The effect of nitrogen and sulphur on the nutrient use efficiency, yield and quality of canola (*Brassica napus* L.) grown in the Western Cape

by

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ABSTRACT

There is an increasing demand for canola (*Brassica napus* L.), an emerging oilseed crop in South Africa. Canola thrives in the Western Cape. However, yet low yields are still obtained within the production areas with poor and or variable responses to nitrogen applications. Crop nutrition and specifically the contribution of sulphur (S) to nitrogen (N) use and selection of nutrient efficient genotypes can be strategies of considerable significance in increasing yields.

This study investigated growth, yield and quality responses of canola to different N (0, 30, 60, 90 and 120 kg N ha^{-1}) and S (0, 15 and 30 kg S ha^{-1}) fertilisation rates in field trials at different localities, during the 2009-2011 period. Responses to N and S under optimum growing conditions and responses of different cultivars were investigated in unison in glasshouse trials at the Department of Agronomy of the University of Stellenbosch.

Locality and growing season (year) significantly affected nutrient content in plants at flowering (90 days after planting), dry mass production as well as yield and quality of canola in field trials at five different localities during the 2009-2011 period.

Growth and yield were also affected by N application rate in both field and glasshouse trials. Sulphur applications did not have an effect on vegetative growth, but rather stimulated flower and pod production in glasshouse trials and resulted in higher grain yields in field trials. Response depends largely on rainfall and S content of the soil. Highest yields were, on average, obtained with application rates of 120 kg N and 30 kg S ha^{-1}, while glasshouse trials showed that even higher rates may be considered under optimum growing conditions. High application rates of N and S also improved water use efficiency from approximately 4-5 kg grain yield to about 8-9 kg grain yield mm^{-1} of rain during the growing season. Agronomic efficiencies of applied N decreases with increasing N rates and values of about 8 kg grain yield increase per kg of N applied at N rates of 120 kg N ha^{-1} indicated that high N rates may improve profit margins of canola as long as the cost of N is not more than eight times the producers price of canola. Agronomic efficiencies of N applications are improved if 15 kg S ha^{-1} is applied complimented with high rainfall, but not with applications of 30 kg S ha^{-1}. Improved agronomic efficiencies of S applications shown at higher N rates, confirmed the dependency of S responses to sufficient availability of nitrogen. Sulphur applications, in contrast to N, resulted in an increase in oil content of the grain in field trials.

Yield responses of different cultivars to nitrogen fertilisation under glasshouse conditions differed, with better responses obtained within short and medium season cultivars, than with a late maturing (long season cultivar), in spite of a better vegetative (dry mass) response of the later maturing cultivar. These results may indicate differences in the growth habit of different cultivars, but more research in this regard is needed.

OPSOMMING

Canola (*Brassica napus* L.), ‘n relatief nuwe oilsaadgewas wat goed aangepas is, word in ‘n toenemende mate in die produksiegebiede van die Weskaap verbou. Lae opbrengste en wisselvallige reaksies teenoor stikstofbemesting word egter verkry ten spyte van die gewas se hoë stikstofbehoefte en dit mag moontlik aan swaweltekorte toegeskryf word.

In hierdie ondersoek is die groei-, opbrengs- en kwaliteitsreaksie van canola teenoor verskillende N (0, 30, 60, 90 en 120 kg N ha^{-1}) en S (0, 15 en 30 kg S ha^{-1}) bemestingspeile in droëland proewe op verskillende lokaliteite bestudeer gedurende die 2009-2011 groeiseisoene. Reaksies teenoor N en S onder optimale groeitoestande en vir verskillende cultivars is in glashuisproewe van die Departement Agronomie van die Universiteit van Stellenbosch, uitgevoer.

Die chemiese samestelling van die plante tydens blomstadium (90 dae na plant), asook droëmateriaal produksie, graanopbrengs en kwaliteit het betekenisvol verskil tussen die lokaliteite, maar lokaliteitsverskille is ook deur die seisoene beïnvloed.

Die ontwikkeling, groei en graanopbrengs van die canola is ook beïnvloed deur die stikstofbemestingspeile in beide die veld en glashuisproewe. Swawelbemesting het nie die vegetatiewe groei van canola beïnvloed nie, maar het blom en peulproduksie in glashuisproewe en graanopbrengste in veldproewe verhoog. Die reaksie van canola teenoor die swawelbemesting is grootliks bepaal deur die swawelinhoud van die grond asook klimaatsfaktore soos reënval. In die algemeen is die hoogste canola opbrengste in veldproewe met toedienings van 120 kg N en 30 kg S ha^{-1} verkry, maar glashuisproewe het getoon dat hoër toedieningspeile nodig mag wees onder optimale groeitoestande soos in besproeiingsgebiede.

Hoë toedieningspeile van N en S het veroorsaak dat die waterverbruiksdoeltreffendheid toegeneem het van 4-5 kg graanopbrengs per mm reën tot sowat 8-9 kg graan opbrengs per mm reën. Agronomiese doeltreffendheid van toegediende stikstofbemesting het afgeneem met toenemende N peile, maar waardes van ongeveer 8 kg opbrengsverhoging per kilogram N toegedien met stikstofpeile van 120 kg ha^{-1}, toon dat hoë N toedieningspeile mag steeds winsgrense verhoog mits die prys van een kilogram N nie meer is as agt maal die produsente prys van canola is nie. Agronomiese doeltreffendheid van stikstofbemesting is verhoog deur ook 15 kg S per hektaar toe te dien, maar nie deur die toediening van 30 kg S ha^{-1} nie. Die agronomiese doeltreffendheid van S toedienings het slegs by die gelyktydige toediening van hoë stikstoftoedienings toegeneem, wat die wisselwerking tussen N en S ten opsigte van graanopbrengs bevestig. In teenstelling met stikstof het swawel toedienings die olie-inhoud van canola in die veldproewe verhoog.

In glashuisproewe is gevind dat kort en medium groeiseisoen cultivars, ten spyte van ‘n groter vegetatiewe reaksie van die lang groeiseisoen cultivars, groter opbrengsreaksies teenoor stikstof- en swawelbemesting toon. Meer navorsing word egter in hierdie verband benodig.

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CHAPTER 1

1.1 INTRODUCTION

The production of canola (*Brassica napus* L.), an emerging oilseed crop, has significantly increased, to become second only to soybean in world production (Hirel *et al.,* 2007). Oilseed rape (which includes *B. napus*, *B. rapa* and *B. juncea* species) is also the world's second largest source of protein meal, although only one-fifth of the production of the leading soybean (USDA, 2005). The increased interest in canola is mostly due to the use of the healthy oil in end-products and use as biofuel (Rayner, 2002).

As a relatively new crop in South Africa production is still low compared to the global major producers. Local production is at present largely limited to the Western Cape Province, which is characterized by a mediterranean climate. Estimates for the production of canola during 2011 was 59 490 tons on 43 510 ha (Crop Estimates Committee, 2011). With the need to reduce oil and oilcake imports, there is potential for growth in both the area under production and yield per hectare. Considering limited land area, to meet the demand, emphasis should be put on increasing yields per hectare. However, according to Van Zyl, (2007), low canola yields (less than 1.5 ton ha^{-1}) are generally obtained.

Low yields may be the result of various factors. It is well known that canola has a much higher requirement for nutrients, especially Nitrogen (N) and Sulphur (S) compared to cereals such as wheat (Oplinger *et al.,* 2000; Gan *et al.,* 2008). Optimum management of these nutrients may therefore be important to ensure high yielding canola crops, with high oil contents as well. In contrast to results obtained in other canola production areas of the world, where considerable responses in yield with addition of N were reported (Hocking *et al.,* 1997; Jan *et al.,* 2002; Svečnjak & Rengel, 2006; Tatjana *et al.,* 2008), generally poor and or variable responses to increases in N application rates have been reported in the production areas of the Western Cape (Hardy *et al.,* 2004). This, however, may be due to insufficient supply in S, because canola producers of the Western Cape, who are traditional wheat producers, almost never applied S fertilisers. Both N and S availability and uptake, and therefore fertilisation requirements, are affected by soil and climatic conditions (Malhi *et al.,* 2008). For this reason, research is needed to study the effect of N and S fertilisation on growth, yield and quality of canola in various soil and climatic conditions of the Western Cape Province of South Africa.

1.2 OBJECTIVES OF THE STUDY

The general objective of this study was:

> To determine optimum N and S fertiliser rates to maximize grain yield and quality of canola in the Western Cape Province of South Africa.

The specific objectives of this study were to:

1. Determine the nutrient content of canola in response to N and S fertilisation in diverse environments.
2. Determine the effects of N and S fertilisation on vegetative growth of canola in diverse environments.
3. Determine the effects of soil and climatic conditions (years and localities) on the yield and quality response of canola to N and S applications rates.
4. Evaluate the N/S fertiliser and water use efficiencies of canola in response to N and S application rates.
5. Determine the growth response of canola to N and S fertilisation under controlled (glasshouse) conditions.
6. Determine the morphological and physiological responses of different canola varieties to N and S fertilisation.

1.3 HYPOTHESES

Poor and variable responses to N fertilisation are due to insufficient S supply and or ineffective N and S use.

1.4 DISSERTATION OUTLINE

This dissertation will be presented as scientific publications, with the FIRST CHAPTER being a general introduction and objectives of the research carried out. CHAPTER 2 reviews the literature of canola with emphasis on N and S fertilisation and their use efficiency.

CHAPTERS (3-8) were in sequence of objectives outlined in Section 1.2 above and were written with their own abstracts, introductions, methodology, results and discussions, and conclusions. CHAPTER 9 lastly form general conclusions and recommendations based on all the work done. Considering the outline here, the duplication of methodology can be seen in chapters 3, 4, 5 and 6. However certain details were omitted in chapters 4, 5 and 6 (full experimental layout).

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CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Canola (*Brassica napus* L.) is increasing in demand because of its oils' high nutritional value and lowest saturated fat compared to any oil on the market. South Africa imports a considerable quantity of canola to meet its food and feed needs even though the crop thrives well in the Western Province. The canola crop's climatic requirements, plant density, fertilisation and irrigation needs in the country has been clearly reviewed in the Canola production manual (undated) and by Seetseng (2008). The Canola production manual pamphlet also covered the production practices with an inclusion of general critical levels of both macro and micronutrients of the crop. Of all the nutrients, considerable high response in yield with addition of N is possible but generally poor and or variable responses to N occur in the Western Cape Province (Hardy *et al.*, 2004). However, there is still need of understanding the uptake and use efficiencies of these nutrients as yields within the province are reportedly lower than the possible potential (Van Zyl, 2007).

Canola yields can be increased by use of appropriate production practices including N and S input and considering the right genotypes. Magnitude of response to nitrogen can vary among genotypes (Svečnjak & Rengel, 2006). Besides the high influence of N on canola development, sulphur also plays a very crucial role affecting the crop's growth and yield (Jan *et al.*, 2010). The optimum S supply and uptake depends on N application rate which can be influenced by the inherent nutrient content of the soil. However fertilisers should be used efficiently, considering global increases in the need of fuel, hence fertilisers. Through understanding the mechanisms that increase N and S uptake, application on the appropriate localities and utilization efficiencies and selecting the appropriate genotypes, South Africa has potential to increase canola production with efficient use of fertilisers.

2.2 DEVELOPMENT IN CANOLA VARIETY TYPES

Canola is one of two genotypes of rapeseed namely *B. napus* L. and *B. campestris* L. Canola was developed through conventional plant breeding from rapeseed, an oilseed plant already used in ancient civilization. A brief description of the rapeseed origin, evolution and relationships between members of the genus *Brassica* could be best described through the Triangle of U (Woo, 1935). It says that the genomes of three ancestral species of *Brassica* combined to create three of the common contemporary vegetables and oilseed crop species. The development of the canola plant can be divided into the following growth stages: germination and emergence; production of leaves; stem elongation; flower initiation; anthesis; and pod and seed development (Canola production manual, undated).

Brassica oilseeds have been grown by humans for more than a thousand years. Records indicate early cultivation of vegetable forms of the crop, in India, in 1500 BC (Prakash, 1980) and in China more than 1000 BC (Li, 1980). Cultivation extended across Europe in the middle ages and by the fifteenth century rapeseed was grown in the Rhineland as a source of lamp oil and also for cooking fat (Booth & Gunstone, 2004). There have been changes in quality aspects of both rapeseed oil and meal through breeding in the later twentieth century (Booth & Gunstone, 2004).

Early varieties of canola contained high levels of erucic acid and glucosinolates, which are sulphur bearing compounds which, when consumed in high amounts, were associated with goitrogenic, liver and kidney abnormalities and fertility problems of livestock. The first low glucosinolates trait, from the variety Bronowski was successfully incorporated into the spring varieties of *B. napus* and *B. rapa* and later into the winter varieties of *B. napus* by the Canadian breeders in the 1970s (Booth & Gunstone, 2004). There are still efforts to improve other species like *B. juncea*. The breeding programmes have resulted in double low varieties, termed so because they are low in erucic acid in the oil and glucosinolates in the meal. The negative associations due to the homophone "rape" resulted in creation of the more marketing-friendly name "Canola" and it was licensed as the first canola double low variety in 1974. The change in name also serves to distinguish it from regular rapeseed oil, which has much higher erucic acid content. The Canola trademark is held by the Canola Council in Canada and is permitted for use in describing rapeseed with less than 2 % erucic acid in the oil and less than 30 micromoles g^{-1} glucosinolates in the meal (http://www.canolacouncil.org).

Oil is the most valuable component of the canola seed and it is primarily influenced by the variety, but the environment has a significant influence on the final oil content of the seed (Anon, 2008). Canola oil contains 6 % saturated fat and is high in monounsaturated fat (Canola Council of Canada, 1990). Subsequent progress in breeding for quality of both oil and meal ensures that use as edible oil now greatly exceeds all other uses, although industrial uses are many and are likely to become more significant (Booth & Gunstone, 2004).

The status of the oil has improved in recent years with the discovery that it has beneficial nutritional properties. Its value for industrial purposes and as a fuel is enhanced by the perceived benign effect on the environment. Many advances have been made in canola breeding including tolerance to certain herbicides. However, conventional varieties typically have up to 2 % higher oil than the triazine tolerant varieties (Anon, 2008). According to Canola Council of Canada (2005), there are many varietal characteristics to be considered when choosing a variety for production but basically the maturity, relative to the length of the growing season (maturity), disease resistance, seed yield and oil content should be considered. In Australia, a concerted breeding effort has led to development of improved cultivars adapted to local environments and resistant to the destructive blackleg disease (Yau & Thurling, 1987).

South Africa imports all canola (*B. napus*) varieties currently in production from Australia, though various trials are carried out for climatic and agronomic suitability by the Western Cape Department of Agriculture. Canola varieties tested are a mix of herbicide tolerant e.g. triazine or imidazolinone tolerant and conventional types.

2.3 NITROGEN UPTAKE AND UTILIZATION

Generally, canola is a heavy nutrient feeder, and the requirements of various macronutrients, including N, are higher in canola compared to cereals (Gan *et al.*, 2008). Plants absorb nitrogen from the soil as NH^{+}_{4} and NO^{-}_{3} ions but uptake by canola is mainly in the form of NO^{-}_{3} ions (Hirel *et al.,* 2007). Uptake and utilization of N is usually divided into two main phases of plant development: vegetative and grain filling. In these two phases N is utilized in various components of many important structural, genetic and metabolic compounds (Hirel *et al.*, 2007). During the vegetative phase, young developing roots and leaves are mainly the sink organs for the assimilation and synthesis of amino acids originating from the N taken up before flowering and then reduced via the nitrate assimilatory pathway (Hirel & Lea, 2001). When N is taken up, it forms a major component of chlorophyll, the compound by which plants use sunlight energy to produce sugars from water and carbon dioxide (photosynthesis). Hence nitrogen increases the plant leaf-area and the net assimilation rate (Yau & Thurling, 1987) which becomes a major influence during the reproductive stage especially grain filling (Rossato *et al.*, 2001).

Nitrogen is a major component of amino acids, the building blocks of proteins. Proteins act as structural units in plant cells and some as enzymes making many biochemical reactions possible that take place for plant growth and development. Moreover, nitrogen is an important component of energy-transfer compounds, such as ATP (Adenosine triphosphate) which allows cells to conserve and use the energy released in metabolism (Hirel *et al.*, 2007). It forms a significant component of the nucleic acids such as DNA, the genetic material that allow cells to grow and reproduce, hence growth of the whole plant.

Development of a large sinks lead to increased N uptake, hence increasing growth. While effects on development are usually small, growth is affected through protein synthesis, leaf expansion and growth of all components of the crop (Yau & Thurling, 1987). Hocking & Strapper (2001) showed that leaf number, as well as, area could be increased. The effects of nitrogen on growth have been shown to be expressed normally in the components of yield as extra pods per meter squared, with little effects on later formed components (Hocking & Strapper, 1993). As the plant develops into flowering and grain filling, the N tend to accumulate in the grain. A large amount of the N taken up during the vegetative growth phase is lost due to the shedding of the leaves (Malagoli *et al.*, 2005). However, pod walls could act as a temporary resource for N supplying up to 25 % of the requirements of the seed (Hocking & Strapper, 1993).

In canola, the requirement for N per yield unit is higher than in cereal crops (Hocking & Strapper, 2001; Sylvester-Bradley & Kindred, 2009). According to Laine *et al.*, (1993), the crop has a high capacity to take up nitrate from the soil, hence accumulating large quantities of N that is stored in vegetative parts at the beginning of flowering. However, yields in canola are half that of wheat, due to the production of oil, which is costly in carbohydrate production (Hirel *et al.*, 2007). Hirel *et al.* (2007) concluded that most of the N stored in the vegetative organs is not used, only an average of 3 % N, in canola seed. The amount of N taken up by the plant during the grain-filling period apparently remains very low (Rossato *et al.*, 2001) considering the loss through leaf fall (Malagoli *et al.*, 2005).

In the European Union, after sowing, to allow maximum growth at the beginning of winter, N fertiliser application may be necessary when there is a shortage in available soil N (Booth & Gunstone, 2004). Fertilisation is again necessary in spring, during the full growth period when large amounts of N are required and up to 70 % of the plant N requirement must be satisfied

(Hirel *et al.*, 2007). This is achieved by the application of N fertilisers, which may be fractioned according to the size of the plant and yield objectives (Brennan *et al.*, 2000). Peak seed yield usually occurs when 180-200 kg N ha^{-1} is applied (Jackson, 2000).

High rates of N may lower oil content (Jan *et al.*, 2002), if not primarily used in previous crop growth. The canola seed protein content increases with addition of N, however, protein and oil have an inverse relationship such that an increase in protein content can significantly lower the oil content of the crop. Application of nitrogen to 120 kg ha^{-1} did not have any significant effect on the protein content but the oil content decreased significantly (42.62 to 42.10 %) (Jan *et al.*, 2002). However, overall grain yields are generally increased with addition of higher levels of N. (Yau & Thurling, 1987; Svečnjak & Rengel, 2006; Tatjana *et al.*, 2008).

2.3.1 Nitrogen deficiency symptoms in canola

Though nitrogen is one of the most abundant elements on earth, its deficiency is probably the most common nutritional problem affecting most crops, including canola. Nitrogen deficient canola plants are usually dwarfed and the foliage is pale yellow (Fismes *et al.*, 2000). Nitrogen in older leaves is redistributed to the younger leaves, and the lower older leaves wither. The remaining leaves often show purple discoloration with the canopy remaining thin and open. Basically, this would lower the pod number, with a reduction in yield. To alleviate the deficiency of nitrogen, many forms of nitrogen can be added to plants. However, N is immediately available if applied in the form of nitrate, though organic manure or the ploughing in of legumes.

2.3.2 Nutrient Use Efficiency

Nitrogen fertilisation of canola has been singled out by the ARC of South Africa as the largest production input item under dry land conditions in the country with this likely also be the case for irrigated canola (ARC, 2007). Scientifically determined guidelines for N fertilisation rates of irrigated canola in South Africa are currently not available and guidelines from other sources are confusing yet N accounts for the largest energy input in oilseed production. For this reason, nutrient use efficiency (NUE) in canola is specifically biased towards nitrogen as the main nutrient; hence NUE in the discussion relates more to nitrogen use efficiency. Understanding N use characteristics of canola will help to improve N use efficiency and minimize production costs (Masson & Brennan, 1998; Fismes *et al.*, 2000) especially when production regions are characterized by different soil and climatic properties.

According to Sylvester-Bradley and Kindred (2009), NUE is generally defined as the yield of grain achieved per unit of nitrogen available to the crop, from soil or applied fertiliser. It can further be defined to Nitrogen Fertiliser Use Efficiency (NFUE) which is the seed yield produced per unit of fertiliser N, and crop N uptake. Nitrogen Use Efficiency is, conventionally, considered as the product of both N capture (often called 'N uptake efficiency'), the proportion of N taken up by the crop of that available to it, and N conversion (often called 'N utilization efficiency'), the amount of DM produced per unit of N taken up by the crop.

Nitrogen efficiency can further be extended to agronomic use efficiency which is the increase in grain yield obtained when N is applied as a fertiliser (Smith *et al.*, 1988) because it is difficult to determine how much N is in the soil and how much is taken up. This Nitrogen Use Efficiency can be expressed as mass of dry matter produced per N added according to Novoa & Loomis (1981) using the following equation:

$$\mathrm{NUE}\,(\mathrm{kgDM\ kg\,N^{-1}}) = \frac{\mathrm{DM\ (kg\,m^{-2})} - \mathrm{DM\ control\ (kg\,m^{-2})}}{\mathrm{N\,added\ (kg\,m^{-2})}}$$

Many studies have shown the importance of nitrogen nutrition to growth and yield of canola with many authors reviewing NUE and its improvement in many crops, setting ideal plants (Sylvester-Bradley & Kindred, 2009). Several rates of N application have been reported, varying with locality, soil types, production practices and varieties, but mostly, in the European Union, a crop yielding 3 t ha^{-1} will require a N application input of 150-210 kg ha^{-1} (Pouzet, 1995). A high rate of N application increases leaf development and leaf area duration (LAD) after flowering and finally increasing overall crop assimilation, thus contributing to increased seed yield (Wright *et al.*, 1988).

Allen & Morgan (1972) concluded that N increases yield by influencing a variety of growth parameters such as the leaf area index (LAI), the number of branches per plant (plasticity), the total plant weight, and the number and weight of pods and seeds per plant. In an experiment done by Cheema *et al.* (2001) on the effect of time and rate of nitrogen and phosphorus application on the growth and seed and oil yields of canola (*B. napus* L.), the highest rates of fertiliser application significantly increased LAI relative to the control and the lower rates of application throughout the period of the trials.

Amongst other factors, excess N, however, can reduce seed oil yield and quality appreciably (Ahmad *et al.*, 2007). The possible reason for a decrease in oil content with an increase in nitrogen may be due to the fact that nitrogen is the major constituent of proteins. Hence, N increases the percentage of protein of the seed as a result there might be a decrease in the percentage of oil considering the inverse relationship between oil and protein (Zhao *et al.*, 1993; Jan *et al.*, 2002). However, the highest N level resulted in the highest value for protein (23.5 %) and glucosinolate (19.9 µmol g^{-1}) contents (Ahmad *et al.*, 2007). The relatively high protein content of the rapeseed meal, in combination with a well-balanced amino acid combination, makes rapeseed meal a valuable source of protein in animal diets, especially non-ruminants.

2.3.3 Genetic variability in Nitrogen Use Efficiency of canola

Some plant species and genotypes have a capacity to grow and yield well on soils with low fertility; these species and genotypes are considered tolerant to nutrient deficiency (Rengel, 1999) hence they are nutrient efficient. Efficient genotypes grow and yield well on nutrient deficient soils by employing specific physiological mechanisms that allows them to gain access to sufficient quantities of nutrients (uptake efficiency) and or more effectively nutrient taken up (utilization efficiency) (Sylvester-Bradley & Kindred, 2009). However, performance of current crop cultivars in temperate regions is far from this ideal (Fageria *et al.*, 2008), though a wide literature exists on improved fertiliser use efficiencies of crops; canola (Yau & Thurling, 1987, Svečnjak & Rengel, 2006; Gan *et al.*, 2008; Tatjana *et al.*, 2008), wheat (Foulkes *et al.*, 1998; Goodlass *et al.*, 2002; Dampney *et al.*, 2006). It is also evident from literature that some crop plants have a higher Nitrogen Use Efficiency, with the extract from Sylvester-Bradley & Kindred (2009) in Table 2.1 showing canola amongst other crops with its NUE (kg DM kg^{-1} N available) of about 9.

Table 2.1 Average overall Nitrogen Use Efficiency of some of the main arable crops in the UK

Crop	**Harvested DM (t ha^{-1})**	**N applied or fixed (kg ha^{-1})**	**N capture (kg N up kg^{-1}N avail.)**	**N conversion (kg DM kg^{-1}Nup)**	**NUE (kg DM kg^{-}N avail.)**
Sugar beet	12.7	105	1.07	64	69
Potatoes: main crop	9.5	155	0.81	50	40
Potatoes: seed	6.7	120	1.09	31	34
Spring wheat: milling	4.9	132	0.68	34	23
Potatoes early	6.3	194	0.71	32	23
Spring oats	4.3	109	0.61	37	22
Winter wheat: milling	6.2	209	0.65	33	22
Spring barley: malting	4.1	119	0.39	53	21
Winter barley: malting	4.6	143	0.45	46	21
Oilseed rape : winter	2.9	207	0.85	12	10
Oilseed rape: spring	2.0	134	1.10	8	9
Peas: harvested dry	3.1	265	0.56	16	9
Faba beans: winter	3.2	285	0.51	17	9
Peas: vining	1.6	165	0.41	16	6

Extract from Sylvester-Bradley & Kindred (2009)

Hirel *et al.* (2007), states that there is a paucity of data on the genetic variability for NUE at low N fertilisation input in canola, though other work has shown genetic variability (Tatjana *et al.*, 2008). In spring rape, it has been shown that cultivars with the lowest yields at the lowest N concentration generally responded more to increased N application rates than cultivars with a higher yield at high N supplies (Yau & Thurling, 1987). This is presumably due to a greater ability for uptake and translocation of N (Grami & LaCroix, 1977).

As plants require large amounts of N from the soil, an extensive root system is essential to allow unresisted uptake. Plants with roots affected by compaction may show signs of N deficiency even when adequate N is present in the soil. A plant supplied with adequate N grows rapidly and produces a large amount of green foliage, hence increasing the photosynthetic capacity. More recently, in spring canola, differences in NUE were found resulting in a greater biomass production (Svečnjak & Rengel, 2006) and due to differences in the root to shoot ratio and harvest index. However, no major impact on plant biomass, N uptake, and seed yield were found across two contrasting N treatments (Svečnjak & Rengel, 2006). These observations confirmed earlier findings showing that there was no interaction between Qualitative Trait loci's for yield and N treatments (Gül, 2003).

When Yau & Thurling (1987) evaluated the variations in fertiliser N response among spring rape cultivars and its relationship to N uptake and utilization, they noted a cultivar difference. Their work showed the ability of genotype to yield adequately where a low N input is partly depended on heritable capacity to utilize N efficiently for dry matter production prior to flowering. Through noting the cultivars and their origin, introgression of genes for more efficient N utilization from earlier varieties to the latter was suggested (Yau & Thurling, 1987).

As recently reviewed by Rathke *et al.*,(2006), it is clear that to improve seed yield, oil content, and N efficiency in winter oilseed rape, the use of N-efficient management strategies are required, including the choice of variety and the source and timing of N fertilisation adapted to the site of application. In a study by Gan *et al.* (2008), five oilseed species investigated for NUE showed similar response patterns of seed N uptake to N fertiliser rates, while the magnitude of response varied among the species; *Sinapis alba*, *B. juncea*, *B. rapa*, *B. napus*, and various varieties within the species. Gerath & Schweiger (1991) have shown that some cultivars may differ in nitrogen uptake and translocation. They classified the cultivars based on nitrogen uptake with; a) higher nitrogen, higher output, b) those which increases yield with increasing rate up to a stable point, then final decrease in yield and c) the third type shows a marked decrease in oil content as nitrogen levels are increased. This correlation between oil and protein content has been documented by several workers.

Hirel *et al.* (2007) considered N harvest index (NHI), defined as N in grain/total N uptake, as an important consideration in crop plants. It reflects protein content within the grain, hence the nutritional quality. Studies on identifying the genetic basis for grain composition showed that breeding progress has been limited by an apparent inverse genetic relationship between grain yield and protein or oil concentration in most cereals (Hirel *et al.*, 2007), as well as canola (Brennan *et al.*, 2000; Jackson, 2000), where the concentration of oil in the canola seed decreased with an increase in protein.

2.3.4 Mechanisms for Nitrogen Use Efficiency in Canola

Efficiency in N application reduces excessive input of fertiliser whilst increasing acceptable yields and quality. Review on the mechanisms with relation to growth, N uptake, patterns of dry matter (DM) and N allocation, grain yield, photosynthetic (PS) rates and N-use efficiency would be important so that assimilation and use can be controlled to meet the crop end-use needs. According to Jackson *et al.* (2008), there is high inefficiency in the N nutrition of plants. The ultimate crop in terms of N use efficiency (NUE) would be expected to maintain maximum photosynthetic production throughout the period of high irradiance and water availability with a photosynthetic canopy formed by the capture of only that N becoming available from the soil (and atmosphere), and with minimal or no fertiliser additions. Since the performance of current crop cultivars in temperate regions is far from this ideal (Fageria *et al.*, 2008), there is a massive challenge in understanding all the inefficiencies and in finding appropriate genetic stock or other innovations that will increase NUE without slowing improvements in crop productivity.

When an excess of N cannot be totally avoided, it should also be important to search for species or genotypes that are able to absorb and accumulate higher concentrations of N, at the same time keeping the N levels at acceptable levels for the end-use of the crop without negatively affecting grain quality. The genetic variability in maximum N uptake in crop plants and the physiological and genetic basis for such variability has never been thoroughly investigated. Variability could confer on some genotypes or species the ability to store greater quantities of N.

Analysis of the genotypic variability of canola N uptake capacity allows the selection and use of those with greatest capacity to accumulate N either during excessive N or under limited N levels.

2.4 SULPHUR UPTAKE AND UTILIZATION

Sulphur availability has been identified as a key factor critical for canola production; with deficiencies frequently lowering canola yield (Fismes *et al.*, 2000). Its concentration in canola plants varies between 1 and 16 g kg^{-1} dry mass, depending on the external supply (Balint & Rengel, 2009). Sulphur is a constituent of certain amino acids needed for protein synthesis in canola. It improves the quality of canola seed, including oil content. Deficiencies will greatly reduce N uptake hence the application of S needs to be balanced with N for optimum yields (Ceccoti, 1996; Fismes *et al.*, 2000). The N:S ratio is diverse (Zhao *et al.*, 1993; Ahmad & Abdin, 2000; Fismes *et al.*, 2000; Balint *et al.*, 2008), but the typical ratios range from 7:1 to 5:1.

With declining atmospheric deposition, due to cleaning up of sulphur dioxide from the burning of fossil fuel and other emission sources, and a changing practice in moving away from nitrogen and other fertilisers containing sulphur (eg ammonium sulphate), sulphur levels in the soil generally have been declining (Booth & Gunstone, 2004). Canola is one of the most sensitive arable crops to sulphur deficiencies, as it has a higher demand (McGrath & Zhao, 1996; Zhao *et al.*, 1993). This effect has been recognized and many crops are now receiving a sulphur dressing though it is recommended that many farmers do not apply enough to prevent deficiencies from limiting yield potential on a sandy soil (where sulphur levels will be low due to leaching loss).

A yield response of 0.7-1.6 t ha^{-1} was reported to an application of 40 kg S ha^{-1} (McGrath & Zhao, 1996). Some work have recommended 16 kg S per ton of seed, thus three ton crop requires around 50 kg S ha^{-1} (Kimber & McGregor, 1995). However, as the effects of sulphur are related to nitrogen levels, such recommendations would be based on nitrogen recommendations of an appropriate variety, also considering inherent soil sulphur levels, climatic regions and yield potential.

Sulphur fertilisation enhanced nitrogen efficiency in canola, leading to increased N assimilation into leaf protein, thus using N efficiently. Canola has a high demand of S because of its high content of S-containing proteins. According to Good & Glendinning (1998), the N:S ratio in plant tissue, which is widely used in assessing the S nutrition in the winter type canola varieties grown in Europe, seems to be of little value to the spring varieties grown in Australia.

There is a diverse range of sulphur fertilisers available including sulphates and elemental S as well as blended products that include various ratios of elemental and sulphate S. Usually, each form of sulphur fertiliser, requires a different management system to maximize the nutrient potential of the product (Canola Council of Canada, 2006). Generally, for immediate crop uptake, sulphate formulations are recommended. Sulphur fertilisers containing elemental S must be managed differently to those containing sulphate based fertilisers but mainly, the disadvantage with elemental forms is that its availability is delayed until soil bacteria oxidize it into the sulphate form (McKenzie, undated).

Besides the formation of proteins during growth and development of canola, naturally occurring compounds called glucosinolates can also be synthesized (Zhao *et al.*, 1993). Sulphur application can increase seed glucosinolates (Jan *et al.*, 2002), with the glucosinolate content of high glucosinolate lines more responsive to sulphur than that of low glucosinolate lines. Several studies have also shown that S supply may increase glucosinolate (GLS) content of canola (Fismes *et al.*, 2000) however the high level of glucosinolate hydrolysis products can adversely reduce the feeding value of rapeseed meal rendering the meal unpalatable. Therefore addition of high S levels contradicts the effect of the addition of high N, where the later decreases glucosinolate levels (Arora & Bhatia, 1970). Thus, an insufficient S nutrition leads to a decline in seed yield whilst an excessive S supply can affect meal quality by increasing seed GLS content, meaning there should be a balance in S and N levels in order to maintain desirable yields of good quality. According to Rosa & Rodrigues (1998), glucosinolates are hydrolysed by the myrosinase enzyme upon seed processing to form undesirable tasting, toxic and goitrogenic compounds. Fismes *et al.*, (2000) observed a significant response of GLS content to S application in calcareous soils when S supply was above 30 kg ha^{-1}, and an application of 75 kg S ha^{-1} increased the GLS content by 52 %. However, with the general widespread use of cultivars low in both glucosinolates and erucic acid (double low cultivars), reasonable levels of GLS can be achieved owing to the ability of these cultivars to store (Zhao *et al.*, 1993) and to regulate (Fismes *et al.*, 1999) excessive S in pod walls.

As nitrogen and sulphur are both involved in plant protein synthesis, the shortage in S supply for crops decreases the N-use efficiency of fertilisers (Ceccoti, 1996). Consequently, the poor efficiency of N caused by insufficient S needed to convert N into biomass production may increase N losses from cultivated soils (Schnug *et al.*, 1993). Plants assimilate N and S in amounts proportional to that incorporated into amino acids and proteins, which suggest that N and S requirements are closely interrelated (Fismes *et al.*, 2000). Increasing N fertiliser rates aggravate S deficiency of oilseed rape and reduce seed yield when available S is limiting (Janzen & Bettany, 1984). Nitrogen addition increases seed yield in S-sufficient conditions, and an optimum oil quality and maximum yield responses to both N and S applications are obtained when the amounts of available N and S are balanced (Josh *et al.*, 1998).

2.4.1 Sulphur deficiency symptoms in canola

There is currently a high requirement of S especially with the environmental cleanup of power stations, since the mid-1980s, reducing atmospheric supply of S (Booth & Gunstone, 2004), to the extend that deficiencies of this nutrient is now pronounced. Chlorosis of the leaves and reduction of yield has been widely observed. Sulphur is involved in photosynthesis and, deficiencies decreases chlorophyll content and leaves turn yellow showing inter-veinal chlorosis (Pouzet, 1995). Generally S deficient plants have short and or spindly stems with yellowing of the young top leaves. With N deficiency, yellowing affects the older, lower leaves first. Sulphur deficiency can also have a purpling and upward cupping of young leaves, delayed and prolonged flowering, pale colored flowers, and fewer, smaller pods.

Sulphur mainly enhances the reproductive growth, and the proportion of the reproductive tissues (inflorescences and pods) to total dry matter was found to be significantly increased by S during pod development (McGrath & Zhao, 1996). Under S deficient conditions, the amount of amino acids and nitrates in leaves increases dramatically and protein degradation within chloroplasts occurred (Fismes *et al.*, 2000). Besides, sulphur affects photosynthetic characteristics. Sulphur deficiency limits protein synthesis by limiting the amount of methionine and cysteine available for the assembly of new proteins (Fismes *et al.*, 2000).

2.4.2 Genetic variability in Sulphur Use Efficiency in Canola

Sulphur requirements depend on plant species (Balint & Rengel, 2009). A canola crop grown under United Kingdom conditions has high S requirements (16 kg of S for 1 ton grain) compared with cereals (3 kg of S for 1 ton of grain) (McGrath *et al.*, 1996). Fismes *et al.* (2000) highlighted cultivar sensitivity to imbalanced N/S ratios and recommended further studying to gain a better understanding of the different sensitivities between cultivars. Ahmad *et al.* (2005) hypothesized these differences to be due to differential S uptake kinetics at the root cell plasma membrane, such that there could be existence of a biphasic transport system (combination of high and low-affinity transporters). Through S starvation, some high-affinity sulphate transporters are regulated in *Arabidopsis thaliana* (Rouached *et al.*, 2008).

Balint *et al.*, (2008) also confirmed genetic variation of canola genotypes during the vegetative growth stage. Such variation in efficiency is due to increasing the rate at which the nutrient is transported within the plant or compartmented in cells (Rengel & Hawkesford, 1997), maybe as a result of the different transport systems (Ahmad *et al.*, 2005).

Lappartient and Touraine (1996) hypothesized that glutathione is responsible for mediating responses to S availability through demand-driven processes that involve the translocation to roots of a phloem-transported message that provides information about the nutritional status of canola leaves. Under such circumstances, S-efficient genotypes are likely to contain larger amounts of glutathione and/or phloem-transported messages compared with inefficient S genotypes (Balint & Rengel, 2009).

Hence, with demand driven processes playing a role in uptake and use efficiency, varietal and environmental differences becomes very important as demand may become related to the growth rates and biomass of the plant. The concentration or amount of these compounds is most likely variable during plant development, contributing to differential S efficiency in a given genotype. Differential S efficiency may also be due to differential remobilization of sulfate reliant on differential efficiency of transporters involved in remobilizing vacuolar sulfate. Balint & Rengel (2009) commented on having genetic modifications on such transporters to increase S efficiency in plants.

