DOES ZINC INFLUENCE GERMINATION, VEGETATIVE GROWTH OR YIELD OF WHEAT (*TRITICUM AESTIVUM*)?

by

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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third-party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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ABSTRACT

Nutrients are essential to all life on earth. Nutrients are divided into macro and micro-nutrients. Macro nutrients such as proteins and carbohydrates for example are needed by organisms in large quantities to remain healthy while micro nutrients such as vitamins and minerals for example are needed in very small quantities. A lack in sufficient uptake of either macro or micro nutrients by organisms can lead to serious health problems, due to the occurrence of nutrient deficiencies. Emphasis has been laid on the importance of zinc (Zn; a micro nutrient) as more than 50% of soils on which staple foods (e.g., wheat) are produced globally are considered as being Zn deficient. Various secondary advantages such as decreased emergence rate, increased disease resistance, better stand density and yield have been linked to an improvement in wheat vigour due to an increase in the Zn concentration [Zn], either in wheat seeds, in the soil or due to foliar applications of Zn. The main aim of the study was to determine what the causal effects of increased [Zn] are on these various parameters. The influence of seed [Zn], priming and foliar applications of Zn fertilisers were either studied in uncontrolled or controlled environmental conditions. Various stress aspects, including water stress, increased planting depth and weed competition were also incorporated into some of the controlled environmental experiments. It is noteworthy that no Zn deficient ($\leq$ 22 mg kg^{-1}) seeds were used during these experiments as none could be found. An increase in wheat seed [Zn] did have a significant positive influence ($p < 0.05$) on the germination percentage of wheat seeds during the germination experiment. Soil moisture and planting depth had a significant influence on seedling growth ($p \leq 0.05$). Seemingly, insufficient amounts of soil moisture led to decreased seedling growth while an increase in planting depth led to a decrease in seedling emergence. Wheat seed emergence was also significantly ($p \leq 0.05$) improved due to an increase in wheat seed [Zn]. Wheat seed germination and seedling growth was not influenced by the presence of ryegrass, but the presence of only one wheat plant had a significant influence on the dry mass (DM) production of ryegrass ($p \leq 0.05$). Wheat stem length and DM actually increased in one of the experiments as the number of wheat plants decreased and the number of ryegrass plants increased. This finding was corroborated by other similar studies and also by two of the controlled environmental condition experiments in this study. Seed [Zn] and different fertilizer treatments had an indifferent influence on plant growth and nutrient composition at all

three localities where field trails were conducted. Results were inconclusive as to whether seed [Zn] did have a positive influence on growth and vigour of wheat, which may have been attributed to the absence of truly Zn deficient wheat seeds in this instance.

UITTREKSEL

Nutriënte is noodsaaklik vir die bestaan van alle lewende organismes op aarde. Nutriënte word verdeel in makro- of mikronutriënte. Makro nutriënte soos bv proteine en koolhidrate word in groot hoeveelhede benodig deur organismes om gesond te bly terwyl mikro nutriënte soos bv vitamienes en minerale slegs in klein hoeveelhede benodig word. 'n Gebrek aan voldoende opnames van makro- of mikronutriënte deur organismes kan ernstige gesondheidsprobleme veroorsaak weens voedingstekorte. Klem word tans gelê op die belangrikheid van sink (Zn; 'n mikronutriënt) aangesien meer as 50% van grond, waarop stapelvoedsels (byvoorbeeld koring) wêreldwyd geproduseer word, beskou word as gronde met ‘n tekort aan Zn. Verskeie sekondêre voordele soos vinniger saailing opkoms, verhoogde siekteweerstand, beter plantdigtheid en opbrengs is gekoppel aan 'n verhoging in koring se lewenskragtigheid as gevolg van 'n toename in die Zn konsentrasie [Zn]. Hierdie verhoogde [Zn] kan deur verskeie metodes bereik word insluitend, om gebruik te maak van koringsaad met hoë [Zn], om te plant op gronde met hoë [Zn] of deur gebruik te maak van blaar voedingsstowwe wat Zn bevat. Die hoofdoel van die studie was dus om te bepaal wat die werklike effek van verhoogde [Zn] op hierdie verskillende parameters is. Die invloed van saad [Zn], “priming” en blaar toedienings van Zn bevattende kunsmis was onder beheerde of onbeheerde omgewingstoestande ondersoek. Die invloed van verskeie stremmingsaspekte soos waterstremming, verhoogde saad plantdiepte en onkruidkompetisie is ook in sommige van die beheerde omgewings eksperimente ondersoek. Dit moet genoem word dat geen ware Zn gebrek ([Zn] $\leq$ 22 mg kg^{-1}) saad tydens hierdie eksperimente gebruik is nie bloot as gevolg van die feit dat geen sulke saad opgespoor kon word nie. 'n Toename in koringsaad [Zn] het gedurende die ontkiemingseksperiment 'n beduidende positiewe invloed ($p \leq 0.05$) op die ontkiemingspersentasie van die koringsaad gehad. Grondvog en plantdiepte het 'n beduidende invloed op saailinggroei gehad ($p \leq 0.05$). Dit is vanselfsprekend dat onvoldoende hoeveelhede grondvog tot verlaagde saailinggroei gelei het, terwyl 'n toename in plantdiepte gelei het tot 'n afname in saailing opkoms. Koringsaad opkoms is ook aansienlik ($p \leq 0.05$) verbeter as gevolg van 'n toename in koringsaad [Zn]. Koringsaad ontkieming en saailing groei is nie beïnvloed deur die teenwoordigheid van raaigras nie, maar die teenwoordigheid van slegs een koringplant het 'n beduidende invloed gehad op die droëmassa-produksie van raaigras ($p \leq 0.05$).

Koring stamlengte en droëmassa het eintlik in een van die eksperimente toegeneem namate die aantal koringplante afgeneem het en die aantal raaigras plante toegeneem het. Hierdie bevinding word nie net deur verskeie ander wetenskaplikes ondersteun nie, maar is bevestig in twee van ons beheerde omgewingseksperimente. Koringsaad se [Zn] en die gebruik van verskillende kunsmisbehandelings het selde 'n beduidende invloed op plantegroei en die samestelling van voedingstowwe in die saad gehad by al drie lokaliteite waar veldproewe plaasgevind het. Ons neem aan dat 'n toename in saad [Zn] 'n positiewe invloed op die groeikragtigheid van koring het, maar dit was nie duidelik tydens hierdie proewe nie as gevolg van die afwesigheid van koringsaad wat werklik ‘n Zn tekort het.

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PREFACE

This thesis is presented as a compilation of 5 chapters.

Chapter 1 Introduction

Chapter 2 Literature review

Chapter 3 The influence of seed zinc concentrations on viability, vigour and growth of wheat.

Chapter 4 The influence of a higher zinc concentration on the vigour of wheat seed, when subjected to environmental stressors.

Chapter 5 Wheat zinc biofortification in the Western Cape Province, South Africa.

Chapter 6 Conclusion and recommendations

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LIST OF ABBREVIATIONS

Anon	Anonymous
CRD	Completely randomised design
CRBD	Completely randomised block design
diethylenetrinitrilopentaacetic acid	DTPA
Ed	Edited by
Eds	Multiple editors
Etc.	Et cetera
Ethylenediamine tetraacetate	EDTA
Ha	Hectares
HLM	Hectolitre mass
IWM	Intergrated weed management
MND’s	Micronutrient deficiencies
NPK	Nitrogen, phosphorous, potassium
OM	Organic matter
PATH	Program for appropriate technology in health
RDA	Recommended dietary allowance
RfD	Reference dose
SOM	Soil organic matter
UNICEF	united Nations international children’s emergency fund

CHAPTER 1

General introduction

1.1 Introduction and background

All organisms, from the smallest creatures (nanobes) to the largest animals (whales), on earth are dependent on sunlight, water, air, nutrients and a specific climate or habitat for survival (Anitei 2007). Nutrients play a critical role in the survival and health of organisms, and is therefore defined as any chemical or food that helps humans, animals and plants to live, develop and remain healthy (https://en.oxforddictionaries.com/definition/nutrient). Plants derive their nutrients from their environment, mostly the soil that they grow in, whereas animals and humans derive nutrients from the food that they consume (Biology online 2011).

Nutrients are divided into macronutrients (carbohydrates, fats and proteins) and micronutrients (vitamins and minerals) (Warne 2014). Macro- and micronutrients are present in soils due to a process called decomposition. Decomposition is the breakdown or rotting of organic material (dead plant and animal material) into its most basic forms (nutrients). Decomposition mainly occurs due to the activity of fungi and bacteria in the soil (The American Heritage® Student Science Dictionary 2014).

Most of the world's soils are currently being depleted of important nutrients and soil microorganisms (fungi and bacteria), which play a critical role in nutrient production and soil fertility. The depletion of these organisms and nutrients mainly occur due to unsustainable farming practices and an exponential increase in the human population. An increase in the global human poulation increases the risk of soil degradation due to an increased planting density or increased inorganic fertilizer use for instance that may be harmefull to soil microbes (Drechsel et al. 2001). Topsoils rich in nutrients and microorganisms are being lost due to natural processes such as erosion and farming methods, including overuse of inorganic fertilizers, overgrazing, intensive monoculture and many more. Nutrient poor soils might lead to nutrient poor crops, which could lead to nutrient deficiencies in those that consume these low nutrient crops on a daily basis (Marler and Wallin 2006).

The human immune system is dependent on sufficient amounts of nutrients for the production of enzymes and other anti-agents to keep the body healthy (Marler and Wallin 2006). A lack of sufficient amounts of nutrients in the diets of people is also referred to as malnutrition. Malnutrition is also referred to as hidden hunger due to the fact that some may eat sufficient quantities of food to feel satiated, but their bodies are actually becoming increasingly deprived of essential nutrients without them realising it (Saltzman et al. 2013). Malnutrition is the leading cause of immune deficiency diseases worldwide due to immune dysfunction. Mortality due to diseases and viruses such as HIV, malaria, tuberculosis and measles, are much higher in areas where malnutrition is more common than in areas where it is not that common (Niedzwiecki and Rath 2005).

Micronutrient deficiencies (MNDs) have become a great concern to the scientific community. Micronutrients are defined as any element needed in relative small amounts for any plant or animal to remain physiologically healthy (Katyal and Randhawa 1983). Conservative estimates put the number of people that are currently affected by MNDs at two billion people worldwide. These deficiencies in humans are linked to a lack of micronutrients in their diets due to place of residence, dietary habits, religion and/or recreational activities (Tulchinsky 2010). Recent studies have indicated that deficiencies in the following four micronutrients, iodine (I), iron (Fe), zinc (Zn) and vitamin A, are of greatest concern to human health (Tulchinsky 2010).

Zinc deficiency in soils lead to decreased yields and lower nutritional quality of agricultural products (Cakmak 2008). It is estimated that more than 17.3% of the world population is at risk of Zn deficiency, and it is most commonly found in low income regions of the world. Zinc is an important micromineral needed by the human body as it acts as a component of many enzyme systems. Zinc is needed by the human body to regulate normal development and to maintain body tissue, vision, the immune system and many more (Anonymous 2010). Disease due to Zn deficiency is found in all countries around the world, especially in countries in Africa, Eastern Mediterranean and South-East Asia.

Staple foods of people in these regions of the world include cereals such as wheat and rice (Caulfield and Black 2004). More than 50% of agricultural land used for cereal production has low levels of plant available Zn, which contribute to Zn deficiencies in crops (Ram et al. 2015). Moreover, these plant-based diets include large amounts of Zn inhibitors such as fibres and phytates. Fibres and phytates allow the body to take up Zn, but prevent the body from absorbing the Zn and putting it to good use (Caulfield and Black 2004).

Higher zinc concentrations [Zn] in the seeds of wheat have been linked to an improvement in the nutritional status of those that consume these seeds (Temple and Masta 2004). Currently, the HarvestPlus organisation, working on biofortification of staple foods with various micronutrients lead the research charge to improve the nutritional value of these globally consumed foods (Anonymous 2010). An example of one of their studies on the influence of Zn on crop growth is currently ongoing in seven countries including Pakistan, South Africa, China, Brazil, Thailand, India and Turkey. The main purpose of this study is to determine if it is possible to successfully biofortify wheat and rice via enhanced fertilisation with Zn, which application method would be most successful and what advantages, if any, foliar applications would hold for the crop itself.

Biofortification, according to Anonymous (2002), is the breeding of staple crops with higher micronutrient levels in order to fight micronutrient deficiencies in the crops and in those that consume those crops on a daily basis. Velu et al. (2014) believes that biofortification of wheat with Zn would be the most economical method to decrease global Zn deficiencies. In South Africa, however the high incidence of Zn deficiency has been addressed by fortification of all wheat and maize products with specific nutrients including Zn, Fe and I (The Department of Health South Africa and UNICEF South Africa 2007).

As mentioned earlier, South Africa also take part in worldwide trials, biofortifying wheat with Zn fertilisers to increase the Zn contents of foods. One of the secondary objectives of the study in South Africa is to investigate what advantages Zn biofortification hold for the crops when they are grown from seeds bio-fortified with Zn. Increases in crop yields and increases in crop vigour have been observed when seed was bio-fortified with Zn (Haslett et al. 2001; Bodruzzaman et al. 2005; Cakmak 2009). Increased vigour may improve the water use efficiency (WUE) of wheat and has been shown to lead to increased competitive abilities and better resistance against pests and diseases of other crops (Rebetzke and Richards 1999). Vigorous seeds may also improve wheat seedling competitiveness for resources in the presence of weeds, and in particular to *Lolium spp*., which are hard to control due to herbicide resistance problems.

1.2 Purpose of the study

From a South African perspective, the secondary benefits, such as improved disease resistance, of more Zn in the grains may be more important than the role that higher [Zn] play in human nutrition due to the fact that fortification is a must according to law in South Africa. Early physiological growth plays an important role in the quality and strength of wheat seedlings. Normal physiological growth is dependent on the [Zn] in seeds, due to the role that Zn plays in enzymatic activities, cell reproduction and gene expression (Frassinetti et al. 2006).

The objective of this study is therefore to investigate if a higher wheat seed [Zn] can improve wheat seed vigour, early plant growth and eventually, yield. Another objective would be to determine if Zn foliar applications together with urea can increase the quality of seeds produced at harvest.

The information gained will be used to determine if Zn biofortification is cost effective in terms of the vigour advantages that it conveys to the resulting seeds and plants.

1.3 Hypotheses

1.3.1 Null hypothesis

The null hypothesis states that increased [Zn] in wheat grains, due to priming or biofortification of wheat will not have any significant influence on germination, growth rate, vigour, yield or grain [Zn] of wheat.

1.3.2 Alternative hypotheses

A total of 5 alternative hypotheses were tested during the study. These include:

(i) Higher Zn levels of wheat seeds would lead to increased germination rates and thus also growth of those seeds;

(ii) Higher Zn levels of wheat would increase vigour of juvenile wheat plants;

(iii) Higher Zn levels would improve the competitive abilities of wheat;

(iv) Higher Zn levels of wheat would lead to increased yields produced by seeds; and

(v) The use of a combination of Zn and urea as a foliar application would lead to an increase in the overall grain quality of wheat.

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Chapter 2

Literature review

2.1 Importance of cereals

Cereals are defined as any species of the grass family, Gramineae, that have edible seeds and are also refered to as grains according to Mckevith (2004). Figure 2.1 below depicts a family tree of the Gramineae family, which gives an indication of the different subfamilies, tribes, genus's and species of cereals that is currently recognised. Cereals are mainly grown for the high amounts of energy derived from them, but they also have many other uses (Mckevith 2004).

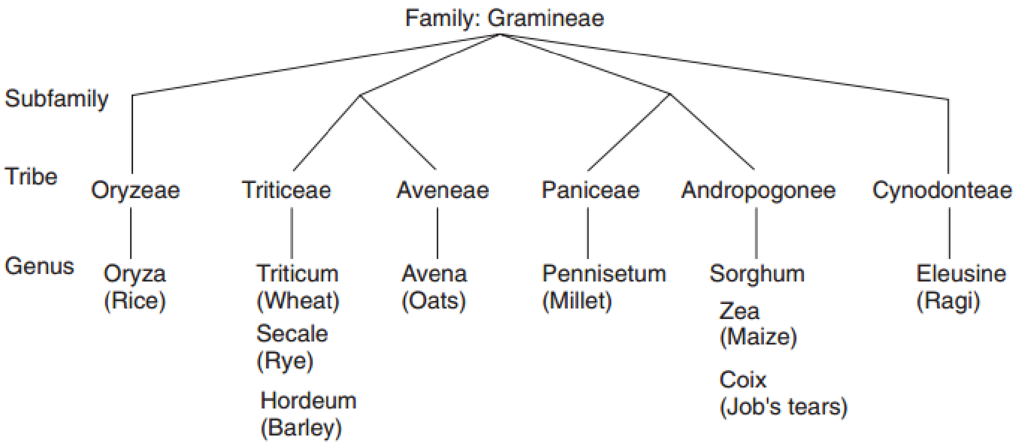

Figure 2.1: Family tree of the grass family (Gramineae) and all its subfamilies (Source: Mckevith 2004)

Cereals are a staple food for mankind, a food source for livestock, used in the production of alcohol, and more recently the production of bio-energy. All cereals are annual plants meaning that they are planted, grown and harvested within less than a year from planting (Koehler and Wieser 2013). Dietary fibre is a component mainly found in plant foods such as cereals and plays an important role in keeping the digestive tract clear as well as satisfying our appetites. Many people in developing countries cannot afford other sources of dietary fibre which is one of the reasons for cereals becoming a well-known staple food (Dvorak 2009). The eight cereals most commonly produced include wheat, maize, sorghum, rice, barley, millet, oats and rye

(Koehler and Wieser 2013). Wheat, corn and rice are produced in larger quantities than the other aforementioned cereal species (Table 2.1).

Table 2.1: Global cereal production in 2010 (Source: Koehler and Wieser 2013).

Species	Cultivated area (Million ha)	Grain production (Million tons)
Corn	162	844
Rice	154	672
Wheat	217	651
Barley	48	123
Sorghum + millet	76	85
Oats	9	20
Triticale	4	13
Rye	5	12

2.1.1 Importance of wheat

Wheat is produced on all 7 continents of the world and is the second most consumed cereal grain in Asia (Makgoba 2013). Wheat has a special set of properties and can therefore not be replaced by any other cereal grain (Dvorak 2009). This special set of properties include the previously mentioned fibres, carbohydrates for energy, B-vitamins for increased health and iron (Fe) for the development of strong muscles and healthy nerves (Anonymous 2011).

Wheat covers more land surface than any other crop and is the second most important crop produced after maize (Anonymous 2008). Anonymous (2008) also states that although wheat is not the most important crop it can be produced in regions with climates not ideal for the production of maize (most important crop worldwide) and rice (3rd most important crop worldwide), which increases the importance of wheat as a staple crop to various continents, countries and religions.

Different wheat types are used to make different food products. Hard wheat is used to make bread, whereas soft wheat is used for baking cakes and making cereals. Durum wheat is used to produce pasta, spaghetti, macaroni and other products with very hard textures (Anonymous 2011).

2.1.2 Wheat growth and development

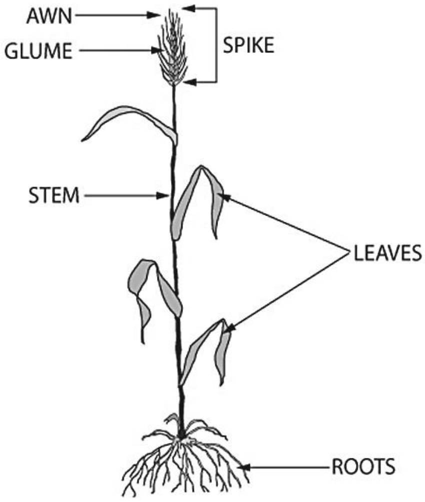

Figure 2.2: Morphological features of a mature wheat plant (Source Thomason et al. 2009).

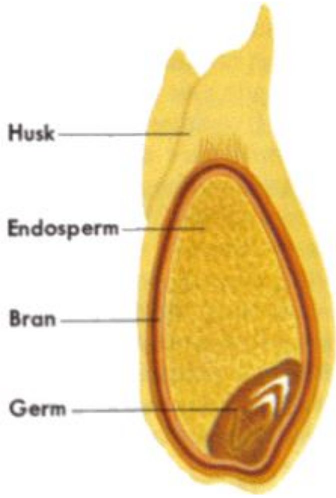

Figure 2.3: Morphological features of a wheat grain/kernel. (Source the Robinson library 2015).

