IMPROVEMENT OF GROWTH AND YIELD OF BREAD WHEAT BY MEANS OF CHEMICAL MANIPULATION UNDER GLASS HOUSE CONDITIONS

By

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DECLARATION

I declare that the thesis hereby submitted by me for the Master of Science in Agriculture degree at the University of the Free State is my own independent work and has not previously been submitted by me at another University. All sources referred to in this study have been duly acknowledged. I furthermore cede copyright of the thesis in favour of the University of the Free State.

Signed on June 2004 at the University of the Free State, Bloemfontein, South Africa.

Signature----------------------
Md. Jahangir Alam

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CHAPTER 1

INTRODUCTION AND RATIONALE FOR THE STUDY

Most populations in the developing world are still rapidly growing and it is estimated that, by the year 2020, an extra 2.5 billion people will require food. This is in addition to the three-quarters of a billion people who are chronically undernourished in this part of the world today. According to recent estimates, over 800 million people, equal to 15% of the world's population, receive less than 2000 calories per day. Particularly in Sub-Saharan Africa and South Asia, these people live a life of permanent hunger and are chronically undernourished (FAO, 1992).

Until well into the next century the potential exists for a further 80 million people to be added to the world population each year and this is close to a quarter of a million people per day (Bos *et al,* 1992; UN, 1993). If the proportion of those deprived of an adequate diet remains the same, the number of undernourished people could be greater than 1.4 billion by the year 2020.

In light of the preceding statistics, what is the prognosis for feeding the world's population in the 21st century? Unfortunately predictions of the population numbers and food requirements are complicated due to numerous variables and many unknowns that interrelate in complex and often circuitous ways. Nevertheless, based on current population growth predictions, it seems that a significant increase in food production will be necessary over the next 30 years.

The following statistics not only show current nutritional problems but also confirm the need for increased food production. Approximately 4.75 billion tons of food is produced worldwide each year, yet more than 400 million people suffer from chronic nutritional deficiencies. More than a billion, one out of every six people are considered to follow an inadequate diet. Further, the world population is estimated to increase from the current 6 billion to about 8.3 billion by the year 2025 and hopefully stabilizing at about 11 billion towards the end of the century (Dibb and Darst, 2000).

Africa, South America, and Asia account for more than 75% of the world's population. The greatest population pressure is in Asia, where there is less than 0.15 ha of arable land per person. As the world population increases, about 90% of the increase will be in developing countries, including much of Asia, where food deficits already exits. For example, China represents more than 22% of the world's population but only 7% of the arable land is utilized by a population that is increasing by more than 15 million per year. India, the world's second most populous country, is expected to reach a population of 1.0 billion during 2003. Although Bangladesh and Pakistan lag behind India in numbers, both countries are already highly populated. The following table supplies interesting information with regard to future yield requirements and the pressure on crop production systems:

Table1: Current and projected world cereal production and demand (million tons) and yield requirements

	Current production	Project demand		Yield t/ha		
				Actual	Required	
	1990	2000	2025	1990	2000	2025
Wheat	600	740	1200	2.4	2.8	4.4
Rice	520	640	1030	2.4	3.1	5.3
Maize	480	620	1070	3.7	4.1	5.8
Barley	180	220	350	2.3	2.7	4.1
Sorghun	85	110	180	1.5	1.8	2.6
All Cereals	1970	2450	3970	2.5	2.9	4.5

(FAO, 1997)

As in the past, humankind will rely largely on plants, and specially the cereals, to supply in virtually all of our increased food demand. Even if current per capita food consumption stays constant, population growth would require that world food production increase by 2.63 billion gross tons, or 57%, by 2025 as compared to 1990 (Table 1). However, if diets improve among the hungry poor, estimated to be at least 1 billion people, the annual world food demand could increase by 100%.

To meet the projected food demands, the average yield of all cereals must be increased by 80% between 1990 and the year 2025. Fortunately, there are still many improved agricultural technologies, either already available or well advanced in the research pipeline, that are currently only partially being exploited and that can be employed in future years to increase crop yields. Further, large unexploited "yield gaps" exist in virtually all low income or food deficit developing countries as well as in the former Soviet Union and Eastern Europe. The potential exists for yields to be increased by 50%-100% in many areas of Asia, Latin America, the former USSR and by100%-200% in much of Sub-Saharan Africa, only by applying existing knowledge more efficiently (Borlaugh, 1997).

The latter potential for increasing crop yields prompted this study and supplied the rationale for investigation the application of ComCat®, a natural biostimulant, at different fertilizer levels in order to determine the role chemical manipulation can play in improving vegetative growth and yield of a Bangladeshi wheat cultivar. In chapter two a comprehensive literature review on the current and predicted world food security situation as well as possible ways to improve it is supplied, while the methods supplied in this study are outlined in chapter three. Chapters four and five deal with the response of a Bangladeshi wheat cultivar, Sonalica, to treatment with different N-levels and a natural bio-stimulant, ComCat®, in terms of vegetative growth and yield during the 2001 and 2002 planting seasons respectively. In conclusion, a general discussion of the findings of this study, as well as recommendations for future research, is supplied in Chapter six.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Despite the impressive advances that have been made over the years in improving the yields of food crops, including wheat, there is little reason to become complacent about the food supply, especially in the developing world. Between 70 and 80 million people will be added to the world's population every year between now and 2020, increasing the world's current population of 6 billion by a third to reach almost 8 billion (Heidhues, 2001). To produce and provide the food needed for the additional 2 billion people is possible but probably not without a special effort.

It is estimated that, during the next three decades, the population of developing countries will increase by at least 1.6% and this addition will probably become urban-based (FAO, 2000). As the individual income increases and consumers substitute rice with course grain cereals, the demand for wheat will increase by 2020 while two thirds of the world's wheat consumption will occur in developing countries (CIMMYT, 1997). Most of the demand will come from Asia, especially over populated countries such as India, China, Bangladesh, Pakistan and Sub-Saharan Africa. To meet these demands in the Asian subcontinents and Sub-Saharan Africa, especially in light of the fact that the area under cultivation is expected to remain minimal or even decrease, wheat yield increases will have to be maintained at 2.5% per year over the next 30 years (FAO, 2000). Some countries, including Bangladesh and Pakistan, with 50% or more of the poor population involved in agriculture, have a negative land balance already. It is therefore expected that the cultivated area in Asia will start to contract (Young, 1998).

The challenge is to increase both farm productivity and sustainability. However, the related requirements are sometimes conflicting at the physiological, agronomic and economic levels, so that an exclusive focus on one aspect will not yield the optimum solution (Penning de Vries, 2001). The question that needs to be answered is how this will be achieved? The general consensus is that it cannot merely come from expanding the cultivated area by simply removing forest to make more agricultural land available because of obvious secondary problems that might arise. Of these the effect on the ozone layer and global warming (Heidhues, 2001) is probably the most important. However, other aspects such as the discovery of new soils and the need for comprehensive research to develop proper cultivation practices need to be considered.

Theoretically, it is also possible to achieve higher yields or increase food production by increasing the land under irrigation. However, most of the world's irrigatable land is probably already in use and chances to expand are slim (Penning de Vries et al., 1995). Future food production growth will have to come from productivity increases and, without a doubt, this is the more complex or more difficult way of increasing food production (Heidhues, 2001). Accurate prediction of food supply and demand is therefore pivotal for the prioritization of research and at least three factors will have to be considered: 1) the rate of population growth, 2) the rate of economic growth and 3) the amount of investment in research to improve productivity and the environmental quality of agricultural systems (Cassman and Dobermann, 2001).

2.2 The food security situation in Sub-Saharan Africa (SSA) and Asia

Among all the regions of the world, over the past two decades, only in Sub-Saharan Africa (SSA) has the food output increased slower than the population. If present trends continue, the number of malnourished children in SSA could increase to 53 million by the year 2020 (Rosegrant *et al*, 1995). Clearly, one of the major challenges facing the agricultural profession is to assure that this calamitous situation does not become a reality. Between now and the year 2020, SSA's population will more than double to over 1.1 billion people (Bos, 1992).

In most regions of the world, increases in food supply over the past two decades have resulted mainly from increasing yields. The only major exception is SSA, where most of the growth in production has occurred due to expansion of the cultivated area. It is widely perceived that technology-based agriculture has bypassed SSA on a significant scale. Instead, where land is still plentiful, "slash and burn" shifting cultivation persists. Further, where population pressures have reduced the fallow period, sedentary low-yield agriculture has arisen (Borlaugh, 1995).

Production per capita continues to grow in previously famine plagued South and West Asia as well as North Africa. However, while these regions became essentially self-sufficient in the supply of basic foods in recent years, a decrease is observed in East Asia and Latin America with a continuing rapid decline in SSA (Hazell, P. 1998). The main reasons for the situation in Asia and SSA are similar and include undesired agricultural practices such as cultivation without manure or fertilizer, overgrazing and deforestation but often unfavourable climate conditions (Rosenzweig & Parry, 1994; Donovan, 1996).

For these regions the current prognosis is that the production of food on available arable land will simply have to be increased by applying techniques that will not further deplete the natural resource base on which agriculture depends. Based on a review of more than eighty case studies, including data from the 1980's, at least 16% of all agricultural land in developing countries is seriously degraded, implying that crops cannot be grown profitably (Scherr, 1999). Crop yield increase is, therefore, no small challenge for agricultural research, but there are reasons to be optimistic. At least one is that researchers will be able to develop technologies that can improve crop yields and at the same time preserve the resource base.

2.3 The potential to increase food production per unit area

Total crop production, including wheat production, can be increased in two ways: a) by increasing the area cultivated or b) by increasing the production per unit area. With regard to a): most of the opportunities for acquiring new agricultural land for crop cultivation have already been exploited (Penning de Vries, 2001). This is certainly true for densely populated Asia and Europe. Only in sub-Saharan Africa and South America large unexploited areas of land still exist, but only some of this land will probably come into agricultural production. In populous Asia, home to half of the world's people, there is very little uncultivated land left to bring under the plow. Apparently, in west Asia, some 21 million hectares of poor soil is currently being cultivated, but that actually shouldn't have been. Most likely, such land is either too arid or the topography is so vulnerable to erosion that they should be removed from cultivation (Buringh & Dudal, 1987).

Further, the last major land frontiers include acid soil areas of the Brazilian Cerrado, the llanos of Colombia and Venezuela as well as the acid soil areas of central and southern Africa and parts of Indonesia. However, these soil types are highly leached and made unproductive by natural causes in geologic time (FSSA Journal, 1997). Nevertheless, these areas are now being made productive by science and technology. Bringing these unexploited potentially arable lands into agricultural production poses formidable, but not insurmountable, challenges (Borlaugh, 1995). The opening of the Brazilian Cerrado and the other acid-soil areas mentioned above, can contribute greatly towards the adequacy in world food production for the next three decades.

In 1990, roughly 10 million hectares of rain-fed crops were grown in the Brazilian Cerrado with an average yield of 2ton ha^{-1} and a total production of 20 million tons. The irrigated area is still relatively small, only 300 000 hectares, with an average yield of 3ton ha^{-1} and a total production of 900 000 tons. In this area there is also 35 million hectares of improved pasture with an annual meat production of 1.7 million tons (Macedo, 1995).

If improved technology, now available, were widely and properly utilized in the areas of the Brazilian Cerrado currently under cultivation, it would be possible for farmers to attain 3.2 ton ha^{-1} in rain-fed crops and 64 million tons of total production. Moreover, the irrigated area can also be increased to 5 million hectares, with an expected average yield of 6ton ha^{-1} and a total crop production of 30 million tons. Meat production could be more than doubled. Thus, in total, food production can be increased from 23 million to 98 million tons, through widespread adoption of the improved technology already available (Macedo, 1995). It is believed that the opening to cultivation of additional vast tracts of the Cerrado will play a central role.

In order to assure the adequacy in world food supply for the next three decades, wise policies to stimulate production on the non-acid soil agricultural lands already in production in other parts of the world, will have to continue. Eventually, technology similar to what is being used in Brazil will hopefully also be applied in the llanos in Colombia and Venezuela as well as in countries of central Africa, Southeast Asia and Indonesia where similar soil types are found

(Bourlaugh, 1997). However, a better opportunity for rapid pay off in the near future will be created if current problems in terms of the unavailability of fertilizers as well as seeds of improved high-yielding and acid resistant wheat, maize, sorghum and cassava varieties can be overcome. Lastly, more sound and adequate weed control management systems need attention in these areas (Rajaram and Dubin, 2000).

With regard to b): since there are only limited prospects for expanding cropland significantly, most food production increases will have to come from increased yields (Heidhues, 2001). This can be made possible by the more efficient use of fertilizer (at least N, P and K), the cultivation of improved varieties and probably the use of new yield improvement agents (e.g. natural biostimulants such as ComCat®, Maxiflo®, Penac® and Kalpak®). Additional factors for improving crop yields on available land that need to be considered by developing countries include a change in attitude towards the cultivation of improved genetically engineered cultivars. Developing countries need to take cognizance of the fact that improved crop production systems, in terms of employing improved varieties and the increased use of chemical fertilizer as well as more effective weed, disease and insect control measures, have allowed world food production to increase more rapidly than the global population over the past three decades (Borlaugh, 1997).

Already in 1987, Wortman and Cummings stated that sensible application of fertilizer and genetic improvement of cultivars have been in the forefront of the struggle to increase world food production and, perhaps more than any other input, has been largely responsible for the success that have been achieved. It is in a certain sense unfortunate that so much resistance towards the use of inorganic fertilizer and genetically modified crops by producers and consumers alike exists today. The need to reconsider what is implied by the latter statement must be seen against the background that a complete package of improved management practices, including the use of hybrid seed and inorganic fertilizer, have contributed towards worldwide yield increases of up to 65% (Dyson, 1995). It is also estimated that approximately 50% of this increase can be attributed to the application of inorganic N fertilizer alone (Dyson, 1995).

Biotechnology, and especially genetic engineering, offers a faster route and also the means of tackling the particularly intractable problems of drought, salinity and toxicity that typically face the poorest farmers on marginal lands. A good start in improving rice varieties, using these technologies, has been made during the 1980's. In 1984, the Rockefeller Foundation launched its "International Program on Rice Biotechnology", with the aim of facilitating the creation of a number of Asian centers of excellence in biotechnology. To date, over $80 million has been spent on collaborative programs with laboratories in the industrialized world, involving a network of some 700 researchers, fellows and advisers. Practical results include the development, through tissue culture, of a new rice variety in China, named La Fen Rockefeller. This variety is now widely grown in the Shanghai area and produces yields of 25% above that of previous varieties (Conway, 1998).

Until recently, from a genetic perspective, it has been generally assumed that increases in the yield potential in plants (and animals) is controlled by a large number of genes, each with minor additive effects. However, the work of recent years shows that there may also be a few genes that are sort of "master genes" that affect the interaction, either directly or indirectly, of several physiological processes that influence yield. For example, BST (Bovine Somatotropin) production is apparently controlled by such a "master gene". BST can increase milk production up to 30% by improving the efficiency of milk produced per unit of feed intake. It now appears that the dwarfing genes, Rht1 and Rht2, used to develop the high yielding Mexican wheat that launched the Green Revolution, also act as "master genes".

Insertion of these genes resulted in reduced plant height, increased tillering, increased number of fertile florets and number of grains per spike as well as the harvest index. Biotechnology may open a new window for searching for new “master genes” and higher yield potential cultivars by eliminating the confounding effects of other genes (FSSA Journal, 1997).

This technology can be rewarding in light of limited uncultivated land being suitable for production in many countries and in light of the fact that increases in food production over the next three decades must come from increasing yields on the land now under cultivation.

2.4 Wheat cultivation in Bangladesh

Wheat is a non-traditional crop in Bangladesh. Before independence in 1971, no attempts were made to introduce wheat to the country. At the time wheat was cultivated on only about 0.129 million hectares with a production of 0.103 million tons (BADC, 1975). After independence, a few Mexican varieties were tested under Bangladeshi environmental conditions. Surprisingly, the yields obtained with these varieties were more than 3 ton ha^{-1}, which encouraged the agricultural industry to introduce wheat to Bangladesh on a larger scale. Policy makers also felt the need of introducing wheat as an alternative cereal during the winter due to the persistent and serious food deficit in the country.

Accordingly, a well-organized government-sponsored wheat expansion program was undertaken in 1975. Systematic research on wheat was also initiated in the same year. Since then, a number of high yielding varieties were released and appropriate production technologies were developed and disseminated to the farmers. As a result, wheat has been established as an important cereal crop in the country and the production program has been very successful. Between 1970 and 1981, the area under cultivation and production increased about 5 and 10 fold respectively (Personal communication; Bangladesh Wheat Research Centre, 2000) that had a significant impact on the national economy. A rapid dietary change also took place so that wheat has become an important food item in the average Bangladeshi household within a time span of only a decade. Now wheat has established itself as the second most important cereal crop in Bangladesh and it is gaining popularity by the day.

As a result, wheat research and extension programs were strengthened in 1980. A number of high yield potential varieties and modern production technologies were developed in the country and disseminated to the farmers through extensive demonstrations and training. Impressive advances in improving the yield and production of wheat have been made ever since. A record quantity of 2.02 million tons of wheat was produced on an area of 0.79 million hectares during the 2000/2001 season that was about 16% higher than that of the 1999/2000 season (Department of Agricultural Extension,Bangladesh, 2000).

However, the present production growth is not sufficient enough to meet the cereal demand of the growing population as the consumption rate of wheat is also increasing. Currently the per capita consumption of wheat is 33 kg/person/year (USDA, 1998). Bangladesh is currently importing about 2 million tons of wheat annually to meet the national demand. This import has almost doubled in some years due to flooding or drought (WRC, 2000). Therefore, to keep pace with the future demand of the growing population, the current trend of wheat production and productivity should be raised and an appropriate research and production plan should be undertaken to boost the wheat production in the country.

2.5 Future challenges and frontier research in Bangladesh

The future demand of cereals is expected to increase due to the growing population of the country. During the next three decades, it is estimated that the population will grow by at least 1.6% per annum while the demand for wheat is expected to grow at 1.8% per annum (IFPRI, 1994). The current trend in production growth is too low to keep pace with the future demand. Therefore, future research should be directed towards the development of economically viable and sustainable technologies to increase the wheat production by narrowing down the gap between potential yield and the current yield in rice-wheat intercropping systems. Possible techniques and/or technologies that can be applied with the aim to improve yields on existing arable land include cultivar improvement, planting time optimization, zero tillage, sustainable disease control, sustainable fertilizer application and the use of a new generation of bio-stimulants.

2.6 Technologies to be considered in future research

2.6.1 Cultivar improvement

In the past, introduction of dwarfing genes in both rice and wheat has allowed for tremendous advances in yield improvement. Recent advances in wheat yield improvement achieved by CIMMYT include the introduction of new spring wheat cultivars. Because of the short winter period in Bangladesh, as well as the rather long growing time to maturity, none of the winter cultivar germplasm could be selected. However, the possibility exists to maximize the potential to introduce winter cultivars by changing the plant architecture genetically through engineering and/or hybridization practices (Razzaque, 2002).

Very recently, China and India have developed a “super head” dwarf variety with a 5-6 g spike weight that is the equivalent of three normal spikes. Modification of plant architecture with larger heads, more grains and fewer tillers could lead to an increase in yield potential (Rajaram and Dubin. 2000). Further yield improvements are also possible by combining high yielding genes from various sources into locally adapted varieties. Besides, biotechnological approaches should also be adopted where conventional breeding would be difficult.

2.6.2 Yield Improvement by means of Chemical manipulation and management practices

The average national yield level of wheat in Bangladesh is about 2.3 tons ha^{-1} currently. The potential to increase this average exists by merely applying the recommended production practices. However, the best environment for wheat production is situated in the northern regions of Bangladesh (including the Dinajpur, Rajshahi, Rangpur and Jessore areas) where the average yield is about 3.2 tons ha^{-1}. The yield target for other medium regions is 2.6 tons ha^{-1} that can easily increase the present national average to 3.0 ton ha^{-1} by 2004 (WRC, 2000). Adopting the existing package of production technologies at farm level should minimize the gap between the national yield and the average on-station and on-farm yields. A short description of available technologies and expected outputs are supplied below.

