MORPHOLOGICAL, PHYSIOLOGICAL AND YIELD RESPONSE OF MAIZE (*Zea mays* L.) TO SEED TREATMENTS

by

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Submitted in partial fulfilment of the requirements for the degree

Magister Scientiae Agriculturae

(Agronomy: Crop and Stress Physiology)

in the

Department of Soil, Crop and Climate Sciences
Faculty of Natural and Agricultural Sciences

UNIVERSITY OF THE FREE STATE
BLOEMFONTEIN

November, 2009

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DECLARATION

I declare that this dissertation hereby handed in for the qualification Magister Scientiae degree at the University of Free State, is my own work and that I have not previously submitted the same work for a qualification at/in another University/faculty**.**

I also agree that the University of Free State has the sole right to the publication of this dissertation.

Signed: _____ **Date:** _____

DEDICATIONS

I would like to dedicate this dissertation to my father “God”. Thank you Lord Jesus for granting me life full of sings and wonders. You have granted me knowledge and wisdom and I know that I can do all things through Christ who strengthens me. Again I am confident that the Holy Spirit lives in me and it always activates, vitalizes, strengthens and makes me to be victorious. Secondly, I am dedicating it to Pastor Jackson Kgopolo and Mpho Wisisani Gunda for their spiritual upliftment.

ACKNOWLEDGEMENTS

I wish to acknowledge the following:

- Professor Seef Pretorius for his thorough supervision, advice and guidance during the entire course of my study. He was not only a supervisor but also a parent through his words of encouragement and always availing himself when I needed help. His guidance and critics yielded direction in my entire life.

- Dr. Elmarie van der Watt for her laboratory technical expertise and always compromising her time ensuring that sample analysis are carried out efficiently and effectively.

- National Research Foundation (NRF) and Department of Science and Technology (DST) of South Africa for the financial assistance. Thank you very much for supporting me financially towards my entire studies in terms of tuition and accommodation fees.

- Dr Geleta Legesse Fite for encouraging me to enroll for this MSc and providing assistance with reference to data analysis.

- Above all my God was the founder of everything, hence he was always my leader, provider and vitalizes me from within.

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CHAPTER 1

INTRODUCTION AND RATIONALE

Maize (*Zea mays* L.) is the most important grain crop grown in South Africa and is one of the most important crops in the milder subtropical and tropical regions of the world (Arnon, 1975 as cited by FSSA, 2007). It is grown under diverse environmental conditions compared to other important grain crops such as wheat and rice (Du Plessis 2003; FSSA, 2007). According to Abulrahaman & Kolawole (1988), maize serves as staple food in Africa and is also used for medicinal purposes. The grain is prepared and consumed in a multitude of ways which vary from region to region and from one ethnic group to the other. For these reasons maize was chosen as crop of importance in this study.

Increasing yield for an ever growing world population has currently become a topic of great concern with regard to food security. Especially in Africa, agricultural productivity has not been able to cope with population growth, leading to increased annual imports and food insecurity (Mugo *et al*., 2005). Food insecurity has been exacerbated by consecutive years of below normal seasons and poor harvests Ósince 2002 in Southern Africa (SADC & RVAC, 2006). Though there has been a maize surplus in South Africa for the 2005/06 season, maize imports for most of the SADC countries have increased. This is either the result of reduced production or the increased demand for maize as part of the current bio-diesel project (Global Crisis Solution for Actionaid Southern Africa Region Management, 2006). The author maintains that 12 million people are facing food insecurity in Southern Africa. This situation is chronic, contributes to increased vulnerability at household and community level and clearly shows that the expected maize green revolution in Africa did not take off (Mugo *et al*., 2005; Gichuki, 2005).

In Africa one of the most important underlying factors to below average maize yields is poor plant stands. This is closely related to poor seed germination and seedling

establishment resulting from interaction with the environment and, in terms of food security, this remains a concern. Poor or reduced seed germination and crop establishment, leading to poor ultimate grain yield, can be attributed to either low vigour seed or result from biotic and abiotic stress conditions pertaining in a specific cultivation area (Kerr *et al*., 2007). As seed germination and seedling establishment are the first critical stages in the life of any crop, a strong rationale exists to focus on these initial stages in order to find solutions for the underlying problem.

Germination is a complex metabolic process that starts with water imbibition and ends when the radicle or primary root protrudes the testa. Subsequently, growth of the young seedling is equally critical. Both events are subjected to precise regulation, the complexity of which originates from both the action of external factors and the characteristics within the seed themselves (Côme & Corbineau, 1989). Seed treatment has been reported by various authors to hold potential in manipulating either seed germination or seedling establishment or both, hence the final grain yield (Kaya *et al*., 2006).

Seed germination involves closely regulated biochemical processes starting with reserve mobilization (Côme & Corbineau , 1989). Studies conducted by Chuanren *et al.* (2004) suggested that seed treatment has the potential to trigger and manipulate these physiological and metabolic activities within the seed resulting in improved germination and seedling growth. The major changes in a seed during germination is a rapid increase in respiratory activities including glycolysis, the tricarboxylic acid (Krebs) cycle and the oxidative pentose phosphate pathway (OPPP) (Muscolo *et al.*, 2001). These respiratory pathways are essential for both energy provision in heterotrophic cells and a wide range of other physiological functions (Fernie *et al*., 2004). All of the mentioned respiratory pathways consist of a series of enzyme controlled reactions. Some of these enzymes are referred to as regulatory enzymes and their activities are closely controlled depending on the energy need of the plant under specific environmental conditions (Akita, 1993; Rakhmankulova *et al.,* 2001). These regulatory enzymes are most probably the best target points in the search for

manipulation techniques that can improve both seed germination and subsequent seedling establishment.

Treatment of seeds with a variety of inorganic and/or organic compounds, some of which are synthetic, has been successfully demonstrated to improve germination and seedling establishment in seeds of many field crops such as wheat, soybean, sunflower and maize (Kaya *et al*., 2006). Inglis *et al*. (2004) concurred that treatment of seeds with the right products has the potential to improve seedling emergence and establishment as well as plant stands. However, more than a decade ago, Jacobsen & Backman (1993) expressed their concern about the use of synthetic chemicals in agriculture and the potential hazards associated with their use. This can probably be regarded as an echo of public concern in this regard. Hence, there is elevated interest in finding alternative measures to manipulate either seed germination or seedling growth or both in an attempt to address both the plant stand problem and consumer concern.

The general aim of this study was, therefore, to determine the morphological, physiological and yield response of maize (cultivar *Bt* 7815) to seed treatments with a variety of products under laboratory, glasshouse and rain fed field conditions. These included a commercially available plant growth regulator (PGR), ComCat®, a prototype PGR referred to as SS, organic acids such as vulvic, amino and humic acid, fertilizer products such as Teprosyn®, Seniphos® and Zumzil® as well as micro-organism products such as EcoFungi® and EcoFlora®. Specific objectives were:

Phase 1: Screening the different products as seed treatments in the laboratory in order to identify the best performing ones in terms of root and coleoptile growth, and to follow below and above soil biomass production by seedlings in the glasshouse (Chapter 3).

Phase 2: To determine the morphological and physiological response of maize seedlings to seed treatment under glasshouse conditions (Chapter 4) with those products that were most promising according to the laboratory screening procedure

Phase 3: To determine the morphological and yield response of maize to seed treatments under field conditions over one season for those products that were most promising according to laboratory and glasshouse screening procedures (Chapter 5).

Phase 4: To evaluate the acquired data and determine the best seed treatment(s) in terms of morphological and yield parameters and to speculate on the possible physiological mechanism(s) involved (Chapter 6).

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CHAPTER 2

LITERATURE REVIEW

2.1 Maize crop and its importance

Maize (*Zea mays* L.) is a grain crop belonging to the grass family *Poaceae* (Paliwal, 2000) and is the only cultivated species of importance in the tribe *Mydeae* (Salian, 2007). Its origin has been a matter of controversy (IITA & CIMMYT, 2007) but it is generally agreed that its evolution into modern forms took place primarily in Central America (Rouanet, 1992) and that it originated through domestication of the wild grass teosinte (*Zea mexicana*), which is native to Mexico, Guatemala and Honduras (IITA & CIMMYT, 2007). According to Maize Industry's Versatile (1984), maize is thought to have originated in South America and has been introduced to Southern Africa by the Portuguese who started cultivating it on the east coast. It is believed that maize has been introduced to South Africa in the 17th century and since the 1950's maize farming has developed into one of the largest and most important branches of agriculture in this country.

According to Harris *et al*. (2007), maize is the third most important crop in the world, after rice and wheat, but in a report by Du Plessis (2003) it was rated as the most important grain crop in South Africa. It is also one of the most important crops in the milder, subtropical and tropical regions of the world. The crop is of significant economic importance worldwide as human food, animal feed and as a source for a large number of industrial products (Paliwal, 2000). In developing countries maize forms part of the staple diet (Du Plessis, 2003).

Rouanet (1992) deliberated that maize is an industrial raw material for a growing range and variety of food and non food products as it is used for human consumption, animal feed and for industrial purposes. Maize starch is involved in the enzymatic conversion into sorbial, dextrine, sorbic and lactic acid and also appears in

household items such as beer, ice-cream, syrup, shoe polish, glue and cosmetics (Du Plessis, 2003). Maize furnishes a large percentage of the calories, protein and fats required for energy and body building as a direct food and, moreover, it is a relatively cheap source of energy which has become a staple food of large sections of the population. It is also an important earner of foreign exchange for South Africa as the country export millions of tons of maize to other countries and earns millions of rands annually (Maize Industry Versatile Resource, 1984). In addition to the traditional uses of maize in South Africa, the country is considering maize fuel, an alcohol based alternative fuel produced by fermenting and distilling the starch rich grains of the crop. The production of ethanol in the country is said to bring huge socio-economic benefits through job creation and the industry will also make a significant contribution to the South Africa's GDP.

In light of the above, this study has been undertaken in an attempt to evaluate the potential of seed treatments to increase maize yield while the effect of these treatments on selected physiological parameters were monitored. According to Richardson (2007), optimization of the genetic potential of crop varieties will be a major contributor to improving yields and productivity to meet the global food, fuel and fibre demands of the future. Chemical manipulation of plant growth and development is an approach which is thought to have a considerable potential for both quantitative and qualitative improvement of crop performance.

Furthermore, plant growth regulators play strategic roles in the regulation of physiological processes and entail a culmination of metabolic enzyme activities (Roberts & Hooley, 1988). Manipulation of plant metabolism by means of compounds of both plant and non-plant origin can be applied to enhance both resistances towards stress factors and/or the final yield. Apart from crop breeding, there is consequently a possible alternative to genetic manipulation in achieving the same goals of yield increasement and the enhancement of resistance towards abiotic and biotic stress factors. Van der Watt (2005) pointed out that bio-manipulation using natural compounds extracted from plants is a possible alternative to plant breeding.

Natural products with the potential to manipulate crops in terms of growth, production and systemic induced resistance are already commercially available; some of which have been tested in this study.

2.2 Cultivation of maize in Africa

According to FAO statistics (FAO, 2004), the area planted to maize in West and Central Africa alone increased from 3.2 million in 1961 to 8.9 million in 2005 (IITA & CIMMYT, 2007). This phenomenal expansion of the land area devoted to maize resulted in increased production from 2.4 million metric tons in 1961 to 10.6 million metric tons in 2005. However, while the average yield of maize in developed countries can reach up to 8.6 tons per hectare, production per hectare is still very low (1.3 tons per hectare) in Africa.

According to Foster (2006), South Africa potentially has an area of 4.5 million hectares that are suitable for cultivation of maize. Furthermore, South Africa produces 6-9 million tons of maize per annum, including genetically modified cultivars (2-3% of the USA production), with a yield potential of approximately 4 tons/ha (12 tons/ha in USA and 16-18 tons/ha in Europe). This is a clear indication that both the production and yields for South Africa are far much less in respect to the USA and European countries which are the most developed countries. However higher yields and multi-cropping are achieved in the few relatively small areas that grow maize under irrigation. Du Plessis (2003) reported that approximately 8.0 million tons of maize grain is produced on approximately 3.1 million hectares of land in South Africa. The total production area (ha) planted and average yield of South Africa for the period from 2000 to 2005 indicates fluctuations between years. The average total production and the average yield varied significantly with the highest being in the period of 2004 to 2005 (Table 2.1).

Table 2.1: Total production area (ha) planted and average yield of the Republic of South Africa for the period 2000/01 to 2004/05 (Anonymous, 2008)

Year	Production (million ton)	Area (million ha)	Average yield (t ha^{-1})
2000/01	7.772 1	3.189	2.44
2001/02	0.077	3.533	2.85
2002/03	9.705	3.651	2.66
2003/04	9.737	3.204	3.04
2004/05	11.749	3.223	3.65
Average	**9.808**	**3.36**	**2.92**

2.3 Food security situation in Africa

Food insecurity has currently become a topic of great concern due to the ever escalating population. Food demand is estimated to double in 2020 if factors such as HIV/AIDS are not taken into consideration; hence food production globally should double in the next 20 years (Penning de Vries, 2001; Alam, 2004)

Sub-Saharan Africa faces problems of food security because of decreasing per-capita food production (Bekunda *et al.,* 2004). As a result, increasing yield for an ever growing population has currently become a topic of great concern with regard to food security. Agricultural productivity in Africa has not been able to cope with population growth, leading to increased annual imports and food insecurity (Mugo *et al*., 2005).

Food insecurity has been exacerbated by consecutive years of below normal seasons and poor harvests since 2002 in Southern Africa (SADC & RVAC, 2006). According to Bekunda *et al.* (2004), extreme poverty, widespread malnutrition and alarming environmental degradation are in part, consequences of a farming environment that results in large-scale nutrient mining from generally old and nutrient poor soils. Though there has been a maize surplus in South Africa for the season 2005/06, maize imports for most of the SADC countries have increased. This might have been as a result of reduced production and the increased demand for maize as part of the current bio-diesel project (Global Crisis Solution for Actionaid Southern Africa Region Management, 2006). The author maintained that 12 million people are already facing food insecurity in Southern Africa. The situation is chronic and contributes to increased vulnerability at household and community level. This clearly shows that the expected maize green revolution in Africa did not take off (Mugo, *et al*., 2005).

2.4 The growth cycle of maize

Different authors have described the growth cycle of maize in different terms and, as a result, allocated different numerical designations as representative of different growth phases. As a matter of interest and according to Hill (2007), subdivisions of the vegetative stages are designated numerically from V1 up to VT through V(n), where (n) represents the last leaf stage before VT for the specific hybrid under consideration. The first and last V stages are designated as VE (emergence) and VT (tasseling) respectively. The (n) will fluctuate with hybrid and environmental differences. Alternatively both the IITA & CIMMYT (2007) designated the symbols R1 through R6 for the reproductive phase. The first reproductive stage is the anthesis or male flowering stage when pollen shed begins while the last reproductive stage is referred to as physiological maturity which is identified by a black layer visible at the base of the grain. However, in this study the extended BBCH-scale (Biologische Bundesantstalt, Bundessortenamt and Chemical Industry) (Meier, 1997) was used.

This is a system for uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species. It resulted from teamwork between the German Federal Biological Research Centre for Agriculture and Forestry (BBA), the German Federal Office of Plant Varieties (IVA) and the Institute for Vegetables and Ornamentals (IGZ). The phenological growth of maize, as described by Meier (1997), includes 97 stages of which more than half is categorized as vegetative while the rest is categorized as reproductive. In this study, where the yield and physiological response of maize to seed treatments were followed, emphasis was placed on the quantification of the early vegetative growth stage (seedling growth; stage 00 through 09) under laboratory conditions while later vegetative growth stages (stage 30 through 69) and final yield (stage 99) were quantified under field conditions.

2.5 Plant respiration pathways as important processes involved in seed germination, seedling establishment and yield

Understanding the plant's metabolic pathways and its regulation is an important tool in chemical manipulation of the plant growth and development processes with the aim to improve germination, seedling establishment and finally the harvestable yield. It goes without saying that photosynthesis is the primary metabolic process that lays the foundation for carbohydrate production eventually determining the yield outcome in starch containing grains such as maize. However, cell respiration supplies the energy necessary for growth and development. The overall process of respiration involves a series of oxidation-reduction reactions during which compounds are oxidized to carbon dioxide (CO_2) while the oxygen (O_2) absorbed is reduced to form water (H_2O). Starch, sucrose, fructose and other sugars, fats, organic acids and even protein can serve as respiratory substrate. Respiration and plant growth are interrelated as respiration supplies the adenosine triphosphate (ATP) necessary for growth while adenosine diphosphate (ADP) and inorganic phosphate (Pi) are released only to be utilized during respiration to produce ATP again. In addition to

ATP, the high energy co-enzyme nicotine amide dinucleotide in its reduced form (NADH), also produced during respiration, is essential for reduction reactions, e.g. the reduction of nitrate to nitrite. The role of cell respiration in the inter-conversion of carbohydrate, protein, lipids and even nucleic acids has been well researched (Salisbury & Ross, 1991).

The respiration rate of plant tissues is related to both substrate supply and energy demands (Dwivedi, 2000), while sugars serve as the main source of energy and carbon flux (Buysse *et al*., 1993). Rakhmankulova *et al.* (2001) maintained that respiration plays a special role in plant adaptation to adverse conditions.

Seed germination and seedling development involves internal biochemical regulation activities of the respiration phases such as the oxidative pentose phosphate pathway, glycolysis and ethanol metabolism (Côme & Corbineau , 1989). Seed germination and seedling establishment, therefore, involves a series of enzymatic activities that are finely regulated during respiration (Rakhmankulova *et al.,* 2001). The authors further explained that alternative respiration pathways are associated with the synthesis of various secondary metabolites which are believed to be involved in plant protection against biotic and abiotic stress factors.

One of the major physiological changes during both the seed germination and seedling establishment phases is a rapid increase in the rates of respiratory pathways such as glycolysis, the oxidative pentose phosphate pathway (OPP) and the tricarboxylic (Krebs) cycle (Muscolo *et al*., 2001). These pathways are essential for both energy provision in heterotrophic cells and a wide range of other physiological functions (Fernie *et al*., 2004). Cell functioning, therefore, involves the combined activity of biochemical reactions catalyzed by an equally large number of enzymes (Côme & Corbineau, 1989). The interaction and coupling of metabolic pathways ensures a unified and regulated system of energy flow both in the cell and in the whole plant (Popova & de Carvalho, 1998). The authors further explained that cellular metabolism can be characterized by the existence of pathway branch-points

where the coordinated distribution of metabolites follows between different processes.

2.5.1 Biochemical regulation of the glycolysis cycle

Glycolysis is the first of the three closely related phases of respiration (Salisbury & Ross, 1991) and is of crucial importance in plants because it is the predominant pathway (Nardi *et al*., 2007). It is a metabolic pathway that involves a series of reactions leading to the breakdown of glucose-6-phosphate to two molecules of pyruvic acid while energy is transferred to high energy bio-molecules such as ATP and NADH that act as cellular energy sources. Pyruvate is completely broken down during the citric acid cycle, in the event that sufficient O_2 is available. The overall reaction of the glycolysis is summarized as follows (Orphardt, 2003):

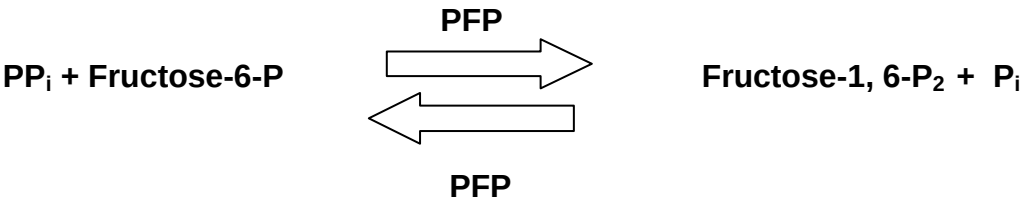
$$C_6H_{12}O_6 + 2NAD^+ + 2ADP + 2Pi \longrightarrow 2\,CH_3(C{=}O)\,COOH + 2ATP + 2NADH + 2H^+$$

More specifically phosphofructokinase (PFK; EC 2.7.1.11), involved in the phosphorylation of fructose-6-phoshate to fructose-1,6-bisphosphate, is regarded as the main regulatory enzyme of glycolysis (Gahan *et al*., 1983; Wong *et al*., 1987). It acts as the main control point in the glycolytic pathway as it is immediately downstream of the entry points for hexose sugars (Pretorius & Small, 1992).

$$ATP + \text{Fructose-6-P} \xrightarrow{PFK} \text{Fructose-1, 6-}P_2 + ADP$$

The above reaction is reversible due to the presence of PFP (PPi: D-fructose-6-phosphate 1-phosphotranferase; EC 2.7.1.90) that catalyses both the phosphorylation of fructose 6-phosphate (Fru-6-P) to Fructose 1,6-bisphosphate (Fru-1,6-P_2) in the glycolytic direction but also the reverse dephosphorylation step in

the gluconeogenesis direction (Mertens, 1991; Gahan *et. al*., 1983; Wong *et al*., 1987).



As is the case with PFK, PFP is also a highly regulated enzyme making this reaction (Gl-6-P $\Longrightarrow$ Gl-1,6-P_2) the most important regulatory point of the glycolysis cycle (Stitt & Vasella, 1988). The authors reported that both inorganic pyrophosphate (PPi) and fructose-2,6-bisphosphate (fructose-2,6-P_2) act as activators of PFP. Maize contains both ATP and PPi dependent phosphofructokinases (i.e. PFK and PFP) (Mertens, 1991). The activity of the ATP-dependant PFK is inhibited when citric acid, phosho-enol pyruvic acid (PEP) and ATP levels are high and activated when ADP and AMP levels are high (Salisbury & Ross,1991). In this way the rate of glycolysis as well as the reverse cycle, gluconeogenesis, is regulated depending on the energy needs of the crop.

2.5.2 Biochemical regulation of the Krebs cycle

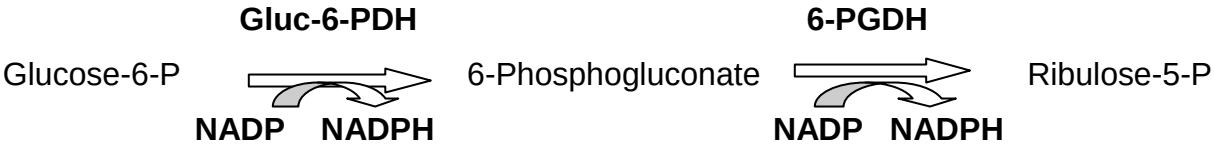
The Krebs cycle is the second step of aerobic respiration that takes over from the glycolysis pathway by completely breaking pyruvic acid down to CO_2 and H_2O (Alisdair *et al*. 2004). Citrate synthase and isocitrate dehydrogenase are the main regulatory enzymes of the pathway (Popova & De Carvalho, 1998; Ophardt, 2003) that are allosterically inhibited by high ATP and NADH levels (Nardi *et al.*, 2007).

Both malate dehydrogenase and pyruvic acid carboxylase are two important regulatory enzymes due to their anaplerotic role in the Krebs cycle (Marchetti *et al*., 1995). The term anaplerotic refers to the ability of these two enzymes to replenish

malate and pyruvic acid respectively during the “bleeding off” of intermediary compounds during amino acid and fatty acid synthesis as a means to ensure that the Krebs cycle will proceed normally.

2.5.3 Biochemical regulation of the pentose phosphate pathway

The oxidative pentose phosphate pathway (OPP) is another plant metabolic pathway which is interwoven with the glycolysis pathway because they have certain intermediates in common (Salisbury & Ross, 1991). The most important regulatory point of the OPP pathway is the first oxidative stage which is the irreversible conversion of glucose-6-phosphate to 6-phoshoglucanate, with an intermediary step, and catalyzed by two enzymes namely glucose-6-phoshate dehydrogenase (Gluc-6-PDH) and 6-phosphogluconate dehydrogenase (6-PGDH) (Debnam & Emes, 1999).



These two enzymes are found in both the cytosolic and plastidic fractions of photosynthetic and non-photosynthetic plant cells (Debnam & Emes, 1999). Especially Gluc-6-PDH controls the flux through the irreversible limb of the OPP pathway by limiting the breakdown of glucose in the early stages of germination (Hauschild & Von Schaewen, 2003). Any treatment that increases the activity of the OPP pathway tends to reduce seed dormancy (Swamy & Sandhyarani, 1986; Pretorius & Small, 1992). Further, Hauschild & Von Schaewen (2003) demonstrated that the cytosolic Gluc-6-PDH activity was affected by different sugars and that elevated cytosolic activity of the enzyme is not a consequence of phosphate sequestration, but depends on the presence of metabolizable sugars. The NADPH is

another factor known to act as a competitive inhibitor of Glu-6-PDH (Hauschild & Von Schaewen, 2003).

2.5.4 A general overview of sugars and its relationship with metabolic regulation and final yield

Soluble sugars such as fructose and glucose, made available directly by photosynthesis or indirectly via the hydrolysis of sucrose (transport form), are the main substrates for respiration (Saglio & Pradet, 1980). According to Couée *et al*. (2006) soluble sugars play a pivotal role in plant structure and metabolism at the cellular and whole plant levels. The authors further maintained that various metabolic reactions and regulatory compounds directly link soluble sugars with mitochondrial respiration or photosynthesis regulation and conversely with anti-oxidative processes such as the oxidative pentose phosphate pathway.

It is very important to understand that a photosynthesizing plant regulates the amount of carbohydrate stored in the form of sucrose, starch or fructans and this is dependent on supply or demand. Measuring sugar levels under research conditions can therefore be a handy indication of the potential energy status of a crop as well as the potential substrate available for normal grain filling and an acceptable yield (Pretorius & Small, 1992).

Equally, the respiration rate of plant tissues is related to substrate supply and demand as well as respiratory energy demand (Dwivedi, 2000). Further, soluble sugars serve as both energy and carbon substrate (Buysse *et al*., 1993), while accumulation of sugars and proteins are believed to promote drought tolerance in plants by maintaining turgor through osmotic adjustment. The regulatory effect of soluble sugars (e.g. glucose) on respiratory pathways via activation of key enzymes, such as Gluc-6-PDH of the OPP pathway, has been well documented (Couée *et al*., 2006). Lastly, as chlorophyll content in the leaves has been shown to be positively

correlated with grain yield in maize (Sorte *et al.,* 2005), it can be deduced that sugar supply via photosynthesis is the principle metabolic activity that determines yield potential. However, more precisely it is the balance between sugar production and consumption (via respiration) that eventually is the determining factor for yield outcome. The latter, and especially the question of whether manipulation of photosynthesis or respiration show the most promise to increase yields, still remains a matter of dispute.

2.6 An overview of plant growth regulating and fertilizer as well as micro-organism products used in the study

2.6.1 Plant growth regulators

Plant growth regulators generally include any synthetic or natural products that promote or inhibit plant growth and development by affecting cell division, elongation or differentiation (Huang, 2007). They are synthesized in one part of the plant and translocated to other parts where they can in very low concentrations cause physiological response of the plant (Salisbury & Ross, 1991). Natural growth promoting ones include phytohormones such as auxin, cytokinins, gibberellins and brassinosteroids, a new generation of phytohormones, while the growth inhibiting ones include abscisic acid and ethylene.

Auxin is responsible for initiation and promotion of cell division in cambium tissue thereby promoting stem and coleoptile elongation, apical dominance and inhibition of leave abscission (Van der Watt, 2005). Cytokinins promote cell division and increase chloroplast production, but can also delay senescence and organ formation. Gibberellins stimulate cell division and elongation (Gallant, 2004), while brassinosteroids (BR's) have been shown to regulate several physiological responses like cell division, cell elongation, synthesis of nucleic acids and proteins and enhancement of yield in cereals and vegetables (Jeyakumar *et al*., 2003). Although these natural phytohormones have not been tested individually in this

study, they form part of the commercially available plant growth regulator, ComCat®, as well as a prototype product (SS) that have been tested.

Optimization of the genetic potential of crop varieties will be a major contributor to improving yields and productivity to meet the global food, fuel and fibre demands of the future (Richardson, 2007). Chemical manipulation of plant growth and development is an approach which is thought to have a considerable potential for both quantities and qualitative improvement of crop performance. Furthermore plant growth regulators play strategic role in the regulation of the physiological developmental processes. The plant growth is a function of cell division and expansion hence changes in the cell wall extensibility induced by plant growth regulators are the culmination of enzyme actions (Roberts & Hooley, 1988). Manipulation of plant metabolism can be used to produce useful quantities of metabolites of both plant and non-plant origin (Ap Rees, 1981). Apart from crop breeding, there is a possible alternative to genetic manipulation in achieving the same goals of yield increase and the enhancement of resistance towards abiotic and biotic stress factors. Van der Watt (2005) pointed out that bio-manipulation using natural compounds extracted from plants is a possible alternative to plant breeding and these may include manipulation of primary metabolites such as respiration and specific secondary metabolites such as sugars and proteins that acts as metabolic intermediates. In addition to natural products (organic products), there are commercial inorganic products/compounds which play the same role as far as abiotic and biotic stress factors are taken into account.

There are commercial available bio-stimulants and inorganic products claimed by the manufactures that they play an important role in complementing varietal resistance. Bio-stimulants are non-fertilizer products which have a beneficial effect on plant growth. Gallant (2004) concurred that bio-stimulants are substances which are neither plant nutrients nor pesticides, but rather are organic material that when applied in small quantities, enhances plant growth and development such that the response cannot be attributed to application of traditional plant nutrients. This gives

an indication that bio-stimulants offer a significant opportunity for farmers in agriculture. In addition to bio-stimulants, there are products which consist of a mixture of macro and micro elements and they also play a pivotal role in plant morphological and physiological growth (Van der Watt, 2005). The detailed description of the different plant growth regulators used in this study follows.

2.6.1.1 ComCat®

ComCat® belongs to a new generation of natural plant strengthening agents and is manufactured from plant extracts with bio-stimulatory properties capable of regulating plant development (Hüster, 1999). According to the author, active substances are obtained from natural donor plants whose genetic potential has not been influenced by artificial breeding or genetic engineering. Moreover, donor plants are multiplied in virgin soils that are not treated with any inorganic fertilizers or agrochemicals. ComCat® consists of a rather crude mixture of all of the natural phytohormones referred to earlier, but also contains free amino acids and flavonoids (Van der Watt, 2005). Of these phytohormones, brassinosteroids is believed to play a pivotal role according to the manufacturers. It has been shown that synergism between these compounds is the key to the successes that have been achieved with the product in the agricultural practice in terms of resistance induction and yield improvement in a series of crops (Schnabl *et al*., 2001).

The manufacturers claim that ComCat® is a unique, new non-toxic plant strengthening agent that increases the plant`s ability to endure stress conditions (Schnabl *et al*., 2001). It is maintained that elicitors or signal molecules in the product coordinate biochemical processes involved in energy production and systemic acquired resistance towards biotic and abiotic stress factors via gene expression (J.C Pretorius, 2008. Personal Communication)[1].

[1] Prof. J. C. Pretorius, 2007. University of Free State, Department of Soil, Crop and Climate Sciences, P O Box 339, Bloemfontein, 9300

Moreover, one of the most promising attributes of ComCat® is its ability to induce flowering in a number of agricultural crops tested, leading to substantial yield increases (Schnabl *et al*., 2001).

2.6.1.2 A prototype plant growth regulator (SS)

A seed suspension (SS) prepared by grinding *Lupinus albus* L. seeds to a fine powder and extracting the contents, has been shown by Van der Watt (2005) to induce root development in seedlings of selected agricultural crops, as well as to improve the yield of these crops. *Lupinus* species belong to the family Fabaceae known to be of great importance in agriculture with regard to their symbiotic biological nitrogen fixation ability and hence the crucial economical role it plays in maintaining adequate nitrogen resources in the plant world (Allen & Allen, 1981). A study conducted by Van der Watt (2005) on the bio-stimulatory properties of SS has confirmed its potential to be developed as a natural product with application potential in agriculture. The active compound isolated, purified and identified was shown to be a triglyceride. In the mean time this compound has been patented and a natural product is currently in the development phase.

Based on the work by Van der Watt (2005), showing significant seedling growth and yield enhancement in maize and selected vegetables following one or more foliar applications, SS is included in this study, but solely as a seed treatment.

2.6.2 Fertilizer products

2.6.2.1 Teprozyn® (Zn/P)

Teprosyn® is a zinc-phosphorus compound with the main attribute of establishing a strong root system during seedling growth and early crop development (Richardson, 2007). According to Vance *et al.* (2003), phosphorus (P) is one of the 17 essential elements required for plant growth. In its phosphate (HPO_4^-) form it plays a role in an

array of processes including energy generation via photosynthesis and cell respiration, enzyme activation or inactivation as well as nitrogen fixation. Orabi and Abdel-Aziz (1982) added that phosphorus exerts many and varied functions in plant metabolism and hence inadequate phosphate supply to the plant seriously affects numerous metabolic processes. The authors maintained that the most important function is its formation of pyrophosphate bonds which allow energy transfer.

Zinc (Zn) on the other hand is an essential micro-element that plays a vital role in the synthesis of the essential aromatic amino acid, tryptophan, and is also involved in enzymatic reactions where it acts as an inorganic co-factor. Tryptophan is a precursor of the auxin, indole acetic acid (IAA), hence the indirect effect of Zn on auxin synthesis and growth regulation (Orabi & Abdel-Aziz, 1982). Zinc also activates the enzyme starch synthase (Jyung *et al*., 1975). The authors demonstrated that a maize cultivar susceptible to Zn deficiency showed a more suppressed activity of starch synthase and finally the number of starch grains in kernels.

Moreover, the relationship between Zn and P are reported to be a positive one (Orabi & Abdel-Aziz, 1982). The authors reported that the application of phosphorus to soil increased the uptake of Zn as well as the Zn-content in maize plants. Seed treatment with Teprosyn® has therefore been recommended by the manufacturers as an option for manipulating seedling growth via increased nutrient use efficiency as well as physiological processes during later growth stages. Research by the manufacturers confirmed this potential in different Teprosyn formulations such as Teprosyn Zn, Teprosyn Zn/O and Teprosyn Zn/P. Significant yield increases following seed treatment with Teprosyn Zn/P have been demonstrated in maize. This is believed to be due to enhanced efficiency of nutrient use by plants following seed treatments (Singh, 2003). According to Richardson (2007), Teprosyn Zn/P seed dressing of two maize cultivars, Dekalb 63-69 and Garst 8581, resulted in enhanced root development and top growth of seedlings leading to more efficient water and nutrient use, as well as drought tolerance. The author concluded that Teprosyn Zn/P

prepared the crop for the coming season thus enabling optimization of their genetic yield potential at harvest.

In support of these findings, Hunter (2001) reported that pea seed treated with Teprosyn Zn/P resulted in vigorous top growth of seedlings, compared to the untreated control, and this accelerated growth was observed in adult plants throughout the growing season.

2.6.2.2 Seniphos®

Seniphos® is another inorganic product included in this study. It basically contains phosphorus and calcium (P/Ca) as main components mixed in specific proportions while it also contains traces of other minerals (Li *et al*., 2002). According to Larrigaudiere *et al*. (1996) this product has been applied to various crops to improve the production of anthocyanin, therefore its effect in other crops such as maize is not yet clear. Barr (2007) demonstrated an impressive improvement in fruit firmness and a drastic increase in sugar content as well as a 17% increase in red coloration in apple fruits. It is usually used as a foliar application and its potential as a seed treatment has not previously been tested.

