African potato.

K levels (meq L^{-1})	N (%)	K (mg kg^{-1})	Ca (mg kg^{-1})	S (mg kg^{-1})	P (mg kg^{-1})	Mg (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	B (mg kg^{-1})	Cu (mg kg^{-1})	Mo (mg kg^{-1})
4.00	2.62±0.03^{a}	47028.55±5493.44^{a}	9003.51±1200.54^{a}	2985.87±327.11^{a}	2341.07±455.59^{a}	2286.36±125.91^{a}	95.63±7.56^{a}	41.41±4.89^{a}	39.81±3.62^{a}	97.61±26.95^{a}	3.52±0.30^{a}	0.36±0.05^{a}
6.00	2.62±0.10^{a}	41408.76±6432.39^{a}	7854.18±1000.40^{a}	3007.14±382.71^{a}	1862.92±265.31^{a}	2115.12±192.72^{a}	101.18±21.35^{a}	43.01±4.91^{a}	32.53±2.47^{a}	108.93±14.96^{a}	3.44±0.47^{a}	0.62±0.25^{a}
8.00	2.55±0.12^{a}	50395.13±1190.80^{a}	8187.40±904.01^{a}	3571.15±206.47^{a}	2051.62±142.47^{a}	2349.02±290.11^{a}	97.27±11.29^{a}	43.12±3.66^{a}	32.92±8.57^{a}	90.00±23.28^{a}	4.08±0.68^{a}	0.72±0.33^{a}
10.00	2.65±0.09^{a}	54292.19±5897.76^{a}	7142.46±617.95^{a}	3740.86±747.46^{a}	1922.37±159.29^{a}	2388.82±397.49^{a}	85.57±9.18^{a}	45.89±5.40^{a}	38.89±16.69^{a}	84.81±31.14^{a}	3.61±0.46^{a}	0.49±0.10^{a}
$LSD_{T\ 0.05}$	**0.20** ^{ns}	**10328.00** ^{ns}	**2022.50** ^{ns}	**884.96** ^{ns}	**613.42** ^{ns}	**608.54** ^{ns}	**28.28** ^{ns}	**9.02** ^{ns}	**18.57**^{ns}	**56.00** ^{ns}	**0.81** ^{ns}	**0.49** ^{ns}

Note: Statistical significance was at 0.05 p value. *** = p<0.0001, ** p < 0.001, * p <0.05, ns = not significant at p<0.05, ± = standard deviation, LSD = Least significant difference at 5% level of significance. Means with the same letter as superscripts within a column are not significantly different (p<0.05).

Table 3.11 Effects of potassium levels on mineral accumulation in the corm of African potato.

K levels (meq L^{-1})	N (%)	K (mg kg^{-1})	Ca (mg kg^{-1})	S (mg kg^{-1})	P (mg kg^{-1})	Mg (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	B (mg kg^{-1})	Cu (mg kg^{-1})	Mo (mg kg^{-1})
4.00	3.12±0.57^{a}	18988.54±1131.42^{a}	9466.59±2006.87^{a}	4158.93±833.74^{a}	4100.17±695.65^{a}	2029.86±313.37^{a}	211.14±58.34^{a}	168.20±74.98^{a}	29.39±6.80^{a}	25.08±1.76^{a}	12.67±9.41^{a}	0.45±0.15^{a}
6.00	3.18±0.27^{a}	17134.43±3456.33^{a}	7633.48±445.60^{ab}	4316.39±204.98^{a}	3741.12±304.25^{a}	2194.31±319.35^{a}	128.18±14.61^{a}	93.78±4.71^{a}	28.69±5.23^{a}	20.20±1.10^{b}	7.09±2.16^{a}	0.58±0.28^{a}
8.00	3.35±0.05^{a}	18709.94±1485.54^{a}	6964.93±381.15^{b}	4239.24±95.61^{a}	3767.79±205.33^{a}	2023.20±102.46^{a}	144.45±49.09^{a}	100.31±16.33^{a}	24.51±1.53^{a}	18.37±2.56^{b}	10.25±5.15^{a}	1.15±0.55^{a}
10.00	2.91±0.13^{a}	19906.96±712.01^{a}	6121.27±641.62^{b}	3656.85±162.58^{a}	3497.94±603.82^{a}	1905.53±321.20^{a}	117.94±43.44^{a}	75.83±1.96^{a}	26.51±1.25^{a}	18.53±0.93^{b}	5.24±1.91^{a}	1.29±0.45^{a}
$LSD_{T\ 0.05}$	**0.65** ^{ns}	**4224.1** ^{ns}	**2133.2***	**949.85** ^{ns}	**778.37** ^{ns}	**532.95** ^{ns}	**101.69** ^{ns}	**75.82** ^{ns}	**8.77** ^{ns}	**3.89***	**10.78** ^{ns}	**0.87** ^{ns}

Note: Statistical significance was at 0.05 p value. *** = p<0.0001, ** p < 0.001, * p <0.05, ns = not significant at p<0.05, ± = standard deviation, LSD = Least significant difference at 5% level of significance. Means with the same letter as superscripts within a column are not significantly different (p<0.05).

3.3.3 Mineral accumulation

There was no significant effect of different levels of K concentration on accumulation of minerals in the leaf of African potato (Table 3.10). However, the accumulation of Ca and B in the corm was affected by nutrient solution of K concentrations (Table 3.11).

Calcium concentration in the corm of African potato was significantly ($p<0.05$) higher at 4.00 meq L^{-1} of K than the concentrations obtained at 8.00 and 10.00 meq L^{-1} K levels. Calcium concentration in the corm at 6.00 meq L^{-1} did not differ significantly from K contents at 4.00 meq L^{-1} K level.

Significantly ($p<0.05$) higher B content was from plants that were grown at 4.00 meq L^{-1} K level where B accumulation was better than plants from 6.00, 8.00 and 10.00 meq L^{-1} K concentration treatments. However, no significant differences occurred at 6.00, 8.00 and 10.00 meq L^{-1} of K levels.

3.4 Discussion

Nutrients application aims at increasing yield and possibly enhancing the quality of plants (Dunn *et al.,* 2018). Macronutrients and micronutrients, for example N, P, K, Ca, S, Mg, and Fe, are vital for plant growth and development, and without them, plants cannot complete their life cycles and accomplish normal physiological functions. Generally, there is slow growth and low yield of plants due to nutrient deficiencies (Dunn *et al.,* 2018; Kalaji *et al.,* 2018). Potassium is vital in plant metabolism, encouraging the production of primary metabolites which increases crop yield and promote quality (Ibrahim *et al*., 2012; Chrysargyris *et al*., 2017). Beside enzyme activation, K addition to plants determines primary and secondary metabolite composition (Ibrahim *et al*., 2012).