2.6.3 Planting time of wheat

The optimum planting time of wheat in Bangladesh ranges between 15 and 30 November. However, in the northwestern zones of the country the optimum planting time can be stretched to the 1st week of December, due to a prolonged winter. Yield losses can be minimized by adhering to the recommended planting time (BARI, 2000).

2.6.4 Seed treatment with Vitavax-200

Seed treatment with Vitavex-200 @ 3g kg^{-1} of seed controls seed borne diseases effectively and other foliar diseases partially. This treatment has been shown to increase yields by at least 10% (BARI, 2000).

2.6.5 Minimum/Zero tillage

Minimum or zero tillage can reduce the turn around time between the rice harvest and wheat planting times. A power tiller driven seeder has been found very effective to plant wheat seed immediately after the rice harvest, which ensures proper depth and good germination. As a result, 20% yield increases can be achieved compared to conventional methods (BARI, 2000).

2.6.6 Use of recommended organic and inorganic fertilizers and proper N management

The national target is to produce 3 million tons of wheat by the year 2005. For this to realize, optimum and balanced fertilizer application is necessary as declining soil fertility and productivity due to intensive cultivation of high yielding varieties is a major constraint in achieving higher yields in Bangladesh (WRC, 2000). The sub-optimal and imbalanced use of fertilizers has also led to a serious depletion of nutrient reserves in Bangladeshi soils. It is also estimated that an amount of 1054 thousand tons of nutrients (N, P & K) is being lost from arable land in Bangladesh annually (Ali, 1994). Nutrient depletion of soil poses a serious threat to soil fertility as well as soil productivity. About 70% of the cultivatable land in the country contains soil organic matter of below 2% (Hoq, 1980). More than 50% of the farmers do not apply nutrients at the recommended rates and both P and K application is especially inadequate. The application of optimum and balanced fertilizer rates has never been as important for Bangladeshi farmers as at present. Nitrogen application is recommended at ±

two thirds of the basal requirement at planting while the remaining one third should be applied as a top dressing.

The recommended use of fertilizer is supplied below (*BARI, 2000)

Nature of Irrigation	Quantity of N / P / K (kg ha^{-1})		
	N	P	K
Full irrigation (3 – 5 times)	100	90	45
Semi irrigation (1 –2 times)	90	65	24
No irrigation	75	65	24

*BARI = Bangladesh Agricultural Research Institute

2.6.7 The use of optimal irrigation and proper drainage

Proper integration of adequate irrigation scheduling and recommended fertilizer dosage is not available for wheat cultivation in Bangladesh. According to the BBS (Bangladesh Bureau of Statistics, 1998), farmers apply medium to high doses of fertilizer but less irrigation than what is required, resulting in lower yields due to the lower availability of both water and nutrients to the plants. In Bangladesh wheat yields under rain fed conditions range from 1.3-1.8 ton ha^{-1} while yields under irrigated conditions vary from 2.0-4.5 ton ha^{-1} depending on the irrigation level and management practices (Harun-ur, 2001). Proper irrigation scheduling might play a pivotal role in improving wheat yield in the future implying that two to three extra irrigation sessions might be needed for sandy soils. However, over irrigation practices must be avoided and excess water should be drained out immediately after irrigation or rainfall to ensure proper growth and yield.

2.6.8 Weed control

It is recommended that weeds should be controlled once within 25-30 days after planting to ensure an estimated yield increase of about 10-15% (BARI, 2000).

2.6.9 Control of foliar diseases by means of chemicals

Spraying of Tilt 100 EC @ 1 ml L^{-1} of water has been shown to control many foliar diseases efficiently and to increase yields by 15-20% compared to untreated controls (BARI, 2000).

2.6.10 Planting in plough pan broken soil

Suitable equipment for breaking the plough pan of soil after tillage is available in Bangladesh and the technique, which compares to minimum tillage practices, has been shown to increase crop yields up to 15%, probably due to improved root systems and nutrient uptake (BARI, 2000).

2.6.11 Yield improvement with natural bio-stimulants at different fertilizer levels

Large scale application of natural bio-stimulants under field conditions is a fairly new enterprise and has not been applied in agriculture as frequently as in the horticultural industry. In the latter instance known plant hormones such as auxin, gibberellins and kinetin have been applied, especially in nurseries, to induce seed germination and root development in seedlings.

ComCat® belongs to a new generation of natural bio-stimulants and is manufactured from plant extracts with bio-stimulatory properties involved in the regulation of plant development (Hüster, 1999). The name ComCat® has been derived from two allelopathic terms, communication and catalyzation, and the active substances identified as brassinosteroids, a new generation of phyto-hormones that act in synergism with auxin and other natural compounds including free amino acids and flavonoids (Schnabl *et al.*, 2001). During the manufacturing process, 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 agrochemical aids.

ComCat® is described and registered as a plant-strengthening agent by the European Union. Claims made by the manufacturers include the enhancement of vegetative growth, especially root growth, of many agricultural crops such as wheat, maize and some vegetables as well as yield enhancement due to improved utilization of soil nutrients, improved flower bud formation and fruit development (Hüster, 1999). If applied at an early growth stage, ComCat® can be beneficial to the farmer providing that sufficient water and nutrients were applied. Other advantages of ComCat® include its ecologically friendly nature, induction of resistance against pathogens (Schnabl *et al.*, 2001) and improvement of product quality without leaving residues in the crop (Hüster, 1999). The product is a water-soluble powder and is applied in small dosages as a seed treatment and/or as a foliar spray.

2.7 Emphasis in this study

ComCat®, was applied at different fertilizer levels in order to determine the role chemical manipulation can play in improving yield and quality of a Bangladeshi bread wheat cultivar, Sonalica. The methods applied are outlined in chapter three. Chapters four and five deal with the response of this wheat cultivar to treatment with different N-levels and two separate ComCat® treatments, i.e. a seed treatment before planting and a foliar spray treatment of seedlings at growth stage 13 (3-leaf stage) in terms of vegetative growth and yield during the 2001 and 2002 planting seasons respectively. In conclusion, a general discussion of the findings of this study, as well as recommendations for future research, is supplied in chapter six.

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant material

Fresh seed of one Bangladeshi bread wheat cultivar, OSD-5 (Sonalica), was used in the study.

3.1.2 Soil

A light sandy loam soil, Bainsvlei, was obtained from the Bainsvlei area, Bloemfontein, South Africa. Soil analysis by the department of Soil, Crop and Climate Sciences, University of the Free State, South Africa revealed a composition of 263 ppm total nitrogen and 19.6 ppm phosphorous while exchangeable and water soluble cations (1N NH_4OAc) measured as follows:

- 649 ppm calcium,
- 161 ppm magnesium,
- 159 ppm potassium and
- 23 ppm sodium.

The soil pH (water) was 5.3.

3.1.3 Fertilizers

Limestone ammonium nitrate (LAN-28%), Maxifos (20%) and Potassium chloride (KCl), used as sources for nitrogen, phosphorus and potassium respectively, were purchased from a local merchant.

3.1.4 Chemicals

ComCat®, a commercial plant biostimulant, was used to investigate its possible effect on plant growth and yield of a Bangladeshi wheat cultivar at different fertilizer levels. Comcat® is a natural product manufactured from donor plants containing active substances that are involved in the regulation of plant growth and development, as well as the activation of inherent plant defense mechanisms against pathogens and biotic stresses (Agra Forum, Germany - personal communication).

All laboratory chemicals used in this study, were purchased from either Merck (Germany) or Sigma (Germany) and were of the highest purity available.

3.1.5 Plastic pots

Plastic pots (cylindrical) measuring 17 cm in height, 20 cm in top diameter and 13 cm in bottom diameter were used in both season's trials and were purchased from the SENWES Co-operation, Bloemfontein, South Africa.

3.2 Methods

3.2.1 Seed treatment

Wheat seeds were treated overnight with ComCat® at 100 g / 100 kg seed by dissolving in 300 ml water. Three hundred seeds were treated for both trials. Before sowing the seeds in pots, all seeds were spread out on a sheet of filter paper and allowed to dry.

3.2.2 Seeding

Four wheat seeds were sown per pot. Thinning was done five days after germination and only the two healthiest plants were left to grow in each pot. Early thinning was done in order to avoid the possible future complication that might have arisen during the measurement of root mass due to root residues that could have remained in the pots.

3.2.3 Pot filling and soil fertilization

Soil was sieved through a 2 mm sieve, in order to remove debris and weed seed before filling the pots to about 10 cm from the top. After filling the pots with soil to this level, fertilizer was broadcasted on top, lightly mixed with the topsoil and covered with another 5 cm soil layer. Four wheat seeds were sown per pot, spaced about 6 cm apart, and slightly pressed into the topsoil in such a manner that it was about 4 cm from the fertilizer to avoid the risk of burning of germinating seedling roots. The seeds were then covered with another 2 cm soil layer leaving a 3 cm space for water application.

3.2.4 Soil fertilization

Limestone ammonium nitrate (LAN-28%), Maxifos (20%) and Potassium chloride (KCl) were used as sources for nitrogen, phosphorus and potassium respectively. In order to ascertain the optimum nutrient requirements of the Bangladeshi wheat cultivar, Sonalica, for N, P and K, application rates as shown in table 3.1 were initially used during the 2001 trial. The medium rate for the N, P and K ratio was based on the standard rate applied in South Africa for winter wheat. Additionally, a down scaled rate (Low) and an up scaled rate (High) was applied in order to ascertain the optimum. After obtaining growth and yield data during 2001, the application rates were adjusted for the 2002 trial (Table 3.1). The three fertilizers were mixed thoroughly in these ratios before mixing it with the topsoil layer.

Table 3.1: Fertilizer application rates for the 2001 and 2002 trials.

Year	Level	Per ha	Element per pot	Fertilizer per pot	Year	Level	Per ha	Element per pot	Fertilizer per pot
2001					2002				
	LOW	N=100 kg	N=3.142 g	11.22 g = LAN		LOW	N=50 kg	N=1.57 g	5.6 g=LAN
		P=25 kg	P=0.785 g	3.925 g = Maxifos			P=25 kg	P=0.785 g	3.925 g=Maxifos
		K=10 kg	K=0.314 g	0.628 g=KCl			K=10 kg	K=0.314 g	0.628 g=KCl
	MED	N=200 kg	N=6.283 g	22.439 g=LAN		MED	N=100 kg	N=3.142 g	11.22 g=LAN
		P=25 kg	P=0.785 g	3.925 g=Maxifos			P=25 kg	P=0.785 g	3.925 g=Maxifos
		K=10 kg	K=0.314 g	0.628 g=KCl			K=10 kg	K=0.314 g	0.628 g=KCl
	HIGH	N=400 kg	N=12.57 g	44.879 g=LAN		HIGH	N=150 kg	N=4.712 g	15.0 g=LAN
		P=25 kg	P=0.785 g	3.925 g=Maxifos			P=25 kg	P=0.785 g	3.925 g=Maxifos
		K=10 kg	K=0.314 g	0.628 g=KCl			K=10 kg	K=0.314 g	0.628 g=KCl

**** During the examination process it was pointed out that ten times more fertilizer was applied per pot than envisaged in table 3.1 as a result of a calculation error. This error could not be rectified except by repeating the trials over two seasons. All interpretations and deductions in this manuscript are based on the envisaged fertilizer levels as indicated in table 3.1 and not the higher rates as miscalculated.**

3.2.5 ComCat® treatments

The influence of ComCat® on the growth and yield of wheat was investigated using different agronomic and yield parameters. ComCat® was applied in two ways, namely as a seed treatment (see 3.2.1) before planting and as a single foliar spray at growth stage 13. Growth stages were based on the description of Meier, 1997.

ComCat® application as a foliar spray at growth stage 13 was at the recommended optimum rate (AgraForum, Germany - Personal communication) of 100 g/ha, and at a volume of 300 l/ha. Foliar spray application was done in a spraying room, fitted with a specially designed automatic spraying machine, over a length of 4 meters. The potted plants were placed in a row under the nozzles, approximately 0.5 m apart, over the 4 m distance that the spray beam moved. The height, pressure and speed of the spraying operation was carefully calibrated according to a volume equaling 300 ha L^{-}, using water, until the desired spray volume (108.8 ml over 4 meters) over a row length was achieved. In this manner all plants received the required dosage. Treatments were replicated four times.

3.2.6 Watering

Plants were kept at field capacity and watered with distilled water throughout the growing period, in order to avoid the mineral content of tap water from influencing the results of the research. Potted plants were shifted around randomly within the blocks every 4 to 5 days in order to prevent exposure to different conditions that could have applied to different areas in the glasshouse.

3.2.7 Control of fungal pathogens

As a precaution against fungal diseases, Spore Kill® was applied on the soil surface at a concentration of 1 ml L^{-1} of distilled water and at 100 ml per pot.

3.3 Experimental design and data measurement

3.3.1 Experimental design

Two sets of trials were run in the green house of the University of the Free State, South Africa, during 2001 and 2002, starting at the same time (30th May), and in the same green house. A randomized complete block design (RCBD) was used in both cases. During both trials vegetative growth and yield parameters were employed to monitor the influence of pattern, yield improvement and quality development of wheat, influenced by the different fertilizer levels and ComCat® treatments. Different sets of data were collected at growth stages 13, 33, 52 and 73 (Meier, 1994).

3.3.2 Data measurement

Various parameters were used to measure the growth and yield response of wheat cultivated in the green house under the influence of different N, P and K levels as well as ComCat® treatments. Parameters included:

3.3.2.1 Vegetative growth

As most of the growth parameters used to follow the growth patterns of the wheat were destructive, extra plants were cultivated for this purpose and replicated four times.

Plant height:

Was measured from the base of the stem to the tip of the longest leaf every second week during the vegetative growth phase and to the tip of the spike, after anthesis using a measuring tape.

Above soil part fresh mass:

After roots were detached from the plant using a sharp knife, the above soil part mass was determined using a digital scale.

Above soil part dry mass:

Above soil plant parts (leaves and stem) were placed in a paper bag and dried in an oven (Labcon, Type FSOE, West Germany) at 70 ºC (±2ºC) for 72 hours. The dry mass was subsequently measured.

Root fresh mass:

The whole root system of each plant was recovered from the soil by washing very gently with running tap water over a 0.5 mm² wire mesh. Clean roots were subsequently wrapped with laboratory grade filter paper for half an hour to remove the excess water from the surface and the fresh mass determined using a digital scale.

Root volume:

Washed roots from a single pot were placed in a 250 ml measuring cylinder. A known volume of water was added and all air bubbles were removed by gently stirring with a glass rod. The volume of the displaced water was taken as the volume of roots (Bohm, 1979).

Root dry mass:

The fresh roots of each pot were placed in a paper bag and dried in an oven at 70 ºC (±2ºC) for 72 hours. The dry mass was subsequently measured.

Leaf area:

Leaf lamina were detached from the plant where it joined the leaf sheath, using scissors, and the leaf area of freshly cut leaves of each replica measured using a digital leaf area meter (Lambda Instruments corporation, Model LI-3000, USA).

3.3.2.2 Yield components

Number of spikes per plant:

The number of spikes on two plants per pot as well as the tillers were counted. Results were expressed as number of spikes or tillers per plant.

Number of kernels per spike:

Kernels were removed from dry spikes by gently rubbing between hands over a large sheet of paper taking care that none went astray. The number of kernels were counted for two plants and expressed as number of kernels per spike.

Number of kernels per plant:

Previous counts were used to also express the number of kernels per plant.

Total yield:

Immediately after threshing dry kernels from the spikes, the mass was determined and the yield expressed as yield per plant (g).

Thousand kernel mass:

One hundred kernels were randomly selected for each replica of each treatment, the mass determined and extrapolated to 1000 kernel mass.

3.3.3 Statistical analysis of data

Analysis of variance (ANOVA) was performed on all data using the NCSS-2000 statistical programme. Tukey’s MSD (mean significant difference) procedure for comparison of means (Steele and Torrie, 1980; Mason *et al*; 1989) was applied to separate means ($p<0.05$). Treatments differing significantly at growth stage 99 were indicated in tables or figures by designating different letters.

3.3.4 Green house conditions

Both trials were conducted in the same green house during both the 2001 and 2002 growing seasons at the University of Free State, Bloemfontein, South Africa. The temperature of the green house was maintained at 23/15 °C±2 °C during day and night time respectively.

CHAPTER 4

EFFECT OF DIFFERENT NITROGEN FERTILIZER LEVELS AND COMCAT® ON THE VEGETATIVE GROWTH AND YIELD OF A BANGLADESHI BREAD WHEAT CULTIVAR UNDER GLASSHOUSE CONDITIONS DURING THE 2001 GROWING SEASON

4.1 Introduction

Fertilizers and its application have been in the forefront of the struggle towards increasing food production over the past century and, perhaps, more than any other input has been largely responsible for the success that has been attained in crop production systems. It is well established that fertilized soils contribute to increases in crop productivity but, where specific plant nutrients are deficient, crop yields can still be low. This confirms that a well balanced fertilizer program is essential for high crop yields and is vital for sustainable crop production (Harre & White, 1985).

The growing population, particularly in Asia, as well as changing diets will lead to a much higher food demand in 2020. This means that food production globally should double in the next 20 years (Penning de Vries, 2001). 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, fertilizer application is not the only factor that needs consideration as sustainable food production is obviously the result of a combination of factors. These include biotic and abiotic stress conditions as well as other possible manipulation techniques such as the use of bio-stimulatory agents (FAO, 1981). In light of the preceding, the underlying study dealt with fertilizer application, separately and together with a commercial biostimulant (ComCat®), as a possible means to increase the production of wheat on available land.

The rationale for this approach is that the application of balanced fertilizer combinations is probably still the first line of defense against low productivity in crop production systems and has contributed to 50 – 75% increase in food production in some developing countries (FAO, 1981). The large increases in the nutrients supplied to crops have been essential to support the agricultural revolution which began in the 1930's in many temperate zones. Rapid adoption to controlled fertilizer practices began in the United States, Europe and Japan after World War ll. During the past two decades, chemical fertilizers used in combination with high-yielding varieties and improved crop management practices have permitted the densely populated nations of Asia to better feed their rapidly increasing populations and lower the real cost of food for everyone, but more importantly for the rural and urban poor (FAO, 1981). In China, chemical fertilizer production has increased from 6000 metric tons in 1949 to over 25 million tons in 1995. Today China is the world's largest producer, importer and consumer of chemical fertilizer (Borlaugh, 1997). Currently, this nation is practically self-sufficient in supplying basic foods and has become the world's largest cereal producer. Although India lags behind China in cereal production, they also became self-sufficient in 1975 and this has been maintained up to the present due to heavy investment in fertilizer application (Borlaugh, 1997).

In particular the increased but accountable application of nitrogen (N) fertilizers, together with advances in plant breeding, has given spectacular results. However, the yield response to increased N levels was smaller than when adequate and balanced amounts of P and K were also applied (Gartner, 1969; Simpson, 1986). The terms "accountable" and "balanced" application of fertilizers is based on research results showing that applying excessive fertilizer too soon may be detrimental to crops by stimulating vegetative growth at the cost of reproductive growth (Mengel and Kirkby, 1987). Already in 1969, Trolldenier also reported that the excessive N application can also stimulate various fungal crop diseases such as brown rust of barley and brown leaf spot of rice.

Despite progress in convincing crop producers in developing countries that fertilizer application is essential for the sustainable production of most crops, many of the more favoured agricultural lands currently under cultivation are still producing food at yield levels far below their potential. The use of chemical fertilizer must be doubled at the very least in South Asia and Latin America, and quadrupled in Sub-Saharan Africa, over the next 20 years (Conway, 1998).

A new and more modern approach is to manipulate crops, besides fertilizer application, with plant growth regulators. Treatment of crops with ComCat®, a new commercial biostimulant manufactured from plants, has improved yield and vegetative growth of many agricultural crops such as wheat, maize and some vegetables (Hüster, 1999). This product is registered with the Federal Biological research Center for Agriculture and Forestry (BBA) in Germany and other European countries, but it has not yet been used extensively in South Africa. According to Hüster (1999; personal communication), ComCat® is a general plant strengthening agent that, when applied to crops as a foliar spray or a seed treatment, increases root development, accelerates nutrient absorption, intensifies nutrient assimilation, induces flower bud formation, increases yields and induces the natural resistance of plants against pathogens.