2.5 CONCLUSION

In general N availability influences several developmental processes within the plant. Sulphur fertilisation is highly depended on N as both elements are needed as building blocks of amino acids and other S and N containing molecules. Sulphur improves the apparent N use efficiency and uptake of both elements is mutually regulated such that they act synergistically during optimum levels. However, the uptake and utilization, and hence fertilisation of the elements need to be coherent with the genotypes of the canola plants being grown, with relevance to the right soil and environmental factors. Being susceptible to both N and S deficiency, knowledge of canola varieties response to fertilisation with different uptake and efficiency will help in using fertiliser in an economic and environmental sustainable manner, whilst potential yields are met.

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CHAPTER 3

The effect of nitrogen and sulphur on macro- and micro-nutrient content in canola (*Brassica napus* L.) plants

Abstract

Soil pH, and nutrient content influences uptake and utilization of nutrients required for plant growth. Soil characteristics in the canola growing areas of the Western Cape Province of South Africa are often very variable. Hence the major aim of this research was to determine effect of soil and climatic differences, as experienced at different localities on macro- and micro-nutrient content in canola plants fertilised with different N (nitrogen) and S (sulphur) application rates. Plants fertilised with 0, 15 and 30 kg S ha^{-1} in combination with N rates of 0 and 120 kg ha^{-1} were sampled at 90 DAP (flowering stage) at Altona, Elsenburg, Langgewens, Roodebloem and Welgevallen localities in the Western Cape during 2009, 2010 and 2011. Nutrient content in canola plants were affected by locality and interactions between locality and nitrogen application rates, but not by S with the result that contents of some nutrients such as B, Ca, Mn, S and N were below critical levels required for optimum growth and development of canola plants. Luxurious consumption has been observed at some localities with regard to K, Na, Fe and Al.

Keywords: Canola, critical nutrient level, nitrogen, nutrient content, sulphur

3.1 INTRODUCTION

Understanding soil nutrient contents and crop nutrient availability is important to increase canola yields. Canola utilizes more nutrients to produce 1 ton of grain than cereals do (Oplinger *et al.*, 2000). Fertiliser applications, especially on nutrient deficient soils, can therefore increase crop yields and quality. Both macro and micronutrients are essential in proper crop growth, but nitrogen (N) and sulphur (S) are the most limiting nutrients (Gan *et al.*, 2008).

Besides N and S, other essential macronutrients for canola growth and development include: phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg), whilst micronutrients include: sodium (Na), iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), boron (B) and aluminium (Al) (Pouzet, 1995). The range between deficient and toxic levels in some micronutrients is narrow (Mengel & Kirkby, 1982), making management very critical.

Proper fertiliser recommendations becomes complex with nutrient interactions due to variability in soil chemical properties. Cayton *et al.* (1985) reported on the absorption and translocation of plant nutrients like Fe, Mg, K, P and Ca and their dependence on Zn concentrations in the soil. Zinc absorption was also highly dependent on N uptake (Cayton *et al.*, 1985), whilst N fertiliser was reported to increase the concentration of excess cations (Hannaway

& Reynolds, 1979). However, N fertilisation has generally been found superior to sulphur in enhancing absorption of nutrients (Jackson, 2000). Antagonistic effects between the plant essential nutrients on absorption sites can also influence uptake of some nutrients over the other (Mengel & Kirkby, 1982). Variability in soil pH can also increase uptake of some nutrients over others. Santonoceto *et al.* (2002) considers factors associated with acidity as causes of poor plant growth, which include deficiencies of K and Ca and the unavailability of other essential nutrients. Uptake of cations from the soil has a recurring effect of increasing soil acidity, therefore the need to efficiently use nutrients.

According to Hocking *et al.* (undated), Australian canola growers face various nutritional problems with the crop as it is grown on diverse soil types that include deep, leached, sandy, highly calcious or acidic soils. The same rings true in South Africa, where the crop is new and little is known about the crop and soil fertility status interaction as well as the effect of soil, climate and production techniques (fertiliser rates). Canola cultivation is on diverse soils ranging both in chemical and physical properties whilst they are distributed within an area characterised by differences in rainfall distribution and intensity. Moreover, poor and or variable responses to increases in N application rates (Hardy *et al.*, 2004) have been noted on canola produced in the Western Cape Province of South Africa.

The aim of this research was to determine the effect of soil and climatic differences, as experienced at different localities on nutrient content of canola plants to different N and S application rates.

3.2 MATERIAL and METHODS

3.2.1 Locality

The field experiments were conducted at Elsenburg (33^{0}51^{I}S; 18^{0}51^{I}E; 117 m.a.s.l.), Langgewens (33^{0}17^{I}S; 18^{0}40^{I}E, 91 m.a.s.l.), Roodebloem (34^{0}22^{I}S; 19^{0}52^{I}E, 132 m.a.s.l) Experimental stations of the Western Cape Province of South Africa during the canola seasons of 2009, 2010 and 2011, as well as at Welgevallen (33^{0}52^{I}S; 18^{0}42^{I}E, 119 m.a.s.l.) in 2010 and at Altona (33^{0}42^{I}S; 18^{0}37^{I}E, 42 m.a.s.l.) in 2011. Originally the study was planned to be conducted at Elsenburg, Langgewens, Roodebloem and Welgevallen, but damage due to birds in 2009 and lesser extend also in 2010 at Welgevallen, necessitated the use of Altona in 2011 instead of Welgevallen.

Rainfall

During the winter seasons of 2009, 2010 and 2011 the rainfall recorded (April to October) at the localities were variable (Fig. 3.1). In 2009, Elsenburg received the highest total rainfall (568.3 mm) whilst Roodebloem and Langgewens received a total rainfall of 339.4 and 285.2 mm, respectively.

The rainfall received in the 2010 season was considerably lower than the 2009 season at most localities except at Langgewens where 366 mm of rainfall was received compared to 285.2 mm of the previous season. Elsenburg received a higher total rainfall (442.9 mm) than Welgevallen (426.6 mm) and Roodebloem (320.9 mm). Higher rainfalls beyond the long term averages for the month were received during May and there has been a drop in rainfall as the season progressed towards spring (September-October) at all localities.

The 2011 rainfall was below the expected at most localities except at Langgewens (308.6 mm) which was higher than in 2009, even though it was below that received in 2010 at the same locality. Elsenburg received the highest total rainfall (325.8 mm) compared to Langgewens (308.6 mm), Altona (295.9 mm) and Roodebloem (241.5 mm). In July, rainfall was low irrespective of locality.

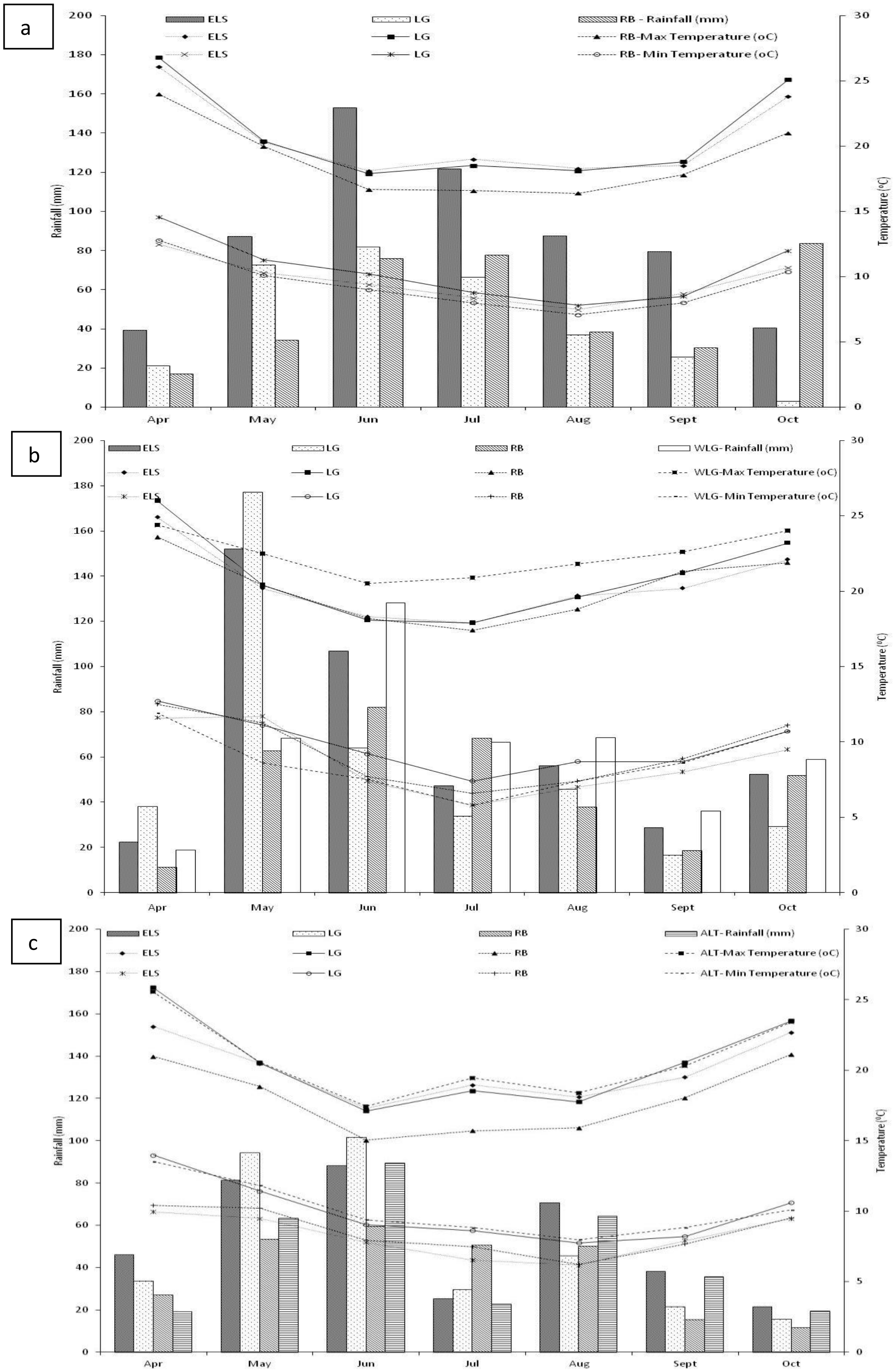

Figure 3.1 Climatic data for Elsenburg (ELS), Langgewens (LG), Roodebloem (RB), Welgevallen (WLG) and Altona (ALT) during the a) 2009, b) 2010 and c) 2011 growing season

Temperature

The mean maximum monthly temperatures for the growing season were 19.7, 19.8 and 18.1 °C for 2009; 19.7, 20.1 and 19.7 °C for 2010; 19.5, 19.7 and 17.4 °C for 2011 at Elsenburg, Langgewens and Roodebloem respectively. The temperature patterns in all three years showed a decrease in both maximum and minimum as the cropping period progressed from April to August, but increased from September at all localities (Fig. 3.1). In all 2009 months, Langgewens was generally warmer than Elsenburg and Roodebloem in all months. In 2010, Welgevallen (22.1 °C) was warmer than the other localities whilst Langgewens was warmer than Roodebloem and Elsenburg only in October. The 2011 canola growing season showed mean maximum monthly temperatures at Altona (19.9 °C) being higher than experienced at Elsenburg, Langgewens and Roodebloem.

In all three years, 2010 was considerably warmer in all localities with temperatures than those in 2009 and 2011. In the three seasons, although maximum temperatures may have risen to about 30 °C on a few days during October, temperatures were generally favourable for canola production.

3.2.2 Soil Characteristics

Soil samples were collected at planting from the plots receiving no N or S and analyzed for pH, acidity, resistance as well as macro and micronutrients at the laboratories of the Department of Agriculture in the Western Cape at Elsenburg using standard procedures (The Non-affiliated Soil Analysis Work Committee, 1990). Soil nutrient content for different localities did not vary much between years, therefore results for different years were pooled (Table 3.1).

Soil nutrient properties differ between localities used for the trials (Table 3.1). Soils at Altona, Elsenburg and Welgevallen had a low pH with values below the ideal of 5.0 and also high acidity values (>1.0 cmol kg^{-1}) compared to Roodebloem and Langgewens which shows values within the pH range (pH 5.0 - 7.0) for optimal canola growth and development. Values of pH at Roodebloem falls in the neutral range, with high Ca, Mg, Na, total cations, B, C, N and lower resistance than the other localities, thus having a higher buffering capacity (Rowell, 1994). However, the soil Mn content of Roodebloem was lower than at other localities even though it was above the critical level.

Besides; N and S, only Mg was below the critical level (<0.4 cmol kg^{-1}) at Elsenburg. Considering the mean values through the soil profile, all micronutrients were above the levels required for canola production except for B (< 0.2 mg kg^{-1}) at Welgevallen and Langgewens. Sulphur and total N levels at almost all localities were low with S below the required critical level of 6 mg kg^{-1} required for canola production, except at Elsenburg and Welgevallen (Table 3.1). The N-mineralisation potential in the 0-200 mm soil profile were as follows: Altona 111.3 kg N ha^{-1} (2011 only), Elsenburg 92.0 kg N ha^{-1} (2009-2011), Langgewens 56.8 kg N ha^{-1} (2009-2011), Roodebloem 59.9 kg N ha^{-1} (2009-2011) and Welgevallen 93.5 kg N ha^{-1} (2010 only). These mineral-N values were calculated from nitrate and ammonium-N contents as measured at planting and mineralized during the first forty days after planting, using the indophenol-blue (Keeney & Nelson, 1982) and salicylic acid methods (Cataldo *et al.*, 1975) to give an indication of the potential of the soil to supply nitrogen to the crop.

Table 3.1 Soil characteristics at planting at Altona, Elsenburg, Langgewens, Roodebloem and Welgevallen. Mean values 2009-2011

Locality	Depth (cm)	pH (KCl)	Acidity (cmol kg^{-1})	Resistance (ohm)	Total cations value (cmol kg^{-1})	Total N (%)	P - citric acid (mg kg^{-1})	K (mg kg^{-1})	Ca (cmol kg^{-1})	Mg (cmol kg^{-1})	S (mg kg^{-1})	C (%)	Cu (mg kg^{-1})	B (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Na (mg kg^{-1})
Altona[1]	15	4.7	0.94	420	5.41	0.11	90	111	3.08	0.85	7	1.27	1.38	0.24	50.37	3.58	60
Altona	30	4.3	0.87	1280	3.74	0.06	60	69	1.93	0.62	3.3	0.59	1.72	0.14	105.4	1.99	34
Altona	45	4.7	0.59	1980	3.97	0.04	34	56	2.24	0.83	2.1	0.36	1.99	0.17	119.8	1.29	38
Elsenburg	15	5.4	1.1	760	4.33	0.06	67	117	3.25	0.33	8.7	0.79	1.94	0.22	44.2	1.53	19
Elsenburg	30	4.6	0.87	13467	3.15	0.05	46	102	1.61	0.27	5.89	0.66	1.57	0.18	43.74	1.01	15
Elsenburg	45	4.6	0.83	1503	2.86	0.05	30	93	1.45	0.28	3.97	0.51	1.52	0.19	28.92	0.74	14
Langgewens	15	5.6	0.37	783	4.11	0.05	83	142	2.92	0.58	6.63	0.48	1.15	0.12	109.07	1.69	28
Langgewens	30	5.4	0.53	1653	3.61	0.04	77	115	2.2	0.66	4.35	0.37	1.07	0.11	117.58	1.26	25
Langgewens	45	5.9	0.45	1890	3.74	0.03	65	98	2.45	0.74	4	0.31	1.11	0.12	112.91	0.86	33
Roodebloem	15	5.4	0.92	763	7.93	0.17	72	107	5.42	1.3	4.01	1.99	0.84	0.3	16.87	1.62	75
Roodebloem	30	5.1	0.92	1090	5.34	0.11	32	66	3.21	1.08	2.18	1.15	0.88	0.21	11.98	1.07	63
Roodebloem	45	5.1	0.81	1403	5.12	0.1	24	65	2.81	1.01	2.07	0.98	0.84	0.21	10.66	0.91	74
Welgevallen[2]	15	4.7	0.97	540	4.91	0.12	201	241	2.57	0.66	11	1.08	1.44	0.14	18.55	3.64	21
Welgevallen	30	4.4	1.11	840	4.45	0.09	189	184	2.29	0.53	6.2	0.99	1.38	0.14	18.43	3.3	12
Welgevallen	45	4.5	1.06	710	4.9	0.09	209	187	2.63	0.67	7.2	0.82	1.55	0.18	13.62	3.36	14
Critical nutrient content[a]		<5[2]	-	-	-	-	<36[2]	<60[2]	<1.0[1]	<0.4[1]	<6.0[1]	-	<0.3[1]	<0.2[1]	<5.0[1]	<0.5[1]	>250[2]

[a] shows the critical soil nutrient levels at which the nutrient became deficient[1] or where the growth of canola plants will be negatively affected[2] (Canola production manual, undated; Peverill, 1999)

[1] 2011 only; [2] 2010 only

3.2.3 Experimental procedure

The experiment was laid out as a randomized block design with a factorial split plot arrangement. Treatments consisted of five nitrogen rates (0, 30, 60, 90 and 120 kg N ha^{-1}) as main plots and three S rates (0, 15 and 30 kg S ha^{-1}) as sub-plots. There were 15 (5N and 3S) subplots in one replication which were repeated in four blocks. The sizes of the subplots were 5 x 3 m^{2}. The N source was Limestone Ammonium Nitrate (LAN) with 28 % N while S was applied in the form of gypsum ($CaSO_4.2H_2O$) with 16 % S. All S treatments were applied at planting whilst N was applied as:

- N 0=0 kg N ha^{-1} (No N applied)
- N 1=30 kg N ha^{-1} (30 kg N ha^{-1}applied at planting)
- N 2= 60kg N ha^{-1} (30 kg N ha^{-1}applied at planting and 30 kg N ha^{-1}at 30 DAP)
- N 3=90 kg N ha^{-1} (30 kg N ha^{-1} applied at planting plus 30 kg N ha^{-1} at 30 DAP and final 30 kg N ha^{-1} at 60 DAP)
- N 4= 120 kg N ha^{-1} (30 kg N ha^{-1} applied at planting plus 30 kg N ha^{-1}at 30 DAP plus 30 kg N ha^{-1} at 60 DAP and final 30 kg N ha^{-1} at 90 DAP)

An early season canola cultivar, Stubby, was sown on 22 April, 25 April and 12 May at Langgewens, Elsenburg and Roodebloem respectively in 2009. The medium season cultivar Bravo was used in the 2010 season and sown on; 17 May, 19 May, 20 May and 26 May at Roodebloem, Langgewens, Elsenburg and Welgevallen respectively. For 2011, cultivar Bravo was planted on 9 May, 10 May, 11 May and 26 May at Roodebloem, Altona, Langgewens and Elsenburg respectively. All planting was done at a planting density of 4 kg ha^{-1}.

3.2.4 Data collection

At 90 days after planting (DAP), a net plot of 0.5 m^{2} per replication was sampled in each of the following six treatment combinations of S (0, 15 and 30 kg ha^{-1}) and N (0 and 120 kg ha^{-1}). It is however important to note that at the time of sampling only 90 kg N ha^{-1} was applied to the 120 N treatments as the last increment of 30 kg N ha^{-1} was applied at 90 DAP. Samples were air dried and thereafter milled and analysed to determine the nutrient concentrations (contents) of N, S, P, K, Ca, Mg, Na, Fe, Cu, Zn, Mn, B and Al (ALASA, 1998).

3.2.5 Data analysis

Data was analyzed, using analysis of variance (ANOVA) (Statistica 11). Because the results of the plant analysis did not show any differences due to the different years in 2009 and 2010, plant samples for different replications were pooled before analysis in 2011 and years were used as replications and locality was regarded as a factor. Interaction effects were compared using least significant difference (LSD) test at 5 % level of probability. Any treatment means found to be significantly different were separated using Fischer's protected $LSD_{0.05}$.

3.3 RESULTS and DISCUSSION

3.3.1 Significance of F values

The nutrient content of N, Ca, Mg, and Na were significantly affected by the interaction of the locality by N application rate. Both locality and N rate as main factors affected the nutrient contents of S, K and Al. The nutrient contents of Ca, Zn, Mn, and B were only significantly affected by locality (Table 3.2).

Table 3.2 Summary of significant effects (F-values) from the Analysis of variance done on data of the plant nutrient contents, and N and S uptake in kg ha^{-1} at 90 DAP.

	Source of variation						
	Locality	**N rate**	**S rate**	**Locality X N**	**Locality X S**	**N X S**	**Locality X N X S**
N (%)	***	***	ns	***	ns	ns	ns
S (%)	***	***	ns	ns	ns	ns	ns
P (%)	ns	ns	ns	ns	ns	ns	ns
K (%)	***	*	ns	ns	ns	ns	ns
Ca (%)	***	ns	ns	**	ns	ns	ns
Mg (%)	***	ns	ns	***	ns	ns	ns
Na (mg kg^{-1})	***	***	ns	***	ns	ns	ns
Fe (mg kg^{-1})	ns	ns	ns	ns	ns	ns	ns
Cu (mg kg^{-1})	***	ns	ns	ns	ns	ns	ns
Zn (mg kg^{-1})	***	ns	ns	ns	ns	ns	ns
Mn (mg kg^{-1})	***	ns	ns	ns	ns	ns	ns
B (mg kg^{-1})	***	ns	ns	ns	ns	ns	ns
Al (mg kg^{-1})	***	*	ns	ns	ns	ns	ns

*, **, *** Significant at P≤0.05, P≤0.01, P≤0.001 respectively, ns denotes non significance at P≤0.05

3.3.2 Nutrient contents

Canola plants at Elsenburg and Welgevallen had significantly higher contents of S, K, Zn, Mn and Al compared to those at Altona, Langgewens and Roodebloem (Table 3.3). Plants at Roodebloem (2.51 %) had a lower N content compared to those at Altona (2.84 %), Langgewens (2.92 %), Welgevallen (3.29 %) and Elsenburg (3.36 %), with S, K, Cu, Zn, Mn and Al contents also lower. Higher rainfall and assumed higher soil moisture levels at Elsenburg and Welgevallen lead to more plant N accumulation because N uptake is improved by sufficient soil moisture (http://www.canolacouncil.org/chapter9.aspx). Thus N overall increased assimilation of other essential mineral nutrients (Santonoceto *et al.,* 2002). Plant Mg and Na contents at Elsenburg and Welgevallen were however less than that at Altona, Langgewens and Roodebloem, because these soils, with the exception of Mg content of Langgewens showed lower Mg and Na. Zinc content at Welgevallen (60.17 mg kg^{-1}) were higher than at Elsenburg (41.34 mg kg^{-1}) and Altona (39.12 mg kg^{-1}) whilst plants at Roodebloem (25.18 mg kg^{-1}) and Langgewens (24.65 mg kg^{-1}) had the lowest contents of Zn. Different responses on Zn content may be due to differences in soil pH (Mengel & Kirkby, 1982) and soil moisture levels at different localities.

Table 3.3 Canola nutrient contents at Altona (ALT), Elsenburg (ELS), Langgewens (LG), Roodebloem (RB) and Welgevallen (WLG) localities and plant response to N fertilisation rates of 0 and 120 kg ha^{-1} at 90 DAP.

	Locality						***N rate* (kg ha^{-1})**				**Critical**[y]
	ALT	ELS	LG	RB	WLG	p	0	120	p	Mean	
N (%)	2.84b	3.36a	2.92b	2.51c	3.29a	***	2.77b	3.20a	***	2.99	>3.5
S (%)	0.28c	0.38a	0.34b	0.30c	0.35ab	***	0.35a	0.31b	***	0.33	>0.5
P (%)	0.52	0.55	0.99	0.44	0.55	ns	0.73	0.49	ns	0.61	>0.3
K (%)	4.62b	4.88b	3.43c	2.99c	6.02a	***	4.17b	4.61a	*	4.39	>2.2
Ca (%)	1.24d	2.11a	2.12a	1.44c	1.79b	***	1.72	1.76	ns	1.74	>1.4
Mg (%)	0.54a	0.34c	0.39b	0.35c	0.31d	***	0.38	0.39	ns	0.39	>0.2
Na(mg kg^{-1})	10066.7a	2173.7c	6193.1b	7516.3b	1295.9c	***	4434.3b	6464.0a	***	5449.15	-
Fe (mg kg^{-1})	450.31	379.15	312.83	867.34	398.32	ns	601.74	361.44	ns	481.59	>19
Cu (mg kg^{-1})	6.69ab	8.22a	5.07b	4.54b	5.88ab	***	5.40	6.76	ns	6.08	>3
Zn (mg kg^{-1})	39.12b	41.34b	24.65c	25.18c	60.17a	***	36.03	40.15	ns	38.09	>20
Mn (mg kg^{-1})	31.59c	40.0bc	43.35b	17.90d	54.75a	***	36.63	38.41	ns	37.52	>30
B (mg kg^{-1})	44.72a	32.09b	28.65c	29.61bc	25.40d	***	31.77	32.42	ns	32.10	>20
Al (mg kg^{-1})	483.33a	575.97a	267.07b	202.25b	520.0a	***	365.62b	453.83a	*	409.73	-

*, **, *** Significant at P≤0.05, P≤0.01, P≤0.001 respectively, ns denotes non significance at P≤0.05. Means in the same row for each one treatment with at least a common letter are not significantly different, LSD $_{0.05}$

[y] shows the sufficient nutrient level for canola growth and development at flowering (Canola Production Manual, undated), Fe critical level is extracted from the manual from Canola Council of Canada

High levels of Al and Mn measured in canola plants sampled at Welgevallen, Elsenburg and Altona may be ascribed to the lower soil pH and hence increased plant availability. Deficient Mn content in plants sampled at Roodebloem, in spite of sufficient levels in the soil, may be ascribed to Ca, which has an antagonistic effect on Mn in the soil solution. The Ca concentration of soil at Roodebloem was fairly high. Relatively low Al contents at Langgewens may be due to the lower rainfall (assumed less soil moisture) and hence reduced uptake of nutrients.

Even though certain nutrients were lower at some localities, almost all micro-nutrients were above the critical levels for canola growth and development. Besides Mn other exceptions were boron which was below the critical level of 29 mg kg^{-1} (http://www.canolacouncil.org/chapter 9.aspx) at Welgevallen and Langgewens because of the insufficient levels in the soil. Similar observations of B deficiency in canola plants have been mainly linked to acid soils (Hocking *et al.*, undated).

In general, fertilisation with 120 kg N ha^{-1} significantly increased the content of K, Na and Al whilst secondary nutrient S was reduced in canola plants sampled at 90 DAP (Table 3.3). Nitrogen increases canola root growth, leading to the increased absorption of nutrients (Chamorro *et al.,* 2002), however N fertilisation did not have any significant effect on P, Cu, Fe, Zn, Mn and B contents of canola plants in this trial.

Consequently, effects of N fertilisation on plant nutrient contents can also be depended on the locality as indicated in the significant interaction found in N, Ca, Mg and Na contents. As shown in Fig. 3.2, interactions showed a general higher plant N content with N fertilisation at Altona, Langgewens and Welgevallen whilst plant N at Elsenburg and Roodebloem were not significantly affected by N addition. Deficient levels (below 3.5 %) of N were found at all localities even with N applications, except in N fertilised plants at Welgevallen which resulted in 3.7 % N (Fig. 3.2) (Canola Production Manual, undated). Sodium content only increased when plants were fertilised with N at Altona. Soils at Roodebloem and Altona have a higher Na content ultimately influencing more plant Na content than at the other localities (Fig. 3.2).

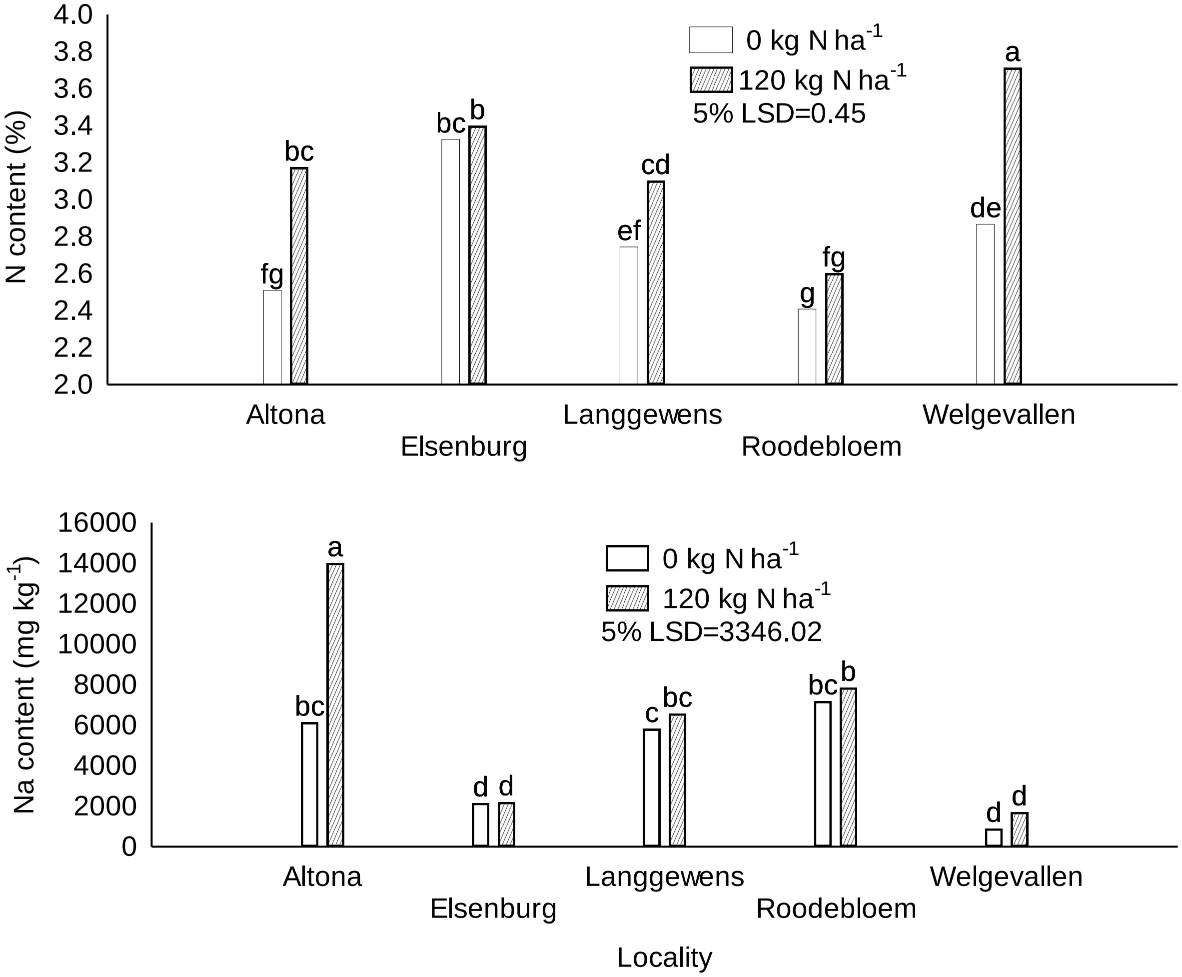

Figure 3.2 Effect of nitrogen fertilisation rates at the different localities on canola NH_4 = N and Na content at 90 DAP.

The significant interaction between locality and N application on Ca and Mg contents generally showed increasing contents of both nutrients when N was applied at Altona (1.10 to 1.38 % for Ca and 0.50 to 0.59 % for Mg) and Welgevallen (1.65 to 1.93 % for Ca and 0.27 to 0.34 % for Mg) only (Fig. 3.3). However at Elsenburg, canola responded with decreasing Ca (from 2.26 to 1.96 %) and Mg (from 0.38 to 0.31 %) content when fertilised with N whilst no significant effects of N addition were found at Langgewens and Roodebloem. The low content of Ca with addition of LAN probably resulted from the competition between NH_4^+ and Ca^{+2} ions for absorption sites on plant roots (http://www.canolacouncil.org/chapter 9.aspx). Though N was applied as LAN, the soil at the various localities had different inherent levels of N (Table 3.1) and hence different N buffering capacities which may be the reason for different responses.

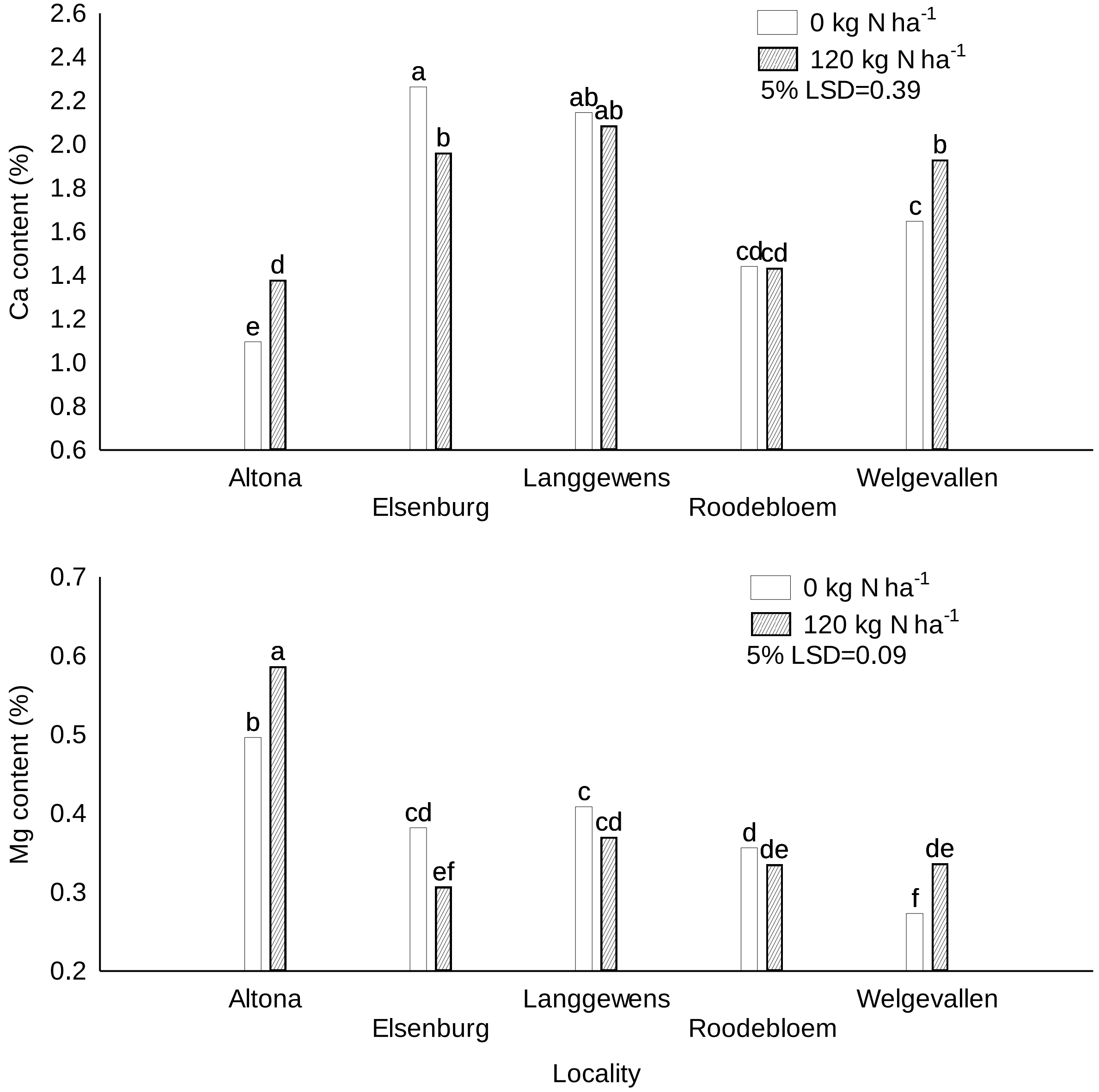

Figure 3.3 Effect of N fertilisation rates at different localities on canola Ca and Mg content at 90 DAP.

Due to a higher soil Ca and higher pH at Roodebloem (Tables 3.1) it was expected that the canola Ca content at Roodebloem would be higher than at Langgewens, Elsenburg and Welgevallen. Even application of N at Roodebloem did not improve plant Ca content. Assumed low moisture level and high soil Na+ content at Roodebloem could have aggravated the situation leading to low Ca content. Sodium disperses soil (Brady, 1974), swelling when it takes up water through hydration blocking the soil pores ultimately leading to low plant water and nutrient absorption.

3.4 CONCLUSIONS

In this study, nutrient content in canola plants were affected by locality and interactions between locality and nitrogen application rates, but not by S. The content of some nutrients such as B, Ca, Mn, S and N were below critical levels required for optimum growth and development of canola plants. At Altona N fertilisation increased plant Ca content to above deficient levels. At some localities such as Roodebloem, canola plants sampled at flowering (90 DAP), showed deficiency levels of elements such as Mn regardless of sufficient levels of Mn in the soils at planting. At Welgevallen and Langgewens on the other hand, deficient levels of B already showed during the soil analysis prior to planting. This indicates that besides a soil analysis at planting, a foliar analysis may also be necessary as some nutrients fall below the critical levels even though the soil shows sufficient levels of the nutrient.

In this study, sulphur concentration within the plants remains a major concern as S remained below the sufficient quantities of 0.5 % regardless of the quantity of sulphur added. More research in this regard is therefore needed.

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CHAPTER 4

The effect of nitrogen and sulphur on seedling establishment, vegetative growth and nitrogen use efficiency (NUE) of canola (*Brassica napus* L.) grown in the Western Cape

Abstract

Variability in soil properties and rainfall distribution is generally high in the canola growing areas of the Western Cape province of South Africa. Rainfall patterns generally influence soil moisture levels and utilization of major nutrients (N and S) required to maximize canola growth and development. The aim of this research was to determine the effect of soil and climatic differences, as experienced at different localities, on seedling establishment, dry matter production and nitrogen use efficiency of canola in response to N (nitrogen) and S (sulphur) application rates. Canola plant populations (plants m^{-2}) were determined at 30 days after planting (DAP), while dry mass was recorded on plots of the treatment combinations of S (0, 15 and 30 kg ha^{-1}) and N (0 and 120 kg ha^{-1}) during flowering (90 DAP) at Altona, Elsenburg, Langgewens, Roodebloem, and Welgevallen localities in the Western Cape in 2009, 2010 and 2011. Plant populations, dry mass production and NUE differed between localities. Nitrogen fertilisation increased plant biomass at most localities over seasons whilst fertilisation with S resulted in increasing plant dry mass only in 2010. NUE measured as gram dry matter gain per gram of nitrogen applied was affected by S at Altona in 2011.