A wheat plant has a very simple morphological structure, which mainly consists of roots, a stem, leaves and a head (Figure 2.2). The lengths and shapes of these plant parts play an important role in availability of moisture to the head and subsequent yield. A wheat plant with morphological features that enables the plant to use as much of its energy to produce larger kernels is sought as this would lead to higher protein and starch production (Thomason et al. 2009). A wheat kernel, grain or seed (Figure 2.3) is divided into three main parts namely the germ, bran and endosperm, each with its own important role. The germ is the part from which the radicle and coleoptile develop, this process is also known as germination. The purpose of the bran is to protect the delicate insides of the seed from harsh environmental conditions and the endosperm is used as storage place for energy in the form of starch and gluten, which is used for growth and development (The Robinson library 2015).

Wheat can either be planted during the start of the winter months or at the start of spring. Ideal conditions for production of wheat includes a cool and moist growing season (between 130-190 days) followed by a warm and dry harvesting season (Erika 2010). Growth of wheat is characterised by (1) a vegetative growth stage which is subdivided into tillering, jointing and booting; and (2) a reproductive phase, which is subdivided into heading and seed ripening (Figure 2.4). During the tillering stage, tillers develop along the stem of the plant where after internodes start to elongate within the jointing or stem extension stage. The booting stage starts at the end of the jointing stage when the head starts to develop inside the sheath of the flag leaf and ends when the spike emerges. The wheat head would also be completely visible at this stage. During the reproduction phase the wheat plant will pollinate itself. After all the anthers have emerged the ripening stage starts, ovaries are pollinated and seeds can start to develop (Dvorak 2009). There are five stages of grain filling that can be physically tested in the field by squeezing a grain kernel between your fingers. The first stage is known as the water stage where a water substance oozes out of the seed when pressed between your fingers; the second is the milky stage thus a milky substance is found within the kernel when broken or squeezed. The third and fourth stages are known as the soft and hard dough stages, respectively and the last stage is known as the ripening stage. The kernel is very hard during the ripening stage and there is no liquid present when the kernel is squeezed. This is an indication that the wheat is dry enough to be harvested (Swanepoel 2016).

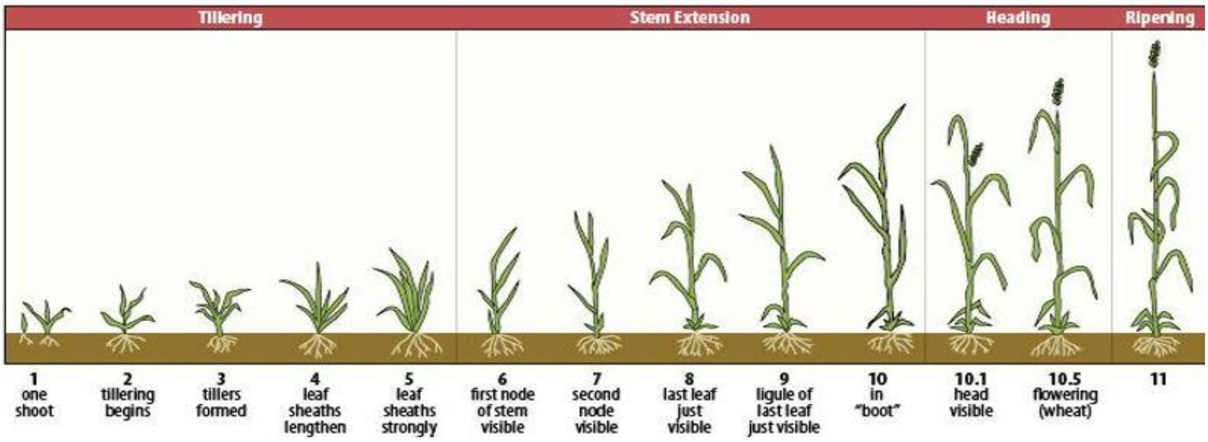

Figure 2.4: Different growth stages of wheat as per the Feekes scale (Source: Thompson 2014).

2.1.3 Cultivation of wheat in South Africa

Wheat is produced under many different environmental conditions in South Africa. It is produced in winter (Western Cape Province) and summer (rest of the country) rainfall regions, under irrigation and dryland conditions. The heterogeneity of South African soils, indicated by the presence of a large variety of different plant species, animals and microorganisms makes farming in South Africa much more challenging than in countries with more homogenous soils and climates (Swanepoel 2016). Wheat is mostly grown under dryland conditions in South Africa with only about 36% being cultivated under irrigation, mostly in the summer rainfall regions (Makgoba 2013). Currently, South Africa imports more than 300 000 tons of wheat annually from countries such as Canada, Germany and Argentina, due to the fact that demand in the country exceeds the domestic supply by South African farmers (Erika 2010; Makgoba 2013). Wheat production in the Western Cape increased from 35% of wheat produced in South Africa in 2000 to 65% of all wheat produced in South Africa in 2013. Increased production in the Western Cape and decreases in other regions of the country is mainly due to the effects of climate change and an increasing concern for sustainable agriculture in South Africa, as large amounts of nutrient rich topsoil is lost every year due to unsustainable farming practices (Swanepoel 2016).

2.1.4 Why fortify wheat?

According to Allen et al. (2006) wheat is one of the best candidates to fortify in order to decrease micronutrient deficiencies in humans. Reasons proposed, include the fact that cereals, dairy products, beverages and sugar are products consumed globally, even by those in less developed countries where malnutrition is a bigger problem than in the developed countries. Since these foods are consumed on a regular basis at relative consistent amounts it can therefore be processed centrally (Allen et al. 2006). Nutrient premixes can also be added to these food sources easily and at low cost, which plays a major role in the process as funds will be one of the main limiting factors when embarking on such a project. These products are then also used very soon after production meaning that vitamin retention would still be optimum when consumed. Tulchinsky (2010) found that so-called ‘food vehicles’ (i.e., foods being fortified with vitamins or minerals are known as food vehicles as they are used to “transport” vitamins and minerals into the bodies of humans) are lacking for zinc (Zn) and that wheat is commonly fortified with Vitamin B complex, folic acid, vitamin B12 and several other minerals considered deficient.

2.2 Zinc - An important micro-mineral

Zinc was first discovered and isolated in 1746 by Andreas Sigismund Margraaf a German chemist who heated charcoal and calamine (Reference 2016). Zinc forms part of the group 2B transition elements of the periodic table and has an atomic weight of 65.39. Zinc does not only play an important role in the health of all living organisms (plants, animals and humans), but is also used in the galvanization process. Galvanization uses Zn to coat steel and Fe to prevent it from rusting. Zinc is also used in the production of coins and many other industrial products such as roof materials. It can also be used to produce chemical compounds such as Zn oxide (ZnO) (Pappas 2015). The term micro mineral means that only small amounts of the mineral (<100 $mg.day^{-1}$) are needed by the human body per day to remain healthy (Frassinetti et al. 2006). Adult males according to Norris (2014) require at least 11 mg $Zn.day^{-1}$ while adult females only need about 8 $mg.day^{-1}$.

2.2.1 Zinc and the use thereof by plants

Anonymous (2004) states that Zn is one of the micro minerals essential for optimal growth in plants. They also found that an increase in soil Zn leads to better germination of both corn and wheat and possibly other plant species as well. Other important uses of Zn by plants include the production of auxins (an important growth hormone), regulation of the activity of enzymes that are responsible for the conversion of carbon dioxide (CO_2) to carbohydrates. Furthermore, it enables plants to withstand lower air temperatures and helps with the formation of chlorophyll and regulates root growth and starch formation (Anonymous 2004). Frassinetti et al. (2006) found that most plants including some cereal grains, vegetables, tree fruits and non-food crops are dependent on Zn. They also determined that Zn play an essential role in protein synthesis, fertility, seed production and protection against diseases. Stanković (2010) found that very high concentrations of Zn in the form of zinc chloride ($ZnCl_2$) reduced germination rates of some plant species and that it also inhibits root and shoot elongation.

2.2.2 Zinc and the use thereof by humans and animals

According to Frassinetti et al. (2006) all living organisms are dependent on Zn for normal growth and reproduction. Zinc plays a fundamental role in cell development, gene expression and cell replication (Hambridge 2000). Zinc is used by the immune system to enhance barriers and non-specific immunity, it further improves the effectiveness of immune components such as monocytes and natural killer cells. Zinc is lastly also involved in maintaining mediators of immune function such as thymulin activity and cytokine function (Caulfield and Black 2004). This information clearly states that Zn plays a vital role in the health of humans and animals and that deficiencies thereof can be linked to diseases such as chronic bronchitis and tinnitus in humans (Marler and Wallin 2006). Schullin et al. (2015) states that adequate amounts of Zn are needed for normal growth, since deficiencies thereof lead to stunting in the growth of children and compromise their learning ability (Ram et al. 2015). It is clear that there is a large number of functions and organs of humans and animals dependent on Zn for normal functioning and that is why this is considered as one of the most important micronutrients that exist.

2.3 Why focus on zinc?

According to Norris (2014) Zn is not stored in the human body and sufficient amounts of Zn should thus be consumed on a daily basis to ensure that the human body remains healthy. This provides the impetus by researchers on improving the uptake of Zn by crops and humans. Zinc is acknowledged as one of the two (vitamin A being the other) micronutrients that are commonly present in low concentrations in the diets of humans (Cakmak 2009). Ozturk et al. (2006) believes that more attention should be given to zinc biofortification of cereal crops as they inherently have low zinc concentrations [Zn]. Some feel that Zn deficiencies are so severe that they classify it as an epidemic both in developed and developing countries. According to Joint FAO and World Health Organization (2005), Zn plays a major role in more than 300 enzyme systems in the human body making it more important than most of the other micronutrients.

Zinc supposedly does not only have positive effects on the health of humans, but provide many advantages to plants. A study by Anonymous (2016) found that lower availability of Zn in the soils that crops are grown in lead to decreased growth and yield of wheat. They further found that yield increased significantly as the amount of Zn soil applications increased while grain [Zn] increased due to foliar Zn applications. This corroborated a previous study in which increased use of Zn containing fertilizers was shown to double grain [Zn] of wheat (Cakmak 2009). A study by Haslett et al. (2001), determined that higher [Zn] can lead to improved vigour of wheat, which would in turn lead to improved resistance against disease, pests and other negative environmental conditions such as drought.

2.4 Causes of Zn deficiency in wheat and humans

Zinc deficiency symptoms to look out for in plants include, yellowing of the middle parts of growing leaves (Haslett et al. 2001) and delayed maturity (Phillips 2015). In addition, leaves can even turn grey and may die or the plant may lose its leaves too early (McCauley et al. 2011). Some symptoms of Zn deficiencies that may occur in humans include hair loss, diarrhoea, weight loss due to loss of appetite, and many more (Anonymous 2016).

2.4.1 Zinc translocation from the environment

Zinc deficiencies within natural soils are closely linked to Zn deficiencies in primary producers as well as primary- and secondary consumers. This makes sense as plants are dependent on soils to take up sufficient amounts of Zn while animals and humans are largely dependent on plants to consume sufficient amounts of Zn. Eventually animals, plants and humans decompose into organic matter facilitated through soil microbe activities to again provide soils with sufficient amounts of Zn and other nutrients. If one of the links are removed, e.g., the plants, a break develops in the chain and Zn can’t be translocated to the next link. This gap eventually leads to decreases of Zn in the chain and would eventually get to a point where there are not sufficient amounts of Zn left to satisfy the requirements of each link, which would then result in the occurrence of Zn deficiency.

2.4.*2* Causes of zinc deficiencies in wheat and other crop species

Wheat is a crop species inherently low in Zn (Cakmak 2008). These low [Zn] can be increased by planting on high [Zn] soils and managing these soils correctly, but a lack in education is currently a problem leading to mismanaged soils and losses of increasingly more Zn. It is estimated that more than 50% of soils on which cereals are produced are low in plant available Zn, which is one of the main causes of Zn deficiency in wheat as well as other food products (Ram et al. 2015). Bhutta et al. (2014) found that more than 80% of the cultivated soils in Pakistan are low in Zn and accordingly may be a major cause of Zn deficiencies in more than 40% of women and children living in Pakistan.

Soluble minerals and nutrients are mainly transported via diffusion in soils to the surfaces of roots. Diffusion is where a particle or substance moves from one area, where it is present in a high concentration, to areas where it is present in a lower concentration. This particle or substance (e.g. Zn) needs to be mobile in order to be taken up by plant roots or to be transported to plant roots, and mobility increases with an increase in solubility. Zinc solubility is influenced by a number of things including soil moisture content, pH, the presence or absence of other nutrients such as Fe and phosphorus (P) in the soil and organic matter (OM) content (Cakmak 2008).

Bagci et al. (2007) found that crops grown under irrigation are less likely to become Zn deficient than those grown under rain fed condition due to the fact that those soils have a higher moisture content which improves solubility and mobility of Zn in the soil. Soil pH has the largest influence on Zn solubility in soils. Cakmak (2008) found that solubility of Zn in soils decreases dramatically (between 30 and 45-fold) when the soil pH goes above 5.5. An increase in pH leads to an increase in adsorption of Zn by other soil particles such as clay minerals and metal oxides, which then decrease the availability of Zn to plant roots. It follows then that an increase in clay or metal particles in the soil decreases the availability of Zn to plants. Eyupoglu et al. (1994) found that there is an inverse relationship between soil organic matter (SOM) and the presence and availability of diethylenetrinitrilopentaacetic acid (DTPA) extractable Zn. This inverse relationship occurs mainly due to the fact that more Zn is adsorbed by the SOM, which decreases the availability for root uptake. This same phenomenon also occurs in soils with very high clay contents. These high clay contents decrease the solubility and mobility of Zn in the soil (Cakmak 2008).

Unsustainable farming practices are some of the main causes of soils becoming depleted of important nutrients and micro nutrients. These practices include the use of new age cultivars to some extent, monoculture, over and under use of fertilizers, overgrazing and the use of fires to remove unwanted material such as weeds and alien invasive species. Cakmak (2008) further explains that problems will also soon occur due to the use of new wheat cultivars that are able to take up Zn better. An increase in the use of these cultivars will become a big problem as Zn reserves in soils will be depleted much quicker. A continuous use of monoculture crop cycles without applying important micro- and macronutrients to the soil is a real threat to soil quality, especially in African countries where Zn deficiency is a widespread and common problem. As the soils become depleted, Zn availability to wheat produced on these soils decreases - this will not only decrease yield, but will also decrease the concentration of Zn found in the grains of wheat, and increase vulnerability to diseases by the plant (Cakmak 2008). Cakmak (2008) found that wheat produced on Zn sufficient soils have an average grain [Zn] of between 20 and 30 mg kg^{-1} while wheat produced on Zn deficient soils only have a grain [Zn] of between 5 and 12 mg kg^{-1}.

2.4.3 Causes of zinc deficiency in humans

Zinc deficiencies in the global human population are linked to Zn deficiencies in the soils on which the crops that they consume are grown. Zinc deficient soils lead to decreased uptake of Zn by crops and therefore also diminished amounts of Zn consumption by those that consume these crops (Ozturk et al. 2006). Other factors such as living conditions or standards, believes and abilities of the human body to utilise Zn also play an important role (Niedzwiecki and Rath 2005).

2.4.3.1 Dietary habits, diseases and alcoholism

The diets of many people, mainly those in developing countries, consist of staple crops such cereals and pulses, which are low in Zn compared to products such as red meat, poultry and fish (Cakmak 2009). These individuals live in great poverty and cannot afford to buy or produce meat products. Zinc deficiencies are also common in developed and wealthy countries. Nriagu (2007a) found that alcoholism, which occur in both wealthy and poor communities, can increase the loss of Zn from the human body. Apparently, alcoholism leads to an increased loss of Zn via the urinary tract. It has been found that 30 to 50% of alcoholics may become Zn deficient as ethanol decreases the absorption of Zn by the body.

People suffering from obesity due to an unhealthy diet require supplemental Zn to remain healthy (Nriagu 2007a). Other unhealthy dietary habits that also play a role in Zn deficiency is the lack in consumption of whole grain products (Slavin 2004). Milling of wheat removes the Zn rich embryo and aleurone layer of wheat. Products produced by milled wheat will on average only contain 15 $mg.kg^{-1}$ of Zn while those produced by wheat grains which were not milled may have concentrations of up to 150 $mg.kg^{-1}$ (Cakmak 2008). Nriagu (2007a) found that many diseases lead to the loss of Zn either due to the disease itself or due to medications provided to fight these diseases.

2.4.3.2 Enzymes and other elements that prevent the body from utilizing Zinc

Bioavailability of Zn in most common “staple” foods such as cereals and legumes are very low and range from 10 to 30% (Nriagu 2007a). These aforementioned crops contain large amounts of substances, which decrease the bioavailability of Zn to the human body (Temple and Masta 2004).

Norris (2014) found that phytates or phytic acids bind to Zn in the digestive system which prevents absorption of Zn by the human body. Accordingly, people with a high phytate diet may require up to 50% more Zn than those with a low phytate diet (Norris 2014). Lignin (an organic complex found in the cell walls of plants) and dietary fibres (the indigestible portion of plant foods) bind to Zn in the human body in such a manner that the body cannot utilise or absorb it efficiently (Nriagu 2007a). The situation is compounded by consuming foods high in calcium (Ca), which complexes phytates that then becomes insoluble. This insoluble complex apparently decreases the ability of the body to absorb Zn efficiently (Lönnerdal 2000). Furthermore, it was found that high rates of folic acid supplementation can influence the availability of Zn to the human body (Nriagu 2007a). This was corroborated by a previous study, which determined that increases of folic acid supplementation of adult men lead to an increase in faecal

[Zn] (Milne et al. 1984). This means that a high concentration of folic acid in the human body will lead to the excretion of Zn and may therefore lead to Zn deficiencies.

2.5 Methods used to increase zinc uptake by humans

Research focus has shifted more to Zn and the deficiency thereof in the last decade as scientist have realised that it plays a major role in the health of a person (Cakmak 2009). Many techniques and supplements have been developed with the main goal of improving or increasing the amount of Zn consumed by people on a daily basis. Zinc supplementation and diet diversification are examples of direct ways to increase the uptake of Zn by humans while food fortification and crop biofortification are examples of indirect ways to increase the Zn uptake by humans. These techniques will be discussed below.

2.5.1 Food fortification and biofortification of crops

Traditional food fortification is when a nutrient or mineral is added to a processed food source such as flour in order to improve the nutrient content of the final product (Allen et al. 2006). Biofortification of crops on the other hand is the breeding of staple crops with higher nutrient levels (Anonymous 2002).

Some advantages linked to traditional fortification include low costs and that it can be done on large scale. In contrast, one problem with traditional fortification is that it requires central processing to ensure effective and good quality control measures. This is of particular concern in less developed countries where it is rather difficult to access such technology and skills that is required to perform fortification tasks (Allen et al. 2006). Vitamin retention may become a problem when these crops are exported across long distances from developed to developing countries. This means that vitamins and minerals added to the crop product, may be lost by the time the food source reaches the mouth of the consumer (Allen et al. 2015). Other problems that may also arise when making use of traditional fortification includes; unwanted changes to the taste of the crop and interactions with other micronutrients, which can either increase or decrease the effectiveness of the fortified micronutrient (Allen et al. 2015).

Biofortification on the other hand shows more advantages over the long-term than traditional fortification (Anonymous 2010). Biofortification may be a complex and costly project to undertake initially, but once a successful biofortified cultivar has been released many advantages will arise. After a large one-time investment upfront biofortified seeds will fortify themselves meaning that costs to produce these nutrient rich crops will decrease significantly. The production of these crops will also remain sustainable if government and international funding is stopped. Other advantages linked to these enriched seeds are that crops may become more resistant to diseases, yields may increase and seedling vigour might improve (Anonymous 2002; Velu et al. 2014). A cost benefit analyses by Nestel et al. (2006) has determined that the benefits reaped from biofortification are much higher than any other technique currently used to increase micronutrient intake by humans.

2.5.2 Zinc supplementation

Zinc supplementation is where people increase their daily Zn intake by making use of Zn supplements (e.g., pills, powders or tablets). Micronutrient powders for example are sprinkled over one's food to increase your micronutrient intake (Winkler 2013). Examples of Zn supplements include zinc sulfate ($ZnSO_4$), zinc gluconate ($C_{12}H_{22}O_{14}Zn$) and zinc acetate ($ZnC_4H_6O_4$) (Tidy 2014). This is an effective way to decrease Zn deficiencies in people who can afford these products and who have access to these products. Most people suffering from Zn deficiencies unfortunately do not have access to or money to buy these products. An advantage of Zn supplementation is that high concentrations of Zn can be ingested per treatment, but there are also serious health dangers connected to the incorrect use of such supplements. One of these dangers is exceeding the recommended dietary allowance (RDA), which could lead to Zn toxicity (Maret and Sandstead 2006). Zinc toxicity could then lead to various other health problems including light-headedness, depression, gastrointestinal toxicity, severe cardiovascular conditions and many many more (Nriagu 2007b). The range between sufficient amounts of Zn intake, Zn deficiency and Zn toxicity are very small. There is also not enough information on what can and cannot be taken with these supplements due to a lack in scientific research at the moment. Other problems associated with Zn supplementation include copper (Cu) deficiency, which may lead to various other health problems such as protein losses, Parkinson's disease and many more (Maret and Sandstead 2006; Angelova et al. 2011).

