

Evaluation of Iron (Fe) and Zinc (Zn) concentration among selected potato (*Solanum tuberosum*) genotypes in South Africa

by

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DECLARATION

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I declare that 'Evaluation of Iron (Fe) and Zinc (Zn) concentration among selected potato (*Solanum tuberosum*) genotypes in South Africa' (Master of Science in Agriculture) is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references.



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DEDICATION

This dissertation is dedicated to my mother and my late father, Joyce and Wilson Managa, who raised me in a poor background but made me strive for the best and change the family background. I also sincerely dedicate this study to the God Almighty for His love, protection and guidance until I reached this point in life. Lastly, I want to dedicate this dissertation to my wife Phumudzo Matibe who supported me in everything I went through during the entire study.

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ABSTRACT

Potato is an important source of energy to most micronutrient malnourished affected population in South Africa. Improvements through bio-fortification can therefore enhance access to essential micronutrients. The study was aimed at determining the level of variability of iron and zinc concentration among 20 potato genotypes as a preliminary step for future breeding program. The materials were evaluated using inductively coupled plasma optical emission spectrometry. Statistical analysis indicated significant ($P < 0.001$) variation of Fe and Zn among the genotypes. The average concentration ranges from 34.67 to 76.67 mg kg⁻¹ and 12.88 to 66.1 mg kg⁻¹ for iron and zinc respectively. The best performing genotypes were cultivar Mnandi, Hertha, Buffelspoort and breeding lines-N105-1, 00-S100-33 and 03-627-50. Iron concentration was positively correlated with Zinc concentration. The study showed that enough variability of Fe and Zn concentration exist among the evaluated genotypes, which can be exploited for use in potato bio-fortification breeding programme.

Key words: Bio-fortification, malnutrition, micronutrient, variability, Fe and Zn concentration, genotypes, potato breeding

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GLOSSARY

ANOVA	Analysis of variance
ARC	Agricultural Research Council
ARC-ISCW	Institute for Soil, Climate and Water
ARC-VOPI	Vegetable and Ornamental Plant Institute
CEC	Cation Exchange Capacity
DAFF	Department of Agriculture, Forestry & Fisheries
DMC	Dry Matter Content
DW	Dry Weight
FW	Fresh Weight
FAO	Food and Agriculture Organisation
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
LSD	Least Significant Difference
NIRS	Near-infrared reflectance spectroscopy
PSA	Potato South Africa
TY	Tuber Yield
WHO	World Health Organisation

CHAPTER 1

1.0 INTRODUCTION

1.1 General background

Micronutrient malnutrition particularly Fe and Zn continues to be a matter of serious concern in the world (WHO & FAO, 2006; Bouis & Welch, 2010). According to FAO, WFP & IFAD (2012), an estimated 14.3% of the people in the developing world are nutritionally insecure.

Zinc plays an indispensable role as a key component of a host of enzymes that are crucial for optimal metabolism and body function (Prasad, 2007). According to Caulfield and Black (2004), the global prevalence of zinc deficiency in humans is estimated to be 31% (range is 4%–73%) with a high prevalence of (37%–62%) found in Southern and Central African regions. Zinc deficiency have been reported to undermine cognitive development in children through alteration in attention, activity and other aspects of neuropsychological function (Black, 1998). Prasad (2007) also reported zinc as an anti-inflammatory and antioxidant agent and that it also functions in cell-mediated immune processes. Zinc deficiency causes stunted growth in children (Brown, Wuehler & Peerson, 2001), as well as morbidity from diarrhoea, pneumonia and malaria (Shankar, 2000). In childhood dietary, zinc ensures optimal physical growth as well as neuro-behavioural and brain development (Gibson, 2006).

On the other hand, during childhood and adolescence, Iron deficiency impairs mental development and learning capacity. In adults, it reduces the ability to do physical labour (WHO, 2008). Iron deficiency can cause severe anaemia, which increases the risk of women dying in child birth. Iron deficiency in children can lead to increased susceptibility to infections, increased fragility, poor physical growth, decreased

appetite, reduced mental performance, retardation of cognitive and psychomotor development and congestive cardiac failure (Haas & Brownlie, 2001).

In South Africa, Zn deficiency is prevalent in poor peri-urban informal settlements with 46 percent of children having less than the recommended daily intake of 70 µg/dL (Samuel *et al.*, 2010). This was attributed to low consumption of food with high bioavailability of zinc, which invariably is a direct consequence of poverty and food insecurity. Zinc deficiency is therefore, a veritable public health concern.

Iron deficiency is the most common micronutrient deficiency in the world. However, information on global data for iron deficiency has not been well documented and as such, anaemia is normally used as an indirect indicator. Globally, anemia affects more than 1.6 billion people, or approximately 25% of the population (ACC/SCN & IFPRI, 2000). In developing countries, approximately 50% of anaemia in the population is thought to be due to iron-deficiency, but the proportion may vary among population groups and in different areas according to local conditions (WHO, 2008).

It is estimated that one out of every two preschool children and pregnant women in developing countries are iron deficient and the highest proportion of individuals affected is in Africa (48 to 68%), with the problem evidently linked to poverty. In South Africa, 21% of children aged between 6 and 71 months were found to be anaemic and that iron deficiency was present in 10% of these children (Labadarios & Van MiddelKoop, 1995).

In less-resourced and poor countries, consumption of commercially fortified foods is minimal (Allen, 2002). This may be due to many factors but not limited to the significant recurrent costs associated with supplementation and industrial food fortification, which

are usually priced beyond the reach of many households (Trowbridge & Martorell, 2002; Bouis & Welch, 2010; Samuel *et al.*, 2010). Achieving dietary adequacy of iron and zinc is therefore a challenge in developing countries including South Africa, particularly in resource-poor communities. There is therefore heavy reliance on cheaper, plant-based diets which are not only poor in micronutrient content, but may also be hindering their bioavailability (Gibson, 1994).

Consequently, the breeding of staple food crops for increased minerals and vitamins (bio-fortification); has recently emerged as upcoming sustainable and cost effective strategy to complement existing nutritional approaches (Bonierbale *et al.*, 2011; Horton, 2006). Improving the nutritional value of food crops that are already consumed by those who are vulnerable to micronutrient malnutrition is a very good strategy to combat micronutrient deficiency.

Potato (*Solanum tuberosum* L.) in terms of consumption is the third most important staple food crop, worldwide and 35% of the total world yield is produced in developing countries, where the crop is regarded as a staple food in the diet of about half a billion people (FAOSTAT, 2013). Since potato is already an important source of energy to resource-limited individuals in developing countries, improvements of the crop through bio-fortification will enhance the availability of essential micronutrients (Mayer, Pfeiffer & Beyer, 2008). Potato crop is also associated with heritable variation for micronutrient concentration. It has low concentration of phytate, and high vitamin C, which makes it a promising crop for bio-fortification (Burgos *et al.*, 2007).

The bioavailability of minerals in potato is also reported to be greater than in cereals and legumes due to the presence of high level of ascorbic acid, which is considered

as a promoter of mineral absorption and low levels of phytatic acid, an inhibitor of mineral absorption (Burgos *et al.*, 2007:668; Bonierbale *et al.*, 2011; Fairweather-Tait, 1983). Continuous flow of new genes and allelic diversity into the commercial gene pool is required when breeding for quality traits in potato. Identification of crop varieties that are naturally high in desired nutrient concentrations is the initial stage for bio-fortification (Graham *et al.*, 2001).

1.2 Objectives of the study

Broad objectives

The main objective of this study was to evaluate 20 selected genotypes, consisting of 15 elite breeding lines and 5 commercially grown cultivars, for minerals and tuber yield traits, as a preliminary step for potato bio-fortification breeding program.

Specific objectives

- (i) To determine the variability of iron and zinc concentration in 20 selected ARC potato genotypes in South Africa
- (ii) To evaluate the genotypes for tuber yield
- (iii) To determine the dry matter content of the 20 selected genotypes in South Africa
- (iv) To determine the correlation between mineral nutrients and tuber yield traits among the genotypes
- (v) To recommend genotypes to be used for biofortification breeding programme in South Africa

1.3 Justification of the study

Prior to this study, the level of Fe and Zn concentrations of potato genotypes in the genebank collections of the Agricultural Research Council (ARC), Pretoria, South Africa was unknown. Hence, it was useful to determine the level of Fe and Zn of those genotypes in order to select parent lines for potato nutritional improvement through plant breeding (Biofortification).

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Introduction

This chapter reviews the literature on various aspects of potato micronutrient concentration. New cultivars of tuber crops, including potato have been developed through crop improvement or plant breeding. One of the key pre-requisites for undertaking cultivar improvement is the selection of appropriate parental cultivars with desirable attributes. Initially, the parental sources are crossed with each other (hybridisation) in order to create recombinant progenies from which the new cultivar can be selected (Acquaah, 2007; Paget *et al.*, 2014). Thus screening of potential parental sources (or crop germplasm) is very useful in crop improvement.

Specifically to nutritional enhancement, selection of micronutrient-dense breeding lines among existing varieties is the first approach (Graham *et al.*, 2001). Subsequently, the selected lines must be combined with high yields and high profitability cultivars (Bouis *et al.*, 2009). Breeding of staple food crops for micronutrient density (biofortification) gained legitimacy when micronutrient deficiencies were recognised as global public health challenge of the 21st century (Pfeiffer, 2010). Currently, biofortification is making good progress and regarded as the most economical and feasible approach to alleviate hidden hunger (Chugh & Dhaliwal, 2013).

2.2 The potato crop

2.2.1 Origin and distribution of potato

Potato (*Solanum tuberosum* L.) was first domesticated in the region of modern-day southern Peru and extreme north western Bolivia between 5000 and 8000 BCE (Spooner *et al.*, 2005). It has since spread around the world and has become a staple crop in many countries. It has been suggested that the introduction of potato was responsible for a quarter of the growth in old world population and urbanisation between 1700 and 1900 (Nunn & Quian, 2011).

The potato is the third most important staple food crop after wheat and rice (www.faostat.fao.org, accessed 15 March 2014). It belongs to the family Solanaceae, genus *Solanum*, subgenus *brevicaule*. The *S. tuberosum* is the cultivated potato while *S. brevicaule* is its wild progenitor. Cultivated potato, *Solanum tuberosum* L., is a highly heterozygous tetraploid ($4x = 48$).

The world potato sector has shown a remarkable growth in the past two decades. Until the early 1990s, most potato were grown and consumed in Europe, North America and countries of former Soviet Union. Since then, there has been a dramatic increase in potato production and demand in Asia, Africa and Latin America, where output rose from less than 30 million tonnes (mln t) in the early 1960's to more than 165 mln t in 2007 (FAOSTAT, 2008). In the year 2005, potato production in the developing world exceeded that of the developed world (FAOSTAT, 2008). The world production of potato in 2010 was about 324 million tonnes harvested from 18.6 million hectares with the average world farm yield of 17.4 t ha^{-1} (FAOSTAT, 2011). Currently, the leading producers of potatoes in the world is Asia, followed by Europe (Table 2.1).