Keywords**:** Nitrogen use efficiency, canola, dry mass, sulphur

4.1 INTRODUCTION

Poor growth of canola and low yields in the Western Cape Province of South Africa has been ascribed to poor nitrogen fertiliser management options (Hardy *et al.,* 2004). In canola, the requirement for N per yield unit is higher than in cereal crops (Oplinger *et al.,* 2000, Hocking & Strapper, 2001; Sylvester-Bradley & Kindred, 2009).The crop has the ability to take up nitrate from the soil and accumulate large quantities of N stored in vegetative parts at the beginning of flowering (Laine *et al.,* 1993).

Nitrogen is utilized in various components of many important structural, genetic and metabolic compounds (Hirel *et al.,* 2007). During the vegetative phase, young developing roots and leaves are mainly the sink organs for the assimilation of inorganic N and the synthesis of amino acids originating from the N taken up before flowering (Hirel & Lea, 2001). When N is taken up, it forms a major component of chlorophyll which together with increases in leaf-area, results in a higher net assimilation rate (Yau & Thurling, 1987). High net assimilation rates are important during grain filling stages to ensure a high yielding crop (Rossato e*t al.*, 2001). During the grain filling stage, N translocated from leaves and stems to the grains may also result in higher yields (Svečnjak & Rengel, 2006; Tatjana *et al.,* 2008).

In addition to N, sulphur fertilisation is also considered a critical factor in high yielding canola crops (Fismes *et al.,* 2000). Sulphur concentration in canola plants varies between 1 and 16 g kg^{-1} dry mass, depending on external supply (Balint & Rengel, 2009). Sulphur is a constituent of certain amino acids needed for protein synthesis in canola. It also affects the quality of canola seed due to its effect on the oil content (Josh *et al.,* 1998). Deficiency of S will reduce N uptake and for this reason, the application of S needs to be balanced with N for optimum yields (Ceccoti, 1996; Fismes *et al.,* 2000). The optimum N:S ratio reported in literature is variable (Zhao *et al.,* 1993; Ahmad & Abdin, 2000; Fismes *et al.,* 2000; Balint *et al.,* 2008), but the typical ratios range from 7:1 to 5:1. In literature 16 kg S per ton of grain yield produced is recommended, so a three ton crop requires about 50 kg S per hectare (Kimber & McGregor, 1995). However, as the effects of sulphur are related to the nitrogen level, such recommendations would be based on nitrogen recommendations in the appropriate variety, with inherent soil sulphur levels, regions and yield potential also playing a major role.

Sulphur fertilisation enhanced nitrogen efficiency in canola (Ceccoti, 1996), leading to increased N assimilation into leaf protein. Nitrogen use efficiency (NUE) is regarded as the amount of vegetative growth produced per unit of N taken up by the crop (Novoa & Loomis, 1981; Sylvester-Bradley & Kindred*,* 2009). Environmental factors can also stimulate the development of large sinks, leading to more N and S uptake which may trigger the uptake of other nutrients and thereby increasing growth. Besides effects of fertilising with N and S, vegetative growth of canola plants can also be influenced by inherent soil fertility and climatic conditions.

Little is known on the uptake and utilization of nitrogen and sulphur by canola in the Western Cape province of South Africa, characterised by varying soil properties and rainfall patterns. This study is aimed at determining the effect of soil and climatic differences, on the growth and nitrogen use response of canola to N and S application rates.

4.2 MATERIAL and METHODS

4.2.1 Locality

Field experiments were conducted during the canola growing seasons of 2009, 2010 and 2011 at Elsenburg, Langgewens and Roodebloem Experimental Stations in the Western Cape province of South Africa as well as on Welgevallen (2010) and Altona (2011). On average, Elsenburg (358.8 mm) received a higher rainfall for the period from planting to 90 days after planting (DAP) per season than Welgevallen (331.8 mm), Langgewens (282.9 mm), Altona (240.5 mm) and Roodebloem (230.2 mm) (Fig. 3.1 in Chapter 3). Temperatures were generally favourable for canola production although the 2010 season was considerably warmer at all localities with higher mean monthly temperatures compared to the 2009 and 2011 growing seasons (Fig 3.1 in Chapter 3).

On average, N-mineralisation potential in the 0-200 mm soil profile were as follows: Altona 111.3 kg N ha^{-1} (2011 only), Elsenburg 92.0 kg N ha^{-1} (2009-2011), Langgewens 56.8 kg N ha^{-1} (2009-2011), Roodebloem 59.9 kg N ha^{-1} (2009-2011) and Welgevallen 93.5 kg N ha^{-1} (2010 only). These values were calculated from nitrate and ammonium-N contents as measured at planting and mineralized during the first forty days after planting to give an indication of the potential of the soil to supply nitrogen to the crops. A detailed soil analysis is presented in Chapter 3.

4.2.2 Experimental procedure

The experiments were laid out in a randomized block design with the factorial split plot arrangement. There were 15 (5 N and 3 S) subplots in one replication which were repeated in four blocks. The sizes of the subplots were 5 x 3 m^2. N was applied in the form of Limestone Ammonium Nitrate (LAN) with 28 % N while S was applied in the form of gypsum ($CaSO_4.2H_2O$) with 16 % S. Treatments consisted of five nitrogen rates (0, 30, 60, 90 and 120 kg N ha^{-1}) and three sulphur rates (0, 15 and 30 kg S ha^{-1}). Full details for experiment layout and agronomic practices are discussed in Chapter 3.

4.2.3 Data collection

Plant densities were recorded at 30 DAP (days after planting) by counting the number of plants in two rows of one meter length per replication and presented as plants m^{-2}. At 90 DAP, a net plot of 0.5 m^2 per replication was sampled in plots of the treatment combinations of S (0, 15 and 30 kg ha^{-1}) and N (0 and 120 kg ha^{-1}) and dried for 72 hours at 80 ^{0}C to determine dry mass production. It is however important to note that at the sampling stage only 90 kg N ha^{-1} was already applied to the 120 N treatments.

Nitrogen use efficiency at 90 DAP (NUE120) was expressed as g dry mass produced per g N added according to Novoa & Loomis (1981) using the following equation:

$$\mathrm{NUE120(gDM\,g\,N^{-1})} = \frac{\mathrm{DM\,(g\,m^{-2})\text{ - }DM\,control\ (g\,m^{-2})}}{\mathrm{N\,added\,(g\,m^{-2})}}$$

4.2.4 Data analysis

Data recorded was analyzed, using analysis of variance (ANOVA) (Statistica 11). To measure response to treatments at different localities, locality was therefore also considered as a factor. Interaction effects were compared using least significant difference (LSD) test at 5 % level of probability. Any treatment means found to be significantly different were separated using Fischer's protected $LSD_{0.05}$.

4.3 RESULTS and DISCUSSION

4.3.1 Significance of F values

A summary of the significant effects (F values) of localities, nitrogen and sulphur application rates as well as their interaction effects on plants m^{-2}, dry mass and NUE (120 kg ha^{-1}) at 90 DAP at Altona, Elsenburg, Langgewens, Roodebloem and Welgevallen during 2009, 2010 and 2011 seasons are presented in Table 4.1. The table clearly shows that plant emergence and growth varies with locality, and nitrogen has an effect on plant biomass accumulation whilst effects of sulphur were mostly shown in 2010. However, responses of nitrogen differ between localities in 2010 and 2011 seasons. Even though there were responses to S shown in 2010 and interaction between S and locality in 2011, there were no significant interactions between N and S in all three seasons on canola biomass accumulation.

Table 4.1 Summary of significant effects (F-values) from the analysis of variance done on plant density (30 DAP), dry mass (90 DAP) and Nitrogen Use Efficiency (90 DAP) (120 kg ha^{-1}) in 2009, 2010 and 2011 seasons.

	Plant density			**Plant dry mass**			**NUE (120 kg ha^{-1})**		
	2009	2010	2011	2009	2010	2011	2009	2010	2011
Locality	***	***	***	***	***	***	ns	***	***
N rate	ns	ns	ns	***	***	**	-	-	-
S rate	ns	ns	ns	ns	***	ns	ns	ns	ns
N X Locality	ns	ns	*	ns	***	***	-	-	-
S X Locality	ns	ns	ns	ns	ns	*	ns	ns	*
S X N	ns	ns	ns	ns	ns	ns	-	-	-
N X S X Locality	*	ns	ns	ns	ns	**	-	-	-

*, **, *** denote significance at P≤0.05, P≤0.01, P≤0.001 respectively and ns denotes non significance at P≤0.05.

4.3.2 Plant density

Plant densities for all three seasons ranged between 36 and 81 plants m^{-2} for the various treatment combinations. The recommended plant densities for canola is between 50 and 80 plants^{-2} (Canola Production Manual, undated) with a seeding rate of 4-6 kg ha^{-1}. In 2009, Langgewens (56 plants m^{-2}) and Roodebloem (55 plants m^{-2}) had on average significantly more plants m^{-2} compared to Elsenburg (48 plants m^{-2}) when counted at 30 DAP (Table 4.2). During the 2010 season, mean plant density at Welgevallen (36 plants m^{-2}) was significantly lower compared to Elsenburg (77 plants m^{-2}), Roodebloem (70 plants m^{-2}) and Langgewens (69 plants m^{-2}) (Fig. 4.1). During the 2011 season, mean plant density at Elsenburg (44 plants m^{-2}) was lower than at Altona (51 plants m^{-2}), Roodebloem (59 plants m^{-2}) and Langgewens (63 plants m^{-2}) (Table 4.3). Germination and emergence of canola seedlings are reduced by low temperatures, very high or low soil moisture contents as well as other physical and chemical soil properties. Lower plant densities at Welgevallen in 2010 and Elsenburg in 2009 and 2011, could therefore be the result of the high rainfall and assumed lower soil temperatures. Amongst other factors, Mendham & Salisbury (1995) also reported on the poor plant establishment of canola during conditions of excessive soil moisture.

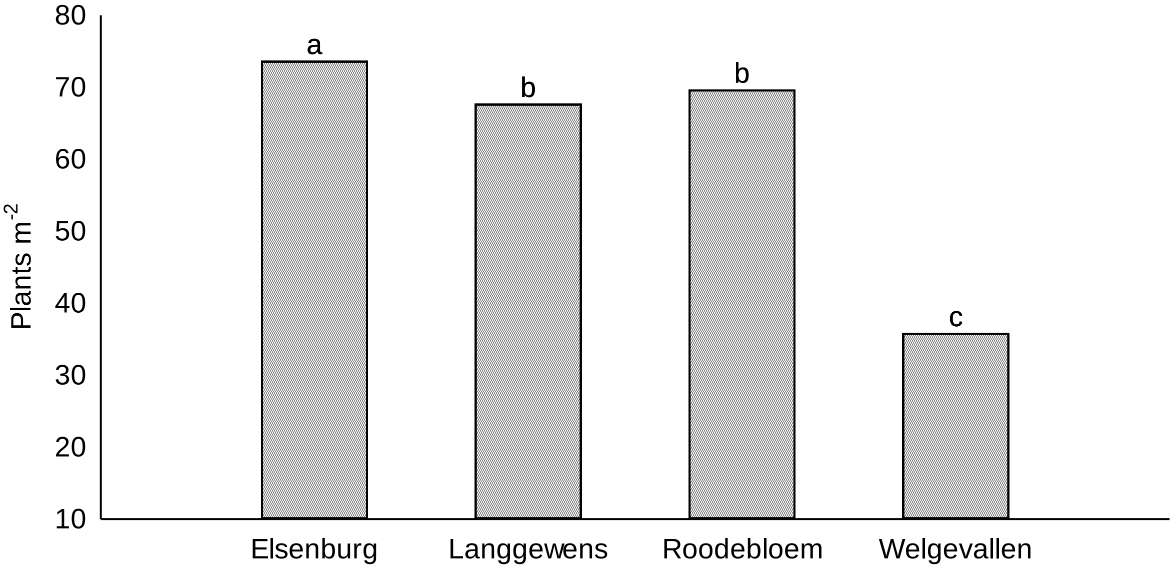

Figure 4.1 Canola plants m^{-2} at Langgewens, Elsenburg, Roodebloem and Welgevallen at 30 DAP in 2010.

A significant interaction effect between localities, N and S rates (in 2009 season) and between localities and N rates (in 2011 season) at $P<0.05$ with regard to plant densities suggested that N and S application rates affected the germination and establishment of canola differently at the individual localities (Table 4.1). Addition of high rates of N fertiliser, especially when soil moisture is limited, can reduce canola seedling emergence and survival (http://www.canolacouncil.org/chapter9), but from this study (Tables 4.2 & 4.3) no clear trends were found in plant population responses with regard to the locality, N and S treatment.

Table 4.2 Canola plants m^{-2} at Elsenburg, Langgewens and Roodebloem localities at S fertilisation rates of 0, 15 and 30 kg ha^{-1} and N fertilisation rates of 0, 30, 60, 90 and 120 kg ha^{-1} at 30 DAP in 2009.

		N rate **(kg ha^{-1})**						
Locality (L)	***S rate*** **(kg ha^{-1})**	**0**	**30**	**60**	**90**	**120**	**Mean**	**Locality Mean**
	0	46	42	51	51	45	47	
Elsenburg	15	48	43	53	45	42	46	**48**
	30	44	46	45	59	55	50	
	N mean	**50**	**44**	**50**	**52**	**47**		
	0	59	45	60	62	55	56	
Langgewens	15	55	43	50	68	61	55	**56**
	30	56	64	57	47	62	57	
	N mean	**57**	**50**	**55**	**59**	**59**		
	0	54	62	49	50	50	53	
Roodebloem	15	58	58	58	53	52	56	**55**
	30	62	44	60	55	57	56	
	N mean	**58**	**55**	**55**	**52**	**53**		
Mean		**54**	49	53	55	53		
$LSD_{0.05}$	**LXNXS: 14**							

Table 4.3 Canola plants m^{-2} at Altona, Elsenburg, Langgewens and Roodebloem localities at N fertilisation rates of 0, 30, 60, 90 and 120 kg ha^{-1} at 30 DAP in 2011.

	N rate **(kg ha^{-1})**					
Locality (L)	**0**	**30**	**60**	**90**	**120**	**Mean**
Altona	51	50	44	54	54	**50**
Elsenburg	39	43	43	47	46	**44**
Langgewens	70	60	65	52	66	**63**
Roodebloem	53	59	59	62	60	**59**
Mean	**53**	**53**	**53**	**54**	**56**	
$LSD_{0.05}$	**LXN: 12**					

4.3.3 Dry mass

Dry mass at 90 DAP differed significantly between localities and were affected by N application rate (Table 4.1) in all seasons, while S as a main factor had a significant effect on dry mass in 2010 only (Table 4.1). During this season the application of 15 kg S ha^{-1} resulted in an increase in plant dry mass from 243.74 to 286.62 g m^{-2} when compared to the control where no S was applied. Significant N x locality (2010), S x locality (2011) and N x S x locality (2011) interactions however indicated that dry mass responses to S and N varied between localities.

In general, higher N application rates resulted, as can be expected, in higher dry mass regardless of season (Fig. 4.2). During 2009, highest dry mass yields at 90 DAP were measured at Langgewens (173.77 g m^{-2}) followed by Roodebloem (138.26 g m^{-2}), with Elsenburg (99.41 g m^{-2}) showing the lowest dry mass at 90 DAP (Fig. 4.3). Considering the mentioned higher N mineralisation potential of the soil at Elsenburg compared to Roodebloem and Langgewens, together with the significantly lower rainfall received at Langgewens and Roodebloem, it was expected that the dry mass produced at Elsenburg would be higher than at the other localities. However, the lower plant populations at Elsenburg were most probably the reason for the lower dry mass observed.

Dry mass produced during the 2010 season at Langgewens, Elsenburg and Roodebloem were generally higher than that of the 2009 season as a result of higher plant populations and responded positively to nitrogen applications (Fig. 4.4). Dry mass at Welgevallen was however significantly less and did not show a significant increase due to N applications. This however does not mean localities with lower plant populations such as Welgevallen and Elsenburg (2009 and 2011) will necessarily have a lower yield potential, as canola has the ability to compensate for lower plant populations as shown by Angadi *et al.* (2003).

During 2011, plants at Langgewens and Elsenburg responded positively to nitrogen application, but a poor response was shown at Roodebloem and Altona (Fig. 4.5). The poor response to nitrogen at Altona can be ascribed to the inherently high soil mineral nitrogen content (111.3 kg N ha^{-1}). At Roodebloem, assumed low moisture levels as a result of the low rainfall, might have result in poor N uptake and plant growth. Although significant interactions between S, N and locality were noted in the 2011 season, the application of S had no effect on plant dry mass during this season and N x S x Locality interaction did not show any clear trends and were for this reason ignored.

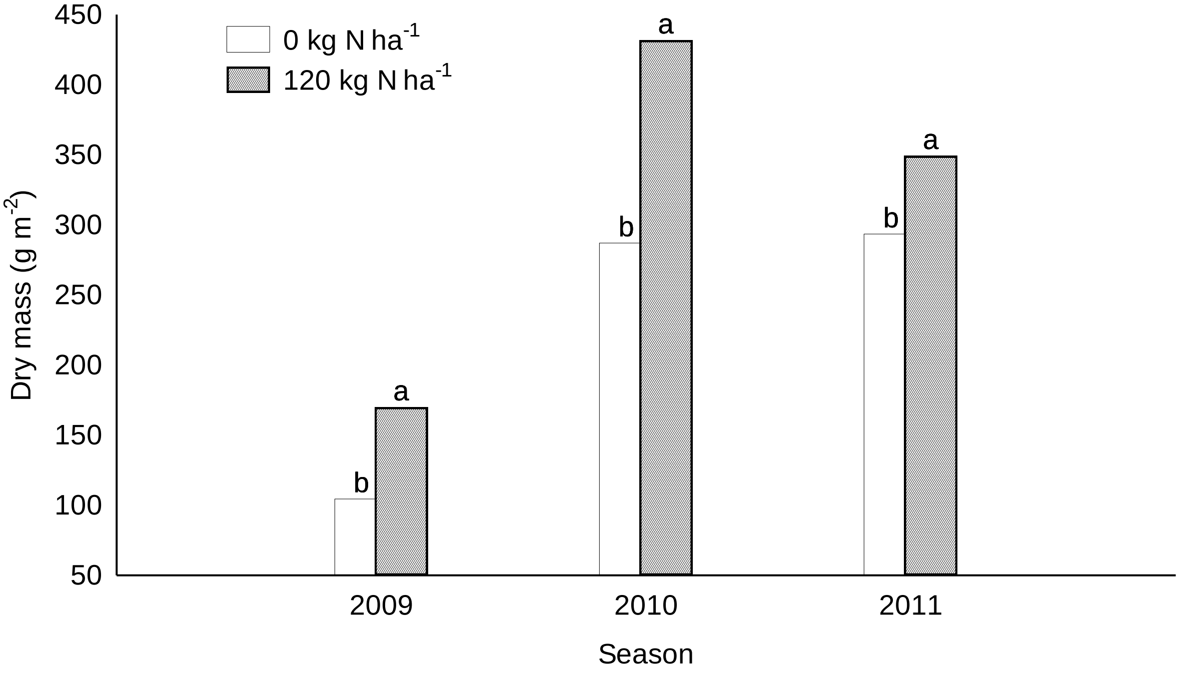

Figure 4.2 Above ground dry mass m^{-2} of canola plants at 90 DAP in response to 0 and 120 kg N ha^{-1} in 2009, 2010 and 2011 seasons.

From the graph, means for each one season with at least a common letter are not significantly different, LSD $_{0.05}$

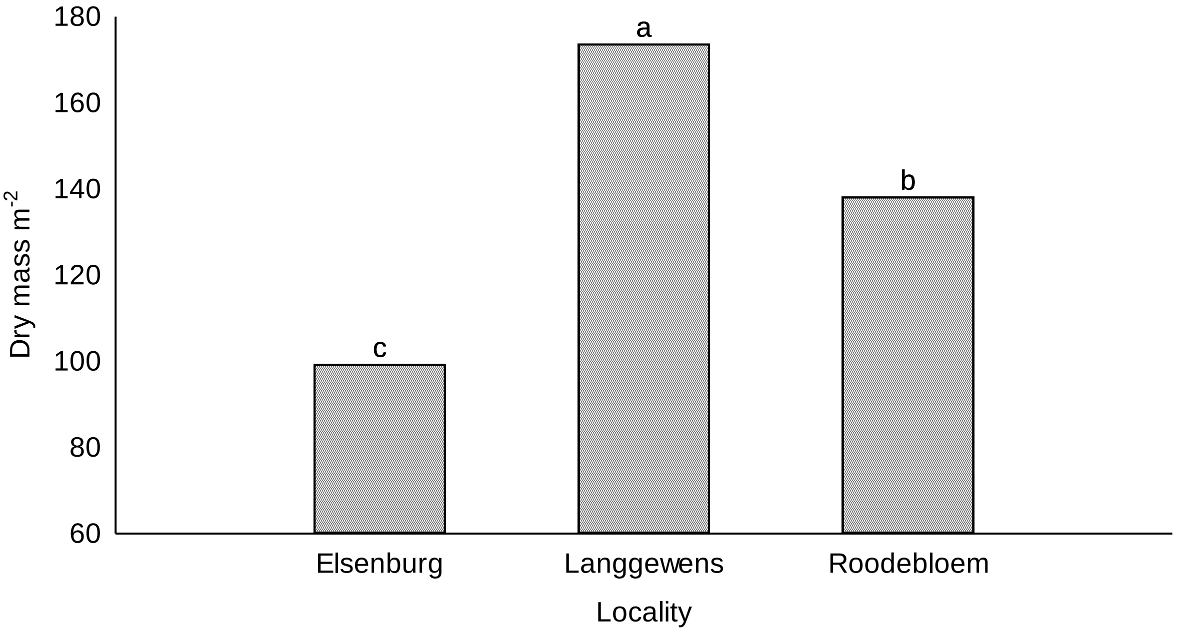

Figure 4.3 Above ground dry mass m^{-2} of canola plants at 90 DAP during the 2009 season at different localities.

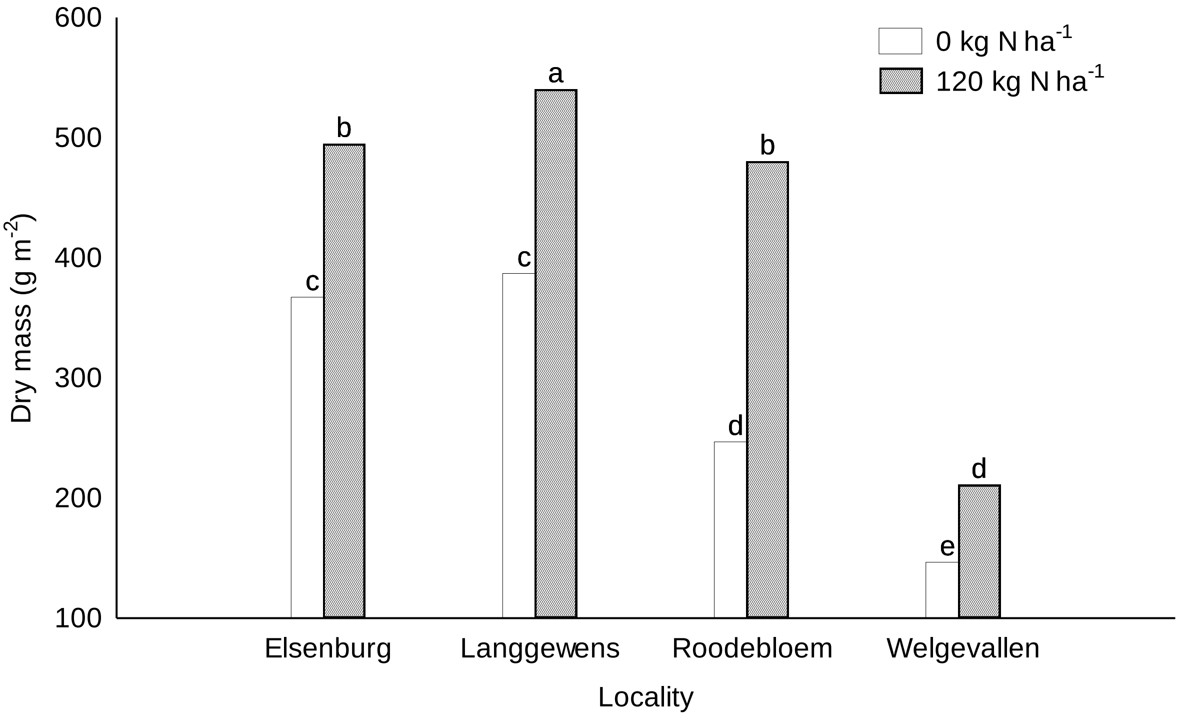

Figure 4.4 Effect of increasing N application rates on plant above ground dry mass m^{-2} at 90 DAP during the 2010 season at different localities.

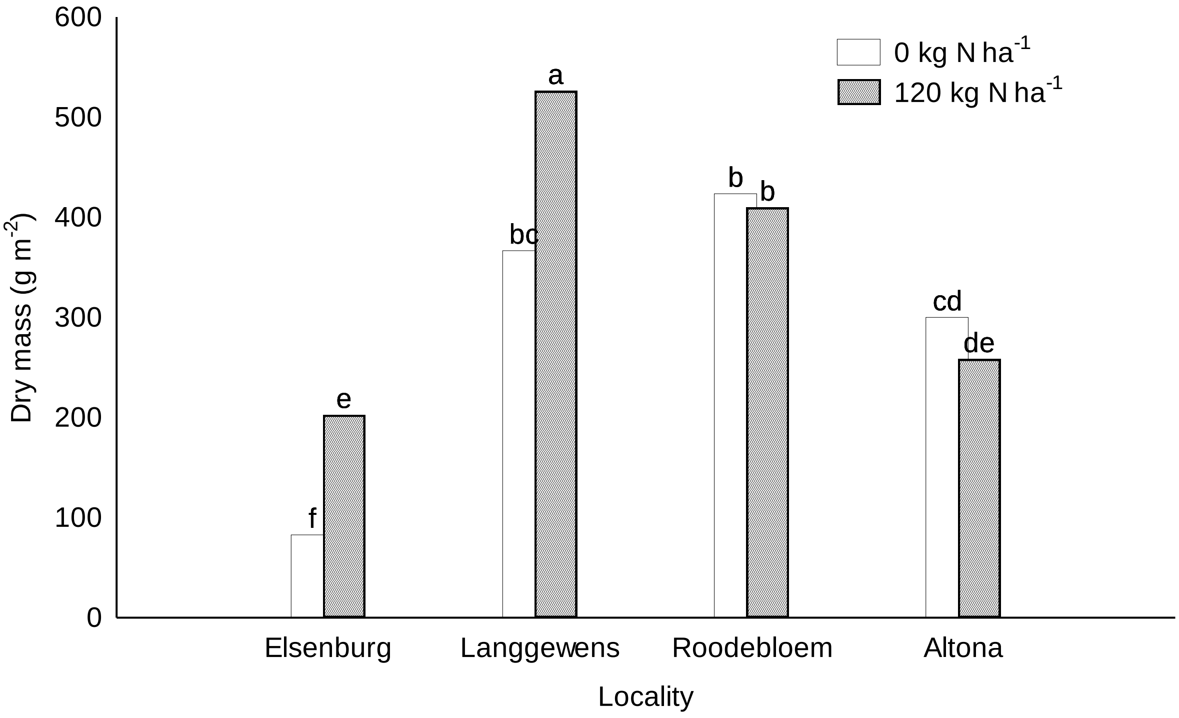

Figure 4.5 Effect of increasing N application rates on plant above ground dry mass m^{-2} at 90 DAP during the 2011 season at different localities.

4.3.4 Nitrogen use efficiency

In spite of nitrogen use efficiency (NUE) values for the 120N treatment, calculated by using the NUE equation of Novoa & Loomis (1981), that ranged between -2.0 and + 15.0 in 2009, no significant differences in the amount of dry mass produced per kg of N added at different sulphur rates were found at any of the localities (Table 4.1). On average higher efficiencies were obtained during the 2010 season (19 g plant dry mass g^{-1} N added) when compared to 2009 (7 g plant dry mass g^{-1} N added) and efficiencies differed significantly between localities. In 2010, Roodebloem showed a significantly higher NUE compared to Elsenburg, Langgewens and especially Welgevallen which had the lowest NUE with a mean value of 7 (Fig. 4.6). In 2011, NUE values at Altona and Roodebloem were significantly lower compared to Elsenburg and Langgewens.

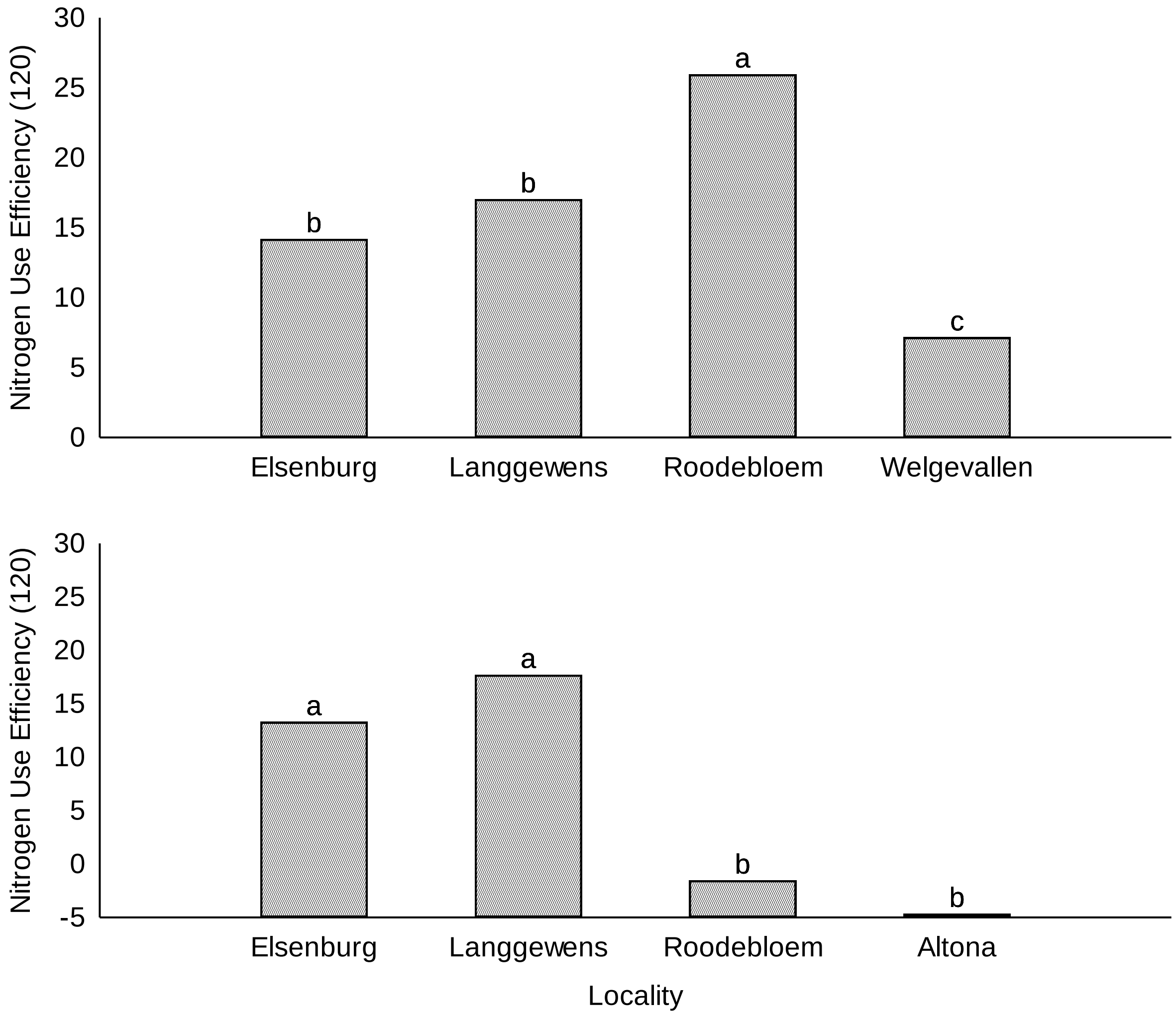

Figure 4.6 Nitrogen Use Efficiency (gram dry matter gain per kg of N applied) at 90 DAP at different localities in 2010 (*top*) and 2011(*bottom*) seasons.

Sulphur application did not influence the nitrogen use efficiency of canola as measured at 90 DAP in any of the seasons, but a significant S x Locality interaction was recorded in 2011. In this growing season, the application of sulphur improved NUE at 90 DAP at Altona, but not at other localities (Table 4.4). This was likely because soils at Altona contain significantly low levels of S compared to other localities. Abdullah *et al.* (2010) ascribed the lack of response in NUE due to application of sulphur in canola to results (Fismes *et al.,* 2000; Jackson, 2000; Jan *et al.*, 2002) which indicate that inadequate S supply are usually shown in a delay and poor flowering of canola which may result in poor seed yield and quality. However, if S is severely lacking, deficient symptoms can be shown during vegetative growth and can ultimately affect the utilization of nitrogen as shown at Altona.

Table 4.4 Effect of Sulphur on Nitrogen Use Efficiency (gram dry matter gain per kg of N applied) at 90 DAP at different localities in 2011 season.

	***S rate* (kg ha^{-1})**			
Locality (L)	0	15	30	**Mean**
Altona	-26.0	8.2	3.9	**-4.6**
Elsenburg	11.8	20.5	7.5	**13.3**
Langgewens	18.2	13.3	21.6	**17.7**
Roodebloem	7.5	-2.3	-9.8	**-1.5**
Mean	2.9	9.9	5.8	

4.4 CONCLUSIONS

Although germination and emergence of canola seedlings varied between localities with some having a plant population of less than the recommended density, nitrogen and sulphur application rates had little or no effect. Variation between localities was most probably due to high rainfall hence assumed very high soil moisture contents as well as other physical and chemical soil properties at some localities. When plants were sampled at 90 DAP, fertilisation with N increased biomass of canola in all three canola growing seasons. Effects of N were however dependent on locality in 2010 and 2011 seasons. However, fertilisation with S resulted in increasing plant dry mass only in 2010. NUE as measured as gram dry matter gain per gram of nitrogen applied were affected by localities in 2010 and 2011 seasons with high efficiency on localities with low inherent N mineralisation potential and lower rainfall (presumably less N loss due to leaching). The application of S had no effect on NUE at 90 DAP, except for Altona during 2011, because literature showed that sulphur is more important for reproductive development (grain yield and quality) than vegetative growth (DM production).

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CHAPTER 5

The effect of nitrogen and sulphur on the grain yield and quality of canola (*Brassica napus* L.) grown in the Western Cape

Abstract

Low canola yields of less than 1.5 tonnes per hectare are obtained in the Western Cape Province of South Africa. Injudicious use of fertiliser has been suggested as the major cause; hence aim of this research was to determine the effect of soil and climatic differences, as experienced at different localities on grain yield and quality response of canola to nitrogen (N) and sulphur (S) application. The study was conducted at Elsenburg, Langgewens and Roodebloem (2009-2011) as well as at Welgevallen (2010) and Altona (2011) localities. Treatments consisted of five N (0, 30, 60, 90 and 120 kg N ha^{-1}) and three S (0, 15 and 30 kg S ha^{-1}) rates laid out in a randomized block design with factorial split plot arrangement. The study showed that rates of N application for maximum grain yields in canola are highly depended on locality (soil and climatic conditions), while responses to S depends on soil conditions. Low rainfall during flowering and pod set may limit yield responses to N applications, while applications of 120 kg N ha^{-1} and 15-30 kg S ha^{-1} may be needed in high rainfall areas to obtain more than 2.0 ton ha^{-1}. Application of S also improves the oil content of canola though this has a compromise on protein content. Increasing N fertilisation reduces the grain size and oil content especially at localities which receive low rainfall during seed-fill.

***Keywords*:** Canola yields, grain size, nitrogen, oil and protein content, sulphur

5.1 INTRODUCTION

Canola (*Brassica napus* L.), an emerging oilseed crop, has become a plant of major economic importance, with yields increasing to almost 60 million metric tonnes worldwide in 2010 (World Oilseed Production, 2010). The crop offers the potential of high quality edible oil, as a source of plant protein meal for feeds, or a potential alternative as biofuel (Rayner, 2002; Abdallah *et al.,* 2010). Recognition of the crop and its potential benefits is bound to increase globally, hence the need to increase production.

Production of the crop in South Africa is still low compared to the major global producers, with production estimated as approximately 45 660 ha in 2009 (Crop Estimates Committee, 2009). The Monthly Food Security Bulletin of South Africa (2012) reports on canola production statistics for the 2011 season with an estimation of 59 490 tonnes on an area of 43 510 ha indicating a national average yield of 1.36 ton ha^{-1} during the 2011 season. Increasing land area for canola production within the Western Cape, where the crop is mostly planted, is restricted due to competition with other winter crops; hence yield increases should focus more on increase per land area. However, research has shown low yields of less than 1.5 tonnes per hectare obtained within the production areas (Van Zyl, 2007). Fertilisation with nitrogen has been reported to increase yield considerably in other canola growing regions of the world (Hocking *et al.*, 1997; Jan *et al.*, 2002; Svečnjak & Rengel, 2006; Tatjana *et al.*, 2008), but has been shown to give poor and or variable responses in the Western Cape production area (Hardy *et al.*, 2004).

Optimizing the yield of canola involves balancing the synthesis of oil and crude protein in the seeds (Ahmad *et al.*, 2007). Canola is a heavy nutrient feeder that requires more macronutrients (particularly nitrogen and sulphur) compared to cereals (Oplinger *et al.*, 2000; Gan *et al.*, 2008). Crop management, N and S fertilisation, and choice of nutrient efficient genotypes can play an important role in modifying oil content in canola seed. Besides nitrogen as a major nutrient required in canola production, sulphur is one of the micronutrients that plays a crucial role in canola, affecting growth and yield, with its requirements about four times higher than that of wheat or maize (Abdallah *et al.,* 2010).