2.6.2.3 Humic and fulvic acids

Humic acids are products of the decomposition of plant tissues and are predominantly derived from lignified cell walls while comprising 60-70% of organic matter in soil (Russo & Berlyn, 1990). Humic substances not only have an impact on the physical, chemical and biological properties of soils, but also have a direct influence on the morphological and physiological properties of crops (Eyheraguibel *et al*., 2007). They, further, have a stimulatory effect on plants including the promotion of seed germination rate and fibrous root growth (Gallant, 2004). These effects are associated with a direct interaction of humic substances with physiological and metabolic processes (Eyheraguibel *et al*., 2007).

Humic acids enter the plant via the root system and are translocated from root to shoot where it increases the respiration rate, enhances mineral nutrient consumption and stimulates hormonal activities (Nardi *et al*., 2007). The authors also reported in recent studies that humic acid products either contain auxin or stimulate the activity of auxin-like molecules. They, further, influence the uptake of minerals by plants and stimulate plant growth with efficiency comparable to that of auxin. Humic acid products have also been reported to activate secondary metabolism in plants, to increase CO_2 uptake by leaves and to accelerate the synthesis of ATP via mitochondrial respiration (Nardi *et al*., 2007). Furthermore, humic acids promote chlorophyll, sugar and amino acid synthesis in plants, stimulate plant growth (higher biomass production) by accelerating cell division, increase the rate of root development and increase the dry matter content.

Fulvic acid is seen as a form of humic acid known as powerful organic electrolytes which help to dissolve minerals and metals making them more readily available for absorption by plants. It is, however, accepted that fulvic acid functions more in plants than in soil (Gallant, 2004).

2.6.2.4 Zumzil®

Zumzil® is a silicon product (Si OH_4), that is a form in which is absorbable by plants (J.C. Pretorius, Personal Communication). Silicon is an essential micro nutrient which is usually absorbed by plants in large amounts exceeding that of other nutrients. Silicon plays an important role in plants under drought conditions. It is deposited in the cell walls of the xylem vessels thereby preventing compression of vessels under high transpiration conditions caused by drought or heat stress (Matichenkov *et al.,* 2001). According to Liang (1999), the effects of silicon on plants have been hypothesized as follows; improvement of photosynthesis activity, increased enzyme activity and enhanced K:Na selectivity ratio.

2.6.3 Micro-organism products

2.6.3.1 EcoFungi®

EcoFungi® is a micro-organism product that consists mainly of different mycohrriza associate with plant roots. It enhances the root volume by improving the uptake of nutrients The product is applied either as seed treatment or soil drench with the aim of improving the soil ecology that usually leads to healthier and more productive crop plants (Dr Douillet, Personal communication)[2].

2.6.3.2 EcoFlora®

Eco-flora® is a product developed by Douillet Eco-microbial (Pty) Ltd and it mainly consists of different Streptomyces and Trichoderma strains. Its main attributes from an agricultural perspective is the ability to induce vegetative growth, improve nutrient uptake and also enhances yield. Additionally the product proved to play a role in the bio-control of a number of fungual pathogens (Dr Douillet, Personal communication)[2].

2.7 Seed treatment as a possible viable manipulation practice with the aim to improve seedling establishment, yield and quality of maize

Seed treatment is an alternative practice which can be used as a manipulating tool to improve the resistance of crops towards abiotic and biotic stress factors (Basra *et al*., 1989). Seed treatment practices have been reported to show potential in protecting plants against these stress factors during radicle emergence and seedling establishment (Inglis *et al*., 2004). It is, therefore, a relative easy and attractive method for introducing biological control agents into the soil-plant environment (Bevivino *et al*., 1998).

[2] Dr. P. Douillet. 2007. North-West University, Potchefstroom Campus, Private Bag X6001, Potchefstroom, 2520.

Seed treatments exhibit a range of different effects on the plant metabolism (Nardi *et al*., 2007). According to Chuanren *et al*. (2004) seed treatments affect both physiological and metabolic activities, resulting in early germination, hence promoting seedling establishment and final harvestable crop yield. Bevivino *et al.* (1998) suggested that the effect of seed treatments may result either from indirect activities such as bio-control of soil borne diseases through competition for nutrients, antibiosis or induction of systemic resistance activity in the plant. El-Naimi *et al*. (2000) maintained that organic seed treatments reduced the common bunt infection due to an increase of the soil borne micro-organism or to the production of toxic metabolites.

The following statement by Johnson (2001) indicates clearly that seed treatments with micronutrients are of great importance to crop production: “Seed coating with micronutrients would show the unique advantage of providing a cost effective application technique compared to soil application. It complements existing methods of counteracting deficiencies and provides an opportunity to enhance fertilizer use efficiency and micronutrient application where traditional methods are not practiced, although the need for treatment exists”.

Treatment of seeds with inorganic and/or organic compounds, both separately and together, has been successfully demonstrated to improve germination and seedling establishment in seeds of many field crops such as wheat, soybean, sunflower and maize (Kaya *et al*., 2006). The elevated micronutrient content due to seed treatment has been associated with improved seedling vigour and final yield (Harris *et al.,* 2007). The authors suggested that the seed treatment approach might have some adVantages for maize production to the effect that uneven application of nutrients to the soil is avoided as each seed is exposed to the nutrient; the initial uptake is guaranteed; the nutrient is available early in the life of the plant; the amount required are likely to be orders of magnitude less and thus less costly than for soil application.

2.8 Morphological, physiological and yield response of crop plants to various seed treatments

Treating maize seeds before sowing have been reported by different authors to affect the morphological growth and physiological metabolism of maize plant in various ways prior to yield. Hameeda *et al.* (2008) reported that treating maize seeds with EB67 and CDB35 phosphate solubilizing bacteria improved the growth and yield of the crop. Their studies on the effect of seed treatments on the growth and yield of maize indicated that seed treatments enhanced root and shoot length growth, plant biomass production and seed vigour index while seed treatment with EB67 and CDB35 increased maize grain yield by 85% and 64% respectively compared to the untreated control.

Seed treatment with humic like substances has been shown to inhibit high proliferation of lateral and secondary roots which in turn induced an increase of total root length and root surface area (Eyheraguibel *et al*. 2007). The authors also demonstrated a significant increase in total biomass which correlated with the increase in fresh and dry weight for each maize plant as well as shoot, leaf and root biomass separately. Similar results were obtained by Nithila *et al*. (2007) who observed an increase in both the dry matter production and grain yield after treating millet with brassinosteroids, a natural phyto-hormone and constituent of ComCat®.

Murungu *et al.* (2003) reported on the effect of seed priming on the growth and yield of maize and cotton across the range of soil aggregate size distribution. Seed treatment with a variety of products inter alia improved seed vigour, seedling emergence and early seedling establishment as well as shoots and root length of maize under low or cold temperature conditions. Treating maize seeds with a fungicide Vitavax®, proline and a bio-stimulant Radiform® promoted germination while proline and the bio-stimulant produced higher seedling fresh weight in comparison to untreated seeds (Vinković *et al*., 2007).

Studies by Kaya *et al*. (2006) concurred that seed treatments may reduce the risk of poor stand establishment in cold and moist soils as their results demonstrated an increase in both germination rate and post germination growth under the same environmental conditions. Vinković *et al*. (2007) confirmed that seed treatment play a role in improving seed germination and seedling emergence under stressful conditions and this contributes to targeted stand establishments.

Treating seeds with either organic or inorganic products has demonstrated to have a positive influence on both the dry matter production and grain yield (Nithila *et al*., 2007). Further, seed treatment with brassinosteroids produced the highest values for all growth components measured leading to yield increase of finger millet (Záborzky, 2004). Additional studies by the author on the influence of seed treatments with a fungicide and an insecticide on the biological value, initial plant development and quantitative and qualitative yield parameters on maize proved that this practice led to increased grain yield and increased stalk strength. Seed treatments can also improve chilling tolerance in crops under field conditions, leading to a significant increase in grain yield due to better field emergence and greater stress tolerance (Sairam, 1994). The author demonstrated an increase in the activities of selected enzymes, chlorophyll content and photosynthesis rate under moisture stress conditions as a result of treating wheat seeds with homobrassinolide. These beneficial effects resulted in higher leaf area, biomass production and increased grain yield.

In contrast to the promotion of plant growth by seed treatments, studies by Kozdroj *et al*. (2004) has shown that treating maize seeds with pseudomonads did not promote plant growth. Rather, this rhizobial strain seemed to induce decreases in shoot and root weights. The results further indicated that the increased levels of inoculant strains to maize seeds resulted in competition for nutrients with maize and this competition might bestow stress to plants, possibly causing the decreased shoot/root ratios. Muscolo *et al*. (2001) observed that treating *Pinus laricio* seeds with phenols extracted from different forest soils inhibited germination and activities of enzymes of glycolysis (aldolase and glucose phosphate isomerase) and the oxidative pentose

phosphate pathway (Glucose-6-dehydrogenase). The failure of treated seeds to germinate strongly correlated to the inhibition of enzymes of glycolysis and oxidative pentose phosphate pathway. This shows that selection of compounds for treatment of seeds should be done cautiously.

Physiological plant parameters can be an indication of the specific physiological activities taking place within the plant cells. Different organic and inorganic products have been reported to affect the metabolism of crop plants. A study on the effect of humic like substances on maize seedlings has shown an increase in enzyme activities such as PPi-PFK (Alisdair *et al*., 2004). According to Swamy and Sandhyarani (1986) seed treatments with growth promoters such as kinetin and abscisic acid have an impact on enzymatic activities. Kinetin and abscisic acid seed treatments decreased the levels of Gluc-6-PDH and 6-PGH activities in the cotyledons and embryonic axes during germination of non dormant lines by inhibiting the production of various mRNA species. It has been concluded that a marked increase in the activities of oxidative pentose phosphate pathway enzymes are closely related to increased germination of seeds and may have significance effect in the termination of dormancy (Atkins & Ross, 1981; Swamy *et al*., 1981; Swamy & Sandhyarani, 1986).

Studies on the effect of seed pre-soaking in AC 94,377, GA_{4+7} and ABA showed that seed treatments can have a remarkable effect on the metabolic activities of germinating maize embryo's under stressing temperature regimes (Basra *et al*., 1989). Results revealed an increase in the accumulation of soluble sugars and proteins after seed treatments as compared to the control. An increase in the activities of enzymes such as peroxidase, acid phosphatase, invertase and catalase was also demonstrated, this may indicate a regulatory effect on the triggering of a mechanism involved in temperature stress alleviation (Basra *et al.*, 1989).

Harman *et al*. (2004) reported on the effect of *Trichoderma harzianum* (T22) strains on the activities of defense related enzymes in maize seedlings. The seed treatment

increased the levels of enzyme proteins in both the roots and shoots. The defense enzymes studied included the pathogenesis resistant (PR) proteins endochitinase, exochitinase and β-1,3 glucanase and their activities have been shown to be significantly increased in maize seedlings after treatment with *T. harzianum.* This is a strong indication that seed treatment with specific elicitors can also increase the resistance of crops towards biotic stress factors. Islam *et al*. (2006) also reported a significant reduction in leaf spot disease and hence an increase in grain yield in field cultivated wheat after seed treatment with garlic and *Bishkatai* extracts. The induced resistance towards fungal attack resulted in an increased number of grains per ear as well as healthy grains.

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CHAPTER 3

SCREENING FOR EFFICIENT SEED TREATMENTS OF MAIZE IN TERMS OF GERMINATION AND SEEDLING GROWTH UNDER LABORATORY AND GLASSHOUSE CONDITIONS

3.1 ABSTRACT

Subsequent to seed germination, seedling establishment is a critical phase in the growth cycle of any crop, but more so for staple food crops such as maize. Treatment of seeds with the proper product offers an attractive approach in addressing both poor germination and seedling establishment. In this study the germination and growth response of maize to plant growth regulators (ComCat® (CC), humic acid, amino acid, fulvic acid, AnnGro™ and a prototype seed suspension (SS) from *Lupinus albus*), fertilizer products (Teprosyn®Zn/P, Seniphos® and Zumzil®) and micro-organism bio-products (Eco-fungi® and Eco-flora®) after seed treatments were investigated under laboratory and glasshouse conditions. Untreated seed was used as negative control. The CC and SS treated seed were initially tested at different concentrations to identify the optimum concentrations. ComCat® at 25 mg kg^{-1} and SS at 12.5 mg kg^{-1} seed optimally stimulated seedling growth. Humic acid, an amino acid mix and fulvic acid, on the other hand, inhibited maize seedling growth. Of the fertilizer products, Teprosyn® stimulated seedling growth while Seniphos® had no effect. However, Seniphos® in combination with CC showed a stimulatory effect. Moreover, addition of AnnGro™ to either Teprosyn®/CC and Teprosyn®/SS or Seniphos®/CC and Seniphos®/SS combination treatments enhanced root and coleoptile growth under glasshouse conditions.

Keywords: maize, seed treatments, plant growth regulators, fertilizer products, micro-organisms, seedling growth.

3.2 INTRODUCTION

According to Kaya *et al*. (2006) poor crop production is, inter alia, a result of suboptimal seedling establishment due to poor weather and soil conditions while Kerr and Hammermeister (2007) maintained that seed vigour is an important underlying factor. Seeds are often the first to encounter stress early in a plant's life cycle and are particularly vulnerable between sowing and seedling establishment (Afzal *et al.,* 2005). Furthermore, poor germination of seeds under stressing environments might be associated with alteration in the endogenous levels of phytohormones (Basra *et al*., 1989).

The overarching goal of crop establishment is to achieve rapid and uniform seed germination as well as uniform seedling growth and autotrophy (Afzal *et al.,* 2005). A new and more modern approach to manipulate seed germination and subsequent seedling growth, besides fertilizer application, is the use of plant growth regulators. Treating seeds with plant growth regulators offers an attractive approach in addressing poor germination and crop establishment as a result of stressing environmental growing conditions (Basra *et al*., 1989). Bevivino *et al*. (1998) elaborated that seed treatment is an attractive method for introducing biological control agents into the plant-soil environment. Furthermore, Vinković *et al*. (2007) maintained that seed treatment with a proper product can improve germination and field emergence under stressful environmental conditions leading to optimal stand establishment. In an attempt to eliminate cold stress and improve seed vigour on maize and soybean respectively, the result demonstrated enhanced germination and seedling biomass production in maize following seed treatment. Germination was found to be significantly higher in the cold test, as compared to constant temperature, while seedling fresh and dry weight were increased by proline and bio-stimulant pre-seed treatments compared to the untreated control.

Numerous studies have demonstrated improvement in seed germination and plant stand of different plant species under both normal and stress conditions in response

to seed treatments with plant growth hormones and other organic/inorganic substances (Basra *et al*., 1989; Afzal *et al*., 2005; Akbari *et al*., 2007). In *Macrotyloma uniflorum* (Lam.), comparisons of seed treatment with an untreated control demonstrated improved germination, root growth, shoot and cotyledon expansion as well as seedling biomass in response to plant growth substances such as Gibberellins (GA3) and Auxin-IBA (Chauhan, *et al*., 2009). Afzal *et al*. (2005) demonstrated improved germination and seedling establishment in wheat under saline stress conditions as a result of GA3 and IAA seed treatments. Still on plant-hormones, Gholami *et al*. (2009) reported a positive effect of seed treatments on maize. The study revealed enhanced seedling emergence as well as tolerance to external stress factors in response to growth promoting rhizobacteria. There is also evidence of growth enhancement in wheat seedlings after using growth hormones such as Auxin as pre-seed treatment (Akbari *et al*., 2007). Treatment of finger millet seed with growth regulators confirmed enhanced seedling growth expressed in terms of increased leaf area index and total dry matter weight accumulation (Nithila *et al*., 2007). The findings supported the results of a study by Akbari *et al*. (2007) by demonstrating increased hypocotyl length, seedling fresh and dry weights as well as hypocotyl dry weight following Auxin seed treatments. However, an antagonistic effect was observed in terms of seed germination percentage at 0.6 salinity level in comparison with the control suggesting that concentration levels also play a role in seed treatment practice.

In addition to phytohormones, a seed treatment with fertilizer products such as zinc stimulated seedling growth and good stand on maize (Harris *et al*. 2007) and in common bean (*Phaseolus vulgaris)* (Basra *et al*. 1989). Moreover, the incidences of pests and diseases can be reduced by pre-sowing seed treatments. Seed treatment with effective micro-organisms (EM) and micro-nutrients has proved reduction in the incidences of diseases and parasite injury in this regard and furthermore prevented nutrient imbalances on wheat and rice. Seed treatments with EM and micro-nutrients (Cu, Zn & Mn) improved maize and wheat from disease and pests infestation as well as increased final yield (Primmavesi, 2009).

In this study the seedling growth response of a hybrid maize cultivar (DKC78-15*Bt*) was followed after treating seeds with a number of products, including plant growth regulators, fertilizer and bio-products (micro-organisms), that are either commercially available or in the prototype development phase. The principle aim was to identify the best performing ones in terms of root and coleoptile growth under laboratory conditions as well as below and above soil biomass production under glasshouse conditions.

3.3 MATERIALS AND METHODS

3.3.1 Seed material

Certified seed of a hybrid maize cultivar (DKC78-15*Bt*) were commercially obtained from Monsanto, South Africa. This is a genetically modified maize hybrid containing the *Bt*-gene.

3.3.2 Chemicals

Chemical products used in this study were sourced from different companies or institutions, Viz. ComCat® (CC) and Seed Suspension (SS) from Agraforum SA Pty Ltd., AnnGro™ from the University of the North West, South Africa and Seniphos® and Teprosyn® Zn/P from Sidi Parani, South Africa, an affiliate of Yara**.** Other products such as humic acid, an amino acid mix and vulvic acid were purchased from Sigma. The chemicals were separated into four groups namely a) plant growth regulators, b) organic acids, c) biological control agents and d) fertilizer products, and tested separately under either laboratory or glasshouse conditions or both.

3.3.3 Methods

3.3.3.1 Seed treatment

Fifty gram maize seed were pre-treated separately with the different test products at either the concentration suggested by the manufacturers or the optimum concentration determined beforehand in the laboratory. In all cases the volume of product used to treat 50 g seed was 1 ml (500 ml kg^{-1}). The seed was placed in a small plastic bag, covered with the product and agitated rigorously for 1 minute. Subsequently, the treated seed was placed on a sheet of filter paper and allowed to dry for 30 minutes.

The seedling growth response of maize to the following seed treatments was quantified against an untreated control (concentrations supplied in brackets):

a) Plant growth regulators:

- ComCat® (CC) [0.5 mg L^{-1} (positive control suggested by manufacturers), 12.5, 25, 50, 100 and 200 mg kg^{-1} seed].
- *Lupinus albus* Seed Suspension (SS) [5 mg kg^{-1} seed (positive control; Van der Watt (2005), 12.5, 25, 50, 100 and 200 mg kg^{-1} seed].

b) Organic acids

- Vulvic acid (1 g L^{-1} seed).
- Amino acid (20 g kg^{-1} seed).
- Humic acid (20 g kg^{-1} seed).

c) Biological control agents:

- Eco-flora® (62.5g kg^{-1} seed)
- Eco-fungi® (62.5g kg^{-1} seed)

d) Fertilizer products:

- Teprosyn® Zn/P (8 ml kg^{-1} seed).

- Seniphos® (4 ml kg^{-1} seed).

Additionally, AnnGro™ (an uptake enhancer) was tested separately at 7.5 ml kg^{-1} seed and later added to specific combination treatments.

3.3.3.2 Laboratory seed treatment screening tests

Two sheets of special germination paper (30 x 30 cm) were used to test the seedling growth response of maize seedlings after seeds were pre-treated with different products. A line, 10 cm from the top, was drawn on the one sheet and 15 seeds spaced evenly on the line. A second sheet of germination paper was placed on top of the first and moistened with distilled water. Both sheets of paper were rolled up together and longitudinally, placed upright in an Erlenmeyer flask containing 200 ml distilled water and kept at 25^{o}C in a growing chamber for 96 h in the dark. Coleoptile and root lengths were measured after 96 h of incubation using a digital caliper. All treatments were replicated three times.

3.3.3.3 Planting and screening procedures under glasshouse conditions

Pre-treated seeds (2 per hole) were planted in a growth medium in seedling trays and kept at field capacity in a glasshouse. Trays were arranged in a complete randomized design and replicated eight times. Three weeks after planting seedlings were removed from the trays, root and above soil parts separated by means of a sharp knife and the fresh mass determined.

3.3.3.4 Statistical analysis of data

Analysis of variance (ANOVA) was performed on the data, using the SAS statistical program to identify differences between treatments. Tukey-Kramer's LSD (least significant difference) procedure for comparison of means was applied to separate means at the 5% ($P<0.05$) probability level. Treatments differing significantly were indicated in figures both as calculated LSD values and by using different letters.

3.4 RESULTS

3.4.1 Maize seedling growth response to seed treatments with plant growth regulators under laboratory and glasshouse conditions

Treating maize seeds with different ComCat® (CC) concentrations prior to subjecting them to a germination test in the laboratory revealed induced root growth at the lower concentrations in the range compared to the untreated control (Fig. 3.1). Although not significantly different from the control, the 25 mg kg^{-1} application emerged as the optimum concentration. Higher concentrations also enhanced the root growth although lower than the optimum. ComCat® concentrations tested did not have a significant effect on coleoptile growth compared to the untreated control.

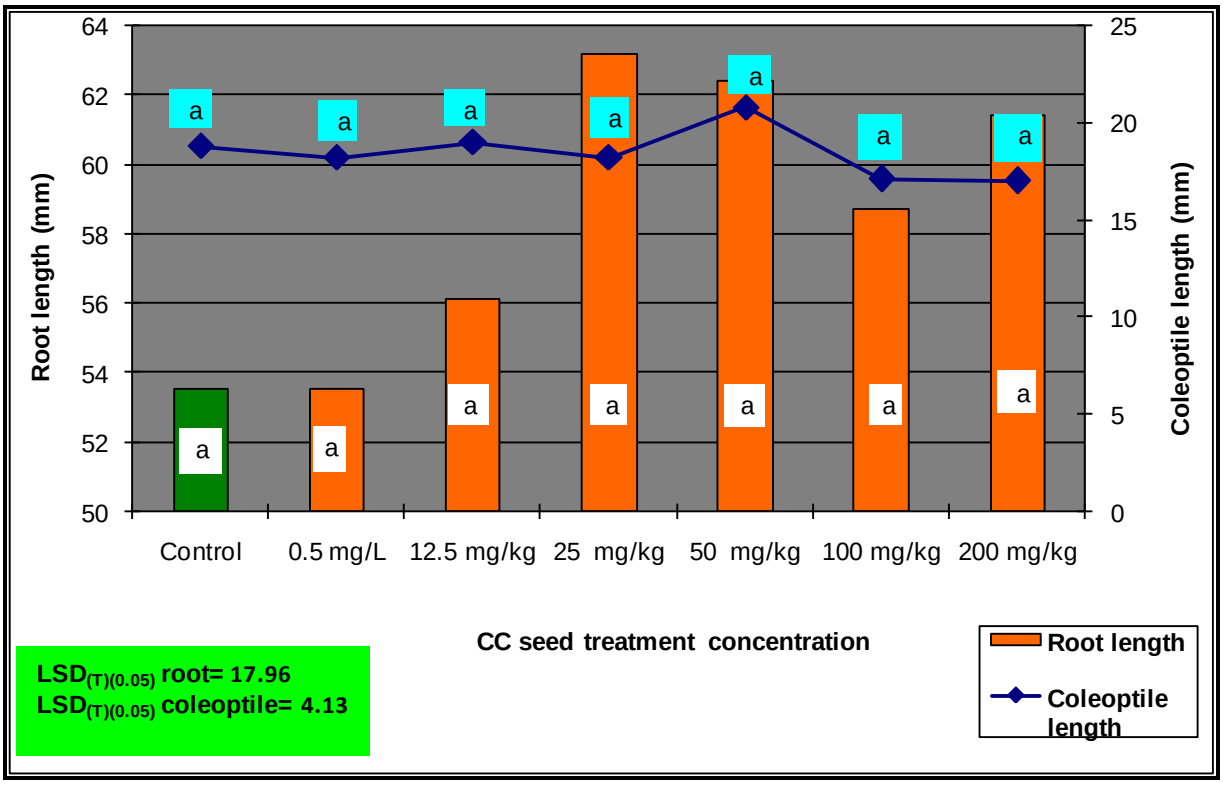

Figure 3.1: Root and coleoptile growth in response to pre-treatment of maize seeds with ComCat® in a concentration range under laboratory conditions. LSD ($P<0.05$) values are indicated in the graph and data differing significantly are indicated with different letters.

The same tendency prevailed for maize seedling root growth under glasshouse conditions as it was for the laboratory conditions from seeds pre-treated with CC in a concentration range (Fig. 3.2). In terms of root fresh mass production, again the 25 mg kg^{-1} concentration proved to be optimal while higher concentrations in the range had an inhibiting effect. The 21.4% root growth induction at this optimum concentration was however not significantly different from the untreated control.

In contrast to observations under laboratory conditions, growth of the seedling above ground parts was significantly improved by seed pre-treatment with CC at 25 and 50 mg kg^{-1} in the glasshouse, compared to untreated control seeds (Fig. 3.2). As the latter fresh mass was increased by 15% and 23.1% respectively, it seems that the 50 mg kg^{-1} CC concentration was more optimal for above soil part growth under

glasshouse condition. In contrast, both below and above growth was significantly inhibited by 12, 5 mg kg^{-1} CC compared to untreated control.

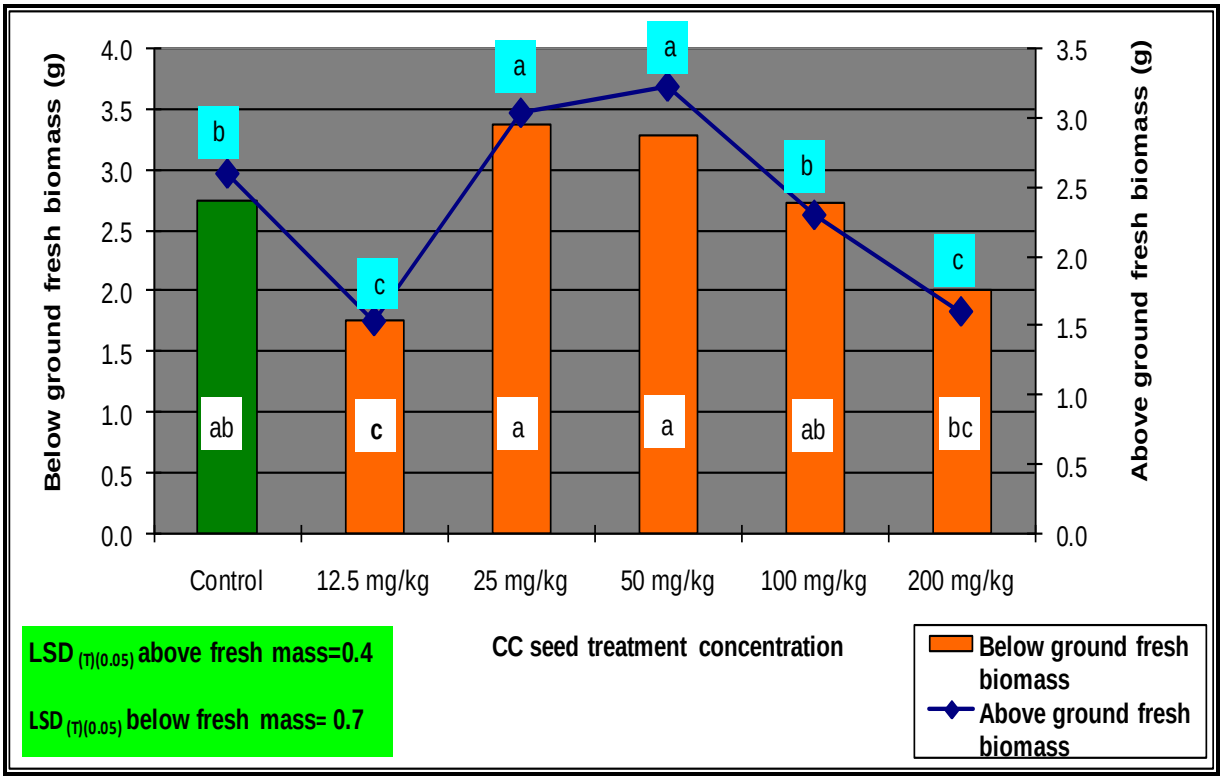

Figure 3.2: Maize seedling growth in terms of fresh mass in response to pre-treatment of seeds with ComCat® in a concentration range under glasshouse conditions. LSD ($P<0.05$) values are indicated in the graph and data differing significantly are indicated with different letters.

Pre-treatment of maize seeds with the *Lupinus albus* seed suspension (SS) at a concentration range revealed the same tendency for induced root growth at the lower concentrations under laboratory conditions, as was the case with CC, but with optimal growth at seeds treated with 12.5 mg kg^{-1}, (Figure 3.3). Although not significantly different from the control, root growth of seedlings was inhibited by SS at the higher concentrations of 100 and 200 mg kg^{-1}. Contrary to stimulated root growth in response to the lower SS concentration range coleoptile growth was not affected.

However, as was the case with root growth, the higher SS concentrations (200 mg kg^{-1}) significantly inhibited coleoptile growth compared to the untreated control.

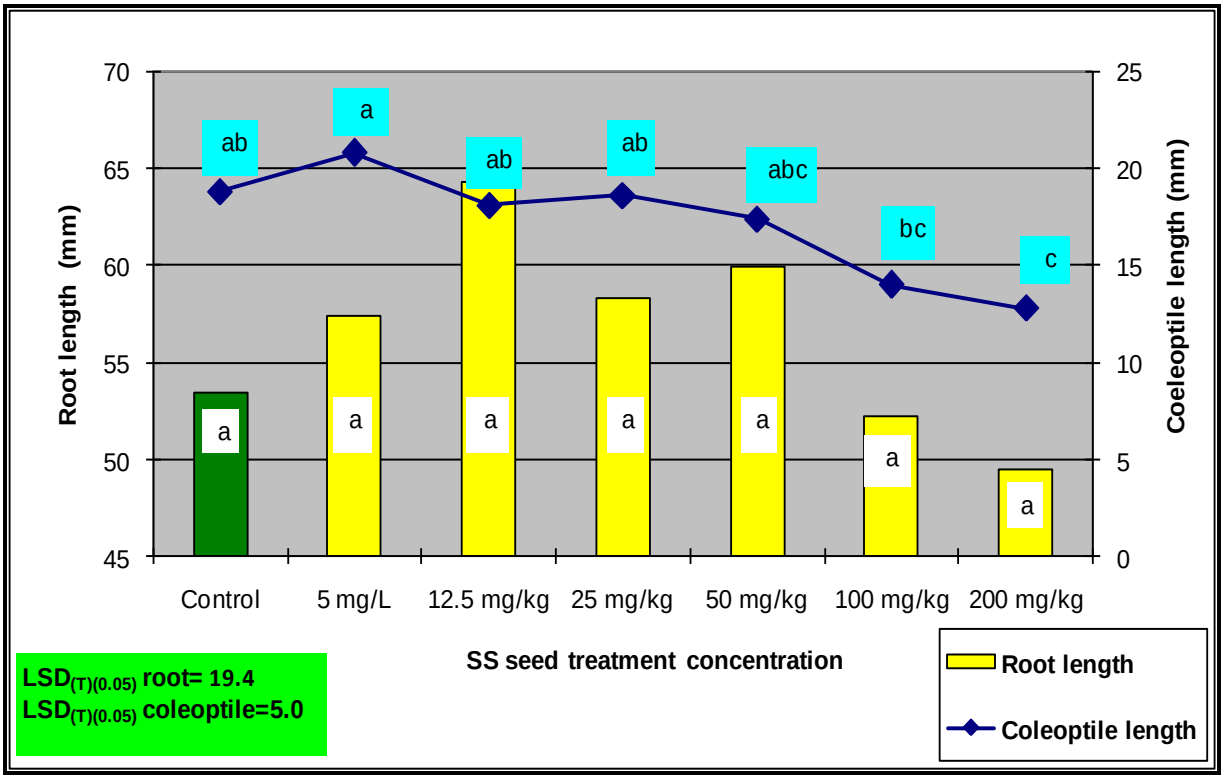

Figure 3.3: Root and coleoptile growth in response to pre-treatment of maize seeds with SS in a concentration range under laboratory conditions. LSD ($P<0.05$) values are indicated in the graph and data differing significantly are indicated with different letters.

Under glasshouse conditions, three weeks after planting, a rather irregular seedling growth response to treatment of seeds with SS in a concentration range was observed and the response of both roots and coleoptiles was similar (Fig. 3.4). Although neither of the concentrations in the range had a significant enhancing effect on root and coleoptile growth, the 25, 50 mg and 100 kg^{-1} concentrations significantly inhibited growth. However, what was rather odd is that the SS seed treatment at 100 mg kg^{-1} had less of an inhibitory effect than the previous two concentrations in the range while double this concentration had no effect on either root or coleoptile growth.

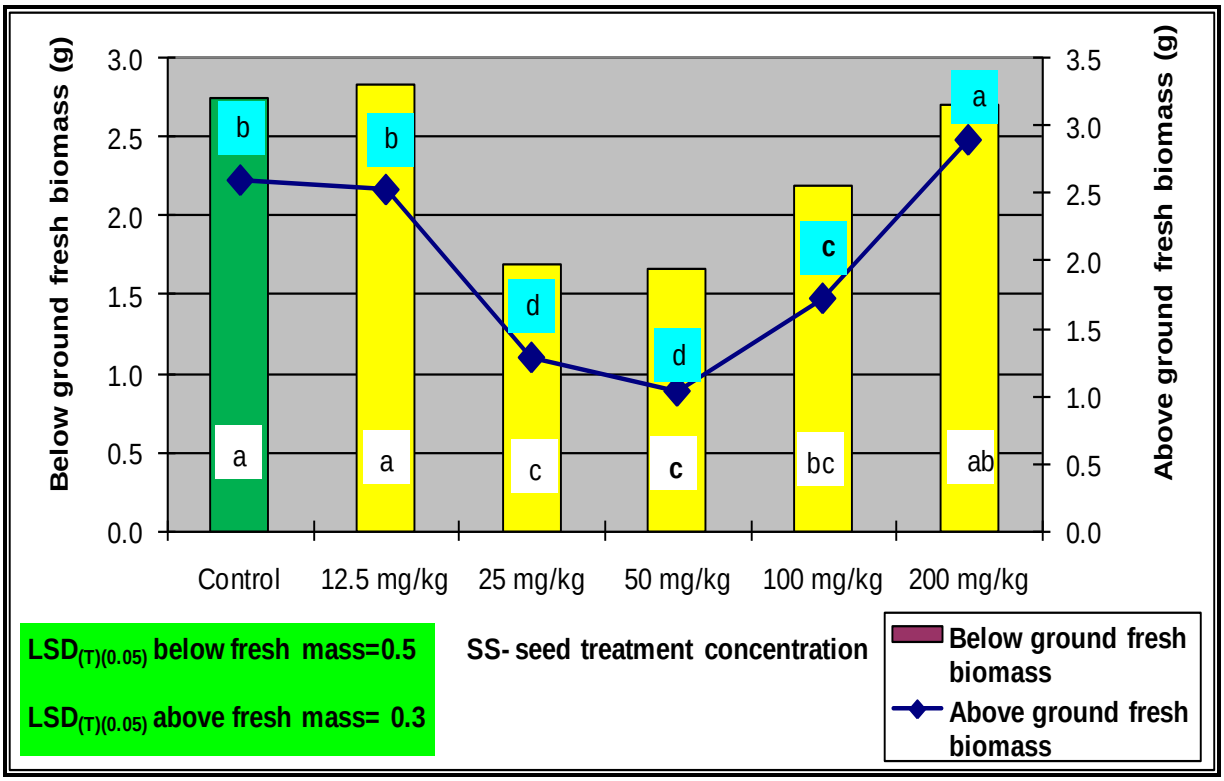

Figure 3.4: Maize seedling growth in terms of fresh mass in response to pre-treatment of seeds with SS in a concentration range under glasshouse conditions. LSD ($P<0.05$) values are indicated in the graph and data differing significantly are indicated with different letters.