Table 2.1 World potato production (tonnes), 2009-2013

	2009	2010	2011	2012	2013
Total Africa	22358707	25527484	27273585	29253748	29731211
Total Americas	40873769	39526018	41506096	43681339	42575317
Total Asia	145836731	159073744	175364623	174086197	180586309
Total Europe	123938512	107684233	129343452	116502463	113023347
Total Oceania	1726742	1805772	1661625	1841620	1836830
Total World	334734461	333617251	375149381	365365367	367753014

(After FAOSTAT, 2014)

2.2.2 Potato production in South Africa

In Africa, the potato arrived late around the turn of the 20th century. In recent decades the production has been in continual expansion, rising from 2 million tonnes in 1960 to a record 16.7 million tonnes in 2007. Currently, potato is cultivated on more than 1.87 million hectares in Africa, producing a total annual crop tuber of about 19.5 million tonnes (du Plessis & van Zyl, 2012). In Africa, potato is produced under a wide range of conditions, which is ranging from irrigated commercial farms in Egypt, South Africa and intensive cultivation in the tropical highland zones of eastern and central Africa, where it is mainly grown by small-holder farmers.

Potato production in South Africa, has grown strongly within 15 years from 1.2 million tonnes in 1990 to a record 1.97 million tonnes in 2007 (FAOSTAT, 2009). Almost 56 000 ha of farm land were planted with potato by more than 2000 producers in 1993, with an average of 27 ha planted per producer (du Plessis & van Zyl, 2012). However, during the same period, the potato farming area actually declined from 63 000 ha to 58 000 ha (FAOSTAT, 2008). In 2011, less than 700 producers planted potato on 52 563 ha (du Plessis & van Zyl, 2012).

The potato in South Africa is produced in all the nine provinces, with the highest production emanating from commercial growers in the Limpopo and Free State provinces. The average yield is about 34 t ha⁻¹ under irrigated farming system (PSA, 2011). South Africa occupies 28th position on the list of world potato producing countries, contributing about 0.6 % of the world's total production on 0.3 % of the country's total surface area (du Plessis & van Zyl, 2012).

In terms of its volume and value of production, the potato is topping all vegetables in South Africa. Its gross value accounts for about 43% of all the major vegetables, 15% of horticultural products and 4% of total agricultural production (DAFF, 2013). The potato industry growth has significantly contributed towards food security and the improvement of the South African economy. The monetary value has grown from R1.3 billion in 1996 to R5.4 billion in 2010, representing a positive growth of 307% (Lekgau & Jooste, 2012). However, South Africa still lag behind in terms of per capita consumption of potato compared to most countries outside the African continent. The per capita consumption of potato in South Africa for example is 38 kg and is much less when compared to that of the British, which is 105 kg (du Plessis and & Zyl, 2012).

There are several constraints that may lead to low level of potato production. These include various biotic and abiotic factors, including heavy frosts, which damages the haulm. Even cold weather makes potato more susceptible to bruising and possibly rotting, which can hugely damage tubers during storage (Kleinkopf & Olsen, 2003). In South Africa, the growth in potato production over the past two decades has been increased by the availability of improved cultivars from the local breeding programme as well as the introduction of foreign cultivars. These cultivars were selected based on their adaptability to South African conditions, improved yields, resistant to disease and

suitability to niche markets, such as the processing industry (Visser, 2012). However, there has been little interest in breeding for genotypes having high nutritional value.

2.2.3 Uses of potato

The potato is used for a variety of purposes apart from being a vegetable cooked or boiled at home for consumption. It is probable that less than 50 % of potatoes grown worldwide are consumed boiled. The rest are processed into potato confectionaries and food ingredients. In many countries including South Africa, part of potato tubers is processed into feed for cattle, pig and poultry. It is also processed into starch for industrial use. Potato is a good source of dietary energy and some important micronutrient minerals such as iron and zinc (Brown, 2005).

2.2.4 Potato genetic characteristics

There are more than 4000 different wild potato genotypes collected at the International Potato Centre in Lima (Peru), indicating huge genetic diversity of the crop. The diversity of genotypes are with regards to yield characteristics, climatic adaptability, pest and disease tolerance ability, as well as quality and nutritional contents (Andre *et al.*, 2007; Burgos *et al.*, 2007; Paget *et al.*, 2014). The existence of genetic variability in the foregoing characteristics pointed out, provides an opportunity to make the breeding or improvement of the potato crop feasible. Precisely, sufficient genetic variability in micronutrients concentration revealed among potato genotypes; has been thought to encourage biofortification of potato to complement the existing nutritional approach (Bonierbale *et al.*, 2011; Burgos *et al.*, 2007; Fairweather-Tait *et al.*, 2011; Paget *et al.*, 2014).

2.3 Potato nutritional content

About 65 to 80 % of the dry matter content of potato tuber is starch (Pedreschi, 2009). The starch packed into starch granules are in the form of amylose and amylopectin (Camire *et al.*, 2009). On a dry matter basis, the protein content of potato is comparable to that of cereals, but it is very high as compared to other roots and tubers (Storey, 2007). Potato is not typically considered as a good dietary protein source. However, its protein quality is to a considerable level. When compared with cereal crops, potato contains more lysine and less sulphur-containing amino acids methionine and cysteine (Camire *et al.*, 2009). It has also been reported that potato produce more protein per unit than any other crop except soya bean (Dale & Mackay, 1994).

Normally, potato tubers have a mineral content of 1.1%, with potassium being the most abundant, then phosphorus, chloride, sulphur, magnesium, iron and zinc being available in moderate quantities (Dale & Mackay, 1994; Camire *et al.*, 2009). The potato is a modest source of iron in human diet, and with just 150 g portion, it may supply 6% of the recommended daily allowance of iron (United State Food & Drug Administration, 2006). Potato is also very important for its high vitamin C content together with other vitamins such as B1, B3, and B6. Vitamin C (or ascorbic acid) also plays an important role as an enhancer of micronutrient bioavailability in the diet (Pfeiffer, 2010).

Fresh potato along with its skin is one of a good source of antioxidant and vitamin C. With just 100 g of fresh tuber, potato can provide 11.4 mg or 20% of daily required levels of vitamin C (United State Food & Drug Administration, 2006). The reported ranges of iron and zinc concentration indicate ample genetic diversity that might be

explored in breeding programs seeking to increase the level of these minerals in the human diet (Andre *et al.*, 2007; Burgos *et al.*, 2007; Ekin, 2011).

2.4 Solubility and accumulation of minerals in potato edible tissues

Plant crops mostly acquire the nutrient from the soil. However, the nutrients must be dissolved in the soil solution before they become available for plant use. The mineral element can be present in the soil as free ions, or ions adsorbed onto mineral or organic surfaces, as dissolved compounds or precipitates (White & Broadley, 2009). Plants have evolved two strategies to take up minerals from the soil. The dicotyledonous and monocotyledonous plants activate a reduction-based strategy when starved for mineral, whereas the grasses activate a chelation-based one (Curie & Briat, 2003; Grotz & Guerinot, 2006; Schmidt, 2003).

Therefore, the most important soil properties such as soil pH, redox conditions, cation exchange capacity, microbial activity, organic matter and water content, play an important role in regulating the availability of these minerals for the crop accumulation (Frossard *et al.*, 2000; Shuman, 1998). The supply and phytoavailability of mineral element in the rhizosphere solution, ultimately limits the accumulation of mineral elements by crops, unless foliar fertilisers are applied (White & Broadley, 2009). From the plant breeding point of view, it is necessary to understand the process and patterns of the accumulation of minerals in edible organs, in order to develop appropriate breeding strategies to enhance the level of desirable minerals in food crops (Subramanian *et al.*, 2011).

With regard to potato or other tuber crops, Subramanian *et al.* (2011) pointed out that the pattern of accumulation in tubers for each mineral depend on an interacting set of

factors. Such factors were found to include the developmental anatomy of the tubers, phloem and xylem loading and unloading, movement across the periderm and mechanism for transport and sequestration within the tuber (Welch & Graham, 2005; Subramanian *et al.*, 2011; Welch & Graham, 2004).

The high concentration of minerals, such as Fe, Zn and Ca occurs in many soils (White & Broadley, 2009). However, as pointed out earlier, the phytoavailability of these mineral elements often restricted by soil properties, in which they predetermine both genetic and agronomic strategies for their effective utilization. Lindsay (1991) suggested that the solubility of iron in most aerobic soils is largely controlled by various Fe (III) oxides, including amorphous forms, maghemite, and ferrihydrite of iron oxidation process that takes place during soil formation under aerobic conditions (Welch & Graham, 1996). On the other hand, the solubility of zinc in soil is highly dependent on the soil solution pH (Grotz & Guerinot, 2006; White *et al.*, 2002; White & Broadley, 2009).

The increase of mineral concentrations without loss of yield can be achieved in edible tissues, but it will depend on increased uptake by roots or leaves, effective redistribution within the plant to the edible portion, and accumulation in edible tissues in a nontoxic form (Welch & Graham, 2005). Genetically, modifying plants in ways that will increase the density of more micronutrient concentrations in edible portions of grain or tuber requires that several barriers to minerals accumulation within the plant be overcome (Welch, 1995; Welch, 1999). These barriers are thought to be the result of tightly controlled homeostatic mechanism that regulate mineral absorption, translocation and redistribution in plant allowing adequate, but nontoxic levels of these nutrients to accumulate in plant tissue (Welch & Graham, 2004). For in-depth

information about these barriers and more on mineral solubility, the readers are referred to the following references (Welch, 1999; Yang & Römheld, 1999).

2.5 Nutritional improvement of potato crop

Since commercial fortification and supplementation may not be sustainable for providing essential micronutrients to many developing countries due to socio-economic constraints; hence, biofortification through breeding for increased mineral and vitamin content in staple food crops may become sustainable upcoming strategy to combat micronutrient deficiencies (Shahriari *et al.*, 2013). The failure of existing nutritional approach is mostly associated with the significant recurrent costs, especially in poor and less-resource countries. Biofortified foods cannot deliver as high as the level of minerals and vitamins per day as compared to supplements or industrially fortified foods (Saltzman *et al.*, 2013). However, it may complement existing interventions to sustainably provide micronutrients to the most vulnerable people in a comparatively inexpensive and cost-effective way (Pfeiffer & McClafferty, 2007; Nestel *et al.*, 2006).

2.5.1 Biofortification in crop improvement

Biofortification is a scientific method of breeding for increasing the nutritional value of food crops already consumed by those suffering from hidden hunger (Bouis *et al.*, 2011). It can be carried out either through conventional selective plant breeding, or through genetic engineering, by means of biotechnology techniques (Paget *et al.*, 2014; Bouis & Welch, 2010). Both traditional plant breeding and biotechnology-based techniques are needed to produce plants with the desired quality traits.

Biofortification relies on the plant's biosynthetic (vitamins) or physiological (minerals) capacity to produce or to accumulate the desired nutrients (Mayer, Pfeiffer & Beyer, 2008). This is an approach that focuses on elevating the concentration of nutrients in edible parts of staple crops (Welch & Graham, 2002). Biofortification differs from ordinary fortification because it focuses on making plant foods more nutritious as the plants are growing, rather than having nutrients added to the foods when they are being processed (Bailey, 2008). Nutrient biofortification of food crops may not only include elevated mineral and amino acid levels, but also enhanced antioxidant levels (Diretto *et al.*, 2007; Rommens *et al.*, 2008). Bouis and Islam (2011) emphasised the biofortification as an improvement on ordinary fortification when it comes to providing nutrients for the rural poor, who rarely have access to commercially fortified foods.

