
THESIS / DISSERTATION SUBMISSION

**Agronomic and economic assessment of
topdressing nitrogen on maize under dryland
in the north-eastern Free State**

Retief Senekal

May 2025

Submitted in fulfilment of the requirements in
respect of the master's degree with specialisation
in Agronomy in the department of Soil, Crop, and
Climate Sciences in the faculty of Natural and
Agricultural Sciences at the
University of the Free State.

Supervisor: Prof. GM Ceronio

Co-supervisor: Dr E Van der Watt

*Inspiring
excellence,
transforming
lives through
quality,
impact, and
care.*

www.ufs.ac.za/centreforgraduatesupport

*Inspiring excellence, transforming lives
through quality, impact, and care.*

Table of Contents

Declaration.....	vii
Acknowledgements	viii
Abstract.....	ix
Chapter 1	1
1. Introduction.....	1
1.1 Background	1
1.2 Objectives.....	3
References.....	4
Chapter 2	6
Literature Review	6
2.1 Origin, Production, and Importance of maize in South Africa	6
2.1.2 Nitrogen Fertilizer	6
2.1.2.1 Nitrogen sources.....	7
a) Urea	7
b) Limestone Ammonium Nitrate (LAN)	7
c) Ammonia gas (NH ₃).....	7
d) Greensulf.....	8
2.2 Nitrogen in the Soil, Plant Uptake, and Role in Plant Physiology	8
2.2.1 The Nitrogen Cycle and Soil Nitrogen Forms.....	8
2.2.1.1 The Nitrogen Cycle.....	8
2.2.2 Forms of Nitrogen in the Soil	9
a) Ammonium (NH ₄ ⁺)	9
b) Nitrate (NO ₃ ⁻).....	9
c) Organic Nitrogen	9
2.2.3 Nitrogen Uptake Mechanisms in Plants	9
2.2.3.1 Root Uptake	9
a) Ammonium Transporters (AMTs).....	9
b) Nitrate Transporters (NRTs)	10

2.2.3.2 Symbiotic Nitrogen Fixation	10
2.2.3.3 Mycorrhizal Fungi and Nitrogen Uptake	10
2.2.4 Nitrogen Assimilation and Transport within the Plant	10
2.2.4.1 Nitrogen Assimilation Pathways.....	10
a) Glutamine Synthetase (GS).....	10
b) Glutamate Dehydrogenase (GDH).....	11
2.2.4.2 Nitrogen Transport in Plants	11
2.2.4.3 Role in Photosynthesis and Growth	11
2.2.5. Role of Nitrogen in Plant Growth and Development.....	11
2.2.5.1 Protein Synthesis.....	11
2.2.5.2 Chlorophyll Synthesis	11
2.2.5.3 Root Development.....	12
2.2.5.4 Reproductive Development	12
2.2.6. Nitrogen Use Efficiency and Agricultural Implications	12
2.2.6.1 Nitrogen Use Efficiency (NUE).....	12
2.2.6.2 Environmental Impact.....	12
2.3 Influence of Nitrogen on Maize Plants	12
2.3.1 Growth and yield	12
2.3.2 Nitrogen and sulphur ratio	14
2.3.3 Maize growth stages.....	14
a) Vegetative growth stages in maize	14
b) Reproductive stages in maize.....	16
2.3.4 Nitrogen uptake by the maize plant	17
2.3.5 Sufficiency ranges of nutrients at different growth stages of a maize plant	20
References.....	22

Chapter 3	27
Materials and Methods	27
3.1 Experimental site.....	27
3.2 Experimental design and treatments	28
3.3 Reason for growth stages and nitrogen sources used	29
3.4 Agronomic practices.....	29
3.5 Data collection and measurements	30
3.5.1 Leaf samples and preharvest measurements	31
3.5.2 Yield and Yield Components	31
3.5.3 Grain quality	31
3.5.5 Return on investment	32
3.6 Statistical analysis.....	32
References.....	32
Chapter 4	33
The influence of nitrogen source, application rate, and growth stage on the leaf nutrient content of maize in the north-eastern Free State	33
4.1 Introduction	33
4.2 Materials and Methods	34
4.3 Results and Discussion	34
4.3.1 Leave analysis.....	37
Why use leaf analysis?.....	37
4.3.1.1 Macronutrient leaf concentration.....	37
4.3.1.1.1 N content.....	37
4.3.1.1.2 S content	40
4.3.1.1.3 P content	44
4.3.1.1.4 K content	46
4.3.1.1.5 Ca content	49
4.3.1.1.6 Mg content.....	52
4.3.1.2 Micronutrient leaf concentration	54
4.3.1.2.1 Cu content	54

4.3.1.2.2 Mn content.....	58
4.3.1.2.3 Zn content	58
4.3.1.2.4 Fe content	60
4.3.1.2.5 B content	63
4.3.1.3 Nutrient leaf ratio	65
4.3.1.3.1 N:S ratio	65
4.3.1.3.2 N:K ratio	68
4.3.1.3.3 Fe:Mn ratio	71
Conclusion	72
References.....	73
Chapter 5	76
The influence of nitrogen source, application rate, and growth stage on the yield components and yield of non-prolific and prolific maize cultivars in the north-eastern Free State.....	76
5.1 Introduction	76
5.2 Materials and Methods	77
5.3 Results and Discussion	77
5.3.1 Yield components and yield of a non-prolific and a prolific cultivar.....	78
5.3.1.1 Number of cobs per plant	81
5.3.1.2. Kernels per plant	83
5.3.1.3 Grain mass per plant	87
5.3.1.4 Grain mass per cob	90
5.3.1.5 Hectolitre mass.....	92
5.3.1.6 1000 kernel weight	94
5.3.1.7 Grain yield	98
5.3.1.8 Nitrogen use efficiency	102
5.3.1.9 Precipitation use efficiency	105
5.3.1.10 Return on investment.....	106
Conclusion	110
References.....	111

Chapter 6	115
The influence of nitrogen source, application rate and growth stage on the grain quality and nutrient content of a non-prolific and prolific maize cultivar in the north-eastern Free State.....	115
6.1 Introduction	115
6.2 Materials and Methods	116
6.3 Results and Discussion	116
6.3.1 Grain quality of a non-prolific and prolific cultivar	117
6.3.1.1 Protein content	117
6.3.1.2 Fat content	124
6.3.1.3 Starch content	127
6.3.1.4 Fibre content	128
6.3.1.5 Milling index (%).....	129
6.3.2 Grain nutrient content	132
6.3.2.1 N content.....	132
Grain nutrient summary of analysis	133
6.3.2.2 P content	136
6.3.2.3 Cu content (mg kg ⁻¹).....	137
6.3.2.4 Mn content (mg kg ⁻¹).....	139
6.3.2.5 Zn content (mg kg ⁻¹)	140
6.3.2.6 Fe content (mg kg ⁻¹)	142
6.3.2.7 B content (mg kg ⁻¹).....	143
Conclusion	144
References.....	145
Chapter 7	149
Summary, conclusion and recommendations	149
7.1 Introduction	149
7.2 Leaf analysis	149
7.3 Yield components and yield.....	151
7.4 Grain quality and grain content.....	152

7.5 Conclusion and Recommendations	153
References.....	154

Declaration

“Submitted in fulfilment of the requirements in respect of the master’s degree with specialisation in Agronomy in the Department of Soil, Crop, and Climate Sciences in the Faculty of Natural and Agricultural Sciences at the University of the Free State.”

A handwritten signature in black ink that reads "R. Senekal". The signature is written in a cursive style and is positioned above a solid horizontal line.

Retief Senekal

14/05/2025

Date

Acknowledgements

My Heavenly Father, for giving me the knowledge, strength, will, and responsibility to complete this study over the two years.

I would also like to express my sincere gratitude to the following persons and institutions:

- VKB Landbou and my colleagues for all their assistance, especially JT Prinsloo, who encouraged me to do my MSc and supported me throughout this study.
- Maize Trust for partly funding this study and my tuition fees. The departments, Plant Sciences (Plant Breeding) and Soil, Crop, and Climate Sciences at the University of the Free State for assisting with the grain sample analysis.
- My supervisor, Prof GM Ceronio, for his valuable guidance and advice throughout the study and for assisting me with the statistical data analysis.
- My co-supervisor, Dr E Van Der Watt, for her valuable advice with the writing of the dissertation.
- My parents, Frikkie and Chárinda, and brothers David and Derik, thank you for your moral support, patience, love, and sacrifice of family time.