Grain yield responses to applied S only occurred when N was applied and tended to increase as more N was applied (Brennan & Bolland, 2008). McGrath & Zhao (1996) reported that low sulphur supply suppresses the development of reproductive organs and may lead to silique abortion and decrease seed yield and oil content. Sulphur forms an integral part of several amino acids, hence affecting seed protein content (Jan *et al.*, 2010). Malhi *et al.* (2007) has shown the effects of S deficiency and applied S being more pronounced on seed than straw. Usually, addition of S within the range of 10-30 kg S ha^{-1} has been found adequate for optimum seed and oil yield with 15-20 kg S ha^{-1} suggested as the norm per tonne yield per year (McGrath & Zhao, 1996). Jackson (2000) showed that about 20 kg S ha^{-1} was adequate for optimum seed and oil yields, where total plant N, P, K, and S uptake averaged 140, 25, 170, and 60 kg ha^{-1}; respectively. These results were obtained at the optimum levels of N and S to give a yield potential of 3 t ha^{-1}.The optimum S supply and uptake however depends on N application rate (N: S ratio of 7:1 is suggested), as well as soil and climatic conditions.

The aim of this research was to determine the effects of soil and climatic differences, as experienced at different localities on yield and grain quality response of canola to N and S application rates.

5.2 MATERIALS and METHODS

5.2.1 Locality

Field experiments were conducted at Elsenburg, Langgewens, Roodebloem Experimental stations of the Western Cape Province of South Africa during the canola seasons of 2009, 2010 and 2011, as well as at Welgevallen (2010) and at Altona (2011). In 2009, Elsenburg received the highest total rainfall (May to October) of 568.3 mm, whilst Roodebloem and Langgewens received a total rainfall of 339.4 and 285.2 mm respectively (Fig. 3.1: presented in Chapter 3). In 2010, Elsenburg received more rain (442.9 mm) than Welgevallen (426.6 mm), Langgewens (366 mm) and Roodebloem (320.9 mm). During the canola growing season, the 2011 rainfalls were far below that of previous years at most localities except at Langgewens (308.6 mm) which was higher than in 2009, even though below that received in 2010 at the same locality. Elsenburg received a higher total rainfall (325.8 mm) than Langgewens (308.6 mm), Altona (295.9 mm) and Roodebloem (241.5 mm). In July, rainfall was quite low at all localities. Temperatures were generally favourable for canola production even though the 2010 season was considerably warmer at all localities with temperatures experienced being above those of 2009 and 2011.

Soil characteristics at the localities were variable and the N-mineralisation potential in the 0-200 mm soil profile was calculated and found to be 56.8, 59.9, 92.0, 93.5 and 111.3 kg N ha^{-1} for, Langgewens (2009-2011), Roodebloem (2009-2011), Elsenburg (2009-2011), Welgevallen (2010 only) and Altona (2011 only) experimental sites respectively. Sulphur was below the required critical level of 6 mg kg^{-1} S required for canola production at all localities except for Elsenburg and Welgevallen (Table 3.1: presented in Chapter 3)

5.2.2 Experimental procedure

The experiments were laid out in a randomized block design with a factorial split plot arrangement. Treatments consisted of five Nitrogen rates (0, 30, 60, 90 and 120 kg N ha^{-1}) and three Sulphur rates (0, 15 and 30 kg S ha^{-1}). Nitrogen was applied in the form of Limestone Ammonium Nitrate (LAN) with

28 % N while Sulphur was applied in the form of gypsum ($CaSO_4.2H_2O$) with 16 % S. Full details for experiment layout refer to Chapter 3.

5.2.3 Data collection

At crop maturity, canola was harvested with a plot harvester on 5 x 3 meter plots and grain yields were converted to kg ha^{-1} (following cleaning and weighing). The mass (g) of 1000 grains was measured using an electronic balance to determine the size of the canola grains. The canola grain protein and oil contents were analysed at Western Cape Department of Agriculture at Elsenburg, using NAR (Near- Infra-Red) technology.

5.2.4 Data analysis

Data recorded was analysed, using analysis of variance (ANOVA) (Statistica 11). To find the response of the treatments on different localities, locality was considered a factor. Considering the fact that different cultivars were planted in the three different seasons and also that three localities were planted in 2009 (Roodebloem, Elsenburg and Langgewens) whilst Welgevallen and Altona were included in 2010 and 2011 respectively, grain yield and thousand grain mass data for the three seasons was analysed separately. Because of the high cost of the analysis for grain oil and protein content of canola no replications were analyzed. For this reason growing seasons were used as replications to do the statistical analysis (only Langgewens, Roodebloem and Elsenburg where data was available for three years). Main and interaction effects were compared using least significant difference (LSD) test at 5 % level of probability. Any treatment means found to be significantly different were separated using Fischer's protected $LSD_{0.05}$. Means from the Statistica analyses were then subjected to best fit regression curves plotted with the Microsoft Office Excel package, using polynomial equations.

5.3 RESULTS and DISCUSSION

5.3.1 Significance of F values

Table 5.1 shows the summary of the ANOVA and significance of F values for nitrogen and sulphur application rates as well as interaction effects between the treatments as measured for canola grain yield and quality at final harvesting at Altona, Elsenburg, Langgewens, Roodebloem and Welgevallen during 2009, 2010 and 2011. From the table it becomes clear that N and S application and interactions between nitrogen and sulphur had a significant effect on yield, grain oil and protein content, but less effect on grain size. Responses also differ between localities and growing seasons.

Table 5.1 Summary of significant effects (F-values) from the Analysis of variance done on canola grain yield, thousand grain mass, grain oil and protein content during the 2009 – 2011 seasons.

	Grain yield			**Thousand grain mass**			**Oil (%)**	**Protein (%)**
	2009	**2010**	**2011**	**2009**	**2010**	**2011**		
Locality	***	***	***	***	***	***	***	***
N rate	***	***	***	ns	ns	*	***	***
S rate	***	***	***	ns	ns	ns	*	ns
N X Locality	*	***	ns	ns	***	ns	***	**
S X Locality	ns	**	ns	ns	***	ns	**	ns
S X N	ns	***	*	ns	ns	ns	ns	ns
N X S X Locality	ns	**	ns	ns	ns	ns	ns	ns

*, **, *** denote significance at P≤0.05, P≤0.01, P≤0.001 respectively and ns denotes non significance at P≤ 0.05.

5.3.2 Grain yield

During 2009, grain yield was significantly affected by locality and N application rate but responses differed between localities (Table 5.1). Without any N fertiliser applied (N0), mean grain yield at Elsenburg (2260 kg ha^{-1}) was significantly higher compared to Roodebloem (1123 kg ha^{-1}) and Langgewens (890 kg ha^{-1}), but although the highest canola yields of 3683 kg ha^{-1} at Elsenburg, 2149 kg ha^{-1} at Roodebloem and 1438 kg ha^{-1} at Langgewens were all measured with the fertilisation rate of 120 kg N ha^{-1}, yields at Langgewens did not increase significantly when more than 30 kg N ha^{-1} was applied (Fig 5.1). This difference in response at different localities are most probably due to differences in soil moisture in particular (rainfall during grain filling period) and to a lesser extent differences in soil nitrogen mineralisation potential, because moisture availability greatly affects the yield response of canola to N fertilisation (http://www.canolacouncil.org/chapter9).

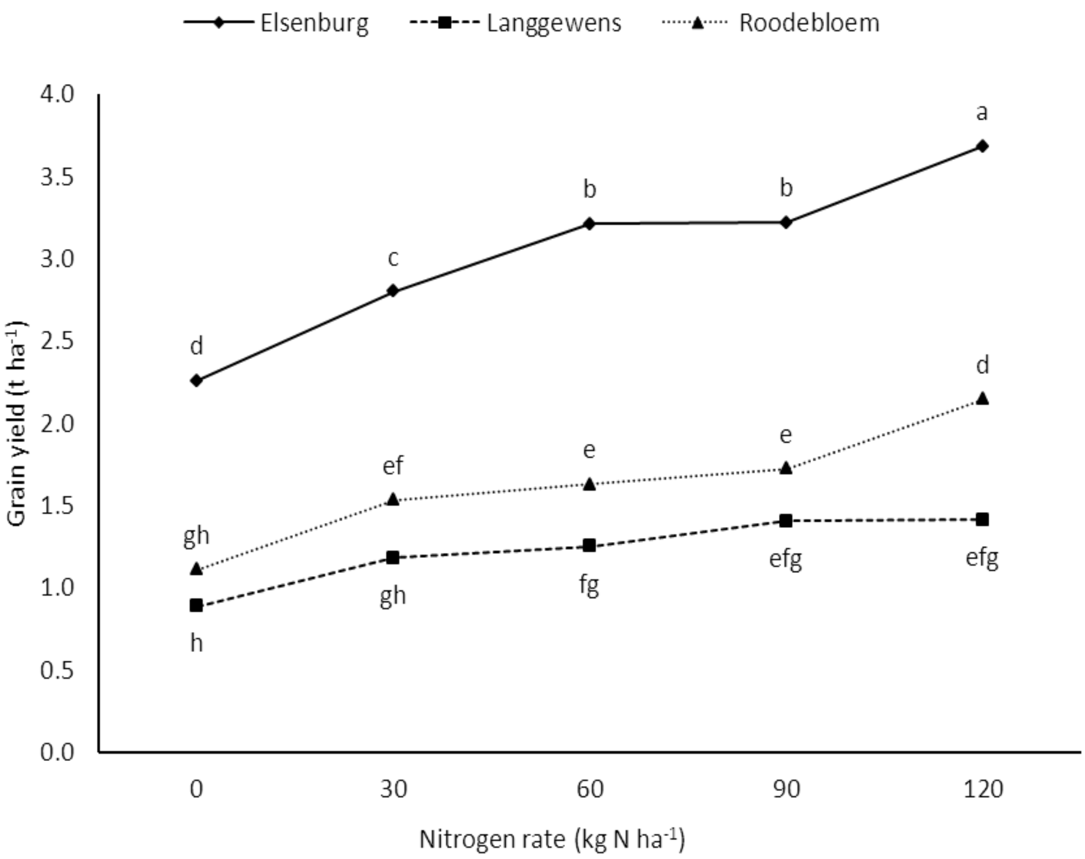

Figure 5.1 Canola yields harvested at Langgewens, Elsenburg and Roodebloem localities as a result of different nitrogen fertilisation rates (0, 30, 60, 90 and 120 kg ha^{-1}) in the 2009 season.

In 2009, Elsenburg received significantly more rain, which was evenly distributed throughout the canola growing season compared to Langgewens where a very low rainfall was experienced during the flowering and seed filling stages (August, September and October) (Fig. 3.1 in Chapter 3). Roodebloem also experienced low rainfall during August and September, but because of the later planting date yields probably benefited from the high rainfall during October. These results support that of earlier studies (Mendham & Salisbury, 1995; Champolivier & Merrien, 1996) which found that most of the yield components were highly affected by water shortage occurring from flowering to the end of seed setting stage.

During 2009, grain yield also differed significantly due to S application rates at all localities because the soil at all localities had low S contents (<6 mg kg^{-1} soil). In this season, the highest S application rate (30 kg ha^{-1}) produced a mean yield of 2115 kg ha^{-1} compared to 1806 kg ha^{-1} when no sulphur was applied. However as shown in Fig 5.2, application of S beyond 15 kg ha^{-1} did not increase yield significantly. Jackson (2000) reported similar findings of increasing grain yields with addition of sulphur, and recommended that optimum seed yield occurred at 20 kg S ha^{-1} in the western USA.

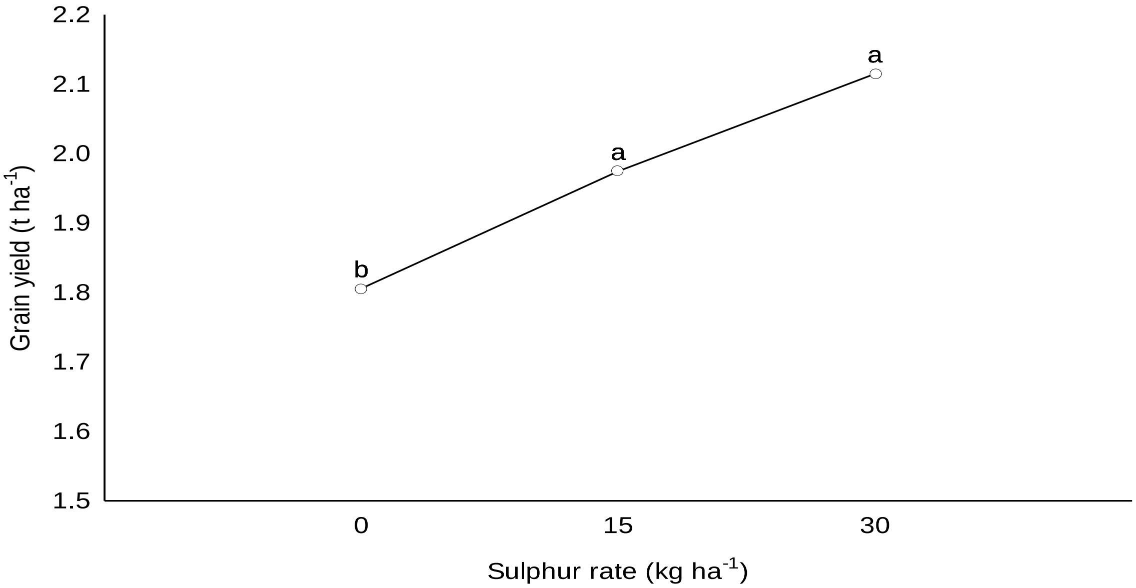

Figure 5.2 Effect of increasing sulphur application on mean canola yield during the 2009 season.

In 2010, grain yield was significantly affected by the locality x N x S interaction (Table 5.1). This significant interaction generally showed that yields tend to increase with an increase in application rate of N and S on all localities, but the degree of response differed between localities (Fig. 5.3). If no S was applied, yields tend to level off or even decrease (Elsenburg) at all localities except Welgevallen at N application rates of 60 to 90 kg N ha^{-1}. At sulphur rates of 15 and 30 kg S ha^{-1}, yields tend to level off with increasing N rates at Welgevallen only, indicating that S requirements differed between localities tested.

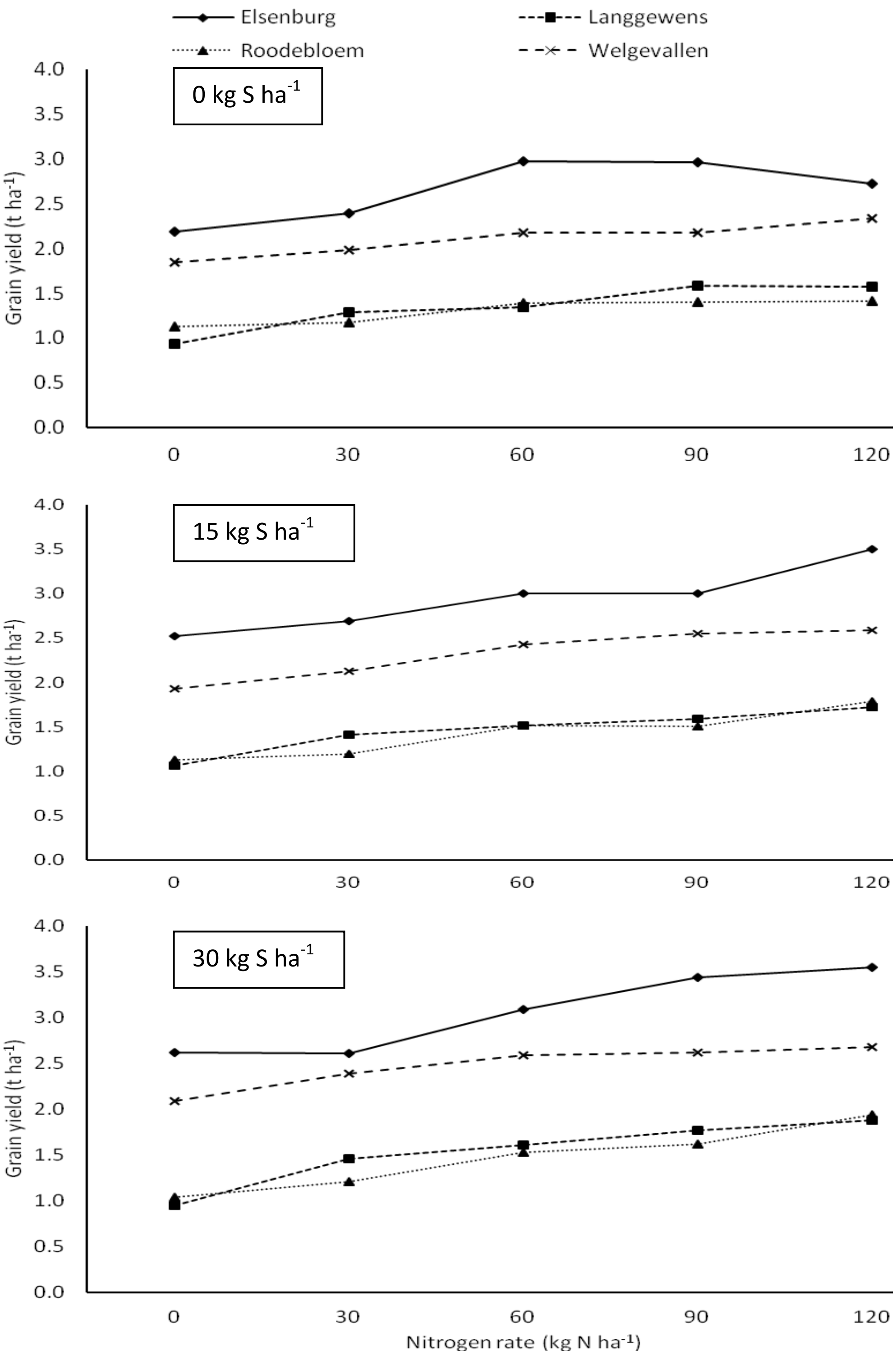

Figure 5.3 Canola yields harvested in 2010 at Elsenburg, Langgewens, Roodebloem and Welgevallen localities as a result of different sulphur (0, 15 and 30 kg ha^{-1}) and nitrogen (0, 30, 60, 90 and 120 kg ha^{-1}) fertilisation rates. (5% LSD=0.20).

In 2011 grain yield again differed between Altona, Langgewens, Elsenburg and Roodebloem localities, with significant responses to the N x S interaction (Table 5.1). On average, grain yields were significantly higher at Roodebloem (2827 kg ha^{-1}) compared to Altona (2634 kg ha^{-1}), Elsenburg (1368 kg ha^{-1}) and Langgewens (1152 kg ha^{-1}) during 2011 (Fig 5.4). Significantly lower yields observed at Elsenburg in 2011 likely resulted from a combination of high rainfall during planting which reduced the plant population (Table 4.3 in Chapter 4) and lower rainfall (Fig 3.1 in Chapter 3) received in July reducing the ability of the crop to compensate (Angadi *et al.,* 2003) for the reduced population. At Langgewens such sensitivity to water stress of canola during flowering, pod setting and grain filling stages (Mendham & Salisbury, 1995; Bagheri & Shahzad*,* 2011) was clearly illustrated by lower grain yields obtained in all the seasons (Fig. 5.4), as this locality showed generally low rainfall from July to October in all seasons when compared to other localities (Fig 3.1 in Chapter 3).

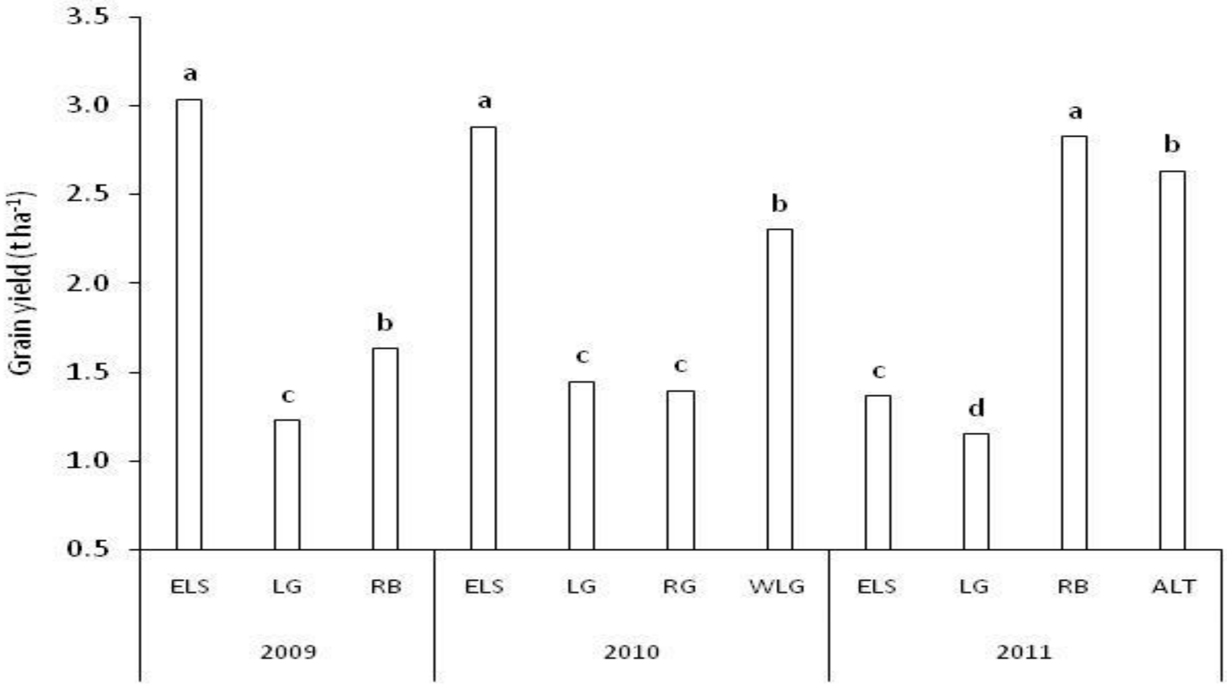

Figure 5.4 Canola grain yields during 2009, 2010 and 2011 seasons at Elsenburg (ELS), Langgewens (LG), Roodebloem (RB), Welgevallen (WLG) and Altona (ALT) localities
From the graph, means for each one season with at least a common letter are not significantly different, LSD $_{0.05}$

The significant N x S interaction results for 2011 showed that increasing N application can significantly increase canola yields, however this should be complimented with S application (Fig. 5.5). In treatments where N was added but with no S, yields increased from 1575 kg ha^{-1} to a maximum of

1878 kg ha^{-1} at 120 kg N ha^{-1}, but yield increases with applications of more than 30 kg N ha^{-1} were not statistically significant. When sulphur rates of 15 kg S ha^{-1} and 30 kg S ha^{-1} were applied, grain yields were significantly increased with increasing nitrogen applications to reach yields of 2310 kg ha^{-1} and 2563 kg ha^{-1} respectively when 120 kg N ha^{-1} was applied.

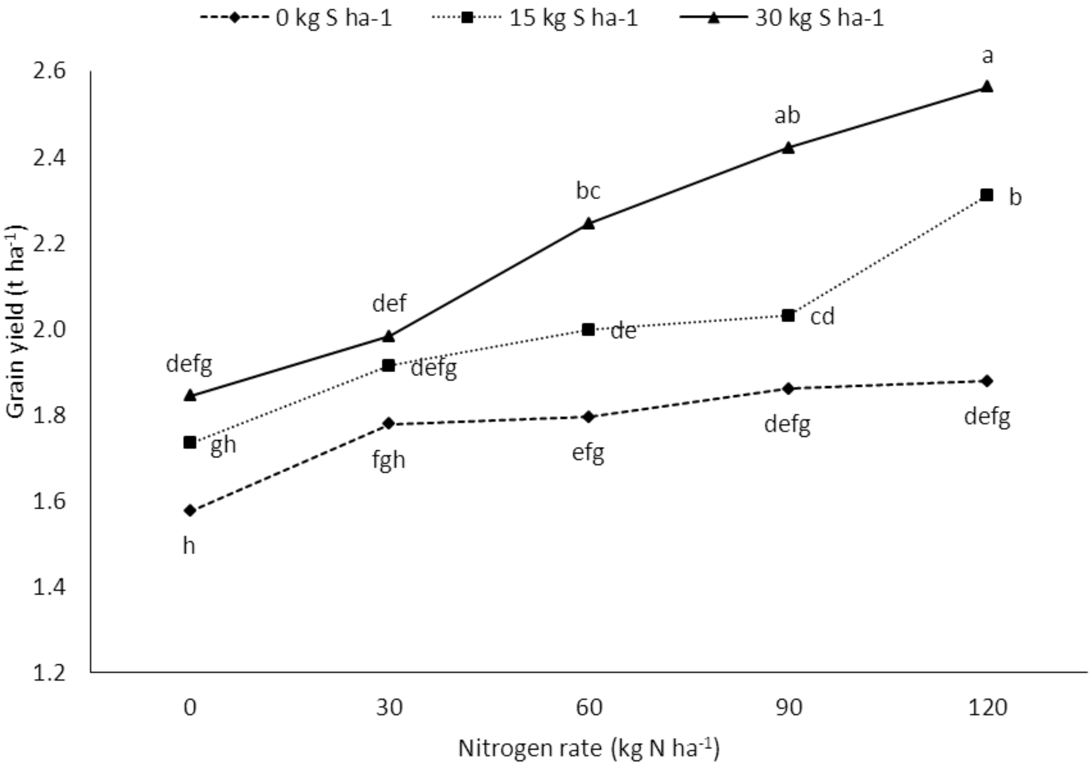

Figure 5.5 Effect of nitrogen and sulphur fertilisation rates on canola yields in 2011 season.

From the results it became clear that the grain yield responses to N were affected by S application. For this reason regression analyses were used to develop N x S response curves for each year. Even though there was no significant interaction shown between S and N at any of the localities during the 2009 canola growing season, regression analysis showed a positive correlation between S and N application (R^2=0.94-0.99), with yields where no S was applied, always less than where 15 or 30 kg S was applied (Fig. 5.6). In 2010 and 2011 significant S X N interaction curves (Fig. 5.6) showed that although S fertilisation increased canola grain yield at all N application rates, yields with 0 kg S ha^{-1} tend to level off at lower nitrogen rates. This clearly illustrates the importance of combined applications of N and S to obtain high grain yields with canola.

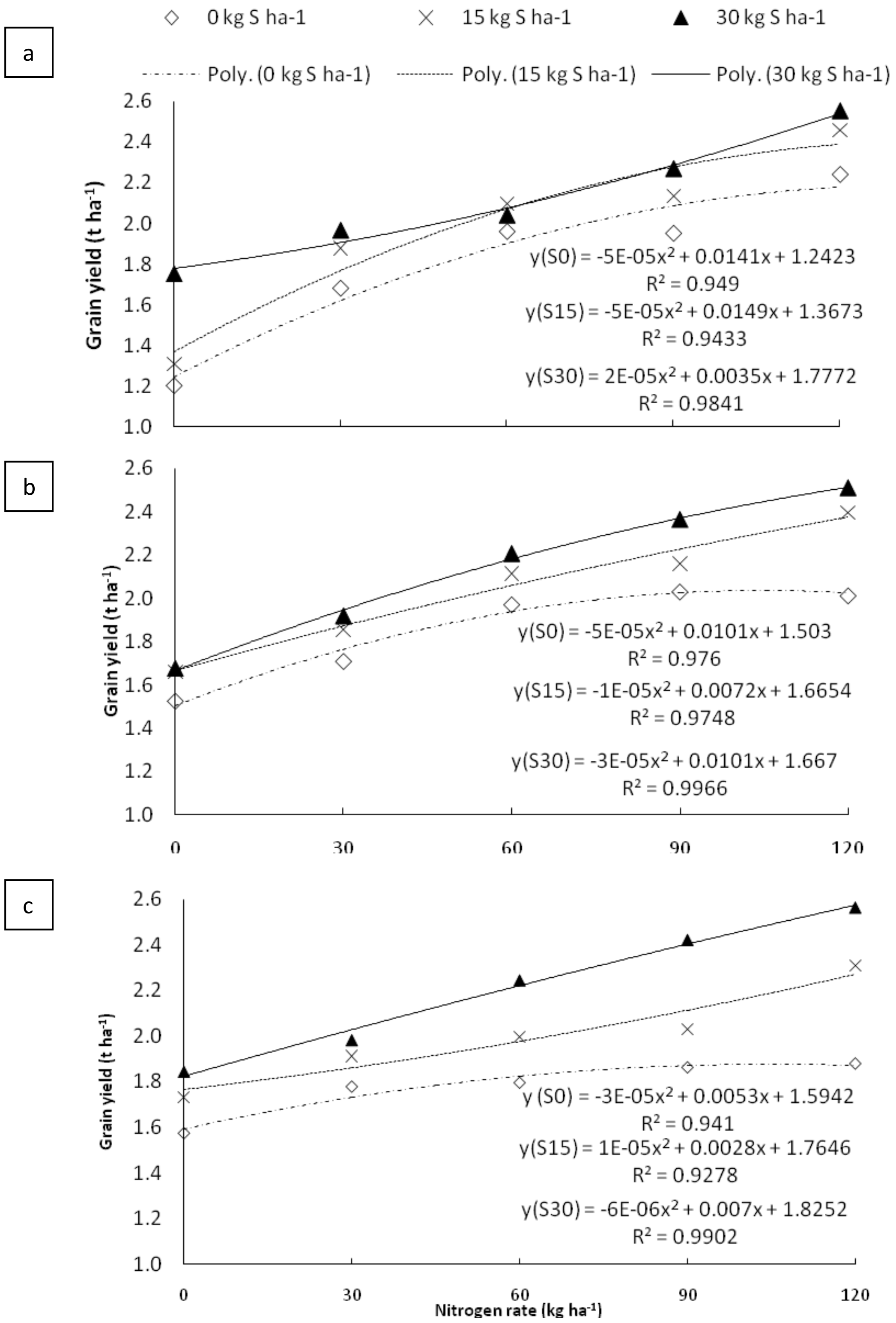

Figure 5.6 Effect of nitrogen and sulphur fertilisation rates on canola yields in (a) 2009, (b) 2010 and (c) 2011 seasons.

5.3.3 Thousand grain mass

In all the seasons evaluated, the size of individual canola grains was affected by locality (Table 5.1), with smaller grains at Langgewens (2.39 g) compared to Elsenburg (2.99 g) and Roodebloem (3.17 g) (Fig. 5.7a) in 2009. As shown in Fig. 5.7b & c, the same trends with regard to locality were reported in 2010 and 2011. As mean seed weight (thousand grain mass) is largely affected by growing conditions and length of the pod filling stage (Cheema *et al.,* 2001), these results clearly illustrated the effect of the dry pod filling period at Langgewens (low rainfall during August, September and October) and the beneficial effect of the higher rainfall during October at Elsenburg and Roodebloem in particular in 2009. Although not measured, it could be expected that less pods and seeds per pod were produced at Roodebloem as a result of the 2009 dry period during the flowering and seed filling (August and September).

The effects of water stress and compensatory behaviour of canola have also been reported in earlier studies (Kimber & McGregor, 1995; Daneshvar *et al.*, 2008). Hence with lower pods formed the plants responded by having higher grain size. These lower pod (seed) numbers will then explain why yields at Roodebloem in 2009 and 2010, were less than that at Elsenburg in spite of a higher thousand grain mass. In 2010, the canola grains at Welgevallen (2.75g) were significantly larger than at Elsenburg (2.56 g) and Langgewens (2.52 g) because of the low plant population, hence compensation through increasing grain size as reported by Angadi *et al.* (2003) and Daneshvar *et al.*, (2008).

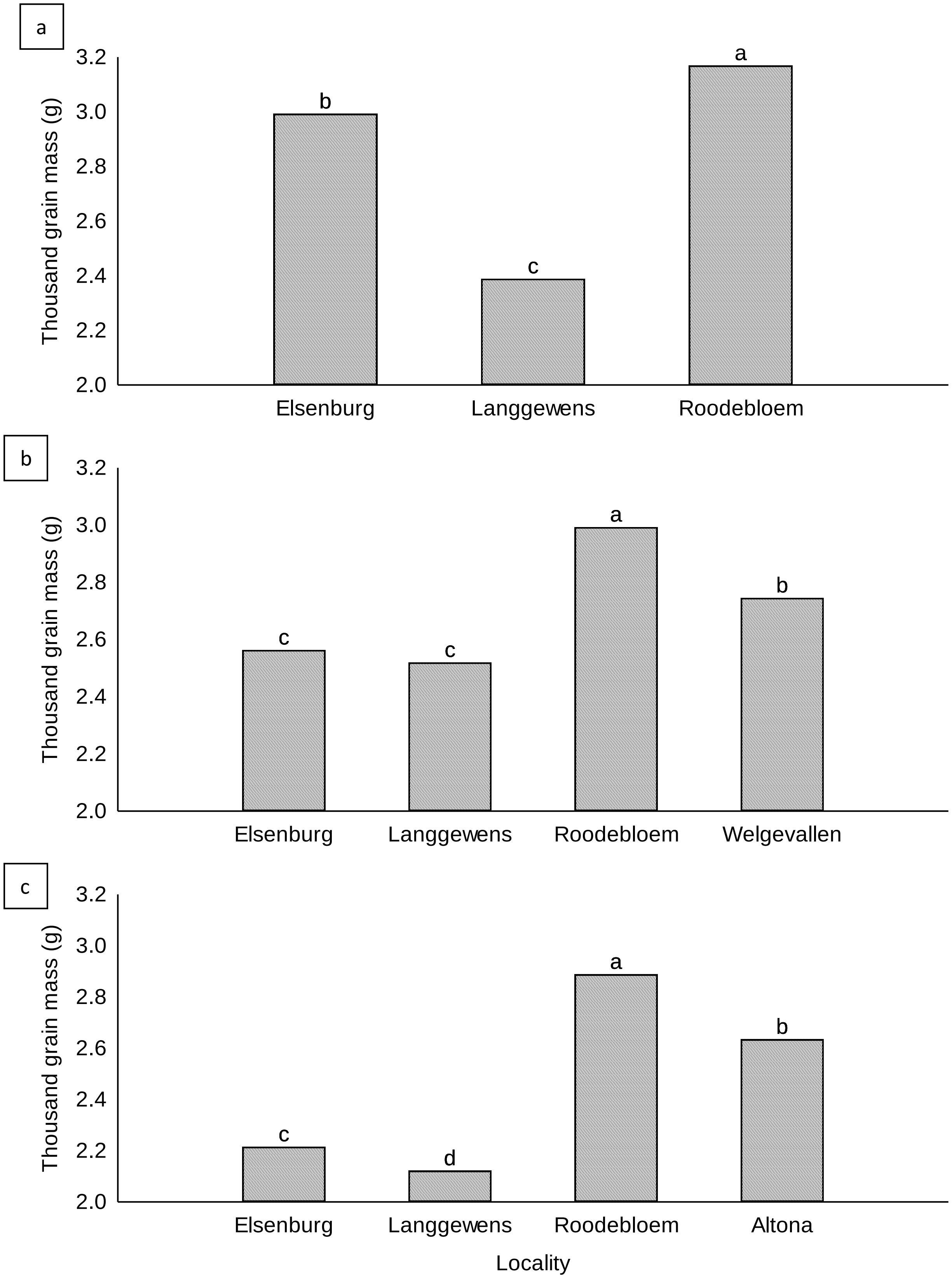

Figure 5.7 Thousand grain mass of canola at Elsenburg, Langgewens, Roodebloem, Welgevallen and Altona during (a) 2009, (b) 2010 and (c) 2011 canola seasons.

The application of S had no significant effect on grain size; whilst N had a significant effect on grain size during 2011 only, where application of 120 kg N ha^{-1} reduced the grain size of canola regardless of the locality (Fig. 5.8). These results suggests that higher grain yields obtained with higher N and S applications during the three canola seasons were the result of larger number of flowers, pods and or seeds produced per pod. The increase in such yield parameters can ultimately reduce the carbohydrates needed for increasing grain size as assimilates tends to be partitioned over a bigger sink. As nitrogen is a major component of protein formation (Hirel *et al.*, 2007), its addition would not influence grain size mostly if there are other environmental factors to consider.

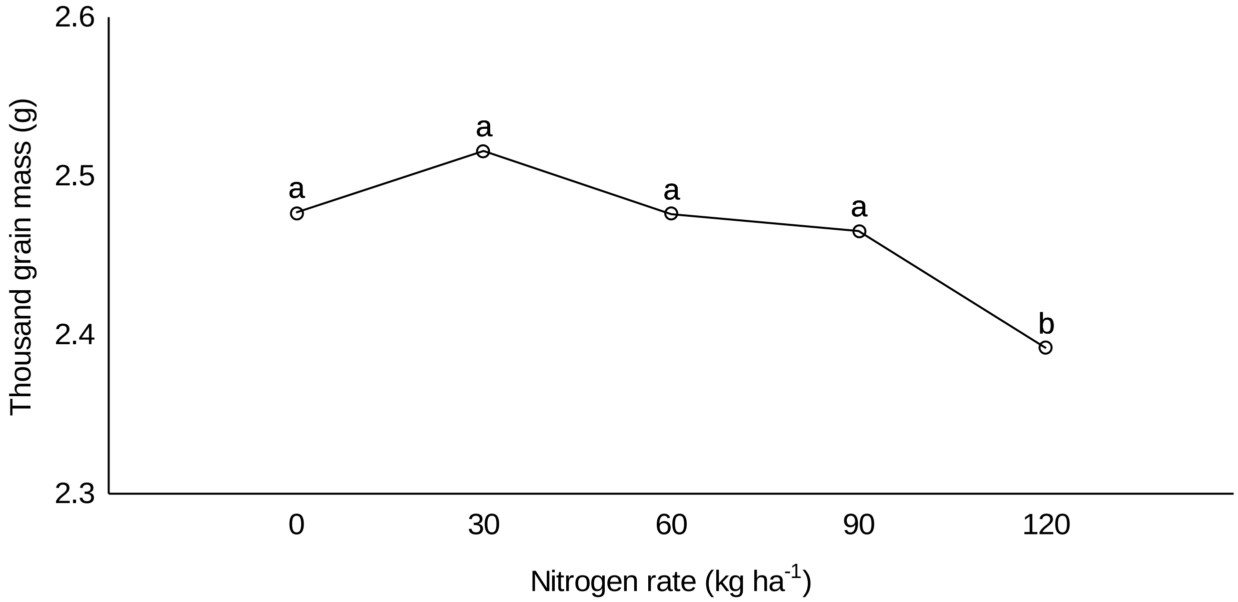

Figure 5.8 Thousand grain mass of canola at different nitrogen (0, 30, 60, 90 and 120 kg ha^{-1}) fertilisation rates during 2011.