In order to identify the optimum N requirements of a new Bangladeshi wheat cultivar, Sonalica, the crop was fertilized under greenhouse conditions with different nutrient rates (chapter 3; section 3.2.3). Additionally, ComCat® was applied in two separate ways namely as a seed treatment before planting or as a foliar spray in the three leaf vegetative growth stage of seedlings, to establish the separate and combined effect of the bio-stimulant on vegetative growth and yield.

4.2 Materials and methods

All methods applied in this chapter have been described previously (see chapter 3; section 3.1 and 3.2)

4.3 Results

4.3.1 The effect of a low N level and different ComCat® treatments on the vegetative growth of bread wheat (cv. Sonalica) under greenhouse conditions

Seed treatment with ComCat® before sowing as well as the foliar spray treatment at the three leaf growth stage (stage 13) showed no significant increase in plant height (figure 4.1A) at growth stage 73. However, at the same growth stage, a statistically significant increase in above soil part fresh mass (Figure 4.1B) was observed in plants treated with ComCat® both as a foliar spray and a seed treatment, as compared to the untreated control. Moreover, the aerial part fresh mass of plants treated with ComCat® by means of a foliar spray was significantly higher than that of plants cultivated from treated seeds. The same tendency was observed at stage 52, but there were no marked differences at growth stages 13 and 33 (Figure 4.1B).

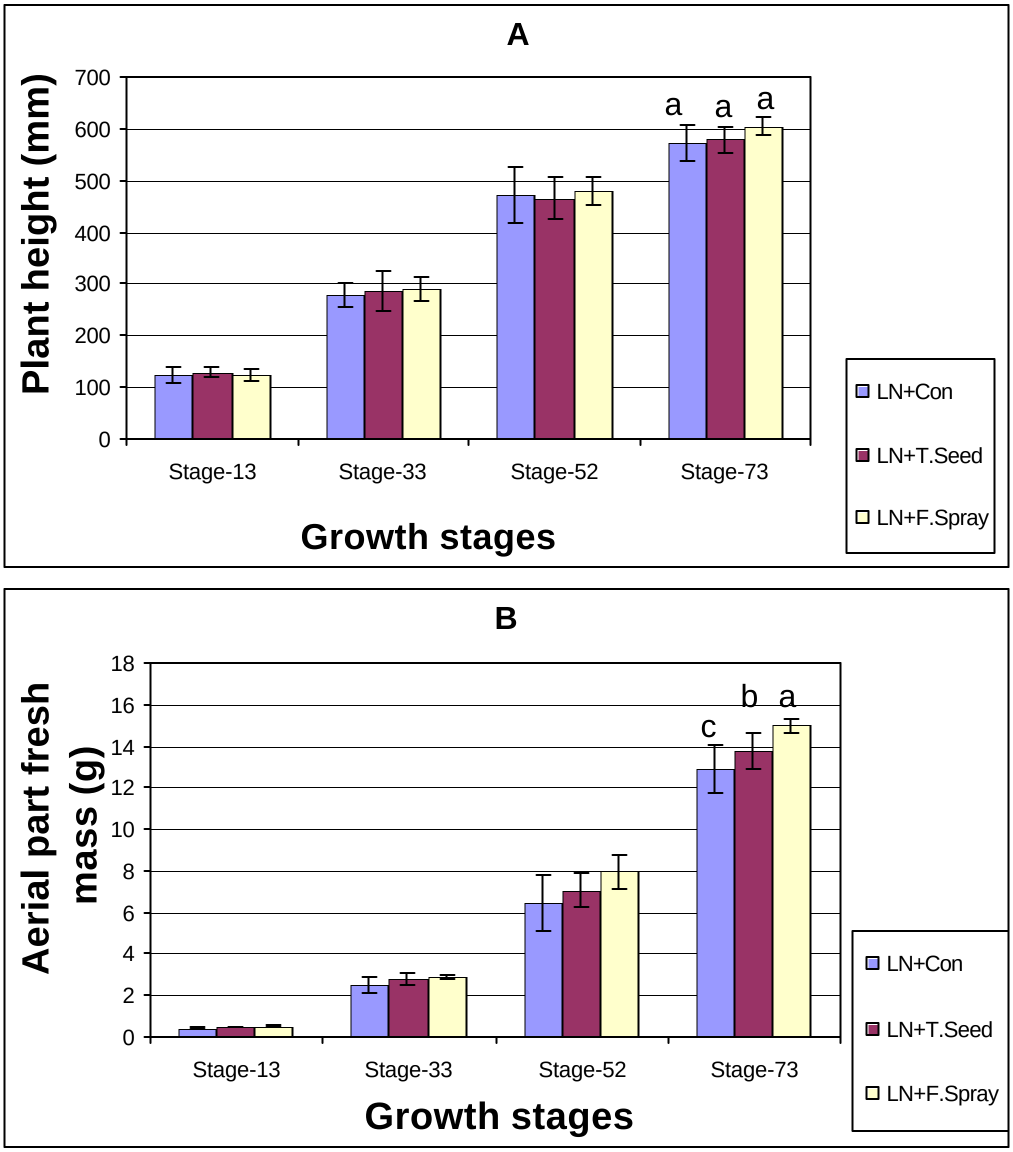

Figure 4.1: The effect of treating bread wheat (cv. Sonalica) with ComCat®, a natural commercial biostimulant, at a low N level by either treating the seeds before planting or applying the biostimulant as a foliar spray on A) plant height and B) above soil plant part mass. Error bars represent standard deviations. Columns assigned with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant difference (MSD) statistical procedure.

A similar tendency as was observed for the effect of ComCat® on the aerial part fresh mass of wheat fertilized at a low nutrient rate (Figure 4.1B), was observed for the aerial part dry mass (Figure 4.2) at growth stage 73.

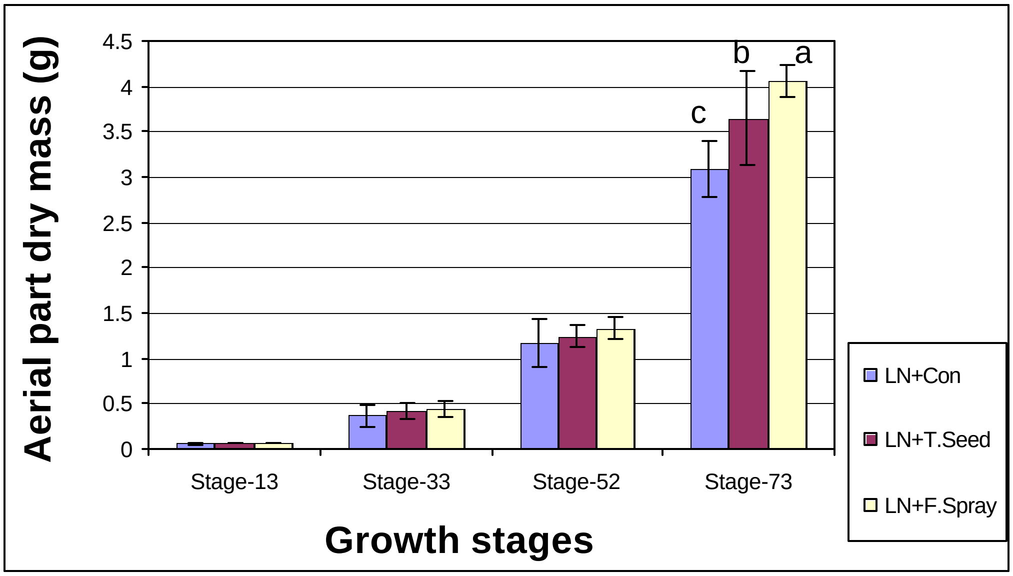

Figure 4.2: The effect of treating bread wheat (cv. Sonalica) with ComCat®, a natural commercial biostimulant, at a low N level by either treating the seeds before planting or applying the biostimulant as a foliar spray on aerial part dry mass. Error bars represent standard deviations. Columns designed with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant difference (MSD) statistical procedure.

Both the foliar spray and seed treatments with ComCat® had a marked increasing effect on the root volume of wheat cultivated under low nutrient conditions (Figure 4.3A) at growth stages 52 and 73, compared to the untreated control. However, probably due to the large standard deviations, these differences were not statistically significant. The same tendency was observed for root fresh mass (Figure 4.3B) and root dry mass (Figure 4.4).

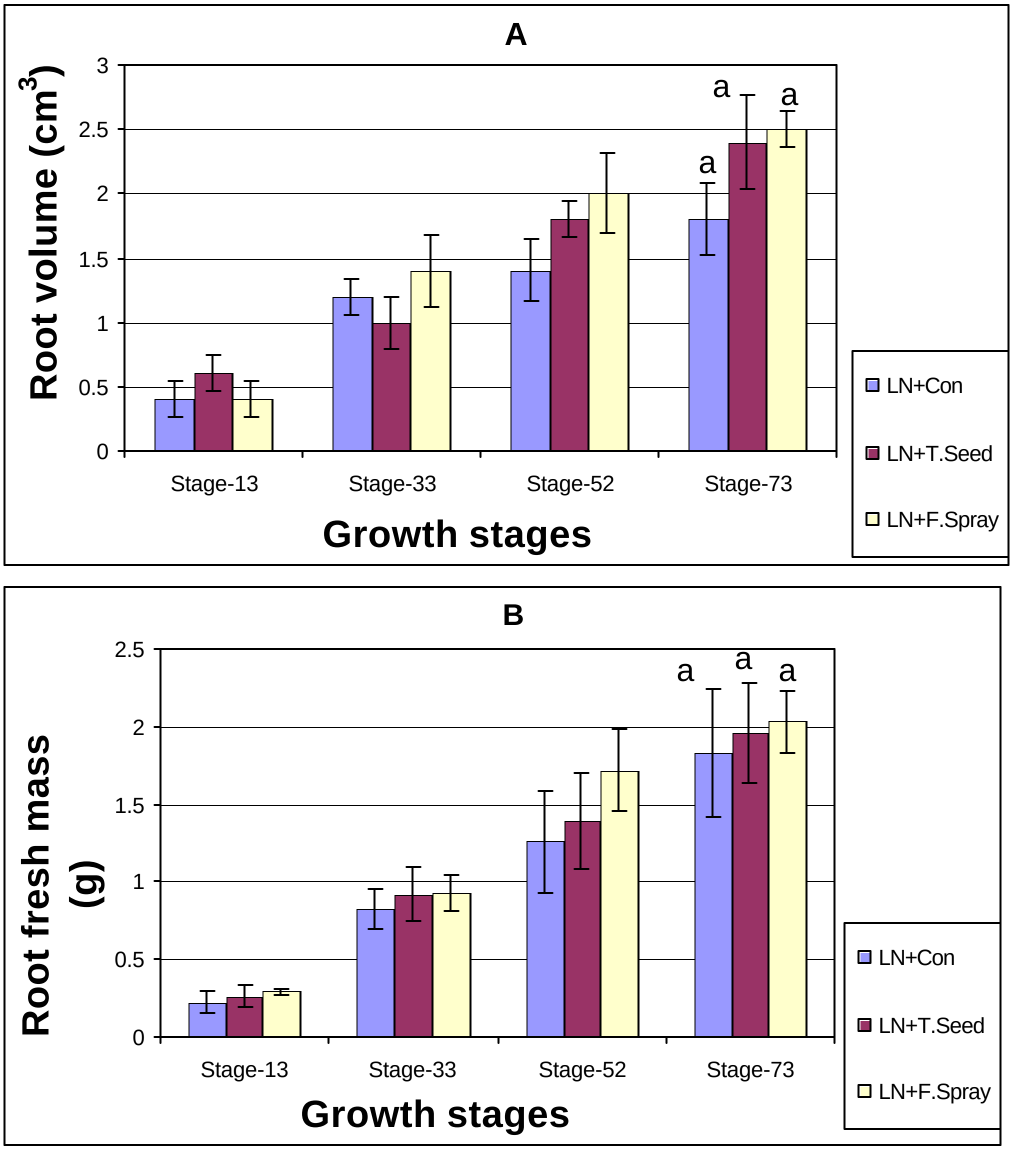

Figure 4.3: The effect of treating bread wheat (cv. Sonalica) with ComCat®, a natural commercial biostimulant, at a low N level by either treating the seeds before planting or applying the biostimulant as a foliar spray on A) root volume and B) root fresh mass. Error bars represent standard deviations. Columns designed with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant difference (MSD) statistical procedure.

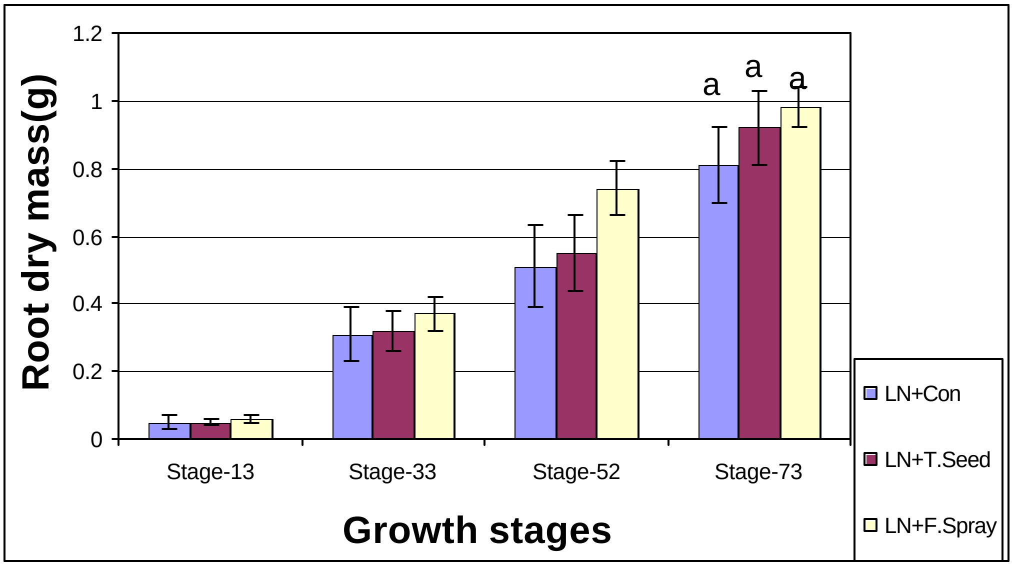

Figure 4.4: The effect of treating bread wheat (cv. Sonalica) with ComCat®, a natural commercial biostimulant, at a low N level by either treating the seeds before planting or applying the biostimulant as a foliar spray on root dry mass. Error bars represent standard deviations. Columns designed with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant difference (MSD) statistical procedure.

Neither of the ComCat® treatments had any significant effect on the leaf area at any of the vegetative growth stages (Figure 4.5).

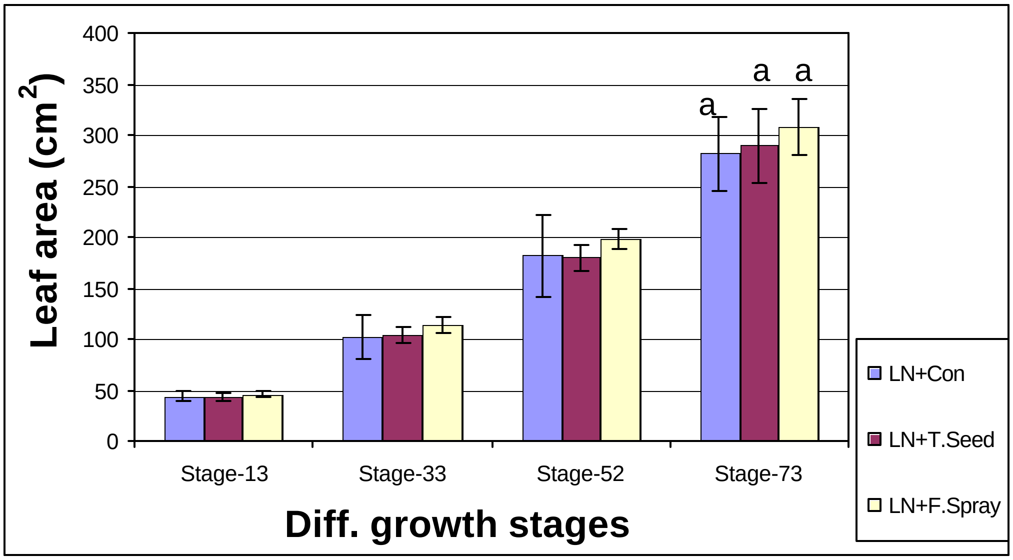

Figure 4.5: The effect of treating bread wheat (cv. Sonalica) with ComCat®, a natural commercial biostimulant, at low N, P and K levels by either treating the seeds before planting or applying the biostimulant as a foliar spray on leaf area. Error bars represent standard deviations. Columns designed with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant difference (MSD) statistical procedure.

4.3.2 The effect of a low N level and different ComCat® treatments on the final yield of bread wheat (cv. Sonalica) under greenhouse conditions

Spike formation was not induced by treatment with ComCat® as compared to the control (figure 4.6A) and neither was the number of kernels per plant (Fig. 4.6B), kernels per spike (Fig. 4.7A) nor the seed weight (Fig. 4.7B).