Abstract

Maize is a vital crop in South Africa, making a significant contribution to the country's food and animal feed production. However, the yield of maize is heavily dependent on nitrogen fertilization, which can be costly and environmentally harmful if not managed responsibly. This study aims to examine the effects of various nitrogen sources, application rates, and growth stages on the grain yield of maize in the north-eastern Free State, South Africa. The experiment was conducted under dryland conditions over two cropping seasons, from 2022/23 to 2023/24, using a randomized complete block design with factorial arrangements. The study involved two maize cultivars (non-prolific and prolific), two nitrogen sources (LAN and Greensulf), three nitrogen topdressing application rates (0, 20, and 40 kg N ha⁻¹), and three growth stages (V4, V9, and V4 & V9).

The results indicate that Greensulf nitrogen fertilizer significantly improved the majority of leaf nutrient content, while LAN was more effective in enhancing the N:S, N:K, and Fe:Mn ratios of leaf nutrients. Leaf nutrient content increased with higher nitrogen application rates, with a 40 kg N ha⁻¹ topdressing yielding the best results. The optimal leaf content for each nitrogen source was achieved when Greensulf was applied at the V9 stage, while LAN was most effective when applied in split applications at V4 & V9.

Greensulf significantly influenced various yield components, including grain mass per cob, 1000 kernel weight (non-prolific cultivar), grain yield, and precipitation use efficiency (PUE). Conversely, LAN significantly impacted the number of cobs per plant and 1000 kernel weight (prolific cultivar). A topdressing application rate of 20 kg N ha⁻¹ generally resulted in the highest yield components and yield, although the control treatment showed the best return on investment (ROI) and nitrogen use efficiency (NUE). The non-prolific cultivar showed improved yield components and yield when nitrogen topdressing was applied at the V9 growth stage. In contrast, the prolific cultivar responded better to split applications at the V4 & V9 stages.

Most grain quality parameters improved with the application of Greensulf, except for protein content and milling index, which did not show a significant response to additional nitrogen applications. A topdressing application rate of 20 kg N ha⁻¹ resulted in the maize grain's highest fat and fibre content. The best grain quality was achieved with a nitrogen topdressing applied as a split application at V4 & V9. Greensulf generally enhanced grain nutrient content, whereas N and Zn content increased with the application of LAN. An application rate of 20 kg N ha⁻¹

yielded the highest grain nutrient content when applied in split applications at the V4 & V9 growth stages. This study emphasizes the importance of selecting appropriate nitrogen sources, application rates, and growth stages to optimize maize yield, nutritional content, and overall crop quality. These findings offer valuable insights for agricultural practices and future research in South Africa's maize industry.

Keywords: Nitrogen source, application rate, growth stage, leaf nutrient content, grain yield, grain quality, grain nutrient content

Chapter 1

1. Introduction

1.1 Background

Maize (*Zea mays* L.) is one of the most important grain crops in the world, primarily used for human consumption. It is also a key ingredient in animal feed and the production of biofuels. Maize, rice, and wheat contributed to about 30% of the food calories for more than 4.5 billion people globally. In some parts of Africa, maize alone accounts for approximately 20% or more of the food consumed (Shiferaw *et al.*, 2011). In South Africa, maize is the most important grain crop, followed by wheat, soya beans, and sunflowers. Maize is the largest locally produced field crop for human and animal consumption in the Southern African Customs Union (SACU), of which South Africa is the leading producer (Prospectus on the South African Maize Industry (PSAMI), 2021).

According to the South African Grain Information Service (SAGIS) (2015), South Africa's maize consumption amounts to 39.8% for animal feed, 37.4% for human consumption, and about 4.8% for starch and glucose products, with almost no production of biofuel products, and around 17.9% of the total maize production is exported. According to SAGIS (2015) and the Bureau for Food and Agricultural Policy (BFAP) (2015), South Africa's exports have declined from 2.2 to 1.96 million tonnes between 2014 and 2024. This decline is attributed to an increase in domestic demand for maize, which is expected to outgrow production growth, particularly for the consumption of yellow maize for animal feed. Exporting one tonne of maize only adds R853 tonne⁻¹ while it could have added on average of R2 662 tonne⁻¹ when processed to maize meal, R3 529 tonne⁻¹ when processed for starch and glucose products or when processed for broiler feed, it can add R 5 531 tonne⁻¹ (BFAP, 2015).

Nitrogen is a vital plant nutrient, and nitrogen fertilizer is globally accepted as a major yield-determining factor that has an optimal effect on almost all the yield components of maize, namely grain yield, biomass, number of rows per cob, and kernels per row only to name a few (Adhikari *et al.*, 2016). The maize plant's response to higher nitrogen fertilizer levels is attributed to being a nitro-positive crop (Adediran & Banjoko, 1995; Shanti *et al.*, 1997). Adhikari *et al.* (2021) reported that grain yield, stover yield, and biomass yield effectively increased as nitrogen levels increased from 160 kg ha⁻¹ to 240 kg ha⁻¹. A nitrogen application rate of 160 kg ha⁻¹ yielded 18.14 tonne ha⁻¹ biomass, and 220 kg ha⁻¹ nitrogen yielded 23 tonne ha⁻¹ biomass, according to Adhikari *et al.* (2021). Abera *et al.* (2013) also reported that an increase in nitrogen application amount significantly increased maize biomass yield.

Nitrogen, therefore, influences the harvest index. Adhikari *et al.* (2021) reported that a nitrogen application rate of 220 kg ha⁻¹ yielded the highest harvest index (43.8), compared to application rates of 160, 180, and 200 kg ha⁻¹ of nitrogen, which had harvest indices of 39.85, 43.08, and 43.97, respectively. Sharifi and Namvar (2016) also found that as nitrogen levels increased, the harvest index increased, with a 42.1% harvest index observed at an application rate of 225 kg ha⁻¹.

An increase in nitrogen application rates on maize fields leads to an increase in grain and biomass yield, but another factor that must be considered is nitrogen use efficiency (NUE). NUE is the amount of grain produced per unit of nitrogen applied to the crop (Abera *et al.*, 2019). Studies have shown that only 50% of the total nitrogen applied to crops is utilised efficiently, while the remaining 50% is lost to the surrounding environment, polluting surface water, groundwater, soil, and air. Chemical nitrogen fertilizers alone account for 34% of NH₃ emissions into the atmosphere, 2-60% of applied nitrogen is lost by leaching when the application is directly followed by heavy rains, and up to 70% of surface applied nitrogen is lost by soil erosion and runoff in this same scenario. Globally, a decrease in NUE over the last five to six decades has been observed, from 68% to 47%, due to a decline in soil fertility, overuse of additional nutrients, monoculture, and intensive management practices (Govindasamy *et al.*, 2023).

Zhou *et al.* (2019) found that NUE, physiological efficiency, nitrogen uptake, and grain yield increased when nitrogen was applied in four split applications at the sowing, V6, V12, and R1 growth stages of maize. When the total nitrogen application was split into four applications at planting, V12, R1, and R2 growth stages, there was an increase in plant uptake, NUE, photosynthetic efficiency, and grain yield in summer maize (Deng *et al.*, 2023). Crop rotation can also improve nitrogen availability and plant uptake when used in a cereal and legume cropping system. Gaudin *et al.* (2015) found in a 20-year trial that there was an increase in the NUE of maize when winter wheat was included in a maize-soybean crop rotation system. With the latest maize cultivars (medium to medium-short growth season length) that produce higher grain yields, the plant's nitrogen demand has increased to support its growth and deliver higher yields. With the higher demand for nitrogen, the maize plant's ability to improve nitrogen uptake did not increase in ratio as the yield component increased. The "new" cultivars do still produce better yields under lower nitrogen application rates than the older cultivars, but can use nitrogen more sufficiently when the total amount is split into more than one application and more time is spent on the specific timing of the agronomic practices (Garnett *et al.*, 2015; Govindasamy *et al.*, 2023).

An increase in domestic use of maize will lead to a change in South Africa from a net export to a net import of maize if production is not increased and a point where the economy can make more on one tonne of maize when it is processed into other products, e.g., ethanol or starch than exporting it. High nitrogen fertilizer prices and input costs can be reduced or maximized by applying nitrogen at the correct rate, at the right time, right source, and understanding the nitrogen use efficiency of the plant. This promoted the research.

1.2 Objectives

The main objectives were to determine which nitrogen source, applied at what rate, and at what growth stage, will affect maize response in the north-eastern Free State.

Sub-objectives include:

- Determining the effect of nitrogen source, topdressing application rate, and application at specific growth stages on leaf nutrient content;
- Determining the effect of nitrogen source, topdressing application rate, and application at specific growth stages on grain yield and yield components; and
- Determining the effect of nitrogen source, topdressing application rate, and applying it at specific growth stages on grain quality and return on investment.