2.5.3 Diet diversification

Diet diversification is when someone changes their diet in order to consume either more or less of a specific food source, nutrient, mineral etc. Diet diversification is seen as a long-term solution to the global Zn deficiency problem but isn't yet viable as many who lack sufficient amounts of Zn are poor or live in regions where they do not have access to a diet that is high in Zn (Anonymous 2010).

Those living in poverty consume staple foods such as wheat, rice and maize. Schulin et al. (2015) states that these cereals or plant-based diets increase the risk of Zn deficiency due to various factors that has already been previously discussed. A list of food sources rich in Zn has been provided (Tidy 2014). According to this list red meat, poultry and fish are the main sources of Zn to humans globally, but can't be afforded by those most commonly threatened by malnutrition due to the high poverty concentration in their home countries. Pulses, nuts and legumes have lower concentrations of Zn, but the bio availability of Zn in these foods are higher than those in cereals and they are more affordable than meat (Temple and Masta 2004). Thus, it is clear that a few other larger problems, such as poverty and a lack of education should be corrected before diet diversification can be implemented in countries where Zn deficiency is an extreme threat to the health of humans.

2.5.4 Sprouting, fermentation and soaking

It has already been mentioned that plant-based diets contain high concentrations of phytates, which decrease the bio availability of Zn and other nutrients to the human body. A few methods have thus been developed to decrease the phytate levels in plant-based diets but these are very complex, costly and could lead to the loss of the advantages linked to the presence of phytates in a diet (Marsh et al. 2012).

The three main methods currently used to decrease phytate contents in the diets of humans include sprouting, soaking and fermenting (Arnarson 2016). Cereals and legumes can be soaked in water for up to 24 hours to reduce their phytate contents. Sprouting is also known as germination. Arnarson (2016) found that phytate levels are lower in germinated seeds of grains and legumes than in those that are not. During fermentation organic acids promote phytate degradation. Yeast (in bread) helps with the breakdown of phytates, which improves the bio availably of Zn and other nutrients to humans.

2.6 Methods used to increase zinc uptake by crops

The recommended daily allowance of Zn per day needed by the human body from whole-wheat grain to have a positive influence on the health of a person is 40 $mg.kg^{-1}$. Average intake of Zn from whole-wheat grain by the world population is currently between 20 and 35 mg kg^{-1}, which is far too low to have a positive influence on their health (Cakmak 2008). Zinc is currently being applied in many different ways to planted crops. It is either applied to the soil, to the leaves as a foliar fertilizer, it can be in an organic or inorganic form, seeds can be primed and it can be applied to the crop in combination with other substances such as fungicides (Velu et al. 2014).

2.6.1 Enhanced fertilisers

Fertilisers are mainly used to increase crop production according to Winkler (2011), but they are currently being developed to not only increase crop production but to also provide additional nutrients to those who consume these crops. Zinc concentration has been successfully increased in crop plants in Thailand by making use of enhanced fertilisers, but high costs to produce fertilisers, transportation costs (as fertilisers are bulky and heavy) and the inaccessibility of some communities may still be major stumbling blocks (Winkler 2011).

According to Haslett et al. (2001) shoot [Zn] was higher when Zn was applied to wheat as a foliar spray in the form of chelated Zn or $ZnSO_4$ than when being applied in the form of ZnO. This may imply that the bio availability of $ZnSO_4$ is higher than that of ZnO. Zinc applied as a foliar spray was a better method to increase shoot growth than applying Zn to the root environment (Haslett et al. 2001). A study by Schulin et al. (2015), which is similar to the previous study found that Zn fertilizers added to the soil had no significant or a low influence on yield, due to low solubility of soil applied Zn fertilizers. Other conclusions when looking at grain [Zn] included; (1) Soil Zn applications do not have a significant effect on grain [Zn]. (2) Foliar Zn applications had a significant effect on the concentration of Zn in grains, (3) Grain Zn levels increased significantly, when crop residues were applied to soils with low Zn availability. (4) Grain [Zn] has a positive relationship to grain nitrogen (N) concentrations.

A study on the different types of Zn application methods found that a foliar application combined with a soil application did not always lead to an increase in yield, and therefore made the assumption that other factors such as soil quality or method of farming could have a greater influence on yield (Zou et al. 2012). Although no significant difference in yield was observed, the combination of these two application methods of Zn did have a significant positive impact on grain [Zn]. A combination of soil and foliar application of Zn to wheat was also found to be the best biofortification method by Cakmak (2008.) He found that a combination of these two methods had a significant positive influence on Zn accumulation in whole-wheat grain. He further determined that grains rich in Zn also lead to better seedling vigour and denser wheat stands.

2.6.2 Conventional breeding

Conventional breeding is also known as hybridisation. Hybridisation is done via cross pollination of two different cultivars. Each of the two have one desired trait and the main aim is to then develop a hybrid cultivar that consists of both these desired traits (Manshardt 2004).

Winkler (2011) states that breeding is currently done all over the globe but is focussed on increasing yields rather than increasing nutrient contents of crops. He found that the HarvestPlus program is currently the most active group when looking at biofortification and how it could help decrease global micronutrient deficiencies. The HarvestPlus program studies three micronutrients (Fe, Zn and pro-vitamin A) and seven staple crops including wheat, rice, maize and sweet potato. The knowledge that they gain from their studies are used in developing countries with the main aim of improving the nutritional statuses of those countries (Winkler 2011). A list of the current studies done by the HarvestPlus program is provided in Table 2.2.

Table 2.2: The various crops and nutrients studied by the HarvestPlus program since 2010 (Source Winkler 2001).

Crop	Nutrient	Countries	Release
Bean	Iron (Zinc)	Congo, Rwanda	2010
Cassava	Provitamin A	Congo, Nigeria	2011-12
Maize	Provitamin A	Zambia	2011-12
Rice	Zinc (Iron)	India, Bangladesh	2012-13
Wheat	Zinc (Iron)	Pakistan, India	2012-13
Sweet potato	Provitamin A	Uganda, Mozambique	2007
Pearl Millet	Iron (Zinc)	India	2011

2.6.3 Nutritional genetic modification

Nutritional genetic modifications are one of the two methods used to genetically modify crops, the other is known as agronomic modifications. Agronomic modifications are mainly used to increase crop yields, resistance to pests, drought and salinity, etc., while nutritional genetic modifications are used to improve the nutrient compilation of a crop or plant species (Winkler 2011). Only agronomic modified cultivars have been successfully produced to date but lots of research is ongoing on the development of ways for nutritional genetic modification.

Some examples of nutritional genetic modification studies that has been done include essential fats in oilseeds, Fe in rice, flavonoids in vegetables and proteins in potatoes (Winkler 2011). No literature could be found of nutritional genetic modifications of wheat, but Zn has been used in many nutritional genetic modification experiments including crops and fruits such as bananas, sorghum, rice and cassava. Nutritional genetic modifications also include research on ways to decrease the presence of substances, such as phytates, which reduce the bio availability of essential nutrients in crops and other plant species (Winkler 2011). Winkler (2011) feels that nutritional genetic modifications would only become successful if farmers accept these new cultivars. This can only be achieved if these genetically modified crops hold advantages for the farmer such as improved yields, better disease resistance etc.

2.6.4 Seed priming

Seed priming is a process where seeds are either soaked in water, or any other solution that contains nutrients before being planted to initiate germination (Singh et al. 2015). Water scarcity is becoming a great concern in all farming communities and some have therefore started to make use of seed priming to reduce water use during pre-sowing of various crop species (Meena et al. 2014). This does not only lead to more sustainable use of water, but has direct benefits on the crop itself. Singh et al. (2015) states that various researchers have already proven that priming of seeds lead to a decrease in the emergence time of young plants, which have benefits such as improved competitiveness and increased resistance to disease connected to it. A study by Aboutalebian et al. (2012) found that wheat seeds primed in water, a $ZnSO_4$ solution, a urea solution and in a combination of $ZnSO_4$ and urea solution all performed better than non-primed seeds in various growth aspects such as tiller number, yield and 1000 kernel mass. Seeds primed in a 0.3% $ZnSO_4$ solution for 10 hours had higher seed [Zn] than those primed with water and those that were unprimed (Harris et al. 2008).

2.7 Why wheat zinc bio-fortification is not applied in South Africa

Wheat Zn biofortification does not yet and possibly won't play a major role in the health and economy of South Africa any time soon. This is due to the fact that fortification of food sources is currently dominating the South African market. According to The Department of Health South Africa and UNICEF South Africa (2007) it is law since 2003 for all milling companies to fortify their flour with Zn and other micronutrients. Sound scientific research proving that a large amount of other advantages comes with the use of biofortification of crops may change the current trend and methods used to fight micronutrient deficiencies in South Africa.

2.8 The influence of zinc on wheat vigour

An increase in seed vigour leads to decreased emergence time and an increase in the growth rate of seedlings (Mondo et al. 2013). An increase in wheat seed [Zn] has been associated with an increase in the vigour of wheat seedlings, which leads to secondary benefits for the seedling (Haslett et al. 2001). A study by Rengel and Graham (1995) came to the conclusion that an increase in wheat seed [Zn] leads to an increase in the growth rate of wheat root and shoot mass. This was corroborated by a later study, which showed priming to increase the [Zn] of wheat seeds (Rehman et al. 2015). They determined that an increase in seed [Zn] improved the rate at which seeds germinate as well as stand density (Rehman et al. 2015). These experiments are proof that Zn has a positive influence on the vigour of wheat. Other advantages associated with improved wheat vigour include improved stand densities and improved tolerance to drought (Carvalho et al. 2012). These advantages can lead to improved yields as a result of increased competitive qualities of such seeds (Sardana et al. 2016). Sardana et al. (2016) determined that crops can even out-compete herbicide resistant weeds if they have any of the above-mentioned characteristics. An example is where barley is more competitive to annual ryegrass than other cereal species due to the fast root growth rate during seedling growth stages. The ability of a crop to out-compete weeds for resources would again lead to various other environmental and health benefits. These benefits occur mainly due to decreased herbicide use, decreased mechanical weed removal, and improved production by those in rural areas where subsistence farmers for example do not have the money or ways to access these important herbicides (Sardana et al. 2016).

In South Africa it is therefore of more interest to investigate the positive effect of higher seed [Zn] on wheat seed and seedling vigour and the potential increase in competitive ability of wheat. Increased competitive ability can possibly be utilised in an integrated management program on herbicide resistant ryegrass (*Lolium* spp.).

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CHAPTER 3

The influence of seed zinc concentrations on viability, vigour and growth of wheat

3.1 Introduction

Seed germination and emergence from the soil is affected by a wide range of abiotic factors. Ghaderi-Far et al. (2010) determined that soil moisture, pH, soil and environmental temperature and planting depth had an influential effect on the germination and emergence success of yellow sweet clover (*Melilotus officinalis*). Germination and emergence is not only affected by these abiotic environmental factors, but also by the nutrient composition of seed, production techniques used and management of the fields that a crop is grown in. Germination and emergence success also directly affect yield and inset costs by farmers and governments. Germination and emergence success is stated as “critical stages” in a plants life cycle by Singh et al. (2015). Singh et al. (2015) further explained that improved germination and emergence: “increases competitiveness against weeds, tolerance to drought periods, yield and avoids the time-consuming need for re-sowing that is costly too”.

A study by Cakmak (2008) determined that higher zinc concentrations [Zn] in the seeds of wheat can lead to improved seed viability and seedling vigour. Viability is defined as the ability to live/germinate under optimal growth conditions, while vigour can be described as the ability of a seedling to successfully grow under stressed or sup-optimal growth conditions (Collins English dictionary 2017). An improvement in both these traits therefor leads to improved germination and emergence success by such seeds/seedlings. Cakmak (2008) further explained that this would lead to other benefits such as improved disease resistance and tolerance to other non-living disturbances, decreasing sowing rates and benefits to the health of humans and animals that consume these crops. The findings of Cakmak (2008) therefor correspond with those of Singh et al. (2015).

Ram et al. (2015) found that more than 50% of soils on which cereals are produced are low in zinc (Zn) or Zn deficient while Bhutta et al. (2014) came to the conclusion that more than 80% of cultivated soils in India are Zn deficient and that this is one of the main causes of Zn deficiencies in Indian women and children. Zinc deficiencies in soils mainly occur due to continued cereal monoculture practices and in specific wheat monoculture practices. Other negative effects of this method of farming include decreased growth, yield and seedling vigour of crops planted on these soils (Marler and Wallin 2006).

Wheat is one of the top 5 staple crops (Allen et al. 2006), consumed on a global scale and it is grown in more climates than any other staple crops including maize and rice (Anonymous 2008). Ozturk et al. (2006) found that [Zn] are very high in wheat seeds during germination, which may indicate that Zn plays an important role during germination of wheat. Anonymous (2004) did a study on the germination success of wheat on Zn deficient soils and came to the conclusion that germination of wheat is positively influenced as [Zn] in the soil increase.

Knowledge on the effects of changes in seed [Zn] on wheat germination and establishment can therefore be beneficial for both developed and developing countries. An increase in seed [Zn] may improve vigour of those seeds. This in turn, may lead to secondary advantages such as increased emergence rates of seeds which according to Clark (2007), would not only lead to a longer growth season (larger yields possibly), but could possibly also lead to improved soil protection and weed suppression by the planted crop.

The aim of the study was therefore to determine the effect of wheat seed [Zn] on germination (vigour and viability), as well as the influence thereof on vegetative and reproductive growth of wheat.

3.2 Methods and materials

3.2.1 Experimental site

Three experiments were conducted at the Welgevallen experimental farm in Stellenbosch under controlled environmental conditions. A germination chamber running at a constant temperature of 20ºC without a light source was used for the germination experiments (viability and vigour experiments) while a glasshouse running at an average temperature of 18/24 ºC night/day was used for the experiment investigating vegetative and reproductive growth of wheat (Wheat vegetative and reproductive growth experiment).

3.2.2 Experimental procedures

3.2.2.1 Wheat viability experiment

Seed batches of the same wheat cultivar (SST 027) with different [Zn] (38 mg kg^{-1}, 49 mg kg^{-1}, 57 mg kg^{-1}, 59 mg $kg^{-1}$and 61 mg kg^{-1}) were exposed to optimal germination conditions whereafter germination rates and percentages were calculated. The seed batches with different concentrations of elements used in the five different treatments were sourced from a separate field trial conducted in 2015 that was funded by the HarvestPlus organisation (See Tables 3.1 and 3.2). The experiments were done to determine if changes in wheat seed [Zn] have a significant influence on wheat viability. Twenty seeds of each treatment (different wheat seed [Zn]) were placed onto two filter papers in a 9-mm diameter Petri dish. The Petri dishes were placed into polyethylene bags to reduce water loss via evaporation and placed into a germination cabinet. The seeds received no nutritional substances, but the filter papers were kept moist with deionized water (dIH_2O) throughout the trial. Petri dishes were inspected daily to record the number of germinated seeds. A seed was considered germinated when the radicle protruded 1 mm from the embryo. The first five seeds that germinated from each replicate were moved into an open Petri dish, containing deionized water and left to grow for an additional five days under glasshouse conditions. Root and coleoptile lengths were recorded by making use of a ruler to determine if different seed [Zn] had a significant influence on the early growth of wheat. Each treatment was replicated ten times, meaning that a total of 200 seeds where used per treatment. The trial was discontinued when no new seeds germinated for 3 consecutive days.

Germination percentage was calculated for each replicate by using the following equation:$GP\ (\%) = \frac{g}{20} * 100$ where g is the number of germinated seeds and 20 the total number of seeds used per replicate (El-Shaieny 2015). The germination rate (*GR*) for each replicate was calculated by using the following equation:$GR = \sum_{i=1}^{k} \frac{ni}{Di.ni} \times 100$ where k = final day, D_i = day of recording, n_i = number of seeds germinated on day D_i and i = day 1 to day k (Pieterse and Cairns 1986).

3.2.2.2 Wheat vigour experiment

The second experiment was focused on the influence of different wheat seed [Zn] on wheat seed vigour. Seeds were subjected to an accelerated ageing test before they were placed into a germination cabinet and exposed to the same optimal germination conditions as previously described (Mavi et al. 2010). The accelerated ageing protocol that was used follows: All seeds of each treatment were placed onto a sieve suspended in a plastic container containing some water (accelerated ageing chamber). The seeds were suspended high enough to prevent any direct contact between them and the water as this would have led to germination. These plastic containers were then closed with a tight-fitting lid to ensure 100% humidity inside and placed into a growth chamber at 42 ºC constant temperature for 72 hours. The same germination procedure was then followed as in the wheat viability experiment.

3.2.2.3 Wheat vegetative and reproductive growth experiment

The same seed with different [Zn] or “treatments” as in the 1st and 2nd experiments were used in the last experiment. During this experiment seeds were planted in a sandy substrate at an approximate depth of 1.5 cm and left to grow until physiological maturity. The goal of this experiment was to determine if there were any significant influences on germination and growth of wheat at different growth and reproductive stages due to different seed [Zn].

Six seeds with the same [Zn] were planted together in a pot under glasshouse conditions with an average day/night temperature of 24 and 18 ºC, respectively. A total of ten pots (each representing one replicate) were used for each of the five treatments. A total of 50 pots were thus used for the third experiment. A sandy growth medium was used instead of a loamy soil as it is more porous, which improves water uptake by the soil. A drip irrigation system was installed to water the pots. Each pot received approximately 120 ml of tap water mixed with a basic nutrient mix per day.

Emergence percentages and rates of the different treatments were recorded on a daily basis to determine seedling vigour. A seed was considered emerged when the coleoptile was clearly visible above the soil surface. The emergence percentage and rate recording of the experiment was discontinued after three consecutive days of no new seedling emergences. Some seedlings were then removed until only two remained per pot. The coleoptile lengths and dry weights of the removed seedlings were recorded as additional information on how the different seed [Zn] influenced growth rates and biomass. Coleoptile lengths were measured from the base of the aboveground parts along the tallest leaf on the plant by making use of a ruler. Dry mass was determined by using an electronic balance after the seedlings were placed into an oven, to dry for 48 h at temperatures of 72 ºC.

The remaining two plants were left to grow until booting stage (when the developing head becomes visible within the sheath). One of the two plants were then removed to record vegetative growth parameters including stem length, leaf area and tiller number. The remaining plant was left to fully mature. Vegetative growth parameters and yield was determined at the end of the ripening stage. Vegetative parameters included stem length, leaf area and tiller number while yield was determined by counting the number of grains in each ear.

3.2.3 Data analysis

All data was analysed by making use of Statistica 13 and Microsoft Excel. Microsoft Excel was used to record all the initial data and to group it correctly. Statistica 13 was then used to determine if there were significant differences in germination rate, germination percentage, coleoptile lengths and radicle lengths between the different treatments. Various one-way ANOVA tests were used to determine if significant differences occurred within each of the recorded parameters. Parameters with p-values smaller than 0.05 are considered to differ significantly between different treatments. Some correlation analyses were also done by means of Statistica 13 to determine whether positive or negative relationships occur between different growth parameters.

3.3 Results and discussion

The seeds from the different seed batches differed significantly in terms of Zn, iron (Fe) and aluminium (Al) concentrations ($p \leq 0.05$). The largest difference was found within the [Zn] (Table 3.1). We therefore assume that one or more of these three nutrients (Zn, Fe and Al) would be the reason if differences occurred in germination and growth, as environmental factors were controlled and remained the same throughout the different experiments and between different treatments.

Table 3.1: The p-values obtained when the concentrations of different elements were compared between the different seed batches used as treatments.

Element	P-value
Zinc -Zn (mg kg^{-1})	0.00
Iron -Fe (mg kg^{-1})	0.013
Copper -Cu (mg kg^{-1})	0.81
Manganese -Mn (mg kg^{-1})	0.10
Aluminium -Al (mg kg^{-1})	0.04
Calcium -Ca (mg kg^{-1})	0.96
Potassium -K (%)	0.38
Phosphor -P (%)	0.83
Sulphur -S (%)	0.56
Magnesium -Mg (%)	0.63

The [Zn] within the different wheat seed batches were compared to one another to determine which [Zn] differed significantly from one another (Table 3.2). The [Zn] of treatments one and two differed significantly from the other three treatments and from each other, while treatments three, four and five did not differ significantly from one another.