However, significant Locality x N and Locality x S interactions in 2010 indicated that thousand grain mass responses to N and S can vary between localities. In 2010 application of N increased the grain size at Welgevallen and Elsenburg, but not at Langgewens and Roodebloem (Table 5.2). El-Nakhlawy & Bakhashwain (2009) reported that the grain size increases as levels of nitrogen fertilisation increase in irrigated canola crops. Consequently, low grain sizes were observed at Langgewens regardless of N application levels because of water stress during grain filling and at Roodebloem grain size actually decreased as N increased. The grain size of canola increased when fertilised with sulphur at Elsenburg, with little or no conclusive results at other localities (Table 5.2).

Table 5.2 Effect of N and S-fertiliser rates on thousand grain mass (g) of canola grown at different localities during 2010.

	N rate **(kg ha^{-1})**					***S rate*** **(kg ha^{-1})**			
Locality (L)	**0**	**30**	**60**	**90**	**120**	**0**	**15**	**30**	**Locality Mean**
Elsenburg	2.47	2.53	2.47	2.64	2.71	2.44	2.61	2.64	**2.56**
Langgewens	2.54	2.57	2.51	2.49	2.48	2.55	2.52	2.50	**2.52**
Roodebloem	3.13	3.07	3.03	2.92	2.82	3.0	3.0	3.0	**3.0**
Welgevallen	2.72	2.69	2.63	2.90	2.78	2.87	2.61	2.76	**2.75**
Mean	**2.72**	**2.71**	**2.66**	**2.73**	**2.70**	**2.71**	**2.68**	**2.72**	
	LSD$_{0.05}$:Locality X N: 0.20					LSD$_{0.05}$:Locality X S: 0.20			

5.3.4 Grain oil and protein content

Grain oil and protein content differed significantly between localities with canola grain at Roodebloem (41.4 %) having significantly higher oil content than at Elsenburg (39.2 %) and Langgewens (36.5 %) localities (Table 5.3). Protein and oil have an inverse relationship such that an increase in protein content can significantly lower the oil content of the crop (Ahmad *et al.*, 2007; Hassan *et al.*, 2007). Such a response was shown at Langgewens, having significantly higher protein content (24.8 %) compared to Elsenburg (22.3 %) and Roodebloem (20.2 %) localities.

Table 5.3 Effect of S-fertiliser rates on grain oil content (%) of canola grown at different localities (Mean values for 2009-2011 period)

S rate **(kg ha^{-1})**	**Elsenburg**	**Langgewens**	**Roodebloem**
0	39.1	36.2	41.3
15	39.3	36.6	41.4
30	39.3	36.7	41.5
Mean	**39.2**	**36.5**	**41.4**
	LSD$_{0.05}$: Locality	Oil: 2.3	
	LSD$_{0.05}$: Locality X S	Oil: 0.4	

Oil content was slightly increased at all localities as a result of applications of 30 kg S ha^{-1} however such increase was only significant at Langgewens (36.2 to 36.7 %) and not at Roodebloem (41.3 to 41.5 %) and Elsenburg (39.1 to 39.3 %)(Table 5.3). However, without any S applied, canola oil content at Roodebloem (41.3 %) was even higher than with 30 kg S ha^{-1} application rates at Langgewens (36.7 %) and Elsenburg (39.3 %). Sulphur application had no significant effect on the protein content at any of the three localities evaluated.

High N application rates lowered the oil content whilst increasing the protein content of canola grains (Table 5.4). Such effects of N on oil content were highly dependent on locality, such that application of 120 kg N ha^{-1} resulted in decreasing canola grain oil content from 40.9 % where no N was applied to 36.7 % at Elsenburg. At the same locality, increasing N application rates resulted in an increase in protein content from 20.5 % where no N was applied to 23.7 % at an application rate of 120 kg N ha^{-1} (Table 5.4). However, even though similar increases in protein and decreases in oil content with N fertilisation were shown at the other two localities (Langgewens and Roodebloem), these changes were not significant. This L X N interaction can, therefore, be ascribed to the inherently different levels of N in the soil and effects of climatic conditions (especially soil moisture) (Fig. 3.1 in Chapter 3) on nutrient uptake and overall partitioning (Hocking, *et al.,* 1997; Jackson, 2000; Malhi *et al.,* 2007).

Table 5.4 Effect of N and S-fertiliser rates on grain oil content (%) and grain protein content (%) of canola grown at different localities (Mean values for 2009-2011 period)

N rate **(kg ha^{-1})**	***S rate*** **(kg ha^{-1})**	**Elsenburg**		**Langgewens**		**Roodebloem**	
		Oil	**Protein**	**Oil**	**Protein**	**Oil**	**Protein**
0	0	40.9	20.4	37.7	23.5	42.8	18.6
	15	40.9	20.6	37.7	23.5	42.7	18.4
	30	41.0	20.4	37.8	23.4	42.9	18.6
	Mean	**40.9**	**20.5**	**37.7**	**23.5**	**42.8**	**18.5**
30	0	40.3	21.6	37.2	24.5	42.4	18.8
	15	40.2	21.5	37.4	24.6	42.4	19.0
	30	40.4	21.8	37.5	24.6	42.5	19.2
	Mean	**40.3**	**21.6**	**37.4**	**24.6**	**42.4**	**19.0**
60	0	39.7	22.2	36.1	24.9	41.6	20.1
	15	39.8	22.6	36.4	25.2	41.7	20.2
	30	39.9	22.9	36.6	25.4	41.8	20.3
	Mean	**39.8**	**22.6**	**36.4**	**25.2**	**41.7**	**20.2**
90	0	38.3	23.1	35.2	25.3	40.6	20.7
	15	38.4	23.1	35.9	25.2	40.7	20.7
	30	38.3	23.3	35.8	25.5	40.9	20.8
	Mean	**38.3**	**23.2**	**35.6**	**25.3**	**40.7**	**20.7**
120	0	36.5	23.8	35.0	25.3.	39.3	22.4
	15	36.9	23.7	35.6	25.6	39.6	22.6
	30	36.8	23.6	35.8	25.6	39.5	22.6
	Mean	**36.7**	**23.7**	**35.5**	**25.5**	**39.5**	**22.5**
Locality mean		**39.2**	**22.3**	**36.5**	**24.8**	**41.4**	**20.2**
Oil -LSD$_{0.05}$: Locality: 2.3		Locality X N: 2.8			Locality X N X S: ns		
Protein- LSD$_{0.05}$: Locality: 1.9		Locality X N: 3.3			Locality X N X S: ns		

5.4 CONCLUSIONS

It can be concluded that the optimum level of N application for maximum grain yields in canola are highly dependent on the locality (soil and climatic conditions), while responses to S depends on soil conditions. Low rainfall during the flowering and pod growth phases may limit yield responses to N applications, but N applications of 120 kg ha^{-1} may be needed in high rainfall areas to obtain grain yields of more than 2.0 ton ha^{-1}. In such areas, S applications of 15-30 kg S ha^{-1} may be needed if soil S contents are <6 mg kg^{-1}. These rates could even improve the canola grain oil content. Though overall grain yield were generally increased with addition of higher levels of N, application of higher N rates significantly decrease the grain oil content hence increasing protein content as nitrogen is the major constituent of proteins, whilst oil content decreases because of an inverse relationship between grain protein and oil content.

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CHAPTER 6

The effect of nitrogen and sulphur on the agronomic and water use efficiency of canola (*Brassica napus* L.) grown in the Western Cape

Abstract

The grain yield per unit of available plant Nitrogen (S) and Sulphur (S) nutrients in the soil is usually low in most crops. Besides the ability of different crops in utilizing nutrients, soil and climatic conditions can also influence uptake and use of major plant nutrients. Hence to determine the effect of soil and climatic differences on canola agronomic efficiency response to N and S application, a factorial split plot of five nitrogen (0, 30, 60, 90 and 120 kg N ha^{-1}) and three sulphur rates (0, 15 and 30 kg S ha^{-1}) was conducted at several localities (Altona, Elsenburg, Langgewens, Roodebloem and Welgevallen) in the Western Cape during the 2009 to 2011 growing season. Agronomic efficiencies of N applications are improved if 15 kg S ha^{-1} are applied, but not with applications of 30 kg S ha^{-1}. Sulphur use efficiency is also improved when high N (120 kg N ha^{-1}) is applied. Application of both nutrients should be complimented with rainfall availability and distribution. High water use efficiency is mostly recorded with both N and S fertilisation. Agronomic N use efficiency at high rates of N are however still low compared to winter cereals and methods to improve it should be investigated.

Keywords: Agronomic use efficiency, canola, water use efficiency

6.1 INTRODUCTION

Nitrogen fertilisation of canola has been singled out by the Agricultural Research Council of South Africa as the largest production input cost under dry land conditions in the country and is probably also the case for irrigated canola (ARC, 2007). Most crop plants are only able to convert 30-40 % of this applied nitrogen (N) to useful food products such as grain (Raun & Johnson, 1999, Hirel *et al.*, 2007). Nutrient conversion challenges are far worse in canola, with N conversion values of even less than 10 % (Malagoli *et al.*, 2005; Sylvester-Bradley & Kindred, 2009). Yet the crop is generally a heavy nutrient feeder (nitrogen and sulphur particularly) (Oplinger *et al.*, 2000; Hocking & Strapper, 2001).

A large amount of the N taken up during the vegetative growth phase is lost through leaf fall in senescence (Hirel *et al.*, 2007, Malagoli *et al.*, 2005), leaving the stems and pods as main sources of assimilates. During grain filling all remaining nutrients tend to accumulate in the grain. Moll *et al.*, (1982) defined nitrogen use efficiency (NUE) as being the yield of grain per unit of available N in the soil (including the residual N present in the soil and the fertiliser). This NUE can be separated into two processes: uptake efficiency and the utilization efficiency (the ability to use N to produce grain yield) (Lea & Azevedo, 2006). Nitrogen efficiency can further be extended to agronomic use efficiency which is the increase in grain yield obtained when N is applied in as fertilisers (Smith *et al.*, 1988) because it is difficult to determine how much N is in the soil and how much is taken up. Besides the differences observed in canola genotypes in N remobilization (Gan *et al.*, 2008a; Tatjana *et al.* 2008), efficiency is also affected by several agronomic factors that have an influence on plant morphology and physiology (Hirel *et al.*, 2007). Increasing N application rates have been shown to reduce its utilisation efficiency, especially, at levels beyond 100 kg N ha^{-1} (Gan *et al.*, 2008a). Amongst the environmental conditions; soil nutrient and moisture levels, temperature and radiation can all influence N use efficiency (Rathke *et al.*, 2006).

Nutrient use efficiency (NUE) in canola is specifically biased towards nitrogen as the main nutrient. However, there are pronounced responses to sulphur (S) by this crop, with its requirements about four times greater than that of wheat or maize (Abdallah *et al.,* 2010). This higher S requirement in canola compared to cereals are ascribed to the higher protein content of these cultivars combined with Brassica's higher proportion of cysteine and methionine which hence contributes to larger sulphur requirements (Durrani & Khalil, 1990). However, the canola grain yield responses to applied S depends on N application rate (N: S ratio of 7:1 is suggested), as well as soil and climatic conditions, which can influence the use efficiencies of the nutrients.

Fertilisation with N and S have been shown to increase yields (Breennon & Bolland, 2008, Gan *et al.*, 2008b; Tatjana *et al.*, 2008) but absorption (Koenig, 2012), translocation (Malhi *et al.,* 2007) and partitioning (Jackson, 2000) of these nutrients have been shown to be greatest where there is available soil moisture (Ahmadi & Bahrani, 2009). The canola crop is very prone to water stress, especially during the period of flowering to grain filling (Faraji *et al.,* 2009) significantly reducing the yielding potential of the crop. Besides the ability of the canola plant to adapt to moisture stress levels (Angadi *et al.,* 2003), available water appears to be better utilised when all conditions are favourable. This utilisation of available moisture can be described as water use efficiency (WUE) which is the grain yield per millimeter of rainfall received (Perry & Hillman, 1991). Evaluation of WUE can be of major importance especially in diagnosing potential constraints to yields, other than lack of water (Cocks *et al.,* 2001). Water use efficiency becomes interlinked with soil available nutrients, such that yield expectations can be established on the basis of received water on appropriate soil nutrients applied.

Even though canola production and yields per hectare in the country are still low compared to the global major producers, nutrient fertilisers should be applied at the correct rates suited to expected rainfall. Disproportionate use of fertilisers where there is both low crop nutrient use efficiency (Malagoli *et al.*, 2005) and water use efficiency, can lead to significant deleterious environmental losses (Hirel *et al.*, 2007). These losses can be mitigated by understanding N and water use characteristics of canola, therefore, improved efficiency and minimize production costs (Masson & Brennan, 1998; Fismes *et al.*, 2000) especially when production regions are characterized by different soil and climatic properties. For this reason, this study is aimed at determining the effect of soil and climatic differences, as experienced at different localities on agronomic N, S and water use efficiency response of canola to N and S application rates.

6.2 MATERIALS and METHODS

The field experiments were conducted at Elsenburg (2009-2011), Langgewens (2009-2011), Roodebloem (2009-2011), Welgevallen (2010) and Altona (2011) Experimental stations of the Western Cape Province of South Africa. Detail of the experiment can be found in Chapter 3.

Climatic data as presented in Chapter 3 and grain yield data as presented in Chapter 5 were used. Agronomic use efficiencies (AE) of N and S were calculated according to Smith *et al.* (1988) using the following equations for N and S respectively:

$$\text{NUE (kg seed kg N applied}^{-1}) = \frac{\text{Grain yield (kg ha}^{-1}) - \text{Grain yield control (kg ha}^{-1})}{\text{N fertiliser applied (kg ha}^{-1})}$$

and

$$\text{SUE (kg seed kg S applied}^{-1}) = \frac{\text{Grain yield (kg ha}^{-1}) - \text{Grain yield control (kg ha}^{-1})}{\text{S fertiliser applied (kg ha}^{-1})}$$

Water use efficiency in form of grain yield per millimeter of rainfall received was calculated by using the following equation (Perry & Hillman, 1991):

$$\text{WUE (kg seed mm water}^{-1}) = \frac{\text{Grain yield (kg ha}^{-1})}{\text{Water Use (mm)}}$$

Water use was calculated as follows:

Water use (mm) = 51.1+ 0.75 (May to October rainfall).

This equation was developed for Western Australian conditions, which are very similar to conditions in the Western Cape and most of the trials in this study were also planted during May. The equation was therefore considered to be relevant for this study.

6.2.1 Data analysis

Data recorded was analysed, using analysis of variance (ANOVA) (Statistica 11). To compare different soil and climatic conditions and measure response to treatments at different localities, locality was regarded as a factor. Considering the fact that different cultivars were planted in the three different seasons and that three localities (Elsenburg, Langgewens and Roodebloem) were planted in all seasons (2009-2011), whilst Welgevallen and Altona were included in 2010 and 2011 respectively, data for the three seasons was analysed separately. Main and interaction effects were compared using least significant difference (LSD) test at 5 % level of probability. Any treatment means found to be significantly different were separated using Fischer's protected $LSD_{0.05}$.

6.3 RESULTS and DISCUSSION

6.3.1 Significance of F values

The summary of the ANOVA and significance of F values shown in Table 6.1 indicates that fertilising canola with different nitrogen and sulphur rates significantly affects agronomic N and S use efficiencies as well as water use efficiency. It is clearly shown that the magnitude of response to nitrogen and sulphur differed between Elsenburg, Langgewens, Roodebloem, Welgevallen and Altona localities as well as between different growing seasons.

Table 6.1 Summary of significant effects (F-values) from the Analysis of variance done on agronomic N (NUE) and S (SUE) use efficiencies as well as water use efficiency (WUE) during the 2009, 2010 and 2011 growing seasons.

	NUE			**SUE**			**WUE**		
	2009	**2010**	**2011**	**2009**	**2010**	**2011**	**2009**	**2010**	**2011**
Locality	**	***	ns	***	***	*	***	***	***
N rate	*	*	ns	ns	***	***	***	***	***
S rate	**	**	ns	ns	*	ns	***	***	***
N X Locality	ns	***	ns	ns	***	ns	ns	***	ns
S X Locality	ns	ns	ns	ns	ns	ns	ns	*	ns
S X N	ns	ns	ns	ns	ns	ns	ns	***	ns
N X S X Locality	ns	ns	ns	ns	ns	ns	ns	**	ns

*, **, *** denote significance at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively and ns denotes non significance at $P \leq 0.05$.

6.3.2 Agronomic N use efficiency

Agronomic N use efficiency (NUE) differed between localities in 2009 with Elsenburg (14.03 kg yield increase per kg N applied) showing higher NUE values compared to Roodebloem (9.53 kg yield increase per kg N applied) and Langgewens (6.50 kg yield increase per kg N applied) (Fig. 6.1), probably due to the better moisture supply (higher rainfall) at this locality (Fig. 3.1 in Chapter 3). This is because canola needs more water during the flowering and pod growth phases compared to vegetative phases (Morrison & Stewart, 2002; Tesfamariam *et al.*, 2010) and moisture stress during this period significantly reduce yield and agronomic efficiency of crops (http://www.canolacouncil.org/chapter9).

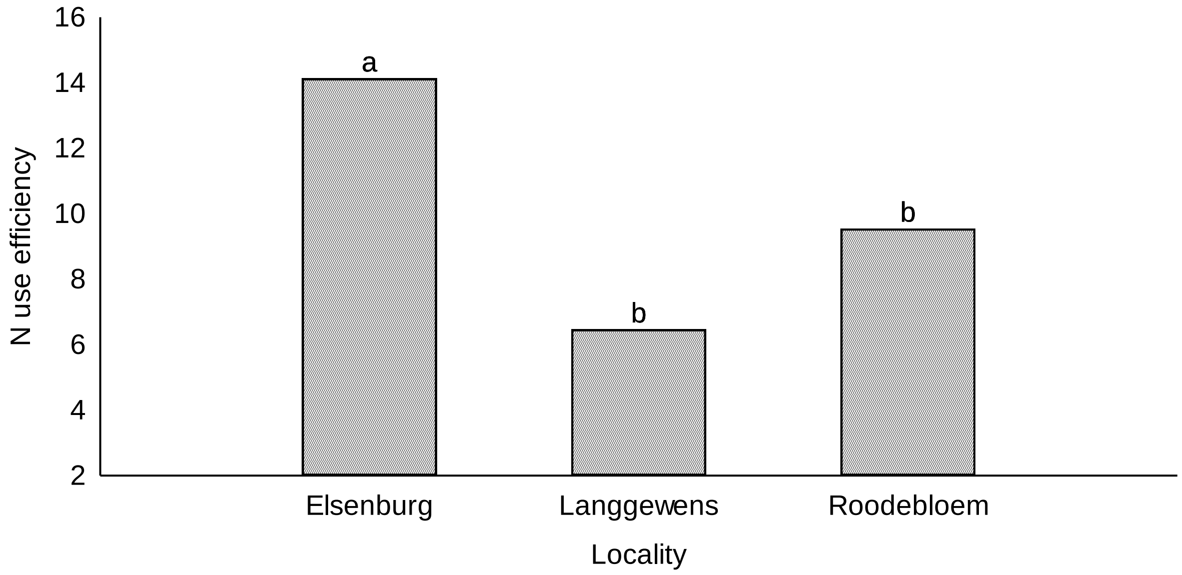

Figure 6.1 Agronomic N use efficiencies of canola at Elsenburg, Langgewens and Roodebloem localities during the 2009 season

Agronomic N use efficiency decreased with an increase in N application rate from on average about 14 kg yield increase per kg N applied at a rate of 30 kg to less than 8 at N application rates of more than 60 kg N ha^{-1} in 2009 (Fig. 6.2). Gan *et al.* (2008b) also showed low nitrogen fertiliser use efficiencies at high N fertiliser application rates.

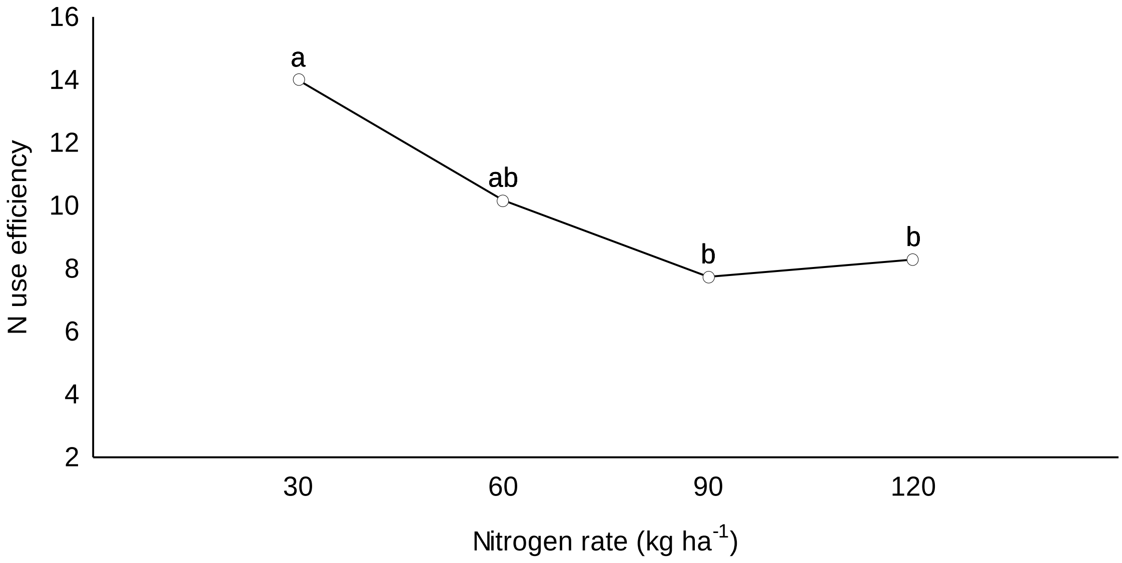

Figure 6.2 Agronomic N use efficiencies of canola with increasing N application rates during 2009 season

In 2009, agronomic efficiency of N fertiliser applications tend to increase with S applications from 11.37 where no S was applied, to 12.71 at rates of 15 kg S ha^{-1}, but showed a significant decrease when more than 15 kg S ha^{-1} was applied (Fig 6.3). This trend was found at all localities because the soil showed deficient levels of S (<6 mg kg^{-1} soil) at all localities (Table 3.1 in Chapter 3).

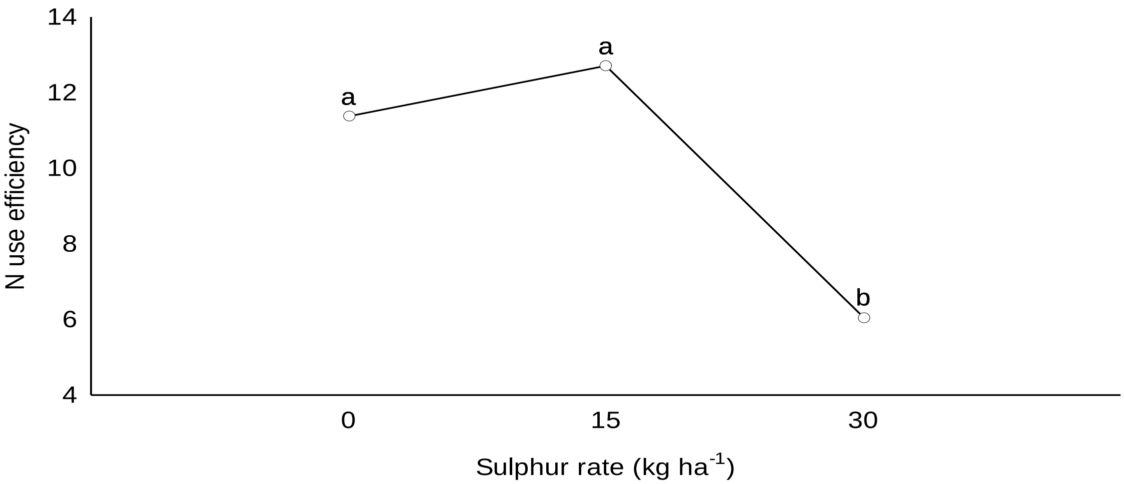

Figure 6.3 Agronomic N use efficiencies of canola with increase in sulphur rates during 2009 season

In 2010 a significant locality X N interaction was shown with regard to NUE (Table 6.1). This was due to contrasting results at different localities (Fig 6.4). At Elsenburg and Roodebloem, NUE tend to increase with an increase in N application rate from 30 to 60 kg N ha^{-1}, followed by a decrease with further increase in N rate. At Welgevallen and especially Langgewens, NUE decreases with increases in N application rates. However, a decrease in NUE with increase in N application (60-120 kg N ha^{-1}) is shown at all localities. The results shown are consistent with those reported by Maman *et al.* (1999) who also found a decrease in N efficiency with an increase in N supply and NUE being interactively affected by soil N supply aside from fertilisation (Gan *et al.*, 2008b) as shown in the locality x nitrogen rate in 2010 season. During 2011, neither N nor S application rate nor their interactions had any significant effect on agronomic efficiency of applied N on any of the localities (Table 6.1).

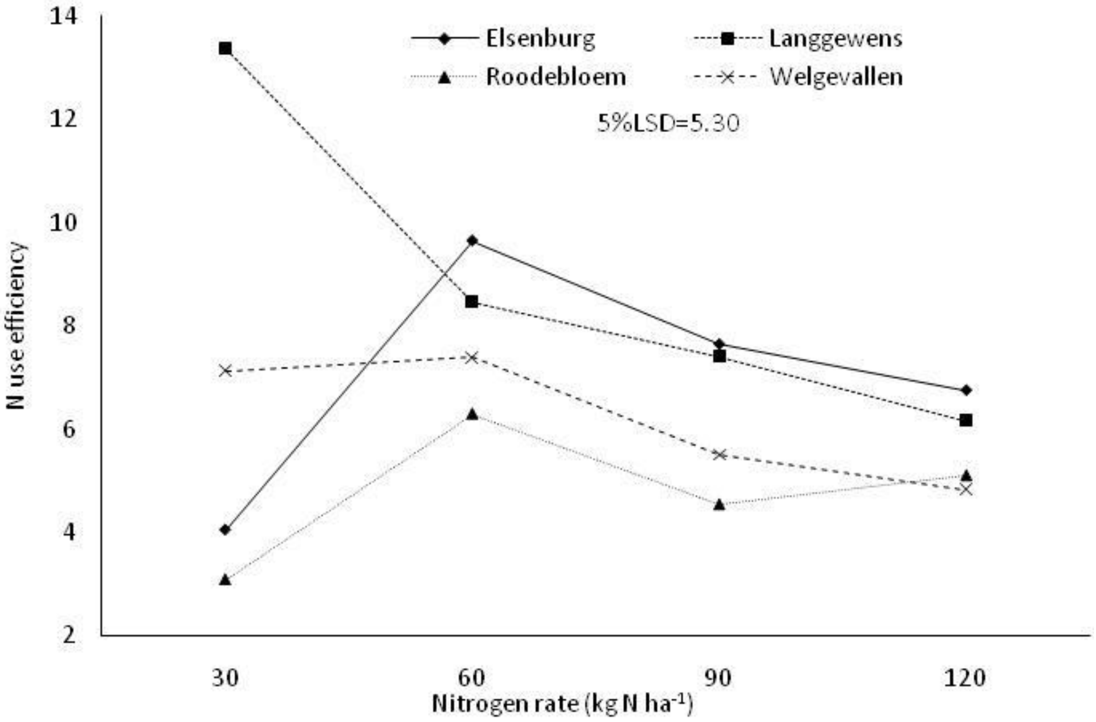

Figure 6.4 Agronomic N use efficiencies of canola at increasing N application rates at Langgewens (LG), Elsenburg (ELS), Roodebloem (RB) and Welgevallen (WLG) during 2010.

The generally low agronomic efficiencies observed (<15 kg seed yield kg^{-1} N added) in this study were within the range observed in earlier studies (Hirel *et al.*, 2007; Fageria *et al.*, 2008), but may also be the result of the high mineral-N content of the soil at experimental sites. According to the Canadian Canola Council, yield response of rainfed canola to fertiliser N is unlikely to be profitable when the soil contains more than 34 to 45 kg nitrate-N ha^{-1} in the top 60 cm (http://www.canolacouncil.org/chapter9). In this study, mineral-N content in the top 30 cm soil was calculated and mean values for different localities were found to be 111.3 kg N ha^{-1}, 92.0 kg N ha^{-1}, 56.8 kg N ha^{-1}, 59.9 kg N ha^{-1} and 93.5 kg N ha^{-1} for Altona, Elsenburg, Langgewens, Roodebloem and Welgevallen experimental sites, respectively. It can thus be said that in this study, both environmental conditions during the critical growth period of the canola crops and soil N supply (Gan *et al.*, 2008b) had an effect on NUE response.

6.3.3 Agronomic S use efficiency

In 2009, agronomic use efficiency of applied sulphur (SUE) was significantly higher at Elsenburg (22.73 kg ha^{-1} yield increase per kg S applied) compared to Langgewens (3.15) and Roodebloem (6.46) (Fig. 6.5). The high SUE values at Elsenburg (2009) may be attributed to the higher and extended period of high rainfall at this locality (Fig. 3.1 in Chapter 3), because adequate availability of moisture and a lengthy grain filling phase ultimately lead to higher grain yield per kg S added (Hassan *et al.,* 2007). Neither N nor S application rate nor their interactions had any significant effect on agronomic efficiency of applied S (kg yield increase ha^{-1} per kg S applied during 2009 (Table 6.1).

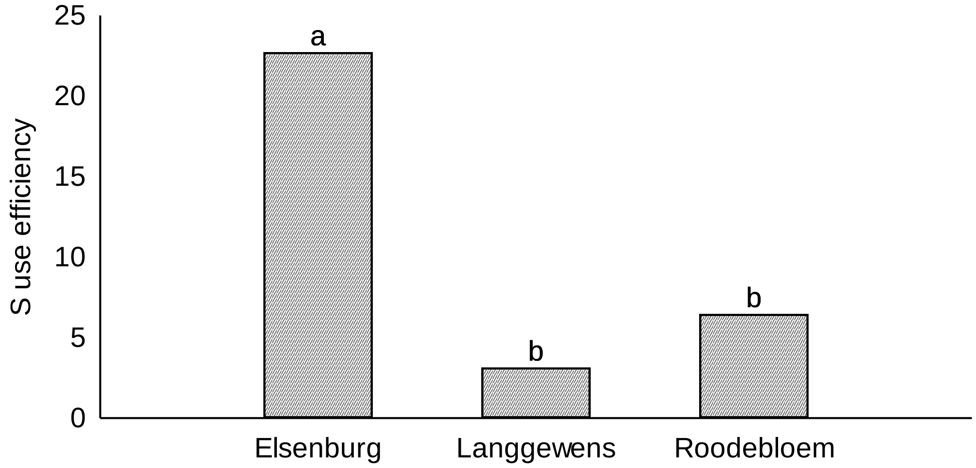

Figure 6.5 Agronomic S use efficiencies of canola at Elsenburg, Langgewens and Roodebloem during 2009 season.

Agronomic S use efficiencies of canola at different localities showed similar trends to that of 2009 in 2010, but results for different localities were highly dependent on N fertilisation levels (Table 6.1). Although SUE tend to increase with increasing N application rates at most of the localities tested during 2010 (Table 6.2), trends were very confusing. At Elsenburg for example, SUE initially decreased with increasing N rates from 0 to 60 kg N ha^{-1}, but increased with further increases in N rate. At

Roodebloem SUE increased with increasing N rates to reach its highest value at 120 kg N ha^{-1}, while the highest SUE value at Welgevallen was obtained with an application of 90 kg N ha^{-1}. The highest SUE (39.21 kg ha^{-1} yield increase per kg S applied) were observed when 120 kg N ha^{-1} was applied at Elsenburg, whilst that at Langgewens were generally low, indicating that a combination of available soil moisture and high N fertilisation can increase S use efficiency (Fismes *et al.,* 2000; Hassan *et al.,* 2007).

Table 6.2 Effect of N on agronomic S use efficiencies (kg ha^{-1} yield increase per kg S applied) of canola grown at different localities during 2010.

	***N rate* (kg ha^{-1})**					
Locality (L)	**0**	**30**	**60**	**90**	**120**	**Locality Mean**
Elsenburg	18.12	13.46	2.96	9.0	39.21	**16.55a**
Langgewens	4.83	7.08	10.25	3.58	9.83	**7.11b**
Roodebloem	-1.08	1.63	6.67	7.38	21.38	**7.19b**
Welgevallen	6.67	11.75	15.21	19.83	14.0	**13.49a**
Mean	**7.15b**	**8.48b**	**8.77b**	**9.95b**	**21.10a**	
LSD$_{0.05}$:L X N -13.20						

The grain yield increased per kg S applied in 2010 was also affected by S application rates (Table 6.1). In 2010, increasing application rates of S from 15 kg S ha^{-1} to 30 kg S ha^{-1} resulted in a decrease in SUE from 12.66 to 9.52 kg ha^{-1} yield increase per kg S applied on average. Although these results suggested that the application of S become less effective at rates higher than 15 kg ha^{-1}, application rates of 30 kg S ha^{-1} may still be economical viable, because grain yield still increase with these rates. Agronomic use efficiencies of S (SUE) values of 9.52 on average indicated that the application of S at these rates will be economically viable as long as the cost to apply a kilogram of S are less than 9.52 times the price of a kilogram of canola.

In 2011, agronomic S use efficiencies of canola were significantly affected by N applications and differed between localities with Roodebloem (19.48 kg ha^{-1} yield increase per kg S applied) having significantly higher SUE values than Altona (14.66), Langgewens (12.07) and Elsenburg (12.05) (Fig 6.6). SUE increased with the addition of N fertiliser with the magnitude of increase the same regardless of locality (Fig 6.7).

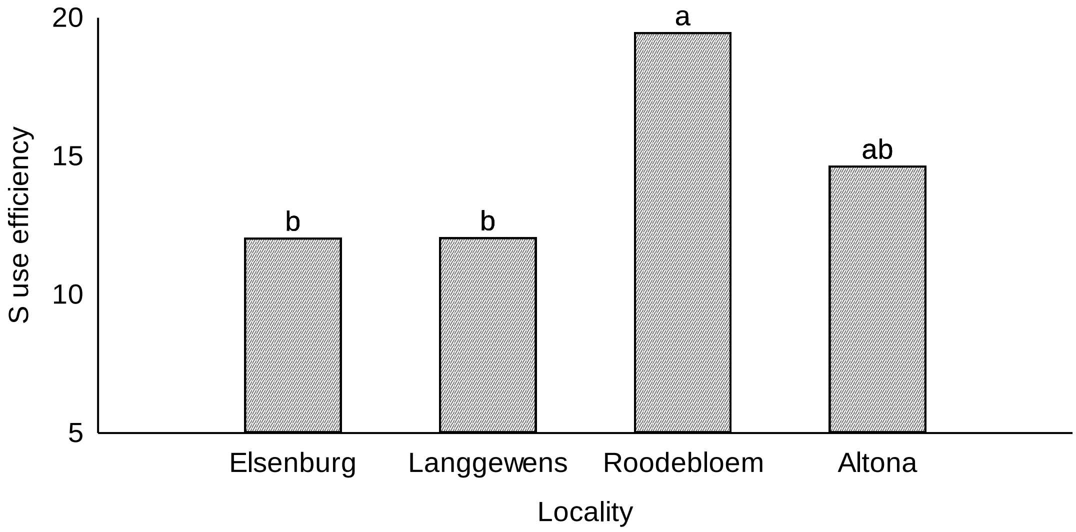

Figure 6.6 Agronomic S use efficiencies of canola at Elsenburg, Langgewens, Roodebloem and Altona during 2011 season.

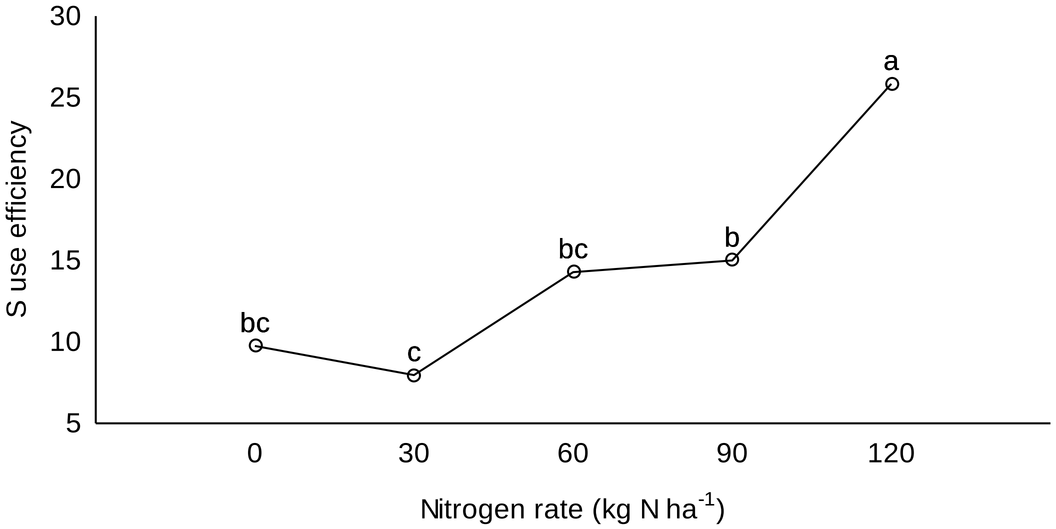

Figure 6.7 Agronomic S use efficiencies of canola at increasing N application rates during 2011.