References

- ABERA, T., WEARY, D. & DEBELE, T., 2019. Nitrogen use efficiency and yield of maize varieties as affected by nitrogen rate in mid altitude areas of Western Ethiopia. *Agric. Sci. Agron.* DOI: 10.20944/preprints201902.0239.v1.
- ABERA, W., HUSSEIN, S., DERERA, J., WORKU, M. & LAING, M.D., 2013. Preferences and constraints of maize farmers in the development and adoption of improved varieties in the mid-altitude, sub-humid agro-ecology of western Ethiopia. *Afr. J. Agric. Res.* 8(14): 1245-1254.
- ADEDIRAN, J.A. & BANJOKO, V.A., 1995. Response of maize to nitrogen, phosphorus and potassium fertilizers in the savanna zones of Nigeria. *Commun. Soil Sci. Plant Anal.* 26(3-4): 593-606.
- ADHIKARI, K., BHANDARI, S., ARYAL, K., MAHATO, M. & SHRESTHA, J., 2021. Effect of different levels of nitrogen on growth and yield of hybrid maize (*Zea mays* L.) varieties. *J. Agric. Nat. Resour.* 4(2): 48-62.
- ADHIKARI, P., BARAL, B.R. & SHRESTHA, J., 2016. Maize response to time of nitrogen application and planting seasons. *JMRD.* 2(1): 83-93.
- DENG, T., WANG, J. H., GAO, Z., SHEN, S., LIANG, X. G., ZHAO, X., 2023. Late split-application with reduced nitrogen fertilizer increases yield by mediating source–sink relations during the grain filling stage in summer maize. *Plants.* 12 (3): 625.
- GARNETT, T.P., PLETT, D., HEUER, S. & OKAMOTO, M., 2015. Genetic approaches to enhancing nitrogen-use efficiency (NUE) in cereals: Challenges and future directions. *Funct. Plant Biol.* 42(10): 1071-1092.
- GAUDIN, A. C., JANOVICEK, K., DEEN, B., HOOKER, D. C., 2015. Wheat improves nitrogen use efficiency of maize and soybean-based cropping systems. *Agric. Ecosyst. Environ.* 210: 1–10.
- GOVINDASAMY, P., MUTHUSAMY, S.K., BAGAVATHIANNAN, M., MOWRER, J., JAGANNADHAM, P.T.K., MAITY, A., HALLI, H.M., SUJAYANANAD, G.K., VANDIVEL, R., DAS, T.K., RAJ, R., POONIYA, V., BABU, S., RATHORE, S.S., MURALIKRISHNAN, L. & TIWARI, G., 2023. Nitrogen use efficiency-a key to enhance crop productivity under a changing climate. *Front. Plant Sci.* 14. DOI: 10.3389/fpls.2023.1121073.
- PROSPECTUS ON THE SOUTH AFRICAN MAIZE INDUSTRY (PSAMI), 2021. Description on South African Maize supplied by PSAMI. Pretoria: The Maize Trust.

- SHANTI, K., PRAVEEN, V.R., RANGA, R.M., SURYANAYANA, M.R. & SARMA, P.S., 1997. Response of maize (*Zea mays*) hybrid and composite to different levels of nitrogen. *Indian J. Agric. Sci.* 67(9): 424-425.
- SHARIFI, R.S. & NAMVAR, A., 2016. Effects of time and rate of nitrogen application on phenology and some agronomical traits of maize (*Zea mays* L.). *Biologija.* 62(1): 35-45.
- SHIFERAW, B., PRASANNA, B.M., HEILLIN, J. & BÄNZIGER, M., 2011. Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. *Food Sec.* 3(3): 307-327.
- SOUTH AFRICAN GRAIN INFORMATION SERVICE (SAGIS), 2015. Total Maize Supply and Demand Table, Published on 24 February 2015. <https://sagl.co.za/wp-content/uploads/Reports/Maize/2015.2016/Maize-Crop-Report-2015-2016.pdf>.
- THE BUREAU FOR FOOD AND AGRICULTURAL POLICY (BFAP), 2015. Adding value in the South African maize value chain. https://www.sagis.org.za/BFAP_2015.pdf.
- ZHOU, B., MING, Z., XUEFANG, S., DAN, W., ZAIKONG, D. & CONGFENG, L., 2019. Integrated agronomic practice increases maize grain yield and nitrogen use efficiency under various soil fertility conditions. *Crop J.* 7 (4): 527–538.

Chapter 2

Literature Review

2.1 Origin, Production, and Importance of Maize in South Africa

Maize (*Zea mays* L.) is an important grain crop belonging to the Poaceae family. *Zea* is an ancient Greek word meaning “sustaining life,” and *mays* is from the Taino language, meaning “life giver” (Shah *et al.*, 2016). Maize was first cultivated in the 1650s in South Africa and has since become one of the most important grain crops in the country, contributing to 25% of the total arable land cultivated under field crops (Kriel, 2022). In 1997, the maize industry of South Africa was deregulated. Producers were able to compete in a free market. Hectares planted annually range from 3.8 to 4.8 million hectares, of which more than 60% were produced in the Free State and the Northwest provinces of South Africa (National Department of Agriculture (NDP), 1999; Kriel, 2022).

Maize is classified into two main types: white and yellow maize. White maize is primarily used for human consumption, while yellow maize is mainly used for animal feed (Kriel, 2022). South Africa produces approximately 11.8 million tonnes of maize annually, of which approximately half of the production consists of white maize for human consumption. Maize in the form of maize meal forms part of 200 million people’s staple diet in developing countries and is also used as starch and fuel in the form of ethanol, which is only a few of the maize uses in the world (Agricultural Research Council of South Africa (ARC), 2003; The Bureau for Food and Agricultural Policy (BFAP), 2015). The human consumption of maize in South Africa is trending at 4.5 million tonnes per annum and 4.8 million tonnes per annum for animal feed consumption. The reason for the higher increase in animal feed consumption is that South Africans in the middle and upper classes consume more maize secondary products, such as eggs, cheese, and meat products, than maize itself. South Africa is a net maize exporter, exporting 17.9% of total maize production, or 2.2 million tonnes (BFAP, 2015).

2.1.2 Nitrogen Fertilizer

Fast-growing crops require high nitrogen inputs to support their rapid growth. If nitrogen is deficient during production, physiological symptoms can occur and reduce yield (Olfati *et al.*, 2014). Nitrogen is a very limited nutrient because of its ability to be lost from the soil in different soil processes, namely nitrate leaching, volatilization, immobilization, and denitrification losses.

Mineral fertilizers are mostly used because of their solubility and relatively fast nitrogen release. Therefore, it is readily available to the crop and can promote yield increases, but it must be managed responsibly to prevent water pollution (Besen *et al.*, 2020). One of the methods to manage nitrogen fertilizers is split applications, where the amount of nitrogen fertilizer is broken up into smaller quantities, when applied at different plant growth stages. When the application is split, the crop can use the nutrient more efficiently and improve yield, while reducing fertilizer losses (Olfati *et al.*, 2014).

2.1.2.1 Nitrogen sources

a) Urea

Urea consists of 46% nitrogen in a concentrated solid fertilizer form. Urea is mainly used as a top dressing because less fertiliser can be applied to the area because of its high nitrogen content compared to other nitrogen granular fertilizers. It dissolves quickly in warm, moist conditions. One of urea's challenges is that it can temporarily lower the soil pH because when the soil takes it up, it changes to ammonium carbonate through the action of the urease enzyme (Finch *et al.*, 2014). Ammonium carbonate is rapidly converted to ammonia and can be lost to the atmosphere through volatilization depending on the air temperature, soil moisture, humidity, soil pH, and wind speed. Up to 50% of the amount of nitrogen can be lost to the atmosphere when urea is applied to the surface and not incorporated into the soil by tillage or sufficient (>10 mm) rain within 24 to 48 hours, especially in high pH (>7 pH) soils (Bremner, 1990; Noble Research Institute, 2006).

b) Limestone Ammonium Nitrate (LAN)

LAN is a granular fertiliser composed of a mix of ammonium nitrate and lime, containing 28% nitrogen, 4.8% Calcium, and 2.6% magnesium. The 28% nitrogen consists of 14% N in the nitrate (NO₃⁻) form and 14% in the ammonium (NH₄⁺) form. Half of the nitrogen is immediately available in the nitrate form for plant uptake, and the other half of the nitrogen is in the ammonium form and will be available later to plants when the nitrification process has taken place. Mostly used on soils with a lower pH because LAN has a lesser degree of acidifying to the soil because of the calcium carbonate content (Finch *et al.*, 2014; Hawksworth, 2017).

c) Ammonia gas (NH₃)

This gaseous nitrogen fertilizer is liquefied under high pressure in special containers and injected 12 to 20 cm into the soil from these pressurised containers with pipes fitted at the back of a tine applicator. The organic matter and clay minerals rapidly absorb ammonia in the soil, resulting in minimal loss if the soil is in a friable condition. The cost of applying ammonia gas is much higher than other nitrogen sources, but when compared to the kilogram of nitrogen

applied per kilogram of source applied, it makes it worthwhile to use since ammonia gas consists of 82% nitrogen and does not have a high acidifying effect on the soil. This fertilizer also has drawbacks because it cannot be used on cloddy or stony soils where losses will be significant (Finch, *et al.*, 2014).

d) Greensulf

Greensulf is an ammonium nitrate in a chemically granulated form that contains 26% nitrogen (50% nitrate and 50% ammonium form) and also contains 5% calcium and 4% sulfur, which is beneficial for plants (Smith, 2020).