Table 3.2: The [Zn] of the seed batches used in the different treatments.

Treatment	{1} 37.545	{2} 48.560	{3} 57.245	{4} 58.966	{5} 61.477
Zn 38 mg kg^{-1}		0.000337	0.000001	0.000000	0.000000
Zn 49 mg kg^{-1}	0.000337		0.002425	0.000560	0.00072
Zn 57 mg kg^{-1}	0.000001	0.002425		0.482097	0.096502
Zn 59 mg kg^{-1}	0.000000	0.000560	0.482097		0.309306
Zn 61 mg kg^{-1}	0.000000	0.00072	0.096502	0.309306	

3.3.1 Wheat viability experiment

No significant differences were found when the germination rates of the different treatments were compared ($p \geq 0.05$) (Figure 3.1). This indicates that the speed at which seeds germinate per day are similar between the different treatments. The combination of optimal growth conditions and good quality seed is probably the reason for the similarity in germination rates between different treatments. A similar study by El Rasafi et al. (2016) came to the conclusion that toxic concentrations of Fe in a growth medium has a negative effect on the germination rate of wheat while this wasn't true for Zn.

Germination percentage differed significantly between different treatments ($p \leq 0.05$). The germination percentages of treatments Zn 61 mg kg^{-1} and Zn 59 mg kg^{-1} were significantly higher than that of the other three (Figure 3.1). It was assumed that the differences in either Zn, Fe or Al concentrations were the cause of differences between the different treatments or that the ratios between these three nutrients could have been the cause thereof.

It was unlikely that Al had a significant influence on the germination percentages, since the variation in aluminium concentrations do not correlate with the variation in germination percentages for example, treatment Zn 49 mg kg^{-1} had the highest concentration of aluminium in the seed, but the second lowest germination percentage (Table 3.3). A study by Tamás et al. (2004) looked at the influence of increased Al concentrations on the germination process of barley seeds during the first 48 hours. They concluded that an increase in Al had a negative influence on germination of barley through root and coleoptile growth inhibition. Their findings do not support the findings of our study, as we found that the treatment containing the smallest concentration of Al (Zn 57 mg kg^{-1}) also had the weakest germination percentage (Table 3.3 and Figure 3.1).

The improved germination percentages from treatment Zn 38 mg kg^{-1} to treatment Zn 61 mg kg^{-1} could partially be due to the interaction and ratio between Fe and Zn. This would definitely have had some influence on the germination percentage since it has been proposed that the interactions between different nutrients within plants and in the soil where they are cultivated are of great importance for successful germination and growth and it has a direct effect on plant quality and yield (Fageria 2001). No literature could be found on what the optimal ratio of Fe: Zn is within wheat seeds but a chart (Mulder's chart) indicating the interaction between different elements in plants and soils showed that there is an antagonistic relationship between the two. The Mulders chart can be accessed via the following link: (http://cultivacegrowth.com/wp-content/uploads/2016/01/MuldersChart.pdf).

Table 3.3: The differences in Zn, Fe and Al concentrations of the different treatments.

Treatment	Zinc concentration	Iron concentration	Aluminium concentration
Zn 38 mg kg^{-1}	37.545	34.084	2.1442
Zn 49 mg kg^{-1}	48.560	36.526	2.4148
Zn 57 mg kg^{-1}	57.245	36.934	1.7173
Zn 59 mg kg^{-1}	58.966	37.374	1.9375
Zn 61 mg kg^{-1}	61.477	39.707	2.0444

A previous experiment by El Rasafi et al. (2016) determined that wheat seed germination actually decreased significantly as the Fe concentration in the growth medium increased from 500 mg kg^{-1} to 750 mg kg^{-1}. They further found no significant influence on germination percentage of wheat as the [Zn] in the growth medium increased from 0 mg kg^{-1} to 1000 mg kg^{-1}. The conclusion was that neither Fe nor Zn had a positive effect on seed germination, but only iron had a negative influence on wheat seed germination. Germination success is very much dependent on the ratios of different elements and whether an element is present in excessive or limited quantities. Yasmeen et al. (2015) has found a positive response of wheat germination to Fe. They determined that wheat germination was positively affected due to the presence of Fe nanoparticles in the growth medium of the wheat seeds.

Zinc probably has a larger influence on wheat germination than does Fe, which is why we conclude that an increase in Zn within wheat seeds do lead to improved germination percentages. One reason for rejecting the possibility of Fe being the most important element in wheat germination is the fact that it plays a more important role in photosynthesis and nitrogen fixing. Both photosynthesis and nitrogen (N) fixing only occurs after a seed has germinated (Kabata-Pendias 2010). Similar observations about the use of Fe in plants were also made in a study by Yasmeen et al. (2015).

Frassinetti et al. (2006) found that Zn is important for normal germination, development and growth of plants. Miransari and Smith (2014) explained that seed germination occurs due to seed maturation. Seed maturation basically occurs due to water uptake by the seed, which leads to an increase in protein storages in the seed. Protein translocation takes place after this, leading to the activation of enzymes known as proteinases. This according to Miransari and Smith (2014) leads to the "mobilization" of storage proteins into the radicle and coleoptile. The radicle and coleoptile then start to grow, activating germination. The germination process is completed as soon as the radicle becomes visible to the human eye. Kabata-Pendias (2010) states that Zn forms an integral part of proteinases (which plays the most crucial role in seed development) and we therefore believe that increases in wheat seed [Zn] do have a significant positive influence on germination percentage.

Ozturk et al. (2006) determined that [Zn] is particularly high in the embryo and aleurone parts of a wheat seed during the start of germination. The embryo is found within the germ and it is from this area where the radicle and coleoptile develop. This could mean that the higher [Zn] within the seeds of Zn 61 mg kg^{-1} and Zn 59 mg kg^{-1} were located in the embryo, which therefore improved germination as radicle lengths were used to determine when a seed had germinated. The study by Yasmeen et al. (2015) reported on the positive influence that Zn priming has on barley, which is considered as proof that an increased seed [Zn] would lead to improved germination percentages.

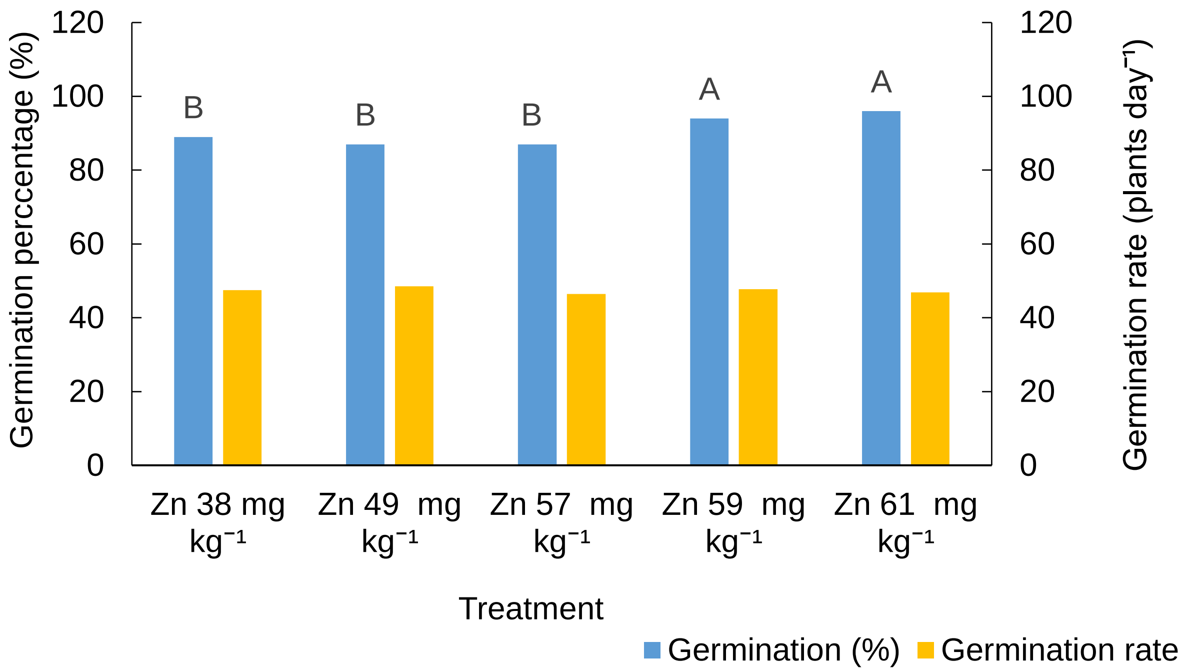

Figure 3.1: The germination percentages and relative germination rates for each of the five different wheat seed batches with different [Zn]. Values that differ significantly at p = 0.05 are indicated with different letters.

Significant differences were found in both coleoptile and radicle lengths between different seeds ($p \leq 0.05$) (Figure 3.2). No relationship was observed between germination percentage, germination rate and early growth of these wheat seedlings due to r- values of 0.10170 and 0.12679 respectively. Reasons for this statement include that treatment Zn 49 mg kg^{-1} had the lowest germination percentage (Figure 3.1) but the second tallest radicle and coleoptile lengths (Figure 3.2). Treatment Zn 59 mg kg^{-1} on the other hand had the second highest germination percentage (Figure 3.1) but the shortest radicle and coleoptile lengths (Figure 3.2). Our findings are supported by a previous study that looked at the influence of different priming substances on wheat seed germination and growth. Zanjan and Asli (2012) found no significant difference in germination percentage of seeds primed with water, pyridoxine and non-primed seeds while significant differences were found between the coleoptile and radicle lengths of the differently treated seeds. We can therefore conclude that an increase in germination percentage or germination rate does not necessarily lead to an increase in the growth rate of a wheat seedling. El Rasafi et al. (2016) determined that [Zn] of up to 1000 mg kg^{-1} in a growth medium where wheat seeds are placed into had no influence on germination percentage and rate while having negative influences on coleoptile and radicle growth.

Figure 3.2 indicates that there is a positive relationship between coleoptile and radicle lengths, due to the fact that there are similar changes in radicle and coleoptile lengths (r= 0.69673). This finding is not supported by other studies that looked at the relationship between radicle and coleoptile growth. A study by Djébali (2012) determined that some priming substances either had a positive or negative influence on either the growth of coleoptiles or radicles alone. This may indicate that there is a negative relationship between coleoptile and radicle growth. The same was found by El Rasafi et al. (2016) who determined that there is a negative relationship between coleoptile and radicle growth of wheat when exposed to different concentrations of cadmium (Cd), Fe and Zn, meaning that an increase in the growth of one would inhibit the growth of the other to some extent.

There is no clear trend in the response of the coleoptile and radicle lengths with regard to the [Zn]. The Zn 61 mg kg^{-1} treatment resulted in the longest coleoptiles and radicles, whereas the Zn 59 mg kg^{-1} treatment resulted in some of the shortest coleoptiles and radicles.

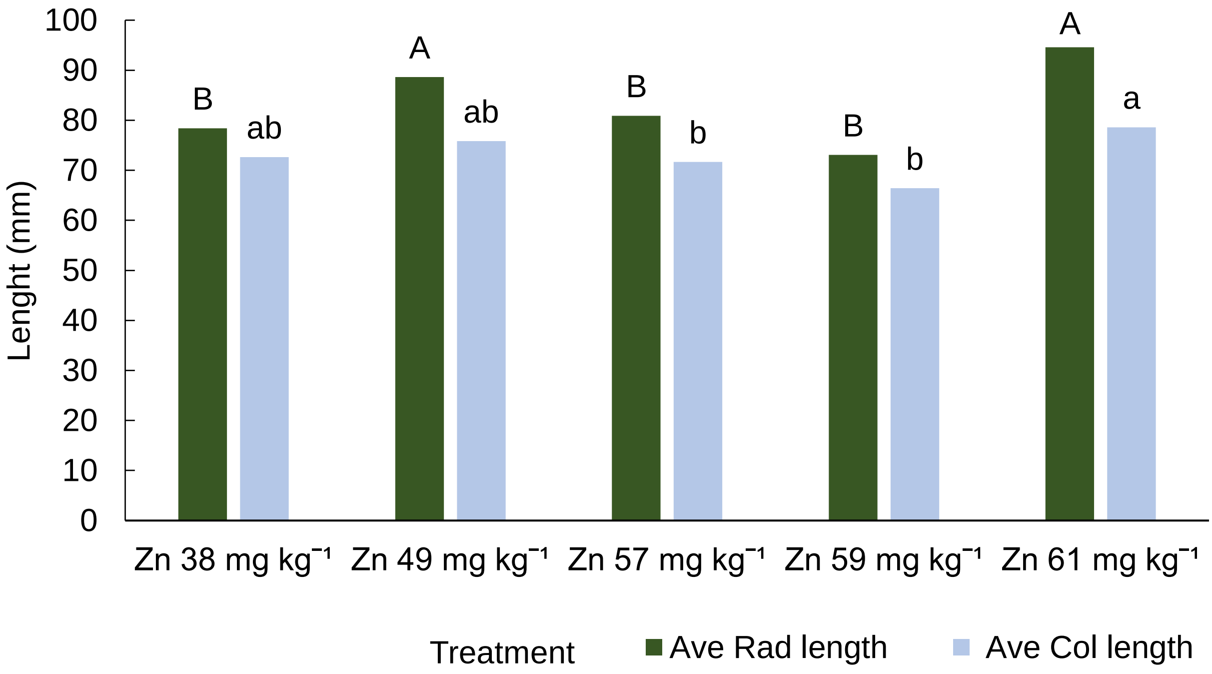

Figure 3.2: The differences in radicle and coleoptile lengths of the different treatments five days after germination of the last seeds. Values that differ significantly at p = 0.05 are indicated with different letters.

3.3.2 Wheat vigour experiment

The wheat vigour experiment did not support our hypothesis that an increase in wheat seed [Zn] would lead to improved vigour. The data recorded, suggests that there were significant differences in both germination percentage and rate ($p < 0.05$) (Figure 3.3). Unfortunately, no obvious trend was present in terms of germination percentage and we therefore cannot conclude that an increase in [Zn] improves germination percentage after seeds have been exposed to an accelerated ageing test. It does, however appear as if germination rate is negatively influenced by higher [Zn]. A study by Stanković et al. (2010) came to the conclusion that germination success decreased as the concentration of zinc chloride ($ZnCl_2$) increased. Stanković et al. (2010) found that wheat seed germination success was 91% when not exposed to $ZnCl_2$ and decreased gradually as the $ZnCl_2$ concentration increased. The reason for this decrease could be linked to the increased chloride (Cl_2) concentration rather than the Zn. Rehman et al. (2015) who looked at different Zn priming substances also determined that $ZnCl_2$ decreases germination success at a certain concentration on and linked this negative effect to the toxic effects of Cl_2 on photosynthesis and respiration. In contrast, to these findings El Rasafi et al. (2016) determined that Zn solutions that differ in concentration had no influence on wheat seed germination percentage and rate.

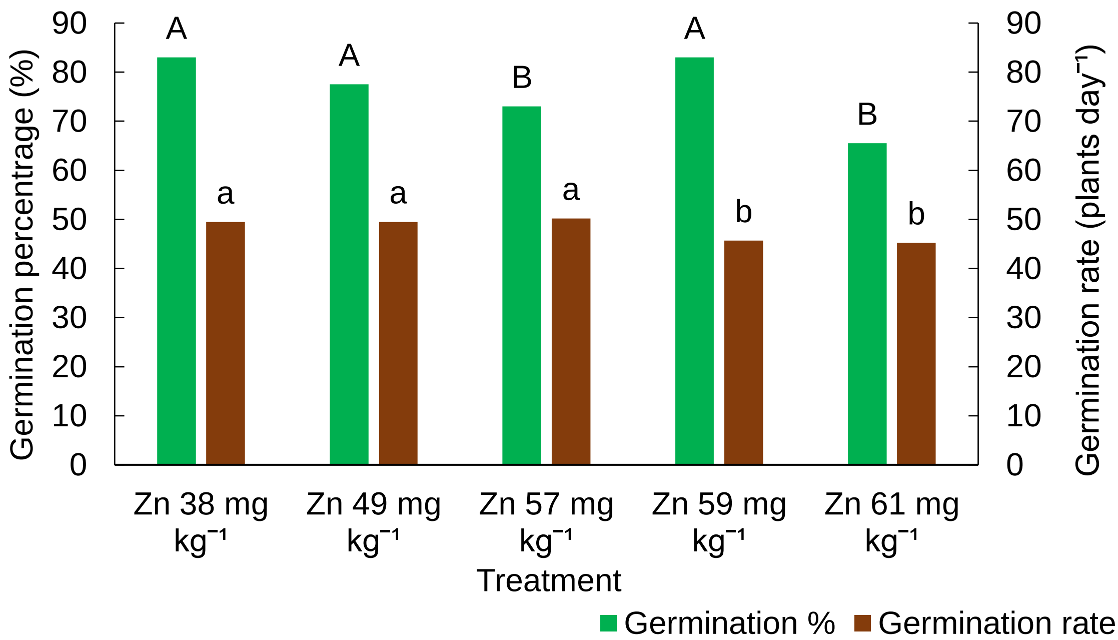

Figure 3.3: The germination percentage and rate data of the seeds that were exposed to the accelerated ageing test. Values that differ significantly at $p = 0.05$ are indicated with different letters.

Stanković et al. (2010) found a decrease in the coleoptile and radicle lengths of wheat as the $ZnCl_2$ concentration increased. This is supported by the findings of Auwal et al. (2016) who determined that an increase in $ZnCl_2$ lead to smaller roots and stems, but this was not the case in our study (Figure 3.4). No clear trend is present when looking at the coleoptile and radicle lengths of our study. This indicates that the increased [Zn] of the wheat seeds did not affect coleoptile and radicle growth rates positively or negatively although significant differences were found between the different treatments ($p \leq 0.05$). Treatment Zn 61 mg kg^{-1} had the tallest average radicle length while treatment Zn 38 mg kg^{-1} had the shortest average radicle length. The tallest average coleoptile length was found in treatment Zn 57 mg kg^{-1} while treatment Zn 38 mg kg^{-1} again had the shortest average coleoptile length.

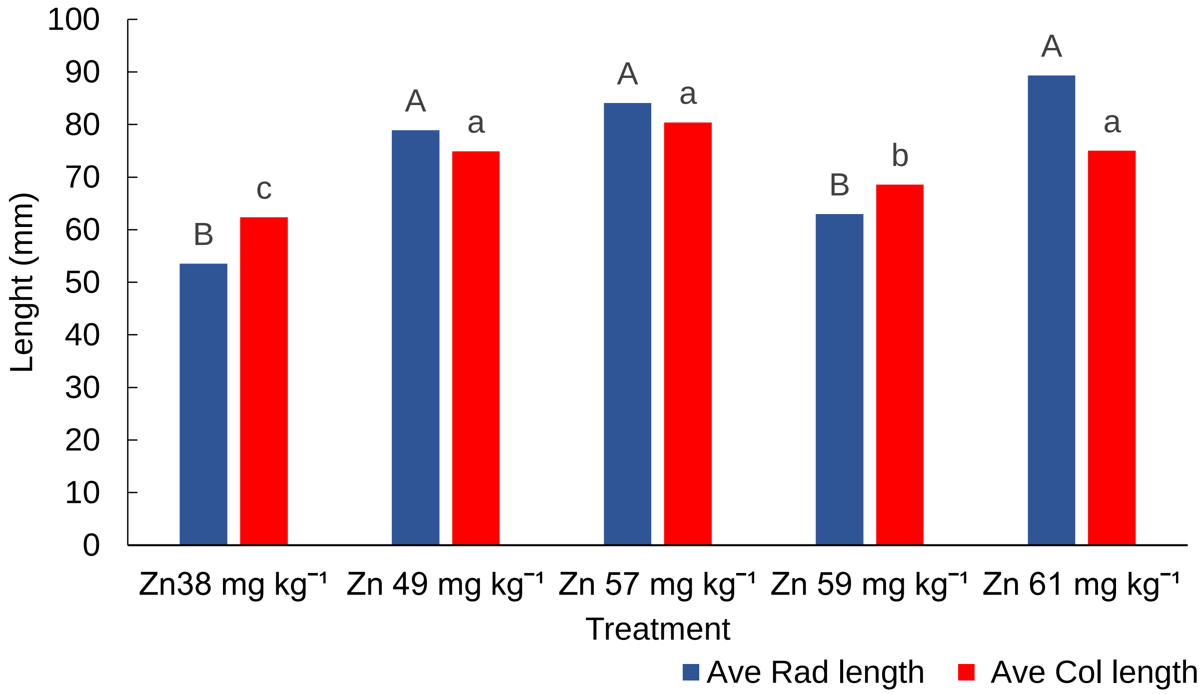

Figure 3.4: The differences in coleoptile and radicle lengths when the accelerated ageing test seeds were left to grow for five days after they have germinated. Values that differ significantly at $p = 0.05$ are indicated with different letters.