The effects of most nutrients have not been studied into much detail for all plants (Koch *et al.,* 2019). Nutrient management need to be studied continuously on medicinal plants to evaluate its effects on mineral composition, primary and secondary metabolite composition (Laekemariam *et al.*, 2018). Steiner's universal nutrient solution consists of 7.00 K, 9.00 Ca and 4.00 Mg in meq L^{-1} as major macronutrients

at an EC of 2 mS cm^{-1}. Overtime and up till now, this nutrient solution is being adapted for maximum productivity of specific plants with slight alterations to the original solution (Combrink, 2019).

The effects of K levels on African potato plants studied was significant on agronomic attributes, mineral accumulation and primary metabolite synthesis. Primary metabolites are vital for the existence, growth, development and response to the environmental pressures of living things (Hong *et al.,* 2016; Shakya, 2016; Khan *et al.,* 2020). Accumulation of primary metabolites such as amino acids help plants to cope with stress thus improving yield and desirable plant quality (Beauvoit *et al.,* 2018).

This study showed a positive relationship between chlorophyll and plant age. As plants grew, the chlorophyll content increased however leaf area reduced over time except for 10.00 meq L^{-1} K level (Table 3.7). This finding is in agreement with the results of Dunn *et al.* (2018) that chlorophyll content increased with increasing plant age and corresponded to an increase in nitrogen content over time. The increased chlorophyll and leaf area observed at K level of 10.00 meq L^{-1} might be attributed to the high K content of this nutrient solution which possibly enhanced enzyme activity for increased plant growth and development as K is known for enzyme activation in plant metabolism (Chrysargyris *et al.,* 2017; Koch *et al.,* 2019). This is evident in the current study as fresh corm mass in the second measurement, fresh root and dry root mass in the third measurement increased with increased K levels of 10.00 meq L^{-1}.

Potassium deficiency can negatively influence several metabolic activites in plants (Chrysargyris *et al.,* 2017). The reduced leaf area in K treatments of 4.00, 6.00 and 8.00 meq L^{-1} might be due to less enzyme activity which affected growth and metabolism during the winter months of the second measurement when the African potato plants were experiecing leaf die back. Karavin (2014) noted that in adverse situations, nutrient resorption from leaves to the durable organs such as shoots, stems and roots occur in plants before defoliation causing leaf mass deacrease. Nutrient resorption might have occured in African potato leaves into the corms before their leaves die back in winter.

Karavin (2014) and Yu *et al*. (2019) reported a positive association between leaf area and leaf dry mass per unit area which is usually related to the rate of photosynthesis in plants however, there were no significant differences in leaf dry mass measurements in the current study. Similarlly, leaf age and plant age jointly did not significantly influence leaf area, leaf dry mass and specific leaf area of *Quercus cerris* (Karavin, 2014). Reduced leaf area in soybean under changing carbon dioxide concentrations improved yield (Srinivasan *et al*., 2016) hence decreasing leaf area observed for African potato in the second measurement with K levels might improve yield and metabolite production in these plants.

Increased chlorophyll content at 4.00 meq L^{-1} K concentration and the consequent increase in other agronomic attributes despite the low K levels possibly might be due to the higher levels of Ca and Mg which are also involved in cell growth and enzyme activation (Mugundhan *et al.*, 2011; Jones, 2012). Also Mg is a component of chlorophyll therefore an increased chlorophyll in the presence of higher Mg level in solution with 4.00 meq L^{-1} is justified though other environmental and physiological characteristic might have influence this result (Frageria, 2001).

In the current study, alanine increased as K ratio increased in the nutrient solution. This result concurs with the results of Ibrahim *et al.* (2012) that have indicated that increasing K increased the production of soluble protein and phenylalanine ammonia-lyase activity in *Labisia pumila*. Therefore, increasing K, and the corresponding increase in alanine production, could promote useful secondary metabolite synthesis. In the presence of extra K, there was an enhanced accumulation of organic acids namely malic acid (Ting, 1981). Malic acid acts as counter ion for K and Ca particularly in nitrate-dependent plants (Ghazijahani *et al.,* 2018). The current study has seen a significant difference confirming a rise in Ca at low K level of 4.00 meq L^{-1} increases malic acid, however, increasing K showed decrease in malic acid up to the 8.00 meq K level. This might be due to the combined effects of the nutrients, other environmental conditions present and the growth stage of the plants.

Since primary metabolism also lead to series of specialized metabolites (Ibrahim *et al*., 2012; Beauvoit *et al*., 2018), the significant differences observed with alanine and

malic acid might yield useful secondary metabolites which could promote the medicinal value of African potato. More systematic studies integrating metabolism and growth conditions are needed (Beauvoit *et al.,* 2018) to maximize the production of quality plants for human benefit.

Potassium levels in the nutrient solution did not significantly affect K concentration in the leaf and the corm of African potato. This finding is in line with Chrysargyris *et al.* (2017) who found out that the application of K in different concentrations did not affect the accumulation of the K into the tissues of spearmint, but rather stimulated the absorption, and thus the content of other minerals such as Ca and nitrogen. Many other studies have reported strong antagonistic relationship between K, Ca, P, Mg and other nutrients (Fageria, 2001; Hochmuth and Hochmuth, 2015; Nguyen, *et al.,* 2017; Li et *al.,* 2018).

In this study, increased Ca and B accumulation in the corm at the lowest K concentration of 4.00 meq L^{-1} might be due to the genetic ability of African potato to accumulate nutrients in the corm even at low soil fertility levels as observed by McAlister and van Staden (1995). The increased Ca accumulation in African potato tissues at 4.00 meq L^{-1} K level agrees with the results of Chen *et al*. (2018) who observed an increasing Ca accumulation in leaves with increasing Ca supply to *Eustoma* plants. Increased application of Ca antagonized the B concentration in shoot of four maize cultivars (Kanwal *et al.*, 2008). Antagonistic effects of Ca on B concentration in wheat was also reported (Turan *et al*., 2009) however, increased Ca at lowest K level 4.00 meq L^{-1} in this study increased B concentration in the African potato corm. This result reveals that higher Ca and Mg with low K supply (4.00 K: 7.66 Ca: 2.07 Mg in meq L^{-1}) could be favourable for optimal performance of African potato plants.

3.5 Conclusion

From the results, it is clear that successful cultivation of African potato could be achieved through the use of modern technique of nutrient management in a controlled environment. Seemingly, African potato performs better with a low nutrient level of K and a slight increase in Ca and Mg. The significant positive results obtained on the agronomic attributes, mineral accumulation in the corm and primary metabolite synthesis of African potato at a low K level of 4.00 meq L^{-1} might yield useful secondary metabolites responsible for the medicinal properties of African potato. Therefore low K, slightly high Ca and Mg levels (4.00 K: 7.66 Ca: 2.07 Mg meq L^{-1}) could be used in cultivating African potato. Further studies should be conducted with more K levels to establish the ideal K level for optimum production. Future studies should also focus on the effects of K levels on secondary metabolite production. Research should also be done on other major cations and their effects on metabolite synthesis of African potato.