2.2 Nitrogen in the Soil, Plant Uptake, and Role in Plant Physiology

One of the most essential nutrients for plants is nitrogen, which is involved in many vital biological functions. Although about 78% of the Earth's atmosphere is nitrogen gas (N_2), plants are unable to use atmospheric nitrogen directly. Instead, they rely on nitrogen available in the soil, which exists in several forms, such as ammonium (NH_4^+), nitrate (NO_3^-), and organic nitrogen compounds. Efficient nitrogen uptake and assimilation are vital for plant growth, photosynthesis, and overall productivity (Forde & Clarkson, 1999; Aczel, 2019).

2.2.1 The Nitrogen Cycle and Soil Nitrogen Forms

2.2.1.1 The Nitrogen Cycle

The nitrogen cycle is a complex biogeochemical process that governs the transformation of nitrogen compounds in the soil and atmosphere. Nitrogen is first fixed into a usable form by nitrogen-fixing bacteria through symbiotic relationships (e.g., *Rhizobium* in legumes) or free-living bacteria. This process converts atmospheric nitrogen (N_2) into ammonium (NH_4^+), a form that plants can directly absorb (Streeter, 2007). Once in the soil, ammonium can be further oxidised by nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*) into nitrate (NO_3^-), which is highly mobile in the soil and more easily taken up by most plants (Schimel & Bennett, 2004; Bernhard, 2010).

The process of nitrification is a two-step process: ammonium is first oxidised to nitrite (NO_2^-) by *Nitrosomonas*, and then nitrite is oxidised to nitrate (NO_3^-) by *Nitrobacter*, a *nitrite-oxidising bacterial* species. Denitrification, performed by facultative anaerobic bacteria, reduces nitrate to nitrogen gas (N_2) or nitrous oxide (N_2O), releasing it back into the atmosphere (Haynes, 1986; Bernhard, 2010). Thus, nitrogen moves continuously between its various chemical forms in the soil and atmosphere, impacting soil fertility and plant growth (Aczel, 2019).

2.2.2 Forms of Nitrogen in the Soil

Soil nitrogen is present in various forms for plant absorption:

a) Ammonium (NH_4^+)

Plant roots directly absorb this cationic form of nitrogen. Ammonium is less mobile in the soil than nitrate due to its positive charge, which causes it to bind to soil particles. While ammonium is a readily available nitrogen source for plants, excessive accumulation can lead to soil acidification (Loqué *et al.*, 2007).

b) Nitrate (NO_3^-)

Nitrate is the most abundant form of nitrogen in soils, especially in well-drained and neutral to slightly alkaline soils. It is highly soluble and can leach from the soil, making it a primary nitrogen source in areas with high rainfall or excessive irrigation. While plants easily absorb nitrate, its mobility can lead to nitrogen loss and environmental contamination (Schimel & Bennett, 2004).

c) Organic Nitrogen

Organic nitrogen sources, which include amino acids, peptides, and proteins, are present in decomposed plant and animal material, soil organic matter, and microbial biomass. This form of nitrogen must undergo mineralization, a microbial process that breaks down organic nitrogen into inorganic forms (ammonium or nitrate) that plants can use. Organic nitrogen is essential in long-term nutrient cycling and soil fertility (Haynes, 1986; Aczel, 2019).

2.2.3 Nitrogen Uptake Mechanisms in Plants

2.2.3.1 Root Uptake

Plants primarily absorb nitrogen through their roots, with specialized transporters facilitating the uptake of ammonium (NH_4^+) and nitrate (NO_3^-). These transport mechanisms are regulated by the availability of nitrogen in the soil and the plant's internal nitrogen status (Pokapū Akoranga Pūtaiao, 2019).

a) Ammonium Transporters (AMTs)

Ammonium uptake is facilitated by Ammonium Transporters (AMTs), membrane proteins that actively transport NH_4^+ from the soil into the plant cells. The transport process is driven by the electrochemical gradient of protons (H^+) and the concentration gradient of ammonium ions. Ammonium uptake is favoured in more acidic soils where NH_4^+ is more abundant. However, high ammonium concentrations can lead to toxicity, as excess ammonium accumulates in plant tissues and may acidify the cytoplasm (Sivaguru *et al.*, 2003).

b) Nitrate Transporters (NRTs)

Nitrate uptake is facilitated by Nitrate Transporters (NRTs), which are divided into two prominent families based on their affinity for nitrate: NRT1 (low-affinity transporters) and NRT2 (high-affinity transporters). NRT1 is responsible for nitrate uptake under low-nitrogen conditions, while NRT2 functions more efficiently under high-nitrogen conditions (Forde & Clarkson, 1999; Masclaux-Daubresse, 2010). Nitrate uptake is also influenced by the plant's internal nitrogen status, with plants adjusting the expression of NRT genes in response to changing nitrogen availability (Loqué *et al.*, 2007).

2.2.3.2 Symbiotic Nitrogen Fixation

In legumes, symbiotic nitrogen fixation is a crucial mechanism that makes nitrogen available to plants. This process involves the formation of root nodules, where nitrogen-fixing bacteria such as *Rhizobium* species convert atmospheric N₂ into ammonia (NH₃) in exchange for carbon provided by the plant. The ammonia is then assimilated into amino acids, which the plant uses for growth (Streeter, 2007). Nitrogen fixation is a highly energy-intensive process, and the symbiotic relationship between legumes and nitrogen-fixing bacteria significantly reduces the need for synthetic nitrogen fertilizers (Zayed *et al.*, 2023).

2.2.3.3 Mycorrhizal Fungi and Nitrogen Uptake

Mycorrhizal fungi form mutualistic associations with many plant species, particularly in nutrient-poor soils. While mycorrhizal fungi are most associated with phosphorus uptake, recent studies suggest that these fungi can also aid in nitrogen acquisition, especially in low-nitrogen environments (Smith & Read, 2008). The hyphal network of mycorrhizae extends the plant's root surface area, allowing it to access nitrogen in forms such as ammonium and nitrate that may not be available to the plant through its root system alone (Grunewald *et al.*, 2009).

2.2.4 Nitrogen Assimilation and Transport within the Plant

2.2.4.1 Nitrogen Assimilation Pathways

Nitrogen must be incorporated into organic compounds after the root system has absorbed it. Two important enzymes play a major role in this assimilation process:

a) Glutamine Synthetase (GS)

GS converts glutamate and ammonium into glutamine, a vital nitrogen donor for several biosynthetic processes. Glutamine is an essential building block for proteins and other nitrogen-containing compounds, such as hormones and nucleic acids (Zayed *et al.*, 2023).

b) Glutamate Dehydrogenase (GDH)

GDH also contributes to nitrogen assimilation by transforming ammonium and glutamate into glutamine. Under stressful circumstances or when there is a high availability of nitrogen, GDH is especially crucial (Zayed *et al.*, 2023).

2.2.4.2 Nitrogen Transport in Plants

Once nitrogen is assimilated, it is transported throughout the plant, primarily through the xylem and phloem. Nitrogen is translocated as amino acids (e.g., glutamine and asparagine) and other organic nitrogen compounds synthesised in the roots and transported to leaves, stems, and reproductive tissues. In the xylem, nitrogen is transported as inorganic compounds (ammonium or nitrate) from the roots to the rest of the plant. On the other hand, the phloem is involved in transporting organic nitrogen compounds, especially during periods of rapid growth or reproductive development (Vanderhoef, 1985; Zayed *et al.*, 2023).