Contamination by a fungus was observed in several of the petri dishes of the wheat vigour experiment. We did not at the time record in which petri dishes contamination occurred. It is therefore recommended that this specific experiment should be repeated and data thereof should thus be interpreted with caution. The experiment could not be repeated immediately due to a lack in sufficient amounts of the particular batches of seed.

3.3.3 Wheat vegetative and reproductive growth experiment

A large number of parameters were studied during this experiment, in an effort to determine if changes in seed [Zn] have an influence on the vegetative and reproductive growth of wheat grown under controlled environmental conditions. The experiment was divided into three sections each looking at the influence of different seed [Zn] on different growth stages of wheat. No conclusive data was recorded and we believe that no significant changes were observed in most parameters due to the optimal growth conditions.

3.3.3.1 Parameters investigated from planting until 18 days after plant

No significant differences were observed in emergence percentage and rate between the different treatments ($p \geq 0.05$) (Table 3.4). The emergence percentage and rate trends for this experiment was however very similar to germination percentage and rate of the viability experiment (Figure 3.5), which support our conclusions that an increase in seed [Zn] does improve germination percentage although it was not demonstrated conclusively during this experiment. Optimal germination conditions in the greenhouse may have led to the similarities in emergence rate and percentage.

Table 3.4: P-values of emergence percentage and germination rate in the wheat vegetative and reproductive experiment.

Parameter	P-value
Emergence percentage	0.082302
Emergence rate	0.837827

Anonymous (2004) found that wheat germination is improved as the [Zn] in the soil increases. This means that Zn is either absorbed by the seeds or utilised in some other way to improve germination success. An increase in germination success due to higher [Zn] in the soil therefor indicates that Zn plays a crucial role during the germination of wheat seeds. Rehman et al. (2015) determined that an increase in Zn due to $ZnSO_4$ and $ZnCl_2$ priming did improve both germination percentage and rate of wheat, but equilibrium was reached for both of these substances, most probably where the substance becomes toxic to the seeds or seedlings. We did not come to a similar conclusion during this experiment but we believe that our findings differ from their findings due to the fact that we looked at the [Zn] of the seeds while they studied the influence of soil [Zn].

We also investigated the similarities in germination/emergence percentage and rate between the viability and growth experiments (Figure 3.5) Germination and emergence percentages were very similar between the two experiments. The emergence rate of the growth experiment on the other hand was much slower than the germination rate of the viabilty experiment.

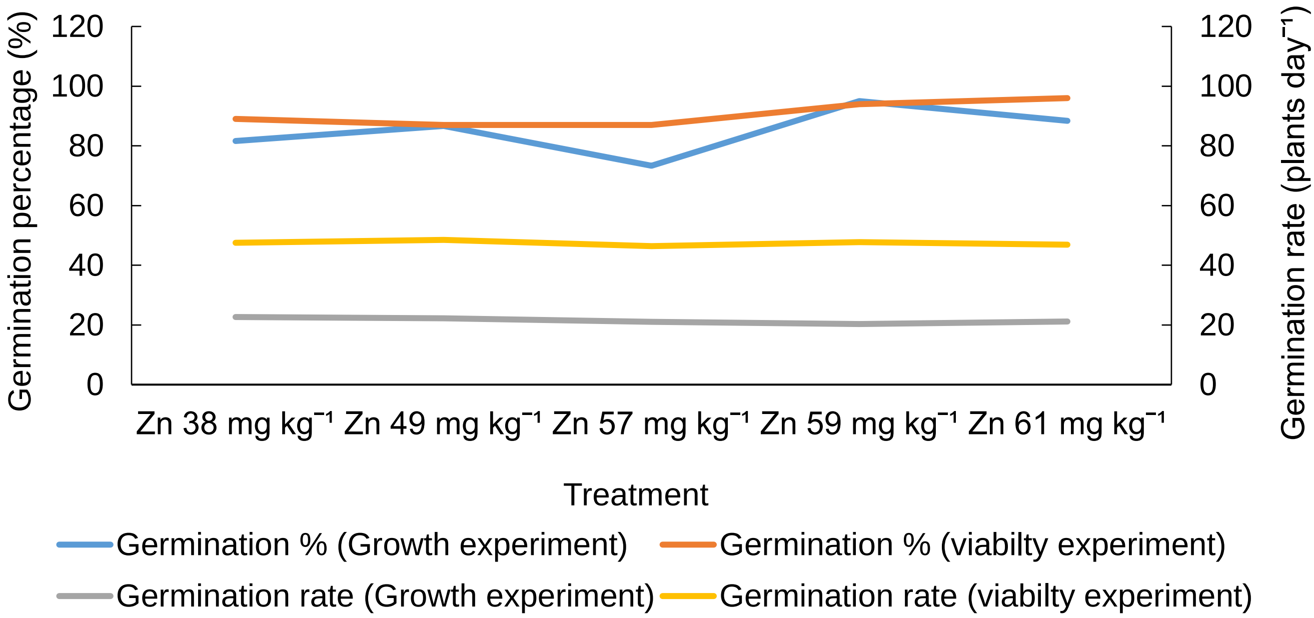

Figure 3.5: The similarities in germination percentage and rate between the viability and growth experiments that were conducted during this study.

An obvious explanation for the slower emergence rate of the growth experiment is the fact that it took longer for the coleoptiles to become visible above the soil surface versus the radicle being immediately visible in a petri dish.

3.3.3.2 Parameters investigated at booting stage

No significant differences were recorded between treatments in any of the parameters investigated ($p \geq 0.05$) (Table 3.5). We again suspect that the optimal growth conditions are the reason for the similarities between the different treatments. A study by Cakmak et al. (2010) found that wheat seeds with a [Zn] of between 11 and 22 mg kg^{-1} are considered as Zn deficient while Zn sufficient seeds have concentrations of Zn averaging between 28 and 58 mg kg^{-1}. All seeds used by us had [Zn] of between 38 and 61 mg kg^{-1} which according to Cakmak et al. (2010) are then Zn sufficient seeds. The combination of optimal growth conditions and Zn sufficient seeds are probably the main reason for the similarities found between the various treatments.

Table 3.5: The p-values of the various parameters investigated during the booting growth stage.

Parameter	P-value
Number of heads per plant	0.490246
Leaf area per plant	0.319740
Dry mass per plant	0.056786
Stem length per plant	0.372614

3.3.3.3 Parameters investigated after harvest

No significant differences occurred between the different treatments when the number of tillers, dry mass of plants, seeds per plant, yield and 1000 kernel mass were compared ($p \geq 0.05$) (Table 3.6). Similar to the parameters recorded during the booting stage (see previous section), no significant differences occurred between treatments, possibly due to the optimal growth conditions.

Table 3.6: The p-values of the various parameters investigated after harvest.

Parameter	p-value
Number of tillers	0.953128
Dry mass	0.142037
Number of seeds	0.524496
Yield weight	0.207576
1 000 kernel mass	0.480724

3.4 Conclusion

Some evidence has been found during these trials that support the notion that Zn may play a role in wheat growth, particularly during early emergence, i.e, germination. Wheat seed [Zn] is considered sufficient when in excess of 28 mg kg^{-1} and this minimum amount would lead to optimal growth under greenhouse or optimal growth conditions. This may have compromised results interpretation in this study, since the lowest seed [Zn] was 38 mg kg^{-1}. Recommendations to improve the validity of this study include that, these experiments should be repeated under normal field conditions where other factors such as planting depth, competition from weeds, water stress etc. may have a significant impact on the germination, growth and yields produced by seeds with different nutrient compilations.

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CHAPTER 4

The influence of a higher zinc concentration on the vigour of wheat seed, when subjected to environmental stressors

4.1 Introduction

Wheat is the only cereal produced on all 7 continents and is the second most commonly consumed cereal grain in Asia (Anonymous 2008; Makgoba 2013). Approximately 217 million hectares (ha) of agricultural land, both in developed and developing countries, are cultivated with wheat leading to the production of 651 million tons per annum (Koehler and Wieser 2013). Wheat is not only a cereal crop with inherently low zinc (Zn) availability to the plant (Cakmak 2008), but more than 50% thereof is produced on Zn deficient soils (Ram et al. 2015). This could have detrimental effects on the growth of wheat, as Zn plays a crucial role in the synthesis of auxins, a well-known plant growth hormone (Sauchelli 1969).

Evidence has demonstrated that increased zinc concentrations [Zn] via foliar sprays can increase wheat vigour (Haslett et al. 2001). Improved vigour has several benefits, including improved chances of survival of that plant due to a smaller risk of damage that can be caused by environmental factors such as wind or hail, increased resistance against pests and diseases due to quicker development of defence mechanisms such as trichomes and waxes and lastly increased competitor exclusion qualities, which impairs or slows the growth and establishment of weedy competitors (Mondo et al. 2013). Excessive [Zn] in the seeds of wheat could also be beneficial to farmers through improved production, who may subsequently earn more from sales, increased food availability and a decrease in global malnutrition due to consumption of more nutritious foods (Cakmak 2009).

The main aim of this study was to compare the influence of different planting depths, different priming methods and varying levels of competition stress on wheat seeds with different seed [Zn]. We hypothesised that wheat seeds with a higher [Zn] would germinate and grow better under sub-optimal conditions than those with a lower [Zn].

4.2 Methods and materials

4.2.1 Methods

Three pot experiments were conducted under greenhouse conditions at Welgevallen experimental farm in Stellenbosch. The first experiment investigated the influence of different planting depths and different wheat seed [Zn] on the establishment and early growth of wheat, while the second experiment investigated the influence of different seed priming methods on wheat-ryegrass interactions in a replacement competition experiment. The third experiment, very similar to the second experiment, was an addition experiment where the number of wheat plants remained the same in pots while the number of ryegrass plants increased. A water stress factor was also implemented during the third experiment. The wheat and rye grass seeds were planted at the same time in the second and third experiments thus simulating conditions where wheat would be planted prior to the first rains.

4.2.1.1 Planting depth experiment

Wheat seeds containing “low” (38 $mg.kg^{-1}$) and seeds containing “high” (61 mg kg^{-1}) [Zn] were planted in different pots at four different depths. Seeds were planted 1 cm, 2 cm, 4 cm and 6 cm below the soil surface and left to germinate. Eight different treatment combinations are thus present in this experiment, arranged as a 2x4 factorial Completely Randomized Design (CRD), each being replicated four times. Ten wheat seeds were planted per replicate, resulting in a total of 160 seeds with a low [Zn] and 160 with a high [Zn] being used. A ruler was used to make markings at different depths on the inside of each pot. The pot was filled with soil up to the mark. After this, ten seeds were sown onto the soil and the pot was then filled to the top. A mixture of sand and gravel was used as a planting substrate to ensure that water would infiltrate and drain well. The pots received water by hand on a daily basis, up to soil water capacity, as wheat seed germination is dependent on sufficient amounts of soil moisture (Acevedo et al. 2002).

The number of seedlings that have emerged from the soil surface were recorded each day to determine emergence success per replicate. Emergence rates and percentages where determined separately for each replicate. Emergence percentage was determined by using the following equation: $GP\ (\%) = \frac{g}{10} * 100$ where g is the final number of emerged seedlings and ten the total number of seeds used per replicate (El-Shaieny 2015). Emergence rate was determined by adapting the formula of $GR = \sum_{i=1}^{k} \frac{ni}{Di.ni} \times 100$ where k = final day, D_i = day of recording, n_i = number of seedlings emerged on day D_i and i = day 1 to day k (Pieterse and Cairns 1986). The trial was discontinued when no new seedlings emerged for three consecutive days. The soil of each pot was then carefully sieved to determine the number of germinated seeds that did not emerge. The same mathematical formula as used to determine emergence percentage was then used to determine germination percentage.

4.2.1.2 Wheat and ryegrass competition replacement experiment

The second experiment investigated how different wheat seed [Zn] and different priming substances (Seed treatments) influence the competitive ability of wheat with rye grass in a replacement (substitutive) series experiment. Five different seed treatments where combined with 5 different competition replacement sub treatments. Seed treatments consisted of: 1) seeds with a [Zn] of 38 mg kg^{-1} primed in 100 ml of distilled water, 2) seeds with a [Zn] of 61 mg kg^{-1} primed with distilled water, 3) seeds with a [Zn] of 61 mg kg^{-1} primed with a 0.3% zinc sulphate ($ZnSO_4$) solution, 4) seeds with a [Zn] of 61 mg kg^{-1} primed with a 0.9% urea solution and 5) seeds with a [Zn] of 61 mg kg^{-1} primed with a compound $ZnSO_4$and urea solution. To prepare the urea and $ZnSO_4$solutions 100 ml of distilled water were used. The compound $ZnSO_4$ and urea solution were prepared by mixing 50 ml of the 0.9% urea solution and 50 ml of the 0.3% $ZnSO_4$solution. The seeds were primed for eight hours in petri-dishes at room temperature (20°C) where-after it was stored in a cool dry place until planting.

The five competition replacement sub-treatments included sowing wheat and ryegrass seeds into small 8 x 8 cm pots at a depth of 1 cm and in wheat to rye grass ratios of: 8:0, 6:2, 4:4, 2:6 and 0:8. Some seedlings were removed after three consecutive days of no new germinated seeds, to narrow the ratios down to 4:0, 3:1, 2:2, 1:3, 0:4. This was done to compensate for those seeds that never germinated and to ensure that each replicate had the correct wheat to ryegrass ratio. Twenty-five different treatment combinations are thus present in this experiment, arranged as a 5x5 factorial arranged CRD, each being replicated four times. Garden soil was used during the experiment and pots where equally watered once or twice daily depending on the overall dryness of all the pots. No extra nutrients were applied to the pots. Seeds were planted on the 5th of September 2016 and harvested on the 14th of November 2016, subsequently the dry mass (DM) was determined for each species in each replicate by cutting the plants at the soil surface and drying the aboveground parts of the plants at 60 ºC for 48 hours. The DM was thereafter determined on an electronic balance scale.

4.2.1.3 Wheat and ryegrass addition experiment

Wheat seeds harvested during a field experiment in 2015 funded by the HarvestPlus organisation on a government farm (Langgewens) in the Swartland region of the Western Cape Province, South Africa were used for this experiment. Microsoft Excel was used to randomise the outlay of the experiment. All seeds used were hand selected to ensure good physical seed condition.

Seeds with a [Zn] of 32 mg kg^{-1} and 72 mg kg^{-1} where used in this addition experiment to determine the influence of four different ryegrass population sizes on a set population size of wheat. An additional environmental stressor, water shortage/drought aspect, was also integrated into the experiment to determine the influence thereof on the growth of wheat and ryegrass. Half of the replicates of each treatment (various planting ratios) received sufficient amounts of water throughout the course of the experiment. By this is meant that the soils were kept at a constant water saturated state, while the other half only received 100 ml of water when the soil became extremely dry. The 100 ml of water was given to these pots only if the top 3 cm or more of the soil was completely dry and moisture free. We determined this by digging into the substrate. All pots had holes in the bottom and was placed into 5 L buckets. The water for the pots that received optimal amounts of water was poured into the 5 L buckets so that the soil can take up the water by capillary action throughout the entire soil profile. The pots that received the water stress treatment received the 100 ml directly, by pouring the water into the pot, rather than pouring it into the buckets, meaning that only the top soil would become water saturated. All the pots received normal tap water. A basic nutrient solution was added to the water when plants started to display deficiency symptoms to ensure that sufficient amounts of nutrients where available for the plants to perform optimally.

Pots with a diameter of 15 cm and a depth of 15 cm were used during this trial. The pots were filled with an uncontaminated gravely sandy soil up to 13 cm. The selected seeds for each pot were then randomly sowed onto the soil, where-after another layer of soil of about 1 cm was used to completely cover the seeds. All 5 L buckets where filled with 500 ml of water two days before planting to ensure that the soils were moist enough for all seeds to germinate. The water stress treatment therefor only had an influence on the growth of the germinated seedlings rather than on the germination of the seeds.

The wheat seeds with the two different [Zn] was planted at ratios of 5-0, 5-5, 5-10 and 5-20 with ryegrass. Sixteen different treatment combinations were thus present in this experiment, arranged as a 2x2x4 factorial arranged Completely Randomized Block Design (CRBD), each being replicated six times. The factors were [Zn] (high and low), moisture level (high and low) and wheat: ryegrass ratios (5-0, 5-5, 5-10 and 5-20).

The plants were left to grow for 6 to 8 weeks where-after all plants of each species were taken out of the pots, the roots were removed and the above ground parts placed into a brown paper bag and dried for 48 hours at a constant temperature of 60 ^{0}C. The dry weights were then determined by using a scale and subjected to statistical analysis to determine if there were any significant differences between the wheat and ryegrass plants of the 12 different treatments.

4.2.2 Data analysis

All data was analysed by using Statistica 13 which is a statistical program. The program was used to determine if there were significant differences in emergence rate, emergence percentage and germination percentage between the different treatments. A two-way ANOVA test was carried out to determine the influence of planting depth combined with seed [Zn] on emergence percentage and -rate. Another two-way ANOVA test and a three-way ANOVA test were used during the second and third experiments respectively to determine if there were any significant differences in wheat and ryegrass dry matter production due to the different priming methods used, presence of water stress and different wheat to ryegrass ratios.

4.3 Results and discussion

4.3.1 Planting depth experiment

No significant interaction between [Zn] and sowing depth occurred in terms of emergence % of seeds. Emergence % was not influenced by [Zn] at different planting depths ($p \geqslant 0.05$). A reason for not finding any differences in emergence success by the different seed types may be that the seeds were not planted deep enough These planting depths were chosen as most farmers plant at a depth of between 2-4 cm.

Emergence % improved significantly ($p \leq 0.05$) as seed [Zn] increased (Figures 4. 1 and 4.4), while no significant differences were found when the number of germinated seeds of the different seed types were compared ($p \geq 0.05$) (Data not shown). This indicates that wheat seeds with a higher [Zn] are more vigorous than those with a lower [Zn].

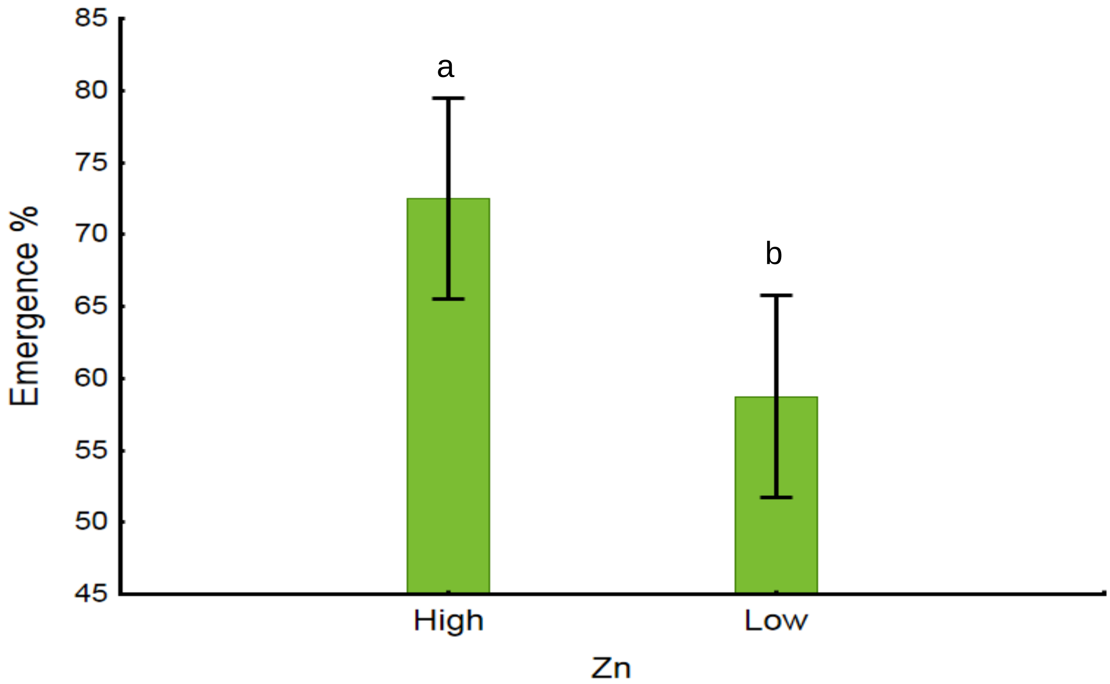

Figure 4.1: The effect of high and low [Zn] on emergence percentage of wheat seeds sown in pots. Vertical bars indicate 95% confidence intervals. Values that differ significantly at p = 0.05 are indicated with different letters.