Although, genetic variability in micronutrient-dense trait has been documented among available potato varieties (Andre *et al.*, 2007; Burgos *et al.*, 2007), it is therefore not only the abundance of the nutrients that need to be considered. The heritability to which those variability transferred from parents to progenies, also determines the efficiency of crop biofortification (Bisognin *et al.*, 2010; Brown *et al.*, 2010; Paget *et al.*, 2014). Lastly, biofortification become sustainable when the sufficient nutrient are retained during processing and cooking.

Therefore, the need for nutritionists to work together with breeders in establishing nutritional breeding targets taking into consideration the average food intake and habitual food consumption patterns of target population group is imperative (Saltzman *et al.*, 2013). Furthermore, the assessment of nutrient losses during storage and processing together with nutrient bioavailability is also imperative (Hotz & McClafferty, 2007).

2.5.1.1 Micronutrient-dense trait heritability

In general, heritability could be described as the proportion of observed differences on a trait among individuals of population that is due to genetic differences. Heritability thus analyses the relative contributions of differences in genetic and non-genetic factors to the total phenotypic variance in a population (Brown *et al.*, 2014). It measures the fraction of phenotypic variability that can be attributed to genetic variation. It has been suggested that micronutrients-dense trait in some genotypes of food crops including potato is genetically controlled (Brown *et al.*, 2010). This also includes other potato tuber traits, such as tuber shape and fresh weight (Bisognin *et al.*, 2012). Genetically controlled traits become unreliable on environmental factors and to be stable across the environments (Paget *et al.*, 2014).

However, significant genotype x environment interaction and low broad sense heritability has also been observed for iron and zinc variation among some potato genotypes (Brown *et al.*, 2010; Burgos *et al.*, 2007; Salas *et al.*, 2012). This suggested that their mineral contents will be less heritable across environments. Thus, the important step in breeding for enhanced concentration should be testing for the stability of iron and zinc accumulation across selection processes and target environments or a better understanding of environmental and management factors that influence the traits (Salas *et al.*, 2012).

Plant breeders' high estimate of trait heritability is ideal, because it means that the tendency of that trait being passed from parent to progeny is high (Paget *et al.*, 2014). Plant traits or attributes are estimated either as broad sense or narrow sense heritability. Estimation of both broad and narrow sense heritability shows how much of the genetic variation is due to additive or non-additive genetic effects (Brown *et al.*,

2010). For nutritional improvement, Paget *et al.* (2014) pointed that exploiting additive gene effects particularly in diploid population will result in the genetic improvement of important micronutrients in potato tubers. In addition, predominates of narrow sense or additive genetic variance, then genetic gains in breeding for micronutrients would be very rapid.

This may be due to the suggestion that the mechanisms controlling the uptake, transport and loading of micronutrients in potato is additive (Graham *et al.*, 1999).

Though, if non-additive genetic variance predominates, the high broad-sense heritability also suggests that varieties with high mineral concentrations will remain high in different environment (Brown *et al.*, 2010). The uses of heritability enable the plant breeders to decide which method should be used for selection, and to decide minimum population required for selection as well as to know response of various traits to selection (Bisognin *et al.*, 2002). Apart from being one of most important food crops worldwide, potato, its heritable genetic variability for micronutrient concentration, low concentration of phytate and high ascorbic acid, makes it a promising crop for biofortification (Bonierbale *et al.*, 2011; Paget *et al.*, 2014).

2.5.1.2 Breeding strategies for biofortification

Micronutrient-enriched food crops can be developed either through traditional plant breeding methods or through molecular biological techniques (Bouis, 2000; Combs *et al.*, 1996). Using selective plant breeding for biofortification, plant breeders search seed or germplasm banks for existing varieties and accessions of crops, which are naturally high in specific desired nutrients. Subsequently, they cross breed these high-nutrient varieties with high-yielding varieties of crops, to provide a seed with high yields and increased nutritional value (Welch, 2001; Saltzman *et al.*, 2013). This method is

prevalent at present, as it is cheaper and was believed to be less controversial than genetic engineering crops by McClafferty and Islam (2008).

Breeding can be made more cost-effective using marker-assisted selection to breed high levels of several minerals and vitamins in a single variety and transgenic methods may prove to be more effective in accomplishing this than conventional breeding (Slater *et al.*, 2008). Transgenic approaches are more advantageous when the micronutrient concentrations does not naturally exist in a crop (for example, provitamin A in rice) or when sufficient amounts of bioavailable micronutrients cannot be effectively bred into the crop (Saltzman *et al.* 2013; Lemaux, 2008). This approach is a key for improving the phytoavailability of mineral elements in the soil, their uptake from rhizosphere, translocation to the shoot and accumulation in edible plant tissues (Davies, 2007; Puig *et al.*, 2007; Zhu *et al.*, 2007). Moreover, transgenic approaches may also be beneficial in reducing the concentrations of anti-nutrients and increase the concentration of promoting substances (White & Broadley, 2009).

In case of vegetatively propagated varieties (such as cassava and potato), conventional breeding has also thought to be difficult due to the scarcity of genetically well-defined breeding lines (Shahriari *et al.*, 2013). Additionally, conventional breeding can change important traits of crops desired by consumers, such as taste (Shahriari *et al.*, 2013). Nevertheless, according to Saltzman *et al.* (2013), several years of conventional breeding is needed after transgenic lines are obtained. This is to ensure that the transgenes are stably inherited and to incorporate the transgenic line into varieties that farmers prefer. Otherwise, plant breeders can use both conventional plant breeding and transgenic methods to reach their breeding targets (Bouis & Welch, 2010).

Graham *et al.* (2001) indicated that “potential is much obviously greater for nutritional enhancement within the breeding programme by deliberate use of nutrient-dense parents followed by selection in segregating populations”, as compared to the selection among currently available varieties which were pointed out in earlier studies (Graham & Welch, 1996). On the other hand, the suggestion that the mechanisms controlling uptake, transport, and loading of micronutrients could be additive (Graham *et al.*, 1999); indicated that emphasis should be placed on an approach of population improvement from recurrent selection.

Biofortified crops can be obtained through breeding, provided sufficient genetic variation is present in the diversity spectrum or by exploiting transgressive segregation (Meyer *et al.*, 2008); but in the absence of such variability, then genetic modification may offer a valid alternative, Saltzman *et al.* (2013) suggested. Moreover, development of molecular markers for micronutrient-dense trait may help to facilitate breeding to a great extent. Up to date, continuing improvements in molecular and genomic technologies has proven to be very important in plant breeding towards acceleration of new varieties development.

Most staple food crops including maize, wheat, rice and potato have already been genetically modified with macro-and micronutrient traits, intended to provide health benefits to consumers (Lai & Messing, 2002; McCue *et al.*, 2003). In addition, biofortification through plant breeding intend to provide a feasible means of reaching undernourished populations in remote rural areas. Delivering naturally fortified foods to people with limited access to commercially marketed fortified foods (Chugh & Dhaliwal, 2013).

2.5.1.3 Feasibility and limitations for biofortified crops

Various research works suggests that the potential to increase the micronutrient contents of staple foods by conventional breeding exist (Graham & Welch, 1996; Graham *et al.*, 2001). In some staple crops, micronutrient-concentration traits are also stable across a wide range of growing environments (IFPRI, 2002; Brown *et al.*, 2010). In most crops studies, it is possible to combine the high-micronutrient-density trait with high yield, unlike protein content and yield, which were reported to be negatively correlated (Bártová *et al.*, 2009). Moreover, the genetic control of those traits is simple enough to make breeding to be economic (Nestel *et al.*, 2006). Therefore, it will be possible to improve the content of several limiting micronutrient together, thus pushing populations toward nutritional balance.

The potato could be among staple food crops which may be considered most efficient and effective for iron and zinc biofortification. This has been confirmed by huge variation in these mineral concentrations reported in several research studies (Andre *et al.*, 2007; Burgos *et al.*, 2007; Ekin, 2011; Tekaligne & Hammes, 2005). Studies on the iron and zinc concentrations among the ARC potato breeding lines were yet to be carried out prior to the commencement of this current study.

There are some limitations in the course of screening the variability of micronutrient concentrations for selection of appropriate breeding lines. Salas *et al.* (2012) remarked that heterogeneity of the concentration of iron and zinc in the soil can cause genetic differences among genotypes, thereby preventing the identification of genotypes with genetically superior Fe and Zn contents. It has also been indicated that given the strong genotype x environment interaction (GxE), screening in the course of breeding

for enhanced micronutrient concentrations could be highly unreliable (Salas *et al.*, 2012).

Abebe *et al.* (2012); and Bonierbale *et al.* (2011) affirmed that the concentrations of mineral elements in potato tubers is influenced by both environmental and genetic factors. It thus implies that irregular distributions of minerals nutrient in the soils that can obscure the genetic differences among genotypes can be minimised under controlled environmental experiment, or by conducting multiple trials on different location.

Lastly, a successful biofortification strategy requires widespread adoption of the crops by farmers and consumers and this presents several important challenges (Powell, 2007). Public acceptance is also essential, especially if the new trait perceptibly change perceptibly the qualities of the crop, such as colour (like in Golden Rice), taste, and dry matter content (Shahriari *et al.*, 2013). Adequate information programs will play an essential role in ensuring acceptance. Wide dissemination of the technology, a requisite for success, also relies on good market networks and channels for the dissemination of agricultural information.

The lack of agricultural infrastructure in some developing countries, especially in Africa, might be a significant challenge for adoption of new biofortified varieties.

2.5.1.4 Benefits and impact of biofortification

Biofortification can be regarded as a cost effective and sustainable strategy of malnutrition management (Horton, 2006; Bonierbale *et al.*, 2011). The introduction of biofortified crop varieties bred for increased mineral and vitamin contents would complement existing nutrition approaches by offering a sustainable and low-cost way

to reach people with poor access to formal markets and/or health care systems (Bouis & Welch, 2010; Saltzman *et al.*, 2013; Welch & Graham, 2004)

Recent research has proven that added micronutrients have a measurable impact on human micronutrient status (Hass *et al.*, 2005; van Jaarsveld *et al.*, 2005; Low *et al.*, 2007). This assessment was done on some mandate crops of Harvest-Plus biofortification programme, such as sweet potato, maize, cassava, bean, for the enhancement of iron, zinc, and provitamin A (Pfeiffer, 2010). Biofortification can potentially provide ongoing benefits throughout the developing world at a fraction of the recurring cost of either supplementation or post-production fortification throughout the developing world (Nestel *et al.*, 2006). Given that, with just one investment in the research and development of germplasm, multiple flow benefits can be obtained by disseminating these new varieties in other regions and countries. Moreover, farmers can multiply the seed for use in the next production cycle, making this a potentially sustainable alternative in the long term (Stein *et al.*, 2005).

Biofortified seeds are also likely to have an indirect impact in agriculture, as a higher trace mineral content in seeds, confers better protection against pests, diseases and environmental stresses. Hence, results in increasing crop productivity, especially when these seeds are sown in micronutrient deficient soils (Welch & Graham, 2004; Welch, 1999). In addition, breeding crops with an increasing ability to acquire and accumulate minerals in their edible portion, will complement the idea of increasing the concentrations of essential mineral elements in produce through the application of mineral fertiliser (White & Broadley, 2009); thereby reduce the cost of production especially for smallholder growers. Biofortification is not a solution in itself but a very important complement to dietary variety and to supplementation. High mineral and

vitamin densities could be bred into the edible portions of staple foods while maintaining high yield, resistance to pests and diseases and other desirable agronomic traits (Saltzman *et al.*, 2013; Gregorio, 2002). Without desirable agronomic traits, farmers will not adopt biofortified staple crops; each variety released must be at least competitive with what is available in the market.