2.2.4.3 Role in Photosynthesis and Growth

Nitrogen plays a vital role in photosynthesis, as it is a significant component of chlorophyll. Nitrogen deficiency often leads to chlorosis (yellowing) of leaves due to decreased chlorophyll content. Reduced chlorophyll directly impairs the plant's ability to capture light energy for photosynthesis, ultimately leading to reduced growth and yield (Guldan *et al.*, 2016). Adequate nitrogen also supports the synthesis of proteins involved in the electron transport chain and other processes critical to photosynthesis (Tezara *et al.*, 2011).

2.2.5. Role of Nitrogen in Plant Growth and Development

2.2.5.1 Protein Synthesis

Nitrogen is essential for plant protein synthesis since it is a fundamental component of amino acids. Proteins serve as structural elements, transport molecules, and enzymes. They are necessary for cellular functions like growth regulation, energy metabolism, and pathogen defence. A lack of nitrogen results in lower protein content, leading to poor leaf and root development, stunted growth, and decreased productivity (Haynes, 1986).

2.2.5.2 Chlorophyll Synthesis

Nitrogen is a molecule found in chlorophyll that is a regulator of absorbing light energy during photosynthesis. Chlorophyll content is decreased by nitrogen deficiency, resulting in chlorosis and lowering the plant's photosynthetic efficiency. This decrease in photosynthesis directly impacts growth, as it limits the plant's capacity to produce the carbohydrates necessary for growth (Tezara *et al.*, 2011).

2.2.5.3 Root Development

Excessive nitrogen can cause unbalanced growth, where the plant allocates more resources to developing its shoots and fewer to developing its roots, despite the fact that nitrogen is essential for both shoot and root growth. Ultimately, this imbalance may jeopardize the plant's health by limiting its access to water and other nutrients (Forde & Clarkson, 1999).

2.2.5.4 Reproductive Development

Adequate nitrogen supply is crucial for reproductive development, including flowering, fruit set, and seed production. Nitrogen is necessary for the synthesis of nucleic acids and proteins, which are necessary for cell division and differentiation during the formation of flowers, seeds, and fruit. Nitrogen deficiency can delay flowering, reduce seed size, and lower overall reproductive success (Vanderhoef, 1985).

2.2.6. Nitrogen Use Efficiency and Agricultural Implications

2.2.6.1 Nitrogen Use Efficiency (NUE)

Nitrogen Use Efficiency (NUE) refers to the ability of plants to take up, assimilate, and utilize nitrogen efficiently. High NUE is essential to maximize crop yields while reducing the amount of nitrogen fertilizer applied. Plant genetics, soil conditions, and nitrogen input management are among the key factors that influence NUE (Haynes, 1986). Because they can lessen the environmental impact of fertilizer use, genetic advances in crops targeted at increasing NUE are a potential field of research (Schimel & Bennett, 2004).

2.2.6.2 Environmental Impact

Overfertilization with nitrogen can lead to eutrophication, resulting in an excessive growth of algae in water bodies, and nitrogen leaching can contaminate groundwater. Nitrous oxide (N₂O), a potent greenhouse gas, is also released by nitrogen fertilizers (Schimel & Bennett, 2004; Aczel, 2019). Sustainable nitrogen management techniques are essential to lessen the environmental impact of nitrogen use in agriculture. These include crop rotation, slow-release fertilizers, and encouraging nitrogen-fixing crops like legumes (Schimel & Bennett, 2004).

2.3 Influence of Nitrogen on Maize Plants

2.3.1 Growth and yield

Maize growth and yield response heavily depend on nitrogen supply during its growing cycle. An efficient supply of nitrogen has a profound impact on the number of leaves produced and the size of the leaves, directly influencing the amount of radiation intercepted over the growing period and the daily rate of photosynthesis of the plant (Vos *et al.*, 2005). This enhanced photosynthetic capacity, a key factor in crop yield, can significantly increase biomass and grain

yield. After anthesis, maize's rate of N uptake decreases, and hybrids with higher N uptake have longer effective leaf area for photosynthesis and delayed leaf senescence. Since ongoing leaf activity promotes nitrogen uptake, maize hybrids with delayed senescence are more able to absorb nitrogen during the grain-filling phase. Dry matter partitioning to grain is reduced when the nitrogen supply declines during grain filling. Longer stay-green maize genotypes retain leaf chlorophyll for longer periods, which can boost grain yield by 10% to 12% (Asibi & Chai, 2019).

Preserving the photosynthate source and nitrogen uptake during the grain-filling phase is responsible for a high maize yield. The ability of the plant to provide the root system with the necessary assimilates for root development and nitrogen uptake, thereby sustaining photosynthesis, is a prerequisite for improving NUE during maize reproductive growth. This involves increasing and maintaining the capacity for nitrogen uptake in soils with low nitrogen supply (Yu *et al.*, 2021). Increases in grain production among maize genotypes at a given amount of nitrogen supply are sometimes the consequence of improved nitrogen remobilization and assimilation. However, they can also be attributed to increased NUE (Asibi & Chai, 2019). Grain yield is influenced by the vegetative development and establishment of the reproductive sink capacity, which is facilitated by maize's early growing season nitrogen remobilization. Enhancing NUE requires a stronger relationship between crop nitrogen uptake and nitrogen supply (Borrás *et al.*, 2004). Even with a significant sink capacity for grain protein synthesis, physiological constraints can cause a shortage of nitrogen uptake, leading to nitrogen loss, increased remobilization of nitrogen from vegetative tissues to grain, and low NUE (Rizzi *et al.*, 1996). Soil nitrogen supply affects the relationships among nitrogen accessibility, late-season nitrogen uptake by maize, and nitrogen remobilization. Since grain formation needs more nitrogen than vegetative tissue maintenance, a low nitrogen supply after anthesis can cause early leaf senescence (Sattelmacher *et al.*, 1994).

In maize, there is a significant correlation between photosynthesis and nitrogen intake, leading to an inverse association between nitrogen remobilization and nitrogen assimilation. Reduced nitrogen remobilization is linked to growing conditions that support post-anthesis photosynthesis, as they increase nitrogen intake. The grain's nitrogen content is directly correlated with the amount of post-anthesis nitrogen uptake and assimilation, and the amount of nitrogen remobilization is positively correlated with aboveground nitrogen content at anthesis when growing conditions are unsuitable for photosynthesis. Variations in total aboveground maize nitrogen uptake after anthesis are the primary cause of variations in post-anthesis nitrogen uptake in grain (Asibi & Chai, 2019).

2.3.2 Nitrogen and sulphur ratio

Following nitrogen, phosphorus, and potassium, sulphur is the fourth primary essential plant nutrient. Sulphur is vital for plant growth and related chlorophyll formation, protein production, enzyme activation, and ultimately increased crop yields, to name a few. In the past, when the yields produced per hectare were not as high as nowadays, the amount of atmospheric sulphur (SO₂ gas) was enough to support the plant's sulphur demands. Still, it alone is not enough anymore to supply the necessary amounts required by the plants (Olson, 2021). According to Liu *et al.* (2020) and Li *et al.* (2019), the nitrogen and sulphur ratios (N:S), which are suitable for optimal growth of maize, are in the range of 14.4:1 to 18.7:1 when using sap analyses. In the case of grain protein concentration, an N:S range of 12.2:1 to 14.0:1 is the optimum ratio in grain analyses.

2.3.3 Maize growth stages

Understanding a maize plant's different growth stages and development is crucial in optimizing yield and profit in a maize farming practice (Figure 2.1 & Figure 2.2). This may assist a producer in timing top-dressing fertilizers, fungicides, insecticides, and even herbicides (BAYER Crop Science, 2020). The growth stages of maize are divided into two main stages: the vegetative stage (V) and the reproductive stage (R) (Figure 2.1).

a) Vegetative growth stages in maize

The number of collars on the maize plant defines the vegetative stage. The leaves are counted from the lowermost first to the topmost fully expanded leaf. The collar connects the leaf sheath and the leaf blade (Bell, 2017).

VE is the emergence stage, and a soil temperature of at least 10 °C is required for a maize seed to germinate. For the seed to emerge, it takes an average of 6 to 10 days, but can take up to two weeks (in cool and dry conditions), depending on soil temperature (MasSeeds, 2022). In this stage, maize emerged from the soil, but no leaf collars had formed. The plant has reached the V1 stage, at which one leaf collar is visible. During stage V1, nodal roots elongate, and by V2, the maize plant will be 5 to 10 centimetres (cm) tall and still rely on the energy of the seed (Figure 2.1) (Bell, 2017; Dekalb Asgrow, 2020).