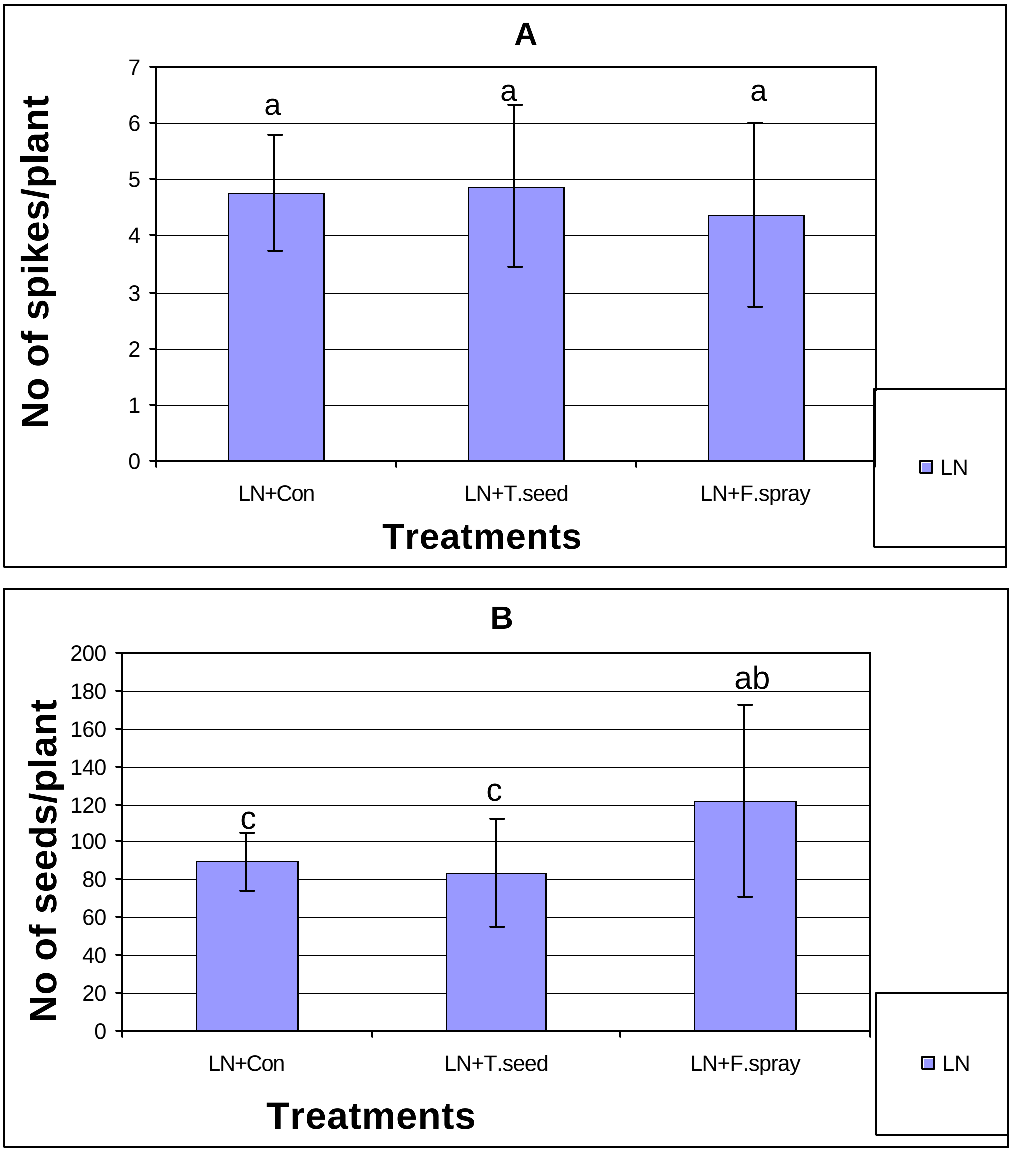

Figure 4.6: The effect of treating bread wheat (cv. Sonalica) with ComCat®, a natural commercial biostimulant, at a low N level by either treating the seeds before planting or applying the biostimulant as a foliar spray on A) number of spikes per plant and B) number of seeds per plant. Error bars represent standard deviations. Columns designed with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant difference (MSD) statistical procedure

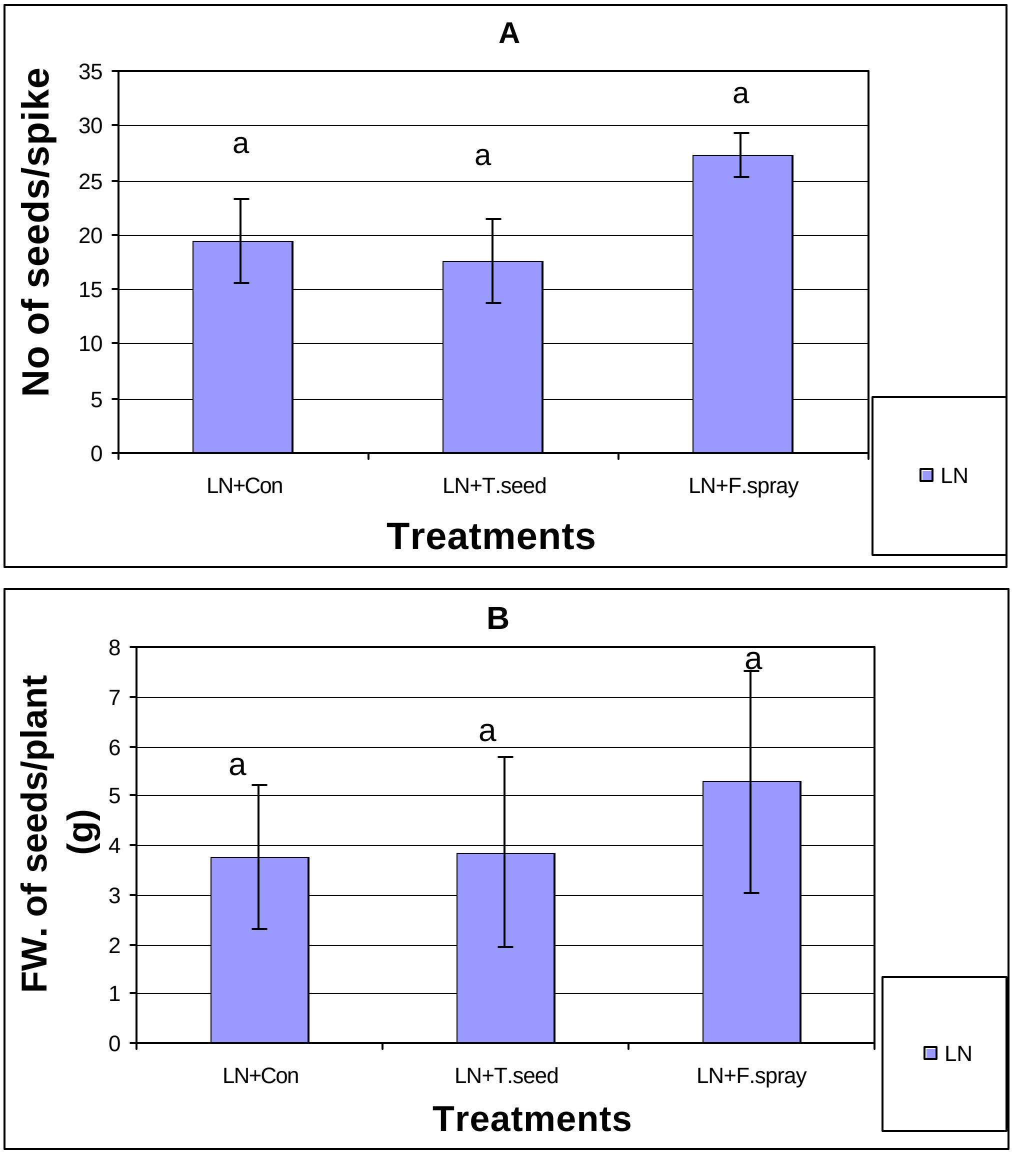

Figure 4.7: The effect of treating bread wheat (cv. Sonalica) with ComCat®, a natural commercial biostimulant, at a low N level by either treating the seeds before planting or applying the biostimulant as a foliar spray on A) number of seeds per spike and B) fresh weight of seed per plant. Error bars represent standard deviations. Columns designed with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant difference (MSD) statistical procedure

Under the influence of especially the foliar spray treatment with ComCat®, the 1000 kernel dry weight did not differ significantly from that of the untreated control (Figure 4.8). Nevertheless, both the seed and the foliar spray treatments contributed to a 10% increase in kernel dry weight.

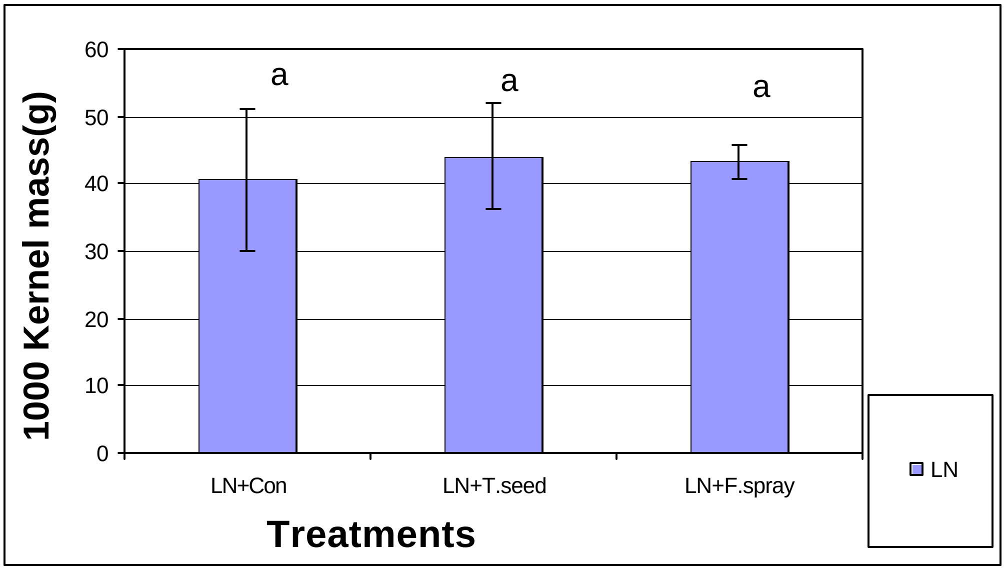

Figure 4.8: The effect of treating bread wheat (cv. Sonalica) with ComCat®, a natural commercial biostimulant, at a low N level by either treating the seeds before planting or applying the biostimulant as a foliar spray on the 1000 kernel dry mass. Error bars represent standard deviations. Columns designed with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant difference (MSD) statistical procedure.

4.3.3 The effect of medium and high N levels and different ComCat® treatments on the vegetative growth and yield of bread wheat (cv. Sonalica) under greenhouse conditions

All plants fertilized with the medium and high nutrient rates during the 2001 season failed to develop normally and died off before they reached the reproductive stage (results not shown). This indicated that the normal fertilizer rate applied to winter wheat in South Africa (applied in this study as the medium rate) was already too high for the Bangladeshi cultivar (Sonalica) investigated in this study.

4.4 Discussion

In this study, attempts were made to investigate the growth and yield performance of a Bangladeshi bread wheat cultivar (Sonalica) under the influence of three different nitrogen fertilizer levels and one concentration level of ComCat$^{®}$ applied either as a seed treatment before planting or as a foliar spray on seedlings at the 3-leaf growth stage. Initially the South African (RSA) standard N-level for winter wheat was taken as the medium N-level together with a down scaled (low) and an up scaled rate (high) during the 2001 season in order to ascertain the optimum level. However, it was observed that the Bangladeshi cultivar could not tolerate the RSA standard (medium rate) for nitrogen application. After germination all plants fertilized with the medium and high nitrogen rates, died off. Subsequently, data on vegetative growth and yield could only be obtained from plants fertilized at the down scaled (low) N-level.

At all growth stages monitored (stages 13, 33, 52 and 73), plants treated with ComCat$^{®}$ either before planting as a seed treatment or as a foliar spray in the 3-leaf seedling stage, tended to enhance the growth and yield of bread wheat as compared to the untreated (only fertilized) control. As it was noticed that the growth pattern of this wheat cultivar was similar for the growth stages of all treatments, only growth stage 73 was chosen for statistical data analysis. Of the two ComCat$^{®}$ treatments, the foliar spray treatment constantly had a statistically significant effect on the plants in terms of increasing most of the vegetative parameters monitored, including aerial part fresh and dry mass as well as root volume and root dry mass. Additionally, especially the foliar spray treatment increased the fresh weight of kernels by >30% and the 1000 kernel dry mass by 10% compared to the untreated control. Although these differences were not statistically significant, probably due to the large standard deviations between replicates, the percentage yield increase observed strongly indicates that the simultaneous application of the commercial bio-stimulant ComCat$^{®}$, together with a rather low N-level, can be a promising enterprise in increasing the production of wheat on the available arable land.

The observed enhancement of root fresh mass and root volume under the influence of ComCat®, was in agreement with the findings of Pretorius (1998; personal communication) in a South African wheat cultivar (SST825) over three seasons under field conditions. This strongly indicates that the effect of the commercial bio-stimulant on the final yield of wheat can be via the induction of root development and the subsequent improvement of nutrient uptake from the soil.

In considering the individual effects of ComCat®, it generally appeared that the application of this bio-stimulant contributed less to the vegetative growth of the wheat plants at earlier growth stages (13 and 33). Most of the remarkable differences were observed at growth stages 52 and 73. At the latter growth stage, the application of ComCat® appeared to have the most significant impact on shoot and root growth. This is difficult to explain, but is probably related to induction of growth at earlier growth stages that only manifests itself at later growth stages.

According to the manufacturers of ComCat® (Hüster, 1999), a group of newly discovered phytohormones, known as brassinosteroids (BR's; Schnabl *et al*., 2001) is one the active substances. BR's stimulate root growth (Davidtchuck, 1999) in crops and increases the yield as well as starch and vitamin C content in potatoes (Khripach *et al*, 2000). Krishnan *et al.* (1999) reported that rice treated with foliar sprays of BR's resulted in the highest number of fertile tillers, filled spikelets and 1000 kernel weight as compared to treatments with either GA_3 or Kinetin.

Due to the fact that the bread wheat cultivar under investigation only survived in the lowest level of nitrogen fertilizer applied during the 2001 season, it was decided to take this level as the medium level for the 2002 trial. A new down scaled (low) and up scaled (high) N-level was calculated (see chapter 3; Table 3.1) and applied together with the two ComCat® treatments (seed and foliar treatments) at the three-leaf growth stage (stage 13) of wheat.

CHAPTER 5

EFFECT OF LOWER NITROGEN FERTILIZER LEVELS AND COMCAT® ON THE VEGETATIVE GROWTH AND YIELD OF A BANGLADESHI BREAD WHEAT CULTIVAR UNDER GLASSHOUSE CONDITIONS DURING THE 2002 GROWING SEASON

5.1 Introduction

The response to nitrogen is crop specific and can also be altered by plant density (Boiffin et al., 2001). This emphasises the necessity to ascertain the fertilizer requirements of crops, especially in the case of new cultivars, in order to optimize the fertilizer applications of the crop. Moreover, in the event of a bio-stimulant applied in combination with different fertilizer levels, especially if root growth stimulation is claimed by the manufacturers, it is possible that the fertilizer requirements of the crop might be altered. In this light it will be essential to monitor the crop response in terms of growth and yield under the influence of the combined treatment. This study was an attempt to address this issue.

During the 2001 season, the RSA fertilizer standard was used initially in an attempt to ascertain the fertilizer requirements of a new Bangladeshi bread wheat cultivar, Sonalica. However, after germination all seedlings that received both the RSA standard (medium) and a higher nitrogen rate died off. Only plants that received nitrogen at a rate lower than the RSA standard, survived. For this reason, nitrogen fertilizer rates were modified during the 2002 season. The low N-level used during the 2001 season was applied as a medium rate during the 2002 season, while a new down scaled (low) and up scaled (high) N-level was applied. The two ComCat® treatments (seed treatment before planting as well as a foliar spray treatment at growth stage 13) was applied at the same concentrations as during the 2001 season.

5.2 Materials and methods

All methods applied in this chapter have been described previously (see chapter 3; Section 3.1 and 3.2). Only the fertilizer rates were modified (see Chapter 3, table 3.1).

5.3 Results

5.3.1 The effect of three different N-fertilizer levels and a ComCat® seed treatment before planting on the vegetative growth of bread wheat (cv. Solanica), at growth stage 73, under greenhouse conditions

A significant difference in plant height was observed between plants treated with different N-levels (low, medium and high) at growth stage 73 (figure 5.1A, B and C) where the low application rate tended to enhance growth in length while the two higher rates (medium and high) had the opposite effect. The ComCat® seed treatment had no effect on plant height except that, in the case of the combined treatment at the highest N-rate, plant height was slightly reduced compared to the untreated control (figure 5.1C).

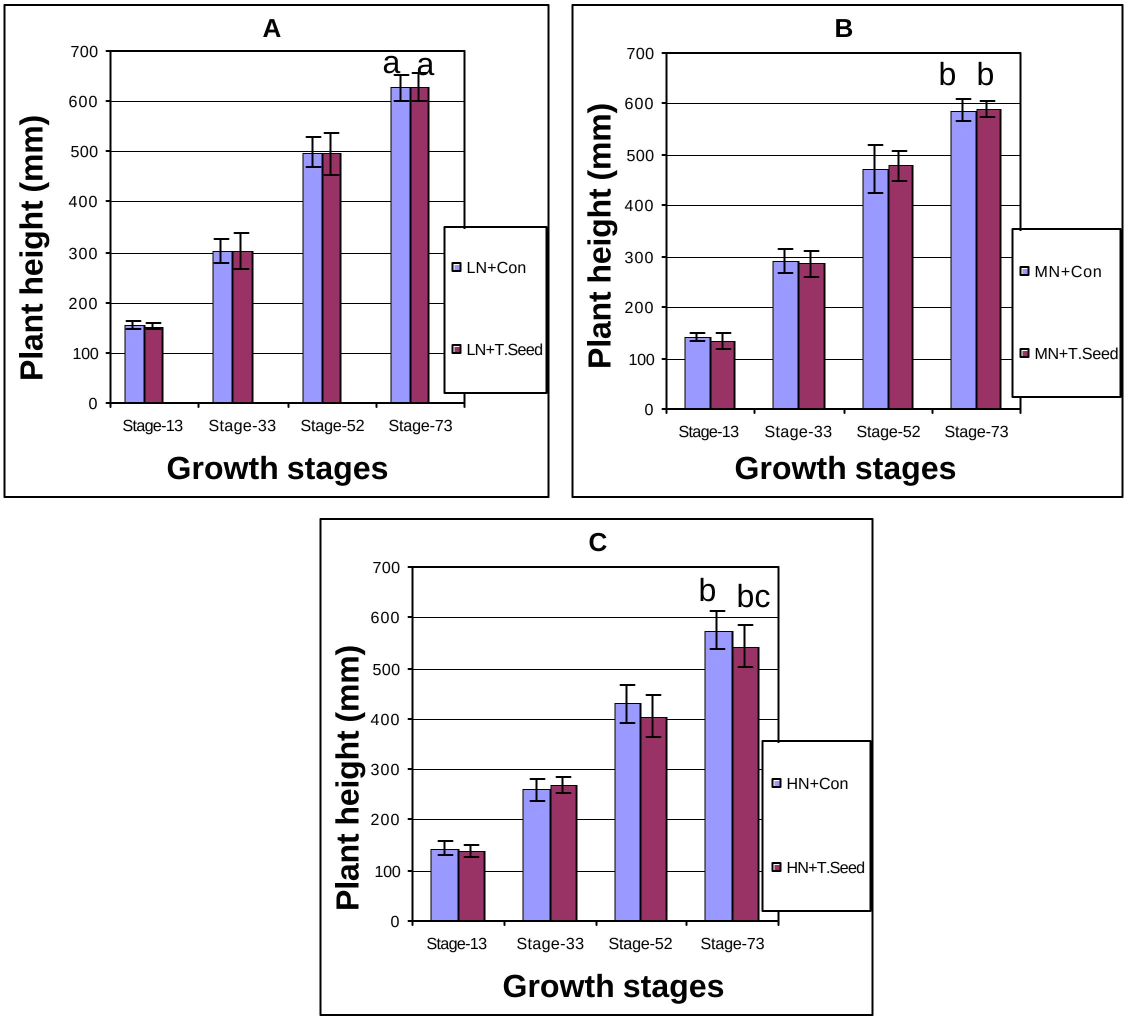

Figure 5.1: The effect of a seed treatment with ComCat® before planting on plant height of bread wheat cultivated at A) a low B) a medium and C) a high N-level. Error bars represent standard deviations. Columns designated with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant Differences (MSD) statistical procedure.

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The aerial part fresh mass (figure 5.2A, B, C) followed a similar trend as for plant height (figure 5.1) in the sense that an increase in the N-level application tended to decrease the fresh mass at all growth stages in the absence of ComCat®. Although not statistically significant, the ComCat® seed treatment contributed to a slight increase in aerial part fresh mass, especially during growth stages 52 and 73, in the case of the low and medium N-level applications (figure 5.2A and B).