Research studies reveal that the level of anti-nutrients and the reduction of food processing tend to be lower for biofortified food with relatively higher micronutrient density compared to non-biofortified varieties (La Frano *et al.*, 2014). Thus, evidence is encouraging to the effort to breed plants with increased micronutrient concentrations in order to decrease the influence of inhibitors and offset losses from processing, which results in higher total absorption rates. These have much to do with the nutrients bioavailability which is described comprehensively in the next section.

2.5.2 Bioavailability of nutrients in diet

Bioavailability can be defined as the efficiency of absorption and utilisation or retention of the nutrients that are present in food (Srini, 2001; Welch, 2002). Researchers such as van Lieshout *et al.* (2001) further alluded to bioavailability, as the amount of the nutrient that is accessible for utilisation in normal physiological functions, metabolism, and storage. Bioavailability can be enhanced or inhibited by the presence of food component and food-processing techniques (La Frano *et al.*, 2014). Even though the concentration of iron and zinc in potato is low as compared to its levels in the cereals and legumes (Storey, 2007), as previously stated above, the bioavailability of these minerals in potato can be greater than in cereals and legumes, and that is due to the presence of high level of ascorbic acid, which is considered as a promoter of mineral

absorption and low levels of phytatic acid, an inhibitor of mineral absorption (Burgos *et al.*, 2007; Bonierbale *et al.*, 2011; Fairweather-Tait *et al.*, 1983).

The amount of micronutrient present in the ready-to-eat portion of the plant and that which is available for absorption, must be assessed. This allows the proper estimation of the minimum micronutrient concentrations that breeders must reach, as well as to predict the ability of these interventions to be successful (La Frano *et al.*, 2014). Bioavailability in terms of the amount of nutrient released from the food matrix and accessible for absorption, usually measured by *in vitro* method such as simulated digestion and dialyzability (van Lieshout *et al.*, 2001).

The Caco-2 cell model, an *in vitro* method, can also measure the amount of nutrient that is taken up the enterocytes. However, its use is limited since it is only considered to provide data on bioavailability if it is coupled with simulated digestion or dialyzability testing (Etcheverry *et al.*, 2012). For further description of these methods to estimate bioavailability, readers are therefore referred to (La Frano *et al.*, 2014; Fairweather-Tait *et al.*, 2005; Failla & Chichumrironchokchai, 2005).

With respect to potato tubers, Burgos *et al.* (2007) assessed iron and zinc retention during processing and found that there were no losses due to cooking. This information is very important in that determination of the micronutrient content on uncooked potatoes may be used directly in comparisons among varieties and calculations of potential impact on the diet. However, cooked potatoes cannot be compared to uncooked one for vitamin C (or ascorbic acid) content; since the changes had been observed during cooking (Burgos *et al.*, 2009). Furthermore, the contamination of soil minerals should be avoided during the processing of potatoes.

For example, iron from the soil is poorly soluble in gastric juices, hence its bioavailability is also expected to be poor (Pfeiffer & McClafferty, 2007).

2.5 Nutritional analyses

Concentration of minerals and vitamins in plant edible parts are usually chemically analysed using one of the following instruments: spectrophotometry, high-performance liquid chromatography (HPLC), and inductively coupled plasma optical emission spectroscopy (ICP-OES) (Zasoski & Barau, 1997; Burgos *et al.*, 2007; Ekin *et al.*, 2011). Since 2005, more potato genotypes have been analysed through chemical analyses, mostly at CIP Quality & Nutrition Laboratory in Peru (Bonierbale *et al.*, 2011; Burgos *et al.*, 2007).

The ICP-OES instrument is one of the most effective and recognised analytical tools for the determination of trace minerals, such as iron and zinc in a myriad of sample types (Zasoski & Burau, 1997). The technique is based on the spontaneous emission of photons from atoms and ions that have been excited in a RF discharge. Liquid samples may be injected directly into the instrument, while solid samples require extraction or acid digestion, so that the analytes will be present in a solution.

The sample solution is converted to an aerosol and directed into the central channel of the plasma. At its core, the inductively coupled plasma (ICP) sustains a temperature of approximately 9726 °C, so that the aerosol is quickly vaporised (Hou & Jones, 2000). The ICP-OES technique has been widely used for mineral determination of several vegetable (Chaves *et al.*, 2010; Naozuka *et al.*, 2011).

Although, the chemical methods are appears to be more precise, the high costs of these methods and the time required for analysis limits their use to small number of

samples relative to those required in extensive screening and breeding programs (Alishahi *et al.*, 2010). Near-infrared reflectance spectroscopy (NIRS) analysis is a rapid and relatively inexpensive technique that facilitates analysis of several traits simultaneously (Alishahi *et al.*, 2010). It does not need chemical agent and avoids contamination with chemical waste.

The NIRS calibration was developed to estimate the carotenoid and phenolic content of freeze-dried and milled potato together with sweet potato samples (Bonierbale *et al.*, 2009). These calibrations are precise and useful for selecting varieties with high, medium, or low concentrations of micronutrients and selecting varieties for starch, protein and individual sugars. To date, more than 10, 000 potato samples from different breeding programs have been analysed through NIRS technique, thus facilitating the breeding progress for nutritional improvement of potato (Bonierbale *et al.*, 2009). Consequently, due to slow adoption of this instrument by the South African institutions of higher learning or research, mineral concentrations of many plant samples are still analysed chemically, mostly by ICP-OES.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Plant materials

A total of 20 potato genotypes composing 15 elite lines and 5 improved cultivars grown commercially in South Africa were utilised in this study. The genotypes were obtained from the Agricultural Research Council, South Africa and Michigan State University, in the USA (Table 3.1). The varieties were selected based on their quality characteristics such as early maturity, high yield, disease tolerance, good tuber quality factors and other desirable morphological traits. The parental sources of some of these breeding lines are presented in Figure 3.1 (ARC-VOPI, Plant Breeding Division).

3.2 Site information

The study was carried out at the ARC-Roodeplaat, Vegetables and Ornamental Plant Institute (VOPI), Pretoria (S 25° 36. 187' E 28° 21. 240'), during 2014/2015 growing seasons at an altitude of 1159m above sea level. The experiment was conducted under greenhouse condition with the controlled temperature ranging from 15 °C to 25 °C.

3.3 Greenhouse experiment

The choice of using greenhouse experiments was to minimise influence of non-genetic factors on minerals variability among the genotypes. Burgos *et al.* (2007) noted that environmental condition may influence the variation of trace mineral concentration among the potato genotypes.

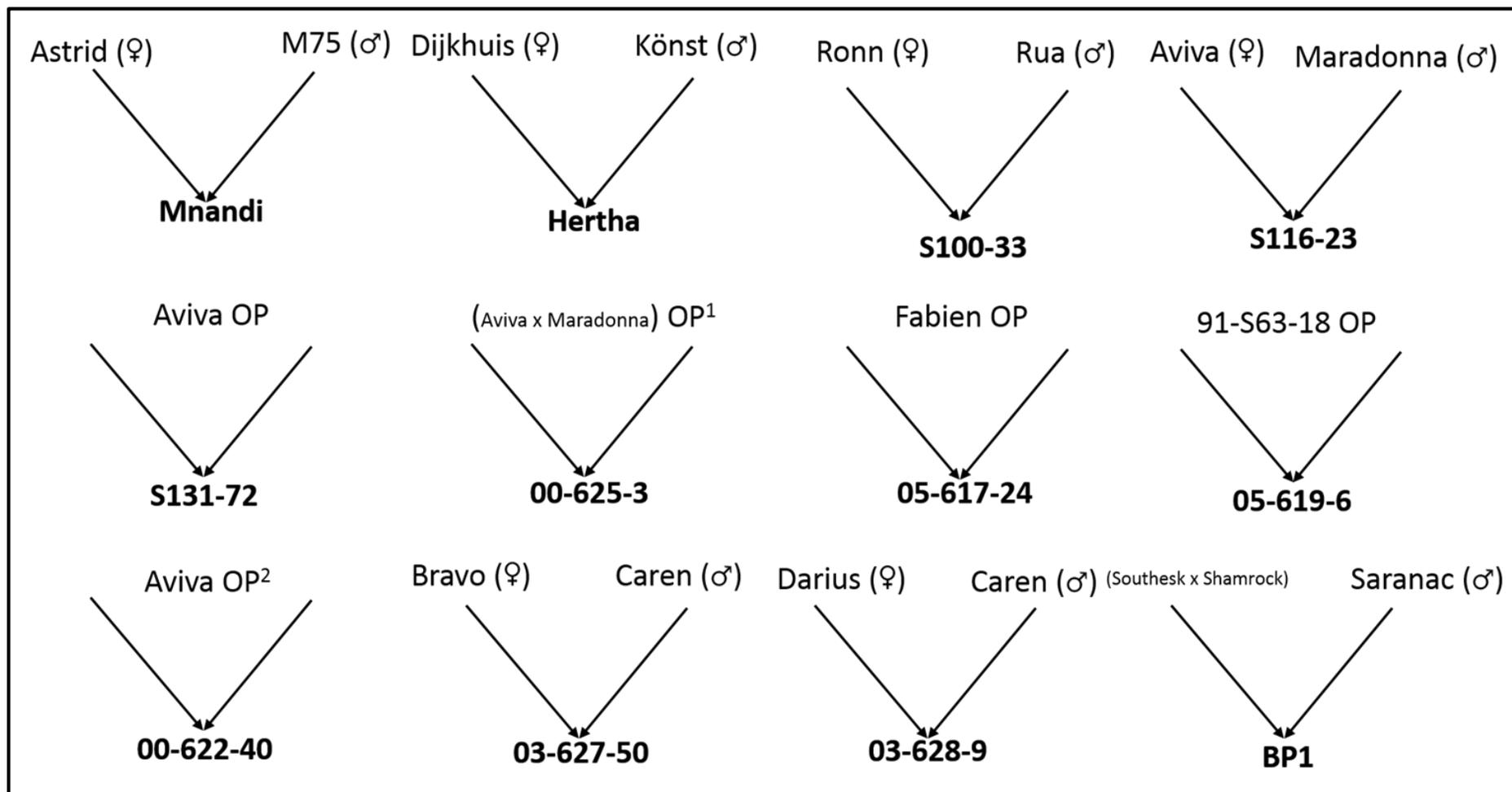
Thus, greenhouse experiments were suggested to be more reliable for preliminary evaluation of such minerals among potato genotypes in this study.