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CHAPTER 4

Multivariate analysis of mineral and primary metabolite accumulation as well as agronomic attributes affected by potassium levels in African potato (*Hypoxis hemerocallidea* Fisch., C.A.Mey. & Avé-Lall.)

Abstract

Principal component analysis (PCA) is a commonly used adaptive multivariate technique for reducing the dimensionality of large datasets, increasing interpretability but at the same time minimizing information loss. The objective of this study was to evaluate the relationship between potassium (K) levels, agronomic attributes, mineral and primary metabolite accumulation in African potato. Principal component analysis revealed the strong synergistic and antagonistic relationship between K levels and all minerals used in the nutrient solution. Potassium showed positive relationship with S, Mg, Zn, Mn and Cu in the leaf, whilst K association with S, Mg and N was negative in the corm. Two principal components accounted for most of the variabilities. The two principal components (PC) explained the relationships that accounted for 80.36% of the total variation in mineral accumulation with PC1 accounting for (51.70%) whilst PC2 accounted for (28.65%). More minerals were accumulated in the corm at K level of 4.00 meq L^{-1}. Rotated PC loading of agronomic attributes was 68.02% with PC1 accounting for 55.17% and PC2 12.84%. The PC loading of primary metabolites totalled 36.55% with PC1 accounting for 21.70% whilst PC2 has 14.85% of the total variation. Most of the primary metabolites positively loaded on PC1 except carnitine, glycine and creatine, which loaded on both PC1 and PC2 negatively. The results from this PCA indicate low K level of 4.00 meq L^{-1} with increased level of Ca and Mg (4.00 K: 7.66 Ca: 2.07 Mg meq L^{-1}) could be adequate for the cultivation of African potato in a controlled environment.

4.1 Introduction

African potato is a useful medicinal plant in South Africa known to cure many diseases in both humans and farm animals including infertility, cardiac diseases, internal parasites, healing of septic wounds, testicular cancer, prostate hypertrophy, for immune boosting, gall- sickness, prevent abortion in cattle, heartwater (ehrlichiosis), redwater and even as food during famine (Bassey *et al*, 2014; Pereus *et al.*, 2018; Kumar *et al.,* 2019).

The demand for the widely esteemed African potato is rising therefore, there is a need for cultivation to meet the growing demand (Kumar *et al*., 2019). Cultivation of African potato in a controlled environment could improve the growth rate and phytochemical content however, nutrient relationships are not well understood for the steady production and the optimization of desirable metabolites in these plants (Mofokeng *et al.,* 2020).

Soil and soil nutrients play an important role in the growth and development of plants and consequently affects the secondary metabolism of plants (Gouvea *et al.,* 2012; Ávila-Juárez *et al*, 2017). Nutrients are usually absorbed by plants in ionic forms and due to the similarities in the uptake forms of some elements, antagonistic and synergistic interactions exist preventing the plants from taking up the right amount of nutrients. Understanding these interactions well is important for the application of balanced nutrient solutions for efficient cultivation of plants (Fageria, 2001; Bindraban *et al*., 2015).

Principal component analysis (PCA) is a commonly used adaptive multivariate technique for reducing the dimensionality of large datasets, increasing interpretability but at the same time minimizing information loss. It does so by creating new uncorrelated variables that successively maximize variance by solving eigenvalue/eigenvector problem (Jolliffe and Cadima, 2016; Westfall *et al*., 2017). The PCA can be based on either the covariance matrix or the correlation matrix. In either case, the new variables (the PCs) depend on the dataset and so are adaptive in the broad sense (Jolliffe and Cadima, 2016). The objective of this study was to evaluate

the relationships between K levels, agronomic attributes, mineral and primary metabolite accumulation in African potato.

4.2 Materials and methods

All materials and methods used are well detailed in chapter three of this study except for the Princicipal Component Analysis (PCA) which is the focus of this chapter.

As part of multivariate analysis, a PCA was done using XLSTAT 2015.04.36025 statistical software. The analysis was based on eigenvalue of the correlation and covariance matrices. The Pearson correlation matrix shows the strength and the direction of the relationship between variables. The Pearson correlation coefficients (r) range from -1.00 to +1.00. The positive or negative shows the direction of the relationship. A perfect correlation of -1.00 or +1.00 shows that all the data points lie precisely on the straight line whilst zero (0) designates no linear relationship between the variables. The correlation range of 0.80-1.00 is very strong, 0.60-0.80 is strong, 0.40-0.60 is moderate and 0.20-0.40 is weak (NurseKillam, 2014; Schober *et al*., 2018).

The PCA revealed synergistic and antagonistic relationships between nutrients in growing African potato and the effects these nutrients have on primary metabolite yield. The synergistic and antagonistic relationships between nutrients in the leaf and the corm of African potato were revealed.

4.3 Results and discussion

A very strong, strong and moderate significant positive and negative relationships were observed in both the leaf and the corm among the 12 nutrient elements used. The correlation matrix and the summary PCA of mineral composition of the leaf and the corm are described on Tables 4.1 - 4.3 and rotated principal component loadings of mineral accumulation in plants due to K levels is shown in Figure 4.1.

In the leaf K showed a very strong synergistic relationship with S (r = 0.88) and Mg (r = 0.97) whilst Fe (r = -0.87) and B (r = -0.99) correlated negatively. Moderate positive relationship occurred between K and Zn (r = 0.64), Mn (r = 0.47) and Cu (r = 0.48). A

very strong positive association occurred between Ca and P (r = 0.87) but a very strong negative relationship existed between Ca and Zn (r = -0.95). On a moderate level, Ca correlated negatively with S (r = -0.67) and positively with Fe (r = 0.53). Mg associated very strongly with Fe (r = -0.78) and B (r = -0.98) in the negative direction whilst Zn (r = 0.45), Mn (r = 0.50) and Cu (r = 0.57) associated positively with Mg on a moderate level. In the corm, K showed a very strong negative relationship with S (r = -0.83) and Mg (r = -0.99). Similarly, Li *et al.* (2018) found strong antagonistic link between K and Mg in the growth of tomato plants. Other studies also report antagonism between K and Mg (Rietra *et. al*., 2017). Potassium also has a negative relationship with N (r = -0.56). In contrast, increased K concentration increased nitrate absorption in the leaves of cucumber plants which positively influenced amino acid concentration in the fruits (Ruiz and Romero, 2002). Positively, K associated with Mo (r = 0.58) on a moderate level. Ca exhibited a very strong synergistic association with all minerals used except Mo (r = -0.90) which has a very strong antagonistic relationship with Ca. Mg moderately associated with Mn (r = 0.43) in a positive way; however, relationship of Mg and Mo (r = -0.68) was negative.