3.4.2 Maize seedling growth response to seed treatments with organic acids under laboratory conditions

Both the amino acid and humic acid products had an inhibitory effect on root and coleoptile growth while the vulvic acid product had no effect (Fig. 3.5). Although the inhibitory effect exerted on seedling growth by treatment of seeds with the former two organic products exceeded 20%, this was not statistically significant, probably due to large standard deviations between replicates.

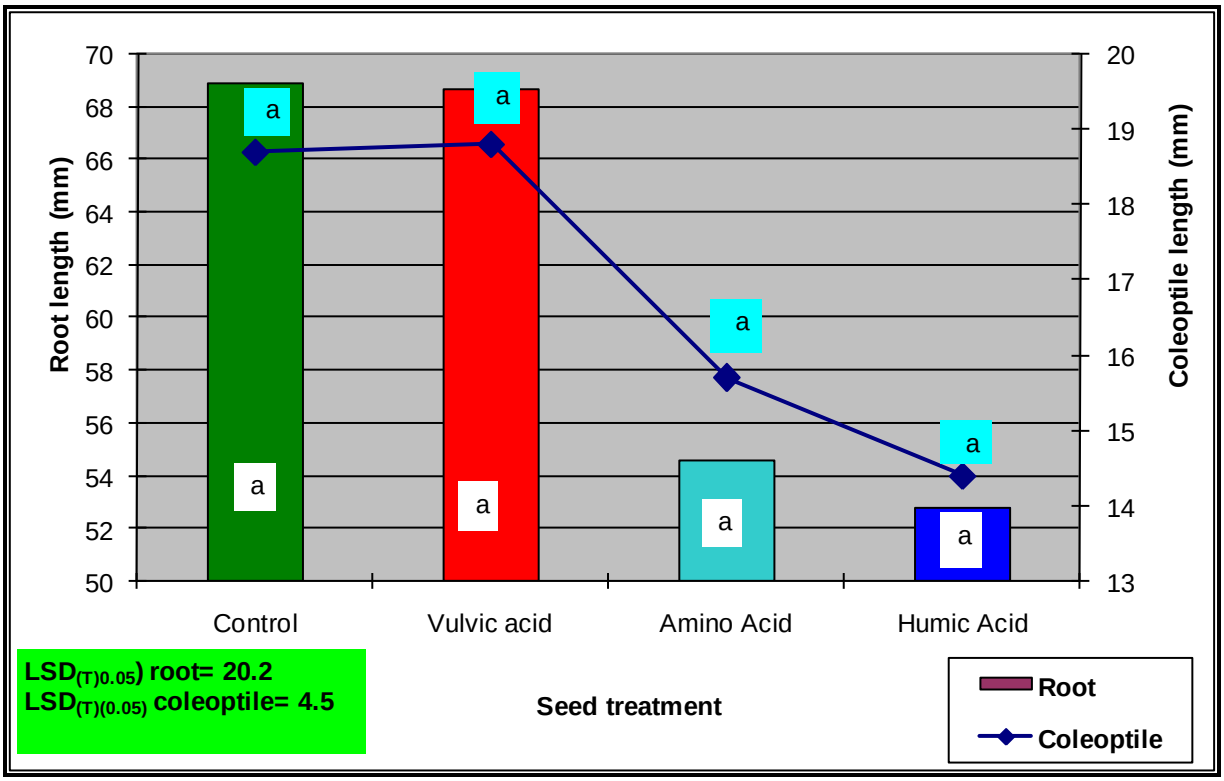

Figure 3.5: Root and coleoptile growth in response to pre-treatment of maize seeds with organic acids under laboratory conditions. LSD ($P<0.05$) values are indicated in the graph and data differing significantly are indicated with different letters.

3.4.3 Maize seedling growth response to seed treatments with two biological control agents under laboratory conditions

Both of the biological control agents, Eco-flora® and Eco-fungi®, had an inhibitory effect on root and coleoptile growth, although statistically not significant (Fig. 3.6) from the control. For this reason the trial was not repeated under glasshouse conditions.

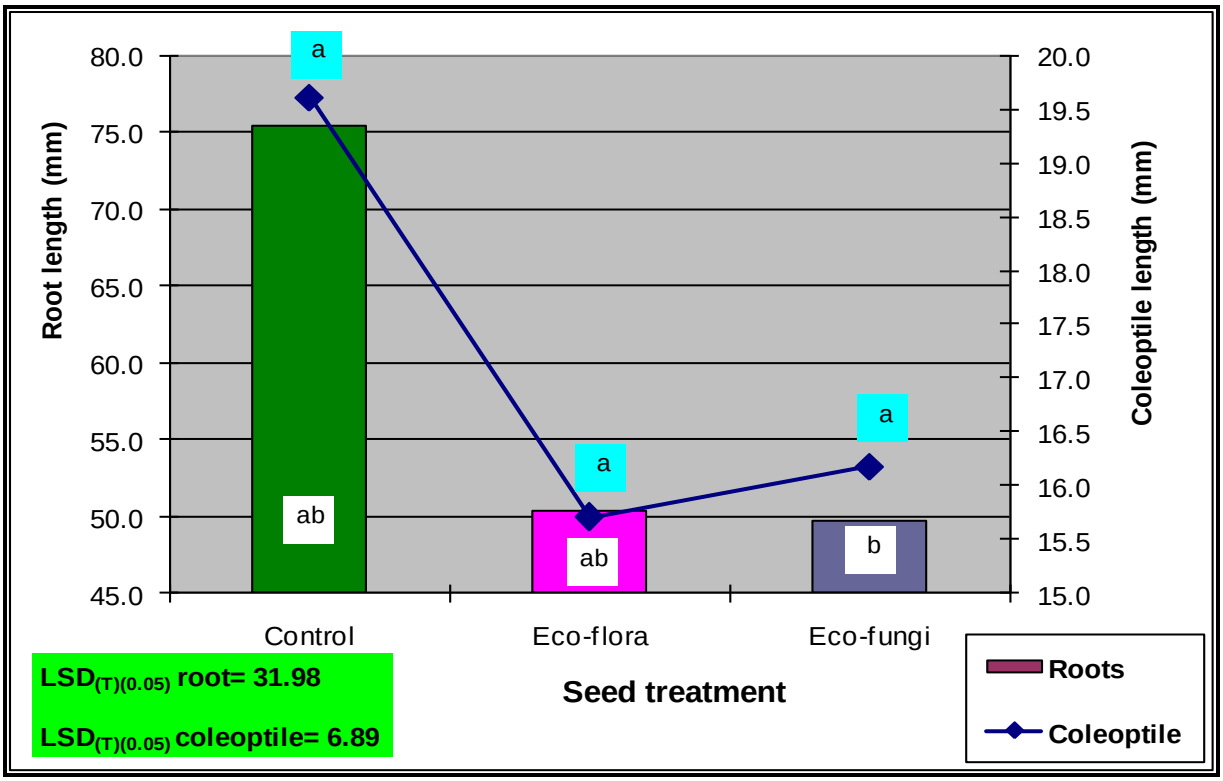

Figure 3.6: Root and coleoptile growth in response to pre-treatment of maize seeds with biological control agents under laboratory conditions. LSD ($P<0.05$) values are indicated in the graph and data differing significantly are indicated with different letters.

3.4.4 Maize seedling growth response to seed treatments with fertilizer products both separately and in combination with plant growth regulators and an uptake enhancer under laboratory and glasshouse conditions

The maize seedling growth response after seeds were pre-treated with a single products viz; AnnGro™, Seniphos® & Teprosyn® separately demonstrated the most enhanced root and coleoptiles growth by Teprosyn® compared to control (Fig. 3.7). Seniphos® on its own had no effect on either root or coleoptile growth. However, where seeds were pre-treated with Seniphos® in combination with ComCat® (CC) significant root and coleoptile growth increases were observed in respect to the

control. All other combination treatments with CC, SS or AnnGro™ either had no effect or a slight inhibitory effect on seedling growth. Though Teprosyn® alone promoted root and coleoptiles growth, this was not the case when it was used in combination with CC, SS and AnnGro™. The most significant inhibitory effect, particularly on coleoptiles growth was observed when Teprosyn® was combined with SS.

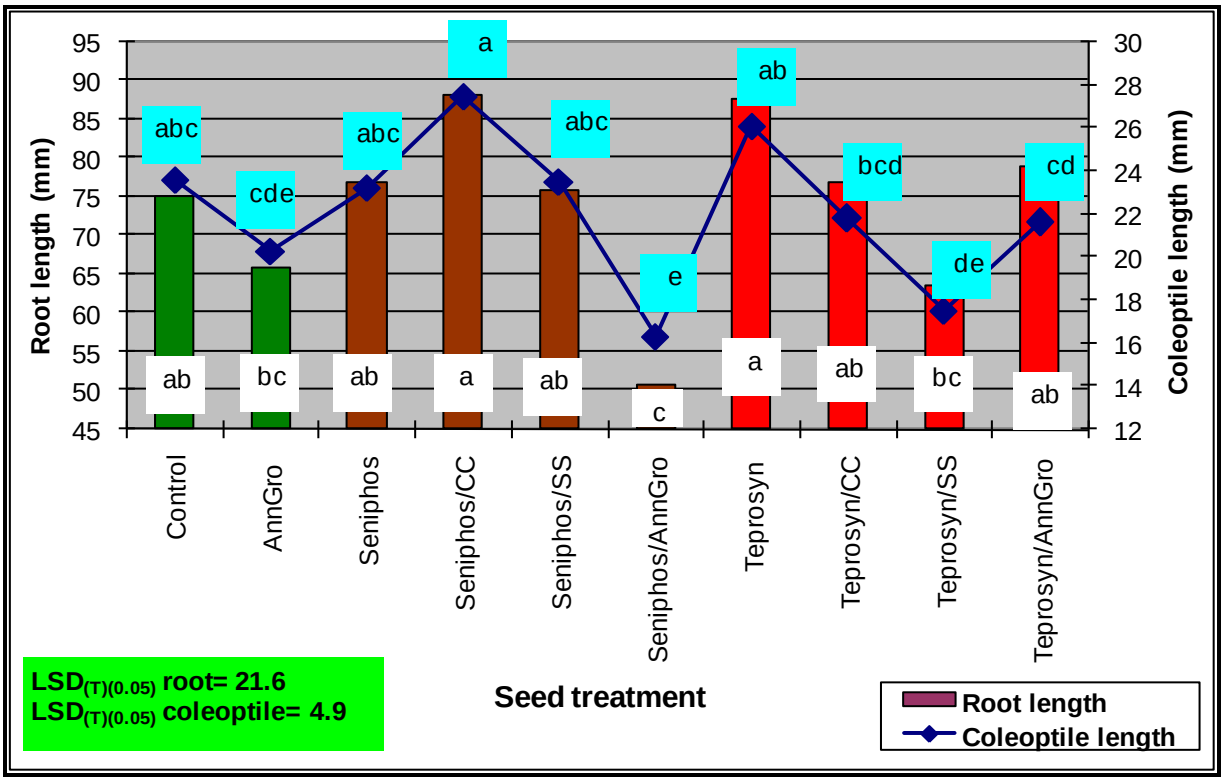

Figure 3.7: Root and coleoptile growth in response to pre-treatment of maize seeds with inorganic fertilizer products, both separately and in combination with plant growth regulators and an uptake enhancer, under laboratory conditions. LSD ($P<0.05$) values are indicated in the graph and data differing significantly are indicated with different letters.

The maize seedling growth response to seeds pre-treated with inorganic fertilizer products did not follow the same pattern under glasshouse conditions as was the case in the laboratory (Fig. 3.8). None of the prodcuts applied on its own had a significant enhancing effect on below ground part (root) growth. However, seeds

treated separately with AnnGro™ and Seniphos® resulted in a significant increase in above ground (coleoptile) extended growth while Teprosyn® had an insignificant inhibitory effect.

Where the fertilizer products Seniphos® and Teprosyn® were tested in combination with CC, SS or AnnGro™ the seedling growth pattern response, subsequent to seed pre-treatment, was altered once again (Fig. 3.8). Seniphos® in combination with CC had no effect on root growth but increased the above ground growth significantly. When Seniphos® combined AnnGro™ a significant inhibitory effect on both below was observed. On the other hand, Teprosyn® in combination with CC, SS or AnnGro™ had no effect on root growth while all three combination treatments increased above soil growth significantly.

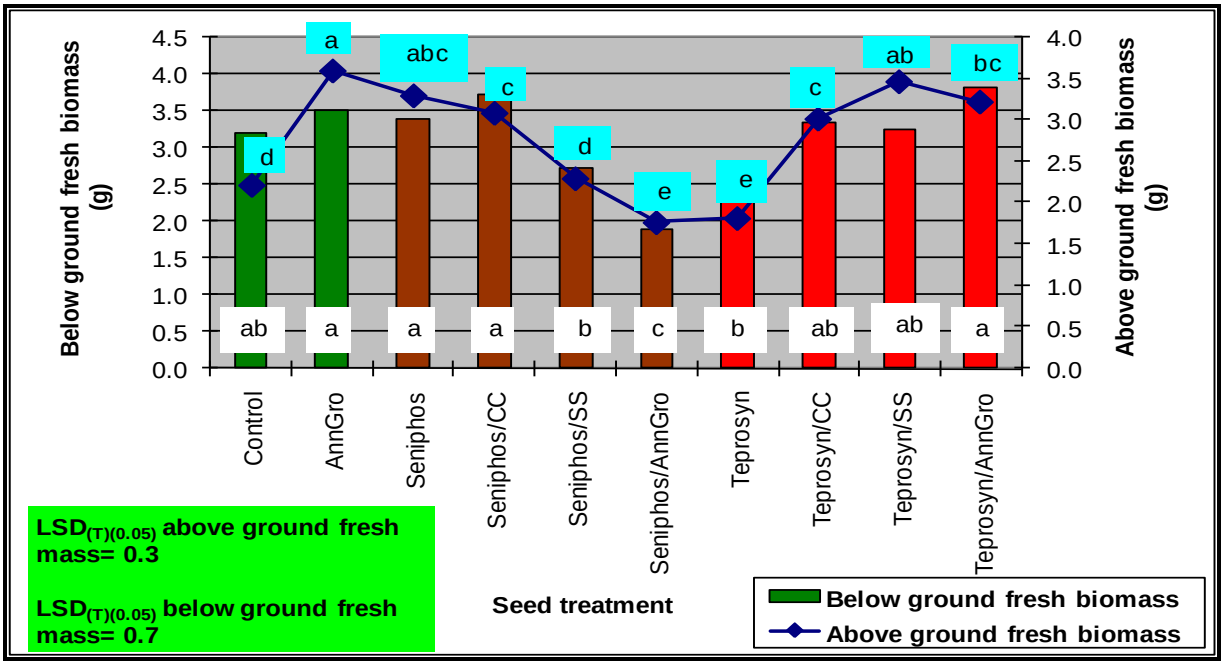

Figure 3.8: Maize seedling growth in terms of fresh mass in response to pre-treatment of seeds with inorganic fertilizer products, both separately and in combination, under glasshouse conditions. LSD ($P<0.05$) values are indicated in the graph and data differing significantly are indicated with different letters.

Subsequently, additional and extended combination seed treatments with inorganic fertilizer products were tested. These included combination treatments of Seniphos® and Teprosyn® with either CC plus AnnGro™ or SS plus AnnGro™ under both laboratory (Fig. 3.9) and glasshouse (Fig. 3.10) conditions.

In the laboratory (Fig. 3.9) the combination treatments containing Seniphos® and both CC and SS together with AnnGro™ had an inhibiting effect on root and coleoptile growth was not though it was not significantly different from the contol. As far as root growth was concerned the inhibition was significant only in the case of the former combination treatment while both combination seed treatments insignificantly inhibited coleoptiles\ growth. Treatment of seed with Teprosyn® in combination with SS and AnnGro™ significantly increased both root and coleoptile growth. Wher e SS was replaced with CC in combination with Teprosyn® and AnnGro™ root growth was also increased, although not significantly, while the seed treatment had no effect on coleoptile growth.

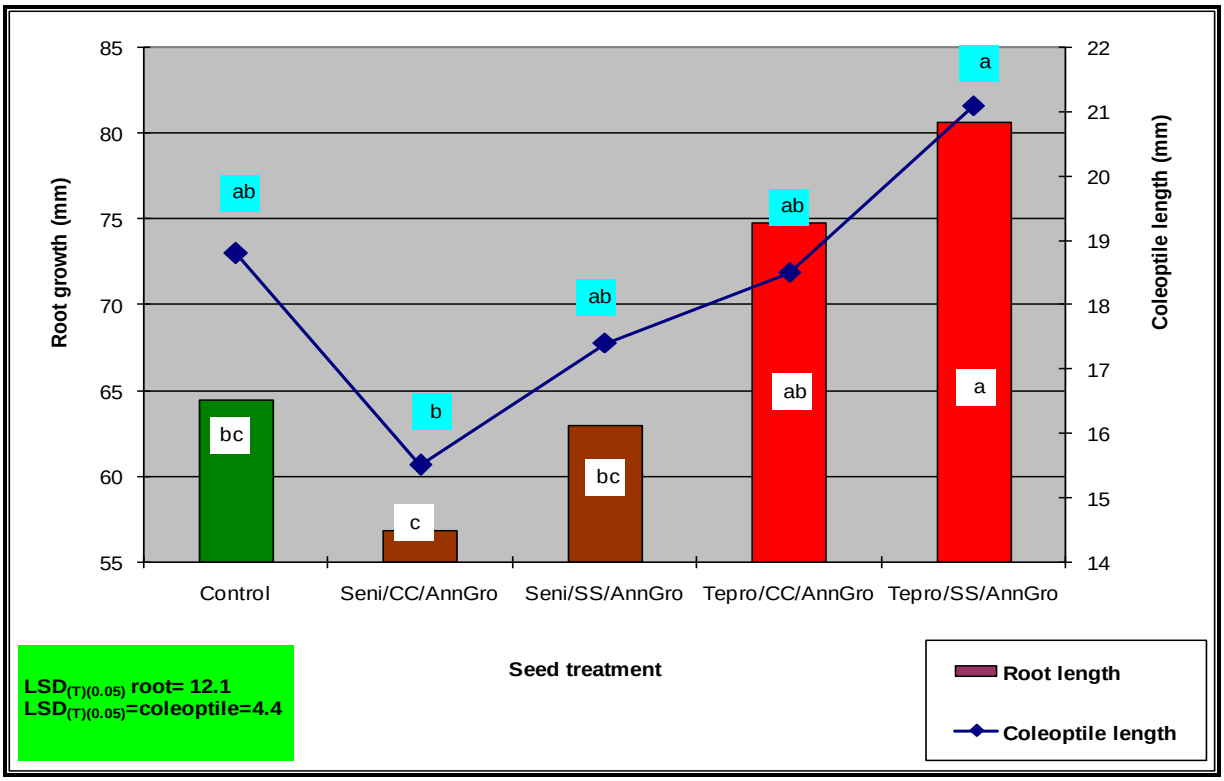

Figure 3.9: Root and coleoptile growth in response to pre-treatment of maize seeds with extended combinations of inorganic fertilizer products with plant growth regulators and an uptake enhancer under laboratory conditions. LSD ($P<0.05$) values are indicated in the graph and data differing significantly are indicated with different letters.

Under glasshouse conditions, three weeks after planting (Fig. 3.10), the results obtained were opposite from those obtained in the laboratory. Here Seniphos® in combination with AnnGro™ and both CC and SS significantly increased root and coleoptile growth. Teprosyn® in combination with AnnGro™ and CC had a slight but insignificant inhibitory effect on root growth and no effect on coleoptile growth. Where CC was replaced with SS in the combination seed treatment with Teprosyn® coleoptile growth was significantly increased while it had no effect on root growth.

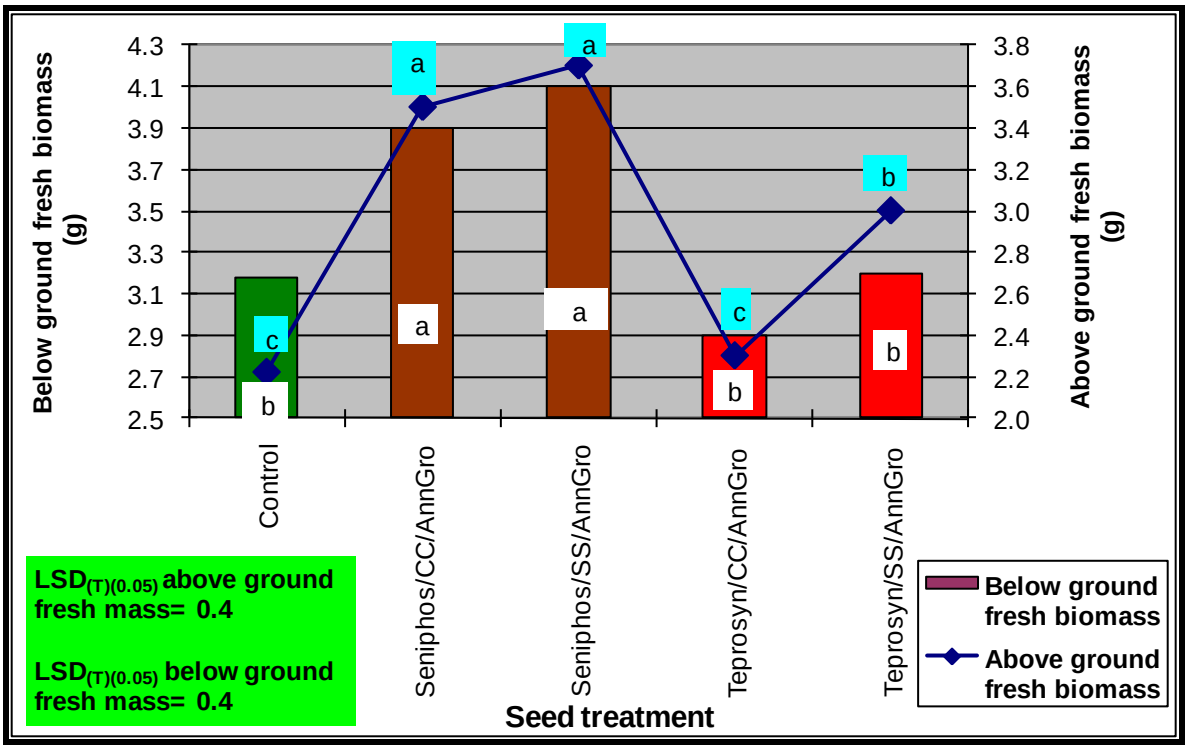

Figure 3.10: Root and coleoptile growth in response to pre-treatment of maize seeds with extended combinations of inorganic fertilizer products with plant growth regulators and an uptake enhancer under glasshouse conditions. LSD ($P<0.05$) values are indicated in the graph and data differing significantly are indicated with different letters.

3.5 DISCUSSION

Seed vigour, or rather the lack of it, is a common problem faced by seed merchants and crop producers alike. For this reason seed coating by seed merchants with a variety of fungicides, nutrients or other products has become common practice in order to ensure acceptable plant stands for most crops. However, fertilizer companies are constantly in search for new alternatives or improved products.

The main aim of the screening procedure followed in this study under laboratory and glasshouse conditions, in terms of the possible effect different seed treatments might have on maize seedling growth, was to identify a) products with application potential

in the agricultural industry and b) products to be used as a premise for further investigation. Seedling growth in terms of root and coleoptile growth during the screening phase in the laboratory as well as in terms of above and below soil part fresh biomass production in the glasshouse revealed different effects of various seed treatments on maize. These treatments included commercial or prototype products in four categories namely plant growth regulators, organic acids, biological control agents and inorganic fertilizer products.

Of the two plant growth regulators (PGR's) tested, ComCat® (CC) was a commercial and the *Lupinus albus* seed suspension (SS) a prototype product. Maize seeds were treated with both PGR's in a concentration range in order to identify the optimum concentration for each in terms of its possible effect on subsequent seedling growth. The seedling growth response to seed treatment with the PGR's was tested under both laboratory and glasshouse conditions.

For CC the optimum concentration was 25 mg kg^{-1} as it contributed to an 18% increase of root length growth in the laboratory, compared to the untreated control. Although the induction of root growth was marked this was not statistically significant, probably due to large standard deviations between replicas. Interestingly, seed treatment with neither of the concentrations in the range had any effect on coleoptile growth. This was in concert with the claims made by the manufacturers (Agraforum, 2006). Under glasshouse conditions the optimum CC concentration of 25 mg kg^{-1} was confirmed, as it significantly increased root growth, but the 50 mg kg^{-1} had the same effect. According to Van der Watt (2005) seed treatment with CC at a concentration of 0.5 mg L^{-1} optimally stimulated seedling growth of other crops, mainly vegetables, in the laboratory. It therefore seems that maize seed reacted differently from vegetable seed to treatment with CC.

Contrary to what was observed in the laboratory, treatment of maize seeds with both 25 and 50 mg kg^{-1} CC concentrations significantly increased root growth under glasshouse conditions. If it is considered that below and above soil part fresh mass

was measured three weeks after planting while growth was measured over only 96 h in the laboratory, indications are that coleoptile growth is slightly delayed by pre-treatment of seeds with CC initially but accelarates with time under glasshouse conditions. As CC contains both brassinosteroids (BR's) and auxin (Agraforum, 2006) as active compounds, it is most probably a sinergystic effect between these two phytohormones that is responsible for induced cell division, cell enlargement and cell elongation (Nithila *et al.*, 2007) leading to enhanced seedling growth.

Treatment of maize seeds with the protype PGR, a seed suspension of *Lupinus albus* (SS), confirmed 12.5 mg kg^{-1} to have an optimal stimulatory effect on root growth in the laboratory. This was in concert with the findings of Van der Watt (2005), although the author tested the effect of SS on cabbage and lettuce seeds. The root growth response of maize seedlings to this optimum SS concentration was not as pronounced three weeks after planting under glasshouse conditions. However, it was the only concentration that had a slight stimulatory effect on root growth and no inhibitory effect on coleoptile growth compared to the other concentrations in the range that was tested.

Except for vulvic acid, treatment of maize seeds with the other two organic acids, viz; humic acid and an amino acid mixture, revealed an inhibitory effect on subsequent seedling growth, in terms of both root and coleoptile growth, in the laboratory. Vulvic acid had no effect on seedling growth. Although humic acid has been reported to have a stimulatory effect on seed germination and plant growth, especially fibrous root growth (Gallant, 2004), it was not the case in this study as it inhibited both root and coleoptile growth by at least 23%. The results obtained in this study supported the findings of Eyheraguibel *et al*. (2008) who reported seed treatment with humic like substances to inhibit high proliferation of lateral and secondary roots in maize seedlings. However, the authors maintained that the latter in turn induced root length growth which was not the case in this study. The two micro-organism bio-products that were tested, Eco-flora® and Eco-fungi®, had a marked inhibitory effect on maize seedling growth and were therefore not considered in any further trials in this study.

Two commercial fertilizer products, namely Seniphos® and Teprosyn®, were tested as seed additives on their own or in combination with PGR's for its possible effects on seedling vigour. Seniphos® is an inorganic liquid fertilizer product that basically contains phosphorous and calcium as main components, mixed in specific proportions, while it also contains traces of other minerals (Li *et al*., 2002). Teprosyn® is a zinc-phosphorous liquid fertilizer product.

Seniphos® applied as a seed treatment on its own had no effect on either root or coleoptile growth in the laboratory, but three weeks after planting in the glasshouse it contributed to a slight increase in root growth and a significant increase in coleoptile growth. Seniphos® has not been developed as a seed treatment but as a foliar supplement of calcium and phosphorous, which probably explains the sustainable effect it had on seedling growth in the glasshouse. Teprosyn®, on the other hand, significantly increased both root (+17%) and coleoptile growth (+9%) of maize seedlings under laboratory conditions after a 96 h incubation period. This was in concert with the findings of Richardson (2007) who maintained that the latter product contributes to establishing a strong root system during early crop development. Surprisingly, the same tendency to promote below and above soil part growth three weeks after planting under glasshouse conditions, where Teprosyn® was applied to seeds on its own, could not be repeated. As a matter of fact, it contributed to a slight inhibition of seedling growth. This strongly suggests that Teprosyn® most probably has a stimulating effect on seed vigour, germination and early seedling growth, but not on sustainable seedling growth following germination. Its inhibitory effect on seedling growth at a later growth stage is difficult to explain at this time, but might be connected to an inhibitory effect on natural growth hormones found in plants. As both Seniphos® and Teprosyn® contain phosphorous, it seems that the Zn contained in the latter is responsible for the improved seedling growth observed in the laboratory. However, according to Orabi and Abdel-Aziz (1982) there is a synergistic effect between P and Zn that has a direct effect on auxin synthesis and growth regulation.

Subsequently, both Seniphos® and Teprosyn® were tested in combination with two hormone containing PGR's [ComCat® (CC) and a *Lupinus albus* seed suspension (SS)] and an uptake enhancer (AnnGro™). Under laboratory conditions Seniphos® in combination with CC significantly increased both root and coleoptile growth. This tendency was repeated three weeks after planting in the glasshouse in terms of below and above soil part fresh mass although it was only significant in case of the latter. Seniphos® used in combination with SS as a seed treatment, however, had no effect on seedling growth. The same applied for Teprosyn® in combination with both CC and SS under laboratory and glasshouse conditions. The uptake enhancer, AnnGro™, in combination with Seniphos® inhibited seedling growth significantly in the laboratory and glasshouse while it contributed to a slight improvement of the Teprosyn® effect on seedling growth in the glasshouse. The latter was statistically non-signifiant.

Interestingly, when Seniphos® was applied as a seed treatment in combination with CC and SS together with AnnGro™, it had a slight inhibitory effect on seedling growth over 96 h in the laboratory, but under glasshouse conditions where growth measurements were taken three weeks after planting, it contributed to significant below and above soil fresh mass accumulation. This strongly indicates that the AnnGro™ might have contributed to enhanced uptake of the Ca and P in the product by the seedlings (Grobler, 2008) which in turn may have contributed to a critical concentration in the plant tissue that lead to stimulated growth. However, when Teprosyn® was applied as a seed treatment in combination with CC and SS together with AnnGro™, it had the direct opposite effect than the previous combination with Seniphos® by stimulating root growth in the laboratory but inhibiting root growth under glasshouse conditions.

Some of the results obtained during this initial screening phase under laboratory and glasshouse conditions complimented each other. However, there also was some inconsistency for some treatments and, as a result; no final conclusions could be drawn from these preliminary results. Subsequently, eight seed treatments that

showed promise in terms of enhancing seedling growth was chosen for further investigation. These included: CC at 25 mg kg^{-1} seed, SS at 12.5 mg kg^{-1} seed, Seniphos®, Teprosyn® as well as the Seniphos®/CC, Seniphos®/SS/AnnGro™, Teprosyn®/SS/AnnGro™ and Teprosyn®/CC/AnnGro™ combination treatments. Further investigation included the physiological response of maize seedlings to these treatments under glasshouse conditions (Chapter 4) and the yield response of maize to some of these treatments (Chapter 5) under field conditions.

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CHAPTER 4

GROWTH AND PHYSIOLOGICAL RESPONSE OF MAIZE SEEDLINGS TO PRE-SOWING SEED TREATMENTS UNDER GLASSHOUSE CONDITIONS

4.1 ABSTRACT

The use of either plant growth regulators or fertilizers or both as external manipulatory measures of metabolic reactions within crops offers an alternative to genetically modified cultivars in an attempt to improve yield. This study was conducted to determine the growth and metabolic response of maize (*Zea mays* L.) seedlings to seed treatment with different commercial products prior to planting. The focus of this study was on growth and respiration rates. Hence, factors such as seedling growth, respiration rate, activities of glycolysis (phosphofructokinase; PFK) and OPP-pathway (glucose-6-phosphate dehydrogenase; Gl-6-PDH) enzymes, respiratory substrate levels (soluble sugars and proteins) as well as chlorophyll content were qualitatively determined. The trial was laid out in a complete randomised block design. The most pronounced and statistically significant enhancement of seedling growth was obtained with the Teprosyn®/CC/AnnGro™ and Teprosyn®/SS/AnnGro™ combination treatments in terms of root growth and plant height. Leaf protein content was enhanced by all treatments except Seniphos®. Soluble sugar content enhancement in roots and leaves was most prominent and consistent for the Teprosyn®/CC/AnnGro™ and Teprosyn®/SS/AnnGro™ combination seed treatments. No significant differences between treatments in terms of chlorophyll content were observed. The Gluc-6-PDH enzyme activity of the OPP pathway was higher than the ATP-PFK enzyme activity of glycolysis. All of the seed treatments stimulated Gluc-6-PDH activity although not significantly. In most cases this coincided with enhanced CO_2 release rates and decreased O_2 uptake rates. Only the CC treatment significantly increased glycolytic PFK activity compared to the untreated control and other treatments.

Keywords: maize, respiration rate, seed treatments, proteins, soluble sugars, chlorophylls, glycolysis, OPP-pathway, ATP-PFK activity, Gluc-6-PDH activity, O_2 uptake, CO_2 release.

4.2 INTRODUCTION

Photosynthesis is the most important primary metabolic pathway in plants and the mechanism of the biochemical pathway can most probably not be altered at this stage (Pretorius & Small, 1992). Respiration, however, shows the greatest potential to be manipulated externally (Akita, 1993). Vegetative growth and metabolic pathways within the plant are reported to be interrelated. Existing literature report on many positive and a few negative correlations between plant growth rates and respiration rates (Hansen *et al.,* 1994). Two decades ago Amthor (1989) reported that the precise nature of the relationship between growth and respiration in plants is largely unknown, and surprisingly to this day little is known about the underlying physiological mechanisms causing the negative correlation between yield and respiration. Two terms have been defined, namely "growth respiration" and “maintenance respiration”. The former is defined as that required for the synthesis of new biomass, and "maintenance respiration" as that required for maintaining biomass. The maintenance component is assumed to provide energy, for instance the turnover of proteins, nutrient uptake and ion fluxes. These processes are also required for growth, therefore, growth and maintenance respiration are not physiologically distinct, neither are they biochemically different (Amthor, 1991).