Table 3.1 Potato genotypes used for mineral evaluation and their characteristics during 2014/2015 growing season

Treatments	Genotypes	Tuber flesh colour	Tuber shape	Skin colour	Maturity*	Source
1	Mnandi	Cream	Oval	Yellow	L	RSA
2	Hertha	Cream	Oval	Yellow	M	RSA
3	BP1	White	Oval	White	M	RSA
4	BFP	Cream	Oval	White	S-m	RSA
5	VDP	White	Long	White	S	RSA
6	00-S100-33	White	Oval	Yellow	S	RSA
7	J461-1	White	Round	Yellow	M	USA
8	N105-1	Cream	Oval	Yellow	S	USA
9	00-615-3	White	Oval	Yellow	L	RSA
10	02-S131-72	White	Oval	White	L	RSA
11	00-S116-23	Cream	Oval	Yellow	L	RSA
12	00-622-40	White	Oval	White	L	RSA
13	03-628-9	Cream	Oval	White	L	RSA
14	00-619-29	White	Oval	White	M	RSA
15	03-627-50	White	Oval	White	M	RSA
16	J036-A	White	Round	White	S	USA
17	05-619-55	White	Oval	White	S	RSA
18	05-619-68	White	Oval	White	S	RSA
19	05-617-24	Cream	Oval	Yellow	L	RSA
20	05-619-6	White	Oval	White	M	RSA

*According to the classification at Agricultural Research Council, Roodeplaat (Gauteng, South Africa).
 RSA= South Africa; USA= United States of America; L= Long; M= Medium; S= Short

Figure 3.1 Parental sources of locally developed potato genotypes (ARC-VOPI Roodeplaat, Plant Breeding Division)



OP= open pollinated in field conditions; ♀= male parent plant; ♂= female parent plant

Note: the open pollinated crosses were considered due to insufficient flowering of particular genotypes in the greenhouse condition, but sufficiently flowering in field condition

3.3.1 Planting and fertilisation

The experimental treatments were laid using Completely Randomised Design (CRD) with each treatment having three replicates (Figure 3.2). The details of the soil pH, cation exchange capacity (CEC), available N, P, K, Fe, Zn and texture of the soil are indicated in Table 3.2. The soil sample was analysed at the Agricultural Research Council, Institute for Soil, Climate and Water (ARC-ISCW). Twenty five centimetre plastic pots were filled with a red topsoil, sand and vermiculite mixture (3:2:1), and with NPK fertiliser in the ratio 3:2:1 (25). Red topsoil and sand was sterilised before it was mixed with fertilisers and vermiculite. Well sprouted, medium sized seed tubers of 20 potato genotypes were planted at a depth of 10 cm. The growing medium was added in stages as the plants grew taller, in order to increase the yield and prevent the potato tubers from turning green.

Table 3.2 Physicochemical properties of soil mixture used for greenhouse planting

Soil physical and chemical properties								
Soil types	Texture	Soil pH (H ₂ O)	Total N (%)	Available P (mg.kg ⁻¹)	Available K (mg.kg ⁻¹)	Available Fe (mg.kg ⁻¹)	Available Zn (mg.kg ⁻¹)	CEC cmol(+). Kg ⁻¹
Red top soil	Clay-loamy	5.56	0.0083	8.96	18.98	1.91	0.32	2.58
River sand	Sandy	6.56	0.002	6.99	10.98	3.87	0.31	1.81

Analysis by Agricultural Research Council, Institute for Soil, Climate & Water (ISCW)

CEC= Cation exchange capacity



Figure 3.2 Potato genotypes planted in Completely Randomised Design (CRD)

3.4 Trial management

During the early growth phase, before tuber formation, the soil was constantly and uniformly kept moistened to a depth of at least 10-15 cm. The frequency of irrigation cycles during this period was determined according to specific soil mixture and controlled conditions in the greenhouse. Plants were irrigated at least 3 times in a week. During the second growth phase, that is, during tuber development, irrigation was less frequent and applied once every 3-5 days prior to harvest. Throughout the duration of the experiment, all the necessary management practices was carried out to ensure good growth and development. The practice mostly 'included' controlling pest and diseases. The plants were also supported by the stakes to grow upright in the greenhouse (Figure 3.3).



Figure 3.3 Potato genotypes supported by stakes to grow upright in the greenhouse

3.5 Evaluation of minerals

The mineral concentration of potato tubers was evaluated based on both dry weight and fresh weight basis. Only three elements were analysed in this study: namely, iron (Fe), zinc (Zn) and phosphorus (P). The analysis was performed following the method adapted from Zasoski and Buurau (1977).

3.5.1 Method descriptions

Following the tuber sample preparation, the mineral concentration from each genotype was determined by inductively coupled plasma optical emission spectrometry (ICP-OES), at ARC-ISCW. Primarily, both dry samples were digested with 7ml HNO₃ (concentrated nitric acid) and 3ml NClO₄ (perchloric acid) at temperatures of up to 200 °C and brought to volume in a 100ml volumetric flask.

3.5.1.1 Sample preparation

Five tuber samples from each variety were randomly selected, thoroughly washed with tap water and thereafter rinsed with distilled water to remove any soil and inert material on the tubers (Figure 3.4). The tubers were then slightly peeled with a peeler and shredded into pieces. Subsequently, 100 g composite sample of the shredded tubers were weighed and placed in a glass petri dish and then oven dried at 70 °C until they were dry enough to be milled. Samples were milled to pass a 1mm sieve in a stainless cutting mill and stored in plastic containers with tight fitting lids and ready for mineral determination.



Figure 3.4 Thoroughly washed sample tubers from two different genotypes

3.5.1.2 Digestion process

Firstly, 1 gram dry sample was weighed using analytical type balance and transferred to a digestion tube. Subsequently, 7 cc of analytical grade concentrated nitric acid was added and swirled gently to wet the side. Three cc of analytical grade perchloric acid were added in few minutes later after all the dry organic material had thoroughly

absorbed water. Thereafter, this was left overnight. The digestion tube was then placed in cold or cool heating block and heating commenced by increasing the temperature from 50 °C to 180 °C (i.e. once the temperature reached 50 °C, it was left for more than 10 minutes in that temperature before heating to 100°C, then again left at that temperature for more than 10 minutes).

Three types of fumes were used as digestion indicators: water vapour, brown fumes of nitrogen dioxide and heavy white fumes from the perchloric acid. When the last of these fumes appeared, the digestion process was accomplished. Then, 0.5 mL wise drop of analytical grade hydrogen peroxide was added and heated for further 10 minutes. The tubes were removed from the block and cooled with a few drops of de-ionized water. Lastly, the 5 cc of a 1:1 solution of hydrochloric acid was added, shaken to mix properly and transferred to a 100 cc A grade volumetric flask.

3.5.1.3 Mineral content determination

An aliquot of the digest solution was used for the ICP-OES determination of Fe, Zn and P. The sequential instrument (Varian Liberty 200) was used and elements were determined almost simultaneously, with only a few seconds between each element. Each element was measured at an appropriate emission wavelengths, chosen for high sensitivity and lack of spectral interferences. The wavelengths used were 940nm (2059) for Fe, 856nm (213) for Zn and 618nm (2013) for P.

3.6 Tuber yield determination

The marketable tubers of each genotype were weighed to determine the tuber fresh yield. The dry matter content was determined using oven drying method to remove all the moisture content from the tubers.

3.7 Statistical analysis

Analysis of variance (ANOVA) on the data set of each variable was performed using the R statistics version 2.14.1 (2013), followed by treatment mean separation using Fisher's *t*-test least significant difference (LSD). Linear correlation of coefficient analysis was also conducted using the Pearson test to examine the strength of the link between the mineral nutrients and tuber yield traits.

CHAPTER 4

4.0 RESULTS AND DISCUSSION

4.1 Mineral concentrations

4.1.1 Variability of mineral within same genotypes

From the plant breeding perspective, the diversification of micronutrient-dense traits that occur between tubers belonging to the same cultivar or even the same potato plant, would not be conducive for supporting biofortification. In the current study, about 95% of genotypes showed high precision data for Fe concentration, and about 75% of genotypes showed high precision data for Zn concentration as indicated by low relative standard deviation (Table 4.1). This implies that the replicates effect were not significant in the greenhouse and repeatability of three results for each element was obtained in most genotypes, especially for Fe concentration. However, few values were detected as probable outliers and rejected from computing the mean of particular genotypes. The outliers could have been a result of contamination or matrix interference of sample during preparation and digestion process (Welna *et al.*, 2011).

Table 4.1 Summary of data recorded for Fe and Zn concentration of 20 potato genotypes evaluated under greenhouse condition during 2014/2015 growing season

Genotypes/ Breeding lines	Fe Concentration mg.kg ⁻¹ DW					Zn Concentration mg.kg ⁻¹ DW				
	Min	Max	Median	Mean	RSD	Min	Max	Median	Mean	RSD
Mnandi	74	80	75.6	76.67	4.4	48.2	51.8	51.0	50.67	3.8
Hertha	45	54	49.4	49.33	9.9	64	77.8	64.01	66.1	16.3
BP1	48	53	50.5	50.5*	7	46.4	46.4	46.4	46.39*	0.1
BFP	53	65	58.5	59.6	10.7	37.7	51.7	44.7	44.71	15.6
VDP	48	62	49.6	53.33	13.9	25.5	35	28.9	29.8	16.1
00-S100-33	53.8	57.8	54.4	55.53	3.6	41.2	54	43.9	46.33	14.6
J461-1	45	50	47.5	47.5*	6.8	30.9	33.1	31.4	31.8	3.6
N105-1	69.2	73.2	70.4	70.93	2.8	35.3	37.5	28.9	30.57	20.4
00-615-3	34	37	35.5	35.5	6.1	24.2	24.3	24.2	24.24*	0.2
02-S131-72	39	54	47.3	46.67	15.1	24.9	39.8	36.4	33.7	20.9
00-S116-23	39	42	40.5	40.67	3.9	28.4	31.4	28.5	29.43	5.7
00-622-40	42.5	43.8	43.5	43.27	1.5	28.2	46.1	35.8	36.7	24.4
03-628-9	48	51	49.8	49.67	3.2	31.4	37.2	34.3	34.3	8.4
00-619-29	42	47	44.3	44.33	5.4	26.9	30.9	28.9	28.91	9.7
03-627-50	43	64	47.5	51.67	20.9	27.2	29.3	27.7	28.07	0.6
J036-A	41	47	43.5	43.67	6.5	21.1	30.2	21.7	24.33	20.7
05-619-55	39	47	45.8	44	9.7	30.6	37.3	32.7	33.53	10.1
05-619-68	39	56	48.1	47.67	17.8	14.3	19.7	16.9	16.97	15.8
05-617-24	32	38	33.8	35.5*	8.5	12.2	12.88	12.9	12.88	5.4
05-619-6	40	43	41	41.43	3.4	17.4	35.7	25.6	26.23	35.4

DW= dry weight basis; RSD= relatively standard deviation (%); Fe= Iron; Zn= Zinc

Mean values are weighed means of triplicate digestis, with the middle value (median) having four times the weight of each of the extreme values (the maximum and minimum). This weighted mean is equivalent to the average of the mean and median.