This finding is supported by Henriques et al. (2012) who states that productivity of plants can decrease due to Zn deficiencies as a result of altered physiological and metabolic processes. This information could be important to those that live and farm in arid regions of the world, where wheat seeds are planted at depths of 20 cm below the soil surface (mostly in low precipitation areas) to ensure that they have access to optimal amounts of soil moisture (Mohan et al. 2013). One should thus consider using seeds with a higher [Zn] in regions where seeds are planted deeper to improve germination, establishment and yield. Rengel and Graham (1995) found that wheat seeds with a higher [Zn] led to the production of larger root, shoot and above ground dry mass, 3 weeks after planting, than those with a lower [Zn]. A larger and faster growing plant can more easily outcompete weeds for limited resources, which means that these plants have an improved or better vigour than those originating from seeds with a low [Zn]. Such seeds also offer a cheap option in an integrated weed management (IWM) system for better resistance against unwanted plants in farmers' fields (Andrew et al. 2015). This means that less herbicides, manpower and fossil fuels are needed to control weeds, leading to financial savings by the farmer as well as decreased air and soil pollution. These larger roots systems may also improve chances of survival when droughts occur during the seedling growth stage of the plant (Acevedo et al. 2002). Another benefit that could be gained from a larger root system includes decreased lodging of plants, which mainly occur due to strong and persistent winds or heavy rain (Rajkumara 2008).

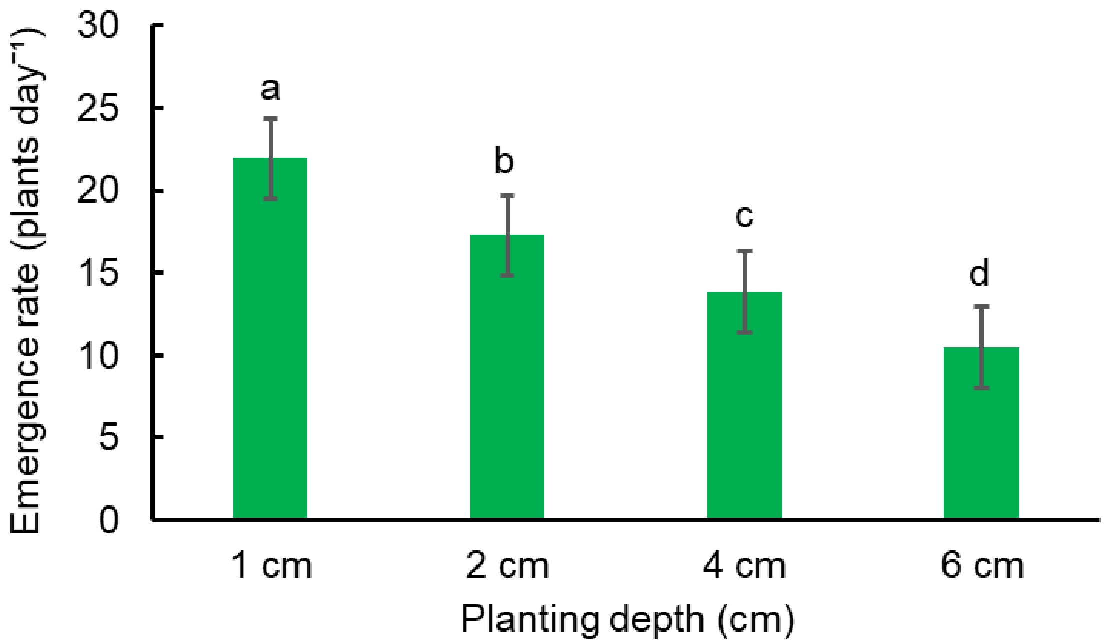

Figure 4.2: The influence of planting depth on the emergence rate of wheat seeds ($p \leq 0.05$). Vertical bars indicate 95% confidence intervals. Values that differ significantly at $p = 0.05$ are indicated with different letters.

Planting depth had a significant influence on wheat seed emergence rate ($p \leq 0.05$). Emergence rate decreased significantly as the planting depth increased. This could be attributed to the fact that it takes more time for a seed to emerge from a deeper planting depth than from a shallower planting depth (Figure 4.2). Seed [Zn] did not have any significant effect on the emergence rate of the wheat seeds from any of the depths ($p \geq 0.05$) (data not shown).

Planting depth influenced emergence percentage significantly ($p \leq 0.05$) (Figure 4.3). Wheat emergence decreased as planting depth increased. The most probable reason for this is that the seeds do not have sufficient amounts of reserve resources to get the coleoptile through the soil surface. Other reasons for decreased emergence success from deeper depths include lower air availability, resulting in decreased germination rates (Hines et al.1991). Secondly, the first leaf formed may be too weak to penetrate the harder and often crusted soil surface. Most of the seeds in the experiment did germinate, suggesting 'aeration' of soils were sufficient at the various planting depths. No crusts formed on these sandy substrates as they were kept moist for most of the time, therefore discarding the crust theory in this experiment.

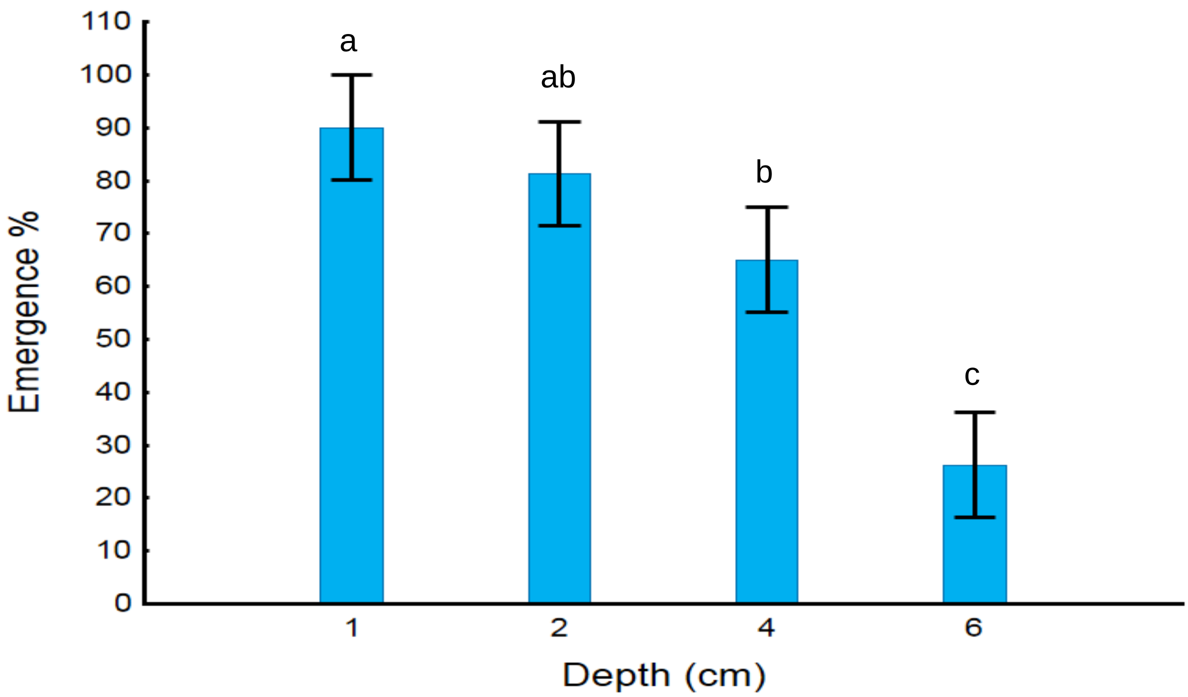

Figure 4.3: The emergence percentage of wheat planted at different sowing depths in pots. Vertical bars indicate 95% confidence intervals. Values that differ significantly at p = 0.05 are indicated with different letters.

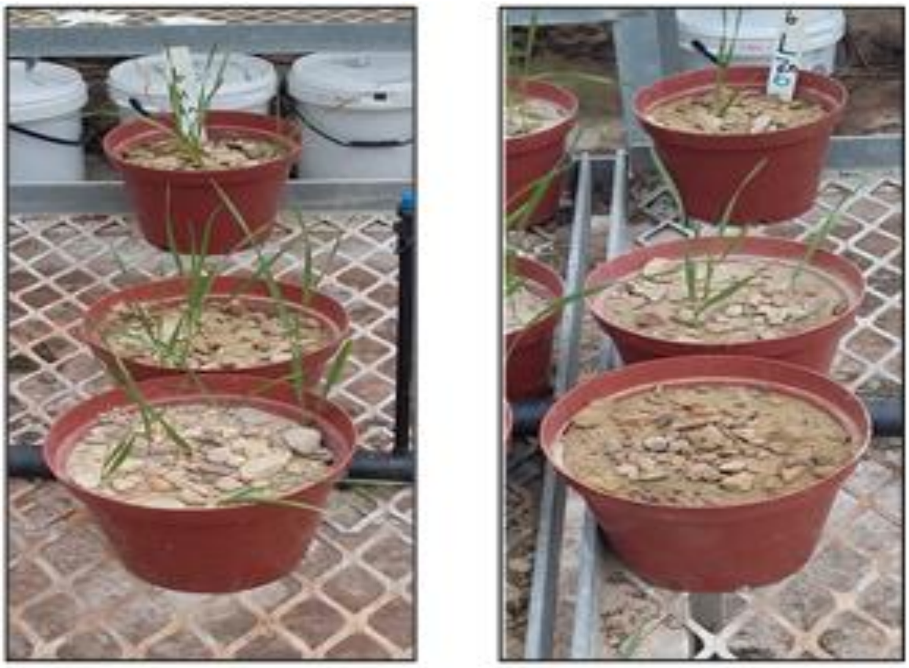

Figure 4.4: More wheat seedlings with a high [Zn] (left) have emerged from a depth of 6 cm than wheat seedlings with a lower [Zn] (right).

4.3.2 Wheat and ryegrass competition replacement experiment

No significant interaction was found between planting ratio of wheat to ryegrass and seed treatment ($p \geq 0.05$) in terms of total dry mass of wheat seedlings per pot (Figure 4.5). Seed treatments had no influence on any of the parameters investigated, contrary to findings about the advantages of priming by other authors. Singh et al. (2015) states that priming methods do have many beneficial aspects to it including, increased germination rates, which leads to improved competition by crops planted in tropical regions of the world. Nawaz et al. (2013) also mentioned the influence that priming has on germination, but did not discuss any other advantages or any of the parameters that were considered for this study. It is therefore suggested that emergence rates should also be monitored in glasshouse experiments such as this one. The period of priming corresponded to the periods recommended in literature (Harris et al. 2008; Aboutalebian et al. 2012; Meena et al. 2014).

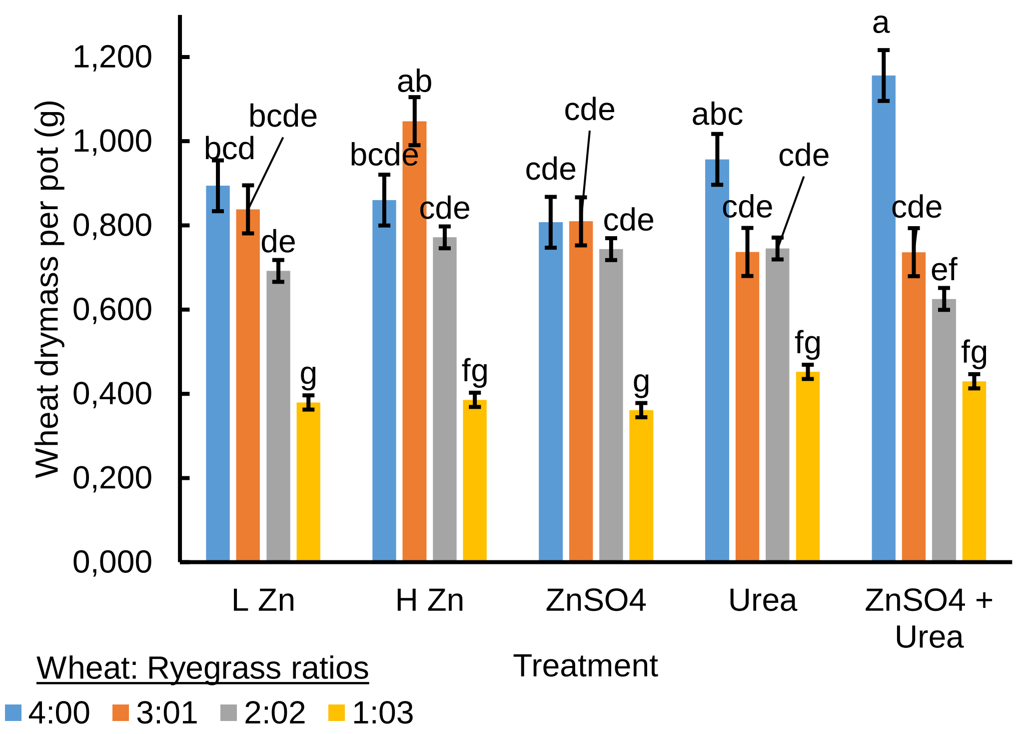

Figure 4.5: An indication of the influences of the different seed treatments and planting ratios of wheat to ryegrass on the dry mass of wheat plants grown in each pot. Values that differ significantly at p = 0.05 are indicated with different letters.

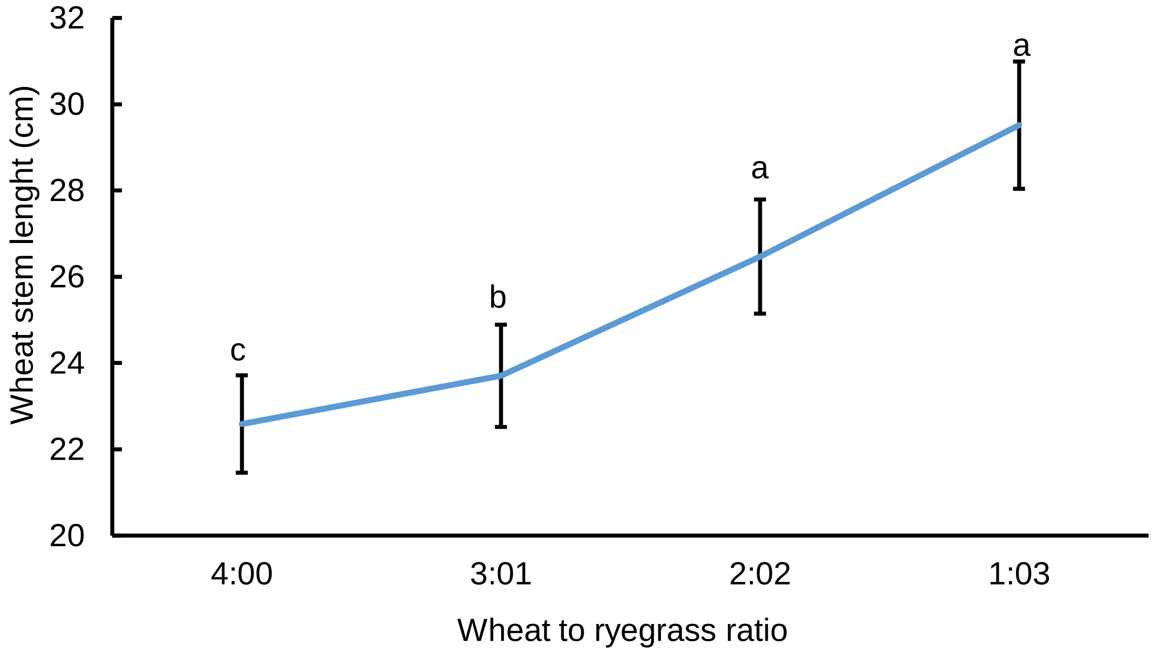

Figure 4.6: Wheat stem length at different planting ratios with ryegrass, a well-known competitor of wheat. Vertical bars indicate 95% confidence intervals. Values that differ significantly at P = 0.05 are indicated with different letters.

However, it was observed that planting ratio had a significant influence on the vegetative growth of wheat ($p \leq 0.05$) (Figures 4.6-4.8). Wheat stem length increased as the number of wheat plants decreased and number of ryegrass plants increased (Figure 4.6). Possible explanations for this include that wheat utilizes more resources than ryegrass or that intraspecific competition (competition between individuals of the same species, in this case between wheat plants) is more severe than interspecific competition (competition between individuals from different species). Fraga et al. (2013) did a similar replacement series experiment and found that intraspecific competition is more detrimental to wheat than interspecific competition with ryegrass. This could be the reason for shorter stem lengths at a higher wheat density. This supports our findings where wheat stem length and mean wheat dry mass per plant decreased as the number of wheat plants per pot increased (Figures 4.6 and 4.7).

Spink et al. (2000) determined that losses due to predation, pests and environmental stressors are higher at higher planting densities and attributed increased intraspecific competition to these losses. A significant difference was found in mean dry weight per wheat plant when the planting ratio was changed from 75% wheat to 50% wheat. The trend shown in Figure 4.7 indicate that as the wheat plant density decreases wheat mean dry mass per plant increases.

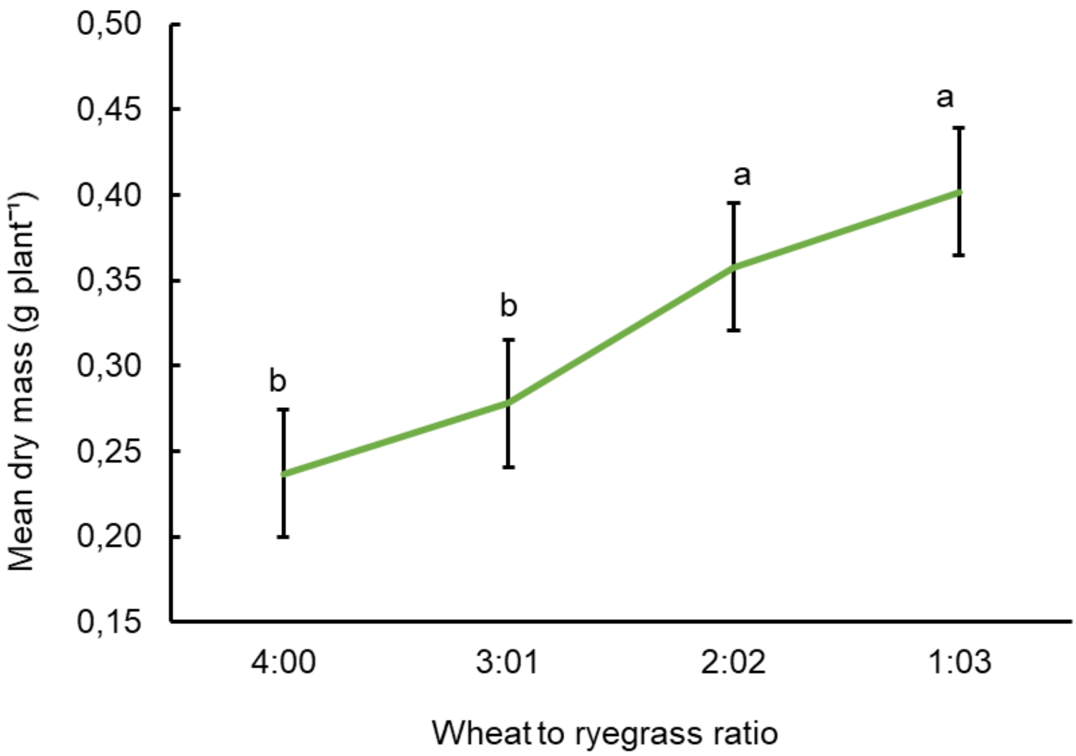

Figure 4.7: Wheat mean dry mass as influenced by the wheat to ryegrass ratio in a replacement series competition experiment. Vertical bars indicate 95% confidence intervals. Values that differ significantly at p = 0.05 are indicated with different letters.

Figure 4.8 indicates that wheat has a stronger competitive ability than ryegrass during the early life stages of these plants as the mean ryegrass dry mass is much lower in the presence of wheat. A previous study found that wheat made use of allelopathy to suppress the growth of annual ryegrass (Wu et al. 1998), which may be the reason for decreased mean ryegrass dry mass during our study. These findings are however contradictory to another study, which found that wheat had no allelopathic effects on Italian ryegrass (Cralle et al. 2003). Most papers however proposed that wheat does have an allopathic effect on the growth of ryegrass. Ferreira (2011) found that the presence of wheat seed leachates leads to decreased radicle lengths of ryegrass that in turn could lead to decreased plant weight and can therefore be considered as an allelopathic method used by wheat to prevent the dominance of and decrease competition by ryegrass. Ryegrass, like any other plant species, also has intraspecific competition within populations, but it is clear from our data that interspecific competition of wheat had more of a detrimental effect on ryegrass dry mass than intraspecific competition. Omondi and Kniss (2014) also came to the conclusion that spring wheat was a better competitor than annual ryegrass in a study where they compared four different grasses in terms of competition for resources such as light, nutrients and water. However, heavy ryegrass infestations are known to markedly inhibit wheat growth and yield. The competitive ability of ryegrass towards wheat is therefore probably dependent on superior numbers of ryegrass in the field.