6.3.4 Water Use Efficiency

Water use efficiencies of canola differed between localities (Table 6.1). In 2009, water use efficiency at Elsenburg (6.36 kg grain mm water^{-1}) was significantly higher than that at Roodebloem (5.34 kg grain mm water^{-1}) and Langgewens (4.64 kg grain mm water^{-1}) (Fig. 6.8), most probably due to the better distribution of rainfall during the growing season at Elsenburg.

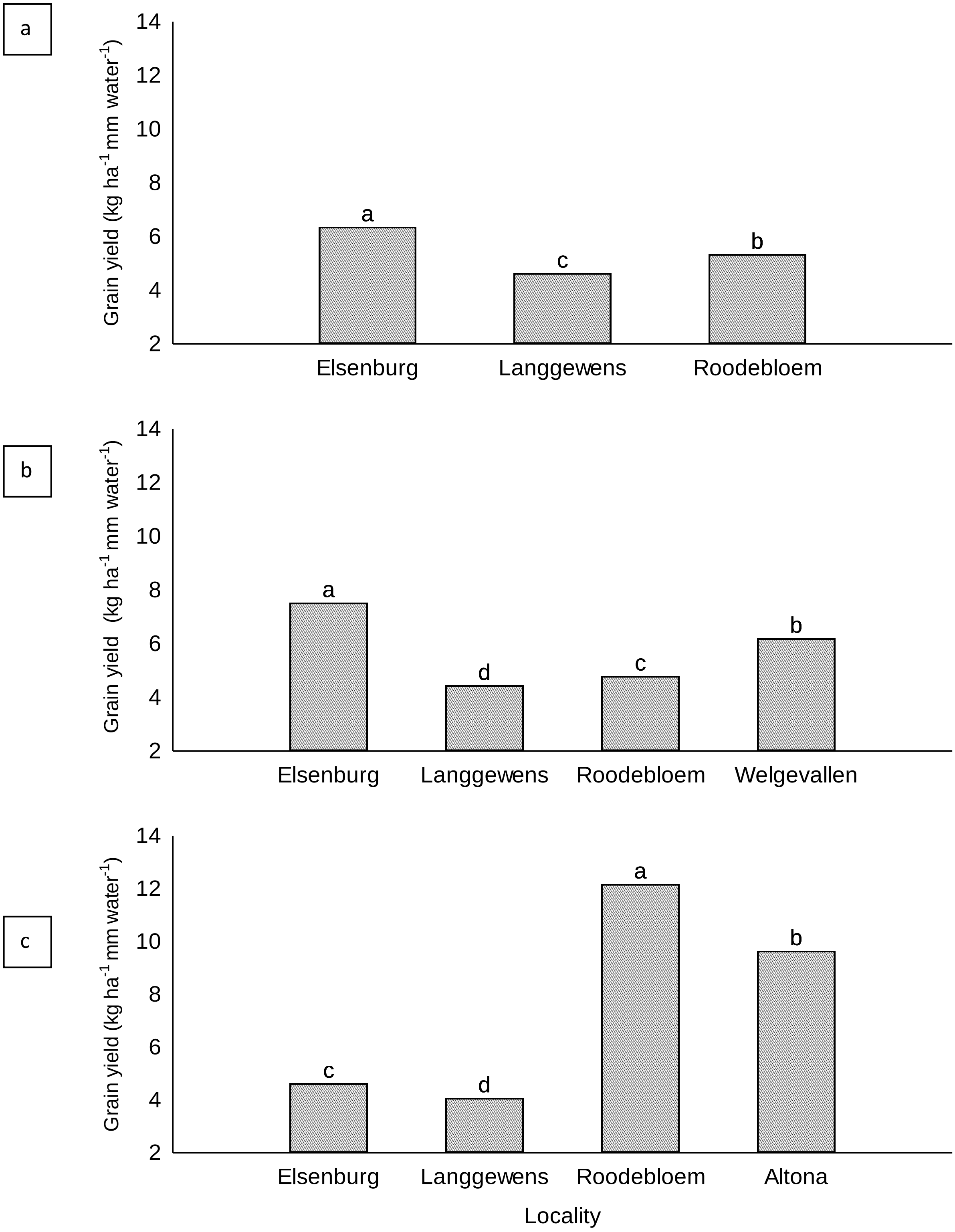

Figure 6.8 Canola yields per mm of water used at Altona, Elsenburg, Langgewens, Roodebloem and Welgevallen during (a) 2009, (b) 2010 and (c) 2011 seasons.

The application of N had a significant effect on WUE during 2009, where fertilisation with higher N rates of 120 kg N ha^{-1} increased yield of canola per millimeter of rainfall received (Fig. 6.9). In this season, WUE increased from 3.91 kg of grain per mm of water with no N (0N) to 6.69 kg grain per mm of water with an application of 120 kg N ha^{-1} (Fig. 6.9)

During 2009 WUE also differed significantly due to S application rates at all localities from 5.04 kg of grain per mm of water when no S is applied to 5.84 kg of grain per mm of water with an application of 30 kg S ha^{-1} (Fig. 6.9) because the soil at all localities had low S contents (<6 mg kg^{-1} soil).

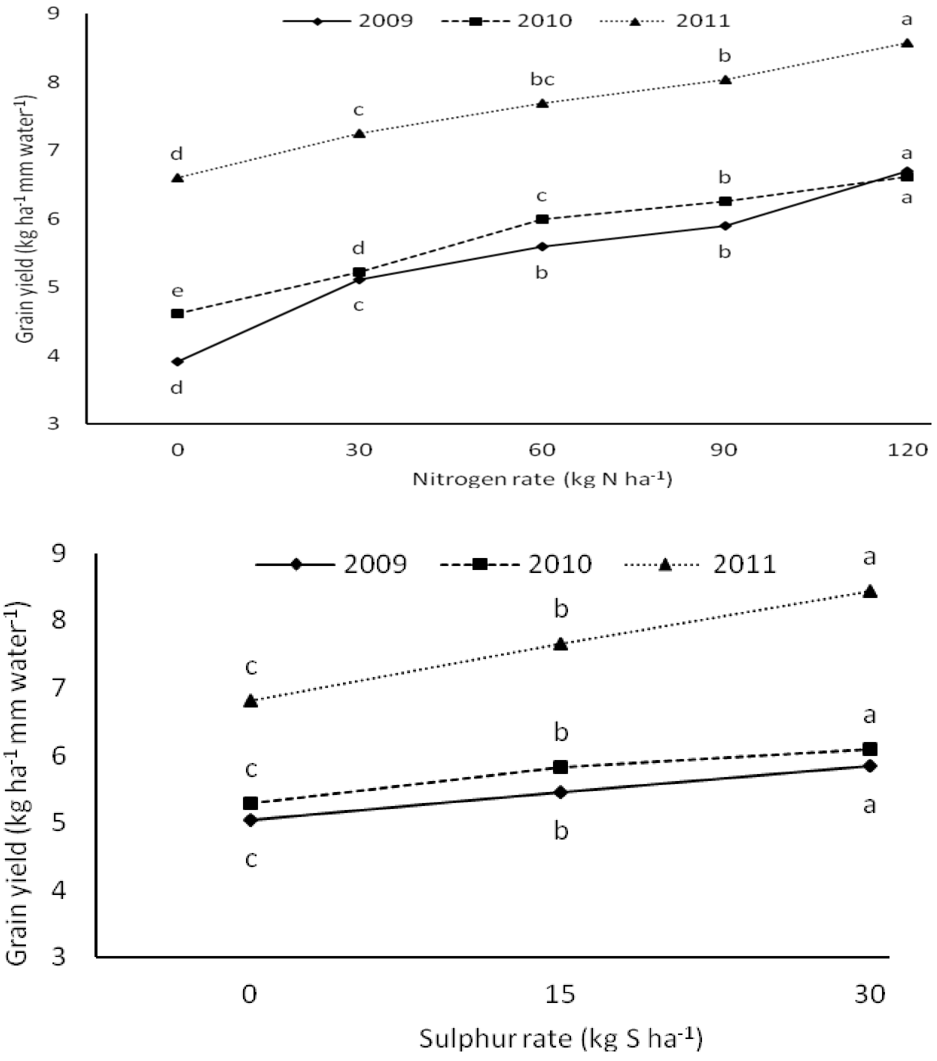

Figure 6.9 Canola yields per mm of water received in 2009, 2010 and 2011 seasons at different nitrogen (*top*) and sulphur (*bottom*) fertilisation rates.

From the graphs, means for each one season with at least a common letter are not significantly different, $LSD_{0.05}$

In 2010 a significant interaction with regard to water use efficiency was shown between locality and N and S application rates (Table 6.1), suggesting different responses at different localities. From Figure 6.10 it is however clear although the degree of response did differ, water use efficiency, expressed as kg grain produced per mm of water, increased at all localities with an increase in N application rate. Responses with increasing N rates also tend to be larger at 15 and 30 kg S application rates compared to where no S was applied. These results confirmed earlier studies (Ahmadi & Bahrani, 2009), which reported that the application of N will increase grain yields to the limits imposed by moisture supply. The addition of fertiliser at localities that receive low rainfall such as Langgewens will for this reason not show the same response than localities that receive high rainfall (Elsenburg).

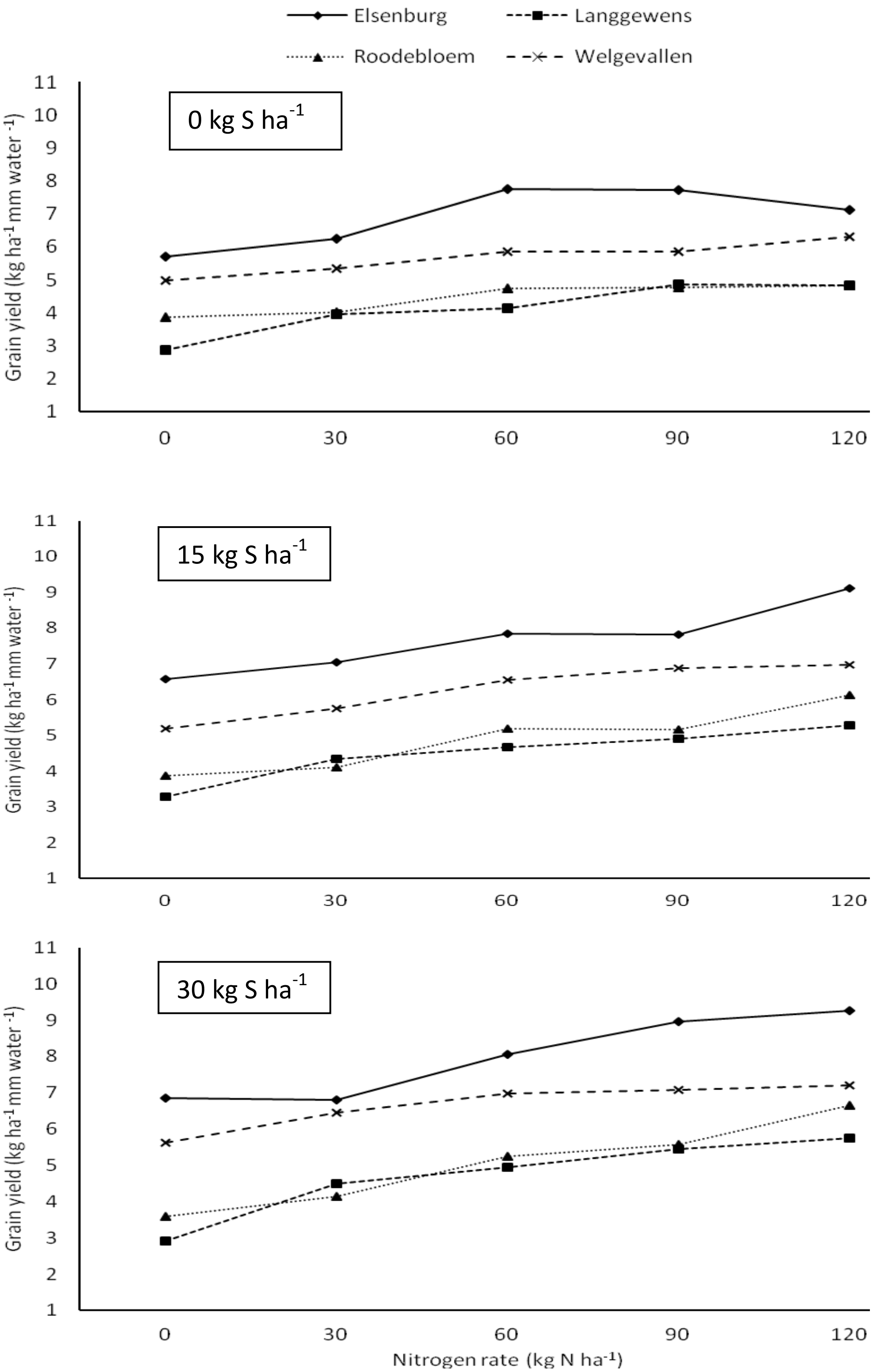

Figure 6.10 Canola yields per mm of water received in 2010 at Elsenburg, Langgewens, Roodebloem and Welgevallen localities at different sulphur (0, 15 and 30 kg ha^{-1}) and nitrogen (0, 30, 60, 90 and 120 kg ha^{-1}) fertilisation rates. (5%LSD= 0.48)

During 2011, Roodebloem (12.18 kg grain mm water^{-1}) had a higher WUE than Altona (9.65 kg grain mm water^{-1}), Elsenburg (4.63 kg grain mm water^{-1}) and Langgewens (4.08 kg grain mm water^{-1}) (Fig. 6.8). This variation in WUE observed was likely due to differences in distribution and intensity of rainfall received during the canola growing season (Fig. 3.1 in Chapter 3) which significantly influenced the grain yield on various localities. Rainfall distribution within the growing season become of major importance, especially considering that the canola crop response to water stress is dependent on growth stage (Ahmadi & Bahrani, 2009). Gan *et al.,* (2004) reported that canola stressed during earlier growth stages exhibit recovery, whereas if stressed during pod development, most of the yield components becomes reduced.

The application of N during 2011 significantly increased the yield of canola per millimeter of rainfall received. In this season, the highest WUE of 8.5 kg per mm of water was observed when 120 kg N ha^{-1} was applied (Fig. 6.9). This increase in WUE resulted from the increase in grain yields with increasing N application. Nitrogen fertilisation resulted in increased absorption and translocation of assimilates and stimulating growth (Wright *et al.,* 1988; Koenig, 2012), hence increasing yield components (Ahmadi & Bahrani, 2009).

Fertilising with S also increased the grain yields observed per millimeter of rainfall received in 2011 with high S application rates, resulting in a WUE of close to 9 kg per mm of water (Fig. 6.9) compared to 6.5 kg per mm of water when no S was applied. Besides N fertilisation, addition of S can have profound effects on canola, increasing the grain yields (Breennon & Bolland, 2008; Abdallah *et al.,* 2010). As with N, S absorption and translocation increased under soil moist conditions increasing the WUE of the canola plant when there is adequate soil S level.

6.4 CONCLUSIONS

Agronomic efficiencies of N applications are improved if 15 kg S ha^{-1} are applied complimented with high rainfall, but not with applications of 30 kg S ha^{-1}. Agronomic efficiencies at high application rates of N are however still low compared to winter cereals and methods to improve it should be investigated. Agronomic S use efficiency is higher when there is adequate soil moisture and can even be improved by high N fertilisation (120 kg N ha^{-1}). More grain yield increase per every millimeter of rainfall is mostly observed with both N and S fertilisation.

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CHAPTER 7

The effect of nitrogen and sulphur on the growth and development of canola (*Brassica napus* L.) grown in a controlled environment

Abstract

Canola is becoming a major source of vegetable oil with increasing demand in South Africa, yet low yields are presently experienced in production areas of the Western Cape Province. Crop response to fertiliser applications in field trials under rainfed conditions are often poor, because of a large number of growth factors that may limit growth and yield. To determine how canola responds to nitrogen (N) with no (low) and high sulphur (S) rates under ideal conditions in a controlled environment, a 5 x 2 factorial experiment, with N (0, 40, 80, 120 and 160 kg ha^{-1}) and S (0 and 40 kg ha^{-1}) fertilisation rates, was conducted. Plants were irrigated with a nutrient solution which contained all nutrients, but with very low N and S contents. Nitrogen application significantly increased leaf area, hence dry mass accumulation and ultimately flowering and pod formation, but high N and S application levels during early growth stages may have a negative effect on growth. Significant interaction between N and S were shown, however the positive effects of S were more pronounced in the reproductive phases. In this experiment, conducted under controlled temperature and watering conditions, but short winter daylight lengths, yield components of canola as measured by the number of flowers and pods at 91 DAP tend to reach a peak at application rates of 120 kg N ha^{-1} and 40 kg S ha^{-1}. This however may be due to a delay in flowering at high N and S rates and did not necessarily reflect the grain yield potential.

Keywords: Canola, controlled growth conditions, nitrogen, sulphur, vegetative and reproductive growth.

7.1 INTRODUCTION

The challenge of increasing crop yield per hectare to satisfy the needs of an ever increasing human population cannot be ignored. The supply of oil and protein is becoming scarce especially in developing countries (Ahmad *et al.,* 2006) and South Africa currently relies on imports for its needs for domestic plant oil and protein production.

Canola is becoming a major source of vegetable oil in the world (Reyes, 2007), but is still a relatively new crop in South Africa and considerable research is still needed for best production practices to optimize yield. Amongst many agronomic factors responsible for low yields, imbalanced and injudicious use of fertilisers also limits crop production (Sattar *et al.*, 2011). Several Western Cape farmers reviewed in the Farmers Weekly (Glennes, 2007) reported yields barely reaching 1.5 tonnes ha^{-1}. These low yields were mainly caused by poor soil management (Hardy *et al.*, 2004). Soil fertility management (especially N and S application) may for this reason be one of the best options to increase canola yields in the Western Cape production area.

Generally, fertilisation with N has previously been shown effectiveness in increasing yields of canola (Allen & Morgan, 1972; Yau & Thurling, 1987; Ahmad *et al.,* 2006; Gan *et al.*, 2008; El-Nakhlawy & Bakhashwain, 2009). Franzen (1997) shown that sulphur is one of the most important soil factors to be considered when growing canola and S deficiency will reduce yield potential (Pouzet, 1995; Fismes *et al.*, 2000). Adequate supply of N encourages leaf development, assisting in retaining leaves in active photosynthesis, hence facilitating development of flowers and pods (Weiss, 1983). Brennan & Bolland (2008) has shown that grain yield responses to S only occurred when N was applied. Results of canola field experiments conducted from 2009 to 2011 (Chapter 5) showed that yields tend to reach a plateau with 15 kg S per ha^{-1}, but did not reach a plateau with nitrogen rates as high as 120 kg ha^{-1}. Crop response in field trials with rainfed conditions, however may often be less accurate, because a large number of growth factors cannot be controlled.

The aim of this study was to determine the response to high nitrogen application rates with no (low) and high S rates under ideal conditions in a temperature controlled glasshouse.

7.2 MATERIALS and METHODS

Pot experiments were conducted at the Welgevallen Experimental Station of Stellenbosch University, during the 2011 winter season (21 May-20 August) in a temperature controlled glasshouse with a 20 ^{o}C day and 15 ^{o}C night temperature. The coarse sandy soil used for the experiment was chemically analyzed and showed low contents for almost all nutrients, as compared to the amounts required for optimum growth and yield of canola (Table 7.1).

Table 7.1 Chemical analysis of the sandy soil at planting and critical nutrient levels for canola

	pH	**Resistance**	**Ca**	**Mg**	**T-value**	**Na**	**K**	**P**	**Cu**	**Zn**	**Mn**	**B**	**S**	**Ca**	**N**
Unit	KCl	ohm	cmol kg^{-1}			mg kg^{-1}								%	
Soil level	6.7	3520	0.97	0.15	1.21	8.0	17	30	0.12	0.23	3.60	0.01	5.8	0.04	0
Critical levels[a]	<5[2]	-	<1.0[1]	<0.4[1]	-	>250[2]	<80[2]	<36[2]	<0.3[1]	<0.5[1]	<5.0[1]	<0.2[1]	<6[1]	-	-

[a] shows the critical soil nutrient levels at which the nutrient became deficient[1] or where the growth of canola plants will be negatively affected[2] (Canola production manual, undated; Peverill, 1999)

Plastic pots of 17.5 cm length, 15 cm width and 35 cm height (Surface area of 0.02625 m^2) were filled with 6 000 g of air dried soil. Mid-season maturing canola cultivar Hyola 61 was planted on 20 May 2011. Five application rates of N (0, 40, 80, 120 and 160 kg ha^{-1}) and 2 rates of S (0 and 40 kg ha^{-1}) were applied to the pots to have a 5 x 2 factorial design. Plants were fertilized with a balanced nutrient solution which contained all nutrients but with low N and S, by means of the irrigation system (Table 7.2). In the fertigation system, micronutrients were added as Microplex mix to give Fe (1.68 ppm), Mn (0.4 ppm), Zn (0.2 ppm), Cu (0.03 ppm), B (0.5 ppm) and Mo (0.05 ppm). The concentrations of the other nutrients in the solution were K (205 ppm), Ca (138 ppm), Mg (37 ppm), N (28 ppm), P (71 ppm), S (16 ppm) and Cl (107 ppm). In order to apply magnesium (magnesium sulphate and magnesium nitrate), nitrogen and sulphur were inevitable also applied, but the levels of N and S were about 20 % of that in a standard solution (Steiner, 1984) and it was assumed that such levels should result in deficiencies.

Table 7.2 Nutrient solution with low N and S applied by fertigation to canola in pot trial

Nutrient solution with minimum N and S	**EC=1.5mS cm^{-1}**
Macronutrient in 900l	
Ca chelaat	446ml
KH_2PO_4	313g
KCl	224g
$MgSO_4.7H_2O$	123g
$Mg(NO_3)_2.6H_2O$	256g
Micronutrients in 900l	
Microplex	18g

Nitrogen was applied as Limestone Ammonium Nitrate (LAN) with 28 % N while S was applied as gypsum ($CaSO_4.2H_2O$) with 16 % S. Although Ca was also applied it is not likely that it would have an effect due to sufficient Ca levels in the nutrient solution. The amounts of gypsum and LAN were calculated by using the pot surface area to supply the required levels of S and N respectively. Both nutrients were added in splits of a quarter of the prescribed treatment at planting, 28 DAP (Days after planting), 49 DAP and 70 DAP hence for N; 0.09, 0.19, 0.28 and 0.38 g of LAN were applied and for S; 0.16 g of gypsum at each application time. Treatment combinations were allotted at random to pots in each replication. Water was added to wet the soil to field capacity a day before planting. Up to crop emergence, the pots were kept moist using a light overhead irrigation, thereafter water was applied through drippers. Irrigation frequency was determined by solar radiation and the amount per irrigation event was adjusted for different growth stages, to make sure that no water stress or leaching of nutrients occurred. Ten seeds were sown in each pot. After emergence, seedlings were hand thinned with the first thinning to 4 plants per pot done at 14 DAP, and the second thinning to maintain a uniform stand of 2 plants per pot, done at 21 DAP. The experiment was laid out in completely randomized design (CRD) having four replications. The experiment was replicated four times for destructive samplings at 28 DAP, 49 DAP, 70 DAP and at flowering (about 91 DAP). Because of the splitted application of N and S, plants sampled at 28, 49 and 70 DAP have at that stage not received their fully allotted rates of N and S (Table 7.3)

Table 7.3 Accumulative quantities of N and S received at different sampling times

	***N rate* (kg ha^{-1})**					***S rate* (kg ha^{-1})**	
Sampling time	0	40	80	120	160	0	40
28 DAP	0	10	20	30	40	0	10
49 DAP	0	20	40	60	80	0	20
70 DAP	0	30	60	90	120	0	30
91 DAP	0	40	80	120	160	0	40

Experiment was terminated at 91 DAP because plants became too tall and tended to lodge. This unfortunately prevented determination of the grain yield, thus number of flowers and pods were used as an indication of the yield potential.

7.2.1 Data collection

The replicated trials were harvested through destructive sampling for both leaf area and dry mass at 28 DAP, 49 DAP, 70 DAP and at flowering (91 DAP). At each sampling date 4 pots per treatment were sampled. Leaf area per plant was measured using a leaf area meter (Licor, Model 3100, LICOR Ltd., Lincoln, NE). Thereafter total above ground plant samples were oven dried to determine dry mass. During the final sampling at flowering (91 DAP), the total number of flowers and pods per plant were counted.

7.2.2 Statistical Analysis

Data recorded was analyzed statistically, using analysis of variance (ANOVA) with nitrogen and sulphur rates considered as fixed factors (Statistica 11). Main and interaction effects were compared using least significant difference (LSD) test at 5 % level of probability. Any treatment means found to be significantly different were separated using Fischer's protected $LSD_{0.05}$. The treatment means from the Statistica analyses were then subjected to best fit regression analyses with the Microsoft Office Excel package, using polynomial equations.

7.3 RESULTS and DISCUSSION

Nitrogen application rates significantly affected leaf area per plant at 28 DAP, 49 DAP and 70 DAP, dry mass at all sampling dates as well as the number of flowers and pods at 91 DAP (Table 7.4). Leaf area per plant and dry mass per plant at 49 DAP, as well as, the number of pods (91DAP) were affected by sulphur, while leaf area and dry mass per plant at 49 DAP as well as number of pods at 91 DAP showed an response due to the interaction between sulphur and nitrogen (Table 7.4).

Table 7.4 Summary of significant effects (F-values) from the Analysis of variance done on plant leaf area (LA), Dry Mass (DM), and number of flowers and pods

	Source of variation		
Yield response	**Nitrogen rate**	**Sulphur rate**	**Nitrogen x Sulphur**
Plant L A (28DAP)	***	ns	ns
Plant L A (49DAP)	***	**	**
Plant L A (70DAP)	***	ns	ns
Plant L A (91DAP)	ns	ns	ns
Plant DM (28DAP)	***	ns	ns
Plant DM (49DAP)	***	**	*
Plant DM (70DAP)	***	ns	ns
Plant DM (91DAP)	***	ns	ns
Number of flowers (91DAP)	***	ns	ns
Number of pods (91DAP)	***	***	***

*, **, *** denote significance at P≤0.05, P≤0.01, P≤0.001 respectively and ns denotes non significance at P≤0.05.

Although with the exception of plant leaf area at 49 DAP, plant dry mass at 49 DAP and number of pods at 91 DAP, no significant interactions between N and S were shown, treatment means were subjected to best fit regression analyses, using polynomial equations (Fig. 7.1-7.3), because that would help to identify any trends in response to N and S treatments.

Vegetative growth as measured by leaf area and dry mass response to N and S fertilisation at the four sampling times (28, 49, 70 and 91 DAP) is shown in Fig. 7.1 and 7.2 respectively, while reproductive growth as measured by number of flowers and pods at 91 DAP, as affected by N and S is presented in Figure 7.3. Regression coefficients for the polynomial equations range between 0.68 and 0.99.

7.3.1 Sampling at 28 DAP

Previously to this sampling date plants had received either 0 (0 kg N ha^{-1} rate), 10 (40 kg N ha^{-1} rate), 20 (80 kg N ha^{-1} rate), 30 (120 kg N ha^{-1} rate) or 40 kg N ha^{-1} (160 kg N ha^{-1} rate) and either 0 or 10 kg S ha^{-1}, which were applied at planting. Both leaf area per plant and dry mass per plant were significantly affected by N application rate (Table 7.4). Although S application had no significant effect and no significant interaction between N and S treatments were found at this stage, the results (Fig. 7.1a and 7.2a) showed that both leaf area and dry mass tended to reach a plateau at 20-30 kg N ha^{-1} (80-120 kg N ha^{-1} rate) at both sulphur levels. At this early vegetative growth stage, both leaf area and dry mass per plant tended to decrease when 40 kg N ha^{-1} (160 kg N ha^{-1} rate) was applied at planting. These results showed that canola do not need much N and S during the early growth stages and higher application rates at planting may even suppress vegetative growth. Moody (2007) also showed that N application in canola can be delayed at least until four leaf stage, without any compromise of dry matter yield. As this experiment was done under irrigation, this suppression may even be more pronounced under rainfed conditions.

7.3.2 Sampling at 49 DAP

Sampling at 49 DAP showed a significant interaction between nitrogen and sulphur rates with regard to leaf area and dry mass per plant (Table 7.4). Increased N application rates resulted in an almost linear increase in both leaf area (Fig. 7.1b) and dry mass per plant (Fig. 7.2b) at high S applications (40 kg S ha^{-1} rate) with no plateau even at 80 kg N ha^{-1} (160 kg N ha^{-1} rate). With low S application rates (0 kg S ha^{-1}), both leaf area per plant (Fig. 7.1b) and dry mass per plant (Fig. 7.2b) reached a plateau at 40 kg N ha^{-1} (80 kg N ha^{-1} rate). The results indicated that canola plants need high amounts of both N and S during the late vegetative (rosette) stage, most probably because of very rapidly expanding leaf area and chlorophyll synthesis, which requires both S and N (McGrath & Zhao, 1996).

7.3.3 Sampling at 70 DAP

At this planting stage, plants had previously received 0, 30, 60, 90 and 120 kg N ha^{-1} respectively and either 0 or 30 kg S ha^{-1} (Table 7.3). Leaf area and dry mass per plant were significantly affected by N but not by S and showed no interaction between N and S (Table 7.4). Vegetative growth as measured by leaf area (Fig. 7.1c) and dry mass (Fig. 7.2c) increased with increasing N rates with no plateau even at 120 kg N ha^{-1} (160 kg N ha^{-1} level), illustrating the high nitrogen requirements during peak vegetative growth stages. Addition of 30 kg S ha^{-1} (40 kg S ha^{-1} level) tends to improve leaf area response to N at higher (90 and 120 kg ha^{-1}) rates (Fig. 7.1c) but this effect was not significant even though the plants were growing very rapidly during this bolting stage. McGrath & Zhao (1996) reported that the effects of S are more pronounced during reproductive stages of canola. Poor responses to S applications at this sampling stage in spite of deficient S contents in the soil used in this experiment (5.8 mg kg^{-1}), may be ascribed to the unavoidable addition of S when Magnesium sulphate was added. Although the amount of S added this way was very low and about 20 % of that in a standard solution (Steiner, 1984), continuous application with the irrigation seemed to be enough to prevent significant S responses.

7.3.4 Sampling at 91 DAP

At the last sampling time, plants had received all allotted N (0, 40, 80, 120 and 160 kg N ha^{-1} rate) and S (0 and 40 kg S ha^{-1}). Plant dry mass was significantly affected by N application rate, but not by S (Table 7.4). Significant increases in dry mass due to increasing N application rates were therefore similar for both s application rates (Fig. 7.2d). Leaf area response to N at 91 DAP (Fig. 7.1d) was much less than at earlier stages, most probably because leaf growth is almost complete at the stage when plants start to flower. When the crop is flowering and podding, assimilates are remobilized from the leaves to the reproductive tissues (Malagoli *et al.*, 2005). Leaves are therefore senescing (leaf area reach a plateau) (Fig. 7.1d), whilst dry mass per plant still show a significant response to N applications (Fig. 8.2d).

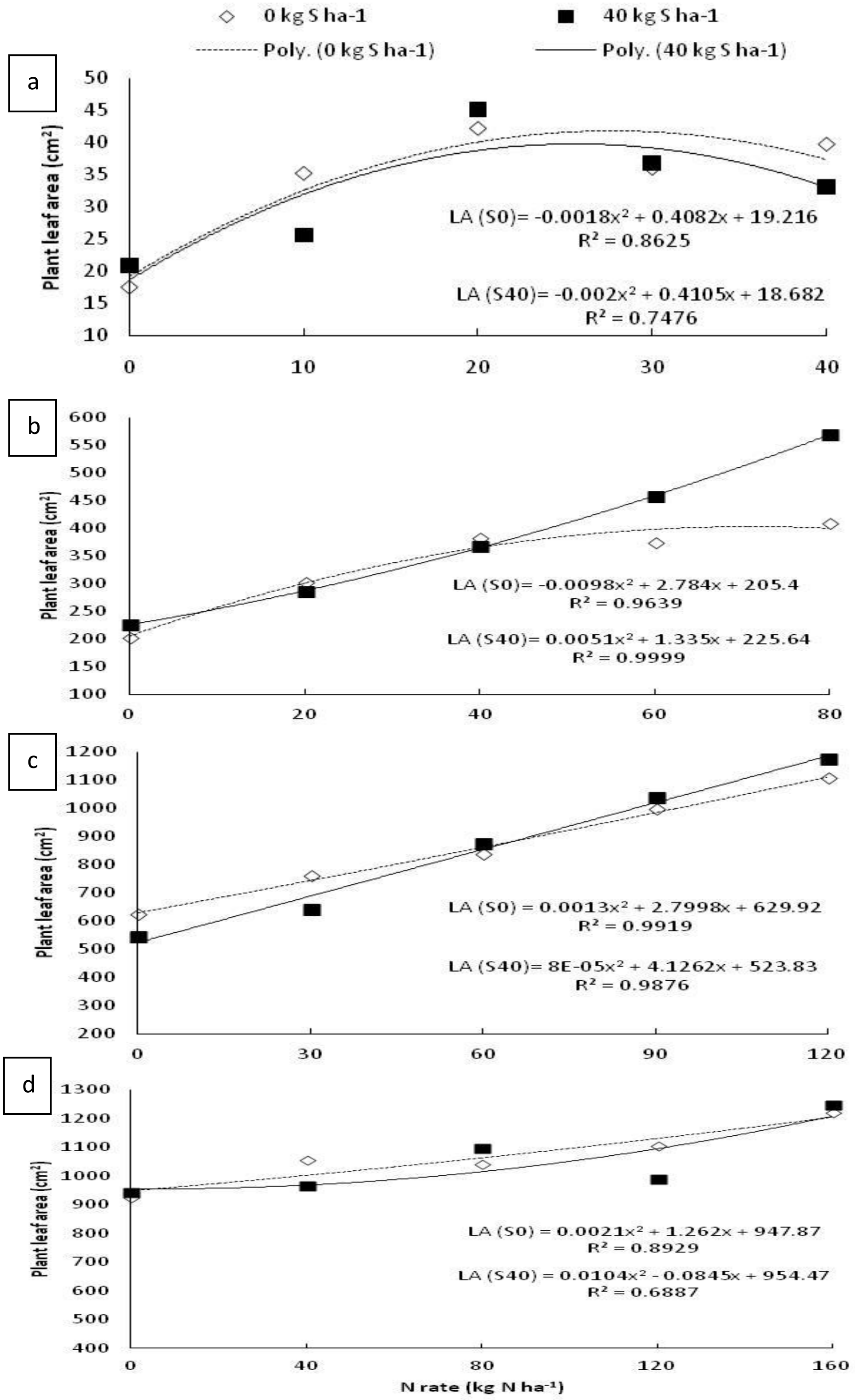

Figure 7.1 Effect of N and S fertilisation rates on leaf area of canola plants at (a) 28, (b) 49, (c) 70 and (d) 91 DAP

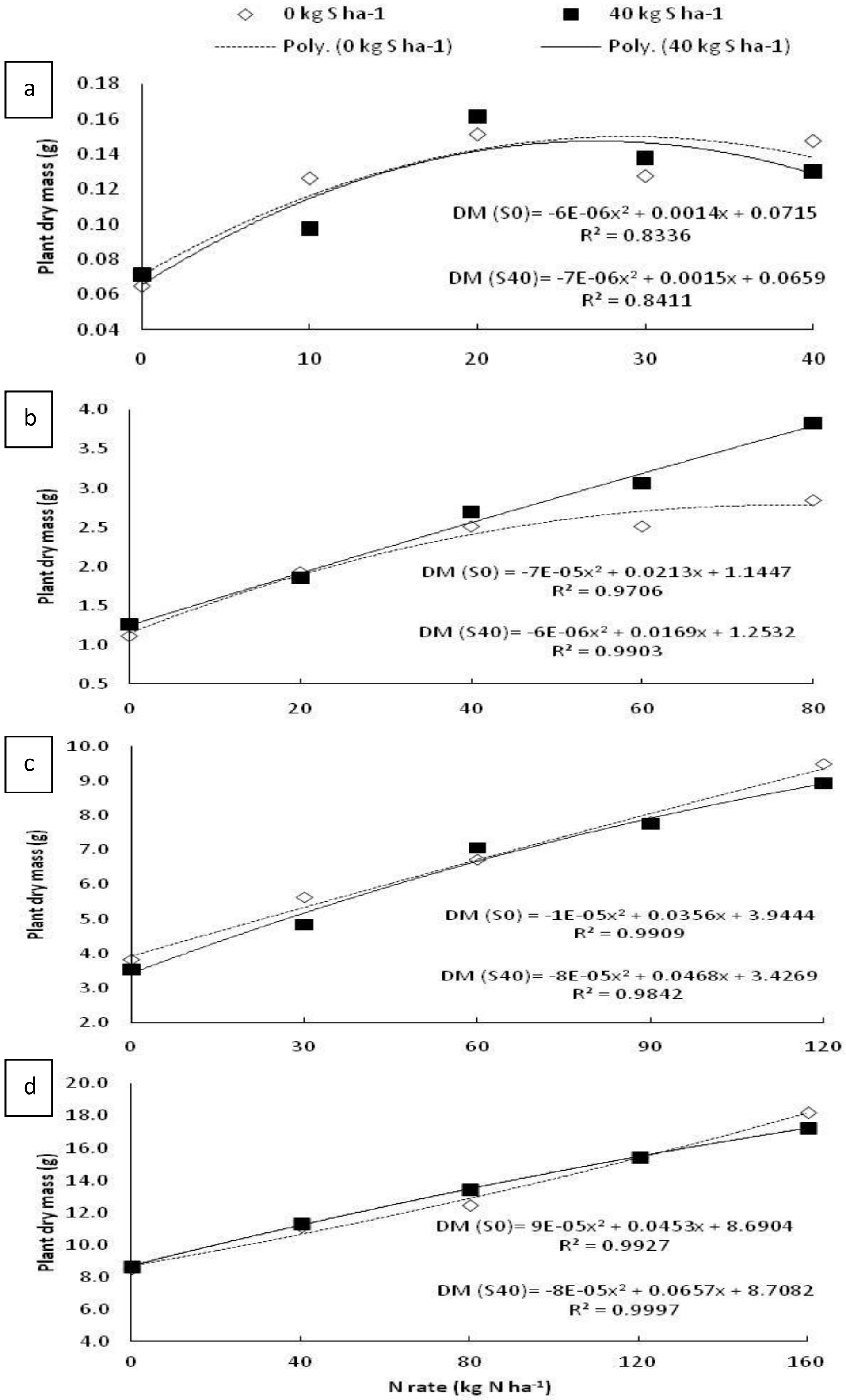

Figure 7.2 Effect of N and S fertilisation rates on dry mass (DM) of canola plants at (a) 28, (b) 49, (c) 70 and (d) 91 DAP

Data regarding flowering and podding as affected by N and S is presented in Fig 7.3. Flowering was significantly affected by N but not by S (Table 7.4). Total number of flowers per plant increased with increasing N application rate to reach a peak at 120 N kg ha^{-1} where-after it tended to decline with high S rates (40 kg S ha^{-1}), but not so with low S rates (0 kg S ha^{-1})(Fig. 7.3a). Although this interactional effect was not significant, such a response may be due to a delay in the onset of flowering at higher N application rates as shown by Brandt *et al.* (2007).