The mineral composition of plants is the combined effect of interactions between endogenous plant processes and the environment. Nutrient imbalances constrain species mineral composition and the yield of plants (Stein *et al.*, 2016; Ávila-Juárez *et al*., 2017). The different synergistic and antagonistic relationships observed in nutrient accumulation of African potato (in the leaf and corm) might be due to the complex biological processes that occur in the plant as well as the nutrient imbalances caused by the K ratios (Pii *et al*., 2015). Since K is vital in plant metabolism (Chrysargyris *et al.,* 2017; Koch *et al.,* 2019), several interactions with other minerals could occur at various stages of growth and development causing changes in accumulation of minerals in different plant organs of African potato. According to Rietra *et al.* (2017), N and K usually have synergistic association which jointly affect growth and yield however, this study showed antagonism between N and K in the corm but very weak synergism in the leaf probably due to cation imbalances. Also, due to the complex source-sink mechanism in plants (Yu *et al.,* 2015), accumulation of minerals and the deposition of photoassimilates in the sink organs could change during the growth

period for specific crops hence the various antagonistic and synergistic relations of minerals observed in the leaf and corm of African potato.

Table 4.1 Correlation matrix (Pearson (n1)) of mineral composition in the leaf of African potato affected by potassium levels.

Variables	N_Leaf	K_Leaf	Ca_Leaf	S_Leaf	P_Leaf	Mg_Leaf	Fe_Leaf	Zn_Leaf	Mn_Leaf	B_Leaf	Cu_Leaf	Mo_Leaf
N_Leaf	1											
K_Leaf	0.007468	1										
Ca_Leaf	-0.37874	-0.39631	1									
S_Leaf	-0.121	**0.887959**	**-0.67408**	1								
P_Leaf	-0.17648	0.045136	**0.872179**	-0.37179	1							
Mg_Leaf	-0.13146	**0.973578**	-0.18177	**0.802061**	0.24681	1						
Fe_Leaf	**-0.49251**	**-0.87391**	**0.536473**	**-0.71792**	0.055287	**-0.78177**	1					
Zn_Leaf	0.379698	**0.641606**	**-0.95572**	**0.81754**	**-0.69526**	**0.451266**	**-0.7489**	1				
Mn_Leaf	**0.588485**	**0.475893**	0.141753	0.057709	**0.563797**	**0.501294**	**-0.6943**	0.08515	1			
B_Leaf	0.082752	**-0.9957**	0.343044	**-0.88633**	**-0.08163**	**-0.98573**	**0.826135**	**-0.59092**	**-0.43495**	1		
Cu_Leaf	**-0.85968**	**0.488045**	0.023619	**0.600212**	0.048631	**0.571671**	-0.00807	0.075243	-0.35697	**-0.56111**	1	
Mo_Leaf	**-0.68219**	-0.09075	-0.3162	0.327258	**-0.59753**	-0.12551	**0.404037**	0.172162	**-0.91876**	0.044213	**0.637212**	1

Table 4.2 Correlation matrix (Pearson (n1)) of mineral composition in the corm of African potato affected by potassium levels.

Variables	N_Corm	K_Corm	Ca_Corm	S_Corm	P_Corm	Mg_Corm	Fe_Corm	Zn_Corm	Mn_Corm	B_Corm	Cu_Corm	Mo_Corm
N_Corm	1											
K_Corm	**-0.56205**	1										
Ca_Corm	0.217853	-0.26594	1									
S_Corm	**0.860514**	**-0.83078**	**0.537604**	1								
P_Corm	0.397602	-0.219	**0.966923**	**0.604813**	1							
Mg_Corm	**0.536378**	**-0.99126**	0.385913	**0.848967**	0.327574	1						
Fe_Corm	0.170369	0.090152	**0.919567**	0.329299	**0.949246**	0.028167	1					
Zn_Corm	0.173889	0.005919	**0.954256**	0.379077	**0.966033**	0.115241	**0.994898**	1				
Mn_Corm	-0.31717	-0.33332	**0.728673**	0.194245	**0.530171**	**0.438818**	**0.496653**	**0.569526**	1			
B_Corm	-0.061	-0.05601	**0.958549**	0.279808	**0.885494**	0.186807	**0.916939**	**0.944801**	**0.800132**	1		
Cu_Corm	**0.533691**	-0.04304	**0.81782**	**0.555999**	**0.935581**	0.129055	**0.915376**	**0.900014**	0.204054	**0.71392**	1	
Mo_Corm	-0.18761	**0.585088**	**-0.9029**	**-0.63374**	**-0.79887**	**-0.68693**	**-0.66222**	**-0.73431**	**-0.86912**	**-0.83843**	**-0.54357**	1

Table 4.3 Summary of the PCA results of mineral accumulation affected by potassium levels.

Mineral accumulation data			
Principal component	**PC1**	**PC2**	**PC3**
Eigenvalue	12.409	6.877	4.715
% Variance	51.702	28.654	19.644
Cumulative % of total variance	51.702	80.356	100.000
Factor loadings			
N_Leaf	0.132	0.397	-0.908
K_Leaf	0.789	0.516	0.332
Ca_Leaf	-0.863	0.285	0.416
S_Leaf	0.950	0.072	0.303
P_Leaf	-0.577	0.702	0.418
Mg_Leaf	0.642	0.586	0.494
Fe_Leaf	-0.756	-0.636	0.155
Zn_Leaf	0.956	-0.030	-0.293
Mn_Leaf	0.055	0.975	-0.213
B_Leaf	-0.762	-0.495	-0.418
Cu_Leaf	0.354	-0.185	0.917
Mo_Leaf	0.279	-0.867	0.412
N_Corm	-0.432	-0.583	0.689
K_Corm	0.583	0.784	0.213
Ca_Corm	-0.936	0.350	0.026
S_Corm	-0.776	-0.561	0.289
P_Corm	-0.906	0.319	0.279
Mg_Corm	-0.679	-0.696	-0.235
Fe_Corm	-0.751	0.597	0.282
Zn_Corm	-0.807	0.551	0.212
Mn_Corm	-0.695	0.272	-0.666
B_Corm	-0.815	0.567	-0.120
Cu_Corm	-0.734	0.330	0.594
Mo_Corm	0.956	-0.044	0.290