Respiration and plant growth are interrelated as respiration supplies the ATP that is utilized for growth while ADP and Pi are recycled in the process (Salisbury & Ross, 1991). Moreover, respiratory pathways of glycolysis, tricarboxylic acid (TCA) and the mitochondrial electron transport chain are essential for both energy provision in heterotrophic cells and a wide range of other physiological functions (Fernie *et al*., 2004). According to Plaxton (2006) plants utilize sucrose and starch as the principal

substrate for respiration via standard pathways of glycolysis, tricarboxylic acid (TCA) and the mitochondrial electron transport chain. Glycolysis pathway is of crucial importance in plants because it is the predominant pathway that “fuels” plant respiration. The bio-energizing role of glycolysis is particularly important in actively growing autotrophic tissues (Plaxton, 1996). The author maintained that the alternative reactions of glycolysis, tricarboxylic acid (TCA) and the mitochondrial electron transport chain are believed to endow plants with crucial metabolic flexibility that facilitates their development and acclimation to avoidable abiotic stresses. According to Kavi-Kishor and Mehta (1988) the glycolysis, Krebs cycle and the oxidative pentose phosphate (OPP) pathways are enhanced during the organo genetic development, hence this enhancement may reflect the need for generation of energy ATP and reducing power in the form of NADPH for developmental processes.

Cell functioning involves the combined activity of many biochemical reactions catalyzed by an equally large number of enzymes (Côme & Corbineau , 1989). The interaction and coupling of metabolic pathways ensure the unified and regulated system of metabolic flows both in the cell and in the whole plant (Popova & De Carvalho, 1998). Phosphofructokinase (EC 2.7.1.11; PFK) in plant cells is the most important regulatory enzyme of glycolysis and it acts as the control point in the glycolytic pathway as it is immediately downstream of the entry points for hexose sugars (Gahan *et al*., 1983; Zimmer, 1992; Figure 4.1).

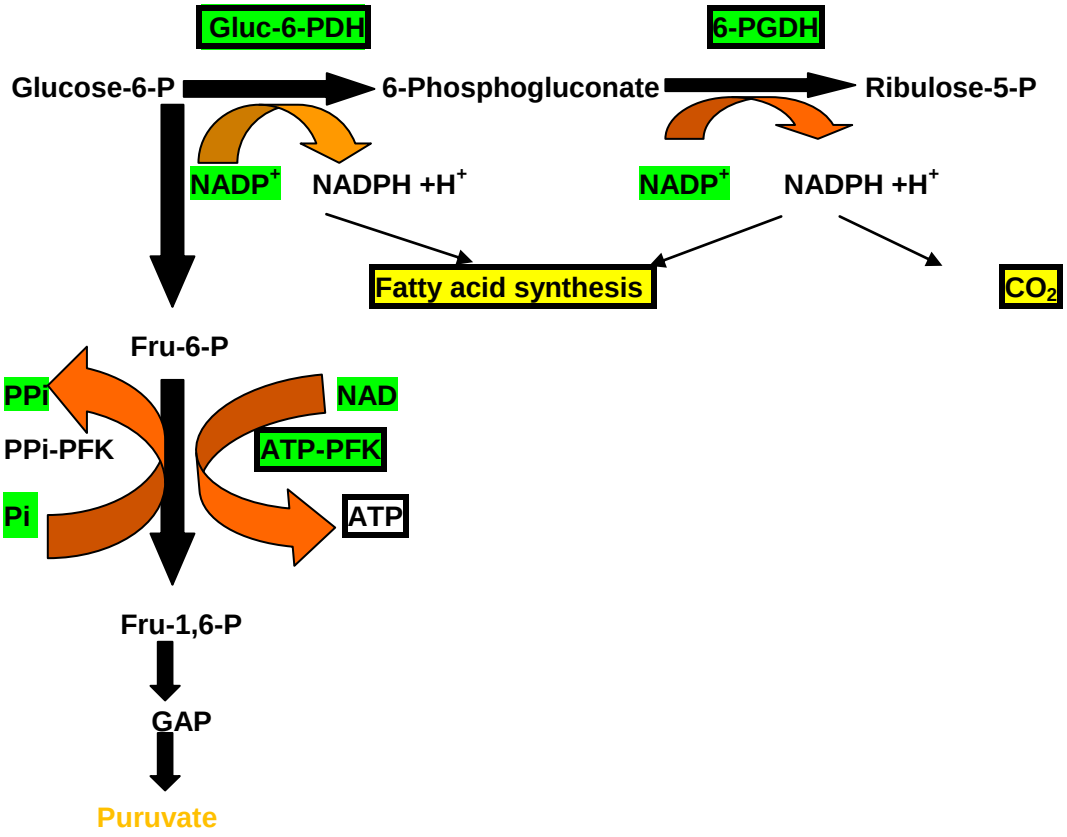

Figure 4.1: Schematic representation of the interwoven processes of glycolysis (vertical process) and oxidative pentose phosphate pathway (horizontal process) assuming glucose-6-phosphate to be the starting points (Adopted from Zimmer, 1992).

The OPP-pathway consists of two stages and the first stage is the irreversible conversion of Glu-6-P to Ribu-5P. This reaction is catalysed by glucose-6-phosphate dehydrogenase (EC1.1.1.4u9; Gluc-6-PDH) (Dennis *et al*., 1997). Gluc-6-PDH is a rate limiting enzyme of the OPP-pathway that controls the flux through the irreversible limb of the OPP pathway (Hauschild *et al*., 2003). This enzyme plays a pivotal role of the oxidative stage which provides reduction power (NADPH) for a wide range of anabolic pathways including synthesis of fatty acids, reduction of nitrite and synthesis of glutamate (Dennis *et al*., 1997). Furthermore, it has been described as an enzyme that determines the level of NADH by controlling the metabolism of

glucose via the OPP-pathway. In seeds, Gluc-6-PDH participates in the control of the OPP-pathway by limiting the breakdown of glucose in the early stages of germination; hence any treatments which increase the activity of the OPP-pathway tend to reduce dormancy (Swamy *et al*., 1981).

Moreover, Gluc-6-PDH has been reported to play an important role in plants as far as biotic and abiotic stress is concerned. Liu *et al*. (2007) reported a pivotal defence role for Gluc-6-PDH under salt stress by elucidating that increase in Gluc-6-PDH protein content and activity results in enhanced NADPH production in plants. Subsequently, an increase in NADPH content stimulates NR-dependent nitrite oxide production. Increase in the latter stimulates the increase in activities of antioxidant enzymes that scavenge the relative oxygen species (ROS) production induced by salt stress as observed on red kidney bean roots.

In addition to respiratory enzyme activities, respiratory substrate levels also determine the respiration rates in plants. Williams & Farrar (1992) postulated that substrate supply can regulate respiration and when substrate is either increased or decreased the rate of respiration responds immediately. Their study showed a 50% increase in respiratory capacity due to glucose supply. However, the regulation is limited by both substrate supply and the demand for ATP. Soluble sugars such as fructose, glucose and sucrose are the main substrates for respiration (Saglio & Pradet, 1980; Chen *et al*., 2004). According to Couée *et al*. (2006) various metabolic reactions and regulations directly link soluble sugars to mitochondrial respiration or photosynthesis regulation and conversely with anti-oxidative processes such as the oxidative pentose phosphate pathway.

Another important parameter in plant growth and productivity is chlorophyll production. Chlorophyll is the main light absorbing pigments embedded in the thylakoid membranes of chloroplasts and its forms include chlorophyll***a***, chlorophyll***b*** and carotenoids which have various roles such as light absorbents and anti-oxidant protectants. Carotenoids are involved in photosynthesis in various ways: they act as

light-harvesting pigments, contribute to the structure of thylakoid membranes and play an important role in photo protection. Perhaps the most important function of carotenoids includes the dissipation of excess energy of excited chlorophyll and the elimination of reactive oxygen species (Lawlor, 2001).

Energy consuming metabolic pathways are adjusted to the availability of oxygen which is involved in more than one regulatory mechanism (Zabalza, 2009). CO_2 is released during the breakdown of sugars by glycolysis and the OPP-pathway under anaerobic conditions and by mitochondrial respiration during aerobic conditions. Moreover, the CO_2 release and O_2 uptake rates are correlated with the production of usable energy (ATP & NADH) which are necessary for the maintenance of growth , as well as for the production of a carbon skeleton necessary for protein synthesis (Atkin *et al*., 2000).

Treatment of seeds has been postulated as a possible chemical way of manipulating physiological and metabolic activities underlying seed germination and seedling establishment. The effect of seed treatments on respiration can be brought about via external manipulation of internal regulation points such as the mentioned enzyme controlled reactions. The energy status of seedlings can be enhanced via manipulation of seedling respiration (Akita, 1993; Pretorius & Small, 1992; Schnabl *et al*., 2001). According to Pretorius and Small (1992) any chemical or biochemical seed treatment will have either a direct effect on glycolysis by activating the ATP-PFK or indirectly by activating Fruc-2.6-BP which in turn regulate fructose 6-phosphate-1-phosphotransferase (PFP; EC 2.7.1.90) in the conversion of fruc-1.6-BP (Figure 4.2) in the gluconeogenesis direction.

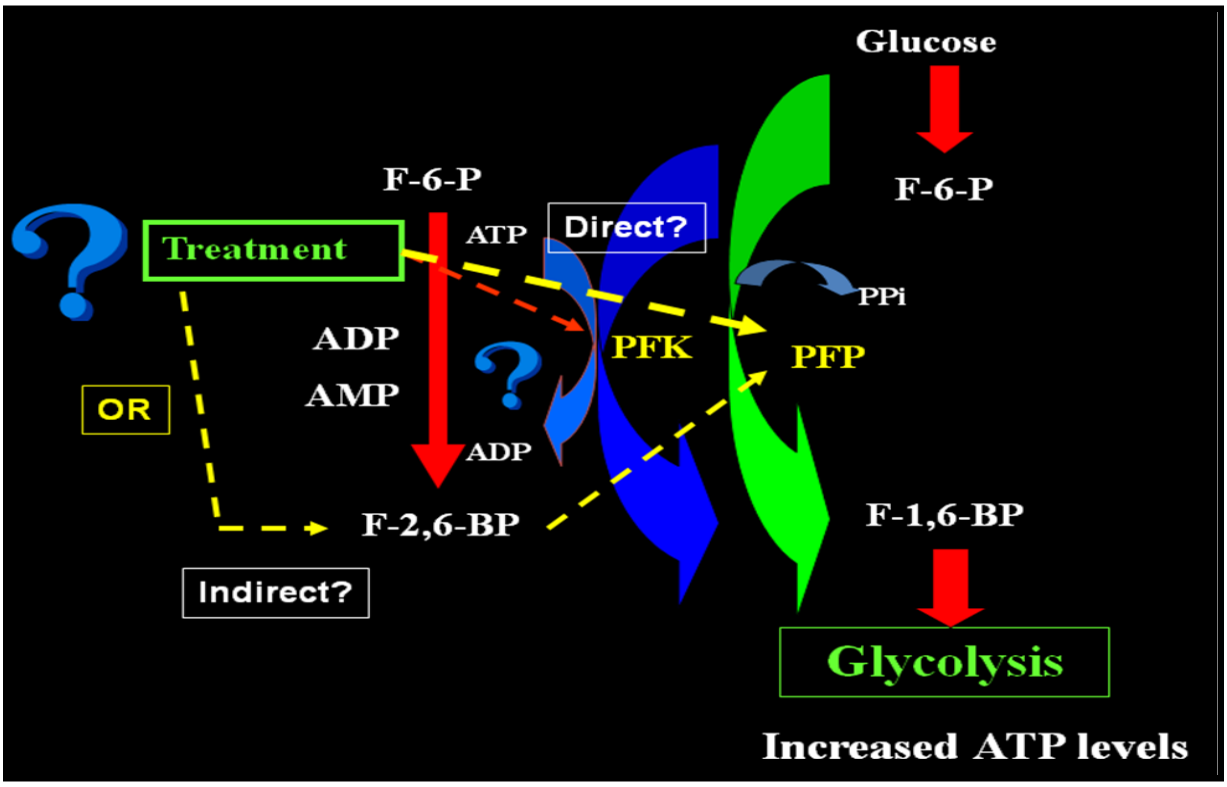

Figure 4.2: Simplified schematic representation of the effect of organic and inorganic seed treatments on glycolysis.

In this study the external manipulation of respiration and growth via internal regulatory points by seed treatments were scrutinized. The general objectives included:

- The influence of seed treatments on the activities of regulatory glycolysis and OPP-pathway enzymes,
- The effect of seed treatments on respiratory substrates (soluble sugars and protein),
- The effect of seed treatments on chlorophyll content and
- The influence of seed treatments on respiratory rate in terms of both O_2 uptake and CO_2 evolution.

4.3 MATERIALS AND METHODS

4.3.1 Materials

See 3.3.1 and 3.3.2.

4.3.2 Methods

4.3.2.1 Experimental design and trial layout

An experiment was conducted under glasshouse conditions at 25 ºC with 15 hrs and 9 hrs day and night growth length period respectively. The experiment consisted of nine (9) inorganic and organic treatments that have shown a repeated positive response in the screening phase. The nine (9) treatments were the fertilizer control, ComCat® (CC), seed suspension (SS), Seniphos®, Teprosyn® Zn/P, Seniphos®/CC, Seniphos®/SS/ AnnGro™, Teprosyn® /SS/ AnnGro™ and Teprosyn® /CC/ AnnGro™.

Cylindrical plastic pots of a size area of 314.15 m^2 were used for the glasshouse experiment. These pots were purchased from the SENWES Cooperation, Bloemfontein, South Africa. The pot experiment was subjected to a complete randomised block design with four replications. Fertilizer NPK (3:2:1) was applied at the rate of 4.71g per pot. Fertilizer was applied about 5 cm below the soil surface of a pot to avoid seed burn and the four seeds planted 2 cm deep. The planting was divided into 5 batches for each data collection, each batch planted weekly over a period of 5 weeks. Plants received equal amounts of irrigation water and were kept at field capacity. Three week old maize seedlings were harvested for the different parameters except for respiration rate (O_2 uptake & CO_2 evolution) where two week old seedlings were used.

Figure 4.3: The randomised block design of the experiment under glasshouse conditions.

4.3.2.2 Morphological parameters under glasshouse conditions

Plant height

Two plants per pot were measured for each replication, thus 8 plants per treatment. Plant height was measured of three week old maize seedlings with a ruler from the base of the stem to the last flagged leaf.

Fresh and dry root mass

The whole root system of each plant was cleaned thoroughly by gentle running tap water to remove all soil and foreign materials. Thereafter, roots were subsequently wrapped with laboratory grade filter paper to remove the excess water from the root surface that might contribute negatively to its mass. Fresh mass was determined using a Metler digital scale. Thereafter, the fresh roots of each pot were placed in a brown paper bag and dried in a digital oven (Labcon, Type FSOE, West Germany) at ±70 ºC. After 72 h the mass was determined using a Metler digital scale.

Fresh and dry mass of above soil parts

Above soil parts and roots were separated using a sharp knife and fresh mass (leaves) determined using a Metler digital scale. The material was placed in brown paper bags and dried in an digital oven (Labcon, Type FSOE, West Germany) at 70 ºC (±2ºC) for 72 hours. The dry mass was determined using a Metler digital scale.

Root volume

A known volume of water was added in a measuring cylinder. Clean dry roots from a single pot were placed in a 250 mL measuring cylinder and bubbles were removed by gently stirring with a glass rod to prevent inaccuracy caused by bubbles. The

displaced water was taken as the volume of roots, thus initial volume subtracted from the final volume.

4.3.2.3 Physiological related parameters

All the sample analyses were done on three week old maize seedlings using the centre part of the leaves with the highest metabolic activities (Amthor, 1989).

4.3.2.3.1 Extraction and determination of sugars (sucrose, D-glucose & D-fructose)

Two g samples from the roots and leaves of two plants separately were used for each replication. The samples were placed in separate test tubes with 15 mL 80 % ethanol and preheated to 80°C in a water bath for 15 minutes in order to stop all enzyme reactions. The volume of ethanol was marked before the samples were preheated and the evaporated ethanol during preheating was restored to the original volume. The tissue samples were homogenized by means of an Oscillating Mill MM400 in order to grind the tissue to a paste. The extracts were placed into separate eppendorf vials (four per replication) and centrifuged at 1200 rpm for five minutes at 25°C. Thereafter 2 mL aliquots of each replication was removed and placed in separate clean eppendorf vials and evaporated at 70°C in an oven until almost dry to get rid of the ethanol. The pellets were then dissolved in 2 mL of distilled water and 50 µL aliquots used for analysis.

The Boehringer Mannheim technique Cat. Nr.10 716 260 035 were used for sugar analysis. The test kit consisted of two solutions and two suspensions. Solution one contained approximately 0.5 g lyophilizate which consists of citrate buffer (pH 4.6) and β-fructosidae (720 U). The second solution contained 7.2 g powder mixture consisting of triethanolamine buffer (pH 7.6) 110 mg NADP, 260 mg ATP and magnesium sulphate. The first and second solutions were diluted in 10 mL and 45

mL distilled water respectively and brought to 37°C beforehand. The third suspension contained hexokinase (320 U) and glucose-6-phosphate dehydrogenase (160 U). The last suspension 4 contained 0.6 mL of phosphoglucose isomerase.
The absorbance of solutions was read at 340 nm wavelength with a Shimadzu spectrophotometer. Solution one (20 µL) was pipette into the blank of the sucrose and sucrose sample wells in an Eliza plate. In addition to that 10 µL of the sample solution was added all the wells on the whole plate. The solutions were then mixed and incubated in a digital oven for five minutes at 37 °C. The plate was removed and 100 µL of solution 2 was added to the whole plate as well as different volumes of distilled water respectively. The solution was mixed and the absorbance of the solution (A_1) was read after three minutes. The reaction was started by the addition of 2 µL of suspension 3 to all the wells. The solutions were mixed and incubated for 15 minutes before the absorbance of the solutions (A_2) were read. Lastly, 2 µL of the suspension 4 was added to the D-glucose/D-fructose part of the Elisa plate, mixed and read after 15 minutes (A_3).

To determine the absorbance difference for both blanks and samples, the absorbance difference of the blank (A_1) was subtracted from the absorbance of the corresponding sample (A_2). Thus means the difference between $\Delta A_{\text{total D-glucose}}$ from the sucrose sample and $\Delta A_{\text{D-glucose}}$ from D-glucose sample yields $\Delta A_{\text{sucrose}}$. The same procedure was adapted for the determination of $\Delta A_{\text{D-fructose}}$ which involved A_3-A_2. The calculations of sucrose, glucose and fructose were done following the general equation:

$$\mathbf{C} = [(V_1 \times MW / (\text{Ɛ} \times D \times V_2 \times 1000) \times \Delta A \ (g\ L^{-1})]$$

V_1= final volume (15 mL)
V_2=sample volume (0.100 mL)
MW= molecular weight (342.3 sucrose; 180.16 D-glucose & D-fructose)
D=light path (1.0124)
Ɛ= extinction coefficient (5.4099)

4.3.2.3.2 Determination of the total water soluble proteins

Two g root and leave samples respectively were harvested and homogenized in 20 mL extraction buffer consisting of 12.5 mM Tris (0.30 g), 2 mM EDTA (0.15 g) and 10mM Mercapto-ethanol (140 µL). After grinding the samples separately into a paste with an Oscillating Mill MM400, the extracts were transferred into 2000 µL eppendorf vials and centrifuged at 12000 rpm for 10 minutes at room temperature. The supernatant was transferred to clean eppendorf vials and 10 µL of each sample diluted 50 times by the addition of 490 µL distilled water. The protein content was determined by using the Bio-Rad method of Bradford (1976). The absorbency of the dilution was determined spectrophotometrically at 595 nm using a Bio-Rad micro plate reader with bovine gamma globulin as a standard. All treatments were replicated four times to increase the accuracy.

4.3.2.3.3 Qualitative determination of chlorophyll content of maize seedlings

Determination of the chlorophyll content was subjected to a modified extraction method of Mackinney (1941). One g leaf samples were cut into small pieces and grinded in 15 ml 80 % acetone with a mortar and pestle followed by an Oscilatting mill MM400. Samples were grinded until the extracts had a deep green colour and then filtered through a whatman number 1 filter paper. The absorption spectra of the different chlorophyll pigments were determined separately using a Shimadzu spectrophotometer at three different wave lengths, 661.6 nm, 644.8 nm and 470 nm respectively. The concentrations of chlorophylls were determined by using Mackinney's (1941) method and calculations as outlined below:

Determination of chlorophyll *a* content

[Constant (11.24) x Absorbance (661.6nm)**]** – **[**Constant (2.02) x Absorbance (644.8)**]**
= Chlorophyll a (µg mL^{-1} extract)
= µg mL^{-1} extract / fresh mass (2) x 15
= total pigment content per gram fresh weight

Determination of chlorophyll *b* content

[Constant (20.13) x Absorbance (644.8nm)] – [Constant (4.19) x Absorbance (661.6nm)]
= Chlorophyll a (µg mL^{-1} extract)
= µg mL^{-1} extract / fresh mass (2) x 15
= total pigment content per gram fresh weight

Determination of total carotenoid content

$$\frac{[1000 \times \text{Absorbance (470)}] - [\text{Constant (1.90)} \times \text{Chlorophyll a}] - [63.14 \times \text{Chlorophyll b}]}{214}$$

= Chlorophyll a (µg mL^{-1} extract)
= µg mL^{-1} extract / fresh mass (2) x 15
= total pigment content per gram fresh weight

4.3.2.3.4 Measuring the respiration rate in maize seedling

Gas exchange (O_2 uptake and CO_2 evolution) was measured simultaneously using a Warburg Pasco respirometer sensor. Each sample was replicated three times and two seedlings (one for CO_2 and the other for O_2) were harvested for each treatment. Each seedling was placed in a glass bottle (one for CO_2 and one for O_2) and the gas exchange measured every minute over a period of 15 minutes for each replication.

4.3.2.3.5 Extraction and assay of respiratory enzyme activities

4.3.2.3.5.1 Phospho-fructokinase (ATP-PFK)

The ATP-PFK enzyme of glycolysis extraction and assays were subjected to a modified method adopted by Nardi *et a*l. (2007). Two g leaf samples were homogenized in 8 mL Tris-extraction buffer (pH 8.0) using an Oscillating Mill MM400. The extraction buffer medium contained 100 mM Tris (12 g), 2 mM $MgCl_2$ (0.406 g), 1 mM EDTA (0.37525 g), 14 mM Mercapto-Ethanol (1000 µL) and 10 % Glycerol (100 µL). The homogenate was centrifuged for 15 minutes at 12000 rpm, the supernatant transferred to a clean eppendorf vial and used to determine any enzyme activities. The enzymatic reaction activity was determined in the reaction medium with a total volume of 1097 µL containing pH 7.5, 100 mM Tris-assay buffer (500 µL), 5 mM $MgCl_2$ .6 H_2O (50 µL), 10 mM Fru-6-P (50 µL), 0.1 mM NADH (20 µL), 1 mM ATP (20 µL), 0.3U α-Gliseraldehyde-3-phosphate dehydrogenase (12 µL), 3U Triosephosphate isomerase (5 µL) and 0.3 U Aldolase (440 µL) in a quarts cuvette. The 20 µL enzyme extract was added last to start the enzyme reaction. The PFK enzyme activity was then determined spectrophotometrically using a Shimadzu UV-2450 visible spectrophotometer by determining the amount of NADH produced over a 10 minute period at a wavelength of 340 nm.

4.3.2.3.5.2 Gluc-6-PDH an enzyme of the Oxidative pentose phosphate respiratory pathway

A method adopted by Botha *et al*. (1992) was used but with some modification in the volumes. Two g leaf samples were cut into small pieces and homogenized in 8 mL of extraction buffer (pH 7.5) using an Oscillating Mill MM400. The extraction buffer consisted of 6 g of 100 mM Tris, 2 mM EDTA (0.375 g), 10 mM mercapto-ethanol (35 µL) and 50 mL of 10 % glycerol. The extracts were placed into eppendorf tubes and centrifuged at 12 000 rpm for 15 minutes at room temperature. The supernatant was

removed and placed into separate clean eppendorf vials and used to determine any enzymatic activities.

The Gluc-6-PDH (EC 1.1.1.49) enzyme activity was determined spectrophotometricaly following the method described adopted by Liu et al. (2007). Six µL of enzyme extract was added to a reaction mixture of 250 µL essay buffer (100 mM Tris-HCL, pH 7.5), 3 µL of 1M $MgCl_2.6H_2O$, 40 mM NADP (7.5 µL), 4.8 µl of coupling enzyme 6-FGDH, 125 µL distilled and 6 µL of 125 mM glucose-6 phosphate which is a substrate in a quarts cuvette. The reduction of NADP to NADPH was measured over a 10 minute's period at 340 nm using a Shimadzu UV-2450 visible spectrophotometer.

4.3.2.4 Statistical analysis of data

Analysis of variance (ANOVA) was performed on the data, using the NC: SAS Institute Inc., Dos statistical program to identify differences between treatments. Tukey-Kramer's LSD (least significant difference) procedure for comparison of means (Mason *et al*., 1989) was applied to separate means at the 5% ($P<0.05$) probability level. Treatments differing significantly were indicated in figures both as calculated LSD values and by using different letters.

4.4 RESULTS

4.4.1 The influence of different seed treatments on seedling growth

- The effect of different seed treatments on various plant growth parameters were measured (Table 4.1). Root length was enhanced by all the treatments but the most pronounced enhancement was by Teprosyn®/SS/AnnGro™ (217%) and Teprosyn®/CC/AnnGro™ (143%) respectively compared to the untreated control. Teprosyn®/SS/AnnGro™ significantly increased the root

fresh mass and plant height compared to the control, Seniphos®, Teprosyn® and combinations of the latter two products. All the treatments increased the root volume, dry root and dry leaf mass non-significantly. The Teprosyn®/SS/AnnGro™ treatment contributed to a lower fresh leaf mass compared to all of the treatments, but the differences were non-significant. Due to the pronounced effect of the different treatments on the growth of maize seedlings some physiological parameters were also measured.

Figure 4.4: Three weeks old maize seedlings at harvest.

Table 4.1: Morphological response of maize to seed treatments under glasshouse pot experiment

Treatments	Root length (cm)	Root volume (mL)	Fresh root mass (g)	Dry root mass (g)	Fresh leaf mass (g)	Dry leaf mass (g)	Plant height (cm)
Control	10.7 ^{c}	1.0 ^{a}	2.0^{bc}	0.2^{a}	9.5^{ab}	1.1^{a}	43.$^{1\,c}$
CC	17.4^{bc}	1.4 ^{a}	1.8 ^{abc}	0.3^{a}	11.4^{ab}	1.3^{a}	46.5^{bc}
SS	15.9^{c}	1.2^{a}	2.0 ^{abc}	0.3^{a}	12.3^{ab}	1.4^{a}	45.7^{bc}
Seniphos®	16.5^{c}	1.4^{a}	1.3 ^{c}	0.2^{a}	11.3^{ab}	1.3^{a}	45.3^{bc}
Teprosyn®	15.9^{c}	1.1^{a}	1.4 ^{bc}	0.3^{a}	13.0^{a}	1.4^{a}	48.7^{b}
Seniphos®/CC	16.5^{c}	1.0^{a}	1.5 ^{bc}	0.3^{a}	12.9^{a}	1.6^{a}	50.0^{ab}
Seniphos®/SS/ AnnGro™	15.0^{c}	1.5 ^{a}	1.3 ^{c}	0.2^{a}	11.9^{ab}	1.4^{a}	46.8^{bc}
Teprosyn®/SS/ AnnGro™	34.0^{a}	1.6^{a}	2.3 ^{a}	0.3^{a}	8.6^{b}	1.2^{a}	54.6 ^{a}
Teprosyn®/CC/AnnGro™	26.1^{ab}	1.7 ^{a}	2.1 ^{ab}	0.3^{a}	11.8^{ab}	1.4^{a}	50.0^{ab}
LSD $_{(T)(0.05)}$	**8.98**	**0.78**	**0.75**	**0.11**	**3.93**	**0.51**	**5.29**

Means with the same letter are not significantly different according to Tukey's LSD procedure at the 5% probability level.

4.4.2 Physiological response of maize seedlings to seed treatments under glasshouse conditions

4.4.2.1 Total water soluble protein content

Compared to the untreated control, all the treatments increased the protein content of the leaves except Seniphos® (Figure 4.5). Teprosyn®/CC/AnnGro™ and Teprosyn®/SS/AnnGro™ significantly increased the protein content in the leaves. The Seniphos®/CC combination treatment decreased the protein content in roots while the Teprosyn®/CC/AnnGro™ combination again notably increased the protein content.

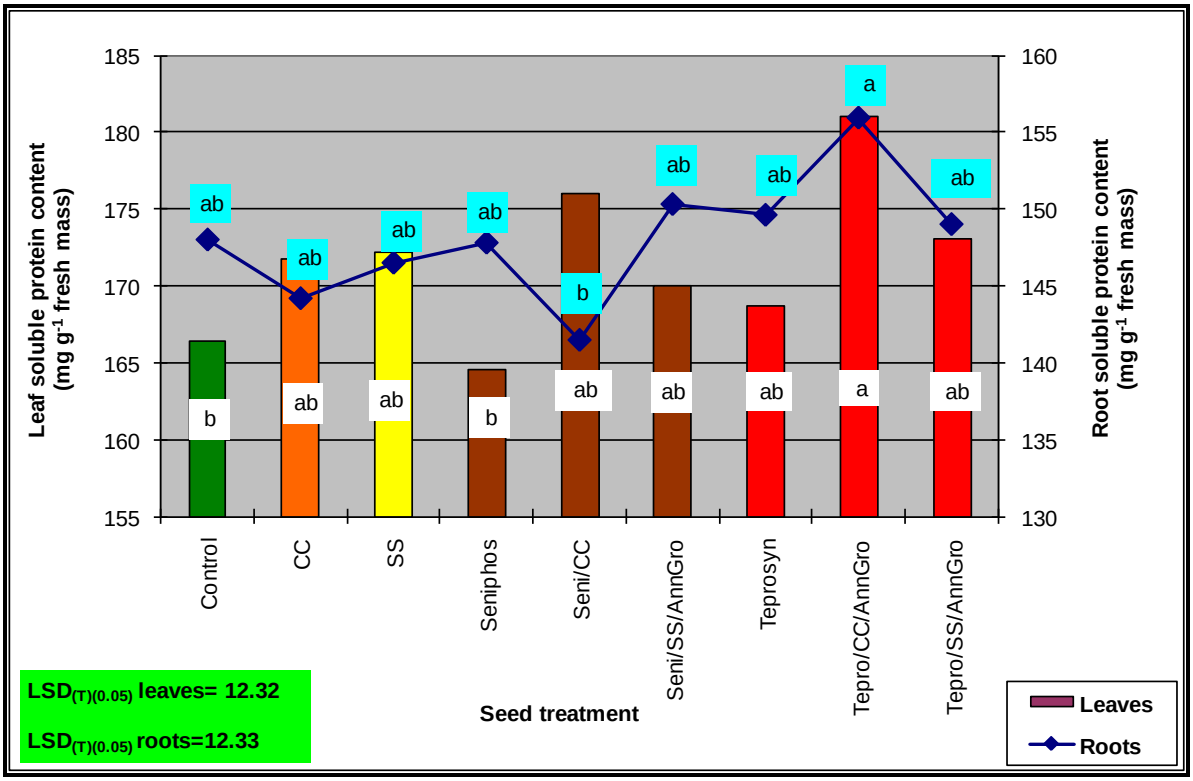

Figure 4.5: The influence of different seed treatments on the total water soluble protein content of three week old maize seedlings. LSD (P<0.05) values are indicated in the graph and means differing significantly is indicated with different letters.

4.4.2.2 Sugar content

The sucrose content of roots under the influence of different seed treatments differed noticeably between treatments (Figure 4.6). Only three seed treatments Teprosyn®/SS/AnnGro™ (2320 µmol g^{-1} fresh mass), Seniphos®/SS/AnnGro™ (2151.8 µmol g^{-1} fresh mass) and Teprosyn®/CC/AnnGro™ (1892.9 µmol g^{-1} fresh mass) increased the sucrose content in roots. Although these increases differed insignificantly from the untreated control, the results indicated a remarkable increase of 94%, 80.6% and 58.9% respectively. The sucrose level in the leaves (Figure 4.7) followed a similar increasing trend only for the Teprosyn®/CC/AnnGro™ (+30.8%) combination treatment, although not statistically significant. All other treatments either had no significant effect or decreased the sucrose content in leaves.

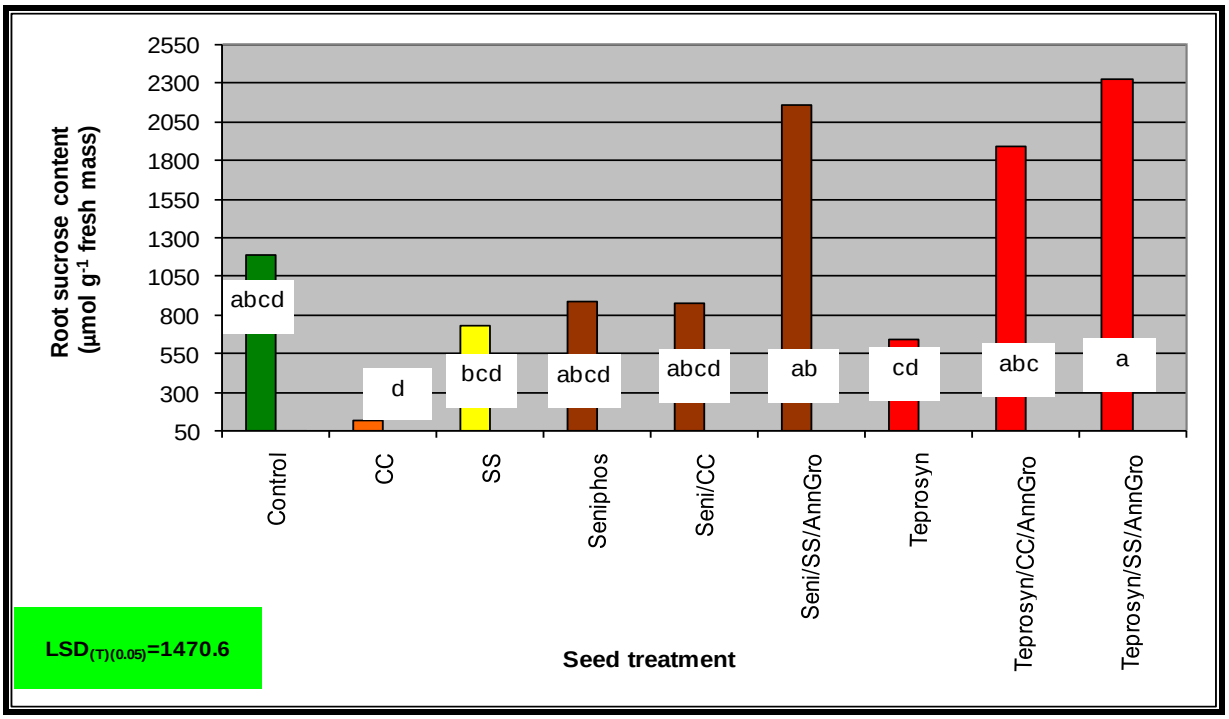

Figure 4.6: Sucrose content in the roots of three week old maize seedlings in response to different seed treatments under glasshouse conditions. LSD ($P<0.05$) values are indicated in the graph and means differing significantly is indicated with different letters.