* mean from two values due to rejection of outlier values

4.1.2 Variability of mineral among genotypes

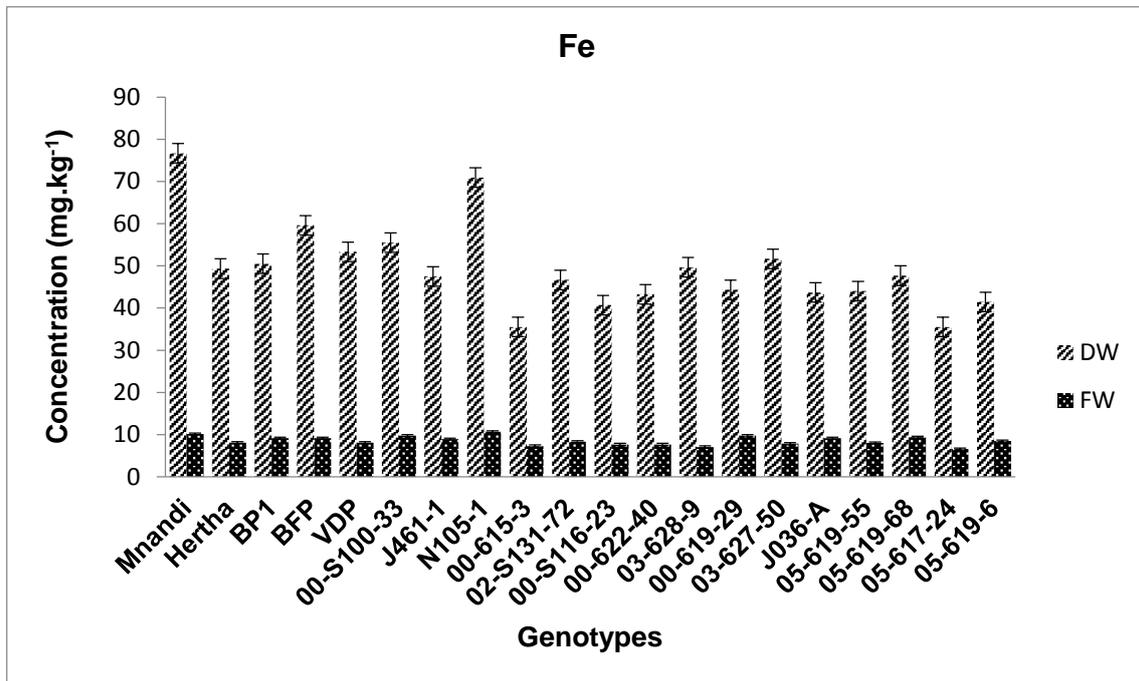


Figure 4.1 Variability of Fe concentration in both dry weight (DW) and fresh weight (FW) basis of 20 potato genotypes tested in the greenhouse condition

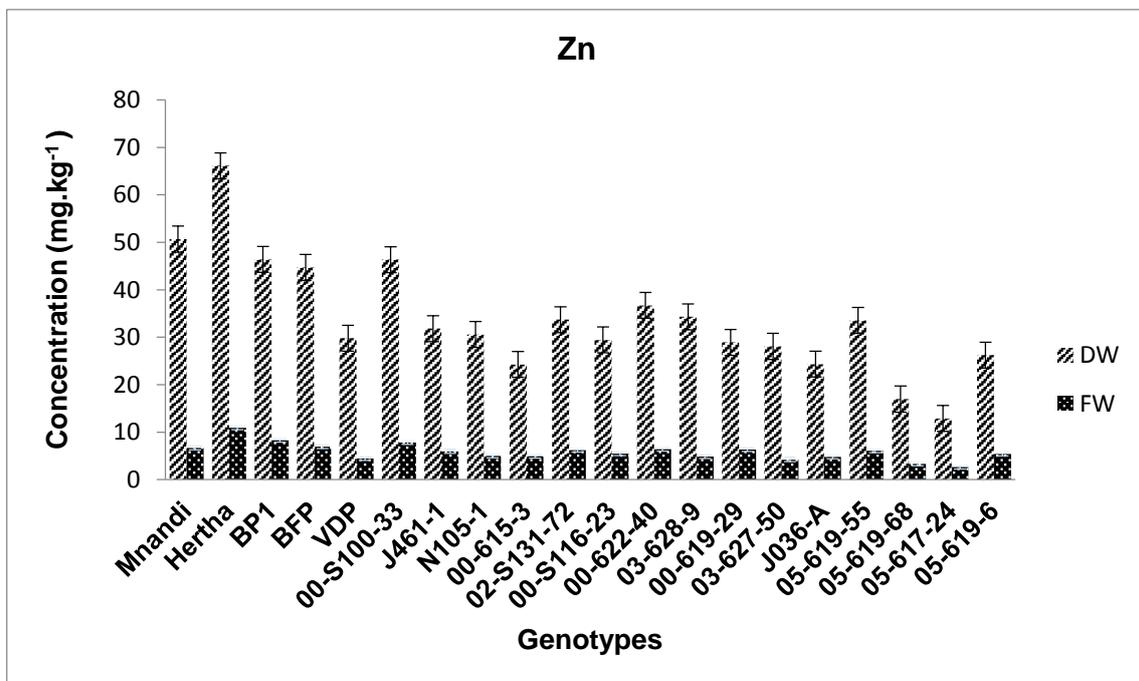


Figure 4.2 Variability of Zn concentration in both dry weight (DW) and fresh weight (FW) basis of 20 potato genotypes tested in the greenhouse

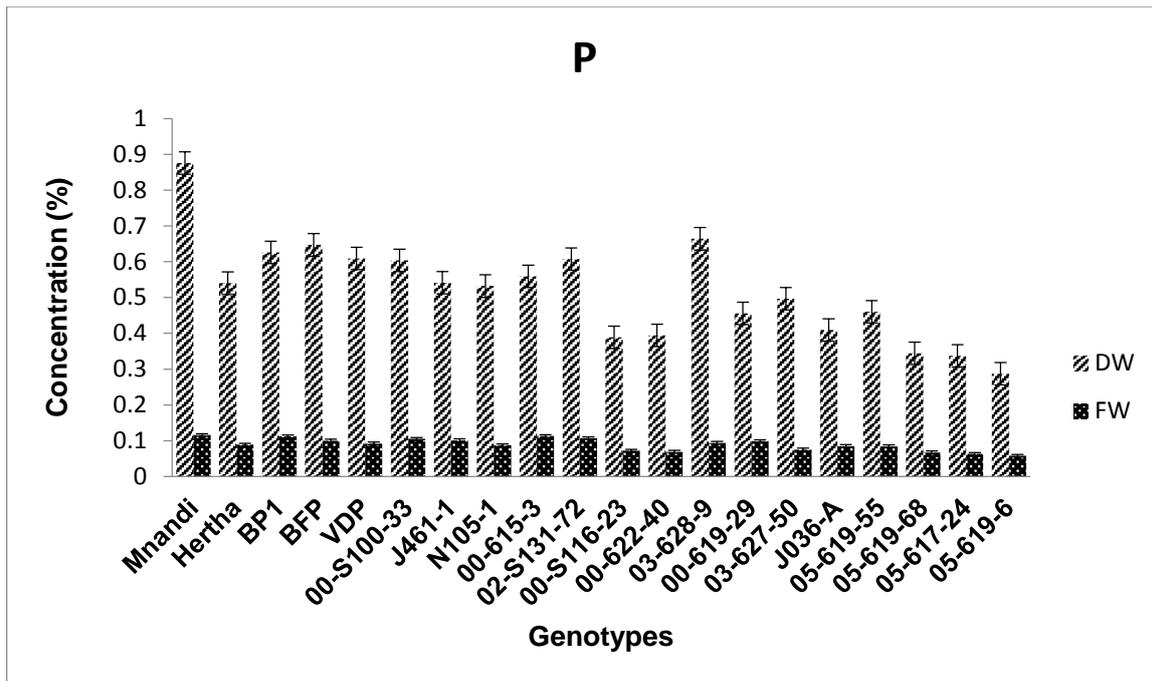


Figure 4.3 Variability of P concentration in both dry weight (DW) and fresh weight (FW) basis of 20 potato genotypes tested in the greenhouse

The result of the study revealed variability in mineral concentration among 20 potato genotypes tested under greenhouse condition. The statistical analysis indicated significant ($P < 0.001$) variation in mineral concentrations among the genotypes. Among the 20 genotypes, Fe concentrations varied from 34.67 mg.kg^{-1} in line 05-617-24 to 76.67 mg.kg^{-1} in cultivar Mnandi on dry weight basis (DW). For the fresh weight the concentration varied from 6.56 mg.kg^{-1} in line 05-617-24 to 10.13 mg.kg^{-1} in cultivar Mnandi (Figure 4.1). Similarly, Zn concentrations varied from 12.88 mg.kg^{-1} in line 05-617-24 to 65.04 mg.kg^{-1} in cultivar Hertha on dry weight basis (DW). For fresh weight, the concentration varied from 2.43 mg.kg^{-1} in line 05-617-24 to 10.89 mg.kg^{-1} in cultivar Hertha (Figure 4.2). The P concentration (%) also greatly varied from 0.287 mg.kg^{-1} in line 05-619-6 to 0.876 mg.kg^{-1} in cultivar Mnandi on dry weight basis and from 0.058 mg.kg^{-1} in line 05-619-6 to 0.116 mg.kg^{-1} in cultivar Mnandi on fresh weight basis (Figure 4.3).

These results suggested that there is variation in the ability of each cultivar to absorb the minerals from the soil, translocate and redistribute those minerals, allowing adequate, but nontoxic levels to accumulate in edible plant tissue (Welch & Graham, 2004). Apart from variation attributed to genetic ability of cultivars; important soil properties such as soil pH, redox conditions, cation exchange capacity, microbial activity, organic matter and water content, also plays important role in governing the availability of these mineral for the crop accumulation (Shuman, 1998; Frossard *et al.*, 2000).

In line with these results, variation of Fe and Zn concentration were also observed in various studies by other researchers, e.g. Abebe *et al.* (2012) reported Fe and Zn concentration ranges from 17.13 to 164.83 and 7.07 to 20.21 mg.kg⁻¹ respectively, on a dry weight basis in potato. Bonierbale *et al.* (2011) reported a range of Fe concentration from 0.27 to 0.75 mg/100g on fresh weight basis and 11.24 to 30.82 mg.kg⁻¹ on dry weight basis among 582 native Andean potato. Zinc concentration in the same accession ranged from 0.20 to 0.67 mg/100g on fresh weight basis and 8.53 to 26.22 mg.kg⁻¹ on dry weight basis. Equally, Ekin (2011) revealed Fe and Zn concentrations of 75.03 to 122.69 and 15.21 to 18.96 mg.kg⁻¹, on dry weight basis among eight potato varieties. Variability of P concentration among potato genotypes was also reported in previous other studies (Damney *et al.*, 2002; Tekaligne & Hammes, 2005; Abebe *at al.*, 2012; Trehan & Sharma, 2003).

Sufficient genetic variability of desirable traits among different genotypes is the key for any plant breeding programme. The results from this study showed that there was considerable variability of both Fe and Zn concentration among the genotypes (Figure 4.1 and 4.2). This indicates that there is a large scope for selection among the evaluated genotypes. As expected, the concentration of both Fe and Zn were much lower on fresh weight basis (FW) as compared to their concentration on dry weight basis (DW) (Figure 4.1 and 4.2). Particularly for tuber crops, this suggested that fresh roots are very poor sources of most minerals because of dilution with large quantities of water (Thacker & Kirkwood, 1992).

Notable in these results was the high level of Fe and Zn concentration in two of the commercially cultivated varieties (Mnandi and Hertha) in South Africa (Figure 4.1 and 4.2). The two cultivars had high concentrations of both minerals. This information is very valuable as they can be recommended to be included as parental genotypes in the biofortification breeding programme in South Africa.