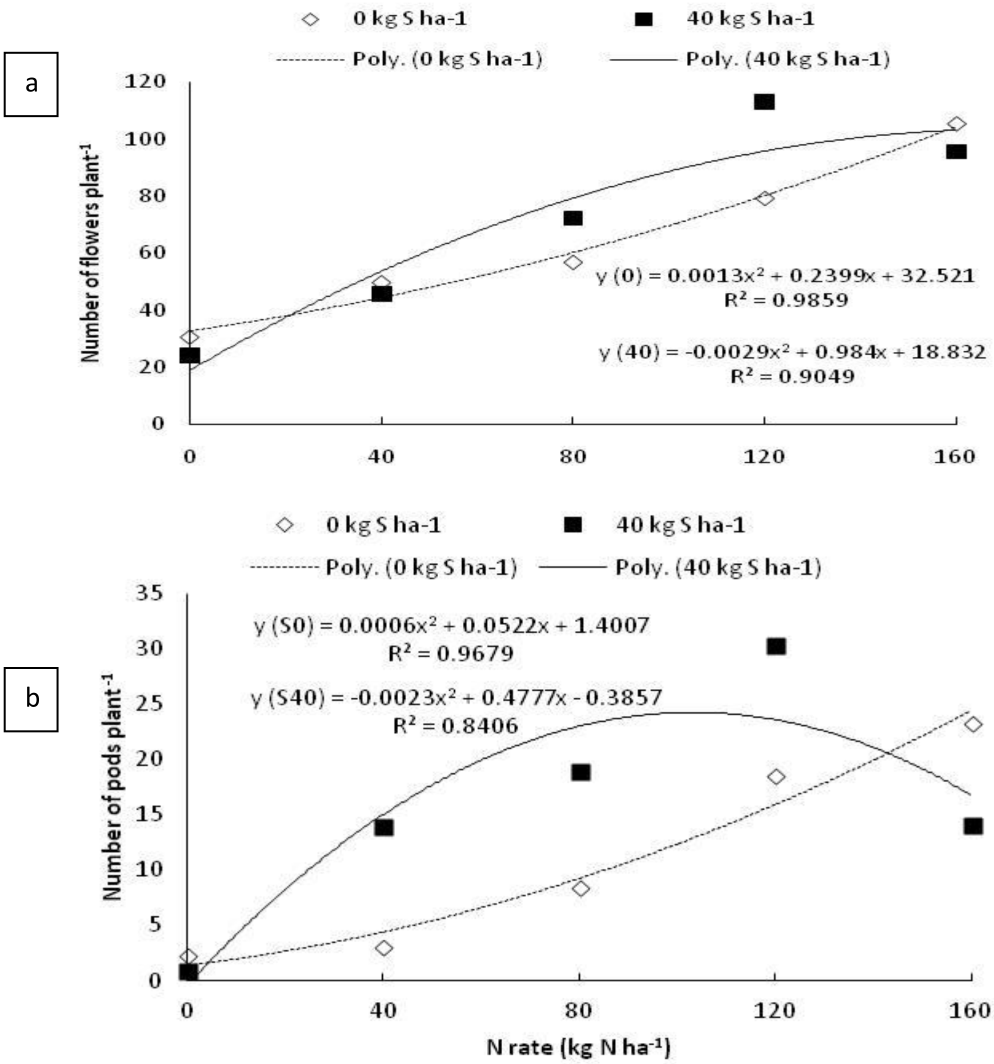

Figure 7.3 Effect of N and S fertilisation rates on total number of (a) flowers and (b) pods per plant at 91 DAP

Number of pods per plant was increased by N applications (Table 7.4) but the response to N was different for different S application rates (Fig. 7.3b). Number of pods per plants showed a continuous increase with an increase in N application rate at the 0 kg S ha^{-1} rate, but as with flowering per plant, declined at high N (160 kg ha^{-1}) rates at the 40 kg S ha^{-1} rate (Fig. 7.3b). This tendency was most probably also due to a delay in flowering during growth conditions that favours luxurious vegetative growth and is not necessarily an indication of the final grain yield (http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex149). From the results, it however became clear that S had a larger effect on reproductive growth than on vegetative (leaf growth). These results supported earlier studies, which showed that the increased availability of N enhance vegetative growth of canola plants (Cheema *et al.*, 2001, Ahmad *et al.*, 2006; Yasari & Patwardhan, 2006). This N which accumulates in early growth stages becomes useful in reproductive stages (Hocking & Strapper, 2001; Malagoli *et al.*, 2005), with S essential in protein synthesis (Fismes *et al.*, 2000; Jan *et al.*, 2002). In addition, naturally occurring compounds called glucosinolates are also synthesized from S (McGrath & Zhao, 1996) and they mostly accumulate in canola reproductive tissues (http://www.canolacouncil.org/chapter9.aspx).

7.4 CONCLUSIONS

In this study, canola grown in a temperature controlled glasshouse and irrigated with a balanced nutrient solution which contained very low levels of N and S, responded positively to N and S applications splitted between planting, 28, 49 and 70 days after planting (DAP). High rates of N and S applied at planting however tend to have a negative effect on leaf area and dry matter production. High application rates of N and S to canola grown in dry areas, under rainfed conditions, should therefore be avoided. At later growth stages vegetative growth as measured by leaf area and dry mass did not reach a plateau even at N rates of 120-160 kg of N ha^{-1}, suggesting that such rates of N may be needed under high rainfall conditions or where canola is grown under irrigation. In this study vegetative growth of canola plants did not respond much to application of S in spite of low S contents in the soil. But this tendency may be because of the continuous unavoidable application of low levels of S during irrigation with the nutrient solution. Reproductive growth and especially number of pods per plant responded positively to S applications as high as 40 kg ha^{-1} splitted between planting, 28, 49 and 70 DAP. Such high S application levels in combination with N applications of 120-160 kg ha^{-1} seemed to delay flowering under favourable temperature and watering conditions, which again may have a negative effect on grain yield of canola in low rainfall areas. More research dealing with splitted applications of N and S on canola may however be needed.

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CHAPTER 8

The effect of nitrogen and sulphur on growth and development of early, mid and late maturing canola cultivars (*Brassica napus* L.) grown in a controlled environment

Abstract

Canola is amongst crops that respond well to nitrogen (N) and sulphur (S) application; however utilization efficiency of the nutrients is low. Variation in nutrient utilization is possible amongst varieties with varying growth habits within the same species. This study was done to determine growth and yield responses of different canola cultivars to N and S fertilisation. Four treatment combinations of low (0 kg S and/or N ha^{-1}) and high (30 kg S ha^{-1} and/or 150 kg N ha^{-1}), alone and in combination were applied on early (Spectrum), mid (Rocket) and late (45Y77) maturing canola cultivars grown in the Western Cape province of South Africa. Increased photosynthetic capacity due to higher leaf area indexes (LAI) as a result of N applications were shown as early as the rosette stages of growth, regardless of cultivar. Spectrum and Rocket yielded significantly higher than cultivar 45Y77 in spite of a better vegetative response of the late maturing cultivar. Short season cultivar (Spectrum) has higher agronomic nitrogen use efficiency. No significant differences in the response of cultivars to S applications were found under controlled conditions.

Keywords: Agronomic nitrogen use efficiency, canola cultivar, yield

8.1 INTRODUCTION

Crop management, Nitrogen (N) and Sulphur (S) fertilisation, and choice of nutrient efficient genotypes may affect the yield and oil content of canola seed. Selecting genotypes that have a high N use efficiency (NUE) to produce a high biomass and grain yield with the minimal amount of N fertiliser (Delmer, 2005) may therefore be an important strategy to improve canola yield and quality.

Oilseed crops such as canola often have NUE values of less than 10 % (Sylvester-Bradley & Kindred, 2009), which are significantly lower compared to winter cereals (Hirel *et al.*, 2007). For this reason higher rates of N fertiliser are usually applied to canola crops, compared to cereals (Yau & Thurling, 1987; Fismes *et al.*, 2000; Gan *et al.*, 2008). An adequate supply of N fertiliser enables rapid leaf growth of the canola crop (Kumar *et. al*, 1997; Gan *et al.*, 2008) which may, together with increased branching and flowering (Yau & Thurling, 1987), hence podding and number of seeds per pod (Al-Barrak, 2006), positively contribute to seed filling (El-Nakhlawy & Bakhashwain, 2009) and grain yield. Sulphur also plays a very crucial role in canola affecting growth and yield (McGrath & Zhao, 1996; Fismes *et al.*, 2000). Grain yield responses to applied S only occurred when N was applied and increased as more N was applied (Brennan & Bolland, 2008). McGrath & Zhao (1996) reported that low S supply suppresses the development of reproductive organs and may lead to silique abortion and decreasing seed yield and oil content. Sulphur forms an integral part of several amino acids hence also affecting protein content of the seed (Jan *et. al*, 2010). The optimum S supply and uptake however depends on N application rate (N:S ratio of 7:1 is suggested), as well as soil and climatic conditions (Malhi *et al.,* 2008).

Differences in optimum N and S requirements may also occur between different canola varieties/species (Tatjana & Zdenko, 2011), because of differences in the nitrogen utilization efficiency in Australian canola germplasm (Balint *et al.,* 2008). When comparing the nitrogen use efficiency and nitrogen uptake of canola and other oilseed species under diverse environments, similar response patterns to N fertiliser rates were shown, but the magnitude of response varied among the species (Gan *et al.*, 2008). Such differences in response may be the result of variations in phenology and growth habit (Maestro, 1995; Sana *et al.*, 2003; Balint *et al.*, 2008).

The aim of this study was to determine the growth and yield responses of different canola cultivars to N and S fertilisation in a temperature controlled glasshouse.

8.2 MATERIALS and METHODS

8.2.1 Experimental site and soil chemical characteristics

A pot experiment was conducted at Welgevallen Experimental Farm of Stellenbosch University during the winter season of 2010 in a temperature controlled glasshouse with a 20 °C day and 15 °C night temperature. The soil used for the experiment was sandy, low in organic N (0.06 %) and available S (1.37 mg kg^{-1}), which made it ideal for the experiment (Table 8.1).

Table 8.1 Chemical analysis of the sandy soil at planting and critical nutrient levels for canola

	pH	**Resistance**	**Ca**	**Mg**	**T-value**	**Na**	**K**	**P**	**Cu**	**Zn**	**Mn**	**B**	**S**	**Ca**	**N**
Unit	KCl	Ohm	cmol kg^{-1}			mg kg^{-1}								%	
Soil level	4.47	2960	2.00	0.39	3.67	14.3	77	153	1.43	3.31	38.9	0.06	1.37	0.70	0.06
Critical levels[a]	<5 [2]	-	<1.0 [1]	<0.4 [1]	-	>250 [2]	<80 [2]	<36 [2]	<0.3 [1]	<0.5 [1]	<5.0 [1]	<0.2 [1]	<6 [1]	-	-

[a] shows the critical soil nutrient levels at which the nutrient became deficient[1] or where the growth of canola plants will be negatively affected[2] (Canola production manual, undated; Peverill, 1999)

8.2.2 Canola establishment

Early, mid and late maturing canola cultivars (Spectrum, Rocket, and 45Y77 respectively) were sown on 28 April 2010. Monopotassium Phosphate (MKP) fertiliser was applied at the rate of 45 kg ha^{-1} prior to sowing to supply potassium and phosphorus. Plastic pots (17.5 cm X 15 cm X 35 cm and a surface area of 0.02625 m^{2}), filled with 6 000 g of air dried soil was used.

8.2.3 Treatments and experimental design

The following four treatment combinations were applied to the three canola cultivars:

- No S and no N (0, 0)
- No S and High N (0 S, 150 kg N ha^{-1})
- High S and No N (30 kg S ha^{-1}, 0 N)
- High S and High N (30 kg S ha^{-1}, 150 kg N ha^{-1})

The treatment which received no N and no S were regarded as a control. Nitrogen was applied in the form of Limestone Ammonium Nitrate (LAN) with 28 % N while S was applied in the form of gypsum ($CaSO_4.2H_2O$). Although Ca was also applied, it is not likely that it would have an effect due to sufficient Ca levels in the soil (2 cmol kg^{-1}). The amounts of gypsum and LAN were calculated by using the pot surface area to supply the required levels of S and N respectively. Both nutrients were added in splits of a third of the prescribed treatment at planting, 45 DAP (days after planting) and 70 DAP. Hence for N, 0.45 g of LAN were applied and for S, 0.16 g of gypsum at each application time. Treatment combinations were allotted at random to pots in each replication. Water was added to wet the soil to field capacity a day before planting and pots were kept moist by covering it with shade netting until emergence. After full emergence, water was applied through drippers and the application frequency were determined by solar radiation, while the amount per irrigation was increased with increase in plant size (hence water usage) to keep the soil moist at all time without excessive drainage. Ten seeds were sown in each pot. Seedlings were hand thinned with the first thinning to 4 plants per pot done 14 DAP. The second thinning, to maintain a uniform stand, of 2 plants per pot was done at 21 DAP. The experiment was laid out in a completely randomized design (CRD) with four replications. The experiment was further replicated three times for destructive sampling at 45 and 70 DAP, with the final harvesting at crop maturity.

8.2.4 Data collection

The replicated trials were harvested through destructive sampling to determine both leaf area and dry mass per plant at 45 and 70 DAP. Leaf area was measured using a leaf area meter (Licor, Model 3100, LICOR Ltd., Lincoln, NE). The LAI was calculated as the ratio of total leaf area to pot area (Watson, 1947).

At crop maturity, flower stems per plant, pods per plant, plant height, total above ground plant mass and grain yield were measured. To evaluate the N-use efficiency; agronomic efficiency was calculated from canola grain yields harvested per plant according to Smith *et al.* (1988) using the following equation:

$$\mathrm{NUE\,(g\,seed\ \ g\,N\,applied^{-1})} = \frac{\mathrm{Grain\,yield\,(g\,plant^{-1}) - Grain\,yield\,control\,(g\,plant^{-1})}}{\mathrm{N\,fertiliser\,applied\,(g\,plant^{-1})}}$$

8.2.5 Statistical Analysis

Data recorded was analyzed statistically, using analysis of variance (ANOVA) with cultivar, nitrogen and sulphur supply considered as fixed factors (Statistica 10) with the exception of agronomic efficiency where nitrogen was not considered as a factor. Main and interaction effects were compared using least significant difference (LSD) test at 5 % level of probability. Any treatment means found to be significantly different were separated using Fischer's protected $\mathrm{LSD}_{0.05}$. On figures, means with at least a common superscript letter were not significantly different at $P<0.05$.

8.3 RESULTS and DISCUSSION

8.3.1 Significance of F values

A summary of the significant effects (F values) of cultivar, nitrogen and sulphur application rates as well as their interaction effects on the leaf area index, dry mass, and yield parameters at different harvesting times are presented in Table 8.2. In contrast to cultivar and N fertiliser rate, S had no effect on any of the recorded parameters, cultivar X N interactions were only shown with regard to plant height and grain yield.

Table 8.2 Summary of significant effects (F-values) from the Analysis of variance done on Leaf area, Leaf Area Index, Dry mass, Grain yield and Agronomic efficiency

	Source of variation						
Variables	**Cultivar**	**N rate**	**S rate**	**N x Cultivar**	**S x Cultivar**	**N x S**	**N x S x Cultivar**
Leaf area (45DAP)	ns	***	ns	ns	ns	ns	ns
Leaf area (70DAP)	ns	***	ns	ns	ns	ns	ns
LAI (45DAP)	ns	***	ns	ns	ns	ns	ns
LAI (70 DAP)	ns	***	ns	ns	ns	ns	ns
Plant DM (45 DAP)	*	***	ns	ns	ns	ns	ns
Plant DM (70 DAP)	*	***	ns	ns	ns	ns	ns
Plant DM (130 DAP)	**	***	ns	ns	ns	ns	ns
Plant height	ns	***	ns	*	ns	ns	ns
Number of flower stems	ns	***	ns	ns	ns	ns	ns
Number of pods	**	***	ns	ns	ns	ns	ns
Grain yield	***	***	ns	*	ns	ns	ns
Agronomic efficiency	*	-	ns	-	ns	-	-

*, **, *** denote significance at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively and ns denotes non significance at $P \leq 0.05$.

8.3.2 Leaf Area

Nitrogen application had a significant effect on plant leaf area at 45 and 70 DAP (Table 8.2), with significant higher values where 150 kg N ha^{-1}, splitted between planting, 45 and 70 DAP, was applied (Table 8.3). Similar trends with high N application rates were shown with regard to leaf area indexes (Table 8.3) resulting in a higher capacity of radiation interception because of a leaf area index of 1.80 m^2 m^{-2} as early as the first sampling at 45 DAP which increased to 4.02 m^2 m^{-2} at 70 DAP. Treatments where no N was applied had a significantly lower LAI of 0.94 and 1.42 m^2 m^{-2} at 45 and 70 DAP respectively, regardless of the cultivar, because nitrogen deficiencies result in fewer and smaller leaves compared to plants receiving sufficient nitrogen (Medham *et al.*, 1981). Cheema *et al.* (2001)

also reported a significant increase in the LAI of canola due to high N application rates. Optimum light interception at a LAI of 3.11 for *Brassica juncea* has been reported by Kumar *et al.* (1997).

Table 8.3 Canola plant leaf area and Leaf Area Index at 45 and 70 DAP at 0 and 150 kg N ha^{-1} fertilisation rates

	***N rate* (kg N ha^{-1})**			
Variable	0	150	**Mean**	**P value**
Plant leaf area at 45 DAP (cm^2)	123.08^b	236.32^a	**179.70**	***
LAI at 45DAP (m^2 m^{-2})	0.94^b	1.80^a	**1.37**	***
Plant leaf area 70 DAP (cm^2)	186.45^b	527.32^a	**356.89**	***
LAI at 70 DAP (m^2 m^{-2})	1.42^b	4.02^a	**2.72**	***

8.3.3 Plant dry mass

There was significant difference (P<0.01) in plant dry mass among the different cultivars with the late maturing cultivar; 45Y77 having a significantly higher dry mass than the early maturing cultivar at all sampling times. At maturity the cultivar 45Y77 has a high dry mass of 13.69 g per plant, whilst dry mass of Rocket (9.10 g plant^{-1}) and Spectrum (10.06 g plant^{-1}) was not significantly different (Fig 8.1). Svečnjak & Rengel (2005) showed similar results with canola cultivars.

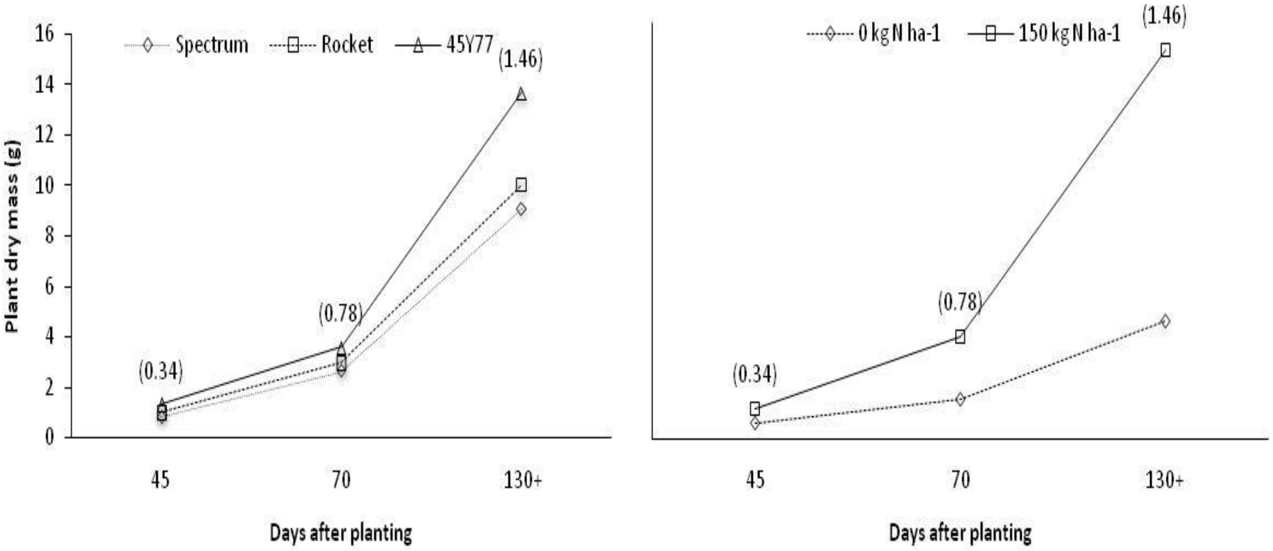

Figure 8.1 Dry mass of canola cultivars and plant dry mass response to nitrogen fertilisation rates at 45, and 70 days after planting and at maturity. 5% LSD = ( ).

Nitrogen applications increased the total dry mass of the canola plants regardless of cultivar, with values of 1.30 and 0.67 g per plant, shown for treatments, receiving 150 kg N ha^{-1} and no N respectively at 45 DAP (Fig. 8.1). This illustrates that canola respond as early as the rosette growth stage (45 DAP) to N nutrition. This response in nitrogen continued throughout the development of the canola plants till maturity, when final harvesting was done between 130 DAP (early maturing cultivar) and 177 DAP (late maturing cultivar) (Fig. 8.1). During the final harvest, treatments where no nitrogen was applied showed a significantly lower plant dry mass of 5.09 g per plant, compared to about thrice as much, where 150 kg N ha^{-1} was applied. Increased dry mass accumulation which correlates with higher LAI values when high N application rates were used, indicate that dry mass accumulation may be used as a physiological index to indicate the photosynthetic capacity of plants (Cheema *et al.*, 2001).

Increases in canola height due to N applications of 150 kg N ha^{-1} have been recorded, but the magnitude of increase depends on the cultivar (Fig. 8.2). In contrast to the late maturing cultivar 45Y77, N fertilisation had no significant influence on the height of the early maturing cultivar Spectrum. While the height of the mid maturing Rocket and late maturing 45Y77 cultivars increased from 84.50 cm plant^{-1} and 72.31 cm plant^{-1} to 132.44 cm plant^{-1} and 158.56 cm plant^{-1} respectively with the addition of 150 kg N ha^{-1} (Fig. 8.2). El-Nakhlawy & Bakhashwain (2009) attributed these significant differences between canola varieties with regard to plant height to differences in genetic background and the genetic x environmental interaction effects.

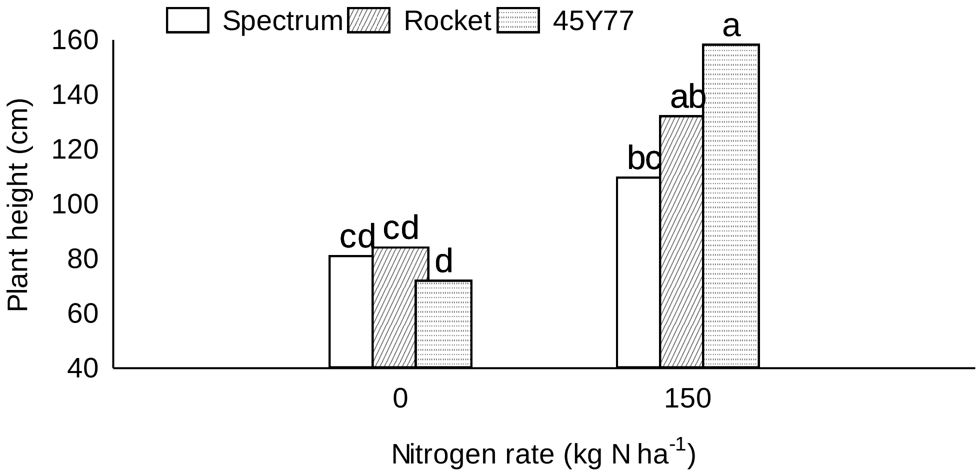

Figure 8.2 Effect of nitrogen application on plant height of canola cultivars grown in pots at maturity

8.3.4 Yield Components

On average, fertilisation with 150 kg N ha^{-1} increased the number of flower stems from approximately 2 to 4 and the number of pods from 17 to 46 per plant (Fig. 8.3). Allen & Morgan (1972) also recorded increases in number of flower stems per plant and number of pods per plant with addition of nitrogen fertiliser. However, due to delayed flowering (visual observation), the number of pods produced by the late maturing cultivar 45Y77 (approximately 19 pods per plant^{-1}) were significantly less compared to the early maturing Spectrum (approximately 34 pods per plant^{-1}) and medium maturing (approximately 42 pods per plant^{-1}) cultivars (Fig 8.3).

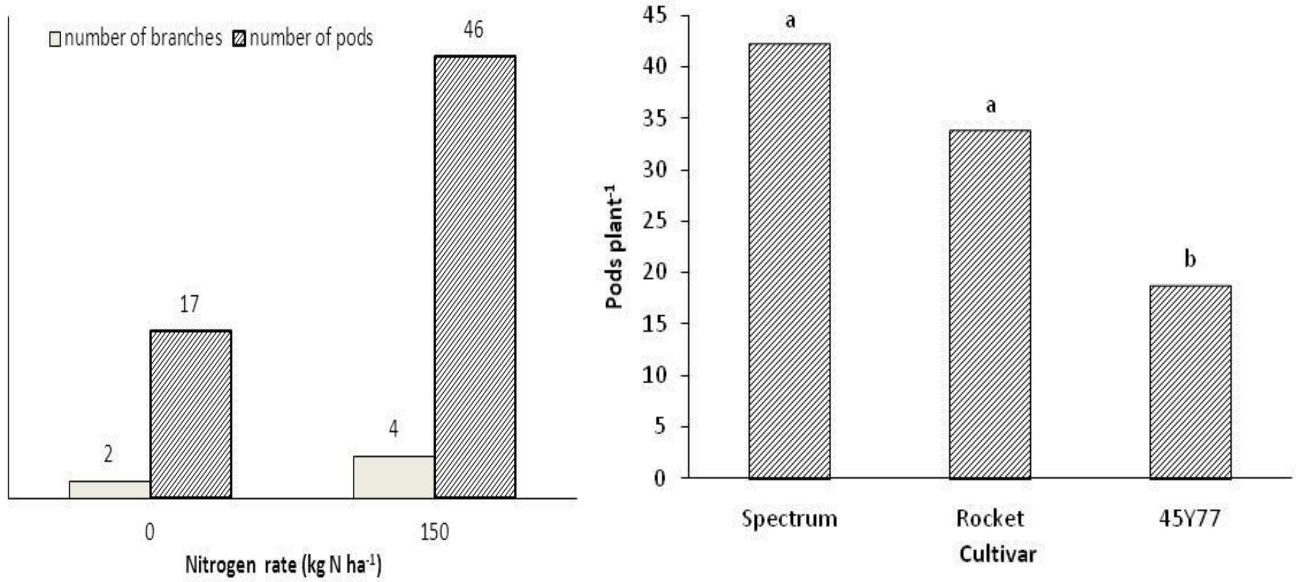

Figure 8.3 Number of flower stems and pods in response to nitrogen fertilisation rates, and differences in number of pods in cultivars

8.3.5 Grain yield

Grain yield was significantly affected by cultivar and N application, but responses differed between cultivars (Table 8.2). Grain yield of the early maturing cultivar, Spectrum, increased from 0.71 g to 2.93 g plant^{-1} and that of the mid maturing cultivar, Rocket, from 0.59 g to 2.04 g plant^{-1} when N fertiliser was increased from 0 to 150 kg N ha^{-1}. No significant response was shown with the later maturing cultivar 45Y77 (0.29 to 0.76 g plant^{-1}) (Fig. 8.4).

This difference in response of different cultivars is most probably due to differences in growth habit of the cultivars. Fertilisation with high nitrogen in late maturing cultivar 45Y77 increased the vegetative growth shown by an increased LAI and dry mass accumulation, but resulted in a delayed flowering, podding and eventually lower grain yields, which was in contrast to the expected higher yield (more photosynthetic days) with late maturing cultivars. Although this trial was done under controlled conditions, these results clearly illustrate the risk of high N applications to late maturing cultivars and especially if grown in areas with a short rainy season.

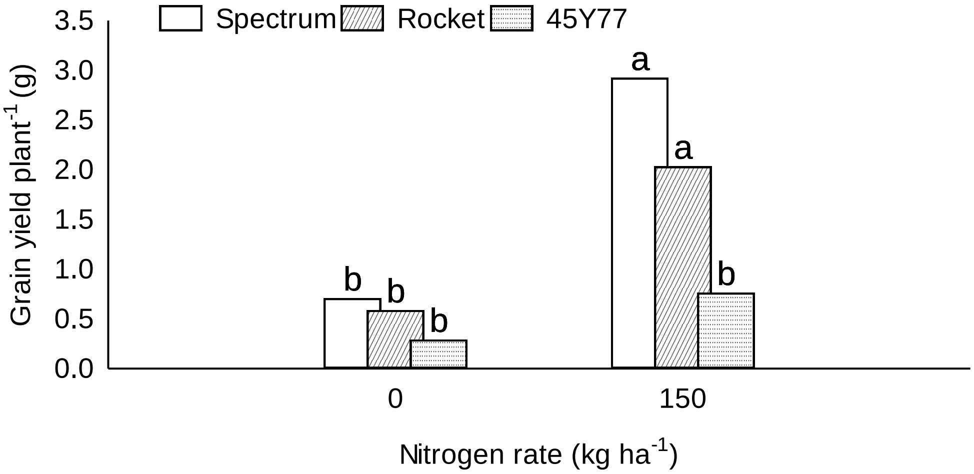

Figure 8.4 Effect of N application on grain yield plant^{-1} of canola cultivars grown in pots

8.3.6 Agronomic N use efficiency

Agronomic N use efficiency (NUE) differed between cultivars (Table 8.2) with Spectrum having a mean value of 11 g yield increase per g N applied, compared to less than 3 g yield increase per g N applied for the late maturing cultivar, 45Y77 (Fig. 8.5). Differences in N use efficiencies of canola genotypes were also found in work done by Balint, *et al.*, (2008), where a large genetic base was used. On average, agronomic N use efficiencies were low (<7 g grain yield g^{-1} N added) when compared to other crops (Sylvester-Bradley & Kindred, 2009) and were not affected by N or S application rate.

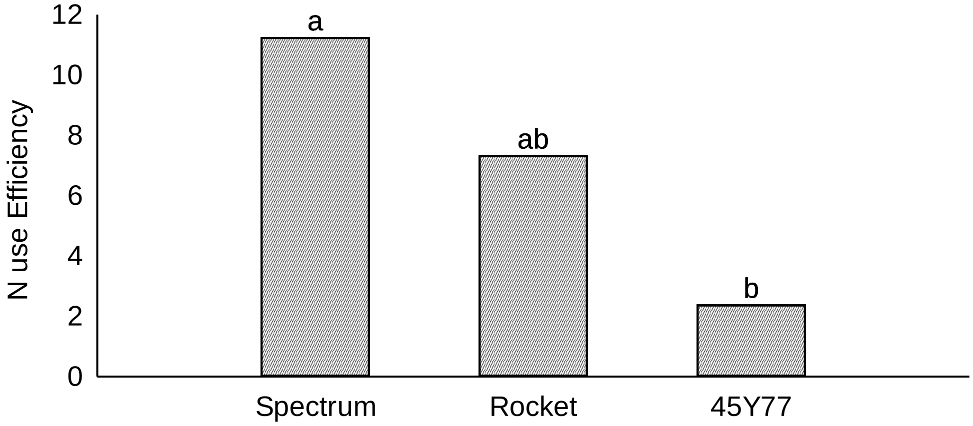

Figure 8.5 Agronomic N use efficiencies of early (Spectrum), mid (Rocket) and late (45Y77) maturing canola cultivars

8.4 CONCLUSIONS

Responses of canola cultivars to N fertilisation, as measured in dry mass production and leaf area supply, were shown as early as the rosette stages of growth (45 DAP). Grain yields differ significantly between early, mid and late maturing cultivars and were increased with addition of nitrogen. However, a better yield response was shown with early and mid-maturing cultivars in this trial. Late maturing cultivars responded to N application by increasing their vegetative phase compromising the reproductive phase (flowering and podding), ultimately producing an unexpected lower grain yield and N utilization efficiency. These results may indicate that short and mid-season cultivars have a more determinate growth habit compared to the late maturing cultivar which (with high nitrogen and sulphur supply) keeps on growing vegetatively. Short and mid maturing cultivars, should, for this reason, be better adapted to areas with a short rainy season, while late maturing cultivars should perform well with high N and S applications rates in high rainfall areas with a long rainy season.

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CHAPTER 9

SUMMARY AND CONCLUSIONS

As a relatively new crop in South Africa, the production of canola is still low compared to the global major producers. Canola production in South Africa is at present to a very large extent, limited to the Western Cape Province which is characterized by a mediterranean type of climate. Estimates for the production of canola during 2011 were 59 490 ton on 43 510 ha (Crop Estimates Committee, 2011). The need to reduce oil and oilcake imports, there is potential for growth in both the area under production and yield per hectare. Considering limited land area, to meet the canola demand, emphasis should be put on increasing yields per hectare. However, according to Van Zyl, (2007), low canola yields (<1.5 ton ha^{-1}) are generally obtained.

In contrast to results obtained in other canola production areas of the world where considerable responses in yield with addition of N were reported (Hocking e*t. al.*, 1997; Jan *et. al.*, 2002; Svečnjak & Rengel, 2006; Tatjana e*t. al.*, 2008), generally poor and or variable responses to increases in N application rates have been reported in the production areas of the Western Cape (Hardy e*t. al.*, 2004). Low yields may be the result of various factors, but because canola is known to have a high nutrient and especially N and S requirement compared to cereals, such as wheat, (Oplinger *et. al.*, 2000; Gan *et. al.*, 2008), optimum management of these nutrients may therefore be important for high yielding canola crops with high oil contents.

Both N and S availability and uptake and therefore fertilisation requirements are affected by soil and climatic conditions (Malhi *et al.,* 2008). Fertilisation with these nutrients have been shown to influence absorption (Koenig, 2012), translocation (Malhi *et al.,* 2007) and partitioning (Jackson, 2000) of other nutrients hence plant biomass which ultimately has an effect on yield. However these effects are greatly affected by soil moisture and inherent nutrient levels. For this reason, a study was conducted to determine optimum N and S fertiliser rates for maximizing grain yield and quality in the Western Cape Province of South Africa.

Specific objectives of the study and results obtained were as follows:

Nutrient content and uptake of canola in response to N and S fertilisation

Nutrient uptake and content as measured at flowering (90 DAP), differed between localities due to climatic conditions (rainfall), soil pH as well as and macro- and micro nutrient levels in the soil. The result was that nutrients such as B, Ca, Mn, and S were below critical levels required for optimum canola plant growth and development at some localities, while luxurious consumption has been observed with regard to Na, Fe, Al and K. These results illustrate the necessity of soil analysis prior to planting. However, at some localities deficient levels of nutrients such as Mn and B were found in plants in spite of sufficient levels in the soil and *vice versa,* which illustrate the need of plant analysis as well. In general, fertilisation with 120 kg N ha^{-1} significantly increased the content of N, K, Na and Al, whilst secondary nutrient S was reduced in canola plants sampled at 90 DAP. Responses differed between localities. Sulphur application rates did not have an effect on nutrient uptake and content in plants at flowering and sulphur concentration within the canola plants remained below sufficient quantities, regardless of fertilisation, prompting for more research in this regard.

Effects of N and S fertilisation on vegetative growth of canola

Increased plant uptake of nutrients due to N application increased the biomass accumulation of the canola plants at flowering, at all localities evaluated in different years. Effects of N were, however, dependent on locality in some of the years tested. Poor response to N in biomass accumulation was most likely a result of poor moisture levels (low rainfall), low plant populations or low inherent soil nutrient levels. Fertilisation with S increased plant dry mass in 2010 field trials only. When nitrogen use efficiency was evaluated based on biomass accumulation it was found that NUE was solely affected by nitrogen application and localities, indicating that biomass production of canola are relative independent of sulphur supply.

Effect of soil and climatic conditions on yield and quality response of canola to N and S application rates

Grain yield potential of canola and optimum level of N application are highly depended on the locality (soil and climatic conditions), while responses to S depends on soil conditions. For this reason, Elsenburg with >400 mm rain, produced the highest yields of more than 2.5 ton ha^{-1} during 2009 and

2010. At Langgewens and Roodebloem with 250-350 mm rainfall, yields of about 1.5 ton ha^{-1} were obtained in the same years. Available rainfall should involve both intensity and distribution throughout the canola growing season. Canola can compensate yields if low rainfalls are received during early growth stages, however low rainfall during flowering and pod growth phases may limit yield responses to N applications. Grain yields at Elsenburg and Langgewens in 2011 were for example less than 1.5 ton ha^{-1}, with a rainfall of more than 300 mm, compared to >2.5 ton ha^{-1} at Roodebloem with a rainfall of less than 250 mm. For effective soil fertility management, N and S application should therefore always match the expected rainfall. Reliability of response to N fertilisation are lower in low rainfall localities, but results showed N applications of 120 kg N ha^{-1} may be needed in high rainfall areas to obtain grain yields of more than 2.0 ton ha^{-1}, though high N levels may reduce the grain oil content. Grain yield responses to nitrogen fertiliser were also affected by S. Results in this study showed that yield responses to N applications were improved by S applications of 15-30 kg S ha^{-1} to such an extent that in contrast to where no S was applied, yields did not tend to level off at rates of 120 kg ha^{-1}. Response curves developed with multiple regression techniques suggested that nitrogen application rates of even greater than 120 kg N ha^{-1} may be needed to obtain maximum grain yields if sufficient S is applied. Sulphur applications are for this reason recommended and especially so if soil contents are less than 6 mg kg^{-1}. Results showed that S applications may also improve the oil content of canola though this has a compromising effect on protein content.