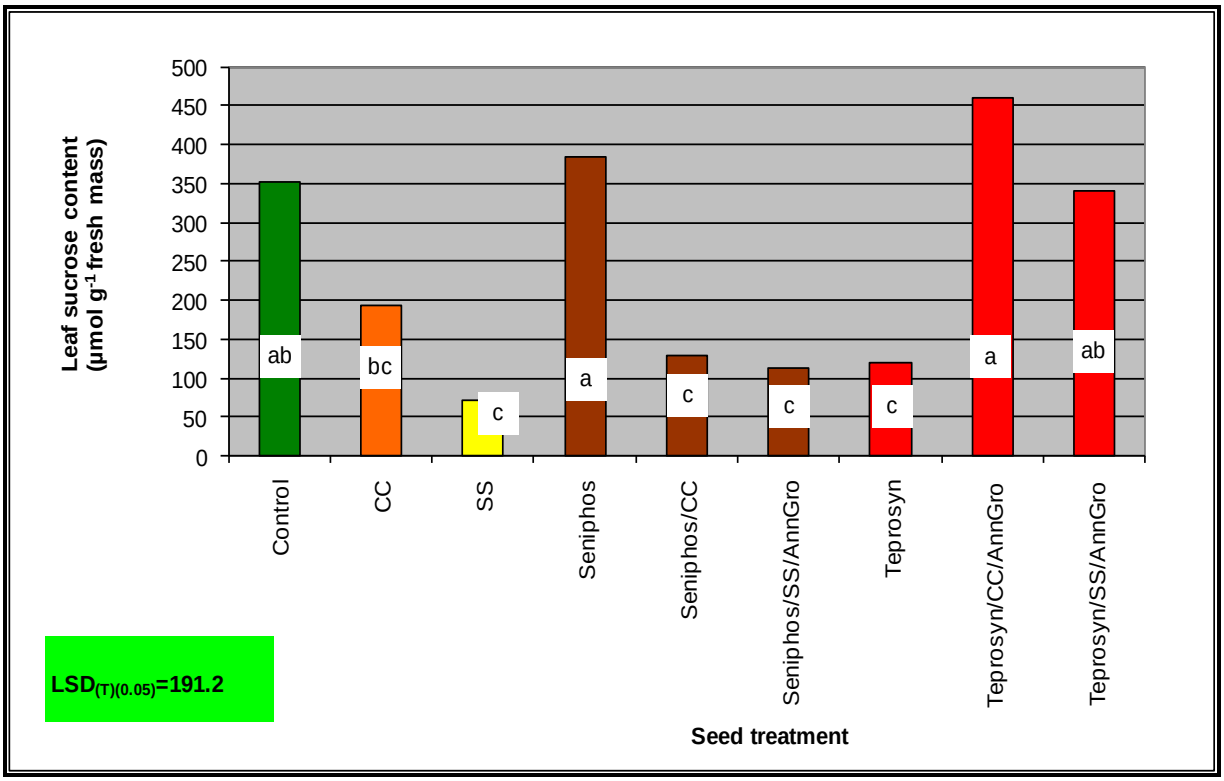

Figure 4.7: The influence of different seed treatments on the sucrose content of leaves under glasshouse conditions. LSD (P<0.05) values are indicated in the graph and means differing significantly is indicated with different letters.

All the treatments, accept CC and Teprosyn® increased the glucose content of roots compared to the control (Figure. 4.8). Teprosyn®/CC/AnnGro™ had a pronounced increasing effect (109 %) on the glucose content of the roots. However, the glucose levels in the leaves (Figure. 4.9) were decreased by all the seed treatments compared to the control and SS significantly inhibited the leaf glucose levels.

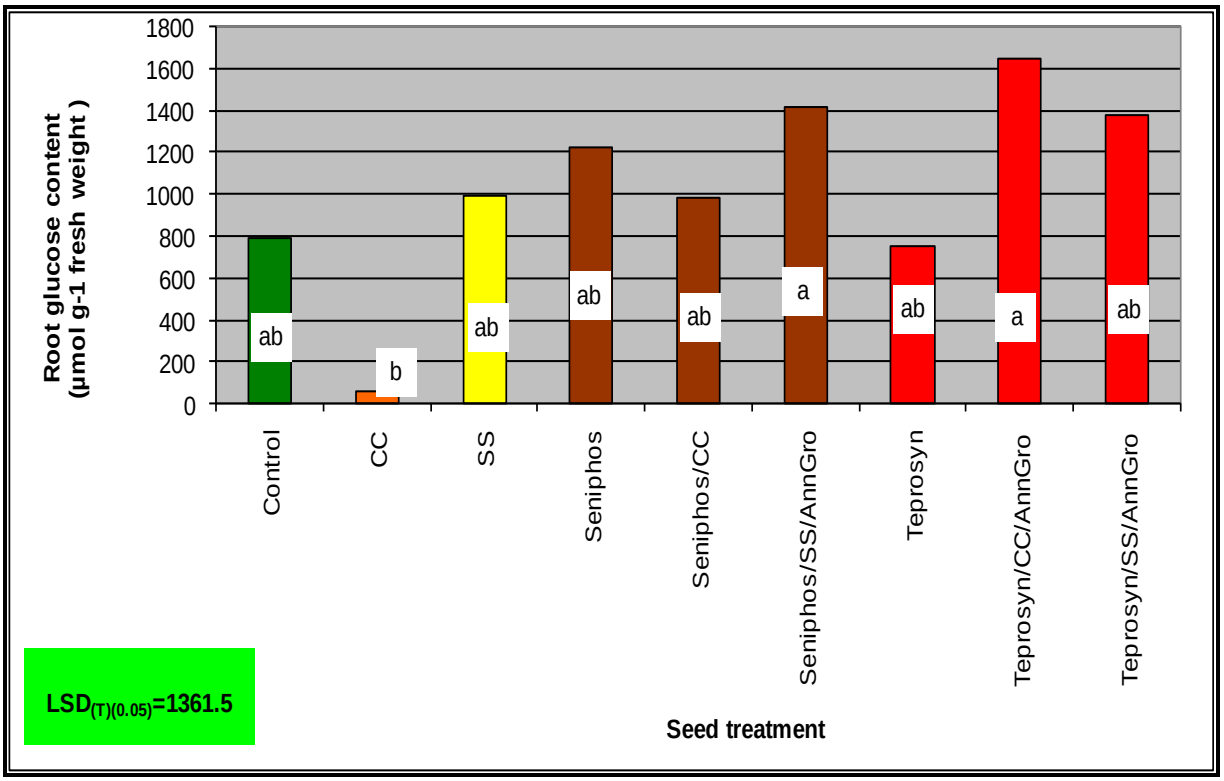

Figure 4.8: Influence of different seed treatments on roots glucose content of three week old maize seedlings under glasshouse conditions. LSD ($P<0.05$) values are indicated in the graph and means differing significantly are indicated with different letters.

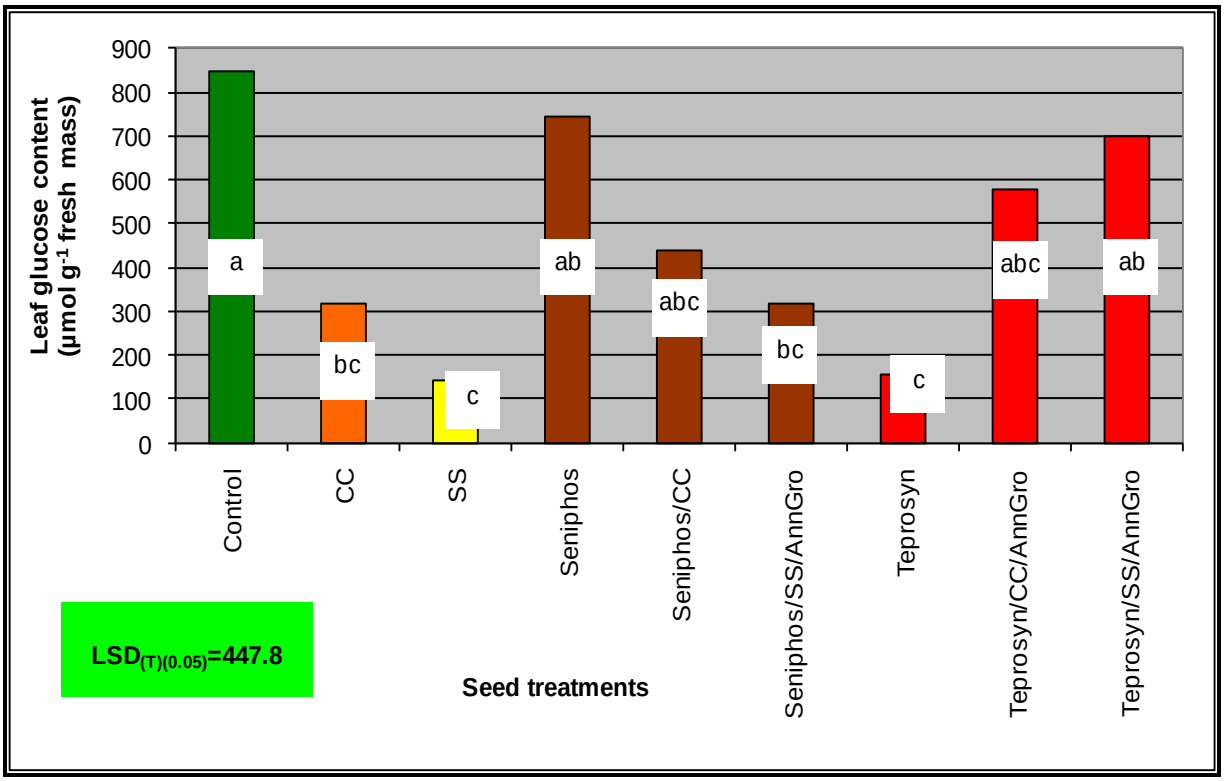

Figure 4.9: Influence of different seed treatments on leave glucose content of three week old maize seedlings under glasshouse conditions. LSD (P<0.05) values are indicated in the graph and means differing significantly is indicated with different letters.

Except for the CC treatment, all other treatments contributed to an increase in the root fructose content while the Teprosyn®/CC/AnnGro™ (+210.3%) and Teprosyn®/SS/AnnGro™ (+225.3%) treatments stood out most prominently in this regard (Figure 4.10). The increase was only significant in the case of the latter combination treatment. In the leaves (Figure. 4.11), the fructose levels were markedly but insignificantly enhanced by Teprosyn®/CC/AnnGro™ (280.0%) and also insignificantly by Teprosyn®/SS/AnnGro™ (150%) and Seniphos® (+109.2 %). The lack of significance was probably due to large standard deviations between replications. CC, SS and Teprosyn® decreased the fructose levels in leaves. It seems that the Teprosyn®/CC/AnnGro™ and Teprosyn®/SS/AnnGro™ had the most pronounced effect on the sucrose, glucose and fructose sugar levels compared to the control and other seed treatments.

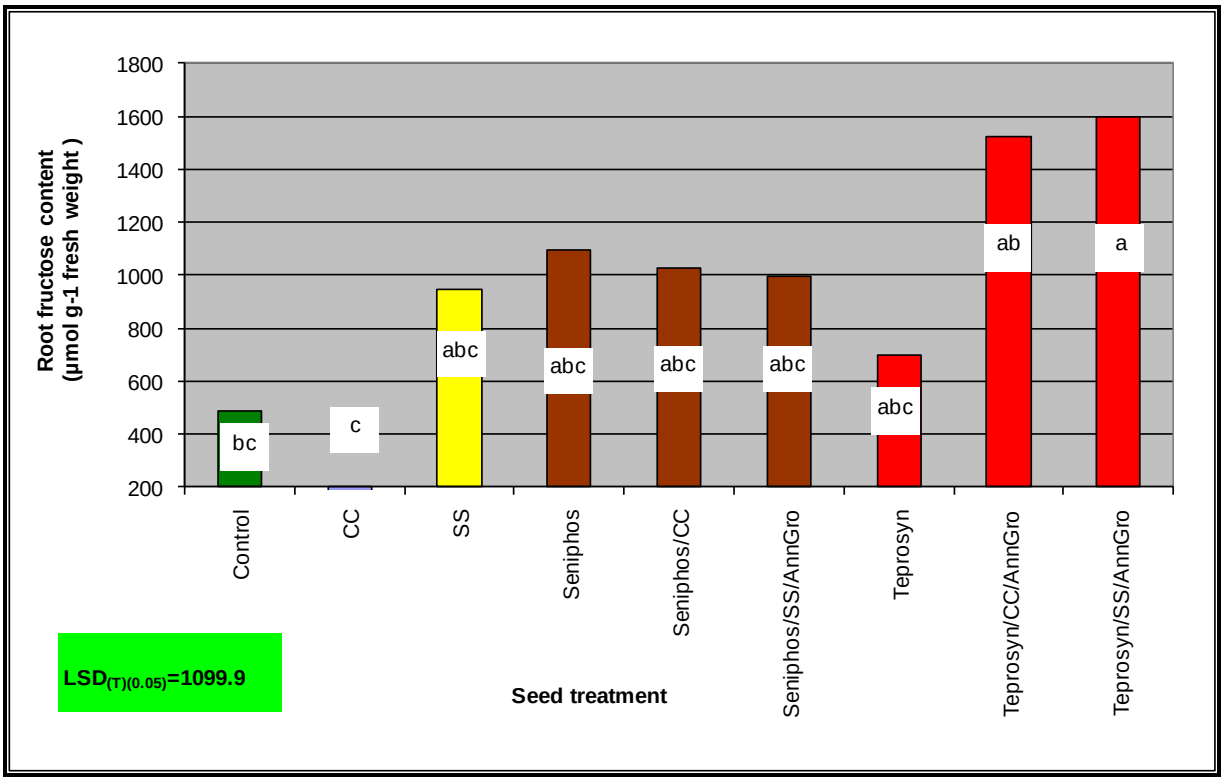

Figure 4.10: Influence of different seed treatments on root fructose content of three week old maize seedlings under glasshouse conditions. LSD ($P<0.05$) values are indicated in the graph and means differing significantly are indicated with different letters.

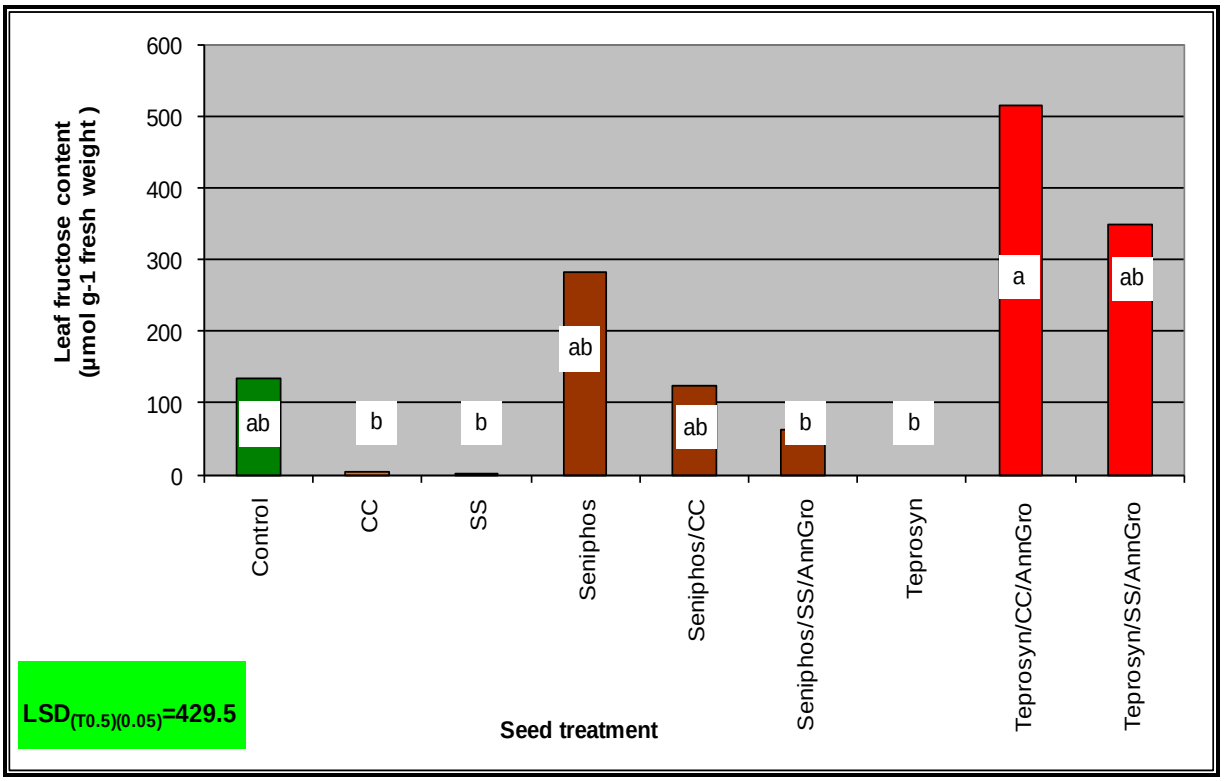

Figure 4.11: Influence of different seed treatments on fructose content of three week old maize seedlings under glasshouse conditions. LSD (P<0.05) values are indicated in the graph and means differing significantly is indicated with different letters.

4.4.2.3 Chlorophyll content of maize leaves in response to seed treatments under glasshouse conditions

Once more the Teprosyn®/CC/AnnGro™ treatment had the most marked effect on the total chlorophyll content in maize leaves, although non-significant compared to the control (Figure 4.12). Interestingly, all treatments had a significant inhibiting effect on the chlorophyll a content and no significant effect on chlorophyll b content compared to the untreated control (Figure 4.13).

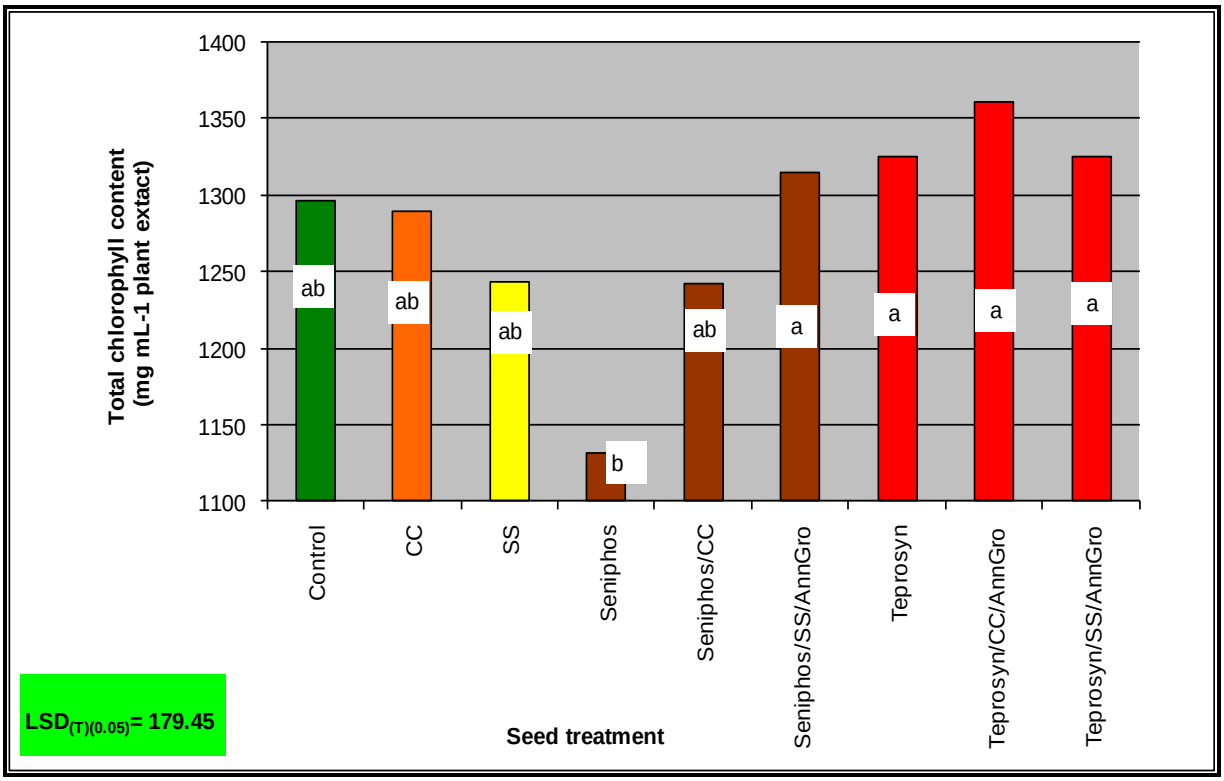

Figure 4.12: Total chlorophyll content of three week old maize leaves in response to different seed treatments under glasshouse conditions. LSD (P<0.05) values are indicated in the graph and means differing significantly is indicated with different letters.

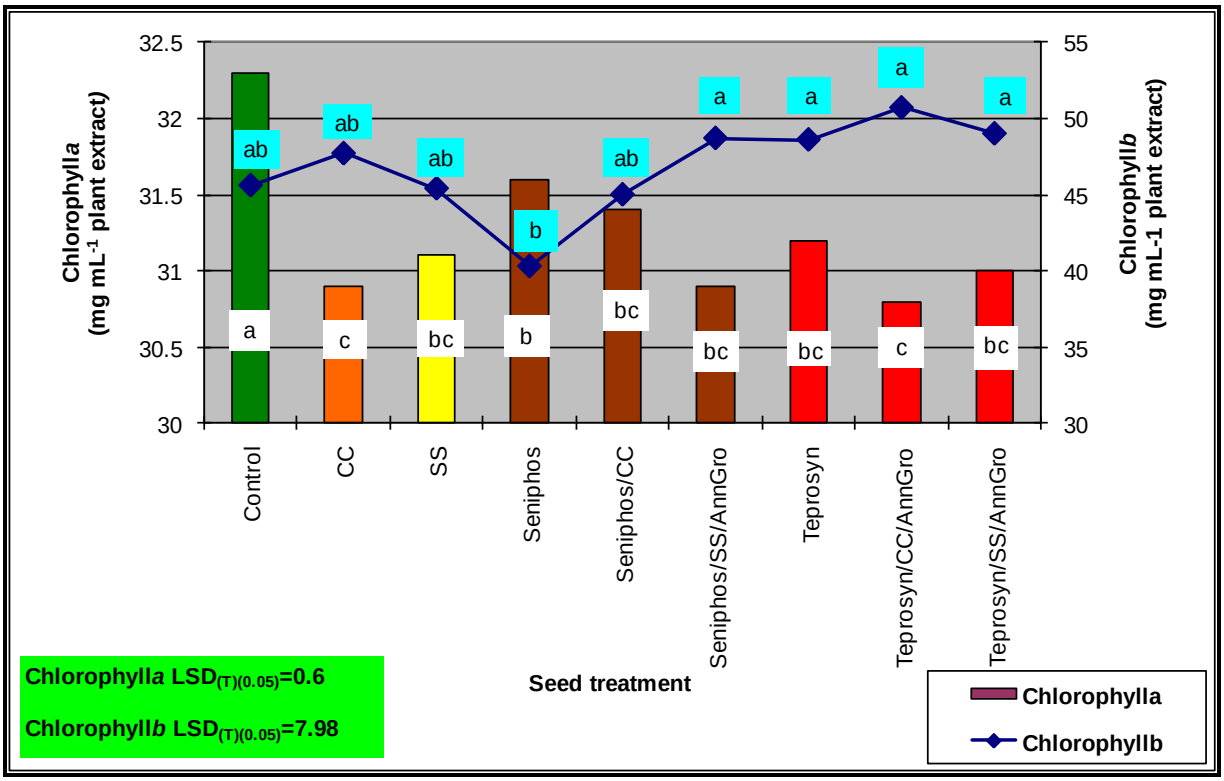

Figure 4.13: Chlorophyll ***a*** and ***b*** content of three week old maize leaves in response to seed different treatments under glasshouse conditions. LSD (P<0.05) values are indicated in the graph and means differing significantly is indicated with different letters.

Yet again all the treatments lowered the carotenoid content in maize leaves, except for Seniphos® which slightly increased the levels by 9.5% (Figure 4.14).

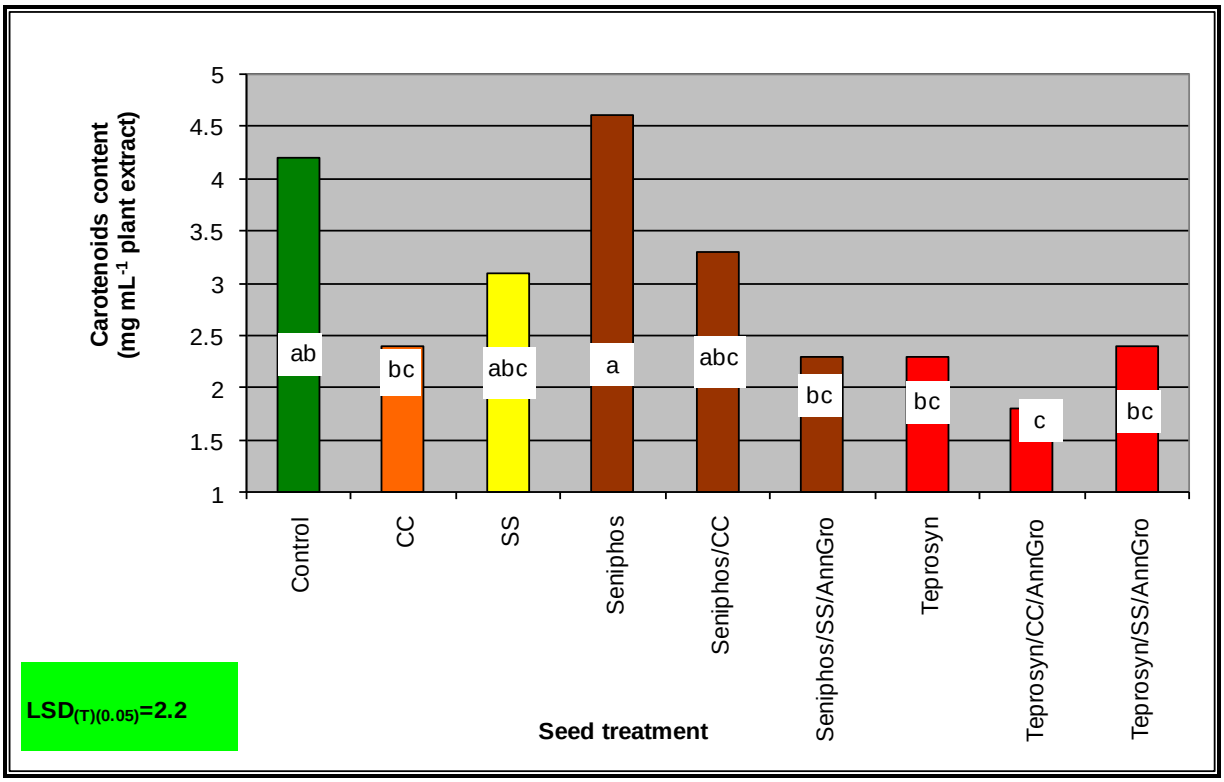

Figure 4.14: Carotenoid content of three week old maize seedlings in response to different seed treatments under glasshouse conditions. LSD (P<0.05) values are indicated in the graph and means differing significantly is indicated with different letters.

4.4.2.4 Respiratory enzymatic activities of glycolysis and OPP-pathway as well as respiratory gas exchange

The Gluc-6-PDH enzyme activity of the OPP-pathway (Figure. 4.15A) in maize leaves was much higher than that of the glycolytic PFK enzyme (Figure 4.15C) in response to different seed treatments. All the treatments increased the Gluc-6-PDH activity insignificantly compared to the control. The Seniphos®/CC (0.21 µmol NADPH g^{-1} fresh mass) combination treatment increased the activity by 100% compared to control, but this was still not significant at the 5% probability level. ATP-PFK activity was significantly stimulated by CC and insignificantly by Teprosyn®/CC/AnnGro™ (50%) as well as Teprosyn®/SS/AnnGro™ (25%). While SS

had no effect, all other treatments decreased the PFK activity but this was only significant in the case of the Seniphos®/CC combination treatment.

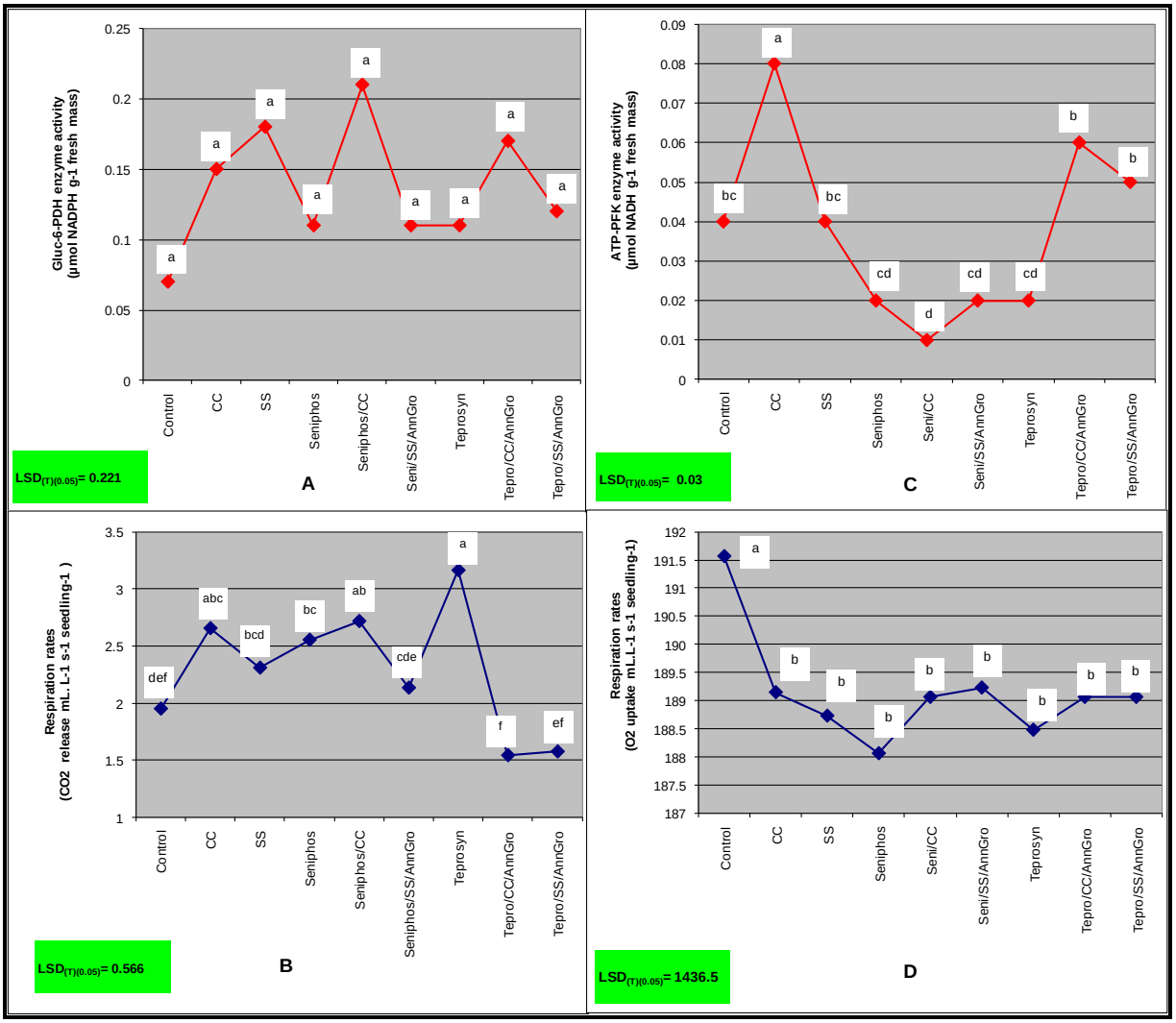

Figure 4.15: The influence of different seed treatments on Gluc-6-PDH enzyme activity of the OPP-pathway(A), CO_2 production(B), ATP-PFK enzyme activity of glycolysis(C), and O_2 consumption (D) of three week old maize seedlings under glasshouse conditions. LSD ($P<0.05$) values are indicated in the graph and means differing significantly is indicated with different letters.

All the seed treatments increased the CO_2 release rate during respiration except the different combinations of Teprosyn® (Figure 4.15B). Teprosyn®, CC, Seniphos® and Seniphos®/CC significantly enhanced the CO_2 release rate compared to the combinations between Teprosyn® and SS/AnnGro™ as well as CC/AnnGro™ and the control. Both Teprosyn®/CC/AnnGro™ and Teprosyn®/SS/AnnGro™ significantly depressed the CO_2 release rate by 20.9% and 19.2% respectively, while Teprosyn® increased the CO_2 release rate significantly by 62.2%. All the treatments markedly decreased the O_2 uptake rate and the decrease was significant compared to the untreated control (Figure 4.15 D).

4.5 DISCUSSION

At the onset it was postulated that seed treatment with the "right" product offers a possible alternative to genetic manipulation of crops with the objective to improve seedling establishment and hopefully also plant growth through the season as well as ultimate yield. However, for the products used in this study the mechanisms of action are not yet fully understood. Hence, this study was undertaken under controlled glasshouse conditions in an attempt to shed some light on the metabolic response of maize seedlings to different seed treatments in terms of selected events underlying seedling growth.

To quantify the influence of seed treatment on the growth of maize seedlings, parameters such as root length, root volume, fresh and dry root mass, fresh and dry leaf mass as well as plant height was determined. Although not significant in all cases, the results revealed a general increase in seedling growth as a response to all the seed treatments when compared to the untreated control. Significantly increased plant height and root length was demonstrated for the Teprosyn®/SS/AnnGro™ combination treatment followed by a pronounced effect by the Teprosyn®/CC/AnnGro™ combination. Overall the most prominent positive seedling growth response to the Teprosyn®/SS/AnnGro™ treatment suggested a synergistic

action between Teprosyn®, SS and AnnGro™. However, as the same tendency was observed in the case of the Seniphos®/SS/AnnGro™ and Teprosyn®/CC/AnnGro™ combination treatments it must be concluded that the only common factor in all these cases was AnnGro™. AnnGro™ is an uptake enhancer that most probably contributed to an increased translocation of the plant growth regulators CC and SS as well as the known Zn/P product (Teprosyn®) into the young seedlings offering a better chance for these products to exert their influences. Seniphos® is not a known seed treatment product but in combination with AnnGro™ it also showed promising results in terms of seedling growth enhancement. . CC (Schnabl *et al*., 2001) and SS (Van der Watt, 2005) are known plant growth regulators whose influences on seedling growth are seemingly enhanced by AnnGro™.