The number of fully unfolded collars determines every vegetative growth stage (V); for example, V3 has three fully unfolded collars. V3 will appear about 2 to 3 weeks after VE, and the maize plant is no longer dependent on the seed's reserves and switches to photosynthesis and nodal roots for energy (Dekalb Asgrow, 2020). From this growth stage, the plant becomes very sensitive to nutrient deficiencies, especially phosphorus. During the V3 growth stage, the

number of rows per ear is already determined, and any form of stress will have a negative impact on the yield (BAYER, 2020; Mas Seeds, 2022). At V5, the plant is about 20 to 30 cm tall, and the growth point remains below the soil surface. At this growing stage, the number of potential leaves and ear shoots are determined (Bell, 2017; Dekalb Asgrow, 2020).

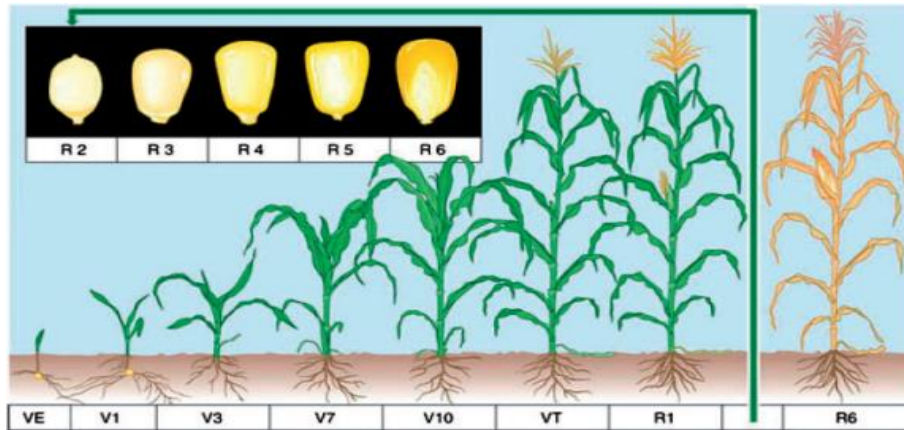


Figure 2.1 Different growth stages of the maize plant, from vegetative (V) to reproductive (R) stages (Zaho et al., 2011).

Growth stages V6 to V8: During these growth stages, 4 to 6 weeks after emergence, the growth point is above the soil surface, making the plant very susceptible to hail and wind damage (Bell, 2017). Stem elongation and a rapid growth phase begin at V7. The number of potential kernels per row is also determined and continues throughout V15 to V16 (Procrop Profitable, Sustainable Cropping (Procrop), 2009; Bell, 2017). When the V8 stage is reached, the plant will be approximately 60 cm tall. From V9 to V11, maize undergoes a steady and rapid growth period, accompanied by dry matter accumulation, which typically occurs about 6 to 8 weeks after emergence. Rapid tassel development appears at the V9 growth stage, but is not visible yet. During this growth period, new leaves appear every 2 to 3 days, and the development of ear shoots also occurs (Procrop, 2009; Dekalb Asgrow, 2020).

Growth stages V12 to V^{nth}: At V12 the plant reaches a length of about 1.2 meters (m) tall or even more, about 6 to 8 weeks after emergence. The demand for nutrients and water is high for growth in this growth period. The leaves are all full-size, and approximately 50% of them are exposed to sunlight. The potential number of kernels and size of the ear are still determined, and brace roots are also developing during this growth stage (Procrop, 2009; Bell, 2017). At V15, the plant will start with silking, and the tassel is almost full size, but not yet visible. This is a critical stage in the plant's life, and any shortages in moisture and nutrients, or insect and hail injury, can reduce the number of kernels per row, shorten ears, and decrease yield (Dekalb Asgrow, 2020).

VT growth stage begins when the last branch of the tassel is visible. The maize plant is almost at its full height, and the pollen shed begins. This occurs approximately 9 to 10 weeks after emergence, when the plant reaches a critical stage that requires pollination to develop viable kernels. The plant is at full size, tassels are visible on top of the maize plant, and silks will emerge within 2 to 3 days. A maize tassel can produce between 2 and 5 million pollen grains. During this stage, pollen shed will begin and continue for about 1 to 2 weeks (Procrop, 2009; Dekalb Asgrow, 2020).

b) Reproductive stages in maize

Following tassel emergence, maize plants transit into the reproductive growth stage (R). This growth stage is determined by kernel development (Figure 2.1). The R1 growth stage is the silking stage, the most critical growth stage in determining the yield potential. Pollination begins at the bottom and proceeds to the tip. Pollen is shed for a couple of days, usually 5 to 10 days, and silks are produced 1 to 3 days after pollen shed has begun and will be ready for pollination within 3 to 5 days (Procrop, 2009). At this stage, potassium uptake is complete, and phosphorus and nitrogen (Figure 2.2) uptake is rapidly occurring. When the silking date is reached, approximately 50 to 55 days can be added to estimate when the plant will reach physiological maturity growth stage (Dekalb Asgrow, 2020).

The blister or R2 growth stage takes place about 12 days after silking when silks darken and dry out. The kernels are white and form a small blister that contains a clear fluid (Procrop, 2009; Bell, 2017). Each kernel develops an embryo and contains 85% moisture. Drought stress during this stage can cause kernel abortion and reduce yield potential (Bell, 2017; Dekalb Asgrow, 2020).

About 20 days after silking the R3 growth stage is reached, which is the milk stage, at this stage, the kernels are yellow and filled with a milky white fluid as starch and protein accumulate (Procrop, 2009). The moisture content in the kernels is about 80%. From this stage onwards, the effect of water and nutrient stress is not as severe as in R1, but it can still impact kernel weight and the abortion of kernels at the tip of the cob (Procrop, 2009; Dekalb Asgrow, 2020).

Growth stage R4 (dough) occurs approximately 26 days after silking, when the milky white liquid in the kernels transitions to a dough-like consistency (Procrop, 2009). At this stage, the moisture content in kernels is about 70%, and the kernels begin to dent at the top. The kernels have accumulated about 50% of their final dry weight. Shallow or unfilled kernels can be produced when the plant experiences any environmental stress (Dekalb Asgrow, 2020).

Growth stage R5 (dent) occurs approximately 38 days after silking, at which point nearly all kernels are dented and contain approximately 55% moisture. Approximately 90% of the kernels are dented, the embryo has nearly reached its full size, and filling begins to slow down, as the kernels approach maturity (Procrop, 2009). The maize is ready for silage harvest at this stage, depending on the desired plant moisture (Dekalb Asgrow, 2020).

Black layer or growth stage R6 begins about 50 to 60 days after silking. At this stage, the plant has reached physiological maturity, and kernels have attained their maximum dry weight, with approximately 30 to 35% moisture content (Bell, 2017). A layer of tissue forms at the tip of the kernel, which conducts sugar and other assimilates into the kernel. This layer collapses, stops functioning and turns black. The total yield is determined, and no stress will impact the yield (Procrop, 2009; Dekalb Asgrow, 2020).

2.3.4 Nitrogen uptake by the maize plant

A plant's nutrient uptake is most significant during the vegetative growth stages. This is consistent with the physiology of maize growth. Maize roots must search the soil for nutrients. Newly formed roots are the most efficient for nutrient uptake; however, the entire root system can also take up nutrients (Strachan & Jeschke, 2018). The rate of nutrient uptake is relatively slow in the early season of a maize plant, but the rate increases as the plant develops and grows (Figure 2.2). Soon after silking, potassium uptake is completed, but nitrogen and phosphorus uptake continue until almost maturity. During grain development, the translocation of nutrients from vegetative tissues to the grain is characterized by higher amounts of nitrogen and phosphorus, whereas potassium is remobilized in comparatively smaller amounts (Hanway & Benson, 1986).

Nitrogen demand increases dramatically about 40 days (V6 to V8) after emergence. Before this time, about 18 to 20% of the total nitrogen demand by the plant had been taken up. By the time the plant reached the end of silking, it should have taken up about 75% of the total nitrogen demand (Table 2.1) (Procrop, 2009; Ross *et al.*, 2013).

During the V10 to V14 growth stages of maize, the plant takes up the highest rate of nitrogen, making it critical to have sufficient nitrogen available during this peak time. Most of the nitrogen is taken up during vegetative growth, with more or less two-thirds of the uptake completed at the beginning of R1. Still, nitrogen uptake does not cease at the start of the reproductive stage. Nitrogen uptake begins to decrease at equal rates for both the vegetative and reproductive stages as it reaches the VT/R1 growth stages. The reproductive growth has a slight increase

until R2, and then there is a sharp decrease until almost R4, when it reaches a steady decrease in nitrogen uptake (Figure 2.3).