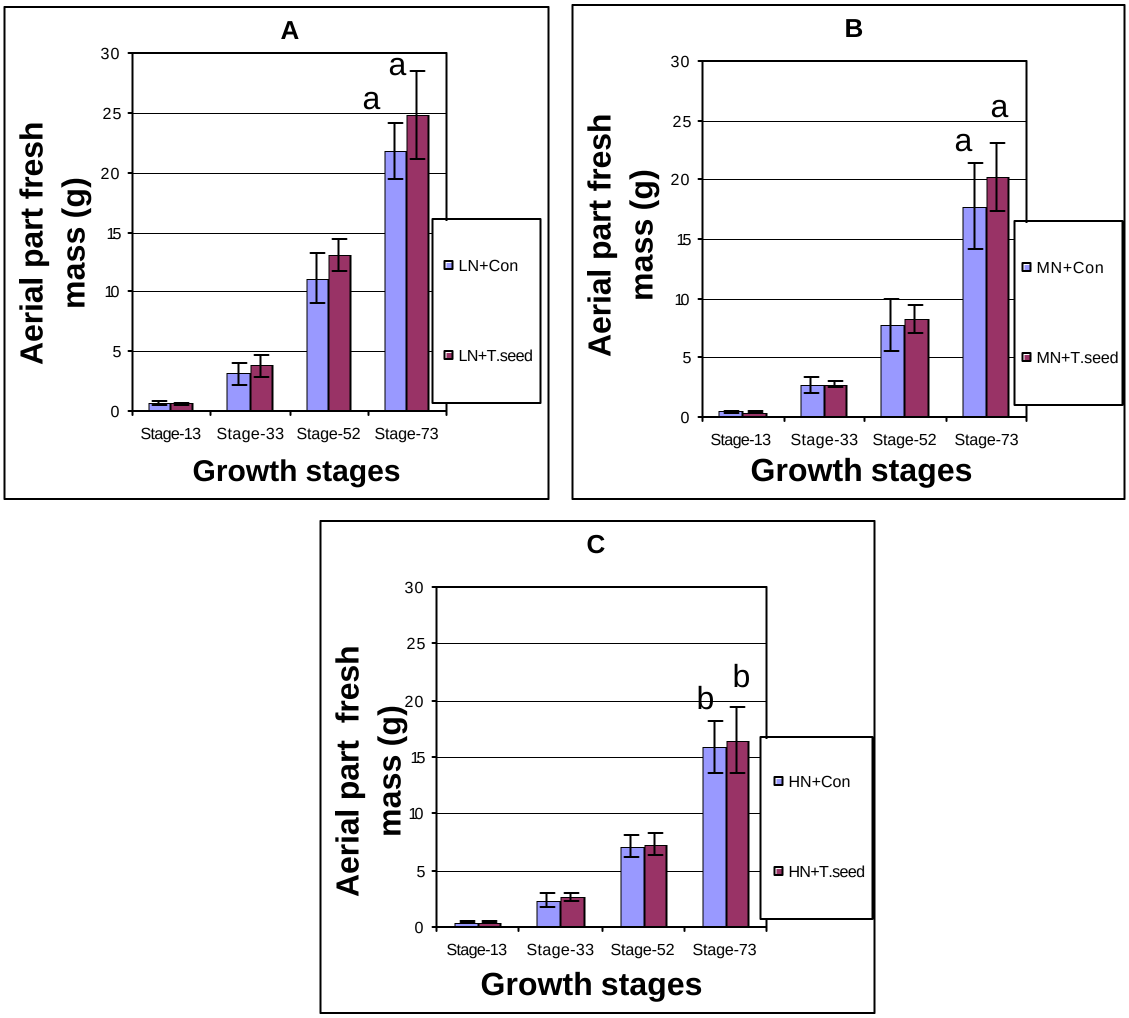

Figure 5.2: The effect of a seed treatment with ComCat® before planting on aerial part fresh mass of bread wheat cultivated at A) a low B) a medium and C) a high N-level. Error bars represent standard deviations. Columns designated with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant Differences (MSD) statistical procedure.

The effect of different N-level applications as well as the combined effect of a ComCat® seed treatment on aerial part dry mass (figure 5.3A, B and C) followed a similar pattern as was observed for aerial part fresh mass (figure 5.2). Again the low N-level, both separate (41.5%) and in combination with the ComCat® seed treatment (45.8%), resulted in an increased accumulation of aerial part dry mass compared to the medium N-level application. However, probably due to the larger large standard deviations, these differences were not statistically significant.

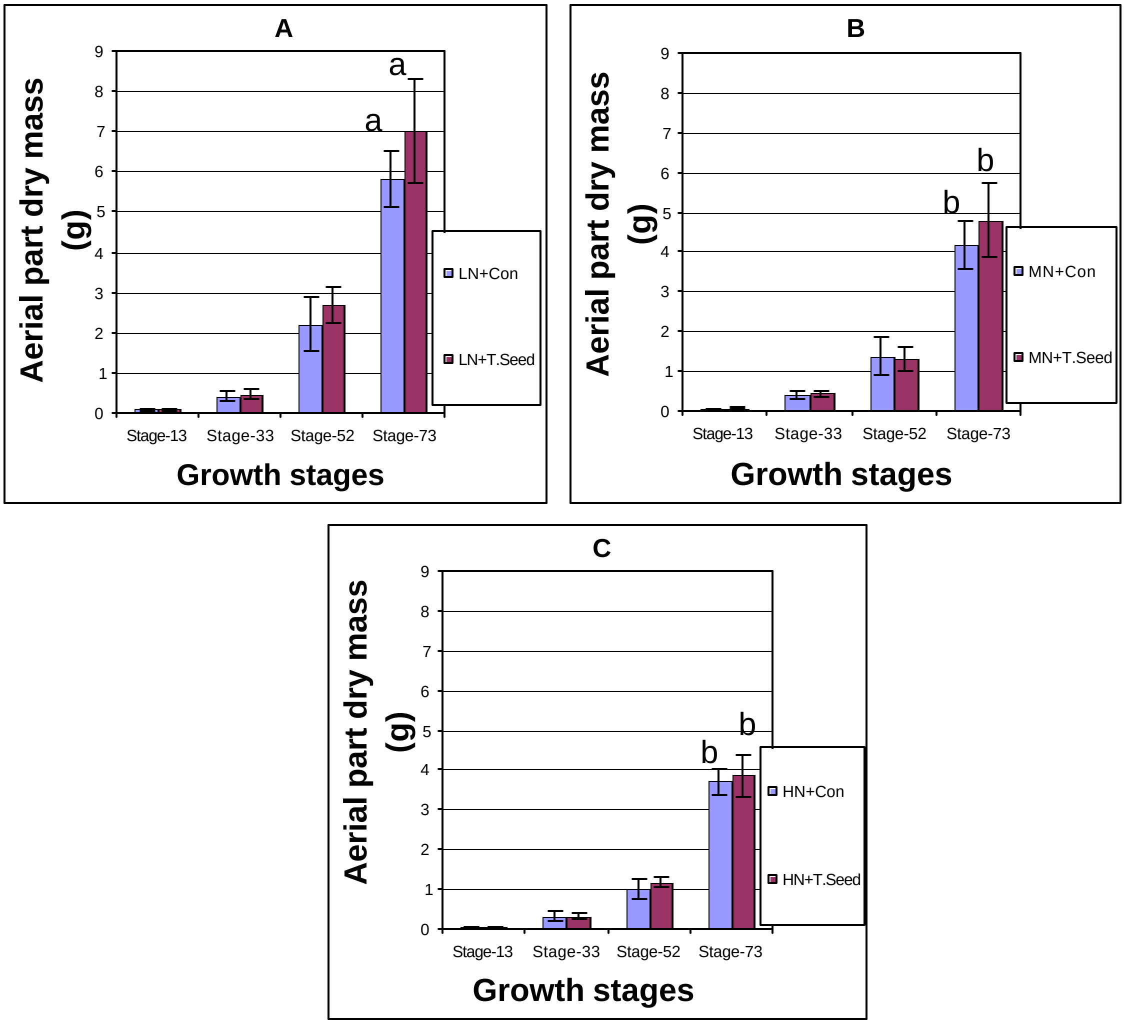

Figure 5.3: The effect of a seed treatment with ComCat® before planting on aerial part dry mass of bread wheat cultivated at A) a low B) a medium and C) a high N-level. Error bars represent standard deviations. Columns designated with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant Differences (MSD) statistical procedure.

In al cases, although not statistically significant, the ComCat ® seed treatment increased the root fresh mass (figure 5.4A, B and C), but more markedly at the low N-level application. No significant differences in root fresh mass were observed in plants cultivated at different N-levels (low, medium and high).

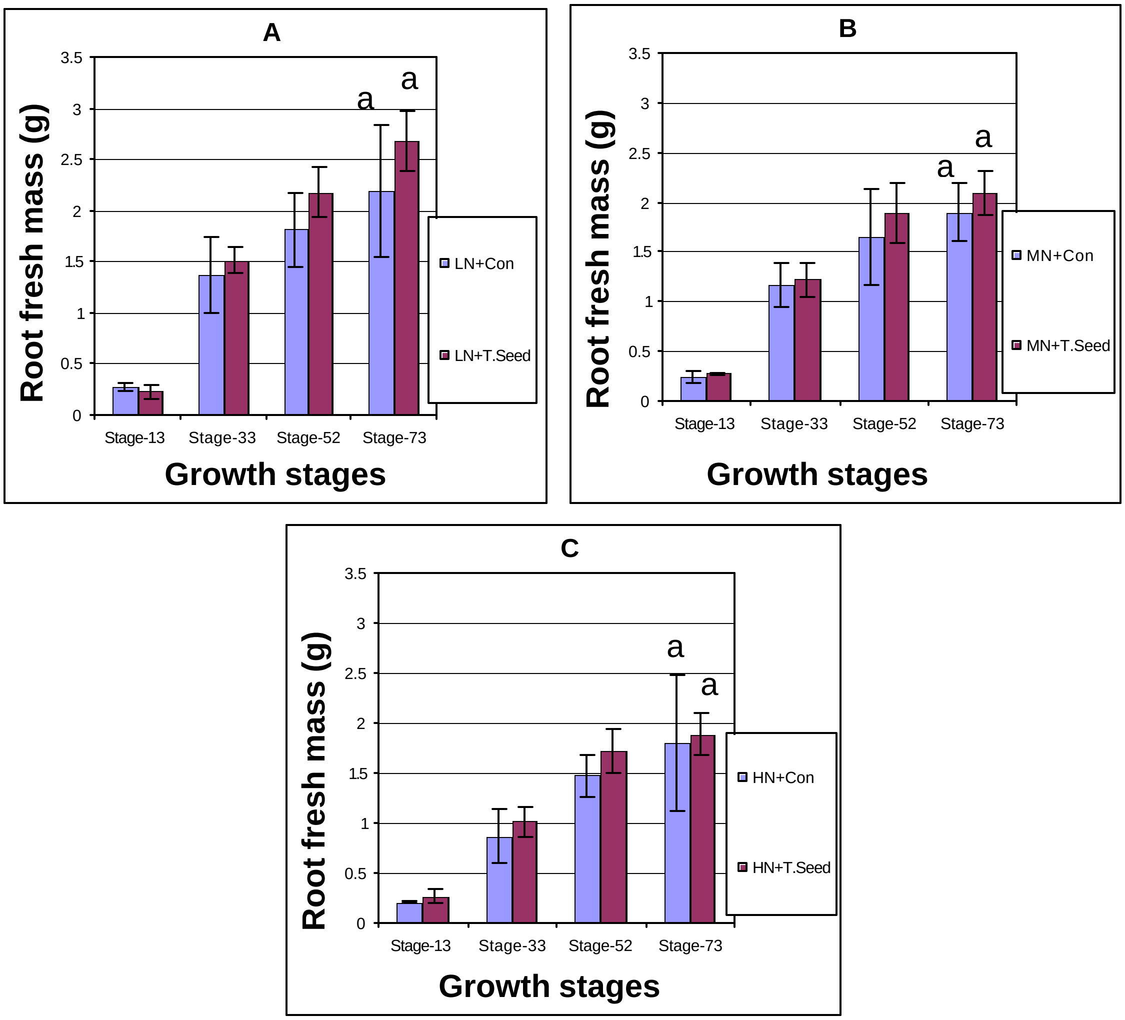

Figure 5.4: The effect of a seed treatment with ComCat® before planting on root fresh mass of bread wheat cultivated at A) a low B) a medium and C) a high N-level. Error bars represent standard deviations. Columns designated with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant Differences (MSD) statistical procedure.

Root dry mass accumulation (figure 5.5A, B and C) followed a similar trend as was observed for root fresh mass (figure 5.4)

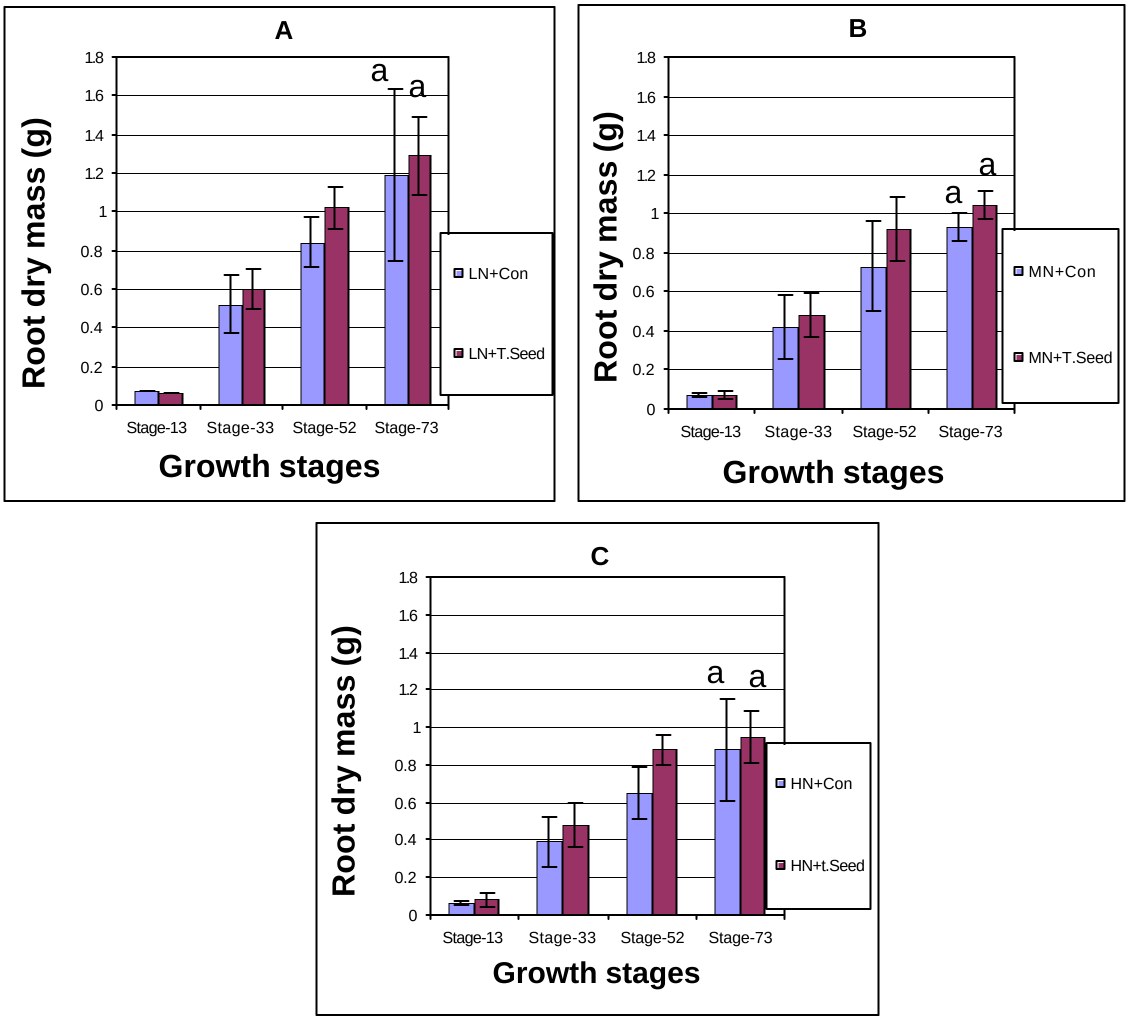

Figure 5.5: The effect of a seed treatment with ComCat® before planting on root dry mass of bread wheat cultivated at A) a low B) a medium and C) a high N-level. Error bars represent standard deviations. Columns designated with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant Differences (MSD) statistical procedure.

In terms of root volume, the analysis of variance statistical procedure revealed significant differences between N-treatments (low, medium and high) and applications (untreated control and ComCat® treated seed; figure 5.6A, B and C). At the low N-level, the root volume of plants cultivated from both untreated and ComCat® treated seeds differed significantly from that of plants cultivated at the medium and high N-levels.

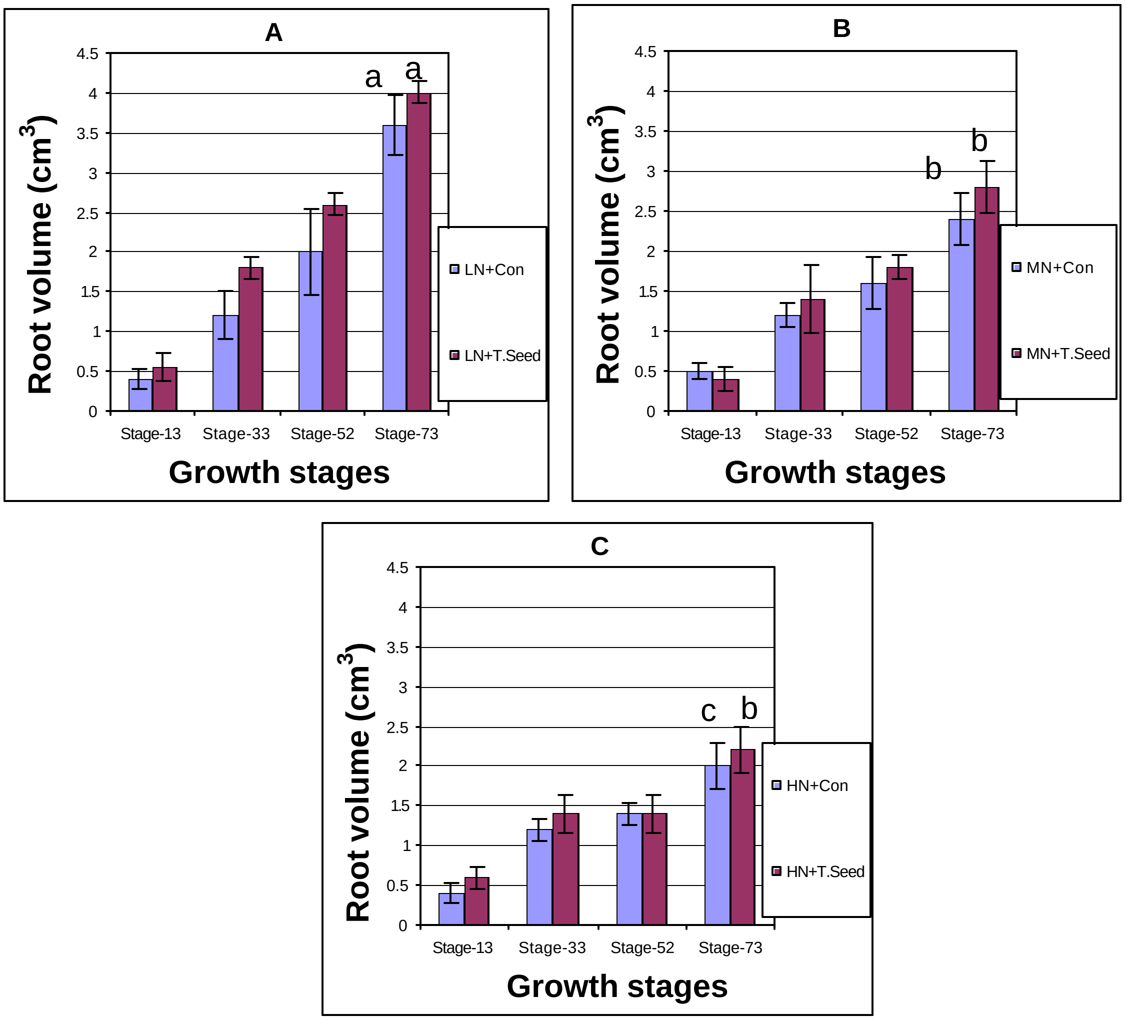

Figure 5.6: The effect of a seed treatment with ComCat® before planting on rot volume of bread wheat cultivated at A) a low B) a medium and C) a high N-level. Error bars represent standard deviations. Columns designated with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant Differences (MSD) statistical procedure.

Although not as defined, the same pattern as was observed by means of other growth parameters was observed for the influence of elevated N-levels on leaf area. In plants cultivated at the highest N-level, the reduction in leaf area was significant compared to that of plants grown in the presence of the least nitrogen fertilizer (figure 5.7A, B and C). The ComCat ® seed treatment had either no effect or slightly reduced the leaf area of plants as the N-level under which they were cultivated increased.