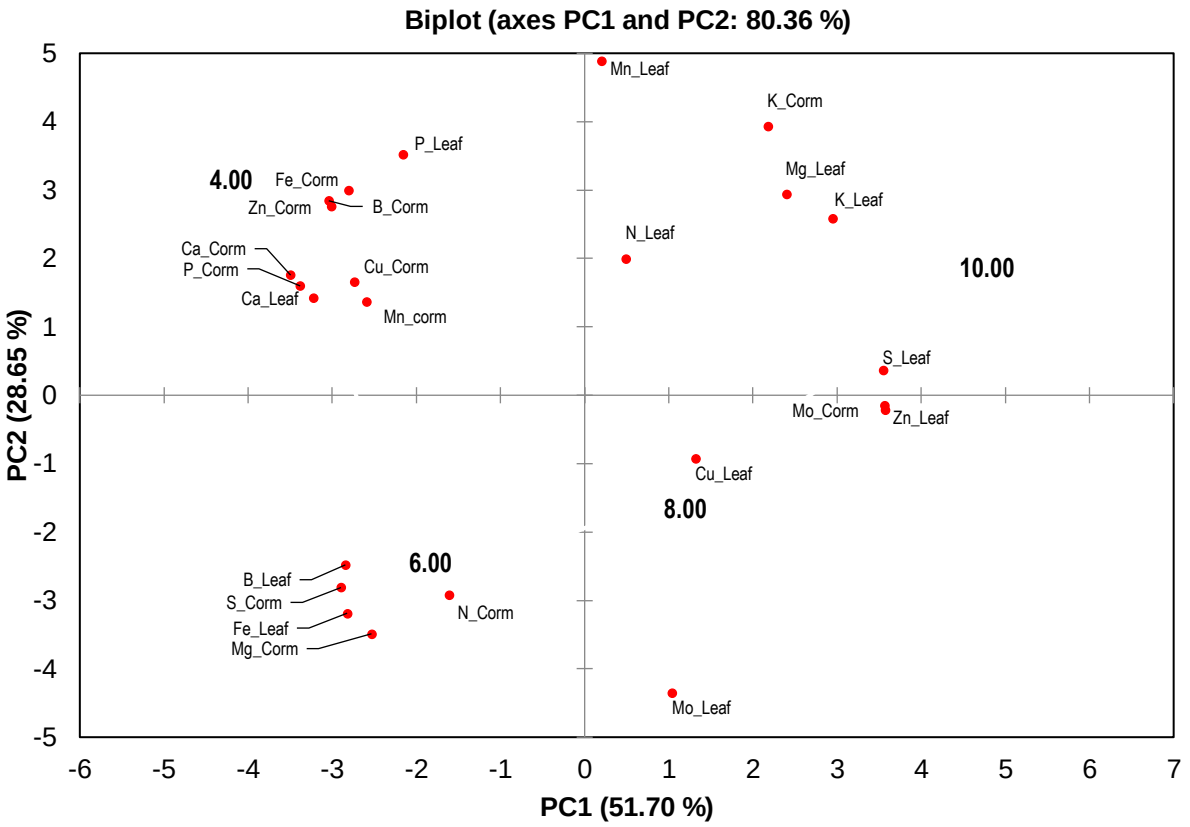

Figure 4.1 Rotated principal component loadings of the mineral accumulation in the leaf and the corm of African potato plants due to potassium levels.

The biplot shows the classification of minerals affected by K levels in the nutrient solution. The two principal components (PC) explained the relationships that accounted for 80.36 of the total variation, PC1 accounted for (51.70%) whilst PC2 accounted for (28.65%). Potassium level of 4.00 meq L^{-1} clustered corm B, Fe, Zn, Ca, Cu, and Mn as well as leaf P and Ca together in one cluster. More mineral accumulation in the corm at this low K level 4.00 meq L^{-1} is a good sign that this level is ideal for growing African potato since the corm is the plant part widely used for medicine. Concentration of 6.00 meq L^{-1} K affected accumulation of corm N, S and Mg besides leaf B and Fe which are grouped in one cluster. Potassium level of 8.00 meq L^{-1} clustered corm Mo and leaf Zn, Cu and Mo whilst K level of 10.00 meq L^{-1} grouped corm K and leaf S, N, K, Mg and Mn together. These clustering reveal the effects of K concentrations on the minerals and their accumulation in African potato.

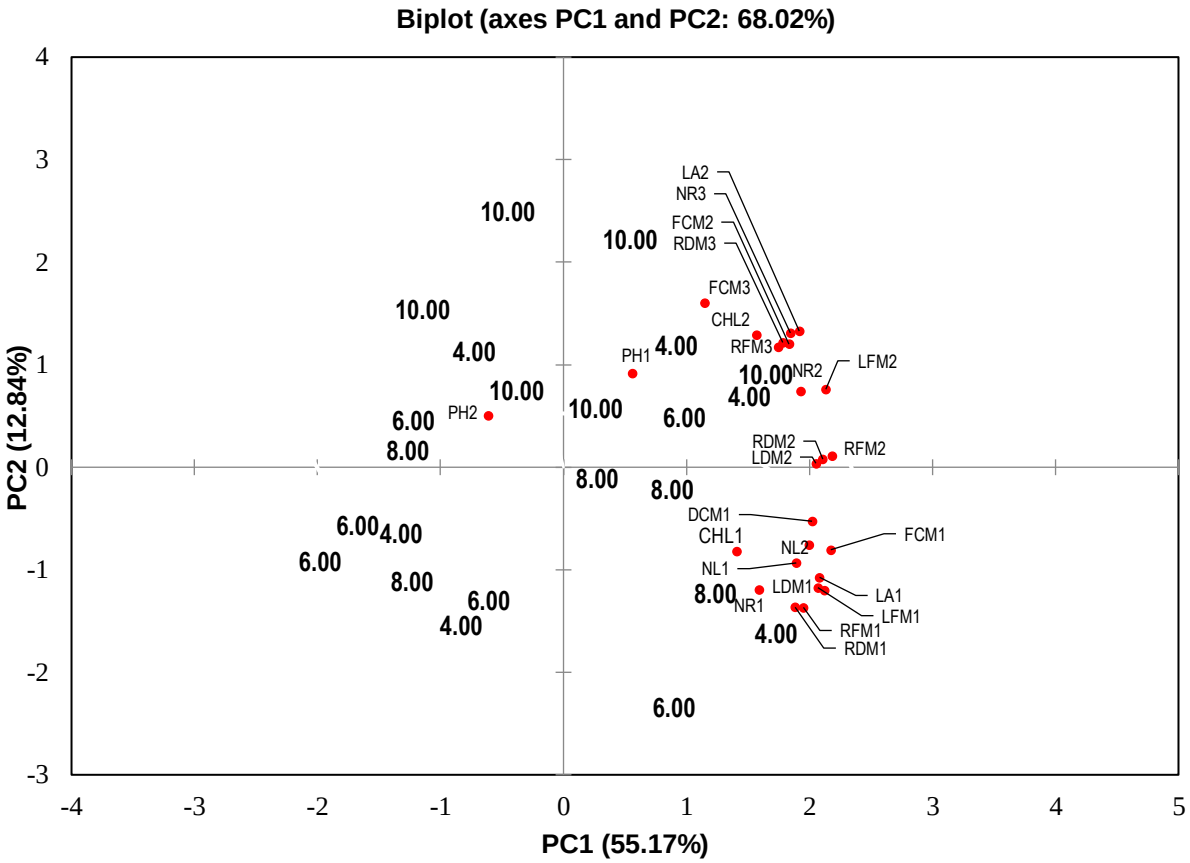

Figure 4.2 Rotated principal component loadings of the agronomic attributes of African potato plants due to potassium levels.