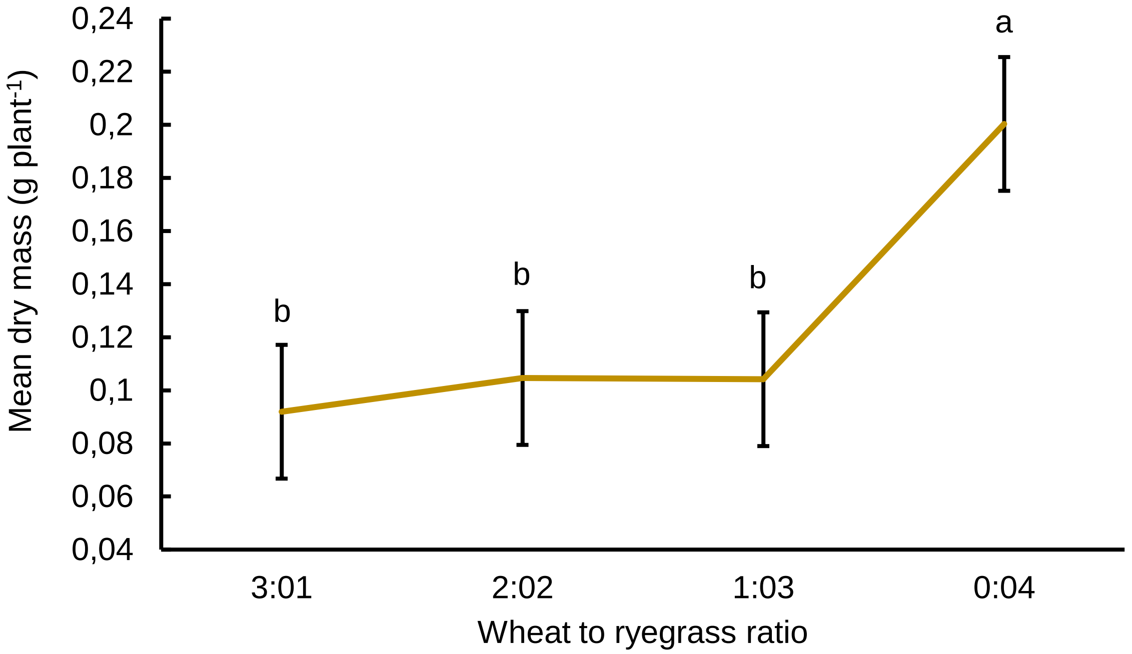

Figure 4.8: Ryegrass mean dry mass production per plant as influenced by the wheat: to ryegrass ratio. Vertical bars indicate 95% confidence intervals. Values that differ significantly at p = 0.05 are indicated with different letters.

4.3.3 Wheat and ryegrass addition experiment

There were no interactions between any of the factors in the design and the only factor that showed a significant ($p \leq 0.05$) effect on dry mass of wheat plants was soil moisture content. Wheat plants that received sufficient amounts of water during the course of the experiment accrued more biomass than those that did not (Figure 4.9)

The effects of limited water availability on growth and development of crops are widely recognised. This might explain why those wheat plants that received insufficient amounts of water developed much slower than those that received sufficient amounts, which subsequently led to decreased dry mass weight at harvest (Figure 4.9). A study by Abdul-Halime et al. (1988) concluded that a soil moisture content of 75% is needed for optimal yield production of wheat. Lehane and Staple (1959) came to the conclusion that the growth rate of plants is significantly affected by the amount of soil moisture available to that plant during the vegetative growth stages. Their findings therefore support ours.

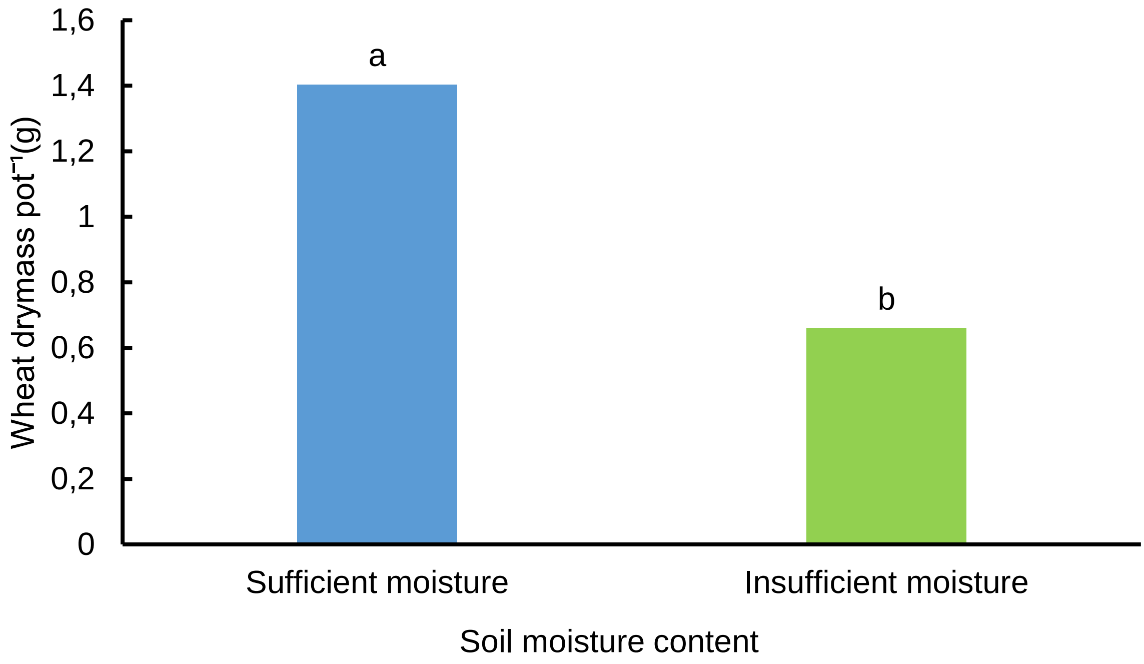

Figure 4.9: The effect of moisture content on the dry mass production of wheat in pots. Values that differ significantly at p = 0.05 are indicated with different letters.

We propose that wheat [Zn] had no significant influence on the growth of these plants (data not shown) (and possibly in the previous two experiments too) due to the fact that all seeds had sufficient concentrations of zinc to develop properly. A study by Cakmak et al. (2010) determined that wheat seeds are only considered as being zinc deficient when the seed [Zn] ≤ 22 mg kg^{-1}, which was not the case in our study as the "zinc deficient" seeds in our experiment had [Zn] of 32 mg.kg^{-1}. No seeds with [Zn] lower than 22 mg kg^{-1} could be found to use during this study. We therefore propose that significant differences might occur under these same experimental conditions if the experiment where to be repeated with seeds that have a [Zn] ≤ 22 mg.kg^{-1}.

Rye grass ratio to wheat yet again had no significant influence on wheat dry weight ($p \geq 0.05$) (data not shown). These findings are similar to those observed in the wheat and rye grass competition replacement experiment. Our findings on the suppressive abilities of wheat on rye grass support the findings of a study by Tanji et al. (1997).

These authors determined that one wheat plant can out-compete 11 Italian rye grass plants under greenhouse conditions and up to 19 rye grass plants under field conditions. Farmers would mainly only plant their crops after the first good rains, as dryland planting commonly lead to uneven plant emergence (Kandel 2017) and therefore decreased yields. Waiting for the first good rains mean that rye grass and other weeds may germinate and start to grow earlier than a crop that is planted in the same field. This could lead to a significant increase in the competition abilities of the weed. Earlier growth by the weed can be seen as an increase in vigour of that weed, and an improvement in vigour would lead to increased competitive abilities by the weed (Andrew et al. 2015). This also holds true for planted crop species. A study by Coleman et al. (2001) states that good seedling vigour leads to improved competitiveness of a crop to both weeds and other crops.

4.4 Conclusion

Increased seed [Zn] may lead to improved emergence success of wheat, especially under suboptimal growth conditions, such as increased planting depth. We therefore conclude that an increased [Zn] in wheat seeds lead to an improvement in the vigour of such a seedling. It was also clear that wheat seedlings had a major suppressive influence on annual ryegrass seedlings when both are present in small populations and planted at the same time. [Zn] did not have any influence on the competitiveness of wheat plants towards ryegrass plants, probably because of the optimal seed [Zn] of all seeds used in these three experiments.

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CHAPTER 5

Wheat zinc and nitrogen biofortification in the Western Cape Province, South Africa

5.1 Introduction

Humans, animals and plants are considered as malnourished when they do not consume sufficient amounts of nutrients and minerals on a daily basis to remain healthy. Malnutrition is also referred to as hidden hunger as organisms suffering from this condition often consume sufficient amounts of food to feel satiated but, their bodies are starved of essential nutrients and minerals (Saltzman 2013). Malnutrition has been linked to mental and physical health problems in both developing and developed countries (Winkler 2011).

Zinc, a trace element, is involved in many enzyme systems and is therefore essential for normal physiological and chemical activity (Anonymous 2010) and (Cakmak 2008). A deficiency in Zn is one of the two most common micronutrient deficiencies that threaten the health of humans with vitamin A being the other. Zinc deficiency often leads to an increase in the occurrence of diseases such as tuberculosis and pneumonia and to other physical impairments such as stunting and poor eye sight (Niedzwiecki and Rath 2007).

Those living in poverty-stricken countries are too poor to buy or produce Zn rich foods. They therefore consume staple crops such as wheat, which are not only low in Zn but they also contain substances like phytates. Phytates according to Lonnerdal (2000) prevent the body from effectively absorbing Zn. These inherently low Zn staple crops are further produced on Zn deficient soils. Ram et al. (2015) explains that more than 50% of soils on which cereals are produced are Zn-deficient. Bhutta et al. (2014) came to the conclusion that more than 80% of cultivated soils in India are Zn deficient. The combined effect of the above-mentioned factors contributing to Zn deficiencies has led to an increased concentration of science on ways to prevent and decrease the occurrence of zinc deficiencies globally.

A large number of methods are used to prevent and treat the occurrence of Zn deficiencies in humans, but a cost benefit analyses done by Nestel et al. (2006)

determined that biofortification is much more beneficial and sustainable than the various other methods. These findings have also been supported by Bouis et al. (2013) who described biofortification as a sustainable, cheap and a long-term method to fight this global phenomenon. Biofortification is defined as the production or breeding of crops containing higher nutrient concentrations (Anonymous, 2002). Biofortification is divided into two sections, the one being genetic bio-fortification and the other agronomic bio-fortification (Schulin et al. 2015).

Enhanced Zn fertilisation via foliar application (part of agronomic bio-fortification) of wheat has attracted attention due to various studies which claim that this can be used as a method to increase the zinc concentration [Zn] within the newly produced grains of wheat. A study by Cakmak (2009) has determined that [Zn] in wheat grains can increase two to three times due to foliar and soil applications of Zn enriched fertilisers.

The main aim of this study is therefore to put the theory of Zn enrichment of wheat by means of foliar application of a Zn containing fertiliser, to the test. A second aim of the study was to determine if foliar application of urea together with Zn would have a positive effect on other quality parameters of the wheat seeds produced. We hypothesised that an application of either zinc sulphate ($ZnSO_4$), urea or a combination of these two substances would lead to increased nutrient concentrations and possibly larger yields produced than plants grown in the absence of these applications.

5.2 Methods and materials

5.2.1 Experimental sites

The experiment was conducted at three localities (farms) within the Western Cape Province. All three farms fall within a Mediterranean climate, which is characterised by hot dry summers and cool, but not freezing, wet winters (Lionello et al. 2006). The three farms where strategically chosen to cover as much of this regions wheat production area, which is also the main wheat production region of South Africa (Goldfus 2017). Altona (33°41’04.9”S; 18°37’09.2”E) and Langgewens (33°16’42.33”S; 18°42’11.62”E) are situated in the Swartland region close to the towns of Philadelphia and Moorreesburg respectively, while Roodebloem (34°13’11.1”S; 19°31’51.0”E) is found in the Overberg/southern Cape region just outside Caledon

(Figure 5.1). Annual rainfall distribution differs between the two regions. The Western Cape Department of Agriculture (2015) states that the Swartland receives up to 80% of its total rainfall during the winter months, while the southern Cape only receives about 60% of its total annual rainfall during the winter months. The soils of these farms are very similar and can be described as rocky and shallow sandy loam soils (Coetzee 2017). Coetzee (2017) examined the % carbon content of the three farms during 2015 and determined that Langgewens had a carbon % of 0.64 while Altona and Roodebloem had carbon percentages of 1.27 and 0.86, respectively.

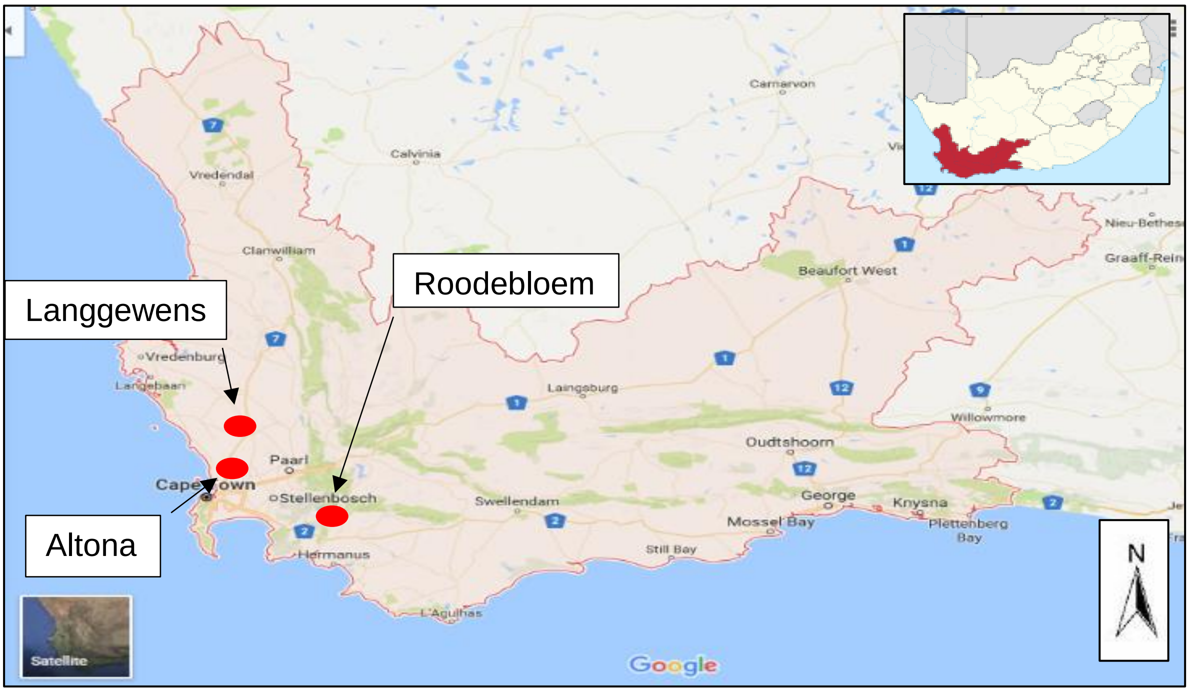

Figure 5.1: The three experimental sites are situated within the Western Cape Province of South Africa. Source: Google maps.

5.2.2 Treatments and layout of the experiment

Two wheat cultivars namely SST 056 and SST 027 were used during this experiment. SST 056 had a [Zn] of 30 mg kg^{-1} while SST 027 had a proportionately higher [Zn] of 45 mg kg^{-1}. The same experiment was repeated on all three farms. These two cultivars, both of which were developed for winter rainfall areas in the Cape region are very similar to one another, with both yielding above average in conservation tillage systems, and also being moderately susceptible to stem rust. (available at: http://www.sensako.co.za/ProductSubCategory.aspx?id=1).

A randomised block design was used to plant a total of three blocks, each comprising of eight plots with a width of 1.5 m and a length of 5 m, resulting in an area of 7.5 m^2 per block, on each of the three farms. A total of 24 plots were thus planted per farm, four of the plots in each of the three main blocks were randomly selected and planted with one of the two wheat cultivars while the remaining four were planted with the other cultivar. Treatments were arranged as a 2x4 factorial with factors Cultivar (SST 056 and SST 027) and four treatments (zero foliar fertilizer, ($ZnSO_4$), urea and urea + ($ZnSO_4$) foliar fertilizers).

5.2.3. Field preparation and planting:

A pre-emergence herbicide (pyroxasulfone) was applied to the fields prior to planting to prevent the emergence of weeds. A conventional tillage method was then used to plough the soil where after a small plot planter was used to plant the seeds. A sowing density of 80 kg. ha^{-1} was used with a row spacing of 170 mm. The wheat received 20 kg of nitrogen (N) per hectare at planting, 50 kg N. ha^{-1} after emergence and 30 kg N. ha^{-1} as a top-dressing. The Langgewens trial was planted on the 6th of May 2017 followed by Altona on May 10th and Roodebloem on May 17th.

5.2.4 Sampling and analysis

5.2.4.1 Six weeks after emergence

The first sampling that was done included determining stand density and removing plants to determine stem length, leaf area and average dry mass. Plants only started to emerge a few weeks after planting due to intense droughts experienced in the Western Cape. Sampling at Roodebloem was done on the 25th of July 2017 while sampling at Langgewens and Altona occurred on the 26th. Stand density was determined by using a 30 x 30 cm quadrant. The quadrant was placed randomly in each plot whereafter the number of plants in it was counted. A plant was considered inside the quadrant if the area where the stem enters the soil was within the quadrant. Five plants were removed per plot and taken to Welgevallen experimental farm at the University of Stellenbosch for further analysis. The five samples of each plot were placed into separate bags to calculate the means per plot of the parameters.

The stem lengths of each plant were recorded by using a ruler. Stems were measured from the base of the stem where the green aboveground parts merge with the white belowground parts and upwards to the tip of the leaf at the top. The roots were removed by using scissors after the stem lengths were recorded, and all the leaves were removed from the stems. The leaves were put through a Li-cor (Li-3100 area meter) leaf area machine. Each of the plants were then placed into separate brown paper bags and left in an industrial drying oven to dry for 48 hours at a constant temperature of 60 °C. The plants were then removed from the brown paper bags and weighed by making use of a three-decimal scale (Mettler PC 440). The dry plant material of each plot was subsequently put through a milling machine and sent to the plant and soil laboratory of the Department of Agriculture: Western Cape at Elsenburg for nutrient analyses.

5.2.4.2 From six weeks after emergence to heading stage

The various farms were visited regularly throughout the duration of the experiment to do visual inspections of the trials to prevent and act on the occurrence of diseases and pests. Esfandiari et al. (2016) found that the most beneficial time to apply $ZnSO_4$ to wheat plants are during the very first seed developmental stages. Most of the plants were in their booting stage during early September 2017, which is the growth stage just before the heads start to become visible and new seeds start to develop. Three fertiliser treatments where applied to the two different wheat cultivars in each of the three main blocks. These fertilisers included $ZnSO_4$, urea (46%) and a combination of the two. Some plots were not sprayed with any fertilisers to act as a control.

Three plants were removed from each plot before applying any of the foliar fertilisers. These plants were taken to the Welgevallen experimental farm at the University of Stellenbosch. Stems lengths were recorded from the base of the tallest tiller to the point where the head start. Each plant was then placed into a separate brown paper bag and dried in an industrial drying oven for 72 hours at a constant temperature of 60 ^{0}C. The dry weight of each plant was recorded by making use of a scale where after the plants were send to a laboratory for nutrient analysis.

A knapsack sprayer was used to spray the wheat plots with the different foliar fertilisers. Zinc sulphate was sprayed at a 0.05% concentration and at a rate of 800 litres (L) per hectare. Thirty grams (g) of $ZnSO_4$ was therefore dissolved into 6 L of water. A total of 0.6 L was then sprayed onto each of the 6 $ZnSO_4$ treatment plots. The urea plots where sprayed with a 20% concentration, and at a rate of 100 L per hectare. Two hundred grams of urea was dissolved into 1 litre of water. A total of 0.075 L of the urea solution was then sprayed onto each of the 6 urea treatment plots. The mixture for the combined $ZnSO_4$ and urea solution consisted 0.5 L of the $ZnSO_4$ solution and 0.5L of the urea solution. A total of 0.3 L of this combined solution was then sprayed onto each of the 6 plots of each farm that were set out to be sprayed with this combined solution. The combined solution was therefore sprayed at a rate of 400L per hectare.

