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Seedling Growth of Maize (*Zea mays L.*) in Response to Seed Treatments

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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), AnnGro™ and a prototype seed suspension (SS) from *Lupinus albus*), fertilizer products (Teprosyn®Zn/P, and Seniphos®) after seed treatments were investigated under laboratory and glasshouse conditions. Untreated seed was used as negative control. ComCat® at 25 mg kg^{-1} and SS at 12.5 mg kg^{-1} seed optimally stimulated 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 : Fertilizer products, maize, plant growth regulators, seed treatments, seedling growth.

INTRODUCTION

Maize (*Zea mays L.*) is the most important grain crop grown in South Africa. It is one of the most important crops in the milder subtropical and tropical regions of the world (Fertilizer Society of South Africa, 2007). It is grown under diverse environmental conditions compared to other important grain crops such as wheat and rice (Du Plessis 2003; Fertilizer Society of South Africa, 2007). 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.

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). 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. 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).

Apart from poor or reduced seed germination and crop establishment concern, more than a decade ago, Jacobsen and 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: In this study the seedling growth response of a hybrid maize cultivar (DKC78-15Bt) was followed after treating seeds with a number of products, including plant growth regulators and fertilizer products 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.

MATERIALS AND METHODS

Certified seed of a hybrid maize cultivar (DKC78-15Bt) were commercially obtained from Monsanto, South Africa. 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. The chemicals were tested separately and in concentration combination under both the laboratory and glasshouse conditions during 2007/2008.

Seed treatments

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*) 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.

Laboratory growth medium experiment

Two sheets of special germination paper (30 × 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°C in a growing chamber for 96h in the dark. Coleoptile and root lengths were measured after 96h of incubation using a digital caliper. All treatments were replicated three times.

Glasshouse seedling tray experiment

Pre-treated seeds (2 per hole) were planted in a growth medium in seedling trays and kept at field capacity in a glasshouse. The glasshouse was set at ±26 ºC. 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.

Analysis of variance (ANOVA) was performed on the data, using the SAS statistical program to identify differences between treatments. Tukey 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 tables both as calculated LSD values and by using different letters.

RESULTS AND DISCUSSIONS

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 2 categories namely plant growth regulators 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. 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. Active substances are obtained from natural donor plants whose genetic potential has not been influenced by artificial breeding or genetic engineering. SS is a prototype seed suspension (SS) prepared by grinding *Lupinus albus* L. seeds to a fine powder and extracting the contents. SS has demonstrated to to induce root development in seedlings of selected agricultural crops, as well as to improve the yield of these crops (Van der Watt, 2005).

Treating maize seeds with different ComCat® (CC) 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 (Table 1). Although not significantly different from the control probably due to large standard deviations between replications, the 25 mg kg^{-1} application emerged as the optimum concentration. It contributed to an 18% increase of root length growth in the laboratory, compared to the untreated control. 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 and this was in concert with the claims made by the manufacturers (Agraforum, 2006). The same tendency prevailed for maize seedling root growth under glasshouse conditions (Table 1) as it was for the laboratory conditions from seeds pre-treated with CC in a concentration range. 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. 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 to the findings by Van der Watt (2005), both below and above growth was significantly inhibited by 12, 5 mg kg^{-1} CC compared to untreated control. The author maintained that 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. 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 synergystic 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.

Table 1: Root and coleoptile growth growth as well as below and above ground fresh mass in reponse to seed treatment with Comcat® in a concentration range under laboratory and glasshouse conditions.