Note: Chlorophyll content is denoted by CHL; Leaf area (cm^2) is denoted by LA; Plant height (cm) is demoted by PH; Fresh mass of corms (g) denoted by FCM; dry mass of the corms (g) denoted DCM; number of roots denoted by NR; root fresh mass (g) denoted by RFM; root dry mass (g) denoted by RDM; number of leaves per plant denoted by NL; Leaf fresh mass (g) denoted by LFM; Leaf dry mass (g) denoted by LDM. 1, 2 and 3 represent the first, second and third data collection respectively.

The PCA showed positive effects of the K levels on the agronomic attributes. Most measured agronomic attributes were loaded positively on PC1 which accounted for 55.17% except PH2 whilst PC2 accounted for 12.84% of the variation. The PC1 and PC2 totalled 68.02%. Most agronomic attributes were positively loaded on K levels of 10.00, 8.00 and 4.00 meq L^{-1} showing positive effect of these K concentrations on agronomic attributes.

Table 4.4 Summary of the PCA results of agronomic attributes affected by potassium levels.

Agronomic attributes				
Principal component	**PC1**	**PC2**	**PC3**	**PC4**
Eigenvalue	22.620	5.266	3.333	2.069
% Variance	55.171	12.845	8.128	5.047
Cumulative % of total variance	55.171	68.016	76.144	81.191
Factor loadings				
CHL1	0.551	-0.322	0.293	-0.124
CHL2	0.616	0.506	-0.194	-0.199
LA1	0.816	-0.421	0.222	-0.023
LA2	0.751	0.521	-0.038	0.250
PH1	0.219	0.358	0.396	-0.356
PH2	-0.240	0.198	0.753	0.060
FCM1	0.852	-0.315	0.082	-0.284
FCM2	0.719	0.471	0.204	0.098
FCM3	0.449	0.627	-0.230	-0.169
DCM1	0.792	-0.207	0.048	-0.374
NR1	0.624	-0.468	-0.249	0.135
NR2	0.756	0.290	-0.277	0.191
NR3	0.723	0.514	-0.223	-0.243
RFM1	0.765	-0.536	-0.072	-0.155
RFM2	0.857	0.043	-0.358	0.231
RFM3	0.685	0.461	-0.113	-0.327
RDM1	0.738	-0.535	-0.084	-0.245
RDM2	0.827	0.031	-0.361	0.295
RDM3	0.699	0.477	-0.091	-0.346
NL1	0.742	-0.366	0.007	-0.232
NL2	0.783	-0.298	-0.099	0.274
LFM1	0.811	-0.460	0.161	0.085
LFM2	0.836	0.298	0.160	0.255
LDM1	0.832	-0.471	0.120	-0.003
LDM2	0.805	0.015	0.154	0.457

Note: Chlorophyll content is denoted by CHL; Leaf area (cm^2) is denoted by LA; Leaf perimeter (cm) is denoted by PM; Leaf ratio; Plant height (cm) is demoted by PH; Fresh mass of corms (g) denoted by FCM; drymass of the corms (g) denoted DCM; number of roots denoted by NR; root fresh mass (g) denoted by RFM; root dry mass (g) denoted by RDM; number of leaves per plant denoted by NL; Leaf fresh mass (g) denoted by LFM; Leaf dry mass (g) denoted by LDM; 1, 2 and 3 represent the first, second and third data collection respectively.

Table 4.5 Summary of the PCA results of phytochemicals (primary metabolites) affected by potassium levels.

Phytochemical (Primary)				
Principal component	**PC1**	**PC2**	**PC3**	**PC4**
Eigenvalue	4.774	3.268	2.843	2.152
% Variance	21.698	14.854	12.921	9.783
Cumulative % of total variance	21.698	36.552	49.473	59.256
Factor loadings				
Alanine	0.303	0.390	-0.460	0.531
Aspartic_acid	0.422	0.647	-0.097	0.422
Carnitine	-0.117	-0.013	0.124	-0.065
Creatine	0.786	-0.492	0.081	0.061
Creatinine	-0.038	-0.614	0.259	0.125
Cytidine_monophosphate	0.608	0.128	-0.159	-0.452
Cytosine	0.521	-0.577	-0.127	-0.148
Dimethylglycine	0.242	0.048	0.346	0.613
Epinephrine	0.408	-0.218	0.350	-0.173
Glutamic_acid	0.681	0.305	0.190	-0.224
Glycine	-0.065	-0.287	-0.727	-0.037
Guanosine	0.334	0.564	-0.003	-0.257
Histamine	0.017	0.503	-0.343	-0.169
Isocitric_acid	0.626	-0.275	-0.061	0.312
Malic_acid	0.066	0.087	0.743	0.020
Methionine_sulfoxide	0.627	0.501	-0.058	-0.475
Norepinephrine	0.270	-0.163	-0.243	0.516
Phenylalanine	0.731	-0.517	-0.118	0.109
Pyruvic_acid	0.320	0.302	0.638	0.250
Serine	0.753	0.287	0.098	0.164
Threonine	0.571	-0.356	-0.107	-0.407
Uracil	0.187	0.075	-0.711	0.188