Agronomic and water use efficiencies of canola in response to N and S application rates

Agronomic efficiencies of applied N in canola are low compared to winter cereals and differed between years and localities. During 2009 and 2010, agronomic efficiencies of N applications on average decreased from about 14 kg grain yield increase per kg N applied at rates of 30 kg N ha^{-1} to about 8 kg grain yield increase per kg N applied at rates of 120 kg N ha^{-1}. These results suggested that high N application rates will still improve profit margins of canola as long as the price of N fertiliser is less than eight times the price of canola. During 2009 and 2010, agronomic efficiencies of S applications on average improved from 7-10 kg grain yield increase per kg S applied if no N were applied, to more than 20 kg yield increase per kg S applied at N rates of 120 kg ha^{-1}. Sulphur applications will therefore only be highly efficient if combined with high N rates. Although water use efficiencies (kg grain yield per mm of May to October rainfall) differed between years and localities, both nitrogen and sulphur fertilisation help to improve water use efficiencies. On average, water use efficiencies were increased from about 4-5 kg grain yield mm^{-1} of rain where no N or S was applied to about 8-9 kg grain yield mm^{-1} of rain with 120 kg of N and 30 kg of S ha^{-1}. These results clearly indicated that these nutrients were indeed very important yield limiting factors to canola production in the Western Cape and although yield responses to applications may be higher in high rainfall areas, low rainfall areas may also benefit from such applications.

Growth response of canola to N and S fertilisation under controlled (glasshouse) conditions

Response curves developed under controlled (glasshouse) conditions confirmed the results of field trials which suggested that the vegetative growth of canola is relative independent of S supply, but effects of N are manifested as early as the rosette stages of growth with the addition of N increasing the photosynthetic capacity of the leaves, hence biomass accumulation. However, high N rates applied during early growth stages (planting) may negatively influence biomass accumulation prompting the need to split application of fertilisers. The results obtained under glasshouse conditions confirmed that higher grain yields obtained with S applications in field trials were due to increased flower and pod development, but also showed that flower and pod development may be delayed if N rates of >120 kg ha^{-1} are applied. This may have a negative effect on grain yield of canola in areas or years when rainfall are low or break-off early.

Morphological and physiological responses of canola varieties to N and S fertilisation

Grain yields differ significantly between early, medium and long season cultivars and were increased with addition of nitrogen. However, yield responses to nitrogen fertilisation differed for the different cultivars with better responses obtained under glasshouse conditions with short and medium season cultivars than with a late maturing (long season cultivar) in spite of a better vegetative (dry mass) response of the later maturing cultivar. Due to the lower yield response of the latter, agronomic efficiency of applied nitrogen were also lower in later maturing than short and mid-season cultivar. Because of the unlimiting water and temperature conditions in the glasshouse, these results may indicate that short and mid-season cultivars have a more determinate growth habit compared to the late maturing cultivar which (with high nitrogen and sulphur supply) which keep on growing vegetatively. This delayed flowering and podding may be the reason for the unexpected lower yield obtained with the long season (late maturing) cultivar.

Future research

- Carry out a cost benefit analysis for evaluation of gross and profit margins at different N and S fertilisation rates in different environmental conditions.
- To match canola crop nutrient requirements and supply, more research dealing with split applications of N and S on canola may be needed.
- Agronomic efficiencies at high application rates of N are still low compared to winter cereals and methods to improve it should be investigated.
- As different canola cultivars were used in each season in this trial and evaluation of water use efficiency in 2011 showed a higher efficiency than 2009 and 2010, it might be worthy investigating whether the differences were caused by different cultivars.

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APPENDICES

APPENDIX 1: ANOVA for chapter 3

Analysis of variance for plant NH_4=N concentration at 90 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	801.4129	1	801.4129	10606.22	0.000000
Locality	8.8072	4	2.2018	29.14	0.000000
N Rate	4.0790	1	4.0790	53.98	0.000000
S Rate	0.0253	2	0.0127	0.17	0.846065
Locality*N Rate	1.8664	4	0.4666	6.18	0.000314
Locality*S Rate	1.1862	8	0.1483	1.96	0.066974
N Rate*S Rate	0.0212	2	0.0106	0.14	0.869409
Locality*N Rate*S Rate	0.2112	8	0.0264	0.35	0.942462
Error	4.5336	60	0.0756		

Analysis of variance for plant S concentration at 90 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	9.756501	1	9.756501	5170.719	0.000000
Locality	0.111453	4	0.027863	14.767	0.000000
N Rate	0.033158	1	0.033158	17.573	0.000092
S Rate	0.000665	2	0.000332	0.176	0.838868
Locality*N Rate	0.018472	4	0.004618	2.447	0.055904
Locality*S Rate	0.004341	8	0.000543	0.288	0.967569
N Rate*S Rate	0.005974	2	0.002987	1.583	0.213812
Locality*N Rate*S Rate	0.003730	8	0.000466	0.247	0.979748
Error	0.113212	60	0.001887		

Analysis of variance for plant P concentration at 90 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	33.40061	1	33.40061	32.21007	0.000000
Locality	3.40045	4	0.85011	0.81981	0.517644
N Rate	1.31951	1	1.31951	1.27247	0.263794
S Rate	2.08551	2	1.04276	1.00559	0.371910
Locality*N Rate	4.06801	4	1.01700	0.98075	0.424924
Locality*S Rate	8.03556	8	1.00445	0.96864	0.469002
N Rate*S Rate	2.00266	2	1.00133	0.96564	0.386577
Locality*N Rate*S Rate	8.28227	8	1.03528	0.99838	0.446825
Error	62.21769	60	1.03696		

Analysis of variance for plant K concentration at 90 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	1731.922	1	1731.922	1688.434	0.000000
Locality	105.174	4	26.293	25.633	0.000000
N Rate	4.412	1	4.412	4.301	0.042379
S Rate	0.256	2	0.128	0.125	0.882869
Locality*N Rate	2.834	4	0.709	0.691	0.601241
Locality*S Rate	2.484	8	0.310	0.303	0.962164
N Rate*S Rate	1.417	2	0.708	0.691	0.505225
Locality*N Rate*S Rate	9.465	8	1.183	1.153	0.342205
Error	61.545	60	1.026		

Analysis of variance for plant Ca concentration at 90 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	272.2057	1	272.2057	4694.966	0.000000
Locality	11.2705	4	2.8176	48.598	0.000000
N Rate	0.0334	1	0.0334	0.577	0.450509
S Rate	0.0798	2	0.0399	0.688	0.506511
Locality*N Rate	1.1135	4	0.2784	4.801	0.001994
Locality*S Rate	0.1625	8	0.0203	0.350	0.942065
N Rate*S Rate	0.0024	2	0.0012	0.021	0.979616
Locality*N Rate*S Rate	0.3351	8	0.0419	0.722	0.670937
Error	3.4787	60	0.0580		

Analysis of variance for plant Mg concentration at 90 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	13.36721	1	13.36721	4899.157	0.000000
Locality	0.61398	4	0.15350	56.257	0.000000
N Rate	0.00030	1	0.00030	0.111	0.740319
S Rate	0.00061	2	0.00030	0.111	0.895217
Locality*N Rate	0.08828	4	0.02207	8.089	0.000028
Locality*S Rate	0.01174	8	0.00147	0.538	0.823410
N Rate*S Rate	0.00131	2	0.00065	0.240	0.787630
Locality*N Rate*S Rate	0.00799	8	0.00100	0.366	0.934395
Error	0.16371	60	0.00273		

Analysis of variance for plant Na concentration at 90 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	2.672411E+09	1	2.672411E+09	636.5241	0.000000
Locality	9.742591E+08	4	2.435648E+08	58.0131	0.000000
N Rate	9.269445E+07	1	9.269445E+07	22.0783	0.000016
S Rate	2.555891E+07	2	1.277946E+07	3.0439	0.055070
Locality*N Rate	1.933560E+08	4	4.833900E+07	11.5136	0.000001
Locality*S Rate	5.946048E+07	8	7.432560E+06	1.7703	0.100958
N Rate*S Rate	5.622904E+06	2	2.811452E+06	0.6696	0.515676
Locality*N Rate*S Rate	1.660789E+07	8	2.075986E+06	0.4945	0.855426
Error	2.519066E+08	60	4.198444E+06		

Analysis of variance for plant Fe concentration at 90 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	20873700	1	20873700	13.63255	0.000482
Locality	3522426	4	880607	0.57512	0.681738
N Rate	1299206	1	1299206	0.84851	0.360664
S Rate	2179442	2	1089721	0.71169	0.494908
Locality*N Rate	6003324	4	1500831	0.98019	0.425226
Locality*S Rate	12437238	8	1554655	1.01534	0.434467
N Rate*S Rate	3658941	2	1829471	1.19482	0.309858
Locality*N Rate*S Rate	11778617	8	1472327	0.96157	0.474366
Error	91869949	60	1531166		

Analysis of variance for plant Cu concentration at 90 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	3327.858	1	3327.858	230.6016	0.000000
Locality	151.263	4	37.816	2.6204	0.043607
N Rate	41.865	1	41.865	2.9010	0.093701
S Rate	19.613	2	9.807	0.6795	0.510709
Locality*N Rate	66.497	4	16.624	1.1520	0.341080
Locality*S Rate	43.107	8	5.388	0.3734	0.930644
N Rate*S Rate	15.443	2	7.721	0.5350	0.588410
Locality*N Rate*S Rate	61.298	8	7.662	0.5309	0.828584
Error	865.872	60	14.431		

Analysis of variance for plant Zn concentration at 90 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	130579.4	1	130579.4	1218.253	0.000000
Locality	15240.4	4	3810.1	35.547	0.000000
N Rate	380.6	1	380.6	3.551	0.064353
S Rate	69.6	2	34.8	0.325	0.724058
Locality*N Rate	557.8	4	139.5	1.301	0.279931
Locality*S Rate	194.2	8	24.3	0.227	0.984656
N Rate*S Rate	22.8	2	11.4	0.107	0.899079
Locality*N Rate*S Rate	616.9	8	77.1	0.719	0.673531
Error	6431.1	60	107.2		

Analysis of variance for plant Mn concentration at 90 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	126701.1	1	126701.1	651.4965	0.000000
Locality	13627.2	4	3406.8	17.5177	0.000000
N Rate	72.0	1	72.0	0.3701	0.545234
S Rate	52.5	2	26.2	0.1349	0.874100
Locality*N Rate	1247.5	4	311.9	1.6036	0.185119
Locality*S Rate	275.7	8	34.5	0.1772	0.993189
N Rate*S Rate	36.6	2	18.3	0.0942	0.910267
Locality*N Rate*S Rate	395.6	8	49.5	0.2543	0.977828
Error	11668.6	60	194.5		

Analysis of variance for plant B concentration at 90 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	92699.67	1	92699.67	5597.276	0.000000
Locality	3998.32	4	999.58	60.355	0.000000
N Rate	9.47	1	9.47	0.572	0.452565
S Rate	52.21	2	26.10	1.576	0.215222
Locality*N Rate	45.90	4	11.48	0.693	0.599772
Locality*S Rate	47.18	8	5.90	0.356	0.939265
N Rate*S Rate	6.65	2	3.33	0.201	0.818542
Locality*N Rate*S Rate	52.07	8	6.51	0.393	0.920162
Error	993.69	60	16.56		

Analysis of variance for plant Al concentration at 90 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	15108712	1	15108712	410.4441	0.000000
Locality	1955041	4	488760	13.2777	0.000000
N Rate	175099	1	175099	4.7568	0.033114
S Rate	115105	2	57553	1.5635	0.217818
Locality*N Rate	27847	4	6962	0.1891	0.943185
Locality*S Rate	134246	8	16781	0.4559	0.881945
N Rate*S Rate	172057	2	86028	2.3371	0.105348
Locality*N Rate*S Rate	196652	8	24582	0.6678	0.717578
Error	2208639	60	36811		

APPENDIX 2: ANOVA for chapter 4

Analysis of variance for plant population at 30 DAP in 2009 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	501600.0	1	501600.0	4907.102	0.000000
Block	334.1	3	111.4	1.089	0.356010
Locality	2493.7	2	1246.9	12.198	0.000014
N Rate	548.4	4	137.1	1.341	0.258013
S Rate	178.7	2	89.3	0.874	0.419682
Locality*N Rate	872.6	8	109.1	1.067	0.390042
Locality*S Rate	124.1	4	31.0	0.303	0.875261
N Rate*S Rate	316.2	8	39.5	0.387	0.926156
Locality*N Rate*S Rate	3675.2	16	229.7	2.247	0.006475
Error	13492.9	132	102.2		

Analysis of variance for plant population at 30 DAP in 2010 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	914764.5	1	914764.5	8534.254	0.000000
Block	25.6	3	8.5	0.080	0.971034
Locality	54645.4	3	18215.1	169.937	0.000000
N Rate	323.2	4	80.8	0.754	0.556717
S Rate	25.2	2	12.6	0.118	0.889164
Locality*N Rate	1863.8	12	155.3	1.449	0.147804
Locality*S Rate	820.3	6	136.7	1.275	0.270827
N Rate*S Rate	719.1	8	89.9	0.839	0.569776
Locality*N Rate*S Rate	3275.7	24	136.5	1.273	0.188092
Error	18972.2	177	107.2		

Analysis of variance for plant population at 30 DAP in 2011 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	695849.7	1	695849.7	4333.127	0.000000
Block	2249.6	3	749.9	4.670	0.003638
Locality	12866.0	3	4288.7	26.706	0.000000
N Rate	414.4	4	103.6	0.645	0.631073
S Rate	412.3	2	206.1	1.284	0.279608
Locality*N Rate	3659.8	12	305.0	1.899	0.037146
Locality*S Rate	950.6	6	158.4	0.987	0.435868
N Rate*S Rate	574.1	8	71.8	0.447	0.891366
Locality*N Rate*S Rate	2668.4	24	111.2	0.692	0.854980
Error	28424.1	177	160.6		

Analysis of variance for plant dry mass at 90 DAP in 2009 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	1354274	1	1354274	433.0444	0.000000
Block	29862	3	9954	3.1829	0.031544
Locality	66398	2	33199	10.6157	0.000140
N Rate	77169	1	77169	24.6758	0.000008
S Rate	1771	2	886	0.2832	0.754564
Locality*N Rate	13042	2	6521	2.0852	0.134748
Locality*S Rate	7735	4	1934	0.6184	0.651435
N Rate*S Rate	8481	2	4241	1.3560	0.266832
Locality*N Rate*S Rate	17170	4	4292	1.3726	0.256496
Error	159494	51	3127		

Analysis of variance for plant dry mass at 90 DAP in 2010 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	12397331	1	12397331	4665.055	0.000000
Block	23012	3	7671	2.886	0.041760
Locality	1167570	3	389190	146.450	0.000000
N Rate	502870	1	502870	189.228	0.000000
S Rate	160776	2	80388	30.250	0.000000
Locality*N Rate	88278	3	29426	11.073	0.000005
Locality*S Rate	29452	6	4909	1.847	0.102647
N Rate*S Rate	8850	2	4425	1.665	0.196676
Locality*N Rate*S Rate	30897	6	5149	1.938	0.086871
Error	183367	69	2657		

Analysis of variance for plant dry mass at 90 DAP in 2011 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	9910443	1	9910443	1567.559	0.000000
Locality	1402166	3	467389	73.928	0.000000
N Rate	74911	1	74911	11.849	0.000965
S Rate	20183	2	10092	1.596	0.209747
Locality*N Rate	175098	3	58366	9.232	0.000030
Locality*S Rate	104350	6	17392	2.751	0.018310
N Rate*S Rate	16232	2	8116	1.284	0.283257
Locality*N Rate*S Rate	140056	6	23343	3.692	0.002964
Error	455199	72	6322		

Analysis of variance for NUE at 90 DAP in 2009 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	1905.420	1	1905.420	22.82918	0.000073
Block	131.727	3	43.909	0.52608	0.668573
Locality	322.034	2	161.017	1.92918	0.167136
S Rate	209.409	2	104.705	1.25448	0.303264
Locality*S Rate	423.950	4	105.987	1.26986	0.309257
Error	2003.141	24	83.464		

Analysis of variance for NUE at 90 DAP in 2010 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	12416.55	1	12416.55	180.8600	0.000000
Block	235.78	3	78.59	1.1448	0.345464
Locality	2179.72	3	726.57	10.5833	0.000050
S Rate	218.52	2	109.26	1.5915	0.218853
Locality*S Rate	762.89	6	127.15	1.8520	0.119163
Error	2265.54	33	68.65		

Analysis of variance for NUE at 90 DAP in 2011 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	1849.645	1	1849.645	10.05716	0.003097
Locality	4323.408	3	1441.136	7.83596	0.000376
S Rate	400.800	2	200.400	1.08965	0.347171
Locality*S Rate	3458.178	6	576.363	3.13389	0.014164
Error	6620.877	36	183.913		

APPENDIX 3: ANOVA for chapter 5

Analysis of variance for grain yield in 2009 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	695.1259	1	695.1259	4157.776	0.000000
Block	1.7647	3	0.5882	3.518	0.017000
Locality	108.0667	2	54.0334	323.191	0.000000
N Rate	19.5150	4	4.8787	29.181	0.000000
S Rate	2.8764	2	1.4382	8.602	0.000308
Locality*N Rate	3.0171	8	0.3771	2.256	0.027200
Locality*S Rate	1.3990	4	0.3498	2.092	0.085393
N Rate*S Rate	1.0194	8	0.1274	0.762	0.636604
Locality*N Rate*S Rate	0.9635	16	0.0602	0.360	0.988776
Error	22.0687	132	0.1672		

Analysis of variance for grain yield in 2010 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	967.6954	1	967.6954	47209.08	0.000000
Block	0.0225	3	0.0075	0.37	0.777830
Locality	92.4933	3	30.8311	1504.10	0.000000
N Rate	14.9055	4	3.7264	181.79	0.000000
S Rate	3.3814	2	1.6907	82.48	0.000000
Locality*N Rate	0.9549	12	0.0796	3.88	0.000028
Locality*S Rate	0.4721	6	0.0787	3.84	0.001267
N Rate*S Rate	0.7464	8	0.0933	4.55	0.000047
Locality*N Rate*S Rate	1.0634	24	0.0443	2.16	0.002362
Error	3.6282	177	0.0205		

Analysis of variance for grain yield in 2011 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	955659036	1	955659036	10745.34	0.000000
Block	1316945	3	438982	4.94	0.002571
Locality	132297490	3	44099163	495.85	0.000000
N Rate	7910921	4	1977730	22.24	0.000000
S Rate	7528529	2	3764265	42.33	0.000000
Locality*N Rate	1408210	12	117351	1.32	0.210856
Locality*S Rate	235278	6	39213	0.44	0.850687
N Rate*S Rate	1509089	8	188636	2.12	0.036071
Locality*N Rate*S Rate	972522	24	40522	0.46	0.987042
Error	15741855	177	88937		

Analysis of variance for thousand kernel mass in 2009 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	1462.449	1	1462.449	36677.97	0.000000
Block	1.231	3	0.410	10.29	0.000004
Locality	20.097	2	10.049	252.02	0.000000
N Rate	0.186	4	0.047	1.17	0.327920
S Rate	0.046	2	0.023	0.58	0.561583
Locality*N Rate	0.389	8	0.049	1.22	0.292141
Locality*S Rate	0.066	4	0.017	0.42	0.796417
N Rate*S Rate	0.284	8	0.036	0.89	0.525945
Locality*N Rate*S Rate	0.464	16	0.029	0.73	0.761540
Error	5.263	132	0.040		

Analysis of variance for thousand kernel mass in 2010 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	1756.843	1	1756.843	71933.63	0.000000
Block	0.119	3	0.040	1.63	0.184801
Locality	8.345	3	2.782	113.89	0.000000
N Rate	0.159	4	0.040	1.63	0.168031
S Rate	0.081	2	0.040	1.65	0.194855
Locality*N Rate	1.703	12	0.142	5.81	0.000000
Locality*S Rate	1.027	6	0.171	7.01	0.000001
N Rate*S Rate	0.166	8	0.021	0.85	0.558643
Locality*N Rate*S Rate	0.648	24	0.027	1.10	0.342771
Error	4.323	177	0.024		

Analysis of variance for thousand kernel mass in 2011 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	1458.541	1	1458.541	46008.93	0.000000
Block	0.106	3	0.035	1.11	0.345023
Locality	23.286	3	7.762	244.85	0.000000
N Rate	0.395	4	0.099	3.12	0.016557
S Rate	0.042	2	0.021	0.67	0.513595
Locality*N Rate	0.586	12	0.049	1.54	0.113762
Locality*S Rate	0.111	6	0.018	0.58	0.744400
N Rate*S Rate	0.225	8	0.028	0.89	0.528290
Locality*N Rate*S Rate	0.891	24	0.037	1.17	0.274322
Error	5.611	177	0.032		

APPENDIX 4: ANOVA for chapter 6

Analysis of variance for Agronomic N use efficiency in 2009 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	14542.07	1	14542.07	136.7774	0.000000
Block	1411.05	3	470.35	4.4239	0.005701
Locality	1428.33	2	714.16	6.7172	0.001798
N Rate	864.95	3	288.32	2.7118	0.048721
S Rate	1184.45	2	592.22	5.5702	0.005021
Locality*N Rate	117.28	6	19.55	0.1838	0.980737
Locality*S Rate	596.39	4	149.10	1.4024	0.238299
N Rate*S Rate	379.98	6	63.33	0.5957	0.733184
Locality*N Rate*S Rate	400.85	12	33.40	0.3142	0.985536
Error	11163.52	105	106.32		

Analysis of variance for Agronomic N use efficiency in 2010 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	8643.688	1	8643.688	603.3506	0.000000
Block	0.619	3	0.206	0.0144	0.997632
Locality	418.731	3	139.577	9.7428	0.000007
N Rate	132.723	3	44.241	3.0881	0.029234
S Rate	140.478	2	70.239	4.9029	0.008740
Locality*N Rate	538.594	9	59.844	4.1772	0.000085
Locality*S Rate	242.429	6	40.405	2.8204	0.012696
N Rate*S Rate	25.696	6	4.283	0.2989	0.936527
Locality*N Rate*S Rate	254.847	18	14.158	0.9883	0.476675
Error	2019.986	141	14.326		

Analysis of variance for Agronomic N use efficiency in 2011 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	4524.147	1	4524.147	151.6917	0.000000
Block	250.456	3	83.485	2.7992	0.042325
Locality	17.024	3	5.675	0.1903	0.902886
N Rate	65.154	3	21.718	0.7282	0.536793
S Rate	120.184	2	60.092	2.0148	0.137162
Locality*N Rate	400.156	9	44.462	1.4908	0.156806
Locality*S Rate	192.224	6	32.037	1.0742	0.380864
N Rate*S Rate	202.815	6	33.802	1.1334	0.346076
Locality*N Rate*S Rate	265.772	18	14.765	0.4951	0.956821
Error	4205.272	141	29.825		

Analysis of variance for Agronomic S use efficiency in 2009 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	13943.66	1	13943.66	32.70913	0.000000
Block	853.86	3	284.62	0.66766	0.574156
Locality	8781.59	2	4390.80	10.29996	0.000097
N Rate	744.03	4	186.01	0.43634	0.782020
S Rate	26.77	1	26.77	0.06280	0.802709
Locality*N Rate	3352.80	8	419.10	0.98313	0.454754
Locality*S Rate	359.82	2	179.91	0.42203	0.657049
N Rate*S Rate	1184.34	4	296.09	0.69456	0.597720
Locality*N Rate*S Rate	1682.26	8	210.28	0.49328	0.857958
Error	37087.45	87	426.29		

Analysis of variance for Agronomic S use efficiency in 2010 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	19676.62	1	19676.62	226.0152	0.000000
Block	110.15	3	36.72	0.4217	0.737756
Locality	2666.20	3	888.73	10.2084	0.000005
N Rate	4138.86	4	1034.71	11.8852	0.000000
S Rate	393.76	1	393.76	4.5229	0.035545
Locality*N Rate	5430.59	12	452.55	5.1982	0.000001
Locality*S Rate	103.69	3	34.56	0.3970	0.755403
N Rate*S Rate	537.30	4	134.33	1.5429	0.194316
Locality*N Rate*S Rate	1613.30	12	134.44	1.5443	0.118101
Error	10185.88	117	87.06		

Analysis of variance for Agronomic S use efficiency in 2011 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	33951.47	1	33951.47	186.3344	0.000000
Block	146.51	3	48.84	0.2680	0.848334
Locality	1466.66	3	488.89	2.6831	0.049937
N Rate	6211.76	4	1552.94	8.5229	0.000005
S Rate	1.81	1	1.81	0.0099	0.920815
Locality*N Rate	3339.52	12	278.29	1.5273	0.123858
Locality*S Rate	645.08	3	215.03	1.1801	0.320417
N Rate*S Rate	795.40	4	198.85	1.0913	0.364149
Locality*N Rate*S Rate	528.40	12	44.03	0.2417	0.995644
Error	21318.24	117	182.21		

Analysis of variance for grain yield per millimeter of rain in 2009 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	5334.855	1	5334.855	5473.713	0.000000
Block	13.727	3	4.576	4.695	0.003789
Locality	90.298	2	45.149	46.324	0.000000
N Rate	152.966	4	38.241	39.237	0.000000
S Rate	19.170	2	9.585	9.835	0.000104
Locality*N Rate	9.599	8	1.200	1.231	0.285818
Locality*S Rate	4.011	4	1.003	1.029	0.394830
N Rate*S Rate	8.097	8	1.012	1.038	0.410617
Locality*N Rate*S Rate	6.142	16	0.384	0.394	0.982067
Error	128.651	132	0.975		

Analysis of variance for grain yield per millimeter of rain in 2010 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	7912.407	1	7912.407	47164.41	0.000000
Block	0.142	3	0.047	0.28	0.837603
Locality	359.117	3	119.706	713.54	0.000000
N Rate	127.369	4	31.842	189.81	0.000000
S Rate	27.254	2	13.627	81.23	0.000000
Locality*N Rate	7.153	12	0.596	3.55	0.000096
Locality*S Rate	2.179	6	0.363	2.17	0.048462
N Rate*S Rate	6.623	8	0.828	4.93	0.000016
Locality*N Rate*S Rate	8.387	24	0.349	2.08	0.003663
Error	29.694	177	0.168		

Analysis of variance for grain yield per millimeter of rain in 2011 season

	SS	Degr. of - Freedom	MS	F	p
Intercept	13982.93	1	13982.93	9644.120	0.000000
Block	23.80	3	7.93	5.472	0.001281
Locality	2780.98	3	926.99	639.353	0.000000
N Rate	109.34	4	27.33	18.853	0.000000
S Rate	105.75	2	52.87	36.467	0.000000
Locality*N Rate	19.19	12	1.60	1.103	0.360349
Locality*S Rate	6.22	6	1.04	0.715	0.637599
N Rate*S Rate	21.80	8	2.73	1.880	0.065795
Locality*N Rate*S Rate	14.98	24	0.62	0.430	0.991268
Error	256.63	177	1.45		

APPENDIX 5: ANOVA for chapter 7

Analysis of variance for plant leaf area at 28 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	44166.33	1	44166.33	940.8938	0.000000
N Rate	2697.50	4	674.37	14.3665	0.000001
S Rate	36.97	1	36.97	0.7876	0.381893
N Rate*S Rate	280.93	4	70.23	1.4962	0.228265
Error	1408.22	30	46.94		

Analysis of variance for plant leaf area at 49 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	5116720	1	5116720	4009.513	0.000000
N Rate	366259	4	91565	71.751	0.000000
S Rate	22255	1	22255	17.439	0.000235
N Rate*S Rate	45406	4	11352	8.895	0.000073
Error	38284	30	1276		

Analysis of variance for plant leaf area at 70 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	29628040	1	29628040	4814.614	0.000000
N Rate	1640863	4	410216	66.661	0.000000
S Rate	1418	1	1418	0.230	0.634695
N Rate*S Rate	54641	4	13660	2.220	0.090580
Error	184613	30	6154		

Analysis of variance for plant leaf area at 91 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	44809301	1	44809301	1044.453	0.000000
N Rate	390889	4	97722	2.278	0.084134
S Rate	4602	1	4602	0.107	0.745563
N Rate*S Rate	48470	4	12117	0.282	0.887024
Error	1287066	30	42902		

Analysis of variance for plant dry mass at 28 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	0.590490	1	0.590490	948.5783	0.000000
N Rate	0.036541	4	0.009135	14.6752	0.000001
S Rate	0.000160	1	0.000160	0.2570	0.615876
N Rate*S Rate	0.002584	4	0.000646	1.0377	0.404171
Error	0.018675	30	0.000622		

Analysis of variance for plant dry mass at 49 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	223.2562	1	223.2562	1882.655	0.000000
N Rate	22.4876	4	5.6219	47.408	0.000000
S Rate	1.3068	1	1.3068	11.020	0.002374
N Rate*S Rate	1.3602	4	0.3401	2.868	0.040037
Error	3.5576	30	0.1186		

Analysis of variance for plant dry mass at 70 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	1717.803	1	1717.803	4556.316	0.000000
N Rate	149.704	4	37.426	99.269	0.000000
S Rate	0.705	1	0.705	1.870	0.181669
N Rate*S Rate	1.530	4	0.382	1.015	0.415620
Error	11.310	30	0.377		

Analysis of variance for plant dry mass at 91 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	6951.923	1	6951.923	7051.297	0.000000
N Rate	405.797	4	101.449	102.899	0.000000
S Rate	0.048	1	0.048	0.048	0.827553
N Rate*S Rate	3.653	4	0.913	0.926	0.461752
Error	29.577	30	0.986		

Analysis of variance for total number of flowers at 91 WAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	180700.8	1	180700.8	342.4269	0.000000
N Rate	31558.4	4	7889.6	14.9507	0.000001
S Rate	339.3	1	339.3	0.6430	0.428939
N Rate*S Rate	2744.5	4	686.1	1.3002	0.292323
Error	15831.2	30	527.7		

Analysis of variance for plant total number of pods at 91 WAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	7120.892	1	7120.892	1436.460	0.000000
N Rate	2507.681	4	626.920	126.465	0.000000
S Rate	203.852	1	203.852	41.122	0.000000
N Rate*S Rate	701.446	4	175.362	35.375	0.000000
Error	148.718	30	4.957		

APPENDIX 6: ANOVA for chapter 8

Analysis of variance for plant leaf area at 45 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	1550051	1	1550051	899.4930	0.000000
Cultivar	247	2	123	0.0716	0.931030
S Rate	554	1	554	0.3212	0.574388
N Rate	153870	1	153870	89.2906	0.000000
Cultivar*S Rate	465	2	233	0.1350	0.874154
Cultivar*N Rate	614	2	307	0.1781	0.837589
S Rate*N Rate	9	1	9	0.0050	0.943749
Locality*S Rate*N Rate	3084	2	1542	0.8943	0.417617
Error	62037	36	1723		

Analysis of variance for leaf area index at 45 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	89.98028	1	89.98028	899.4930	0.000000
Cultivar	0.01433	2	0.00716	0.0716	0.931030
S Rate	0.03213	1	0.03213	0.3212	0.574388
N Rate	8.93213	1	8.93213	89.2906	0.000000
Cultivar*S Rate	0.02701	2	0.01350	0.1350	0.874154
Cultivar*N Rate	0.03563	2	0.01782	0.1781	0.837589
S Rate*N Rate	0.00051	1	0.00051	0.0050	0.943747
Locality*S Rate*N Rate	0.17901	2	0.08950	0.8947	0.417617
Error	3.60124	36	0.10003		

Analysis of variance for plant dry mass at 45 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	46.73840	1	46.73840	634.3906	0.000000
Cultivar	0.68299	2	0.34149	4.6352	0.016173
S Rate	0.00574	1	0.00574	0.0779	0.781706
N Rate	4.75965	1	4.75965	64.6038	0.000000
Cultivar*S Rate	0.00895	2	0.00447	0.0607	0.941184
Cultivar*N Rate	0.04182	2	0.02091	0.2838	0.754575
S Rate*N Rate	0.00413	1	0.00413	0.0560	0.814281
Locality*S Rate*N Rate	0.09807	2	0.04903	0.6656	0.520195
Error	2.65228	36	0.07367		

Analysis of variance for plant leaf area at 70 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	6113583	1	6113583	505.8073	0.000000
Cultivar	1751	2	876	0.0724	0.930248
S Rate	10098	1	10098	0.8355	0.366772
N Rate	1394288	1	1394288	115.3564	0.000000
Cultivar*S Rate	22321	2	11161	0.9234	0.406378
Cultivar*N Rate	44780	2	22390	1.8525	0.171494
S Rate*N Rate	27703	1	27703	2.2920	0.138774
Locality*S Rate*N Rate	15296	2	7648	0.6328	0.536924
Error	435124	36	12087		

Analysis of variance for leaf area index at 70 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	354.8928	1	354.8928	505.8073	0.000000
Cultivar	0.1017	2	0.0508	0.0724	0.930248
S Rate	0.5862	1	0.5862	0.8355	0.366772
N Rate	80.9383	1	80.9383	115.3564	0.000000
Cultivar*S Rate	1.2957	2	0.6479	0.9234	0.406378
Cultivar*N Rate	2.5995	2	1.2997	1.8525	0.171494
S Rate*N Rate	1.6081	1	1.6081	2.2920	0.138774
Locality*S Rate*N Rate	0.8879	2	0.4440	0.6328	0.536924
Error	25.2589	36	0.7016		

Analysis of variance for plant dry mass at 70 DAP

	SS	Degr. of - Freedom	MS	F	p
Intercept	1805.531	1	1805.531	469.8688	0.000000
Cultivar	29.812	2	14.906	3.8791	0.029811
S Rate	0.199	1	0.199	0.0518	0.821305
N Rate	356.049	1	356.049	92.6578	0.000000
Cultivar*S Rate	7.661	2	3.831	0.9969	0.378979
Cultivar*N Rate	2.498	2	1.249	0.3250	0.724624
S Rate*N Rate	1.892	1	1.892	0.4924	0.487372
Locality*S Rate*N Rate	3.059	2	1.530	0.3981	0.674548
Error	138.335	36	3.843		

Analysis of variance for plant height at harvesting

	SS	Degr. of - Freedom	MS	F	p
Intercept	544745.5	1	544745.5	541.7744	0.000000
Cultivar	3210.6	2	1605.3	1.5964	0.216610
S Rate	249.8	1	249.8	0.2484	0.621212
N Rate	35343.9	1	35343.9	35.1510	0.000001
Cultivar*S Rate	228.7	2	114.3	0.1137	0.892841
Cultivar*N Rate	6881.9	2	3441.0	3.4222	0.043587
S Rate*N Rate	3.8	1	3.8	0.0038	0.951340
Locality*S Rate*N Rate	910.0	2	455.0	0.4525	0.639603
Error	36197.6	36	1005.5		

Analysis of variance for number of flower branches per plant at final harvesting

	SS	Degr. of - Freedom	MS	F	p
Intercept	459.4219	1	459.4219	168.0171	0.000000
Cultivar	12.8750	2	6.4375	2.3543	0.109421
S Rate	0.6302	1	0.6302	0.2305	0.634075
N Rate	81.3802	1	81.3802	29.7619	0.000004
Cultivar*S Rate	2.0417	2	1.0208	0.3733	0.691070
Cultivar*N Rate	8.1667	2	4.0833	1.4933	0.238207
S Rate*N Rate	0.2552	1	0.2552	0.0933	0.761741
Locality*S Rate*N Rate	2.0417	2	1.0208	0.3733	0.691070
Error	98.4375	36	2.7344		

Analysis of variance for plant pods at final harvesting

	SS	Degr. of - Freedom	MS	F	p
Intercept	47880.33	1	47880.33	153.8324	0.000000
Cultivar	4528.89	2	2264.44	7.2753	0.002220
S Rate	6.02	1	6.02	0.0193	0.890160
N Rate	9661.69	1	9661.69	31.0416	0.000003
Cultivar*S Rate	130.32	2	65.16	0.2094	0.812088
Cultivar*N Rate	468.78	2	234.39	0.7531	0.478196
S Rate*N Rate	3.00	1	3.00	0.0096	0.922337
Locality*S Rate*N Rate	147.47	2	73.73	0.2369	0.790292
Error	11205.00	36	311.25		

Analysis of variance for plant grain yield

	SS	Degr. of - Freedom	MS	F	p
Intercept	71.37002	1	71.37002	89.23286	0.000000
Cultivar	13.50020	2	6.75010	8.43955	0.000987
S Rate	0.13441	1	0.13441	0.16805	0.684281
N Rate	22.77007	1	22.77007	28.46908	0.000005
Cultivar*S Rate	0.51484	2	0.25742	0.32185	0.726870
Cultivar*N Rate	6.12226	2	3.06113	3.82728	0.031111
S Rate*N Rate	0.27150	1	0.27150	0.33945	0.563776
Locality*S Rate*N Rate	0.22301	2	0.11150	0.13941	0.870337
Error	28.79344	36	0.79982		

Analysis of variance for plant dry mass

	SS	Degr. of - Freedom	MS	F	p
Intercept	5755.649	1	5755.649	425.1953	0.000000
Cultivar	187.543	2	93.771	6.9273	0.002849
S Rate	0.569	1	0.569	0.0420	0.838742
N Rate	1647.656	1	1647.656	121.7197	0.000000
Cultivar*S Rate	1.729	2	0.865	0.0639	0.938238
Cultivar*N Rate	78.926	2	39.643	2.9153	0.067073
S Rate*N Rate	1.362	1	1.362	0.1006	0.752939
Locality*S Rate*N Rate	10.378	2	5.189	0.3833	0.684331
Error	487.313	36	13.536		

Analysis of variance for Agronomic N use efficiency

	SS	Degr. of - Freedom	MS	F	p
Intercept	1174.934	1	1174.934	31.42529	0.000026
Cultivar	315.908	2	157.954	4.22471	0.031312
S Rate	14.009	1	14.009	0.37470	0.548107
Cultivar*S Rate	11.509	2	5.754	0.15389	0.858484
Error	672.987	18	37.388		