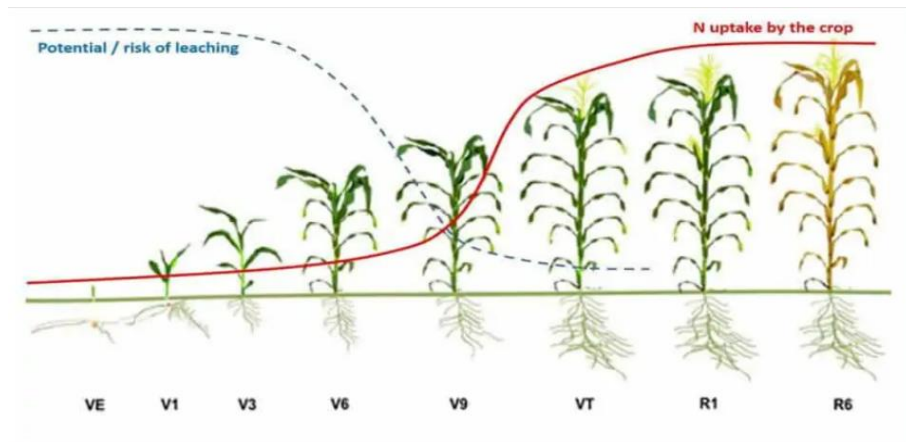


Figure 2.2 Nitrogen uptake at different growth stages (Jones, 2017).

This decrease in nitrogen uptake is due to the increased retranslocation of nutrients from other parts of the plant to accumulate in the developing seeds during the grain-filling stage (Ross *et al.*, 2013). Topdressing of nitrogen should be applied between V4 to V8 to align nitrogen availability with the crop demand. This is also the last opportunity for application, because after this stage, the plants will be damaged by the machinery when cultivated under dryland conditions. In the case of irrigated maize, nitrogen can be supplied through fertigation to time the application more efficiently (Dekalb Asgrow, 2013).

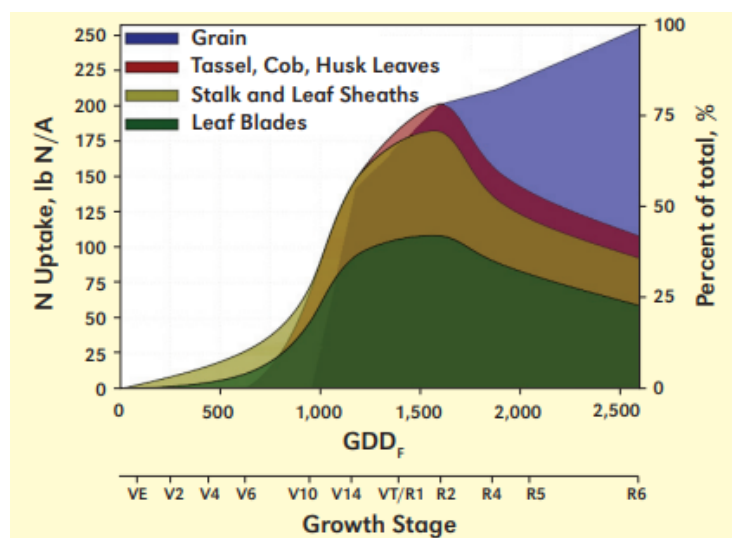


Figure 2.3 Total maize nitrogen uptake and partitioning across four plant stover fractions: Leaf, stalk, reproductive and grain tissues (Ross *et al.*, 2013).

Table 2.1 NPK removal for a short-growth and a medium-growth cultivar as a percentage of the total for different growth stages (Fertiliser Association of South Africa (FERTASA), 2016)

NPK removal as % of total							
(Short grower = 100-117 days and medium grower = 118-130 days to maturity)		N		P		K	
Growth stages		Short	Medium	Short	Medium	Short	Medium
R6 Mature	Physiologically mature	4	4	7	6	2	0
R5	Full dent stage	6	6	9	8	3	8
R3-R4	R3-Soft -to R4-Hard dough stage	5	14	14	16	4	2
R2	R2 water-blister stage	9	8	14	17	18	17
V14-V18/R1	V16-17- Plants approximately 10 days from tassel emergence. V18- Nodes of the ear stem are clearly visible. R1- The end of the vegetative growth stage. First silk appears on the ear.	18	23	14	21	19	25
V11-V14	V12- Tassel is already well developed, while ear development is visible on the stalk. The yield potential is determined at this stage.	28	27	23	24	27	39
V7-V10	V7- The maize plant commences active vegetative growth, and older leaves start dropping. V8- The first five leaves separate from the stalk. The seed coat is still visible. Secondary roots system is already well developed. The radicle is visible and displays greater vertical growth.	17	12	11	4	16	12
V5-V6	V5- The first five leaves form a collar around the stalk. The growing tip is at the soil surface.	4	4	3	4	5	3
V3-V4	V3-V4 – 3 rd and 4 th leaves, respectively, have also developed a collar around the stalk section.	4	2	3	0	3	1
V1-V2	V1± 4-5 days after emergence, leaf with a collar around the stalk section; has an oval-shaped tip. V2- 2 nd leaf has also developed a collar around the stalk section. Radicles are fully developed.	3	0	2	0	3	0
Germination	Emergence of the plant usually occurs 6 to 7 days after planting, depending on heat units. In cold conditions, emergence can be delayed by quite a few days.	0	0	0	0	0	0

Stages represent a specific quantity of fully developed leaves

2.3.5 Sufficiency ranges of nutrients at different growth stages of a maize plant

Nutrient elements are essential for plant growth, as they support critical metabolic functions within plants. The absence of these essential nutrients inhibits the ability of plants to complete their life cycles (Brown *et al.*, 2022). Furthermore, the roles of essential nutrients are specific; thus, the excess of one nutrient cannot compensate for the deficiency of another, as illustrated by the “Law of the Minimum” and the “Law of the Optimum.” Ensuring adequate and balanced plant nutrition is vital for crops to realize their genetic growth potential (Amisshah *et al.*, 2022).

As a high-input crop, maize has significant nutrient requirements and is particularly vulnerable to nutrient imbalances. Such imbalances can negatively impact nutrient uptake, utilization, and overall productivity. Plant tissue analysis is a valuable tool for monitoring the nutritional health of crops (Table 2.2). It complements soil tests by providing insight into the nutrient levels of plants at the time of sampling, thereby facilitating timely nutrient management decisions. For plant tissue analysis to be effectively utilized as a management tool, it is important to have a clear and convenient interpretation of the analysed results (Ruiz Diaz, 2021; Plank & Kissel, 2024).

Table 2.2 Sufficiency ranges of macro- and micronutrients for a maize plant at different growth stages (Campbell & Plank, 2000)

Stage of the plant	Macronutrients (g kg ⁻¹)					
	N	P	K	Ca	Mg	S
Seedling (VE to V4)	4.0-5.0	0.4-0.6	3.0-4.0	0.3-0.8	0.2-0.6	0.18-0.5
Early growth (V5 to before tasselling)	3.0-4.0	0.3-0.5	2.0-3.0	0.25-0.8	0.15-0.6	0.15-0.4
Tasselling (VT)	2.8-4.0	0.25-0.5	1.8-3.0	0.25-0.8	0.15-0.6	0.15-0.6
Maturity (R6)	2.5-3.5	0.25-0.4	1.6	2.5	0.2-0.8	0.12-0.4
	Micronutrients (mg kg ⁻¹)					
	Fe	Mn	Zn	Cu	B	Mo
Seedling (Ve to V4)	40-250	25-160	20-60	6-20	5-25	0.1-2.0
Early growth (V5 to before tasselling)	30-250	20-150	20-70	5-25	5-25	0.1-2.0
Tasselling (VT)	30-250	15-150	20-70	5-25	5-25	0.1-2.0
Maturity (R6)	30-250	15-150	16-50	4-20	3-20	0.1-2.0

Nitrogen is a crucial nutrient for plants, affecting nearly every aspect of their growth and development. From the nitrogen cycle and uptake mechanisms to nitrogen assimilation and transport, plants have developed complex systems to acquire and use nitrogen efficiently. Although nitrogen is necessary to support growth and productivity, environmental problems

such as nitrogen leaching and greenhouse gas emissions make managing it in agriculture extremely challenging. Maintaining global food security and reducing the environmental impact of nitrogen fertilization requires ongoing research into sustainable fertilization techniques and nitrogen use efficiency (NUE).