According to a postulate by Van der Watt (2005) the active compound of SS, glyceroltrilinoleate, induces the production of dihydroepijasmonic acid (DH-JA) and jasmonic acid (JA) as well as dihydro-12-oxo-phytodienoic acid that promote root growth and vegetative growth in crops respectively. Brassinosteroids (BR's), the active compound of ComCat® (CC) consequently also promote plant growth (Schnabl *et al*., 2001). According to Vardhini and Rao (1998) BR treated groundnuts showed an increase in root and shoot growth due to increased levels of soluble proteins, RNA and DNA.

All the seed treatments tended to enhance plant height with the highest significant increase of 26.7% induced by the Teprosyn®/SS/AnnGro™ treatment. Promotion of growth by this combination treatment might have been due to a synergistic effect between the active compounds of SS (glycerol trilinoleate) and the Zn moiety of Teprosyn®. Zinc is indirectly involved in the synthesis of auxin growth promoting hormones (Orabi & Abdel-Aziz, 1982), is also involved in enzymatic reactions where it acts as an inorganic co-factor while the growth promoting action of SS was demonstrated by Van der Watt (2005). According to the author the active compound of SS (triglycerides) play a role in activating metabolic events leading to enhanced seedling growth. The active compound of SS was also demonstrated to enhance the

respiration rate and seedling growth in a variety of vegetable crops (Van der Watt, 2005) and this supplied the rationale for testing this metabolic aspect under glasshouse conditions in this study.

Since respiration plays a central energy releasing role in living organisms, it is pivotal in determining the energy status of plants. The energy status of a crop is also important in determining the growth rate and eventually the production potential of the crop (Pretorius & Small, 1992). According to the authors the maintenance of respiratory substrate levels such as soluble protein and sugars throughout the growing season not only determines growth but also the final yield. It is necessary to elaborate on these two respiratory substrates individually.

A positive relationship exists between protein content and the rate of respiration (Amthor, 1989). According to the author this relationship is simply due to a greater general metabolic rate in tissues with high protein content with all other factors being equal. However, protein breakdown or usage of protein as respiratory substrate is oxygen dependant as the breakdown only occurs in the mitochondrion during the Krebs cycle (Pretorius & Small, 1992). The significant increase in total soluble proteins observed in both roots and leaves of maize seedlings from seeds pre-treated with Teprosyn®/CC/AnnGro™ was probably due to a synergistic effect between CC and the active substances (P & Zn) of Teprosyn®, as the same was not observed when the two products were applied on their own. The role of phosphorus in protein synthesis is well known whereas Zn could have played a role in activating enzymes responsible for protein synthesis (Orabi & Abedel-Aziz, 1982). Interestingly, the same tendency was observed with other combination treatments that contained Teprosyn®. However, the Seniphos®/CC and Seniphos®/SS/AnnGro™ combination treatments also contributed to a marked increase in soluble protein in leaves. In Seniphos® the main active compounds are phosphorus and calcium. In addition to the known metabolic role of P, Ca has been reported to express specific genes involved in protein synthesis (Gulin *et al*., 2003). The total soluble protein measured in maize seedling leaves was higher than in the roots on a fresh mass

basis. This is in concert with reports by Subramanian and Charest (1995) who observed that leaf homogenates generally contained 2-4 times more soluble proteins than root homogenates in developing maize seedlings.

More than a decade ago Riccard *et al*. (1998) reported that proteins induced in plants either artificially by means of external manipulation or by means of stress conditions are known to be involved in stress tolerance and lignin synthesis or are simply broken down via respiratory metabolism, but are not primary respiratory substrate. As the *in vitro* activity of the key regulatory glycolytic enzyme PFK was not increased by the seed treatments that induced soluble protein production in maize seedlings, it might suggest that respiratory metabolism was not dependent on protein levels but rather on other substrates. The latter was confirmed by the low O_2 consumption rate observed in seedlings from seeds pre-treated with all of the products.

Sugars are generally the primary substrates of respiratory metabolism, the respiration rate is closely correlated with the sugar content in plant tissue (Dwivedi, 2000) and soluble sugars play a central role in plant structure and metabolism at a cellular and whole organism levels (Couée, 2005). Sucrose is the form in which carbohydrates are translocated from the leaves to the sink tissue where it is hydrolyzed to its two monosaccharide forms, glucose and fructose (Prado *et al*., 2000), and metabolized. In low vigour seeds a positive correlation between the availability of carbohydrate substrate and respiration rate was reported by Bettey and Finch-Savage (1996) and this is most probably also the case in seedlings and mature plants.

All three forms of soluble sugars were measured in maize seedlings in order to determine the effect of seed treatments on sugar content as well as the correlation between sugar content and respiration rate. Although not significant, the sucrose content in roots were markedly higher on a gram fresh mass basis than in leaves and especially where seeds were pre-treated with Seniphos®/SS/AnnGro™, Teprosyn®/CC/AnnGro™ and Teprosyn®/SS/AnnGro™. Both CC (Schnabl *et al*., 2001) and SS (Van der Watt, 2005) are known to increase sugar production in a

variety of crops. The fact that the root sucrose content was in some cases seven-fold higher than in the leaves is difficult to explain as maize is not known as an accumulator of carbohydrate in the below soil parts. The only explanation at the growth stage where sugar content was measured is probably that the seedling was still in a phase of root development that might have accounted for the high energy need. The latter interpretation is partially supported by the high glucose and fructose levels in roots, again especially where seeds were pre-treated with the above mentioned three combination treatments, indicating a rather active hydrolyzation of transported sucrose. In this regard Amthor (1989) reported that a decrease in soluble sugar content in leaves compared to roots reflect cellular development followed by a period dominated by cell expansion suggesting that more sugar as a source of energy was supplied for root expansion at this growth stage. Further, high sugar concentrations in root tissue have been reported to induce lateral root development (Laby *et al*., 2000; Wittenmayer & Merbach, 2005).

On the other hand, the P-moiety of both Seniphos® and Teprosyn® in the above combination treatments might also have played a role in the distinct increase of sugar levels in maize seedlings from seeds pre-treated with these two products. In barley Wang and Tillberg (1997) showed that short-term phosphorus deficiency had a negative effect on carbohydrate storage in sink and source tissue. In this study where P was additionally applied as seed treatment, taking into account that P plays an essential role in regulating sucrose synthesis in the cytosol via the cytoplasmic enzyme sucrose phosphate synthase, there is the possibility that these two products induced sugar formation either on their own or in a synergistic fashion when combined with CC and SS.

Sustainable sugar levels are essential in the life cycle of plants as it is the primary energy source (Taneja *et al*., 1992). Glycolysis and the OPP-pathway are two important metabolic pathways involved in the metabolism of carbohydrate and are interwoven as the two pathways share intermediary substrates (Muscolo *et al*., 2001). In order to determine the effect of seed treatments on these two respiratory pathways

the *in vitro* activities of the most important regulatory enzymes, namely ATP-PFK (glycolysis) and Gluc-6-PDH (OPP-pathway) were measured spectrophotometrically in terms of NADH and NADPH produced respectively. The Gluc-6-PDH enzyme activity of the OPP pathway was higher than that of ATP-PFK in these young maize seedlings and this was in agreement with the findings of Maciejewska and Bogatek (2002) who also reported low ATP-PFK activity and higher Gluc-6-PDH activity on poplar twigs in early autumn. These findings reflect a shift from glycolysis to the OPP-pathway suggesting the production of triose phosphate sugars by by-passing the PFK regulation point of glycolysis and removing, at least partially, control over phosphorylation of Fru-6-phosphate in young active growing plant tissue (Debnam & Emes,1999).

Although all seed treatments had an increasing effect on Gluc-6-PDH activity the most pronounced increase was observed for CC (114%), SS (150%), Seniphos®/CC (200 %) and Teprosyn®/CC/AnnGro™ (142 %), compared to the untreated control. This correlated positively with the enhanced CO_2 release rate under the influence of the above mentioned seed treatments, except in the case of the Teprosyn®/CC/AnnGro™ combination treatment. CO_2 is released during both the OPP-pathway (Ap-Rees, 1981; Centen *et al*., 2008) and mitochondrial respiration (Krebs cycle) and it is difficult to distinguish between the two. However, the positive correlation between enhanced Gluc-6-PDH activity and the CO_2 release rate strongly suggest that the seed treatments which had a positive effect on growth could have been mainly via OPP-pathway manipulation. NADPH recycling takes place in the mitochondrion where it is oxidized to form ATP essential for growth and development while erythrose-4-phosphate produced during the OPP-pathway is essential for DNA and RNA synthesis in active growing tissue (Pretorius & Small, 1992),

On the other hand, the ATP-PFK activity was significantly promoted by CC (100 %) and non-significantly by Teprosyn®/CC/AnnGro™ (50%) and Teprosyn®/SS/AnnGro™ (25%) indicating an accelerated flux of carbon through glycolysis in these cases. All of the other seed treatments inhibited PFK activity. Interestingly, these were also the

treatments that promoted root length growth and growth in plant height. The latter indicates that a normal respiration rate was probably sufficient to support the utilization of carbohydrate as building material in an active growing maize seedling.

As the supply of photosynthate determines normal energy supply in the life cycle of a crop, chlorophyll content in leaves was quantified with the objective to ascertain whether any of the seed treatments had a manipulatory effect on the most basic pigments underlying the supply of respiratory substrate. Total chlorophyll content in leaves can be used as a sensitive indicator of the cellular metabolic state of leaf tissue as well as of the whole plant (Khosravinejad *et al*., 2008). Interestingly, as was the case with sugar content, increased chlorophyll levels were measured in leaves of seedlings from seeds treated with the three combination treatments, viz. Teprosyn®/CC/AnnGro™, Teprosyn®/SS/AnnGro™ and Seniphos®/SS/AnnGro™ suggesting a sturdy metabolic state. For brassinosteroids, the active compound of CC, the inducing effect on chlorophyll synthesis has been reported (Sairam, 1994; Vigneshwari, 2005). However, in the case of Teprosyn® and SS this is the first indication of its possible role in promoting chlorophyll synthesis.

Contrary to increased chlorophyll production by the latter three combination seed treatments, the total chlorophyll content in the leaves of maize seedlings was strongly inhibited where seeds were treated with Seniphos® on its own. The inhibitory effect by Seniphos® could be ascribed to the presence of Ca^{2+}. This is consistent with results by Tanaka and Tsuji (1980) that showed that Ca^{2+} inhibited the accumulation of chlorophyll in the early phase of greening in cucumber by inhibiting δ-aminolevulinic acid (ALA) formation and by accelerating the destruction of newly formed chlorophyll. Larrigaudiere *et al*. (1996) reported that Seniphos® has been applied to various crops to improve the production of anthocyanin while Arreola *et al*. (2008) maintained that chlorophyll accumulation does not continue when leaves start to accumulate anthocyanin. The decrease in total chlorophyll accumulation by Seniphos® might have coincided with leaf anthocyanin production resulting in a decrease in the activities of the enzymes involved in chlorophyll synthesis (Arreola *et*

al., 2008). This response is similar to that reported by the author for young poinsettia leaves where decreases in chlorophyll levels were observed suggesting that it coincided with anthocyanin synthesis. To the contrary, Ca^{2+} in the nutrient solution was reported to have a positive effect on the chlorophyll concentration, chlorophyll*a*/chlorophyll*b* ratio and on the photosynthetic carotenoids in soybean (Milivojevic & Stojanovic, 2003). In this study the apparent negative effect of Seniphos® on chlorophyll content in maize seedlings was reversed when Seniphos® was applied in combination with SS and AnnGro™.

Interestingly, the only treatment that had a slight improving effect on carotenoid content in maize seedling leaves was Seniphos® applied on its own, while all other treatments tended to reduce the level of this pigment. Carotenoids protect chlorophyll by absorbing and transferring excessive light energy (Arreola *et al.,* 2008) that is beneficial to the plant in preventing chlorophyll from being photo-oxidized. However, the carotenoid increase under the influence of Seniphos® in maize seedlings was negligible and not worthwhile pondering on any further.

In summary, from a growth promoting perspective as well as positive effects on most of the metabolic events followed in maize seedlings in this study, the Teprosyn®/CC/AnnGro™ and Teprosyn®/SS/AnnGro™ combination treatments seemed to be the most consistent of all the products tested for its potential to improve seedling establishment when applied as seed treatments prior to sowing. However, in chapter 5 this postulate will be tested under field conditions and only then final conclusions can be reached.

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CHAPTER 5

GROWTH AND YIELD RESPONSE OF MAIZE TO SEED TREATMENTS UNDER RAIN FED FIELD CONDITIONS

5.1 ABSTRACT

In attempt to evaluate the applicability of selected seed treatments that showed potential under laboratory and glasshouse conditions, a field experiment was conducted during 2008/2009 growing season. The main aim of this study was to determine the influence of selected seed treatments on the growth and ultimate yield of maize under rain fed field conditions. The response of maize to ten seed treatments and one foliar application were evaluated in terms of growth and yield and these included ComCat® , SS, Teprosyn®, Teprosyn®/AnnGro™, Teprosyn®/CC, Teprosyn®/SS/AnnGro™, Teprosyn®/CC/SS, Seniphos®, Seniphos®/CC, Seniphos®/SS/AnnGro™ and CC-foliar. The untreated control was used as the main check. The experiment was laid out in a complete randomized block design with 4 replications. No clear pattern emerged in terms of the association between growth and yield parameters. However, the ComCat® treatment was the only one to stand out in terms of a positive correlation between vegetative growth and yield. For the other treatments this association was rather erratic. However, a positive relationship between dry matter accumulation in roots and leaves existed for those treatments that had an increasing effect on the final maize yield. These included the Teprosyn®/SS/AnnGro™, CC, Seniphos® and Seniphos®/SS/AnnGro™ seed treatments that had the most marked effect on yield enhancement. The maximum total yield of 4.6 t ha^{-1}, although not statistically significant, was 800 kg ha^{-1} higher than the untreated control when seeds were treated with the Teprosyn®/SS/AnnGro™ combination treatment at planting. To the contrary, the Teprosyn®/AnnGro™ treatment contributed to the most significant reduction in final yield compared to the other treatments.

Keywords: Maize, seed treatments, plant growth, yield components, rain fed field conditions.

5.2 INTRODUCTION

Maize (*Zea mays* L.) is the most important grain crop grown in South Africa and is one of the most important crops in the milder subtropical and tropical regions of the world (FSSA, 2007). More than two decades ago Abulrahaman and Kolawole (1988) identified maize as a staple food commodity in Africa and this has not changed since. However, despite the fact that maize provides a high percentage of daily calories in most African households, production is still very low and fluctuates due to the limiting changing climatic and environmental conditions such as poor and unreliable rainfall as well as low soil fertility due to high inorganic fertilizer prices (Lekgari & Setimela, 2001).

Collectively the above mentioned factors have a negative effect on the final grain yield leading to Sub-Saharan Africa facing food security problems mainly because of decreasing per-capita food production (Bekunda *et al.,* 2002). Two years earlier Muflin (2000) predicted that the challenge to feed the world population, that is likely to rise to 8 billion, is alarming particularly since recent analyses suggest that the rate of increase in yields of several crops may have dropped over the last decade. Mwaniki (2006) maintained that 12 million people are facing food insecurity in Southern Africa. As a result, increasing yield for an ever growing population has currently become a topic of great concern with regard to food security.

Since there are only limited prospects for expanding cropland significantly, most food production increases will have to come from increasing yields on existing arable land by, inter alia, improving plant nutrition practices. However, more than a decade ago Marley and Adeoti (1995) identified crop establishment as a major constraint to crop production in the semi-arid tropics. More recently Ulukan (2005) voiced his opinion on

seed pre-treatment as one of the alternative production practices that holds potential for increasing yields. The author based his statement on the potential of specific seed treatments to improve seed germination and subsequent seedling growth via manipulation of physiological processes that may lead to improved utilization of soil water and nutrients and ultimately yield.

Although progress has been made in the transformation of cereals including maize, significant research on yield increase is still limited. However the ease with which genetic manipulation can be made has opened a new range of fertilizer or organic products and both application methods in crop production. Apart from various breeding methods, seed treatment prior to planting offers a great opportunity in complementing varietal breeding. Seed priming permits pre-germination physiological and biochemical changes to occur (Bradford, 1986). This genetic manipulation starts with the seed itself prior to subsequent growth stages thereto improved grain yield. Several studies have demonstrated the effect of plant growth regulators in altering several physiological parameters and hence the yield (Malik *et al*., 1995; Verma *et al.*, 2009). The importance of PGRs has been emphasized in source and sink a relationship leading to enhanced translocation of photo assimilates hence influencing the yield. Application of growth regulators such as gibberellic acid (GA_3) has been reported to manipulate a variety of growth and development phenomena as well as yield as observed in various crops (Hoque & Haque, 2002). Their study indicated that morphological parameters of mungbean can be favorably influenced by the application of GA_3 with a consequent yield increase. According to Ulukan (2005) the maximization of yield from seed treatments result from good seedling emergence and even stands which are the most essential prerequisite for yield maximization. In groundnut plant regulators are reported to have the potential of altering plant archetype; promote photosynthesis; alter assimilate stimulate uptake of mineral ions; enhance promote flowering; uniform pod formation; increase mobilization of assimilates to improve seed quality; induce synchrony in growth and development of reproduction and vegetative flowering and delay senescence of leaves (Verma *et al*., 2009).

In this study morphological and yield parameters were applied to assess the response of maize (cultivar DKC7815 *Bt*) to selected seed treatments under rain fed field conditions. The treatments were selected from data collected under laboratory and glasshouse conditions on the basis of those treatments that showed potential to improve seedling growth in terms of either root development or above soil part growth or both.

5.3 MATERIALS AND METHODS

5.3.1 Materials

See 3.3.1 and 3.3.2.

5.3.2 Methods

5.3.2.1 Experimental design

A field trial was conducted at the Kenilworth experimental farm of the department of Soil, Crop and Climate Sciences, University of the Free State, in the Bloemfontein district (29°01'00"S, 26°08'50"E) during the 2008/2009 growing season. The trial was planted on the 17th of November 2008 and laid out in a complete randomized block design (CRBD) with four (4) replications. The field was divided into four blocks with 12 unit plots per block. Each plot was 9 m x 10 m (90 m^2) and five rows per plot were planted manually using a specially designed hand-held single row planter calibrated to place seeds 0.3 m apart (in-row spacing). As the row width was 1.7 m, the projected plant population was 19647 plants ha^{-1}.

5.3.2.2 Fertilizer applications

The trial was carried out on Bainsvlei soil. Soil samples were collected prior to planting for chemical analysis (Table 5.1). Fertilizer [3:1:0 (28)] was applied at a rate of 200 kg ha^{-1} (21 kg N and 7 kg P ha^{-1}) according to the withdrawal amount for an expected yield of 3 ton ha^{-1}, at a depth of 5 cm below the seed prior to sowing. The amount of K contained in the soil was within the accepted limit for maize. Hand weeding was carried out one week after seedling emergence and again four weeks later.

Table 5.1: Soil analysis

Property	
Soil type	Bainsvlei (clay loam)
pH (H_2O)	6.88
EC (Ohm)	1600
Nutrients	**Ppm**
N ($NaHCO_3$)	530.4
P ($NaHCO_3$)	16.8
K (NH_4OA_c)	112.0
Ca (NH_4OA_c)	1825.8
Mg (NH_4OA_c)	397.5
Na (NH_4OA_c)	67.5
Zn (HCl)	2.2

5.3.2.3 Seed treatments after planting

The trial comprised of 12 treatments viz; 10 seed treatments that were compared to each other as well as to an untreated negative control and a single foliar application of a commercial plant growth regulator that served as a positive control, in terms of the vegetative growth and yield response of maize. The 10 seed treatments were applied at the time of sowing while the foliar treatment was applied six weeks after planting. The selected products or combinations thereof were diluted in 3 L of water and applied directly onto the seeds using a rug bag nozzle spray at a rate of 83 L ha^{-1} (750 ml per plot). Seed treatments with single products included:

- ComCat® (50 g ha^{-1})
- SS (25 g ha^{-1})
- Teprosyn® (0.5 L ha^{-1})
- Seniphos® (1.5 L ha^{-1})
- AnnGro™ (60 mL ha^{-1})

Combination treatments, at the same rates as indicated above, were chosen on the basis of data obtained under laboratory and glasshouse conditions. These included:

- Teprosyn®/AnnGro™,
- Teprosyn®/ComCat®,
- Teprosyn®/SS/AnnGro™,
- Teprosyn®/ComCat® /SS,
- Seniphos®/ComCat® ,
- Seniphos®/SS/AnnGro™.

The foliar treatment (ComCat® /AnnGro™) was a combination of ComCat® at 100 g ha^{-1} and AnnGro™ at 60 mL ha^{-1} that served as a positive control.

5.3.2.4 Collection of growth data

Plant height and stem thickness were collected non-destructively from plants in the two middle rows (3 m x 3.4 m; 10.2 m^2), the area from which final yield was also

determined at the end of the drying cycle, in order to circumvent any side effects. Data was recorded one, two, three and six weeks after emergence and samples consisted of six representative plants per plot, hence 24 plants per treatment. Plant height, defined as height from the soil to the tip of the largest unfolded leaf, was quantified using a measuring tape. Stem thickness was measured just above the soil surface using a digital caliper.

Subsequently, three plants per plot were removed from a row next to the middle two rows that was not earmarked for later yield measurement, and used for determining leaf area, fresh and dry root biomass as well as fresh and dry leaf biomass. This was achieved by uprooting plants from the soil with a spade in similar fashion at the same depth in an attempt to allow as little as possible hair roots to remain in the soil. Prior to fresh root mass determination, the whole root system of each plant was cleaned thoroughly by gentle running tap water to remove the adhered soil and foreign materials. Subsequently, roots were wrapped in laboratory grade filter paper for half an hour to remove the excess water from the root surface. The average fresh mass was determined by weighing leaf and root material separated from three representative plants per plot using a digital BP 3000 Mettler scale.

Leaf area was determined from the three (3) representative plants per plot using a Li-3100C area meter. All leaves from each plant were passed through the calibrated area of the meter and the sum represented the leaf area for the plant.

For dry mass determination the leaf and root samples were dried separately in paper bags in an oven at 70 ºC for ±48 hours and the dry mass determined.

5.3.2.5 Collection of yield data

All the yield parameters were recorded from the 10.2 m^2 (3 m x 3.4 m) harvested area of each plot, i.e. the area of the two middle rows, while the area that was used

destructively to determine growth parameters was left out. The plant population was recorded prior to harvesting by counting the number of plants in the harvested area of each plot. The number of cobs per plot was counted and the weight determined. From this data the average cob weight per plot was calculated. Additionally the percentage non filled stalk ends was calculated by measuring the cob length and the non-filled end of the cob recorded from 10 representative cobs per plot. Subsequently, kernels were removed using a threshing machine where after which the total kernel weight per plot as well as the 100 kernel weight per replicate was determined using a digital mass meter. The number of kernels per cob was also calculated by dividing the total kernel weight by the number of cobs. Total yield was calculated from the total kernel weight per harvested area of each plot and expressed as ton ha^{-1}.

5.3.2.6 Statistical analysis of data

Statistical analyses were performed on 4 replications for all of the collected data. Data was subjected to analysis of variance (one-way ANOVA) using the SAS statistical program and Tukey's least significant difference (LSD) procedure to separate means at the 95% confidence level or 5% ($p>0.05$) level of significance.

5.4 RESULTS

5.4.1 The influence of seed treatments on plant growth under rain fed field conditions

5.4.1.1 Plant height

Although the growth response of maize to different seed treatments at planting did not follow an unvarying pattern at different time intervals in terms of plant height, most but not all of the treatments tended to contribute to enhanced stem length

growth compared to the untreated control (Figure 5.1). Of all the treatments the ComCat® (CC), SS, Teprosyn®/AnnGro™ and Seniphos® applications illustrated this tendency more pronounced one week after seedling emergence (Figure 5.1A). The latter was, however, statistically non-significant compared to the untreated control. This tendency more or less prevailed when plant height was measured two weeks after seedling emergence except that stem length growth in response to the Teprosyn®/AnnGro™ combination treatment was significantly enhanced compared to the SS, Teprosyn®/CC, Seniphos®/CC and CC foliar treatments (Figure 5.1B). Compared to the untreated control and all other treatments plant height was significantly inhibited only by the CC foliar application. As was the case in the first week after emergence, the Teprosyn®/AnnGro™ and CC seed applications stimulated plant height growth compared to the untreated control, albeit statistically non-significant. In addition to the latter two, a tendency to induce plant height growth was also observed for the Teprosyn®, Teprosyn®/SS/AnnGro™, and Teprosyn®/CC/SS treatments.

Interestingly, three weeks after emergence, all treatments contributed to enhanced plant height growth, even the CC-foliar application that had an inhibiting effect in this regard during the previous two weeks (Figure. 5.1C). At this growth stage most pronounced increased plant heights were observed for the CC, Teprosyn®, Teprosyn®/AnnGro™ and Seniphos® treatments which all differed significantly from the untreated control. A similar growth pattern prevailed six weeks after emergence (Figure 5.1D) for all seed treatments except the CC-foliar application that contributed to a reduced plant height. Collectively, the CC, Teprosyn®, Teprosyn®/AnnGro™ and Teprosyn®/SS/AnnGro™ seed treatments at planting can be singled out as those that had the most pronounced effect on subsequent above soil plant height growth compared to the untreated control and the other treatments.

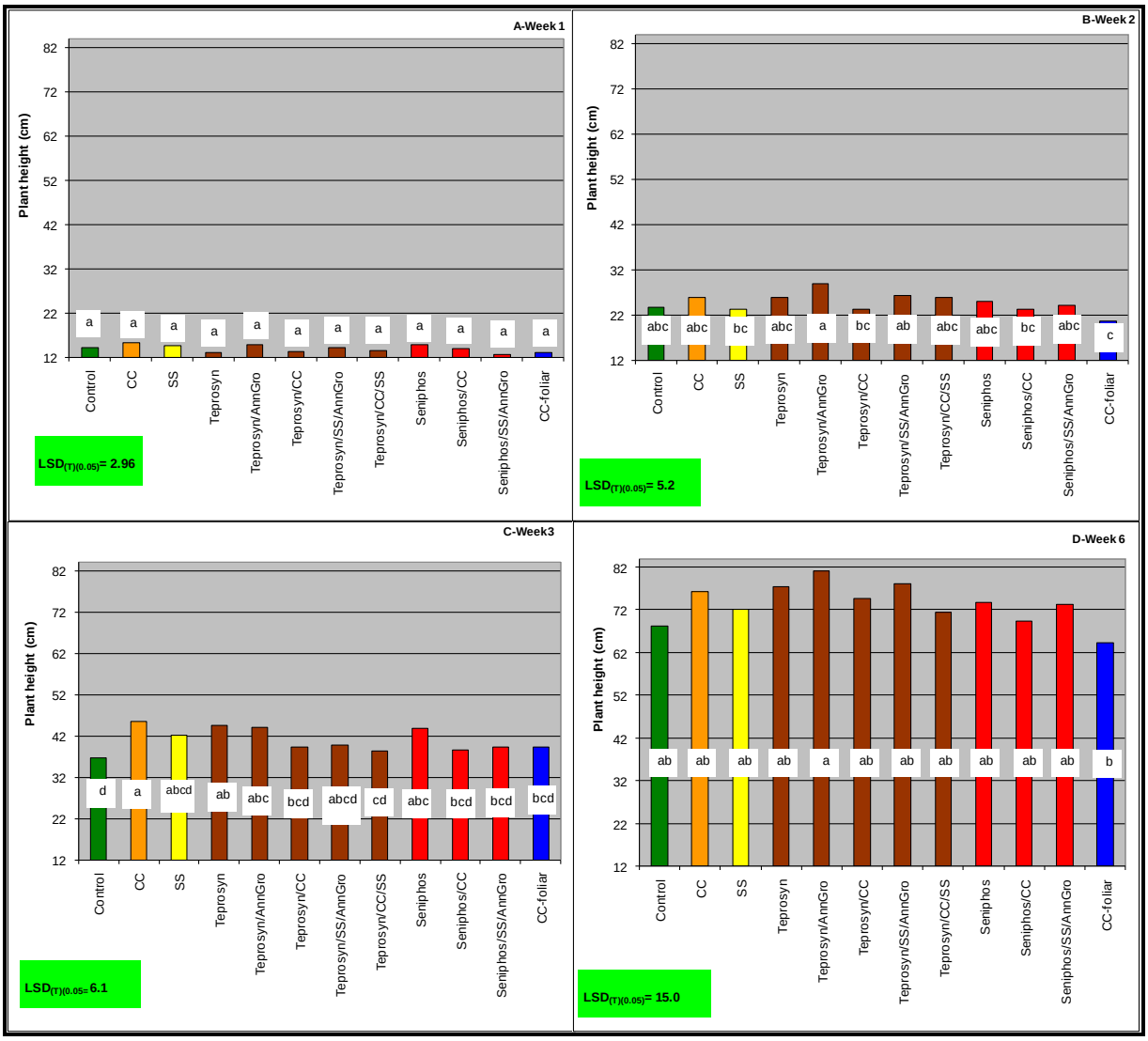

Figure 5.1: Plant height of maize one week (A), two weeks (B), three weeks (C) and six weeks (D) after seedling emergence in response to seed treatments at planting under rain fed conditions. LSD ($P<0.05$) values are indicated in the graph and means differing significantly are indicated with different letters.

5.4.1.2 Stem thickness

Compared to the untreated control, and as was the case with plant height, seed treatment at planting with CC and Teprosyn®/AnnGro™ once again stood out as the two treatments that contributed most to an increase in maize stem thickness one week after emergence (Figure 5.2A). The difference was only significant in case of the latter treatment, which did not differ significantly from the other, while the effect of the Teprosyn®/AnnGro™ application also differed significantly from that of the Seniphos®, Seniphos®/CC, Seniphos®/SS/AnnGro™ and CC foliar applications in terms of stem thickness. Two weeks after emergence this tendency prevailed for the CC and Teprosyn®/AnnGro™ treatments although no significant differences were observed between all treatments (Figure 5.2B). At three weeks (Figure 5.2C) the Teprosyn® and Seniphos® applications had a similar increasing effect on stem thickness as did CC and Teprosyn®/AnnGro™ although the contributions of these four treatments differed significantly only from that of the Seniphos®/CC treatment which clearly had an inhibitory effect on stem expansion. At week six (Figure 5.2D), no significant differences between treatments were observed in terms of stem thickness.

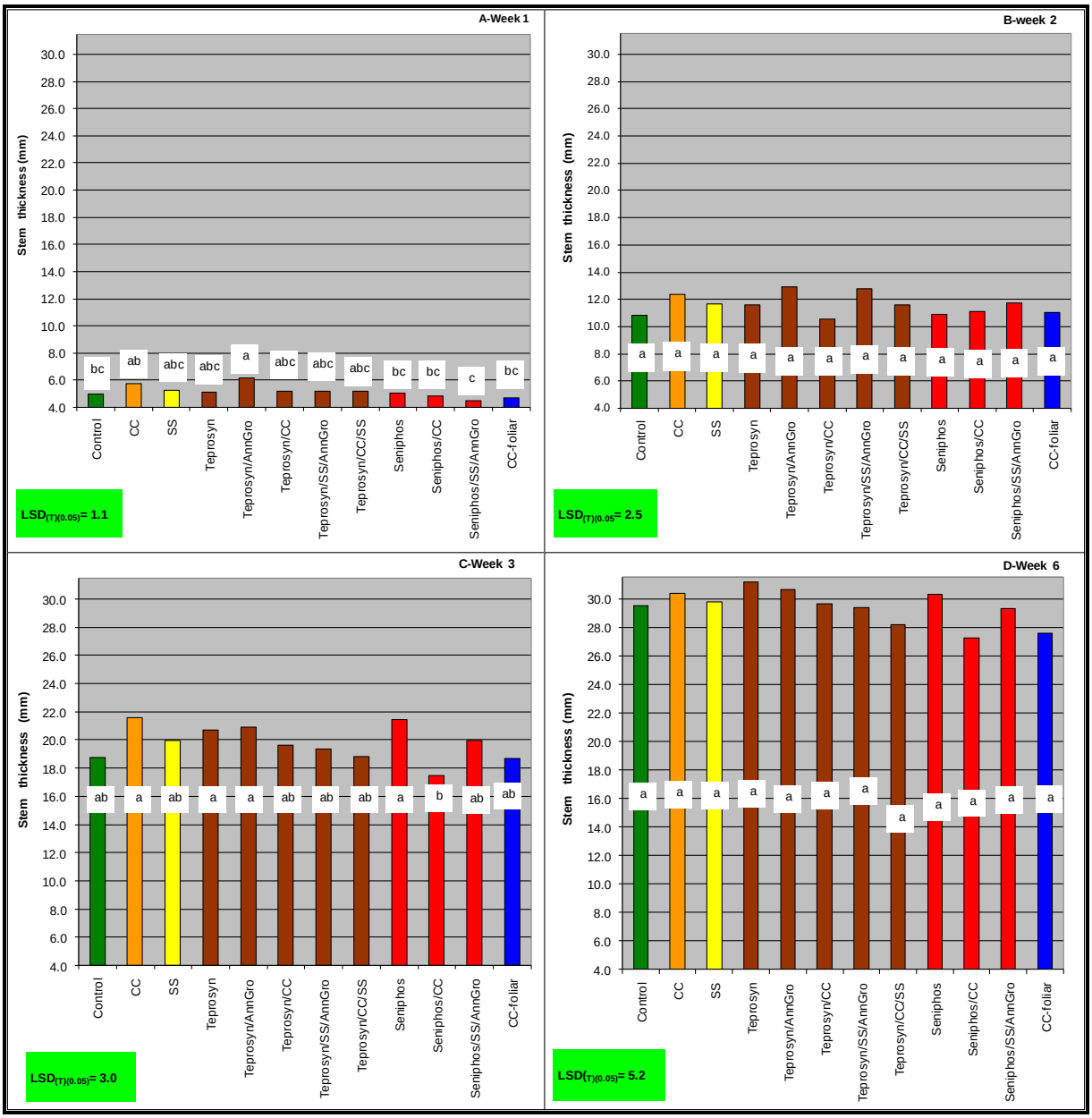

Figure 5.2: Stem thickness maize one week (A), two weeks (B), three weeks (C) and six weeks (D) after seedling emergence in response to seed treatments at planting under rain fed conditions. LSD (P<0.05) values are indicated in the graph and means differing significantly are indicated with different letters.