Although variation in Fe and Zn concentration can be significantly influenced by environmental conditions (Salas *et al.*, 2012; Burgos *et al.*, 2007; Brown *et al.*, 2010; White *et al.*, 2012). Particularly the phytoavailability of soluble soil micronutrients. In this study, it could be assumed that the reported variability among the genotypes was more genetically attributed, since the growing conditions were controlled in the greenhouse. However, knowledge of genotype by environment interaction effects on the micronutrient concentration will be very imperative for further nutritional enhancement of these promising genotypes.

Table 4.2 Dry matter content and tuber yield of 20 potato genotypes evaluated in greenhouse condition during the 2014/2015 growing season

Genotypes	Mean	
	DMC (%)	TY (g)
Mnandi	13.25 ⁱ	29.13 ^{gh}
Hertha	16.45 ^{fg}	120.3 ^a
BP1	17.95 ^{def}	56.2 ^{cdefgh}
BFP	15.6 ^{gh}	68.53 ^{bcdef}
VDP	15.25 ^{gh}	76.33 ^{bcde}
00-S100-33	17.4 ^{ef}	30.55 ^{gh}
J461-1	18.8 ^{cde}	57.13 ^{cdefg}
N105-1	16.45 ^{fg}	66.33 ^{bcdef}
00-615-3	20.35 ^{abc}	26.07 ^{gh}
02-S131-72	17.75 ^{ef}	46.8 ^{defgh}
00-S116-23	18.8 ^{cde}	81.2 ^{bc}
00-622-40	17.75 ^{ef}	45.25 ^{efgh}
03-628-9	14.15 ^{hi}	25.47 ^h
00-619-29	21.85 ^a	49.1 ^{defgh}
03-627-50	15.15 ^{gh}	77.95 ^{bcd}
J036-A	20.85 ^{ab}	81.13 ^{bc}
05-619-55	18.25 ^{de}	37.4 ^{fgh}
05-619-68	19.5 ^{bcd}	28.5 ^{gh}
05-617-24	18.9 ^{cde}	89.13 ^{ab}
05-619-6	20.5 ^{ab}	50.17 ^{cdefgh}
Trial Mean	17.75	57.13
CV (%)	13.05	44.37
LSD	1.58	31.47
Significant	***	***

CV= Coefficient of variation; LSD= Least significant different; TY= Tuber yield; DMC= Dry matter content. Mean in the same column followed by a similar letter are not significantly different from each other at the 5 % probability level

*** = highly significant at P< 0.001

4.2 Tuber yield

The statistical analysis indicated significant ($P < 0.001$) variation in tuber yield (TY) based on tuber fresh mass among the genotypes (Table 4.2). The tuber fresh weight (g) range from 25.47 in line 03-628-9 to 120.3 in cultivar Hertha. The contrast in the tuber yield observed between the genotype 03-628-9 and Hertha in particular was of interest from a potato genetic improvement standpoint. It indicates the enough variability among the selected genotypes that can be useful in selection for good yield in potato improvement. In line with this results, variability of tuber yield among potato genotypes was also reported by Abebe *et al.* (2012). Arvanitoyannis *et al.* (2008) also established that potato yields and tuber quality can vary according to cultivar.

However, there was no significant difference observed between genotype Mnandi, BP1, 00-S100-33 and 05-619-55, this results suggested that there was no genotypic variability among all these genotypes, and without genetic diversity crop selection or improvement cannot be facilitated (Acquaah, 2007). Although this study mainly focussed on nutritional aspect of potato; maintaining the productivity is the most important criteria that must be met before new lines of micronutrient-enriched potato are released and distributed. Welch & Graham (2003) reported that maintaining or increasing the yield of micronutrient-enriched new developed varieties will quickly guarantee widespread farmer acceptance, and ensure that targeted people at risk of developing micronutrient malnutrition will benefit from such a breeding approach.

4.3 Dry matter content

Dry matter content (DMC) in this study also varied among the genotypes at $P < 0.001$ significant level.

The lowest dry matter content (13.25%) was obtained in commercial cultivar Mnandi, while the highest dry matter content (21.85%) was obtained in elite breeding line 00-619-29 (Table 4.2). Again this results revealed enough variability of dry matter content similar to tuber yield observed among studied genotypes. These results are consistent with those obtained by Abebe *et al.* (2012), who reported values ranged from 17.25 % to 27.90 % dry matter content in potato. However, the insignificant between genotype BFP, VDP and 03-627-50 were also observed, which is not of interest in crop improvement perspective (Acquaah, 2007). The breeding lines that showed good results in terms of dry matter content were 00-619-29, J036-A, 05-619-6, 00-615-3 and 05-619-68. The variation in total dry matter yield of crops mostly depends on the size of leaf canopy, the rate at which the leaf functions (efficiency), and the length of time the canopy persists (duration) of each cultivar (Tekalign & Hammes, 2005). Tuber dry matter content trait is the main determinant of quality, both for processing and for cooking (Wilson & Lindsay, 1969). The processing efficiency and the quality of finished product depend on the dry matter content. The lines with high dry matter content from this study are thus recommended for consideration as parents in the breeding programme.

4.4 Correlation between minerals, dry matter content and tuber yield

Table 4.3 Coefficient of linear correlation between the mineral nutrients and tuber yield traits of the potato genotypes evaluated in greenhouse during the 2014/2015 growing season.

	Fe	Zn	P	DMC	TY
Fe	1.00	0.452*	0.678**	-0.587**	-0.02 ^{ns}
Zn		1.00	0.569**	-0.459*	0.068 ^{ns}
P			1.00	-0.682**	-0.168 ^{ns}
DMC				1.00	0.076 ^{ns}
TY					1.00

Fe = Iron; Zn = Zinc; DMC = Dry matter content; TY = Tuber yield
 ns = Not significant; * = Significant (P < 0.05); ** = highly significant (P < 0.01)

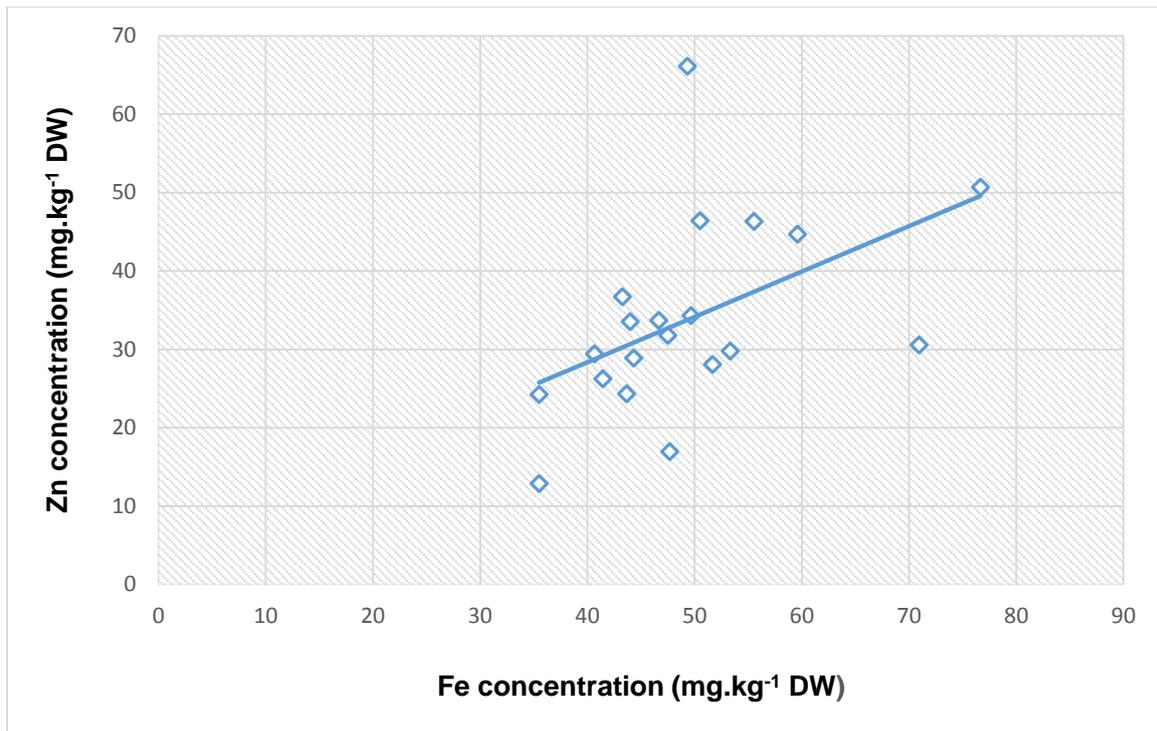


Figure 4.4 The relationship between Fe and Zn concentration of 20 potato genotypes

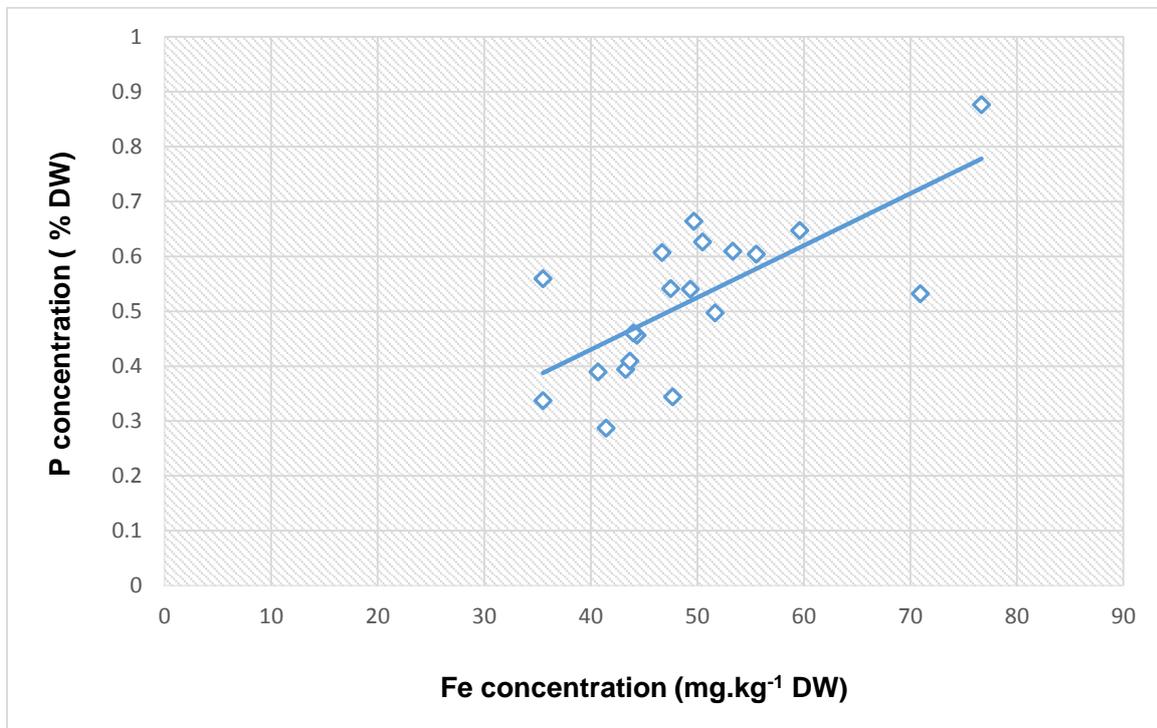


Figure 4.5 The relationship between Fe and P concentration of 20 potato genotypes