CC Seed treatment concentration	*Laboratory root length*	*Coleoptile length*
	(mm)	
Treatment	NS	NS
Control	53.5	18.8
0.5 mg L^{-1}	53.5	18.2
12.5 kg ha^{-1}	56.1	19.0
25 kg ha^{-1}	63.2	18.2
50 kg ha^{-1}	62.3	20.8
100 kg ha^{-1}	58.7	17.1
200 kg ha^{-1}	61.4	17.0
CV (%)	17.3	12.6
STDev	8.9	2.2
$LSD_{(T)\ (0.05)}$	18.0	4.1
CC Seed treatment concentration	*Glasshouse Below ground fresh mass*	*Above ground fresh mass*
	(g)	
Treatments	*	*
Control	2.8^{ab}	2.6^{bc}
12.5 kg ha^{-1}	1.8^{b}	1.5^{d}
25 kg ha^{-1}	3.4^{a}	3.0^{ab}
50 kg ha^{-1}	3.3^{a}	3.2^{a}
100 kg ha^{-1}	2.7^{ab}	2.3^{c}
200 kg ha^{-1}	2.0^{b}	1.6^{d}
CV (%)	14	10.0
STDev	0.7	0.7
$LSD_{(T)\ (0.05)}$	0.7	0.4

Means within the same column followed by the same letter(s) are not significantly different at ($p < 0.05$), * significantly different, NS non significantly different

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 (Table 2). This simulatory effect was probably due to symbiotic biological nitrogen fixation ability of lupinus and hence the crucial economical role it plays in maintaining adequate nitrogen resources in the plant world (Allen & Allen, 1981). The result was in agreement with the findings of Van der Watt (2005), although the author tested the effect of SS on cabbage and lettuce seeds respectively. 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. 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} concentration significantly inhibited growth. 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.

Table 2: Root and coleoptile growth growth as well as below and above ground fresh mass of maize in reponse to seed treatment with a prototype seed suspension (SS) in a concentration range under laboratory and glasshouse conditions.

SS Seed treatment concentration	*Laboratory* root length	*Coleoptile length*
	(mm)	
Treatment	NS	*
Control	53.5	18.8
5.0 mg L^{-1}	57.4	20.8
12.5 kg ha^{-1}	64.3	18.1
25 kg ha^{-1}	58.3	18.6
50 kg ha^{-1}	59.9	17.4
100 kg ha^{-1}	52.2	14.0
200 kg ha^{-1}	49.5	12.8
CV (%)	19.3	16.5
STDev	9.9	3.6
$LSD_{(T)\ (0.05)}$	19.4	5.0

SS Seed treatment concentration	*Glasshouse* Below ground fresh mass	*Above ground fresh mass*
	(g)	
Treatments	*	*
Control	2.8a	2.6a
12.5 kg ha^{-1}	64.3	2.5
25 kg ha^{-1}	58.3	1.3bc
50 kg ha^{-1}	59.9	1.1c
100 kg ha^{-1}	52.2	1.7a
200 kg ha^{-1}	49.5	2.9a
CV (%)	19.3	7.9
STDev	0.7	0.6
$LSD_{(T)\ (0.05)}$	0.5	0.3

Means within the same column followed by the same letter(s) are not significantly different at ($p < 0.05$), *significantly different, NS non significantly different.

Growth response of maize to fertilizer products in combination with plant growth regulators

Two commercial fertilizer products, namely Seniphos® and Teprosyn®, were tested as seed additives on their own or in combination with PGR's for 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. 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). 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.

The maize growth response after seeds were pre-treated with single products viz; AnnGro™, Seniphos® and Teprosyn® demonstrated the most enhanced root and coleoptiles growth by Teprosyn® although improved growth was not significantly different from the control (Table 3). 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 above ground growth compared to untreated control. 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 96h incubation period with respect to the control. This was in accordance with the findings of Richardson (2007) who maintained that it 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 (Fig. 3.0), Seniphos® in combination with CC increased both root and coleoptile growth from both the control and other treatments. 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 response tendency 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 significant from the control.

Table 3: Root and coleoptile growth growth as well as below and above ground fresh mass of maize in reponse to seed treatment with inorganic fertilizer products, both separately and in a combination with a plant growth regulator and an uptake enhancer under laboratory and glasshouse conditions.