5.4.1.3 Leaf area

One week after emergence the measured leaf area of all maize seedlings was either the same for some treatments or less in the case of the rest **(**Figure. 5.3A). As a response to the SS, Teprosyn®/CC/SS and CC foliar treatments the leaf area was significantly lower than that of the untreated control. Leaf area, two weeks after planting (Figure. 5.3B), showed a non-significant increasing response as a result of treating maize seed at planting with CC, SS, Teprosyn®, Seniphos®, Seniphos®/CCSS/AnnGro™. A markedly reduction in leaf area was demonstrated only by the Teprosyn®/CC and CC-foliar treatments. No significant differences in leaf area between treatments were observed three weeks after seedling emergence (Figure 5.3C), most probably due to large standard deviations. However, except for the CC, CC/Seniphos® and CC foliar treatments, all other treatments markedly increased leaf area during this growth stage. Compared to the untreated control, only the Teprosyn® and Teprosyn®/CC/SS combination treatment had a significantly enhancing effect on the leaf area of maize six weeks after emergence (Figure 5.3D). The leaf area expansion response of maize to these two treatments also differed significantly from five of the other treatments viz. SS, Teprosyn®/AnnGro™ , Seniphos®, Seniphos®/CC and CC foliar treatments.

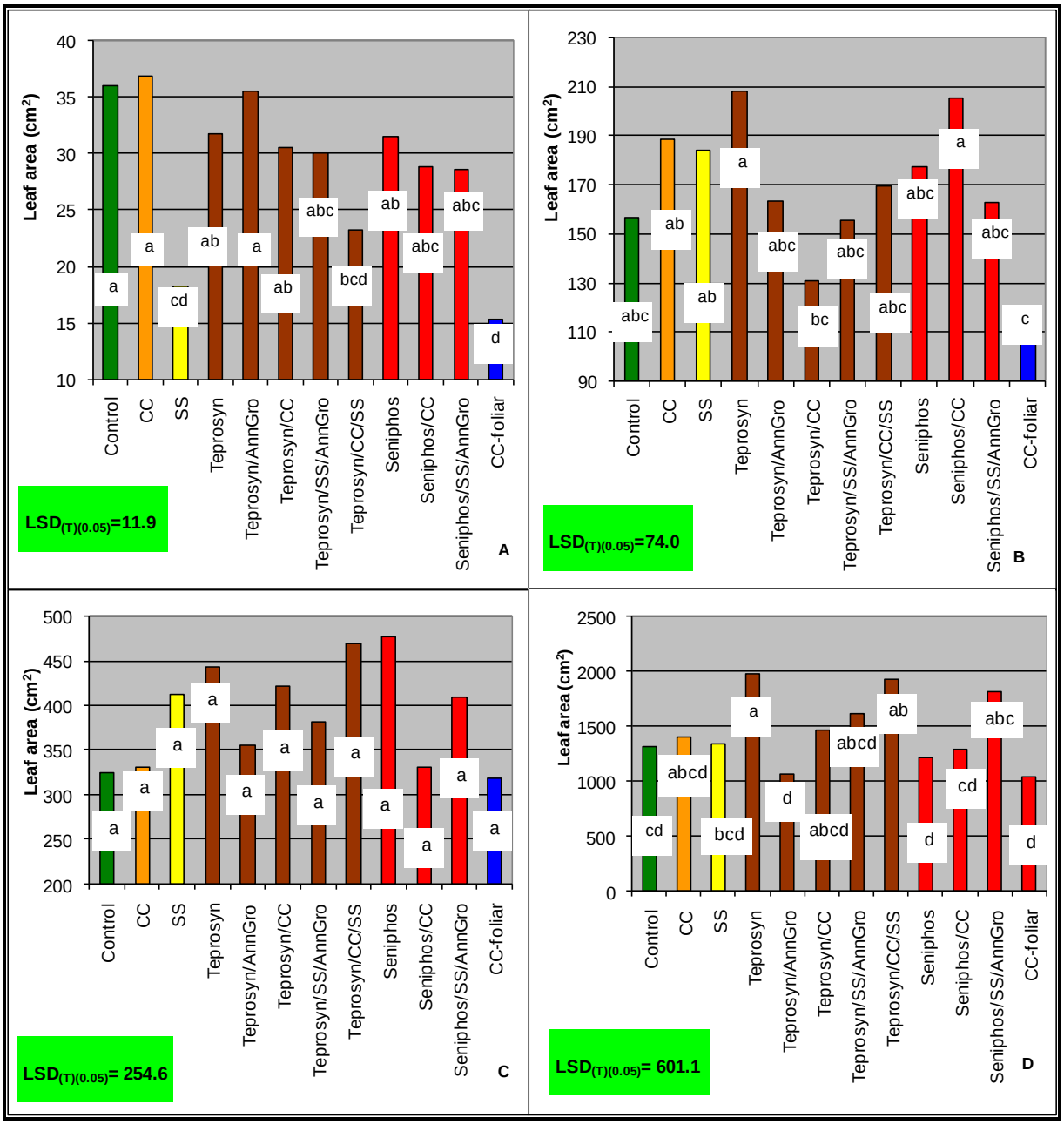

Figure 5.3: Leaf area of maize one week (A), two weeks (B), three weeks (C) and six weeks (D) after seedling emergence in response to seed treatments at planting under rain fed conditions. LSD (P<0.05) values are indicated in the graph and means differing significantly are indicated with different letters.

5.4.1.4 Biomass production

5.4.1.4.1 Fresh and dry leaf biomass production

Biomass production in terms of leaf fresh mass in the first week after seedling emergence showed the most marked increase where seeds were pre-treated with CC followed by the Teprosyn®/CC combination treatment (Figure. 5.4A). This was, however, statistically not significantly different from the untreated control. One week after emergence, all other treatments had either no effect on leaf fresh mass or contributed to a decrease thereof when compared to the untreated control. Actually, seven of the eleven seed treatments, viz. SS, Teprosyn®/AnnGro™, Teprosyn®/SS/AnnGro™, Teprosyn®/CC/SS, Seniphos®/CC, Seniphos®/SS/AnnGro™ and CC-foliar, inhibited the production of leaf fresh mass at this growth stage. However, this decrease in biomass production was only significant in the case of the SS, Teprosyn®/CC/SS and CC-foliar treatments compared to the untreated control and the two treatments that enhanced fresh mass accumulation. Dry mass accumulation more or less followed exactly the same pattern as fresh mass production except that none of the differences between treatments were statistically significant (Figure 5.4A). In the case of the Seniphos®/CC treatment dry mass accumulation remained high despite the drop in fresh mass.

Two weeks after seedling emergence (Figure. 5.4B) a slight change in the pattern of leaf fresh mass production was observed. Compared to the untreated control the consistent tendency to contribute to an increase in leaf fresh mass was only observed as a response to the CC seed treatment. Although not significant, the Teprosyn®/CC combination treatment contributed to a decrease in leaf fresh mass production at this growth stage, where the opposite applied one week earlier. Similarly the leaf fresh mass production response of maize to treatment of seeds with SS, Teprosyn® and Seniphos®/CC showed an increase as opposed to a decrease one week earlier. In the case of all other treatments the response remained more or less the same. Dry mass accumulation followed exactly the same pattern as did fresh mass production (Figure 5.4B).

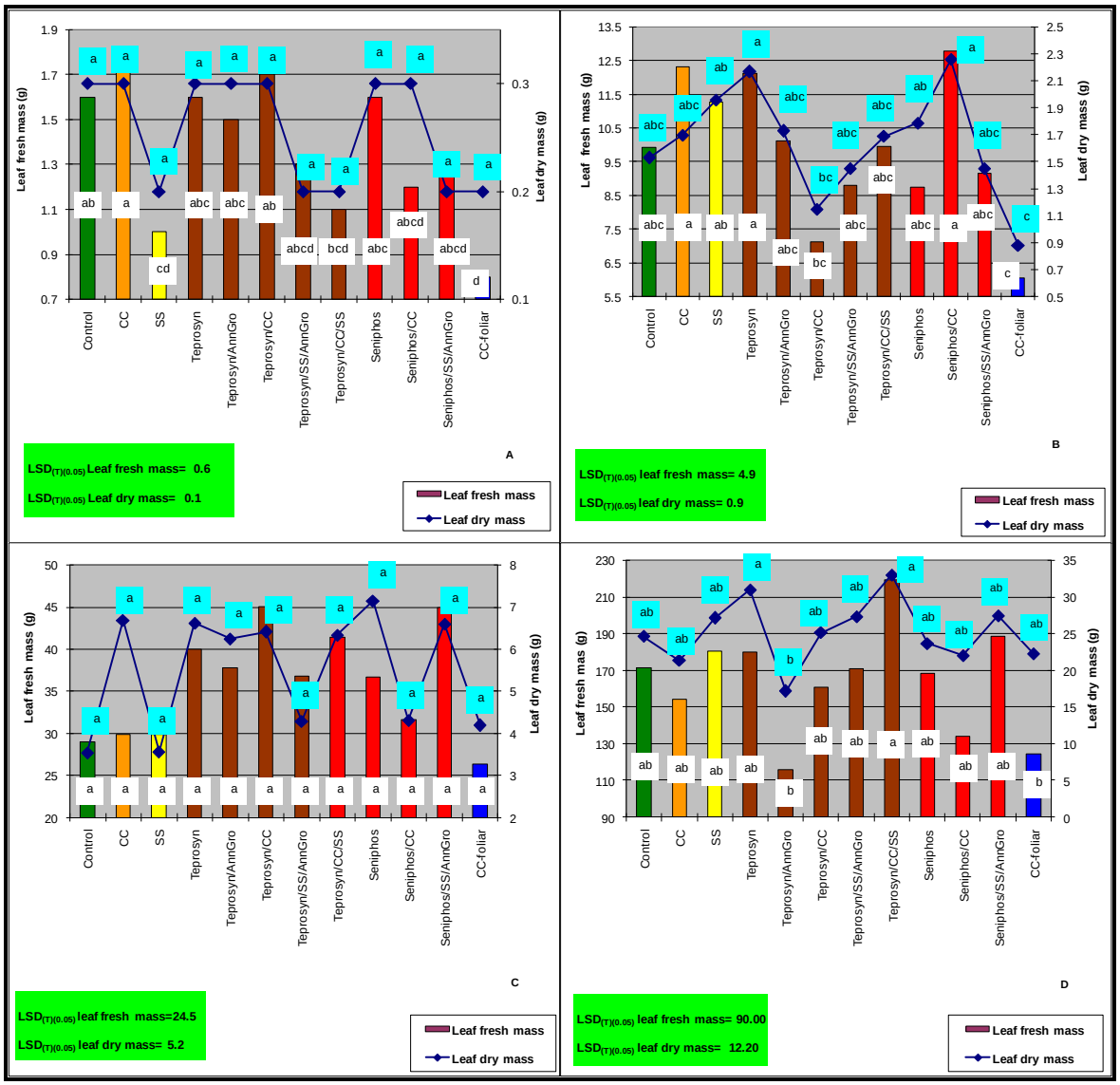

Figure 5.4: Influence of seed treatments on leaf biomass production under rain fed field conditions one week (A), two weeks (B), three weeks (C) and six weeks (D) after seedling emergence. LSD (P<0.05) values are indicated in the graph and means differing significantly are indicated with different letters.

The influence of pre-seed treatment on leaf fresh and dry mass as investigated on the 3rd week after planting (Figure. 5.4C) revealed a slight or marked induced fresh leaf biomass production for all the treatments, with CC-foliar as an exception, compared to the untreated control. Though the increase in fresh leaf mass was not significantly different, the Teprosyn®/CC treatment showed the most prominent stimulatory effect. Although not significantly different, the same

tendency applied for dry mass accumulation at this growth stage as all the treatments contributed to an increase in leaf dry mass (Figure 5.4C). Interestingly, even though there was an insignificant inhibitory effect on fresh leaf biomass by the CC-foliar treatment, the opposite applied for dry mass accumulation. This tendency for the dry mass to remain high even though the fresh mass seemed to decrease was also observed for the CC seed treatment.

Six weeks after planting the only seed treatment that contributed to a marked increase in fresh leaf mass, compared to the untreated control and other treatments, was the Teprosyn®/CC/SS combination treatment (Figure. 5.4D). In the case of all other treatments the leaf fresh mass was either the same or worse than that of the untreated control. Statistically significant differences were only observed between the control and the growth response to seed treatment with Teprosyn®/AnnGro™ and CC-foliar. Although dry matter accumulation more or less followed the same pattern as leaf fresh mass production, it was again interesting to observe that the dry mass remained rather high in cases where the fresh mass tended to be lower than that of the control, viz. the SS, Teprosyn®/AnnGro™, Seniphos®/CC and CC-foliar treatments.

5.4.1.4.2 Fresh and dry root biomass production

One week after seedling emergence no significant differences between either root fresh or dry mass was observed between treatments and the control (Figure 5.5A). One week later four of the seed treatments, CC, SS, Teprosyn® and Seniphos®/CC, tended to increase root fresh mass compared to the untreated control (Figure 5.5B). The other treatments either had no effect or a slight inhibitory effect on root development at this growth stage. The latter inhibitory effect was only significant for the CC-foliar treatment.

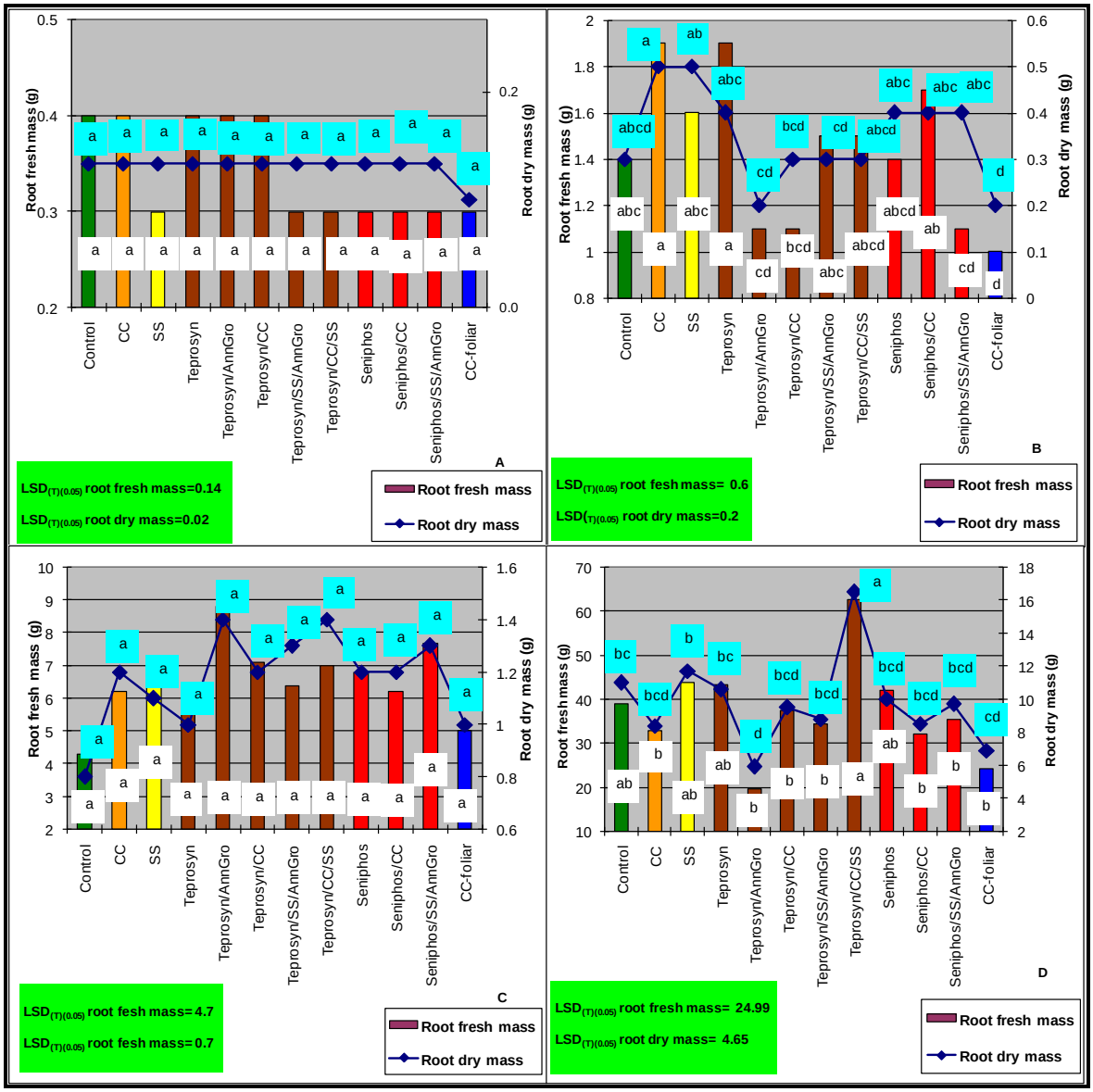

Figure 5.5: Influence of seed treatments on root biomass production under rain fed field conditions one week (A), two weeks (B), three weeks (C) and six weeks (D) after seedling emergence. LSD ($P<0.05$) values are indicated in the graph and means differing significantly are indicated with different letter.

Three weeks after emergence all seed treatments showed a tendency increase in both fresh and dry root mass (Figure 5.5C) although this was not statistically significant. At the six week growth stage after seedling emergence the only treatment that had a statistically significant enhancing effect on dry root mass was the Teprosyn®/CC/SS combination treatment.

5.4.2 The influence of seed treatments on maize yield and yield components under rain fed field conditions

5.4.2.1 Plant population in relationship with cob number

Although the average plant population in the two middle rows harvested ranged between 15 and 19 plants plot^{-1}, there were no significant differences between treatments (Figure 5.6). However, this was not the case for the average number of cobs plot^{-1}. The CC and Teprosyn®/SS/AnnGro™ treatments yielded the highest number of cobs which were significantly different from the response to seed treatment with Teprosyn®/AnnGro™ and Seniphos®/CC but not significantly different from the rest of the treatments including the control (Figure 5.6).

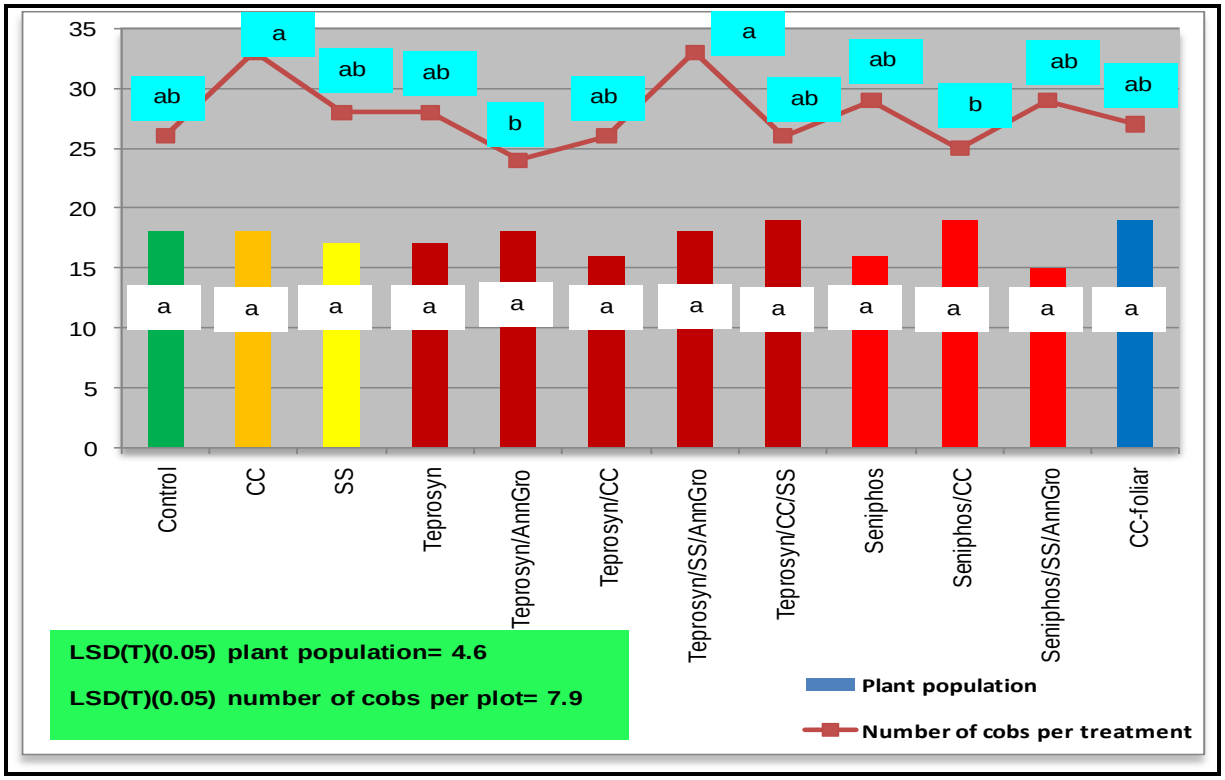

Figure 5.6: Plant population at harvest and its relationship with cob number under the influence of seed treatments and foliar spray of maize under rainfed field conditions. LSD (P<0.05) values are indicated in the graph and means differing significantly are indicated with different letters.

5.4.2.2 Prolificacy

Prolificacy especially expressed as the number of cobs plant^{-1}, slightly differed amongst treatments (Figure 5.7). When expressed as the number of cobs plot^{-1}, the tendency was exactly the same, but not so pronounced. Treating maize seeds with CC and Teprosyn®/SS/AnnGro™ at planting contributed to the most marked increase in the production of cobs plant^{-1} and this differed significantly from the number of cobs produced as a response to treatment with Teprosyn®/AnnGro™, Teprosyn®/CC/SS and CC-foliar, but not from the untreated control or the rest of the treatments.

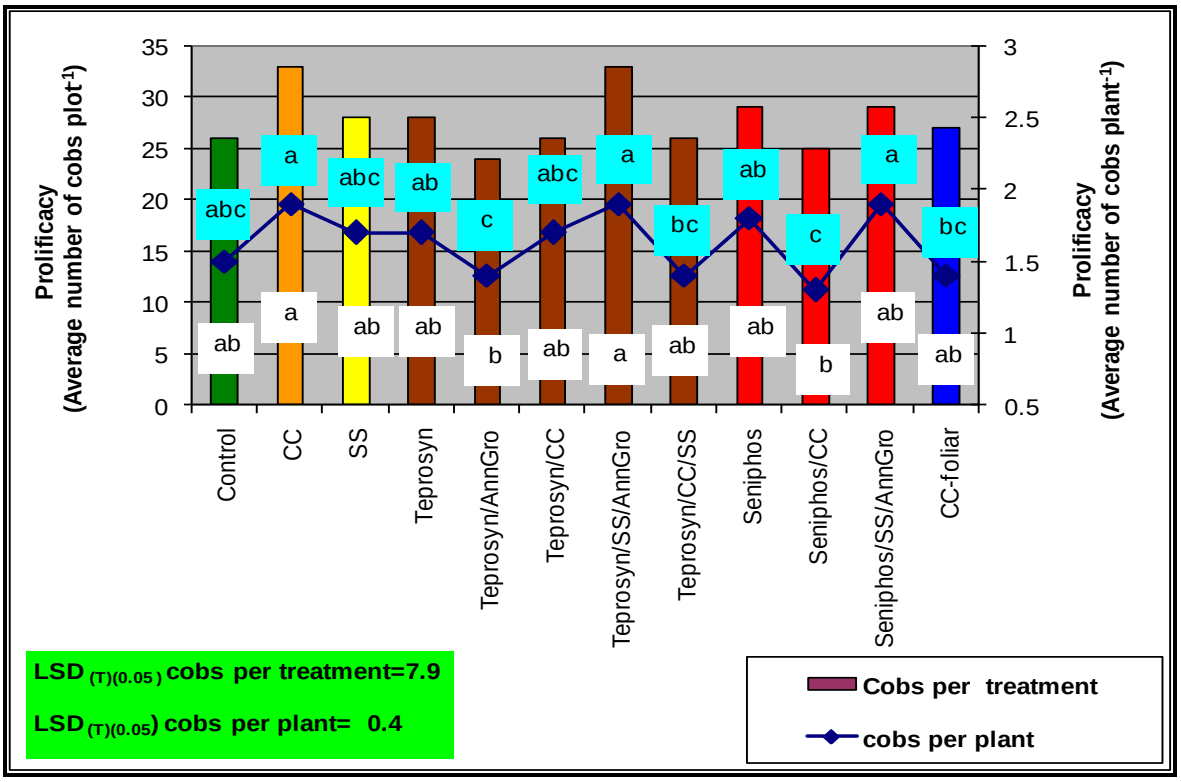

Figure 5.7: Number of cobs in response to seed treatments of maize under rain fed field conditions. LSD (P<0.05) values are indicated in the graph and means differing significantly are indicated with different letters.

5.4.2.3 Average cob weight

No significant differences in cob weight between treatments were observed except the decrease as a response to the Teprosyn®/AnnGro™ treatment which was significantly lower than all other treatments including the untreated control (Figure 5.8).

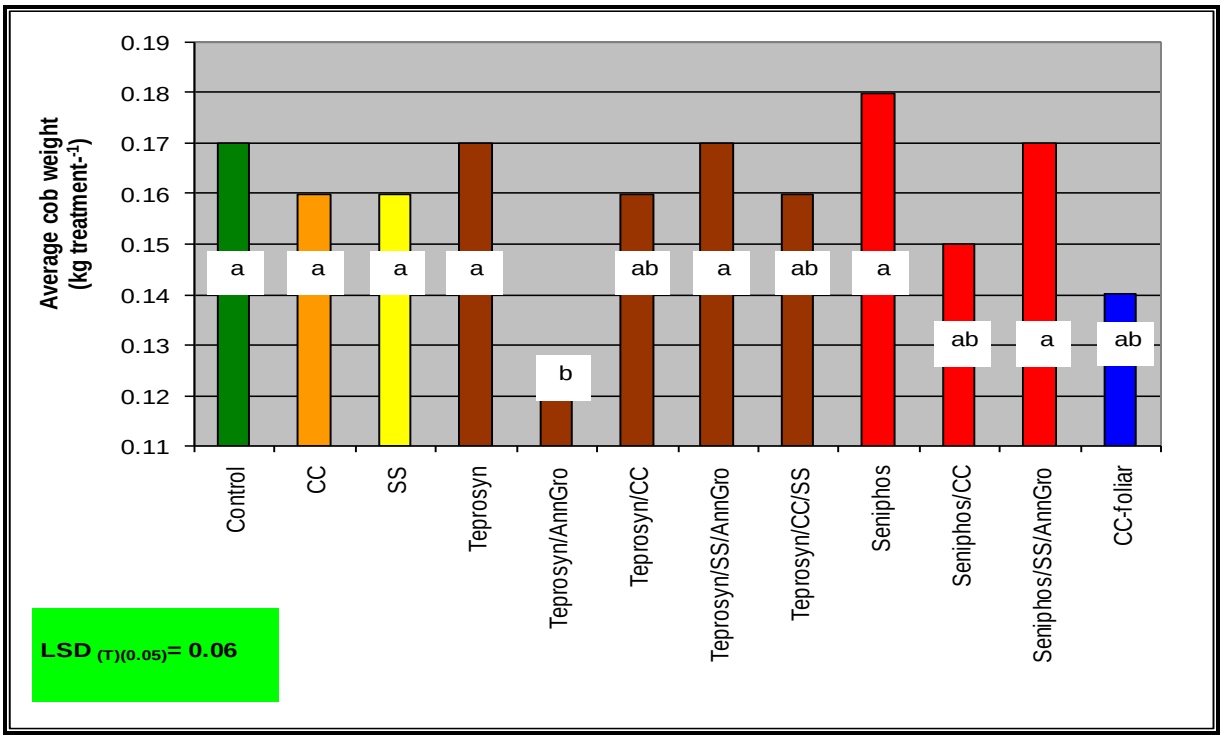

Figure 5.8: Average cob weight in response to different treatments of maize under rain fed field conditions. LSD (P<0.05) values are indicated in the graph and means differing significantly are indicated with different letters.

5.4.2.4 Cob length and percentage non-filled stalk ends

Cob length (Figure 5.9) followed more or less the same pattern as did cob weight (Figure 5.8) in terms of differences in responses to different treatments. The only

significant differences were observed between the Teprosyn®/AnnGro™ as well as the CC-foliar treatments as compared to all other treatments. Cob length in the case of the latter two treatments was well below that of the untreated control.

Similar insignificant differences between treatments were observed in terms of the percentage non-filled stalk ends as a response, except that the response of maize plants to the Teprosyn®/AnnGro™ seed treatment was significantly higher than that of all other treatments except for SS, Teprosyn®/CC and Seniphos®/CC (Figure 5.9).

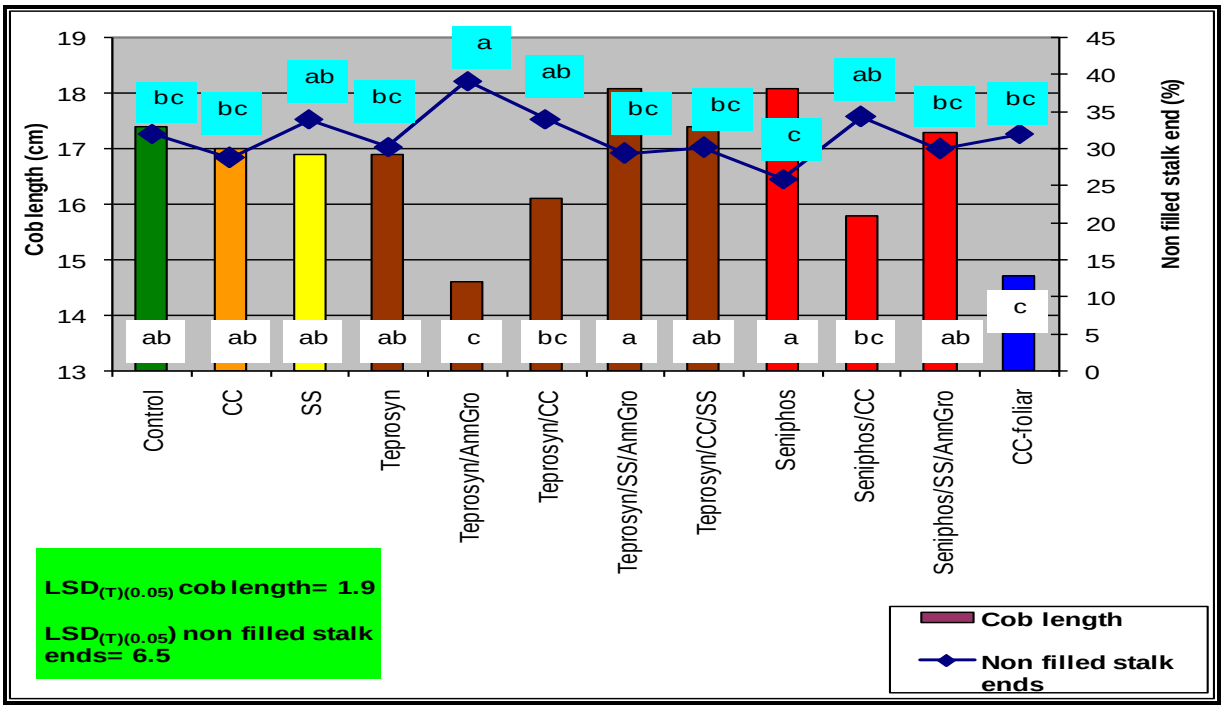

Figure 5.9: Cob length and percentage non-filled stalk ends of maize under rain fed field conditions. LSD (P<0.05) values are indicated in the graph and means differing significantly are indicated with different letters.

5.4.2.5 Number of kernels

Despite the fact that the lowest average number of kernels cob^{-1} was 355 (CC-treatment) and the highest 429 (Teprosyn®/CC/SS treatment) differences between all treatments, including the untreated control, were statistically non-significant (Figure.

5.10). This was probably due to large standard deviations between replications. Nevertheless, the response of maize to seed treatment with Teprosyn®, Teprosyn®/CC/SS and Seniphos®/CC showed a tendency to increase kernel number compared to the untreated control.

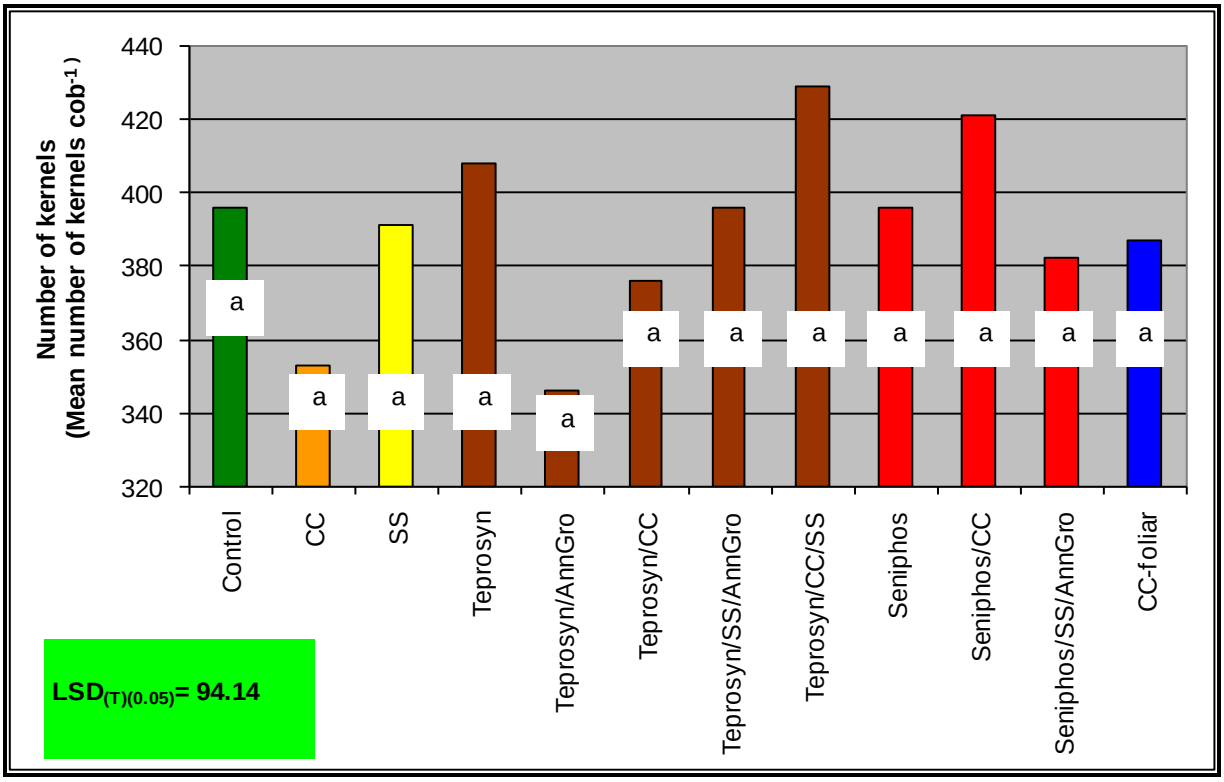

Figure 5.10: Number of kernels per cob in response to seed treatments and a foliar spray under rain fed field conditions. LSD (P<0.05) values are indicated in the graph and means differing significantly are indicated with different letters.