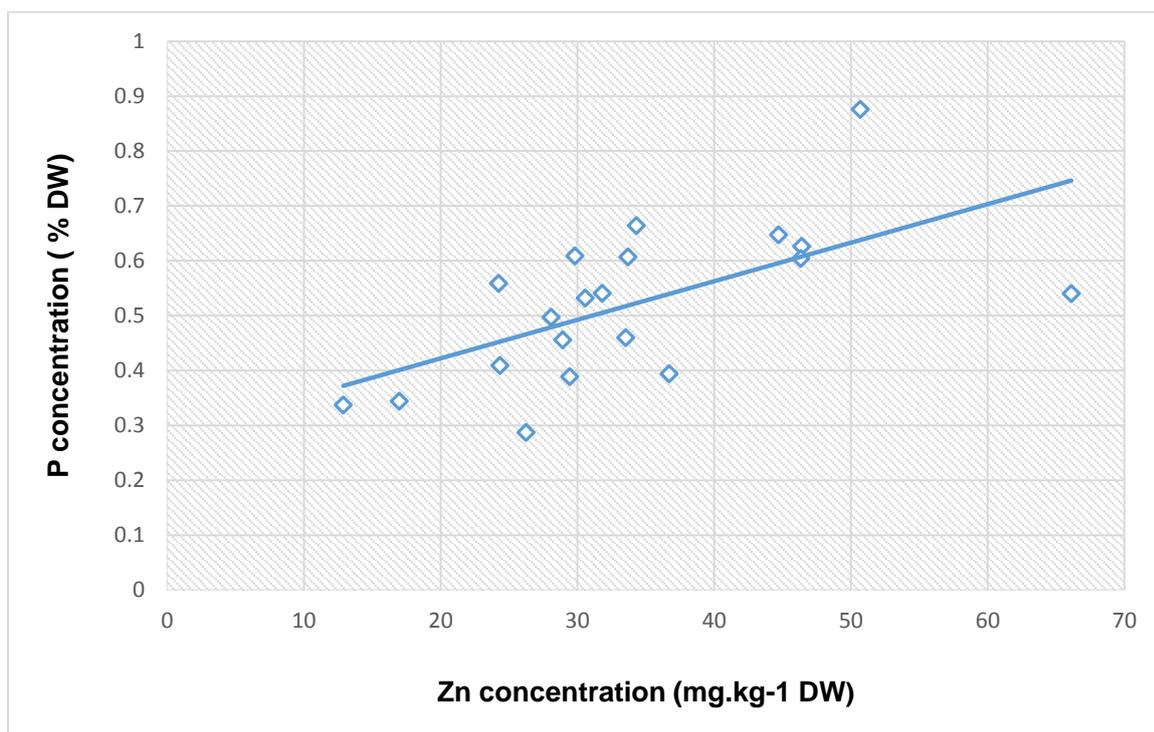


Figure 4.6 The relationship between Zn and P concentration of 20 potato genotypes

Both positive and negative associations between pairs of traits were obtained in the current study (Table 4.3). Positive correlation was observed between Fe and Zn (Figure 4.4). This results are in agreement with those of Paget *et al.* (2014) and indicated that evaluation together with selection of one of these minerals will result in concomitant increase in the other. This is useful particularly where both characters are desirable in a cultivar development programme but one character is relatively easier to measure. Similar association between Fe and Zn was reported by Abebe *et al.* (2012) in potato and this was also emphasised by Reddy *et al.* (2005) in a research work carried out on sorghum. Both Fe and Zn were also positively correlated with P as shown in Figure 4.5 and 4.6.

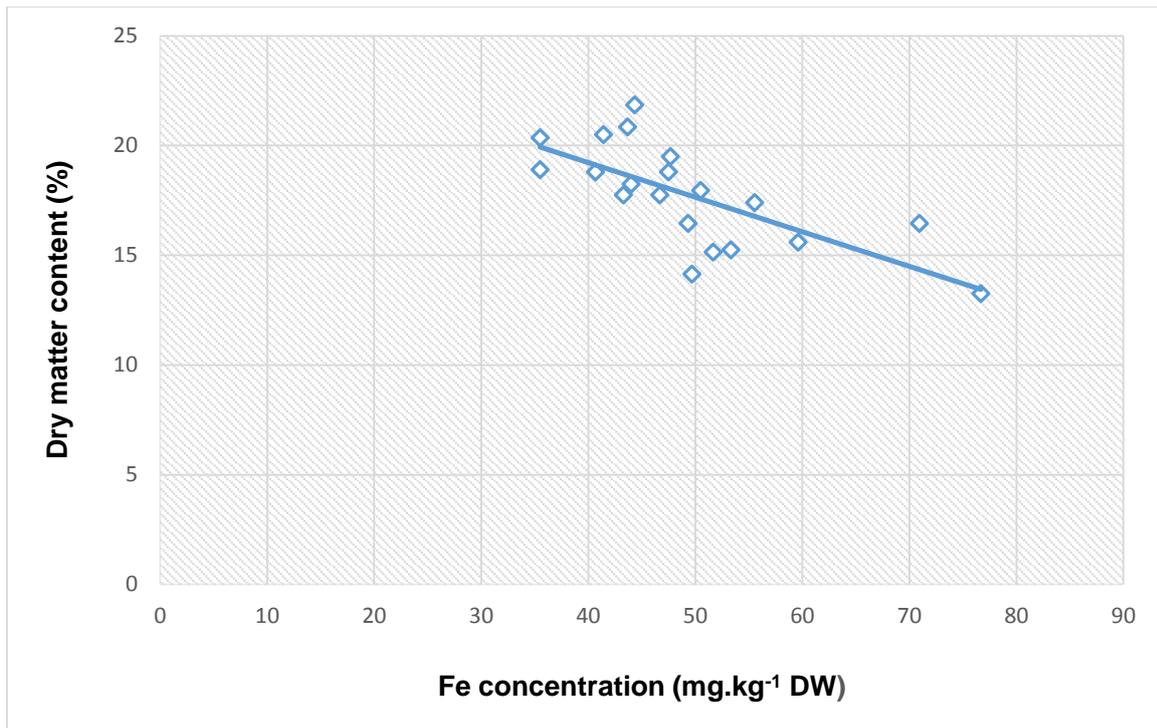


Figure 4.7 The relationship between Fe concentration and dry matter content of 20 potato genotypes

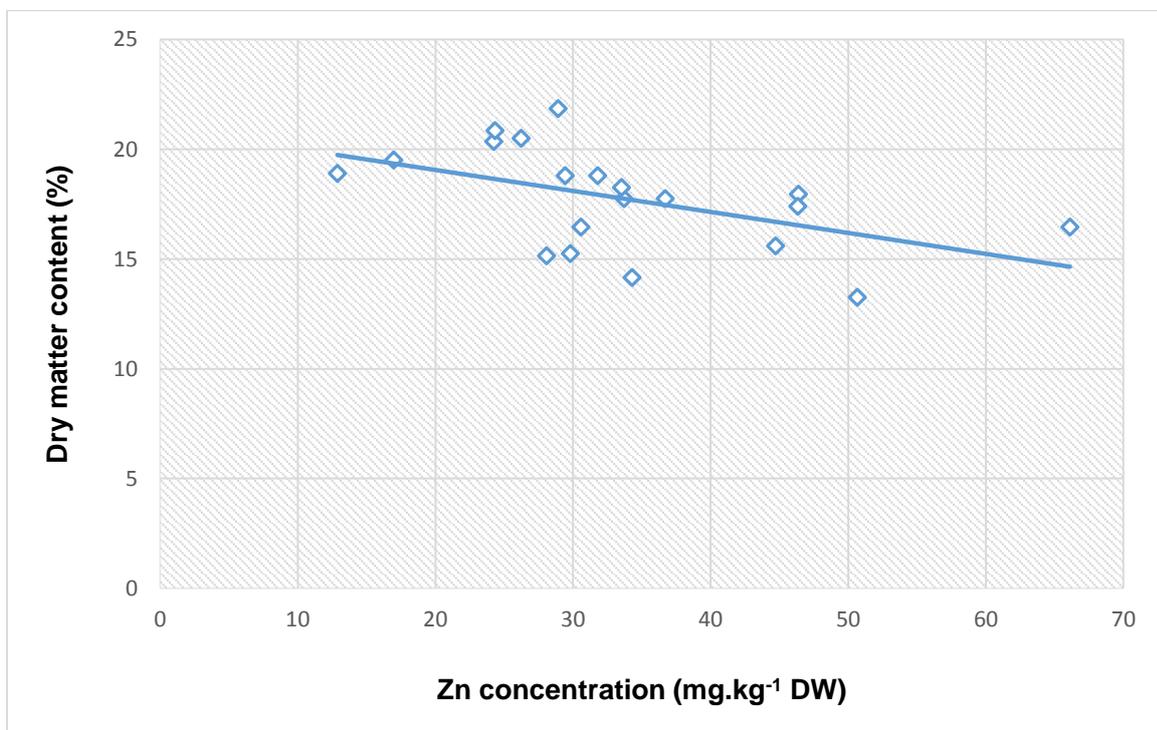


Figure 4.8 The relationship between Zn concentration and dry matter content of 20 potato genotypes

Positive correlation between P and tuber fresh yield was reported by White *et al.* (2009). In contrast, this study showed that Fe and P were negatively associated with tuber yield (Table 4.3). However, the association was not statistically significant. The insignificant correlation between minerals and tuber yield, in this study, suggested that each character could be under the control of independent genes. Genes that are independent of each other are not linked; therefore, they can be selected against each other. Significant negative correlation between essential minerals (Fe, Zn and P) and dry matter content were observed in this study (Figure 4.7 and 4.8). These negative relationships are in agreement with observations reported by other researchers (Abebe *et al.*, 2012; Tekaligne & Hammes, 2005) and can be attributed to a dilution effect caused by the high plant growth rates that exceed the ability of plant to acquire these mineral elements (Abebe *et al.*, 2012; Jarelle and Beverly, 1981).

The main concern in breeding food crops is simultaneously selecting for multiple traits related to yield and quality, as well as other traits, such as biotic and abiotic stresses (Luby, 2009). This is because the successful food crop variety requires several characteristics valued by consumers and providing economic sustainability for producers. Therefore, positive association between two or more desirable traits, such as Fe and Zn concentrations as observed in this study, would be favourable and easily facilitate selection process in the breeding programme.

CHAPTER 5

5.0 SUMMARY AND CONCLUSION

This study revealed significant variability in the concentrations of iron and zinc, among 20 potato genotypes in South Africa. Genotype Mnandi, Hertha and Buffelspoort, as well as the ARC breeding line N105-1, 00-S100-33 and 03-627-50, were found to have higher concentration of both iron and zinc among the evaluated genotypes. Therefore, recommended for use in the bio-fortification breeding programme. In addition, genotypes 00-619-29, J036-A, 05-619-6, 00-615-3 and 05-619-68 were also found to have high dry matter content. Therefore, also recommended for consideration as parents in the breeding programme. However, further investigation is needed to make firm conclusions about the extent of genetic potential of these genotypes as good parental sources. Such investigation could at least include testing the stability for the mineral concentration of selected genotypes at more locations in prospective potato production areas in order to obtain more conclusive results pertaining to the genotypes. In this manner, it would also create opportunity to estimate the genetic gain or level of heritability of the trait and also identify the best breeding strategy to increased micronutrient content of potato.

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