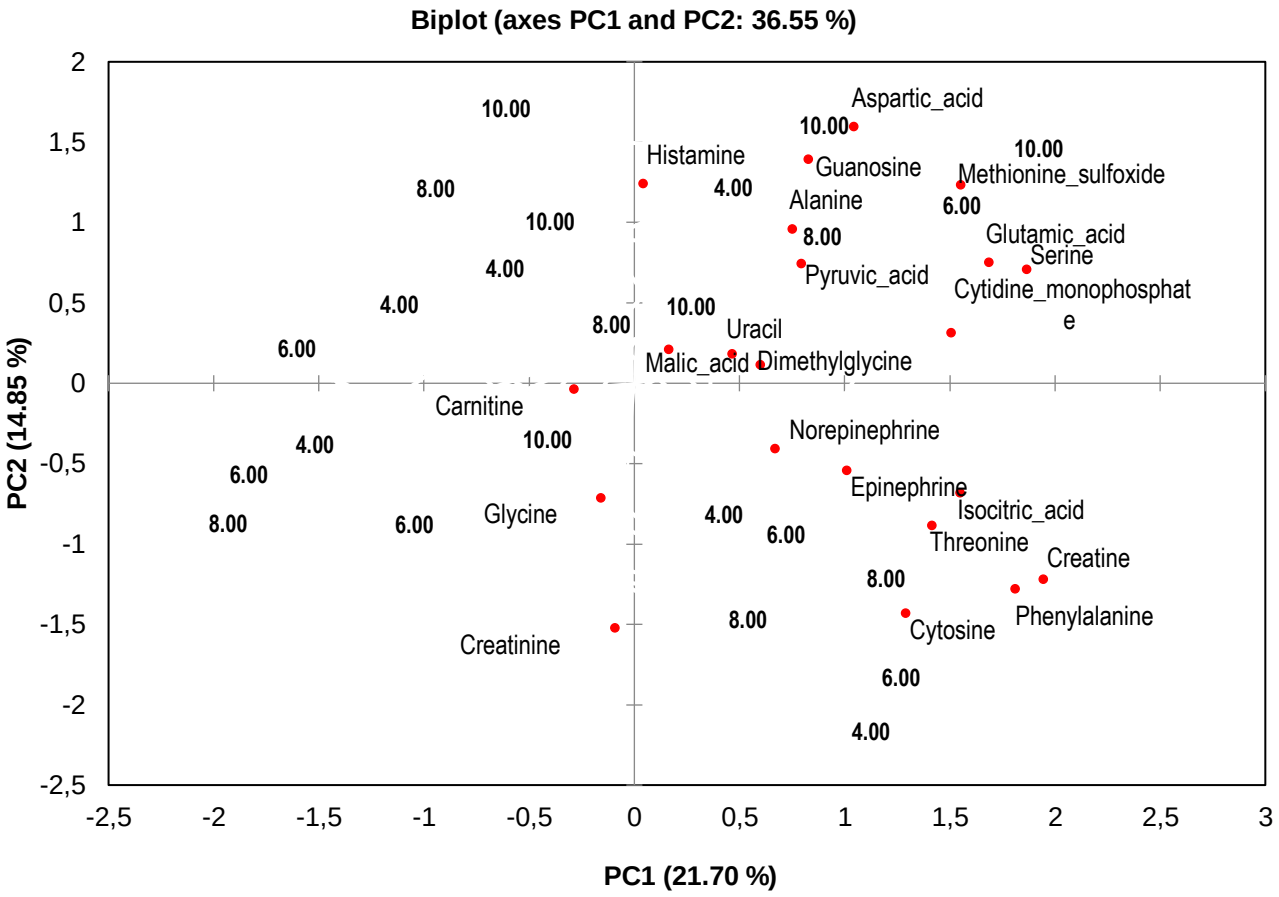

Figure 4.3 Rotated principal component loadings of the primary metabolites of African potato due to potassium levels.

Furthermore, the PCA showed that most of the primary metabolites positively loaded on PC1 except carnitine, glycine and creatine which loaded negatively on both PC1 and PC2. The total percentage of both PCs was only 36.55% with PC1 accounting for 21.70% whilst PC2 has 14.85% of the total variation (Table 4.5 and Figure 4.3).

Aspartic acid, guanosine, histamine, methionine sulfoxide, alanine, glutamic acid, serine, pyruvic acid, cytidine monophosphate, uracil, malic acid and dimethylglycine were class according to level 4.00, 6.00, 8.00 and 10.00 meq L^{-1}. However, norepinephrine, epinephrine, isocitric acid, threonine, creatine, phenylalanine and cytosine were classed according to K level 4.00, 6.00 and 8.00 meq L^{-1}. Carnitine, glycine and creatinine were negatively classed on K level 4.00, 6.00, 8.00 and 10.00 meq L^{-1}. All K levels have some kind of positive effects on primary metabolite synthesis

and since secondary metabolites are biosynthetically produced from primary metabolites (Indrajeet and Rajesh, 2018) the K effects on these primary metabolites could affect secondary metabolite production positively.

4.4 Conclusion

The strong antagonistic and synergistic relationship of K with other elements were revealed through the PCA conducted. Potassium showed positive relationship with S, Mg, Zn, Mn and Cu in the leaf, whilst K association with S, Mg and N was negative in the corm. More minerals were accumulated in the corm at K level of 4.00 meq L^{-1} indicating low K level of 4.00 meq L^{-1} with increased level of Ca and Mg (4.00 K: 7.66 Ca: 2.07 Mg meq L^{-1}) could be adequate for the cultivation of African potato. Positive effects of K levels of 10.00, 8.00 and 4.00 meq L^{-1} were seen on agronomic attributes. All K levels affected most primary metabolites positively which could lead to synthesis of secondary metabolites for the production of good quality African potato plants. This PCA gave more understanding into the effects of K concentration on agronomic attributes, mineral accumulation and primary metabolite synthesis on cultivated African potato plants using nutrient solutions. This research clearly shows that nutrient solutions could be managed effectively in cultivating quality medicinal African potato in a controlled environment. A further secondary metabolite analysis might be ideal for ascertaining the right K level for growing African potato of excellent medicinal quality.

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CHAPTER FIVE

General discussion and recommendations

The increasing demand for African potato necessitated its cultivation to meet demand and to reduce pressure on wild populations. Growing plants with nutrient solutions offer some benefits compared with the conventional methods of farming. Some people think the quality of cultivated medicinal plants might be less compared with wild harvested ones. However, cultivating African potato with nutrient solution in a well managed environment could enhace yield and positively synthesize useful secondary metabolites responsible for medicinal property in these plants.

Potassium (K) is one of the major cations responsible for metabolic activities in plants which can positively influence the production of both primary and secondary metabolites in plants. It also helps plants to survive the exposure to the various biotic and abiotic stresses. Hence the objectives of this study were to determine the effects of different concentrations of K on the agronomic attributes, mineral and the phytochemical contents of African potato (i.e. primary metabolites). The study was conducted at the Bloemfontein campus of the Central University of Technology, Free State. Four levels of K were evaluated at 4.00, 6.00, 8.00 and 10.00 meq L^{-1} of K arranged in a randomised complete block design with six replications.

Results revealed the effects of K levels on the agronomic attributes, mineral accumulation and primary metabolite synthesis. Optimization of agronomic attributes started at 8.00 meq L^{-1} K and heightened at 10.00 meq L^{-1} K though not all parameters were significantly different at these levels. Potassium level of 4.00 meq L^{-1} however showed a positive effect on yield parameters compared with level 6.00 meq L^{-1} despite the low concentration of potassium. Though 10.00 meq L^{-1} of K produced the highest fresh corm mass during the second measurement of growth, this mass was not significantly better than that produced with K level of 4.00 meq L^{-1}. Likewise, root fresh mass and root dry mass produced during the third data collection showed no significant difference at 10.00 and 4.00 meq L^{-1} of potassium. Increasing K level seems to positively affect growth however, low K level encouraged mineral deposition in the

corm, the most widely used African potato plant part for medicine. Potassium levels in the nutrient solution did not significantly affect K accumulation in the leaf and corm of African potato. However, Ca and B were significantly accumulated only in the corm at the lowest K concentration of 4.00 meq L^{-1}. Low K supply with higher Ca and Mg (4.00K: 7.66Ca: 2.07Mg meq L^{-1}) could be favourable for optimal performance of African potato plants.