5.4.2.6 Total kernel weight

The total kernel weight per treatment (Figure 5.11) did not necessarily correspond with the number of kernels counted per cob. As a matter of fact, treatments that contributed to a higher number of kernels cob^{-1} (Teprosyn®, Teprosyn®/CC/SS and

Seniphos®/CC; Figure 5.10) did not show the same effect in terms of total kernel weight indicating possible differences in grain filling. However, despite non-significant differences between treatments, maize tended to respond to the CC, Teprosyn®/SS/AnnGro™, Seniphos® and Seniphos®/SS/AnnGro™ treatments in terms of an increase in total kernel weight, compared to the untreated control. Only the difference between the Teprosyn®/SS/AnnGro™ and the Teprosyn®/AnnGro™ as well as the CC-foliar treatment in terms of kernel weight was statistically significant.

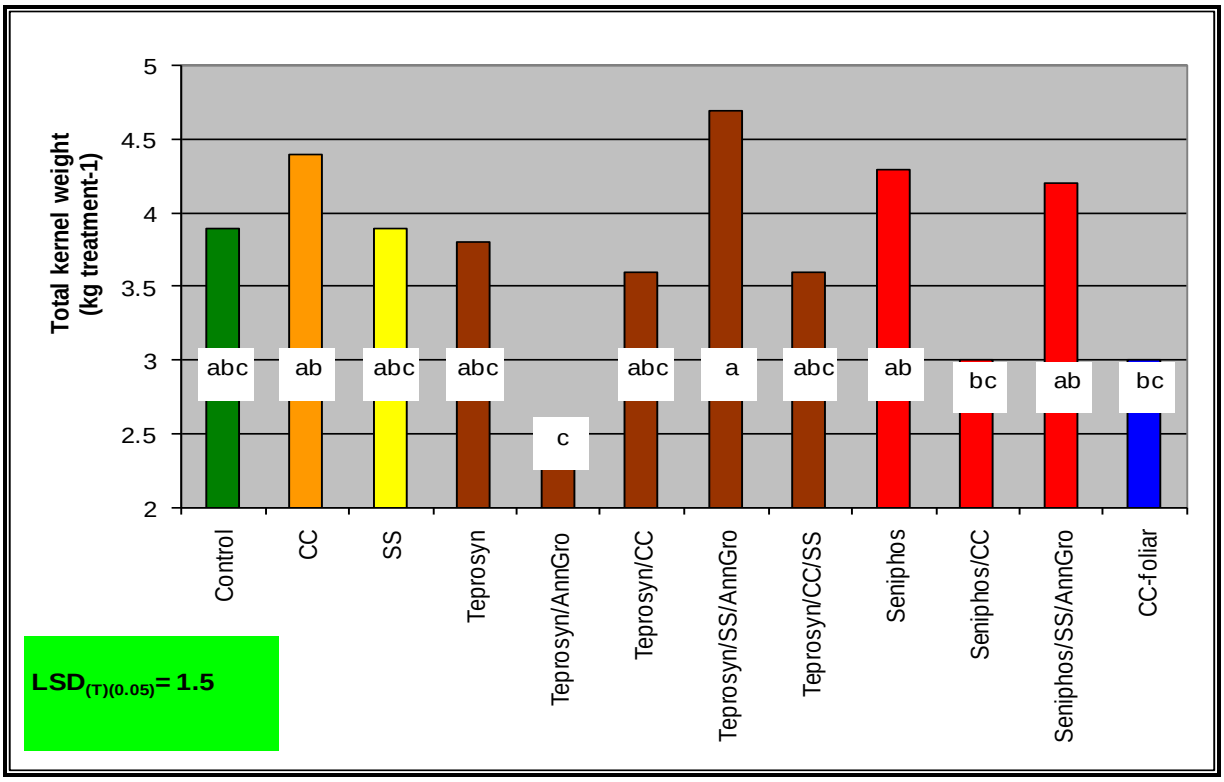

Figure 5.11: Influence of seed treatments on kernel weight of maize under rain fed field conditions. LSD (P<0.05) values are indicated in the graph and means differing significantly are indicated with different letters.

5.4.2.7 Total yield

As expected the calculated total yield (Figure 5.12), expressed in ton ha^{-1}, followed the same pattern as did the measured kernel weight (Figure 5.11) in terms of the

response of maize to seed treatment at planting. Although not statistically different from the untreated control, the four seed treatments that contributed to a marked yield increase were CC (+ 500 kg ha^{-1}), Teprosyn®/SS/AnnGro™ (+ 800 kg ha^{-1}), Seniphos® (+ 400 kg ha^{-1}) and Seniphos®/SS/AnnGro™ (+ 300 kg ha^{-1}). The only two treatments that decreased the final yield markedly compared to the untreated control as well as the former four treatments were the Teprosyn®/AnnGro™ and CC-foliar treatments. The Teprosyn®/CC, Teprosyn®/CC/SS and Seniphos®/CC combination treatments also tended to reduce the final yield of maize when applied as seed treatments at planting.

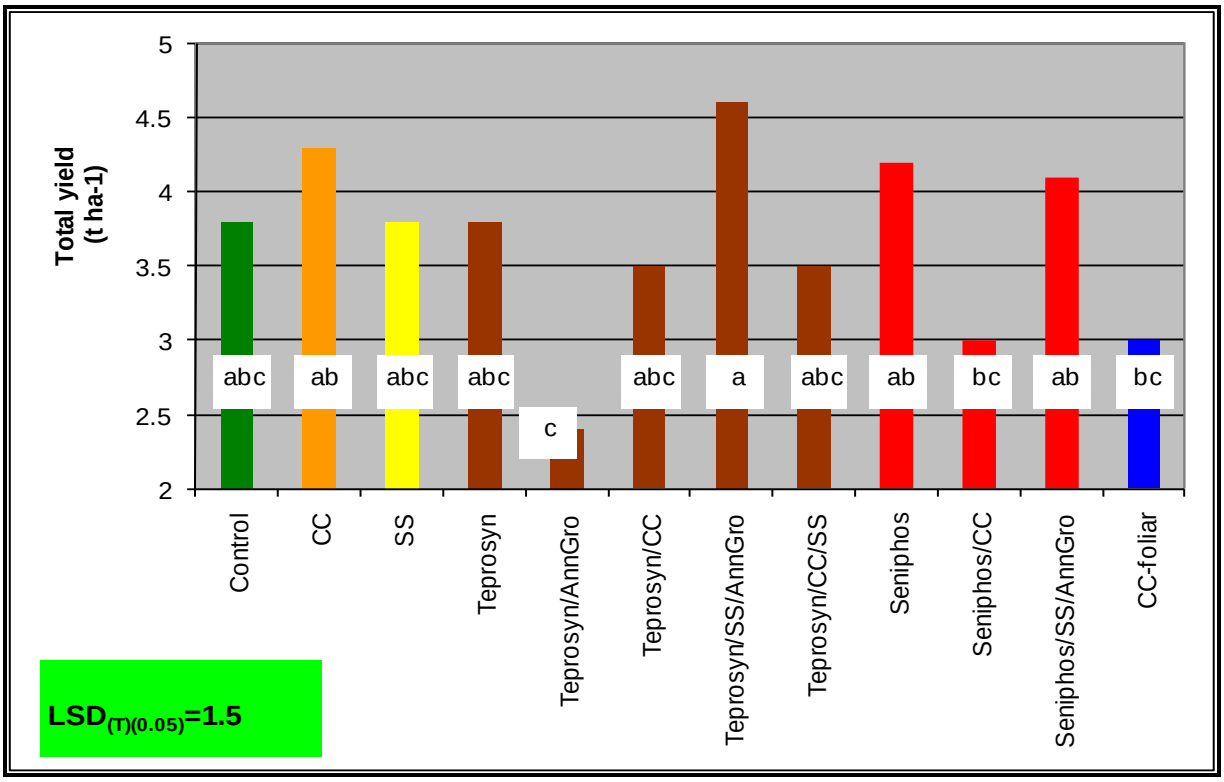

Figure 5.12: Influence of seed treatments on total grain yield of maize under rain fed field conditions. LSD (P<0.05) values are indicated in the graph and means differing significantly are indicated with different letters

5.5 DISCUSSION

Poor seedling establishment and the subsequent ultimate grain yield has become a topic of great concern to farmers and the world at large. Plants are exposed to different abiotic and biotic stresses hence resulting in either crop failure or poor ultimate grain yield and this is very common in grain crops such like maize. This further holds a major world threat due to food insecurity especially in the developing countries. As a result, seed treatment has been identified as one of the agricultural practices that hold the greatest potential in addressing poor seedling establishment and subsequent growth prior to final grain yield. This investigation was a follow up of the glasshouse trials with the objective to identify morphological and yield responses of maize to seed treatment under rain fed field conditions.

The field experiment was conducted during the 2008/2009 growing season under rain fed conditions. Morphological data parameters were collected from a week up to six weeks after planting, which was identified as the initial tasselling stage. These parameters included plant height, stem thickness, leaf area and biomass production in terms of fresh and dry mass for roots and leaves respectively. Subsequently, yield parameters were recorded at harvest.

The stimulatory effect of seed treatments on plant growth was observed as the plant growth stages advanced. Collectively, the CC, Teprosyn®, Teprosyn®/AnnGro™ and Teprosyn®/SS/AnnGro™ seed treatments at planting can be singled out as those that had the most pronounced effect on subsequent above soil plant length growth compared to the untreated control and the other treatments. Meanwhile, of all the above treatments, the CC seed treatment demonstrated the best consistent induced growth throughout the growth sample period. The stimulatory property of Teprosyn® either applied alone or in combination, could probably be attributed to an indirect positive effect of Zn on auxin synthesis and growth regulation as reported by findings by Orabi & Abdel-Aziz (1982). In addition to Teprosyn® applications, the CC effect was probably due to a synergistic effect between the active substance, brassinosteroids, and auxin known to regulate and induce cell enlargement, hence

promoting cell division and cell elongation (Nithila *et al.*, 2007). In addition to Teprosyn®, CC and Teprosyn®/AnnGro™, as it was in the case of plant height, Seniphos® seed applications had a similar increasing effect on stem thickness. Moreover, the contributions of these four treatments differed significantly only from that of the Seniphos®/CC treatment that clearly had an inhibitory effect on stem expansion.

As a response to the SS, Teprosyn®/CC/SS and CC foliar treatments the leaf area was significantly lower than that of the untreated control. A non significant increasing response as a result of treating maize seed at planting with CC, SS, Teprosyn®, Seniphos®, Seniphos®/CC/SS was observed up to three weeks after seedling emergence, most probably due to large standard deviations. However, only the Teprosyn® and Teprosyn®/CC/SS combination treatment had a significant enhancing effect on the leaf area of maize six weeks after emergence and the leaf area expansion response of maize to these two treatments also differed significantly from five of the other treatments viz. SS, Teprosyn®/AnnGro™, Seniphos®, Seniphos®/CC and CC foliar treatments. The collective roles of these treatments resulted into cell expansion hence an increase in plant growth.

Dry mass accumulation followed exactly the same pattern as did fresh mass production i.e. an increase in fresh mass production resulted in an increase in dry mass production, though there were some few exceptions where this pattern was not apparent. The Teprosyn®/CC treatment showed the most prominent stimulatory effect as far as fresh leaf mass is concerned. Although not significantly different, the same tendency applied for dry mass accumulation at this growth stage as all the treatments contributed to an increase in leaf dry mass. Interestingly, even thought there was an inhibitory effect on fresh leaf biomass by the CC-foliar treatment, the opposite applied for dry mass accumulation. This tendency for the dry mass to remain high even though the fresh mass seemed to decrease was also observed for the CC seed treatment. This result corroborated findings by Richardson (2007) who also observed that Teprosyn® Zn/P seed dressing of two maize cultivars, Dekalb 63-69 and Garst

8581, resulted in enhanced root development and top growth of seedlings. As a result, enhanced root and top growth development is suggested to have led to more efficient water and nutrient usage, hence preparing the crop for the coming season and enabling optimization of their yield potential at harvest. Similar results were also reported by Hunter (2001) who observed that pea seed treated with Teprosyn® Zn/P resulted in vigorous top growth of seedlings, compared to untreated control, and this accelerated growth was observed in adult plants throughout the growing season. The enhancement of leaf area by seed treatments contributed to delayed senescence of leaves.

Enhancement of yield components such as prolificacy, cob weight, and kernel weight by seed treatments correlated positively with the total grain yield. Treatments that promoted a high prolific character resulted in the production of high final total grain yield. These treatments, viz. CC, Teprosyn®/SS/AnnGro™, Seniphos® and Seniphos®/SS/AnnGro™, contributed to two (2) cobs per plant. However, the total grain yield did not vary significantly from the control. Surprisingly, in the case of Teprosyn®, a yield reduction was observed probably due to shorter cobs and lower kernel weight. This finding with maize was in contrast to that of Singh (2003) who observed a 30% yield increase on wheat as a result of seed coating with Teprosyn®.

Less prolificacy character suggests fewer cobs with greater grain weight (FAO, 1980; Anderson *et al*., 1984; Mkhabela *et al*., 2001 ;) probably due to less intra-competition during cob production and kernel formation. However, this was not the case in this experiment as less prolific treatments such as Teprosyn®/AnnGro™, Teprosyn®/CC/SS, Seniphos®/CC and CC-foliar resulted in poorly developed cobs with less grain weight, hence lower total grain weight. Prominent yield increase by the CC seed treatment agrees very well with Alam (2004) and Van der Watt (2005) who also recorded increased yield on wheat and maize respectively. Meanwhile it was reported that CC foliar spray had a high yield enhancing effect than the CC seed treatment on dry beans and wheat respectively (Molahlehi, 2000; Gerbremedhin, 2001; Alam, 2004 ;) but this result on maize did not support their findings. The CC

seed treatment (4.3 t ha^{-1}) had a marked yield enhancing effect on maize where the CC foliar application (3.8 t ha^{-1}) rather decreased the final yield.

The number of kernels per cob did not positively correlate to kernel weight. Though Seniphos®/CC and Teprosyn® seed treatments contributed to the highest number of kernels, the total kernel weight was lower. This might probably be due to smaller kernels, which were visually observed. Cob length and non-filled stalk ends also played a role in the final total grain yield. Statistically significant decreases of 8.2% (CC) and 6.8% (Seniphos®) and insignificant decreases for Teprosyn®/SS/AnnGro™ and Seniphos®/SS/AnnGro™ in terms of non filled stalk ends while higher total yields were still measured suggests that kernels with higher phyto mass was produced. To the contrary, a higher percentage (14.7 %) of non-filled stalk ends showed a total yield depression of 2.4 t ha^{-1} by Teprosyn®/AnnGro™. Interestingly, when Teprosyn® and SS were applied as seed treatments on their own their contribution to final yield did not differ from that of the untreated control and this differed from the findings of Kenty (2007) who reported that Zn/P seed treatment of cotton increased the final yield. However, a possible synergistic effect was observed when Teprosyn® and SS were applied in combination as this seed treatment resulted in the highest total grain yield of 4.6 t ha^{-1}. Further, applying Teprosyn® in combination with either AnnGro™ or CC had the opposite decreasing effect on the final grain yield of maize.

Although seed treatment with Seniphos® did not contribute to a significant increase in grain yield compared to the untreated control, the number of cobs was higher than that of the control and the cobs were also well developed in terms of length and non filled stalk ends. The same trend was observed when Seniphos® was applied in combination with SS and AnnGro™. The CC/Seniphos® combination treatment exhibited less of a prolific character and seemed to exhibit an antagonistic effect, leading to a reduction in cob and kernel weight thereby reducing the final total grain yield, though it had the highest plant population. An increase in final tomato yield of two cultivars H9478 and H9997 was reported by Paliyath and Bruulsema (2003).

In terms of plant growth and total yield no clear correlation or pattern emerged and the association was rather erratic. However, ComCat® (CC) stood out in terms of a positive correlation between vegetative growth and yield. Especially the correlation between plant height and final yield was shown by Jamali and Ali (2008) to be positive in terms of spike length, number of grains per spike and final grain yield. Positive association of plant height with grain yield within the major dwarfing gene group was reported by Law *et al.* (1978) as well as Aycicek and Yildirim (2006). The results obtained in this study support the above as taller plants for the Teprosyn®/SS/AnnGro™ and CC treatments correlated with increased total grain yield and yield components. However, contradicting results were observed for Teprosyn®/AnnGro™ and Teprosyn® where increased plant height correlated negatively with total grain yield. This is difficult to explain but Sangoi and Salvador (1997) reasoned that taller plants were the result of vigorous top growth that in turn resulted in an increase of energy usage for growth leading to poor yields. In other words, more assimilates were used for plant growth than for grain filling.

Contrary to the positive correlation between plant height and total grain yield, the shortest plants measured for the Seniphos® and Seniphos®/SS/AnnGro™ combination treatment lead to increases in total yield, compared to the untreated control, albeit it non significantly. Similar contrasting results were reported by Villareal *et al.,* (1992) wherein plant height had a strong negative correlation with grain yield in the single-gene dwarfing group.

In addition to plant height, a positive correlation existed between dry matter accumulation in leaves as well as roots and the final maize yield. This was observed for the Seniphos®, Teprosyn®/SS/AnnGro™, CC and Seniphos®/SS/AnnGro™ treatments. Dry matter accumulation largely depends on the optimal production of photosynthate throughout a growing season and this also correlates with leaf area (Dijak *et al*., 1999; Subedi & Ma, 2005). Leaves serve as source of photosynthate that is ultimately translocated to kernels during grain filling. In this study especially the CC and Teprosyn®/SS/AnnGro™ seed treatments contributed to the highest

increase in final yield but this did not clearly correlate positively with leaf area. However, the higher total grain yield measured for these two treatments compared to the untreated control and other treatments suggests that greater amounts of assimilates (carbohydrates) were probably translocated to the sink (kernels) from the source (leaves). This aspect has been confirmed by Schnabl *et al.* (2001) for ComCat® (CC) who demonstrated increased translocation of sucrose in plants following foliar application of the commercial bio-stimulant.

A negative correlation between leaf area and total grain yield was also observed for the Teprosyn®/CC and Teprosyn®/CC/SS combination seed treatments where increased leaf area resulted in decreased total grain yield. A possible explanation is that a sink limiting situation due to a decreased kernel (sink) number relative to leaf area could have prevailed. Further, kernel arbotion in maize has been linked to a shortage of current assimilate supply to kernels (Lizaso *et al.*, 2003). However, due to the fact that the kernel number measured was lowest for the CC and Teprosyn®/SS/AnnGro™ treatments while these two treatments contributed to the highest increase in final maize yield, possible kernel abortion under the influence of some of the treatments tested is ruled out.

In conclusion, results from this field trial showed that four of the seed treatments, viz. ComCat® (CC), Teprosyn®/SS/AnnGro™, Seniphos® and Seniphos®/SS/AnnGro™ contributed to yield increases in maize, albeit not significant at the 5% probability level. Of these the Teprosyn®/SS/AnnGro™ seed treatment (+800 kg ha^{-1}) contributed to the most marked yield increase followed by CC (+500 kg ha^{-1}), Seniphos® (+400 kg ha^{-1}) and Seniphos®/SS/AnnGro™ (+300 kg ha^{-1}). Although not statistically significant, the yield increase is still substantial from an economic point of view in all cases. Further field experiments over at least two consecutive seasons is needed to supplement this result in order to come to a concrete conclusion.

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CHAPTER 6

GENERAL DISCUSSION

Food security is an issue of great concern in many countries, but particularly in Africa, and especially with regard to the production of cereal staple crops (UNEP-UNCTAD, 2008). Food insecurity is exacerbated, inter alia, by poor soil fertility. Although inorganic soil fertilization still remains the principle cultivation practice to ensure sustainable yields, high fertilizer prices has contributed to food insecurity due to poor financial resources by African farmers (Lekgari & Setimela, 2001). From this perspective, it is critical for scientists to develop alternative, but sustainable, farming practices with the aim to improve crop yields. These practices should preferably adhere to minimum criteria such as low input costs, accessibility to all farmers irrespective of their socio-economic status, sustainability and must show above average potential to contribute to food security by increasing crop yields substantially. Despite crop improvement by breeding, there is still a growing need to seek alternative measures to complement varietal resistance. The latter supplied the rationale for this study. Seed treatment of maize with a range of either organic or inorganic substances and/or existing products offered an opportunity to investigate the potential for this rather simple method to not only promote seedling establishment but hopefully also the final yield.

Germination and seedling establishment are probably the most critical events in the life cycle of crops when all other factors are optimal (Kaya *et al*., 2006). This involves complex metabolic and physiological processes that starts at seed germination and continues in a plant capable of completing a normal life cycle while the interaction between either the seed or the seedling and the environment determines the outcome in terms of seedling establishment (Allen & Meyer, 1998). Poor or reduced seed germination and crop establishment has currently become a topic of great concern and poor establishment can result from the exposure of the crops to different environmental stresses (Kerr & Hammermeister, 2007). Seedling establishment is therefore one of the major challenges of crop production in the semi-arid regions and

its importance has to be recognized by both farmers and researchers alike (Murungu *et al*., 2004).

Seed treatment has been identified as one of the production alternatives to address the problem of poor seed germination, seedling establishment as well as ultimate grain yield in many crops (Khajeh-Hossein *et al*., 2003; Kaya *et al*., 2006). In recent years the trend in agriculture towards greater sustainability as well as public concern over the use of hazardous material associated with the use of synthetic chemicals has increased (Jacobsen & Backman, 1993). Subsequently, the use of small quantities of either organic or inorganic products for treating seeds is believed to help in reducing or combating this problem.

The current study focused on the manipulation of maize seeds via treatments with a range of substances or commercial products with the objective of improving seedling establishment and, ultimately, final yield. The study comprised of three phases: **1)** screening of a product range under laboratory and glasshouse conditions in terms of seedling growth with the objective of identifying the best performing ones for further investigation (chapter 3), **2)** quantification of key physiological events in seedlings under controlled glasshouse conditions as a response to selected treatments in an attempt to partially understand the underlying mechanism(s) involved (chapter 4) and **3)** following the growth and yield response of maize to seed treatments under field conditions over one season (chapter 5).

From the screening phase and in terms of seedling growth under laboratory conditions, an optimal concentration of 25 mg kg^{-1} seed for ComCat® (CC) and 12.5 mg kg^{-1} seed for a *Lupinus albus* seed suspension (SS) was identified. At these optimal concentrations both CC and SS dramatically out-performed both the untreated control and even the 0.5 mg L^{-1} for CC and 5 mg L^{-1} for SS which were identified as optimal foliar applications by Van der Watt (2005). As a result these optimal concentrations for CC and SS seed treatments were applied in follow-up investigations under glasshouse and field conditions. Organic acids including humic acid, vulvic acid and an amino acid mix surprisingly inhibited seedling growth and were therefore excluded from further investigation. Likewise, the two biological

control agents Eco-flora® and Eco-fungi® decreased coleoptile and root growth of maize seedlings under laboratory screening conditions and were also discarded.

Two inorganic products, Seniphos® and Teprosyn®, were tested individually as well as in combination with the two plant growth regulators CC (at 12.5 mg kg^{-1} seed) and SS (at 25 mg kg^{-1} seed) as well as the uptake enhancer AnnGro™. The treatments that showed particular promise initially in terms of seedling growth manipulation under laboratory conditions were Seniphos® and Teprosyn® on their own as well as Seniphos®/CC, Seniphos®/SS/AnnGro™, Teprosyn®/CC/AnnGro™ and Teprosyn®/SS/AnnGro™ combination treatments. These were all included in the follow-up metabolic response investigation (chapter 4) and the field trial (chapter 5). A general discussion on the findings concerning the latter two aspects follows.

Seed germination, seedling establishment and the subsequent growth stages of plants involve different metabolic processes that are interrelated. For instance, according to Hansen *et al. (*1994), existing literature reports on many positive and a few negative correlations between plant growth rates and respiration rates. The latter suggests that manipulation of plant respiration should be a particularly fruitful area of investigation concerning the development of ways and means to increase or control plant growth rates and crop productivity. Rapid growth requires a rapid respiration rate while growth per unit respiration is the greatest when respiration is efficient (Looms & Amthor, 1999). Given this background, the metabolic response of maize seedlings from seeds pre-treated with a product range mainly included respiratory aspects such as respiratory substrate levels, respiration rate and *in vitro* activities of key regulatory enzymes under controlled glasshouse conditions. The point of departure was the postulate that the potential exists to manipulate certain respiratory metabolic events underlying the early seedling energy status and growth via seed treatments.

With regard to SS, the enhancement of seedling growth via seed treatment was previously reported by Van der Watt (2005) on different crops and confirmed in this study. Van der Watt (2005) showed that SS induced dihydroepijasmonic acid (DH-JA), jasmonic acid (JA) and dihydro-12-oxo-phytodienoic acid as intermediary steps

in the Jasmonic acid pathway, possibly leading to the promotion of root and above soil part growth under stress conditions. Moreover, the active compound of SS namely the triglyceride linoleic acid was shown to play a central role in manipulating respiratory metabolic events and seedling growth (Van der Watt, 2005). However, in this study the SS seed treatment did not show a consistent positive effect in terms of respiratory parameters and had no effect on the final maize yield.

With regard to ComCat® (CC), the active brassinosteroid (BR) compounds have been shown to affect many metabolic and growth aspects in crops (Morinaka *et al*., 2006) while, more than a decade ago. Vardhini and Rao (1998) reported on the synergistic effect between BR's and auxin in improving seedling growth in groundnuts. The authors suggested that both compounds regulate and induce cell growth in terms of cell division and cell elongation in a synergistic fashion and this was confirmed by Nithila *et al.* (2007). The results obtained with CC in this study, concurred with the latter findings in terms of seedling growth and the CC seed treatment also contributed to a 500 kg ha^{-1} yield increase under field conditions.

In this study the metabolic response of maize seedlings to seed treatments with CC and SS was rather confusing. For example both contributed to similar marked increases in soluble leaf protein, marked decreases in soluble sugars concomitant with substantial Gluc-6-PDH and CO_2 release rates while the collective effect of these respiratory aspects lead to an increase in final yield only in the case of CC. Hence, CC and SS were included in combination treatments together with fertilizer products Seniphos® and Teprosyn®, separately or together with the uptake enhancer AnnGro™.

The Teprosyn®/SS/AnnGro™ combination seed treatment had the most pronounced effect on early seedling growth and ultimately had the most pronounced yield improving effect (+800 kg ha^{-1}). It was also the treatment that consistently had a positive effect on leaf soluble protein and root sugar levels, but a decreasing effect on respiration rate both in terms of CO_2 release and O_2 consumption. Because of the fact that only the CC and Teprosyn®/SS/AnnGro™ treatments contributed to marked increases in the final maize yield, the following discussion will concentrate on these

two treatments only unless interesting contradictory results from other treatments, that might substantiate a specific point, is deemed necessary.

Previous studies confirmed that dry matter and grain yield are interrelated (Dong *et al*., 1993) and that dry matter is a result of accumulated daily carbon gains from photosynthesis throughout the growing season that is ultimately translocated to the kernels (Lee & Tollenaar, 2007). According to Richards (2000) above soil part dry biomass of crops is positively correlated with grain yield. This was confirmed by Moll *et al*. (1994) as well as Rajcan and Tollenaar (1999*a* & *b*) who attributed the correlation to delayed leaf senescence which in turn suggests a longer duration of photosynthesis, continued nitrogen uptake as well as increased kernel numbers. Increased dry matter accumulation by the two seed treatments that had the most marked effect on kernel yield, viz. CC and the Teprosyn®/SS/AnnGro™ combination treatment was, however, not significant in either roots or above soil parts and could therefore not be regarded as the single most important factor underlying the effect on final yield.

Most of the reports found in the literature concerning the way soluble protein content in crop plants should be interpreted deals with its role under stress conditions (Subramanian & Charest, 1995; Gulin *et al*., 2003; Mohammadkhani & Heidari, 2008). Seeing that the field trial conducted in this study was under rain fed conditions and some drought stress could have been experienced during the growing season, it might be worthwhile to ponder on this aspect briefly. Low and poor rainfall distribution (220 mm) was obserevd for the entire growing season. It has been shown in the past that increases in total protein levels in vegetative plant tissue is associated with the expression of specific genes and the synthesis of stress proteins (Mohammadkhani & Heidari, 2008). Interestingly, both CC and the Teprosyn®/SS/AnnGro™ seed treatments highligthed previously as the most promising in terms of its contribution to final yield, only slightly and insignificantly increased soluble protein levels in leaves. Although this at least correlated positively with the observed yield increases under the influence of these two treatments, once again soluble protein content could not be singled out as a particularly important underlying factor.

Soluble sugars are not only a primary respiratory substrate that provides the energy for growth and development during a crop's vegetative growth phase, but are eventually translocated to the harvestable parts during the productive phase (Amthor, 1989). In terms of the two highlighted seed treatments that contributed to substantial yield increases, viz. CC and the Teprosyn®/SS/AnnGro™ combination treatment, no clear picture emerged in this regard at the early growth stage of seedlings where sugar content was measured. Treatment of maize seeds with CC actually decreased all the different sugar forms in leaves and roots of seedlings at this growth stage. A possible relationship between low sugar content and increased respiration as well as seedling growth at this development stage might explain the low sugar content under the influence of this treatment. However, where seeds were treated with Teprosyn®/SS/AnnGro™ an opposite picture emerged. This treatment increased sucrose and fructose levels in roots while the respiartion rate was depressed. Because of the opposing state of affairs no clear deduction was possible at this seedling growth stage. It is suggested that sugar formation under the influence of these two treatments be followed over the total vegetative growth stage in a future investigation.

Sugar content in plant tissue is closely related to respiration rate and *in vitro* activities of regulatory enzymes involved in primary respiratory events supply a strong indication of the *in vivo* utilization of respiratory substrate (Rakhmankulova *et al.,* 2001). Especially the CC seed treatment had a marked enhancing effect on Gluc-6PDH activity, indicating a strong carbon flux through the OPP-pathway. But, CC also significantly enhanced PFK activity in maize seedlings suggesting that the glycolysis cycle was similarly active which could explain the low sugar levels measured in seedlings treated with CC. The enhanced activities of both these enzymes under the influence of CC correlated positively with an increase in kernel yield. However, in the case of the Teprosyn®/SS/AnnGro™ seed treatment and at the seedling growth stage that these aspects were measured, no clear picture emerged. A slight enhancement of Gluc-6-PDH activity was observed while this treatment had no effect on PFK activity. The opposing picture that emerged between the two treatments in terms of the metabolic seedling response, while they were the only ones to enhance the final

yield, indicates that measurement of the metabolic response at this early growth stage was not sufficient to come to a foregone conclusion. It is suggested that this aspect be investigated over a total growing season in order to follow the metabolic events more closely up to the drying cycle.

- In conclusion, from all the seed treatments tested in this study ComCat® (CC) on its own and the Teprosyn®/SS/AnnGro™ combination treatment showed the most promising potential to not only improve maize seedling growth during the early seedling establishment phase, but also to increase the final yield. Although the metabolic response measured at this early growth stage of maize could not shed much light on the underlying mechanisms involved in the final yield response, it must be accepted that the collective growth and metabolic effects of these two treatments eventually culminated in yield improvement. From a mechanistic point of view it became clear from this study that metabolic events involved in the mechanisms of action of these two products can only be elucidated if an entire study is devoted to this aspect only.

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SUMMARY

Increasing crop yields for an ever growing world population has currently become a topic of great importance for agronomists and plant physiologists alike. The main objective is to find the cheapest and most effective methods to obtain this goal. In this regard seed treatment is one of the approaches that offer great potential. With this in mind, the underlying study was undertaken in order to test a range of products including prototypes and commercially available products. During the laboratory and glasshouse screening phases optimal concentrations for the plant growth regulators ComCat® (25 mg kg^{-1} seed) and SS (12.5 mg kg^{-1} seed) were identified in terms of seedling growth response. Additionally, the two fertilizer products Teprosyn® and Seniphos® initially showed promise in this regard when applied on their own or in combination with the plant growth regulators and the uptake enhancer AnnGro™ . However, of all the seed treatments and purely based on the eventual marked yield increase obtained, ComCat® (+500 kg ha^{-1}) on its own and Teprosyn® in combination with SS and AnnGro™ (+800 kg ha^{-1}) proved to be the most promising. Although the metabolic response of maize seedlings to the different seed treatments were followed in terms of selected events, no clear picture emerged in terms of the mechanisms of action underlying the eventual yield increase. It became clear that this aspect needed special attention in a follow-up study over a total growing season. From this study it is recommended that ComCat® on its own as well as the Teprosyn®/SS/AnnGro™ combination treatment can be considered strongly as seed treatments of maize under rain fed conditions, but this should be followed over more seasons. This recommendation is purely based on the consistent enhancing effect that both treatments revealed in terms of seedling growth and final yield. The potential of these treatments should also be evaluated under irrigation conditions.

Keywords: seed treatments, maize, growth regulators, fertilizer products metabolic response, seedling growth, yield.

OPSOMMING

Die verhoging Van oesopbrengs in ‘n verskeidenheid Van gewasse gemeet teen die agtergrond Van ‘n ewig groeiende wêreldbevolking is huidig ‘n belangrike onderwerp onder beide agronome en plantfisioloë. Die hoofdoel is om die goedkoopste maar doeltreffendste metodes te vind ten einde hierdie doel te bereik. In hierdie verband is saadbehandeling een Van die benaderings wat groot potensiaal toon. Met laasgenoemde in gedagte is hierdie studie onderneem en met die hoofdoel om ‘n reeks produkte, insluitende prototipes en kommersieël beskikbare produkte, in hierdie verband te toets. Gedurende die aanvanklike laboratorium en glashuis siftingstoetse is optimum konsentrasies vir die plantgroeireguleerders ComCat® (25 mg kg^{-1} saad) en SS (12.5 mg kg^{-1} saad) in terme van saailing groeirespons geïdentifiseer. Addisioneel het die twee bemestingsprodukte Teprosyn® en Seniphos® aanvanklik potensiaal in hierdie verband getoon toe hulle op hulle eie of in kombinasie met die plantgroeireguleerders en die opname verhoger AnnGro™ as saadbehandelings toegedien is. Maar van al die saad behandelings, en suiwer gebaseer op die finale oesopbrengsverhoging wat verkry is, het ComCat® (+500 kg ha^{-1}) op sy eie en Teprosyn® in kombinasie met SS en AnnGro™ (+800 kg ha^{-1}) die hoogste potensiaal getoon. Alhoewel die metaboliese respons van mieliesaailinge op die verskillende saadbehandelings in terme van geselekteerde momente gevolg is, kon geen gevolgtrekking gemaak word met betrekking tot die aksiemeganismes wat die uiteindelike verhoging in oesopbrengs voorafgegaan het nie. Dit het duidelik geword dat hierdie aspek spesiale aandag moet geniet in ’n opvolgstudie en oor ’n volle groeiseisoen. Uit die resultate wat met hierdie studie bekom is word aanbeveel dat ComCat® op sy eie sowel as die Teprosyn®/SS/AnnGro™ kombinasie behandeling sterk oorweeg word as saadbehandelings Van mielies onder droëland toestande oorweeg word. Hierdie aanbeveling is suiwer gebaseer op die konsekwente verhogingseffek wat beide behandelings op saailinggroei en oesopbrengs getoon het. Die potensiaal van hierdie behandelings onder besproeiingstoestande behoort ook geëvalueer te word.

Sleutelwoorde: saadbehandelings, mielies, groeireguleerders, bemestings-produkte, metaboliese respons, saailinggroei, oesopbrengs.