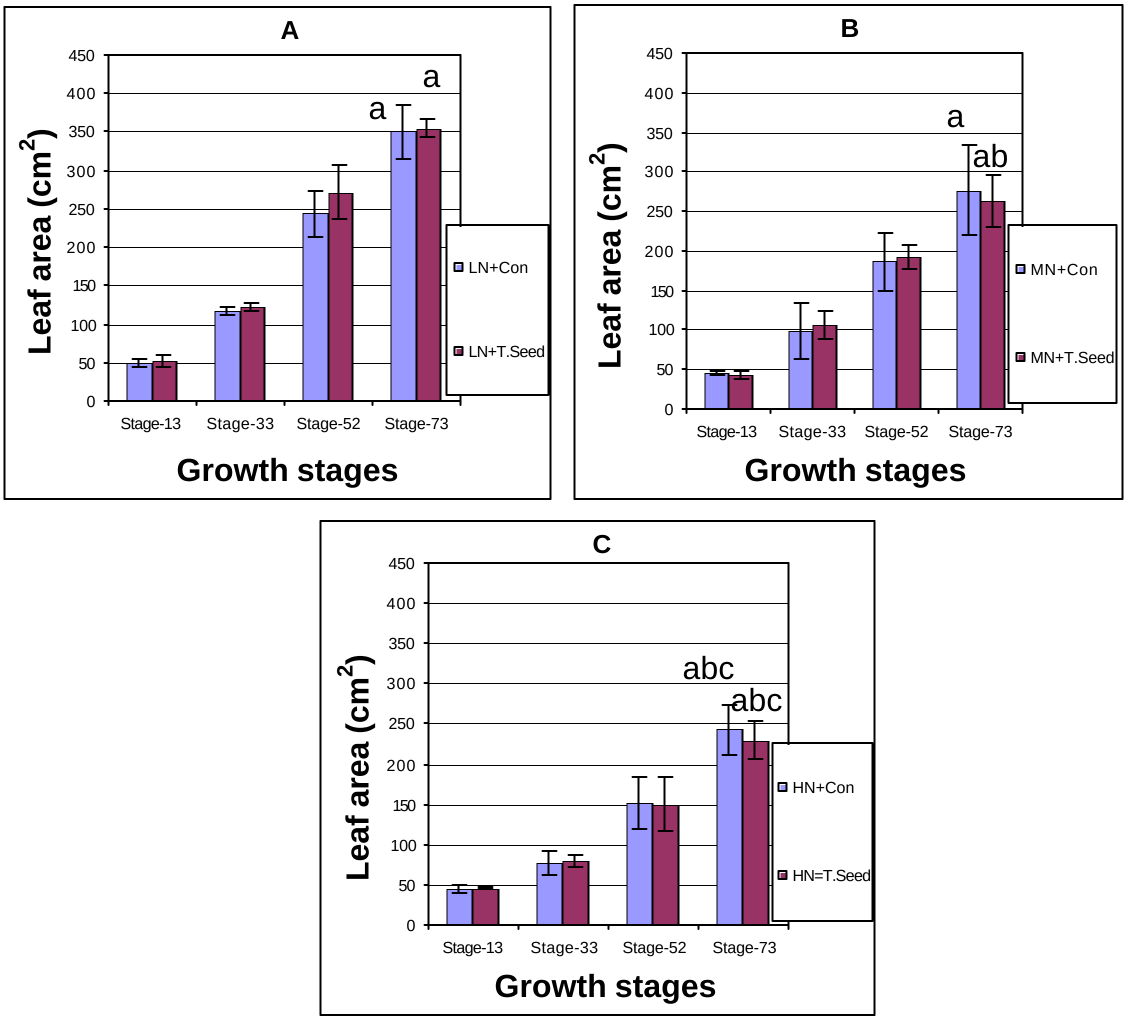

Figure 5.7: The effect of a seed treatment with ComCat® before planting on leaf area of bread wheat cultivated at A) a low B) a medium and C) a high N-level. Error bars represent standard deviations. Columns designated with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant Differences (MSD) statistical procedure.

5.3.2 The effect of three different N-fertilizer levels and a ComCat® foliar spray treatment on the vegetative growth of bread wheat (cv. Sonalica), at growth stage 73, under greenhouse conditions

As was observed in the previous trial, elevated nitrogen fertilization tended to decrease plant growth in terms of height, but statistically these differences were non-significant (figure 5.8A, B and C). A ComCat® foliar spray treatment had no effect on plant height at none of the three different N-levels at which plants were cultivated.

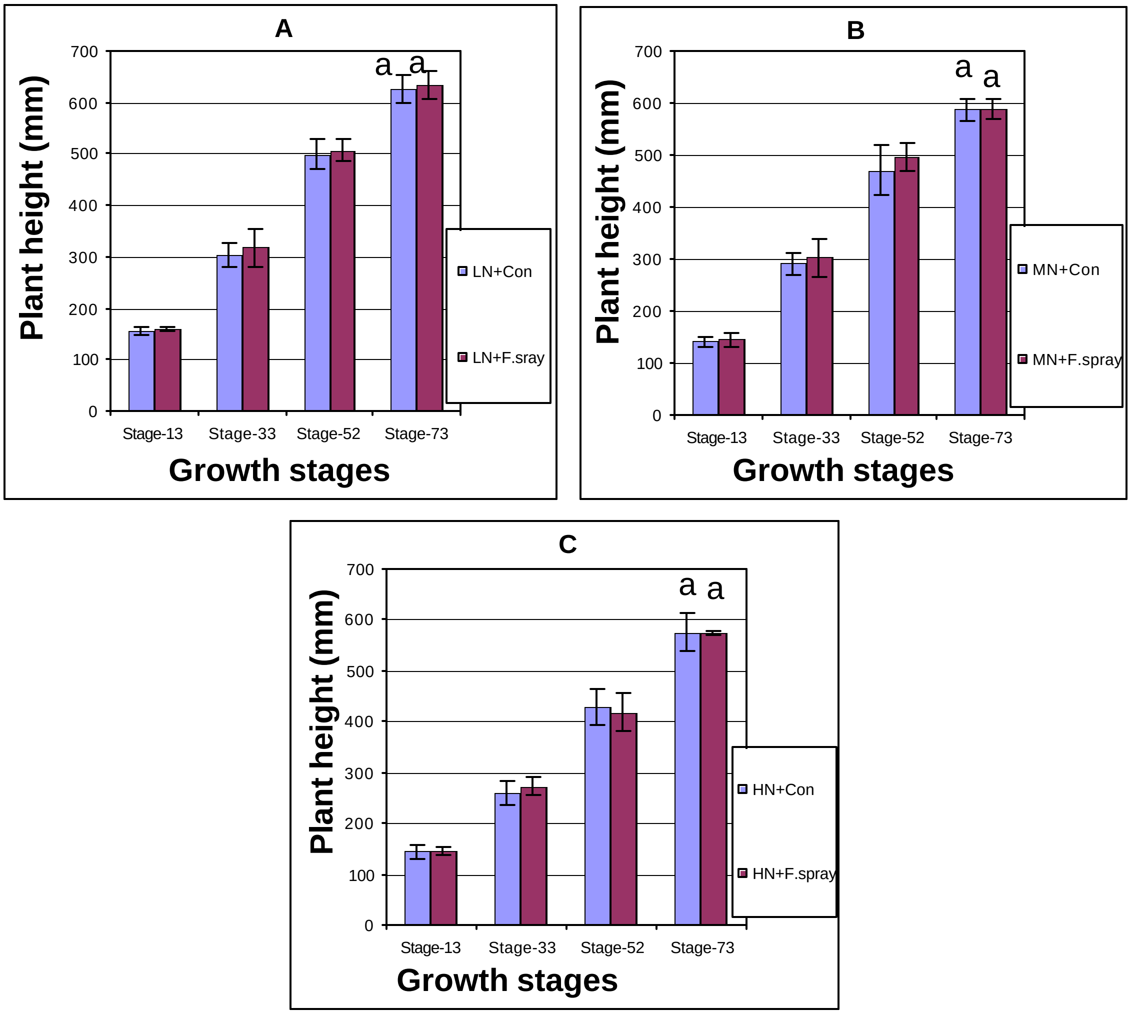

Figure 5.8: The effect of a foliar spray treatment with ComCat® at growth stage 13 on plant height of bread wheat, cultivated at A) a low B) a medium and C) a high N-level, at later growth stages. Error bars represent standard deviations. Columns designated with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant Differences (MSD) statistical procedure.

Interestingly, the analysis of variance statistical procedure revealed significant differences between both nitrogen applications and the ComCat® foliar spray treatment at different N-levels in terms of aerial part fresh mass (figure 5.9A, B and C). The same pattern of reduction in aerial part fresh mass, with increased N-fertilization, as was observed earlier (fig 5.2), was confirmed. In all cases, plants treated with ComCat® by means of a foliar spray, showed a slight increase in aerial part fresh mass compared to untreated plants and this was accentuated in plants cultivated in the lowest N-level (+18.2%).

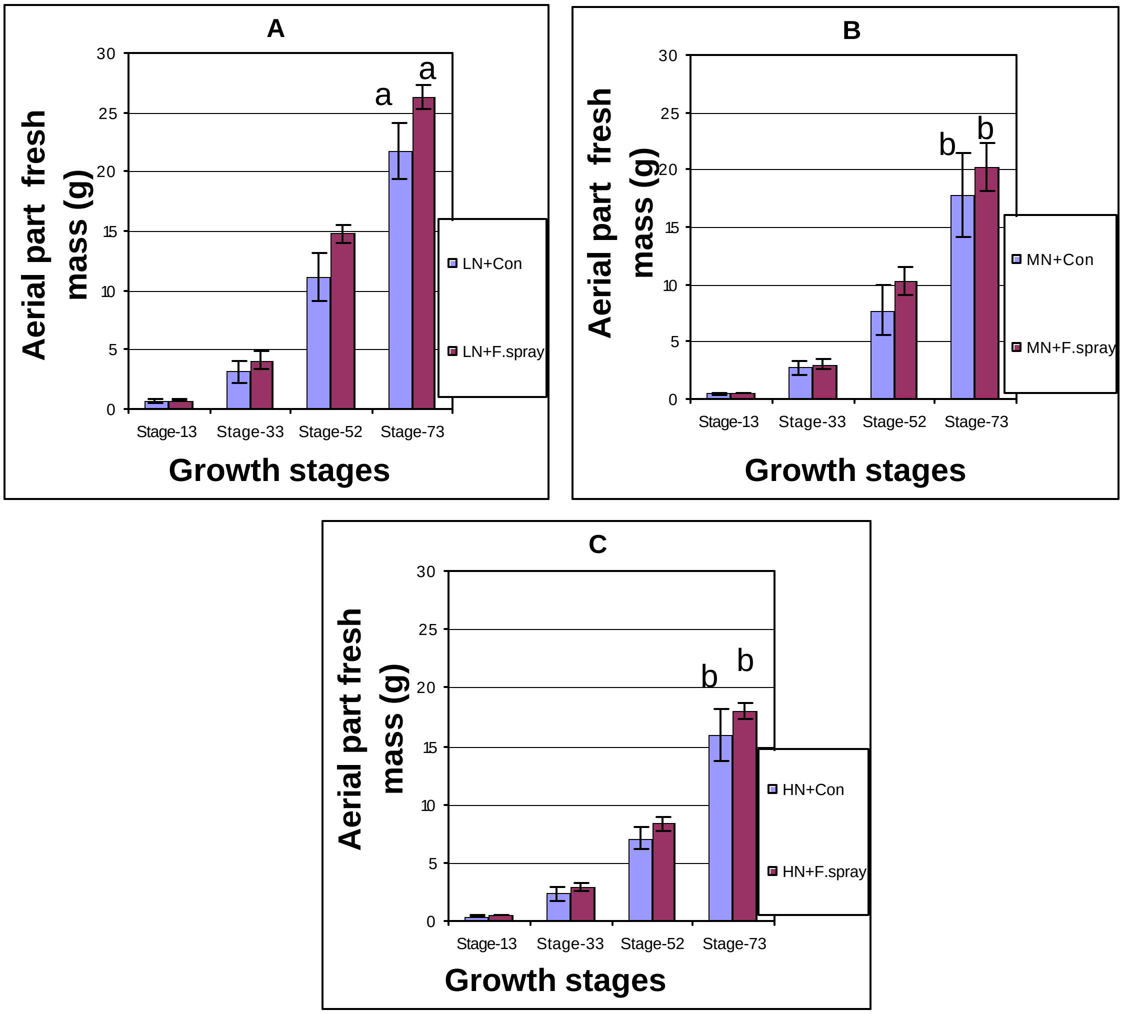

Figure 5.9: The effect of a foliar spray treatment with ComCat® at growth stage 13 on arial part fresh mass of bread wheat, cultivated at A) a low B) a medium and C) a high N-level, at later growth stages. Error bars represent standard deviations. Columns designated with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant Differences (MSD) statistical procedure.

The response of both untreated and ComCat® treated plants followed a similar pattern in terms of aerial part dry mass (figure 5.10A, B and C) as was the case for aerial part fresh mass (figure 5.9). Statistically, an increase in N-level applications had a significant reducing effect on aerial part dry mass. Although plants treated with ComCat® as a foliar spray tended to increase in aerial part dry mass, this was not statistically significant).

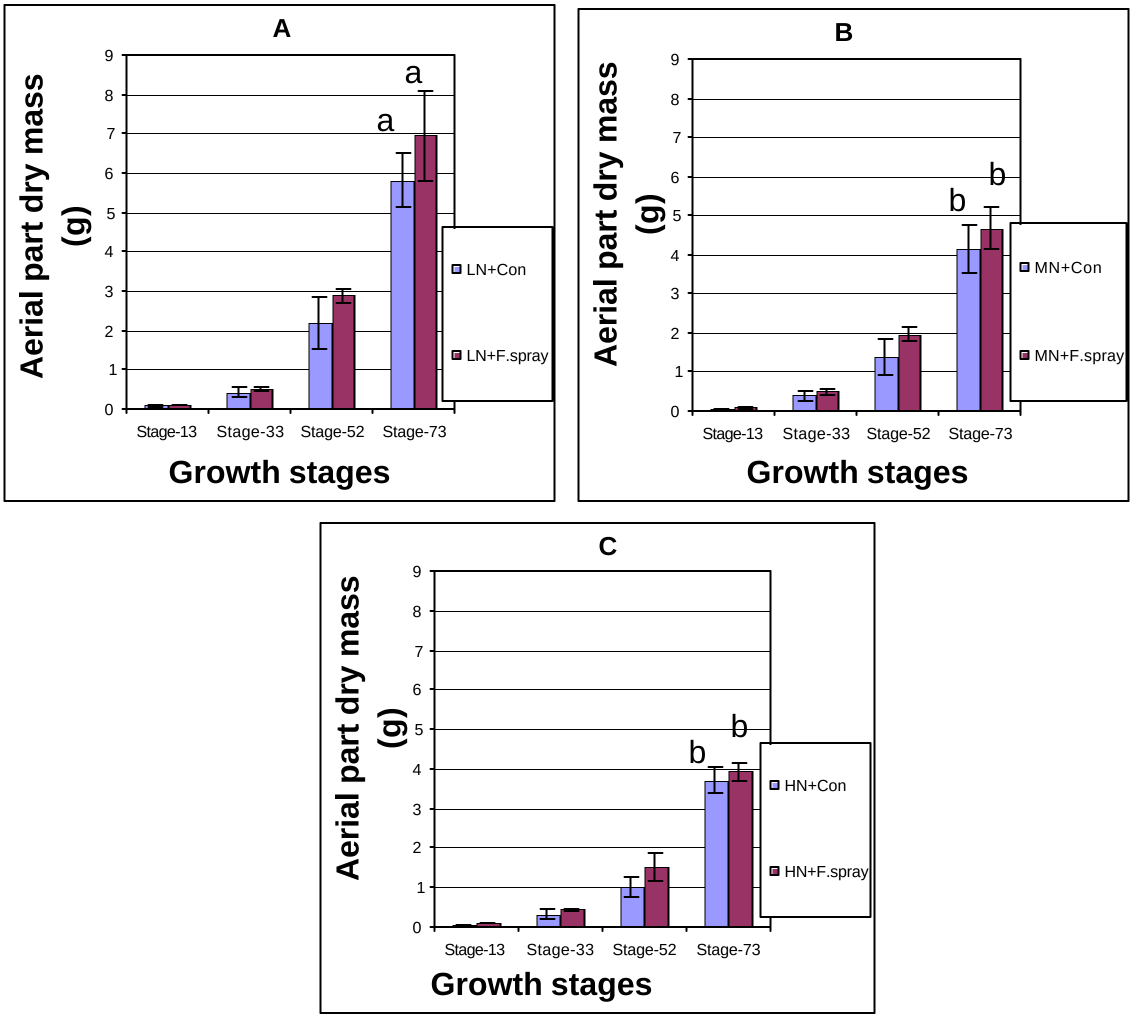

Figure 5. 10: The effect of a foliar spray treatment with ComCat® at growth stage 13 on aerial part dry mass of bread wheat, cultivated at A) a low B) a medium and C) a high N-level, at later growth stages. Error bars represent standard deviations. Columns designated with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant Differences (MSD) statistical procedure.

The ComCat® foliar spray treatment increased the root fresh mass of plants grown under low nitrogen application by 28,9% (figure 5.11A). Probably due to the high standard deviations between replicates this was not statistically significant. Although not as marked, the same tendency was observed in plants cultivated under medium (figure 5.11B) and high (figure 5.11C) nitrogen applications.

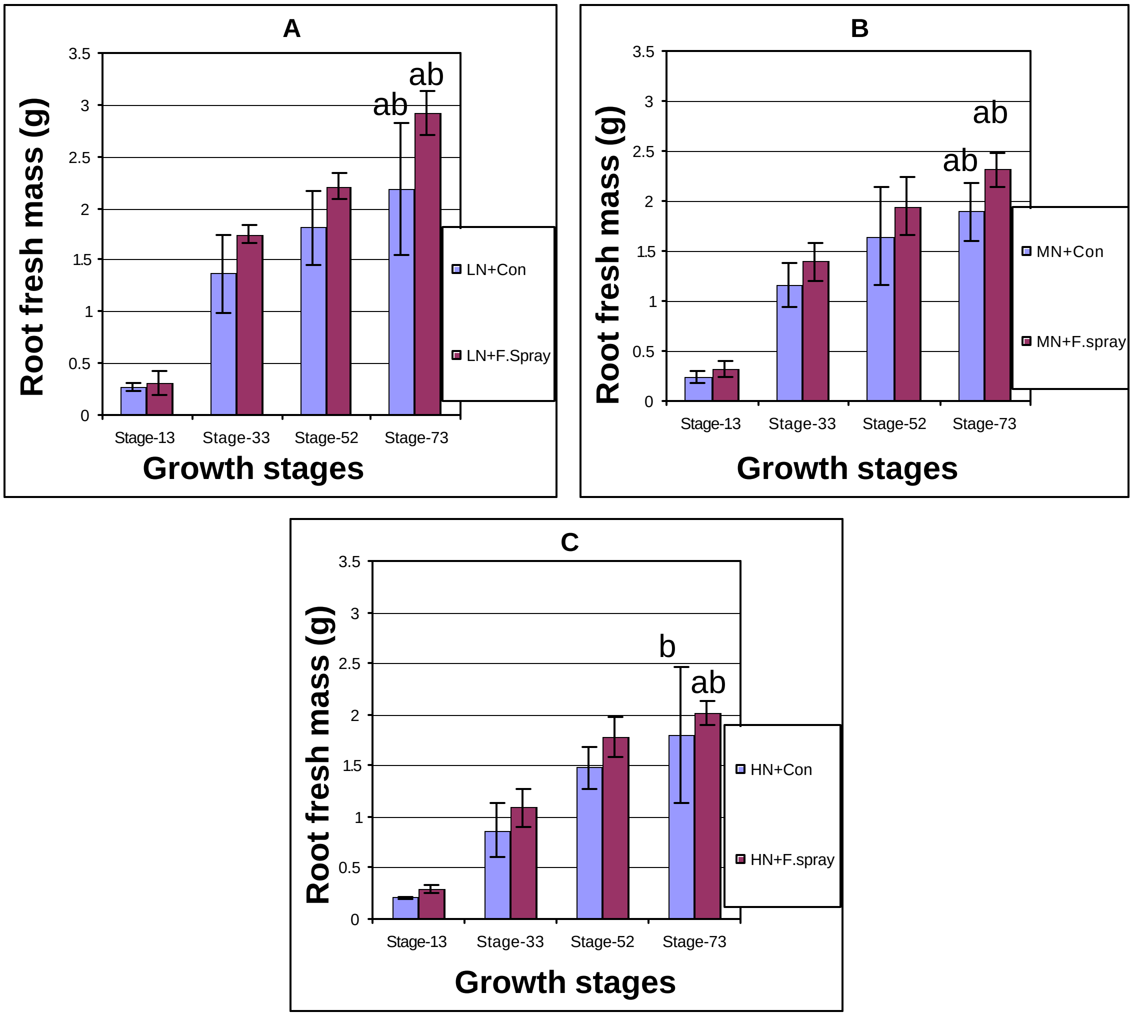

Figure 5. 11: The effect of a foliar spray treatment with ComCat® at growth stage 13 on root fresh mass of bread wheat, cultivated at A) a low B) a medium and C) a high N-level, at later growth stages. Error bars represent standard deviations. Columns designated with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant Differences (MSD) statistical procedure.