Multivariate anlysis showed that K levels had strong positive and negative relationship between the agronomic attributes minerals and primary metabolites. Potassium showed positive relationship with S, Mg, Zn, Mn and Cu in the leaf, whilst K association with S, Mg and N was negative in the corm. These complex associations of K with other minerals in African potato could be due to the genetic adaptation mechanisms of African potato to nutrient imbalances in the environment as well as its internal metabolic activities. Several minerals were accumulated in the corm at K level of 4.00 meq L^{-1} indicating low potassium at this level with increased level of Ca and Mg (4.00K: 7.66Ca: 2.07Mg meq L^{-1}) could be adequate for the cultivation of African potato

Positive effects of K levels of 10.00, 8.00 and 4.00 meq L^{-1} were seen on agronomic attributes which could affect yield and plant quality in terms of metabolite accumulation. Most primary metabolites were loaded positively on PC1 with all the nutrient solutions except carnitine, glycine and creatinine. Secondary metabolites are synthesized from primary metabolites in the presence of both biotic and abiotic stresses hence the numerous primary metabolites produced with different K levels could yield useful secondary metabolites of medicinal importance.

From the observations made in this study, it could be concluded that levels of K have both positive and negative effect on growth, mineral content and metabolite yield of African potato. This study therefore offers an idea of effective fertilizer management that will serve as the basis for future studies on the effective use of nutrient solution in cultivating African potato. It could be recommended from the current study that K level of 4.00 meq L^{-1} is ideal for growing African potato. However, further studies should consider verifying more K levels than the four levels used in the current study and could be extended lower than 4.00 meq L^{-1} and higher than 10.00 meq L^{-1} to ensure

the ideal K level in cultivating African potato. Also, the growth period should be extended to about 18-24 months and data collected seasonally to verify any changes in mineral accumulation, growth and metabolite synthesis. Future studies should also include secondary metabolite analysis to know the effects of K levels on secondary metabolite production in African potato to ensure quality medicinal plant production. The effect of the major cations, Ca and Mg should also be studied to know their effects on the growth, mineral accumulation and metabolite production in African potato.

APPENDICES

Appendix 1 Results of the chemical analysis of the feeding water.

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Sec 5.10 F1

Revision 9

Test Report *Case no: 2018 - 369*

Page 1 of 2

Client/Company name:	CUT
Contact person:	Dr Sedibe
Contact number:	0762919119 / 076414 2374 msedibe@cut.ac.za
Postal address:	
Order number:	

Sample information

Sample type:	1 x water samples (chem)
Delivered by:	Patience Akakpo
Date received:	18/04/2018
Reporting date:	07/05/2018
Final report:	07/05/2018

Subcontracted analysis are indicated by * and **non-accredited** analysis by #

Methods are available on request of client.

Statement (HPC) - All counts below 30 and above 300 per plate will also be reported. Counts above 300 are estimated

Disclaimer: For HPC - zero or no counts means ≤10 in 1ml.

Results marked with # in this report, are not included in the SANAS Schedule of Accreditation for this laboratory.

Results marked "Subcontracted Test"in this report are not included in the SANAS Schedule of Accreditation for this laboratory, unless indicated that it was done by a SANAS Accredited laboratory.

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Sec 5.10 F1
Revision 9

Test Report

Case no: 2018 - 369

Determinand	Units	Methods used	South African National Standard (SANS) 241:2006&2015 for drinking water (partial)	Client sample name: Dr Sedibe water sample
				Laboratory number
			Class 1 (Recommended levels) Class 2 (Maximum allowable for limited time) ** EU standard	388-1
			Value	Value
Chemical report				
pH #	pH units	Chem-TM06	5.5 tot 9.7	8.14
Electrical conductivity #	mS/m	Chem-TM06	≤ 170	16.4
Calcium as Ca #	mg/L	Chem-TM02	≤150 - 300	17.8
Magnesium as Mg #	mg/L	Chem-TM02	≤70 - 100	4.5
Sodium as Na #	mg/L	Chem-TM02	≤ 200	12.1
Potassium as K #	mg/L	Chem-TM02	≤50 - 100	2.2
P-Alkalinity #	mg/L	Chem-TM06		0
M-Alkalinity #	mg/L	Chem-TM06		61.7
Fluoride as F #	mg/L	Chem-TM01	≤ 1.5	0.11
Chloride as Cl #	mg/L	Chem-TM01	≤ 300	9.2
Nitrite as N #	mg/L	Chem-TM01		0.01
Bromide as Br #	mg/L	Chem-TM01	**≤3	<0.04
Nitrate as N #	mg/L	Chem-TM01	≤ 11	0.34
Phosphate as PO_4 #	mg/L	Chem-TM01	*≤15.33	<0.1
Sulphate as SO_4 #	mg/L	Chem-TM01	≤ 500	9.0
Calcium Hardness #	mg/L	calculated	≤375 - 750	44
Magnesium Hardness #	mg/L	calculated	≤287 - 410	19
Total Hardness as $CaCO_3$ #	mg/L	calculated	≤662 - 1160	63
Total Dissolved Solids #	mg/L	calculated	≤ 1200	118
Aluminium as Al #	mg/L	Chem-TM02	≤ 0.300	0.023
Arsenic as As #	mg/L	Chem-TM02	≤ 0.010	<0.020
Barium as Ba #	mg/L	Chem-TM02	≤ 0.700	<0.020
Cadmium as Cd #	mg/L	Chem-TM02	≤ 0.003	<0.003
Cobalt as Co #	mg/L	Chem-TM02	≤ 0.500	<0.020
Chromium as Cr #	mg/L	Chem-TM02	≤ 0.050	<0.020
Copper as Cu #	mg/L	Chem-TM02	≤ 2.000	0.026
Iron as Fe #	mg/L	Chem-TM02	≤ 2.000 (chronic health)	0.039
	mg/L	Chem-TM02	≤ 0.300 (aesthetic)	
Manganese as Mn #	mg/L	Chem-TM02	≤ 0.400 (Chronic health)	<0.020
	mg/L	Chem-TM02	≤ 0.100 (Aesthetic)	
Nickel as Ni #	mg/L	Chem-TM02	≤ 0.070	<0.020

Appendix 2 Some pictures of the custom-made irrigation system.

Appendix 3 *Hypoxis hemerocallidea* plant parts and plants at different stages of development.