5.2.4.3 At and after harvest

Harvesting occurred during the first two weeks of November 2018. A small plot harvester was used during the harvesting of the trails at the various localities. The yield of each plot (replicate) was collected separately. The seeds of each replicate were cleaned by using a seed cleaning machine. Yield weight was determined by weighing each replicate individually where after yield in tons per hectare was calculated. Due to financial limitations, each replicate was not analysed. Instead a composite sample of the three replicates was sent for analysis and the nutrient composition data could therefore not be analysed statistically for each locality. However, an analysis was made where the three localities were considered as replicates in a completely randomised block design (CRBD). The seeds were sent to the SGS Laboratory in Somerset West to determine seed nutrient composition. Seed N percentage was multiplied by 6.25 to determine the seed protein percentages (Maclean et al. 2003).

5.2.5 Data analysis

All data were analysed by making use of Statistica 13 and Microsoft Excel. Microsoft Excel was used to record all the initial data. Statistica 13 was then used to determine if there were significant differences in the different recorded parameters between the different treatments. Both one and two-way ANOVA tests were run depending on the variables being compared to one another. Vegetative growth was only compared between the two cultivars while yield and quality parameters were compared between cultivars and fertiliser treatments. Significant differences occur at $p \leq 0.05$.

5.3 Results and discussion

Spring wheat is mainly produced under dryland conditions during the winter months in the Western Cape Province, South Africa. Drought conditions during planting (April/May) and at seed ripening or harvest (October/November) is a real threat to the success of small grain farmers in the Western Cape (Goldfus 2017).

Table 5.1 contains the nutrient content information of the two wheat cultivar seeds that were used during this study. The magnesium (Mg), boron (B), sulphur (S), calcium (Ca) and phosphorus (P) concentrations are not included in the table, since the concentrations of these nutrients were exactly the same or differed by less than 0.02%.

Table 5.1: The differences in nutrient content of the two wheat seed cultivars (SST 027 and SST 056) that was used during these trials.

	Nutrient content						
Cultivar (SST)	N (%)	K (%)	Na (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})
027	2.16	0.45	58	27	42	3	45
056	1.88	0.50	66	66	53	6	30

5.3.1 Six weeks after emergence

No significant differences were observed in several of the parameters recorded six weeks after emergence at the three localities ($p \geq 0.05$). Table 5.2 indicates which parameters have been analysed and what the p-values were when the two different wheat cultivars where compared to each other. P-values ≤ 0.05 are indicated in red and represent those parameters that differed significantly between the two wheat cultivars. The only significant difference that was observed were plant length on Altona ($p = 0.000$). Cultivar SST 056 had a longer length than cultivar SST 027 on Altona.

Table 5.2: P-values of the parameters tested to compare between two wheat cultivars (SST 027 and SST 056) six weeks after emergence.

Locality:	Plant density	Plant length	Leaf area	Dry mass
Langgewens	0.865997	0.382812	0.351421	0.501002
Altona	1.000000	0.000000	0.075141	0.153613
Roodebloem	0.374997	0.581004	0.384548	0.14874

The lack of significant differences in plant density, leaf area and dry mass on all the farms might have been due to the fact that the nutrient compositions of the different cultivars were very similar and that none of the seeds were deficient in any nutrients. The SST 027 seeds were stored for a year at a temperature of approximately 4 ^{0}C. Strelec et al. (2010) determined that seed germination and vigour decreased significantly when stored for one year at temperatures of 40 ^{o}C than when being stored at 25 ^{0}C. It is therefore unlikely that the storage environment and the age of the different cultivars had a significant influence on the different parameters of the two cultivars.

The reason for the significant difference between the plant lengths of the two cultivars on Altona could be that cultivar SST 027 had much lower concentrations of both manganese (Mn) and iron (Fe) (Table 5.1). Both these nutrients play an important role in photosynthesis, which in turn has an influential impact on the growth rate of a plant (Kabata-Pendias 2010; Mousavi et al. 2011). However, the absence of differences in dry mass does not support the viewpoint that these two elements had an influential impact on plant length or growth of the two cultivars. Both these cultivars are lodge tolerant (available at: http://www.sensako.co.za/ProductSubCategory.aspx?id=1) and the significant increase in plant length ($\pm$3 cm on average) of STT 056 is therefore seen as negligible with no significant influence on the quality of the plant. We assume that this significant increase in plant length can be ascribed to the traits of the cultivar although no information on this could be found.

5.3.2 From six weeks after emergence to heading

Significant differences were observed in plant length on Roodebloem and Altona ($p \leq 0.05$) between the two wheat cultivars. No significant differences occurred between the different cultivars in terms of dry mass at any of the three localities ($p \geq 0.05$) (Table 5.3). The significant increase in plant length of SST 056 on Altona and Roodebloem are ascribed to the phenological trait of the cultivar rather than seed nutrient composition or environmental conditions. The similarity in dry mass between the different cultivars is also ascribed to the phenological traits of the cultivars as these two cultivars share a number of traits (available at: http://www.sensako.co.za/ProductSubCategory.aspx?id=1).

Table 5.3: P-values of the parameters tested to compare between two wheat cultivars (SST 027 and SST 056) at heading.

Locality:	Plant length	Dry mass
Langgewens	0.408847	0.953262
Altona	0.004503	0.448847
Roodebloem	0.011067	0.497932

Plant samples were analysed to determine the leaf [Zn] and protein percentages at heading stage. No significant differences were observed between the two cultivars on any of the three localities both in leaf [Zn] and protein percentage. The average [Zn] and protein percentages of the two cultivars are presented in Table 5.4.

We believe that the similarities in growth (dry mass), Zn and protein concentrations (Tables 5.4 and 5.5) of the two cultivars at the heading stage are due to the fact that none of the seeds were truly Zn deficient and that there was not any great difference in N percentage between the two cultivars, which has a direct effect on protein concentration (Maclean et al. 2003). Wheat seeds according to Cakmak et al. (2010) are only truly zinc deficient when the seeds have a [Zn] of 22 mg kg^{-1} or less.

Table 5.4: The average leaf Zn and protein percentages of the two different wheat cultivars at booting stage.

Locality:	Cultivar			
	SST 027 [Zn] (mg kg^{-1})	SST 056 [Zn] (mg kg^{-1})	SST 027 (Protein %)	SST 056 (Protein %)
Langgewens	17.67	17.67	11.56	11.38
Altona	30.67	29.00	16.56	14.60
Roodebloem	28.33	26.33	15.67	14.69

5.3.3. At and after harvest:

There were no significant interactions ($p \geq 0.05$) between factors in terms of wheat yield at any of the farms. Treatment did not have a significant effect on any of the parameters (yield in tons ha^{-1}, 1000 kernel mass and hectolitre mass) recorded ($p \geq 0$ .05) which is disappointing but the severe drought (particularly at Langgewens) or a late application of the foliar fertilizer might be responsible for the similarities found (Table 5.5 and 5.6). All crops suffered and struggled to grow during this season as the winter rains were delayed and much less has fallen than the long-term average. However, the different fertilizer applications were applied at an optimal time to increase protein content and nutrient composition of the yields produced, which is just after the flowers are pollinated (Bly and Woodard 2003). This was also suggested to be the best time to apply Zn fertilizers (Esfandiari et al. 2016).

Table 5.5: The average yields (tons ha^{-1}) produced at the three localities for the eight different treatments.

TREATMENT	Cultivar	FARMS:		
		LANGGEWENS	ALTONA	ROODEBLOEM
CONTROL	SST 027	1.831	2.979	4.675
ZNSO4	SST 027	1.519	3.093	4.665
UREA	SST 027	2.107	3.395	4.565
ZNSO4 + UREA	SST 027	1.902	3.403	4.362
CONTROL	SST 056	1.483	2.901	4.326
ZNSO4	SST 056	1.558	3.197	4.670
UREA	SST 056	1.694	3.137	4.618
ZNSO4 + UREA	SST 056	1.839	3.110	4.728

Rainfall had a massive impact on the yields produced at each of the three localities (Table 5.6). Roodebloem had a much better rainfall season than Langgewens with sufficient amounts of precipitation during the critical growth stages such as the germination stage in April and heading stage in August for example. Roodebloem had a total of 60.7 mm in August while Langgewens only had 35.5 mm and it can be speculated that this might have been the reason for the increased yields at Roodebloem. A study by Mohan et al. (2013) states that stand establishment is dependent on soil moisture, which under dryland conditions are increased only due to precipitation, and that this has a direct effect on yield produced at the end of the growing season.

Table 5.6: Precipitation data for Langgewens experimental farm and Roodebloem from the start of planting season until harvest in mm (Source: oral interview with Dr. PJ Pieterse).

Localities	Monthly precipitation (mm)						
	April	May	June	July	August	September	October
Langewens	13	10.5	71.5	30	35.5	15	26.5
Roodebloem	23.3	8.5	68.2	47.7	60.7	21.9	31.8

Although a significant difference in hectolitre mass was found only on the Langgewens experimental farm some of the minimum and maximum 1000 kernel masses and hectolitre masses for each of the three localities as well as the treatments that led to these values are shown in Table 5.7 and Table 5.8.

Table 5.7: A summary of the highest and lowest average 1000 kernel masses as well as the treatments that they were exposed to for each of the three localities.

Locality:	Langgewens	Altona	Roodebloem
Treatment	Control	Control	Control
Cultivar	SST 027	SST 056	SST 056
Ave min 1000 kernel mass (g)	17.967	16.610	19.317
Treatment	Urea	$ZnSO_4$ + Urea	Control
Cultivar	SST 027	SST 027	SST 027
Ave max 1000 kernel mass (g)	18.511	18.960	20.285

Table 5.7 indicates that the high zinc content seeds (SST 027) performed better than the low zinc content seeds (STT 056) although they did not perform significantly better ($p \geq 0.05$). The average maximum 1000 kernel mass was derived from high Zn content seeds at all three localities while the average minimum 1000 kernel mass of only Langgewens was derived from high Zn content seeds.

It seems as if the high Zn content seeds also dominated with regards to the hectolitre mass. Two of the three localities derived their average maximum hectolitre masses from high Zn seeds while they derived their average minimum hectolitre masses from low Zn seeds (Table 5.8). It is still important to remember that no significant differences occurred and it is therefore suggested that a similar experiment is repeated under greenhouse conditions. This experiment should include true Zn deficient seeds and no drought aspect to determine the real effects of the seed [Zn] and the different foliar fertilizer applications on the growth of wheat.

Table 5.8: A summary of the highest and lowest average hectolitre masses as well as the treatments that they were exposed to for each of the three localities.

Locality:	**Langgewens**	**Altona**	**Roodebloem**
Treatment	Urea	Control	$ZnSO_4$ + Urea
Cultivar	SST 056	SST 056	SST 027
Ave min Hectolitre mass	381.553	385.847	394.167
Treatment	Control	Control	$ZnSO_4$ + Urea
Cultivar	SST 027	SST 027	SST 056
Ave max Hectolitre mass	389.407	400.300	399.694

A significant influence due to cultivar was observed in hectolitre mass (HLM) on Langgewens experimental farm only ($p \leq 0.05$) (Figure 5.2). The SST 027 seeds had a higher HLM than the SST 056 seeds indicating that the SST 027 harvest had a higher bulking density than the SST 056 harvest (Nel et al. 1998). The SST 027 cultivar would lead to a better milling yield produced after the seeds have been milled due to this higher bulking density. This increased milled yield eventually leads to an increase in the food that is produced per volume of seed and thus to an increased profit produced per volume of seed. The significant influence of seed [Zn] on hectolitre mass on only one of the three localities implicate this finding and it is therefore suggested that this experiment should be repeated in a more consistent and favourable growth environment.

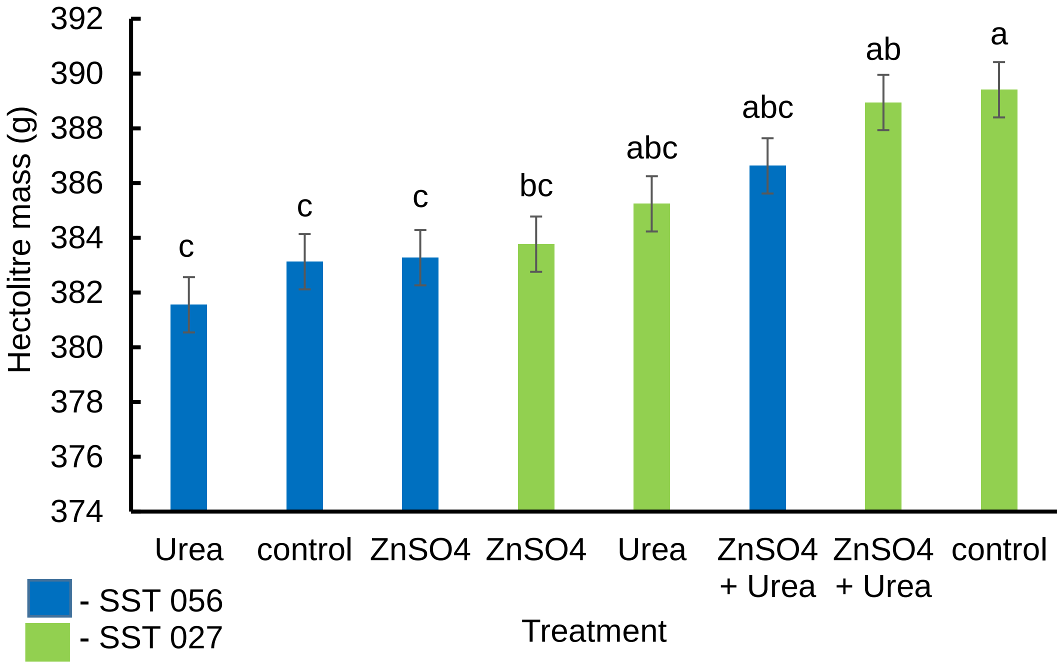

Figure 5.2: Hectolitre mass of wheat as influenced by Cultivar on Langgewens experimental farm ($p \leq 0.05$).

Wheat seed [Zn] were determined on the harvested seeds of the two wheat cultivars of all three the localities (Table 5.9), but no statistical analysis was run to determine if there were significant differences in wheat seed [Zn] between the cultivars due to the financial constraints. A summary of the seed protein data is shown in Table 5.10. No statistical analyses were done on the protein content either. Statistical analysis was however done to compare [Zn] and protein percentages where the three different farms were considered as replicates. No statistical interactions occurred between the factors and no significant differences occurred within factors ($p \geq 0.05$). This could be due to the fact that all crops suffered due to the intense drought experienced in the Western Cape Province. However, some of the data indicated that cultivar could possibly have a significant influence on [Zn].

Table 5.9: The average seed [Zn] of the different wheat cultivars at the different localities after harvest in mg.kg^{-1}. Only one sample was analysed fro each treatment due to financial limitaions.

Locality:	**Treatment**							
	SST 027 [Zn] (mg kg^{-1})				**SST 056 [Zn] (mg kg^{-1})**			
	Control	ZNS04	Urea	UREA - ZNS04	Control	ZNS04	Urea	UREA - ZNS04
Langgewens	42	36	39	30	50	36	41	36
Altona	40	37	38	41	42	46	41	48
Roodebloem	35	45	37	42	41	47	40	43

Table 5.10: The influence of the different foliar applications on the protein content of seeds harvested at the three farms. Only one sample was analysed fro each treatment due to financial limitations.

Locality:	**Treatment**							
	SST 027 (Seed protein %)				**SST 056 [Zn] (Seed protein %)**			
	Control	ZNS04	Urea	UREA - ZNS04	Control	ZNS04	Urea	UREA - ZNS04
Langgewens	17.81	18.44	16.69	17.69	18.19	17.63	17.81	17.31
Altona	15.63	15.25	15.43	14.94	15.69	15.50	15.63	15.44
Roodebloem	15.13	15.25	15.81	16.19	15.19	15.38	15.63	15.44

Tables 5.9 and 5.10 indicate that SST 056, the cultivar with the lower Zn content, had higher [Zn] and protein percentages after harvest in most of the cases. However, the [Zn] and protein percentages between the two cultivars seem neglible irrespective of locality.

5.4 Conclusion

Wheat cultivars (with variable [Zn]) and foliar fertilizer applications had some effect on wheat growth and nutrient compositions during this study, but not to the extent that it is recommended to use wheat seeds with a higher [Zn] or these different foliar fertilizer applications. It is proposed that the severe drought had a negative impact on productivity of all plants at the different localities. However, it may be that wheat seeds with a higher [Zn] could improve the vigour and therefore the growth and development of a plant. This assumption is made due to the fact that most of the maximum values assessing the various quality parameters were derived from seeds high in [Zn], while the lowest ones are mainly derived from low Zn seeds. However, the harvested seeds of cultivar SST 027 had on average a lower [Zn] than those of SST 056 probably due to a dilution effect of the zinc due to the higher yields. It is recommended that this study is repeated under controlled environmental conditions and in the presence of wheat seeds that are truly Zn deficient to see the real impact of higher seed [Zn] and different foliar fertilizers that were applied during this study.

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CHAPTER 6

Conclusion and recommendations

This study ascertained whether zinc (Zn) has an influential effect on the growth, reproduction or qualitative and quantitative qualities of wheat (*Triticum aestivum*) with regard to yield. Zinc is considered as a trace mineral, which means that only small amounts of this mineral is needed on a daily basis for an organism to remain healthy (Katyal and Randhawa 1983). Zinc is one of the four main nutrients currently described as deficient in the diets of humans (Tulchinsky 2010). We have therefore decided to focus on Zn and wheat as wheat is one of the top three staple crops of the world and can be produced in more climates than the other two which is rice and maize (Anonymous 2008).

Both controlled (Greenhouse trails) and uncontrolled (In-field trails) environmental studies were conducted in the Western Cape Province of South-Africa. The influence of various Zn aspects was studied including the influence of wheat seed Zn concentration [Zn], various Zn priming substances and Zn foliar fertilizers. Specific environmental factors were studied under greenhouse conditions to determine if seed [Zn] would improve the vigour of seeds under stressed environments such as increased planting depths, decreased soil moisture availability and competition due to the presence of rye grass, a well-known weed in wheat fields across the Western Cape.

Previous literature has indicated that Zn plays an important role in the germination and therefore vigour and growth of wheat and other plant species (Haslett et al. 2001, Cakmak 2009). We therefore hypothesised that an increase in [Zn] would lead to improved germination, vegetative growth and yield of wheat.

Some obvious observations have been made during the course of this study, including that an increased planting depth and decreased soil moisture has a significant negative effect on the germination and growth of wheat ($p \leq 0.05$). Wheat seedling biomass decreased significantly under controlled environments due to decreased soil moisture. An increase in rainfall led to an improvement in yield produced on Roodebloem close to Caledon while Langgewens experimental farm which had much less rainfall also had much lower yields.

Some notable discrepancies from this study were that priming had no influence on seed germination. Although we adhered to guidelines for seed treatments from other similar studies (Singh et al. 2015), exact reasons for these aforementioned discrepancies remain elusive.

Wheat seed [Zn] had an influence on the vigour of wheat, judging from the marked improvement of seed germination percentage, as well as seed emergence from deeper depths. Wheat growth increased as the number of wheat plants decreased and number of rye grass plants increased. It is plausible that intraspecific competition played a role here. This finding corresponded with the results of a similar study done by Fraga et al. (2013).

Although no significant differences occurred in wheat yield due to treatment during the field trials we found that most of the highest yields, were produced by the SST027 (high Zn seed) wheat cultivar, which indicate that an improved [Zn] lead to an improvement in wheat yield. In addition, it may be that this increased yield was the reason for the lower Zn and protein concentrations produced by the SST027 cultivar due to a dilution effect. Hectolitre mass was significantly improved on Langgewens experimental farm due to the use of cultivar SST 027. This cultivar also had the highest hectolitre mass on Altona although no significant interaction between cultivar and hectolitre mass could be found. These findings may in one way or another support the findings of a few other studies that seed [Zn] does have an effect on wheat germination, vegetative growth and yield.

We believe that the true influence of seed [Zn], priming and foliar Zn applications on the various parameters that have been studied would have been much clearer if seeds have been used with a [Zn] of less than 22 mg kg^{-1}. Seeds with a [Zn] smaller than 22 mg kg^{-1} are consider as seeds that are zinc deficient (Cakmak et al. 2010). Seeds with a [Zn] smaller than 22 mg kg^{-1} could not be found to use during this study and we therefore feel and recommend that the study should be repeated while making use of truly Zn deficient seeds.

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