Exactly the same tendency as was observed for root fresh mass applied for accumulation of root dry mass (figure 5.12A, B and C).

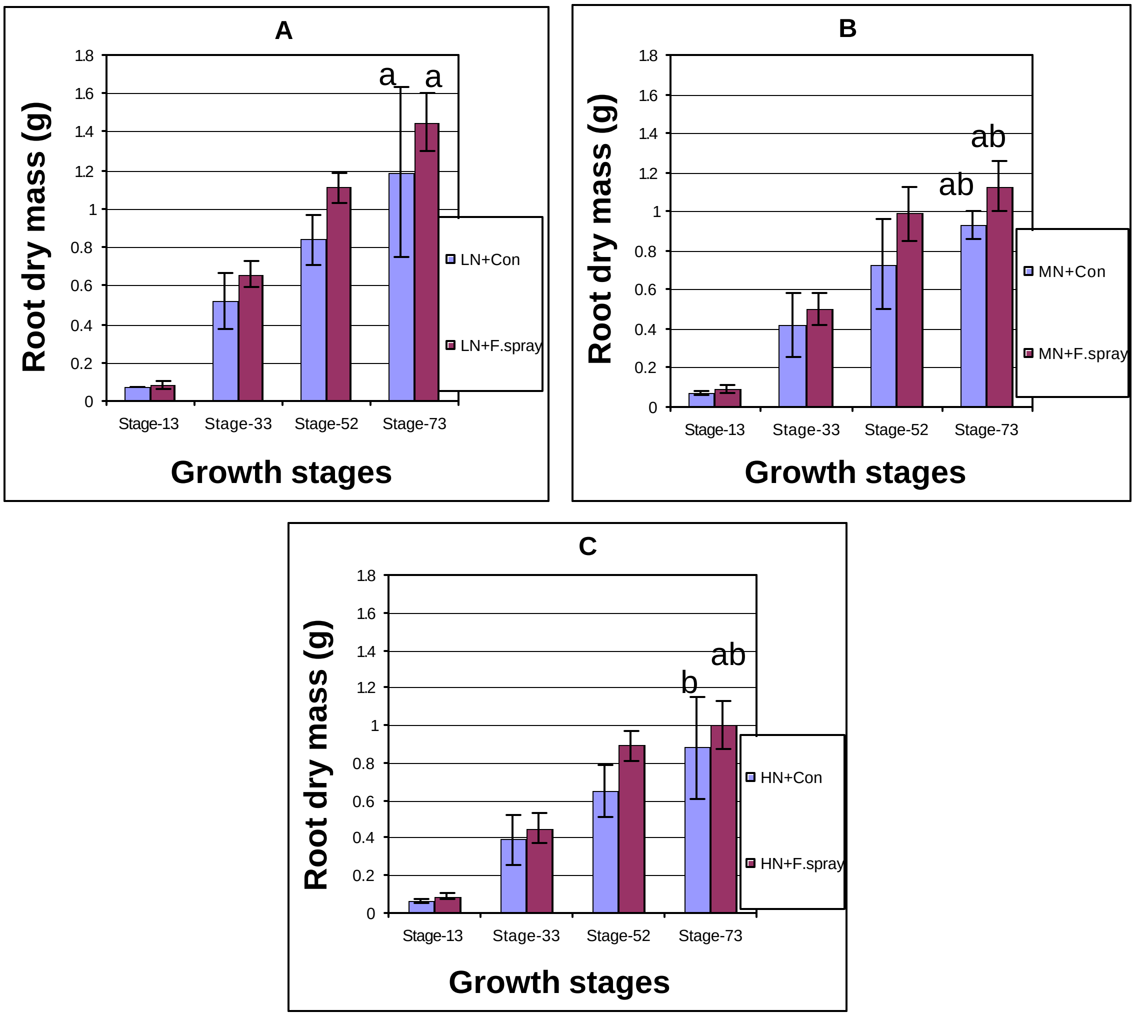

Figure 5.12: The effect of a foliar spray treatment with ComCat® at growth stage 13 on root dry mass of bread wheat, cultivated at A) a low B) a medium and C) a high N-level, at later growth stages. Error bars represent standard deviations. Columns designated with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant Differences (MSD) statistical procedure.

Interestingly, differences in root volume between untreated plants cultivated at different N-levels and ComCat® treated plants were statistically significant at the 5% probability level (figure 5.13A, B and C) while this was not the case for both root fresh and dry mass. An increase in nitrogen fertilization significantly reduced the root volume while it was significantly increased by the ComCat® foliar spray treatment at all N-levels.

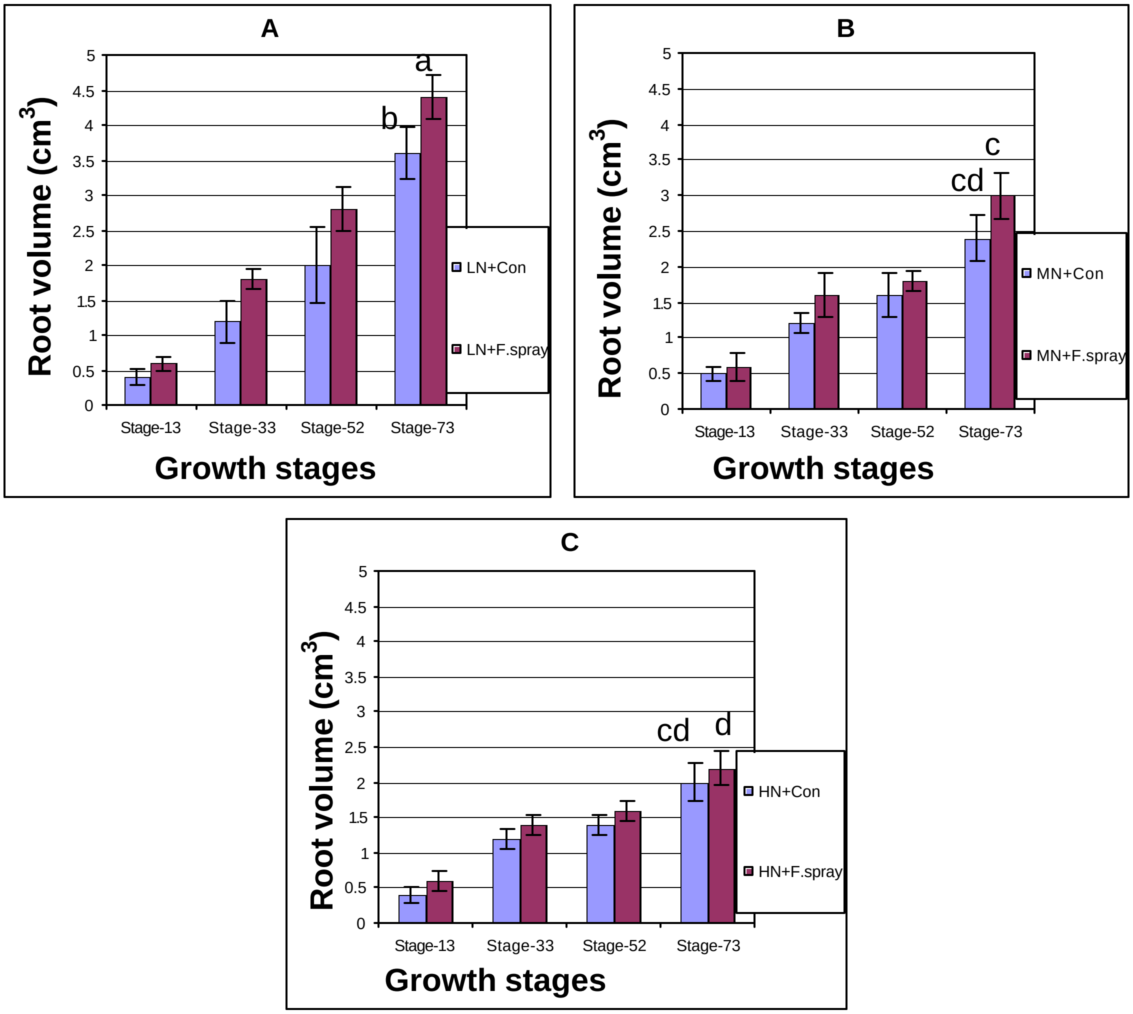

Figure 5.13: The effect of a foliar spray treatment with ComCat® at growth stage 13 on root volume of bread wheat, cultivated at A) a low B) a medium and C) a high N-level, at later growth stages. Error bars represent standard deviations. Columns designated with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant Differences (MSD) statistical procedure.

As was observed before (figure 5.7), elevated nitrogen fertilization significantly reduced the leaf area of plants (figure 5.14A, B and C) and the ComCat® foliar spray treatment had no effect.

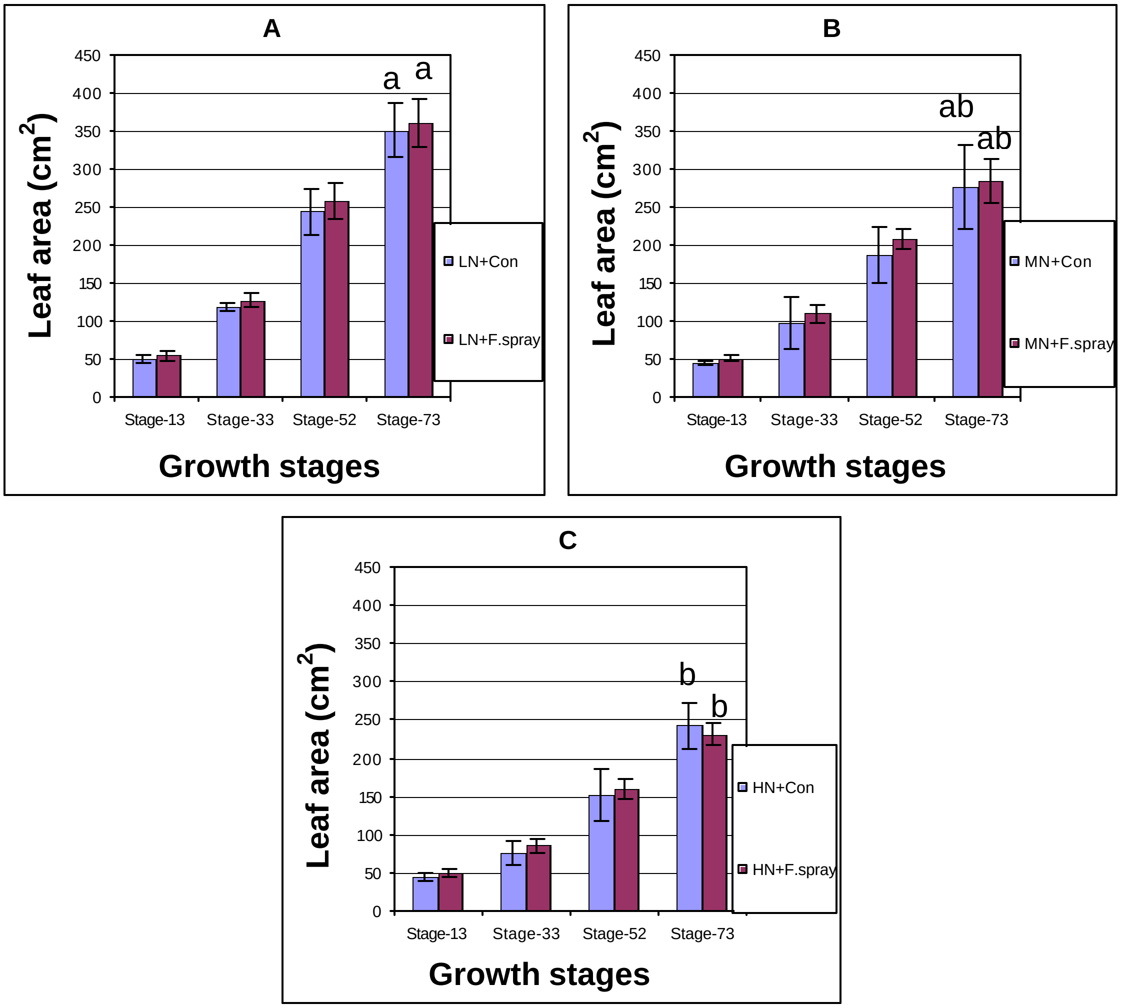

Figure 5. 14: The effect of a foliar spray treatment with ComCat® at growth stage 13 on leaf area of bread wheat, cultivated at A) a low B) a medium and C) a high N-level, at later growth stages. Error bars represent standard deviations. Columns designated with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant Differences (MSD) statistical procedure.

5.3.3 The effect of three different N-fertilizer levels and two ComCat® treatments on spike formation and kernel yield of bread wheat (cv. Sonalica) under greenhouse conditions

Statistically, elevated nitrogen fertilizer application had no significant effect on the number of spikes produced per plant (figure 5.15A). However, plants cultivated at the low N-level showed some increase (20%) in spike formation. In all cases both ComCat® treatments, but especially the foliar spray treatment, showed a tendency to increase spike formation, albeit non-significant. On the other hand, both ComCat® treatments had either no significant effect (at the low and medium N-levels) or had a significant inhibiting effect (at the high N-level) on the number of seeds produced per spike (figure 5.15B).

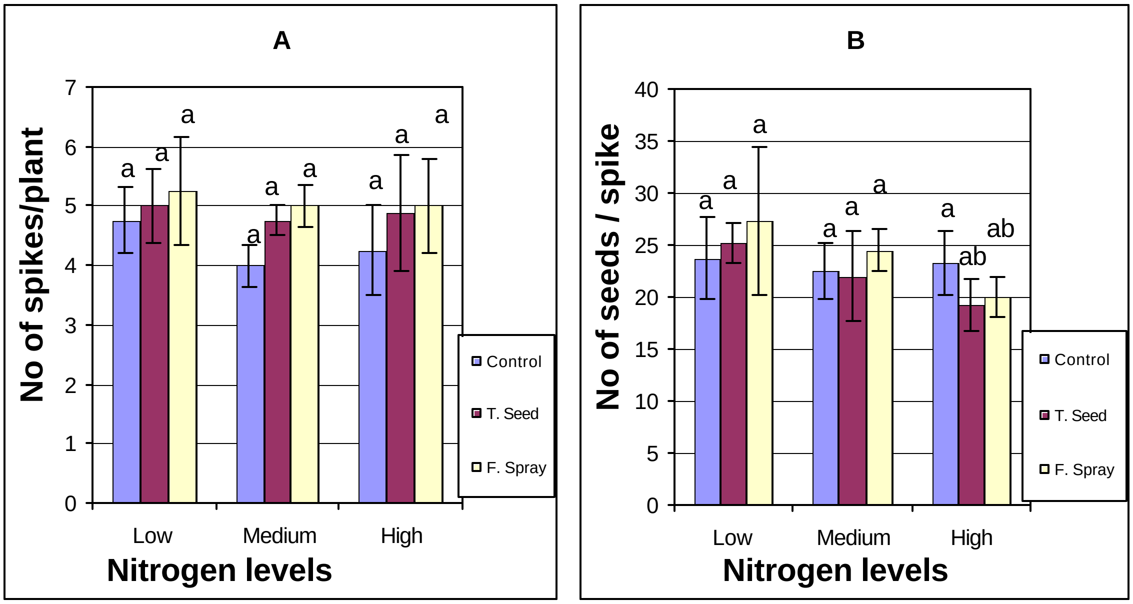

Figure 5.15: The effect of a seed treatment before planting as well as a foliar spray treatment with ComCat® at growth stage 13 on A) the number of spikes per plant and B) the number of seeds per spike of bread wheat, cultivated at a low, medium and high N-level. Error bars represent standard deviations. Columns designated with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant Differences (MSD) statistical procedure.

Although elevated nitrogen fertilization did not have a significant reducing effect on the number of seeds per plant (figure 5.16A) it significantly decreased the dry phytomass of seeds (figure 5.16B). Moreover, especially the ComCat® foliar spray treatment had a statistically significant increasing effect on the final kernel yield in terms of both the number and phytomass of seeds per plant (figure 5.16A and B). The latter was more accentuated in plants cultivated at a low N-level. Exactly the same tendency was observed for the 1000 kernel mass (figure 5.17).

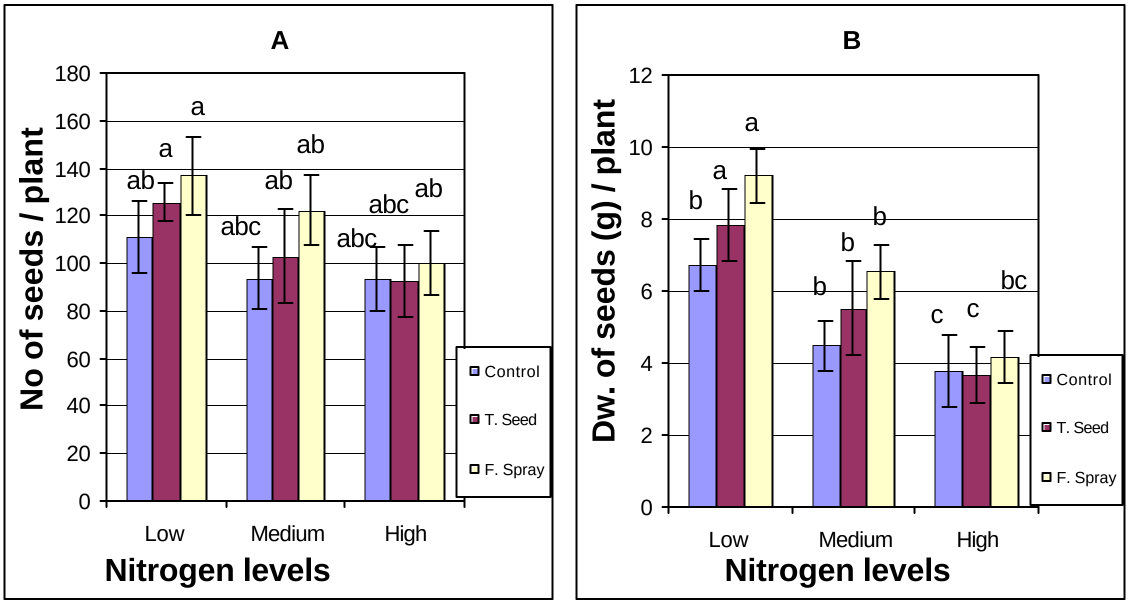

Figure 5.16: The effect of a seed treatment before planting as well as a foliar spray treatment with ComCat® at growth stage 13 on A) the number of seeds per plant and B) the dry weight of seeds per plant of bread wheat, cultivated at a low, medium and high N-level. Error bars represent standard deviations. Columns designated with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant Differences (MSD) statistical procedure.

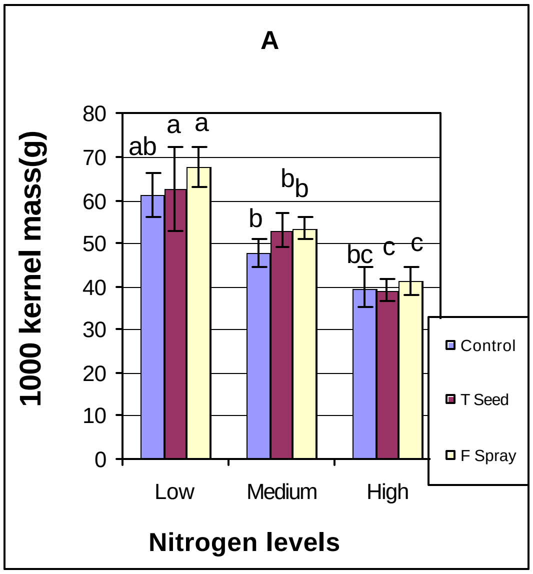

Figure 5.17: The effect of a seed treatment before planting as well as a foliar spray treatment with ComCat® at growth stage 13 on the 1000 kernel mass of bread wheat, cultivated at a low, medium and high N-level. Error bars represent standard deviations. Columns designated with different letters differed significantly ($p<0.05$) according to Tukey's Mean Significant Differences (MSD) statistical procedure.