SS Seed treatment	*Laboratory*	
	root length	*Coleoptile length*
	(mm)	
Treatment	*	*
Control	75.1^{ab}	23.6^{abc}
AnnGro™	65.8^{bc}	20.3^{cde}
Seniphos®	76.9^{ab}	23.2^{abc}3.5^{a}
Seniphos®/CC	88.1^{a}	27.5^{a} 3.7^{a}
Seniphos®/SS	75.8^{ab}	23.5^{abc}
Seniphos®/AnnGro™	50.7^{c}	16.3^{a}
Teprosyn®	87.8^{a}	26.1^{ab}
Teprosyn®/CC	76.8^{ab}	21.8b^{cd}
Teprosyn®/SS	63.5^{bc}	17.5^{ab}
Teprosyn®/AnnGro™	79.1^{ab}	21.6^{bcd}
CV (%)	12.8	17.0
STDev	14.8	4.0
$LSD_{(T)(0.05)}$	21.6	4.9

SS Seed treatment	*Glasshouse*	
	Below ground fresh mass	*Above ground fresh mass*
	(g)	
Treatment	*	*
Control	3.2^{ab}	2.2^{d}
AnnGro™	3.5^{a}	3.6^{a}
Seniphos®	3.5^{a}	3.3^{abc}
Seniphos®/CC	3.7^{a}	3.1^{c}
Seniphos®/SS	2.7^{b}	2.3^{d}
Seniphos®/ AnnGro™	1.9^{c}	1.8^{a}
Teprosyn®	2.7^{b}	1.8^{a}
Teprosyn®/CC	3.4^{ab}	3.0^{c}
Teprosyn®/SS	3.2^{ab}	3.5^{ab}
Teprosyn®/ AnnGro™	3.8^{a}	3.2^{bc}
CV (%)	12.9	7.2
STDev	0.7	0.7
$LSD_{(T)(0.05)}$	0.7	0.3

Contd.

Means within the same column followed by the same letter(s) are not significantly different at ($p < 0.05$), * significantly different, NS non significantly different

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 and glasshouse conditions. In the laboratory the combination treatments containing Seniphos® and both CC and SS together with AnnGro™ had a significant inhibiting effect on root while the inhibitory effect was not significant for coleoptile growth compared to the control. Treatment of seed with Teprosyn® in combination with SS and AnnGro™ increased both root and coleoptile growth significantly compared to the control. Where SS was replaced with CC in the combination of Teprosyn® and AnnGro™ root growth was also increased, although not significantly, while coleoptiles growth was inhibited though non significantly compared to the control. Under glasshouse conditions, three weeks after planting, 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.

Interestingly, when Seniphos® was applied as a seed treatment in combination with either CC or 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 compared to the control and other treatments. This strongly indicates that the AnnGro™ might have contributed to an 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.

Table 4: Root and coleoptile growth growth as well as below and above ground fresh mass of maize in reponse to seed treatment with extended combinations of inorganic fertilizer products, plant growth regulator and an uptake enhancer under laboratory and glasshouse conditions.

SS seed treatment	*Laboratory*		*Glasshouse*	
	Root length	*Coleoptiles lentgh*	*Below ground fresh mass*	*Above ground fresh mass*
	(*mm*)		(*g*)	
Treatment	*	*	*	*
Control	64.5^{bc}	18.8^{ab}	3.2^{b}	2.2^{c}
Seniphos®/CC/AnnGro™	56.8^{c}	15.5^{b}	3.9^{a}	3.5^{a}
Seniphos®/SS/AnnGro™	62.9^{bc}	17.3^{ab}	4.1^{a}	3.7^{a}
Teprosyn®/CC/AnnGro™	74.8^{ab}	18.5^{ab}	2.9^{b}	2.3^{c}
Teprosyn®/SS/ AnnGro™	80.6^{a}	21.1^{a}	3.2^{b}	3.0^{b}
CV (%)	9.5	12.9	5.7	7.1
STDev	10.7	3.1	0.5	0.6
$LSD_{(T)(0.05)}$	12.1	4.4	0.4	0.4

Means within the same column followed by the same letter(s) are not significantly different at ($p < 0.05$), * significantly different, NS non significantly different

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 for the physiological response of maize seedlings to these treatments under glasshouse conditions and the yield response of maize to some of these treatments under field conditions is required to qualify interactions.

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