5.4 Discussion

A statement by Heidhues (2001), that the future supply of food is likely to be based largely on productivity increase, underlines the need and supplies the rationale for identifying future innovation-driven practices in the agricultural industry. These innovative practices include genetic engineering techniques, but also the more efficient use of fertilizers in combination with a new generation of natural bio-stimulants, of which a few have emerged during the past decade. This comparative study not only pursued the objective of ascertaining the nitrogen fertilizer requirements of a new Bangladeshi bread wheat cultivar but also to determine the role ComCat®, a commercial bio-stimulant of plant origin, could play in combination with different nitrogen levels as a manipulative tool.

From the data collected during the 2001 season it became clear that the nitrogen fertilizer requirement of the bread wheat cultivar under scrutiny was much lower than that of South African winter wheat cultivars. Subsequently, the nitrogen fertilizer applications were modified during the 2002 season. ComCat® was applied either as a seed treatment before planting or as a single foliar spray at seedling growth stage 13. Four growth stages (13, 33, 52 and 73) were chosen for data collection. Most of the remarkable differences in vegetative growth were observed at growth stages 52 and 73, and were also very similar. For this reason only growth stage 73 was chosen for statistical data analysis.

Overall, even though the nitrogen fertilizer application was scaled down substantially during the 2002 growing season, the response of this bread wheat cultivar, in terms of growth and yield, was more positive at the lowest N-level. In most cases, both the ComCat® seed and foliar spray treatments tended to enhance the effect of the low N-level application even further indicating either 1) a more sufficient utilization of the available nitrogen or 2) stimulation of the crop by means of another mechanism. Alternatively, the higher nitrogen applications could either have had an inhibiting effect on growth and yield or an outright toxic effect, as was seen during the 2001 season when all of the plants cultivated in much higher nitrogen regimes died off.

The least effect of elevated N-levels was observed for the vegetative parameter plant height as no significant differences in length growth were observed for plants cultivated in either the low or the medium and high N-levels. Moreover, neither the ComCat® seed nor the foliar spray treatment had any effect on plant height. This corresponds with the view of Lupton *et al* (1974) that plant height is not a reliable parameter to predict either vegetative growth or yield potential. However, this was not the case for all of the other parameters used to quantify the vegetative growth of the crop under different nitrogen regimes both separately and in combination with the bio-stimulant treatments.

Although not statistically significant in all cases, cultivation of bread wheat under the lowest nitrogen conditions resulted in elevated aerial part fresh and dry mass when ComCat® was applied both as a seed treatment before planting and as a foliar spray at the 3-leaf growth stage (stage 13) during the 2002 season. This was also the case for root fresh and dry mass as well as root volume. A substantial decrease in vegetative growth observed in plants cultivated under the higher nitrogen regimes, as measured in terms of the mentioned vegetative growth parameters, is rather difficult to explain. As elevated nitrogen levels is known to increase the respiration rate of plants (Akita *et al*., 1993). One would have expected that an increased energy status would have resulted in increased vegetative growth. As this was apparently not the case, the possibility must be considered that the high nitrogen salt concentration in the soil could have either had an osmotic effect, limiting water uptake, or an outright toxic effect (Carter 1967,1969; Campbell and Lindsay, 1998).

Interestingly, both the ComCat® seed and foliar spray treatments tended to enhance the positive growth response of wheat irrespective of the N-level at which the plants were grown. However, the most significant enhancing growth effect was observed in plants cultivated in soil containing the lowest nitrogen level. Regarding the latter, the manufacturers of the product claimed induced root development and subsequent improved utilization of available nutrients as one of the attributes of ComCat® (Hüster, 1999). The significant increase in root fresh and dry mass as well as root volume observed, especially after treating seedlings with a single foliar spray, confirmed this claim and can explain the increased vegetative growth. The fact that this enhancing effect on plant growth was not as marked in plants grown under the medium and high nitrogen regimes is more difficult to explain. In this regard it can be postulated that damage done to either physiological processes or the structural make-up of plants under the influence of sub-toxic nitrogen levels overshadowed the bio-stimulatory effect of ComCat®, by interfering with its mechanism of action. If one can go on claims made by the manufacturers (Agraforum, 2001), namely that ComCat® increases the respiration rate of plant tissue, improves root formation and induces flower bud formation, as being part of the product's mechanism of action, then additional research will be necessary to test the postulate stated earlier.

An additional claim made by the manufacturers of ComCat®, is that its application either as a seed treatment before planting or as a foliar spray enhances yield in many agricultural and horticultural crops and this was confirmed by Schnabl *et al*. (2001). Pretorius (personal communication) also reported similar improvements in wheat cultivated under field conditions during the 1998 and 1999 growing seasons at the experimental farm of the University of the Free State, South Africa. Significant yield increases were observed for both wheat and maize treated with ComCat® at the three leaf growth stage (stage 13) at a similar dosage as was used in this study.

Indeed, in this study both of these treatments increased the phytomass of kernels significantly but, the foliar spray treatment more so than the seed treatment. This was also more marked in plants cultivated under the lowest nitrogen regime. In fact, under the elevated nitrogen conditions, and more so at the highest N-level, the improving effect of ComCat® on yield was totally nullified. The improved yield under low nitrogen conditions could rather be ascribed to both an increase in the number of kernels per spike as well as a possible increase in the translocation of carbohydrate to the kernels during the grain filling period leading to an increase in kernel phytomass, than to an increase in the number of spikes per plant. However, somewhat contradictory was the non-significant difference in the 1000 kernel mass observed under low nitrogen propagation conditions in combination with both Comcat® treatments, indicating a negative correlation between the number of kernels and the 1000 kernel mass. In this regard Fisher *et al.*, (1977) demonstrated that kernel weight decreased linearly with an increase in grain number, probably due to increased competition among developing grains for assimilate accumulation (Clarke *et al.*, 1981). On the other hand, Hay and Walker (1989) maintained that the final grain yield is more dependent on kernel number than kernel mass. In this study the increase in number of kernels per plant was 22.7%, the increase in kernel weight per plant was 32.3% and the increase in 1000 kernel mass was 10.6% under the influence of a single Comcat® foliar spray and low nitrogen conditions. From this it is concluded that the final kernel mass per plant is probably a more reliable parameter to assess final yield than the arbitrarily chosen thousand kernels used to quantify 1000 kernel mass.

According to the manufacturers of ComCat®, brassinosteroids are one of the active ingredients of the product. Brassinosteroids, as pure phytohormones, have been reported to not only increase crop yields but also crop quality (Prusakova *et al.*, 1999a). Similar results were reported from China with a brassinosteroid isomer, Tianfengsu, applied to a number of crops including rice, maize, wheat, cotton, vegetables and fruit (Ikekawa, 1991). Other claims made for this newly discovered phytohormone is its ability to regulate the uptake of ions into the plant cell (Khripach *et al.*, 2000). These claims can explain the yield improvement observed in the bread wheat cultivar under scrutiny in this study, as cultivated under a low nitrogen regime in combination with ComCat®, but not the inability of the bio-stimulant to maintain the same manipulative level under excessive nitrogen conditions. More research is necessary to obtain clarity in this regard.

The underlying results confirmed that nitrogen fertilizer needs to be applied at an optimum level in order to avoid undesirable side effects that can have a negative impact on the sustainable production of crops. Further, as excessive and unnecessary over application of fertilizers affects the economy of crop production systems, the optimum amount of fertilizer to be applied to crops, based on sound scientific information, needs to be calculated accurately. This is important in order to develop recommendation programs that adjust fertilizer rates to crop requirements (Zubillaga, *et al.*, 2002). Moreover, results showed that application of a bio-stimulant such as ComCat®, in combination with an optimum nitrogen regime, can result in the improvement of yield. In future research attention should be given to the combined effect of ComCat® and other macro-nutrients as well as the potential to decrease fertilizer applications when a bio-stimulant is applied in combination.

CHAPTER 6

GENERAL DISCUSSION

High applications of inorganic fertilizer, in particular when applied directly before or at the time of sowing, may be detrimental to seed germination and seedling development. Especially ammonium nitrate (NH_4NO_3) is harmful in this respect (Barker *et al.*, 1970). In this study, results obtained during the 2001 season, showed how sensitive the Bangladeshi bread wheat cultivar, Sonalica, was for nitrogen fertilizer. Shortly after germination, all of the young potted seedlings that received the equivalent of 200 kg ha^{-1} (medium dosage) and 400 kg ha^{-1} (high dosage) died off. Only potted seedlings that were cultivated in the equivalent of 100 kg ha^{-1} (low dosage) nitrogen fertilizer survived and developed normally. Subsequently, data on vegetative growth and yield could only be obtained from plants fertilized at the down scaled (low) N-level during 2001.

At the low N-level, both the ComCat® seed treatment before planting and the foliar spray treatment of seedlings two weeks after emergence at the 3-leaf growth stage (growth stage 13), tended to enhance the growth and yield of bread wheat as compared to the untreated controls during the 2001 season. Of these treatments, the foliar spray treatment constantly had a statistically significant enhancing effect on the vegetative growth of plants in terms of most of the vegetative parameters used to monitor growth. Additionally, a marked increase in kernel yield under glasshouse conditions strongly indicated that the simultaneous application of the commercial bio-stimulant ComCat®, together with a rather low N-level, holds promise for increasing the production of bread wheat. However, this potential needs to be verified under field conditions for the cultivar under investigation.

Based on the response of plants to the N-levels applied during the 2001 growing season, 100 kg ha^{-1} was applied as the medium dosage during the 2002 growing season while 50 kg ha^{-1} represented the low dosage and 150 kg ha^{-1} represented the high dosage nitrogen applications. Phosphate (P) and potassium (K) applications were kept the same during both growing seasons for all nitrogen levels at 25 and 10 kg ha^{-1} respectively. The response of plants to the two separate ComCat® treatments under the altered nitrogen conditions was again followed.

Interestingly, again the bread wheat plants responded more positively to the new down scaled or low (50 kg ha^{-1}) nitrogen application in terms of both vegetative growth and yield, as compared to plants cultivated under medium and high nitrogen conditions during the 2001 growing season. Almost four decades ago, Ota and Yamada (1965) reported that a high application of nitrogen fertilizer greatly increased the percentage of sterile flowers in a low nitrogen tolerant bread wheat variety, cultivated in Ceylon at the time, while the phenomenon was less obvious in a high nitrogen tolerant variety. This can explain the significant decrease in number of seeds counted in plants cultivated under high nitrogen conditions in this study as compared to the low N-level cultivated plants.

On the other hand, Walker *et al*. (1994) reported that applying too little nitrogen usually results in poor vegetative growth and sub-economical yields in wheat, while excessive nitrogen can lead to lush shoot growth but also a decrease in grain yield. This corresponds with the reports of Murata (1966) and Tanaka *et al*. (1966), almost four decades ago, that the more vigorous the growth of rice was before heading, the smaller the dry matter increase was in kernels during the grain filling stage. This did not seem to be the case in the bread wheat cultivar under investigation as both vegetative growth and kernel yield tended to decrease as nitrogen fertilizer was increased above the 50 kg ha^{-1} level, rather indicating that this cultivar is low nitrogen tolerant.

However, what needs to be kept in mind when speculating on the responses mentioned above is that the potted plants received fertilizer in a confined space as a band placement equivalent to a dosage per hectare under field conditions. The same amount of fertilizer applied broad cast under field conditions can probably not be compared to the circumstances under glasshouse conditions. 'Low nitrogen tolerance', in real terms, might therefore have manifested as an artifact of the growth restriction imposed on plants by the small pots and was not necessarily the result of one or other physiological disorder induced by the excessive supply of nitrogen. In this regard Shimshi (1969) warned that, in cereals, excessive application of nitrogen can increase the susceptibility of a crop to fungal disease or excessive application of one nutrient, including nitrogen, can cause a relative shortage of other plant nutrients. Gärtel (1968) also demonstrated that excessive supply of potassium (K) depressed the uptake of magnesium (Mg^{2+}) by wheat while excessive phosphate (P) reduced the uptake of zinc (Zn). In both cases significant yield decreases were recorded. It is suggested that a possible impairment of the uptake of other nutrients under elevated nitrogen conditions be investigated in a follow-up study.

This study confirmed that failure to supply the optimum amount of any one of the nutrients essential to plant growth, and in the optimum ratio to other nutrients can result in serious yield reductions. This corresponds to a statement made by Carter (1992) that, for all new crop varieties, the need exists for determining the production potential at a number of sites in an attempt to identify genotypic differences in tolerance to high and low levels of mineral nutrients. For the bread wheat cultivar investigated in this study, the latter should be verified under field conditions.

The other main variable considered during 2002 was the vegetative growth and yield responses of this bread wheat cultivar to treatment with ComCat®, a commercial bio-stimulant of plant origin, by means of two separate application methods, i.e. a seed treatment before planting and a foliar spray treatment at growth stage 13. As was the case during the 2001 growing season, results obtained during 2002 consistently confirmed that the application of ComCat® affected the vegetative growth of plants. Stimulation of aerial part growth was not as significant as that of root development. In the latter instance, and especially under low nitrogen conditions, root fresh mass was increased by >22% when ComCat® was applied both as a seed treatment and as a foliar spray. Root volume was increased to the same extent when the bio-stimulant was applied as a foliar spray but to a lesser extent (11%) when applied as a seed treatment. This was in agreement with results obtained by Gebremedhin (2001) under glasshouse conditions on two Ethiopian bread wheat cultivars and was also in agreement with claims made by the manufacturers of the natural product (Agraforum, Germany).

Moreover, especially under low nitrogen conditions, the ComCat® foliar spray treatment had a statistically significant enhancing effect on yield in terms of both number of seeds per plant (+22.7%) and the seed dry weight per plant (32.3%). Although the yield enhancing effect of the seed treatment was about 50% less than that of the foliar spray treatment, it was still statistically significant at the 5% probability level, compared to the untreated control. This was also in agreement with the findings of Gebremedhin (2001) with two Ethiopian wheat cultivars as well as that of Molahlehi (2000) with dry beans under glasshouse conditions and standard nitrogen fertilizer rates. The fact that the increase in number of kernels per plant and subsequently also the dry mass was reduced drastically as the nitrogen fertilizer level increased, could possibly be attributed to low nitrogen tolerance of the cultivar under investigation and an increase in the percentage of sterile flowers (Ota and Yamada, 1965).

Interestingly, an increase of the 1000 kernel mass (+11.5%) by the ComCat® foliar spray treatment under low nitrogen conditions was not statistically significant at the 5% probability level and was also 50% lower than the increase in kernel number per plant. The apparent negative correlation between the number of kernels and the 1000 kernel mass is in agreement with the reports of Fisher *et al*. (1977), Shanahan *et al*. (1984) and Gebremedhin (2001) that kernel weight decreases linearly with an increase in grain number. However, an increase in both kernel number and phytomass under the influence of ComCat® must be regarded as positive events from an agricultural perspective.

In summary, the results of this study and several previous unpublished reports strongly indicate that sustainable production of grain crops can be achieved by an integrated approach of applying nutrients at the optimum or even a suboptimal level, together with a bio-stimulant such as ComCat®. As specific reports on the effect of ComCat® on the number and biomass of wheat kernels are not yet available in literature, it is suggested that these aspects be trialed and tested for the bread wheat cultivar Sonalica, as well as other wheat cultivars and crops, under field conditions. According to Ishizuka (1969) it is quite difficult to increase grain yields in regions where yields are already high compared to the world average. For instance, the average grain yield in developed countries is 4-5 ton ha^{-1} compared to the 2-3 ton ha^{-1} in Africa. It should, therefore, probably be kept in mind that when crop yields are relatively low in a specific region, the integrated approach recommended higher up might be more effective in this region than in developed countries such as Europe and the USA. In the latter instance, the effects of optimized nutrient application as well as bio-stimulants on the quality of crops should be included in future studies.

Summary

In this study, attempts were made to investigate the growth and yield performance of a Bangladeshi bread wheat cultivar (Sonalica) under the influence of three different nitrogen fertilizer levels and one concentration level of ComCat®, a commercial bio-stimulant, applied either as a seed treatment before planting or as a foliar spray on seedlings at the 3-leaf growth stage, under glasshouse conditions. Initially the South African (RSA) standard N-level for winter wheat was taken as the medium N-level together with a down scaled (low) and an up scaled (high) rate during the 2001 season in order to ascertain the optimum level. However, it was observed that the Bangladeshi cultivar could not tolerate the RSA standard (medium rate) for nitrogen application. After germination all plants fertilized with the medium and high nitrogen rates, died off. Subsequently, this N-level was taken as the medium level for the 2002 trial and a new down scaled (low) and up scaled (high) N-level was calculated and applied, together with the two ComCat® treatments.

Overall, even though the nitrogen fertilizer application was scaled down substantially during the 2002 growing season, the response of this bread wheat cultivar, in terms of growth and yield, was more positive at the lowest N-level. Results obtained during 2002 consistently confirmed that the application of ComCat®, especially as a foliar spray at stage 13 of seedling development, affected the vegetative growth of plants. However, the enhancing effect of the bio-stimulant was more pronounced on yield, in terms of both the increase in number of kernels and kernel dry mass per plant.

Finally, the results of this study and several previous unpublished reports strongly indicate that application of a bio-stimulant such as ComCat®, in combination with an optimum nitrogen regime, can result in the improvement of yield under glasshouse conditions. In future research attention should be given to the combined effect of ComCat® and other macro-nutrients, as well as the potential to decrease fertilizer applications when a bio-stimulant is applied in combination, under field conditions.

Opsomming

In hierdie studie is ‘n poging aangewend om die respons van ‘n broodkoring cultivar uit Bangladesh, Sonalica, op drie verskillende stikstof peile en twee behandelings met ‘n kommersiële biostimulant ComCat®, naamlik ‘n saadbehandeling voor plant en ‘n blaarbespuiting op groeistadium 13, onder glashuis toestande na te vors. Aanvanklik, tydens die 2001 groeiseisoen, is die RSA standaard vir die N-peil as die medium vlak geneem tesame met ‘n afgeskaalde (laag) en ‘n opgeskaalde (hoog) peil, ten einde die optimum peil te identifiseer. Maar dit het duidelik geword dat hierdie cultivar baie sensitief was vir die medium en hoë stikstofvlakke onder glashuistoestande aangesien alle saailinge kort na opkoms afgesterf het. Gevolglik is die lae N-peil wat in 2001 gebruik is as die medium peil in 2002 geneem terwyl nuwe af- (laag) en opgeskaalde (hoog) peile bereken is en saam met die twee ComCat® behandelings toegedien.

Oor die algemeen, ten spyte van ‘n substansiële afskaling van die N-peil gedurende 2002 groeiseisoen, was die respons van hierdie koring cultivar steeds meer positief op die lae N-peil. Data gedurende 2002 versamel het bevestig dat veral die blaarbespuiting met ComCat® op groeistadium 13 van saailing ontwikkeling die vegetatiewe groei van plante die meeste beïnvloed het. Maar, veral die verhoging van oesopbrengs, in terme van beide die aantal graankorrels en graankorrel massa per plant, was die mees betekenisvolle respons van hierdie koring cultivar op behandeling met die biostimulant.

Opsommend het die resultate van hierdie studie, tesame met vroëre ongepubliseerde verslae, sterk daarop gedui dat die aanwending van ‘n biostimulant soos ComCat® in kombinasie met ‘n optimum stikstofpeil tot verhoogde oesopbrengs in koring onder glashuistoestande aanleiding kan gee. Toekomstige navorsing behoort aandag te gee aan die gekombineerde effek van ComCat® met ander makro-elemente op koring, asook die moontlike afskaling van bemesting toediening in kombinasie met die biostimulant, onder veldtoestande.

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