References

- ACZEL, M.R., 2019. What is the Nitrogen Cycle and Why Is It Key to Life? *Front. Young Minds*. 7(41). DOI: 10.3389/frym.2019.00041.
- AGRICULTURAL RESEARCH COUNCIL OF SOUTH AFRICA (ARC), 2003. *Maize production supplied by ARC-Grain Crops Institute*. Potchefstroom: ARC-Grain Crop Institute. <https://www.arc.agric.za/arcgci/fact%20sheets%20library/maize%20producti%20on.pdf>.
- AMISSAH, S., ANKOMAH, G., AGYEI, B.K., LEE, R.D., HARRIS, G.H., CABRERA, M., FRANKLIN, D. H., DIAZ-PEREZ, C.J., HABTESELASSIE, M.Y. & SINTIM, H.Y., 2022. Nutrient Sufficiency Ranges for Corn at the Early Growth Stage: Implications for Nutrient Management. *Plants*, 12(4): 713. DOI: 10.3390/plants12040713.
- ASIBI, A. E. & CHAI, Q., 2019. Mechanisms of Nitrogen Use in Maize. *Agron*. 9(12): 775. DOI: 10.3390/agronomy9120775.
- BAYER CROP SCIENCE, 2020. The growth stages of maize plant. [Online]. Retrieved from <https://www.cropscience.bayer.africa/za/en-za/resources/articles/the-growth-stages-of-a-maize-plant.html> (Accessed 04/08/2022).
- BELL, J., 2017. Corn growth stages and development. Texas A&M AgriLife Extension and Research Agronomist, Amarillo: Lubbock, TX, USA.
- BERNHARD, A., 2010. The Nitrogen Cycle: Processes, Players, and Human Impact. *Nat. Educ. Knowl*. 3(10):25.
- BESEN, M.R., OGAWA, J.M., RIBEIRO, R.H., GUGINSKI-PIVA, C.A. & PIVA, J.T., 2020. 'Does split nitrogen application increase garlic yield and quality?'. *Rev. Sci. Agrár. Parana*. 19(2): 138-143.
- BORRÁS, L., SLAFER, G. A. & OTEGUI, M. E., 2004. Seed dry weight response to source–sink manipulations in wheat, maize and soybean: A quantitative reappraisal. *Field Crops Res*. 86(2-3), 131-146.
- BREMNER, J.M., 1990. Problems in the use of urea as a nitrogen fertilizer. *Soil Use and Manag*. 6(2): 70-71.
- BROWN, P.H., ZHAO, FJ. & DOBERMANN, A., 2022. What is a plant nutrient? Changing definitions to advance science and innovation in plant nutrition. *Plant Soil*. 476: 11–23.
- CAMPBELL, O.R. & PLANK, C.O., 2000. Reference sufficiency ranges for plant analysis in the southern region of the United States. Southern Cooperative Series Bulletin: 394. <https://aesl.ces.uga.edu/sera6/PUB/scsb394.pdf>

- DEKALB ASGROW, 2013. Corn nutrient uptake. [Online]. Retrieved from <https://www.dekalbasgrowdeltapine.com/en-us/agronomy/corn-nutrient-uptake.html> (Accessed 12/08/2022).
- DEKALB ASGROW, 2020. Determining corn growth stages. [Online]. Retrieved from <https://www.dekalbasgrowdeltapine.com/en-us/agronomy/corn-growth-stages-and-gdu-requirements.html> (Accessed 04/08/2020).
- FERTILIZER ASSOCIATION OF SOUTHERN AFRICA (FERTASA). (2016). Fertilizer handbook. 7th ed., Fertilizer Association of Southern Africa. Lynnwood Ridge, Pretoria, South Africa.
- FINCH, H., SAMUEL, A. & LANE, G., 2014. 4 - Fertilisers and manures. In: H. Finch, A. Samuel & G. Lane (eds.) *Lockhart & Wiseman's Crop Husbandry Including Grassland*. 9th ed., Cambridge: Woodhead Publishing. pp 63-91.
- FORDE, B.G. & CLARKSON, D.T., 1999. "Nitrate and ammonium nutrition of plants: Physiological and molecular perspectives". *Plant and Soil*. 211(1): 1-13.
- GRUNEWALD, W., ISTERDAEL, G. V., BEECKMAN, T., GHEYSEN, G. & MATHESIUS, U., 2009. Manipulation of Auxin Transport in Plant Roots during Rhizobium Symbiosis and Nematode Parasitism. *Plant Cell*. 21(9): 2553-2562.
- GULDAN, S.J., AMUNDSON, S.J. & LIEBERMAN, M., 2016. Nitrogen's role in plant metabolism and development. *Plant Physiol*. 162(1): 168-179.
- HANWAY, J.J. & Benson, G.O., 1986. How a corn plant develops. In: J.C. Herman (eds) Special report no 48. Iowa State University. <https://publications.iowa.gov/18027/1/How%20a%20corn%20plant%20develops001.pdf>.
- HAWKSWORTH, M., 2017. Properties of limestone ammonium nitrate- LAN (28) [online]. Retrieved from <https://agrispex.co.za/properties-of-limestone-ammonium-nitrate-lan28/> (Accessed 12/11/2024).
- HAYNES, R. J., 1986. "Ammonium and nitrate in the soil-plant system." *Soil Sci. Soc. Am. J.* 50(4): 1045-1049.
- JONES, D., 2017. Top Dressing Fertilizer For Maize: Why Timing Is Key. [Online]. Retrieved from <https://croprnuts.com/top-dressing-fertilizer-for-maize/> (Accessed 01/08/2022).
- KRIEL, G., 2022. *Maize Cultivation Field Crops in South Africa*. [Online]. Retrieved from <https://southafrica.co.za/maize-cultivation.html> (Accessed 01/08/2022).
- LI, N., Yang, Y., Wang, L., Zhou, C., Jing, J., Sun, X., Tian, X., 2019. Combined effects of nitrogen and sulfur fertilization on maize growth, physiological traits, N and S uptake, and their diagnosis. *Field Crop. Res.* DOI: 10.1016/j.fcr.2019.107593.

- LIU, S., CUI, S., ZHANG, X., MI, G. & GAO, Q., 2020. Synergistic Regulation of Nitrogen and Sulfur on Redox Balance of Maize Leaves and Amino Acids Balance of Grains. *Front. Plant Sci.* 11. DOI: 10.3389/fpls.2020.576718.
- LOQUÉ, D. & VON WIRÉN, N., 2007. Nitrogen transport and signaling in plants. *J. Exp. Bot.* 58(7): 1439-1452.
- MASCLAUX-DAUBRESSE, C., DANIEL-VEDELE, F., DECHORGNAT, J., CHARDON, F., GAUFICHON, L. & SUZUKI, A., 2010. Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive agriculture. *Ann. Bot.* 105(7): 1141-1157.
- MASSEEDS, 2022. Key growth stages of maize. [Online]. Retrieved from <https://www.masseeds.com/nos-dossiers/key-growth-stages-maize> (Accessed 04/08/2022).
- NATIONAL DEPARTMENT OF AGRICULTURE (NDP), 1999. Maize profile. [online]. Retrieved from <https://www.nda.agric.za/docs/FactSheet/maize.htm> (Accessed 27/07/2022).
- NOBLE RESEARCH INSTITUTE, LLC, 2006. Weigh pros, cons when choosing summer nitrogen source. [Online]. Retrieved from <https://www.noble.org/news/publications/ag-news-and-views/2006/july/weigh-pros-cons-when-choosing-summer-nitrogen-source/> (Accessed 13/09/2022).
- OLFATI, J.A., PIREE, M., RABIEE, M. & SHEYKHTAHER, Z., 2014. 'Fertilizer Amount and Split Application on Fertilizer Efficiency in Garlic'. *Int. J. Veg. Sci.* 20(3): 197-201.
- OLSON, R., 2021. Is sulphur the missing ingredient? The sulphur institute (TSI). Sulphur, no. 392. Retrieved from <https://www.sulphurinstitute.org/pub/?id=be62aa02-1866-daac-99fb-4ae5712bf1c7> (Accessed 11/11/2024).
- PLANK, C.O. & KISSEL, D.E., 2024. Plant analysis handbook for Georgia [online]. Retrieved from <https://aesl.ces.uga.edu/publications/plant/Nutrient.html> (Accessed 12/11/2024).
- POKAPŪ AKORANGA PŪTAIAO, 2019. The nitrogen cycle- Science Learning Hub [online]. Retrieved from <https://www.sciencelearn.org.nz/resources/960-the-nitrogen-cycle> (Accessed 23/11/2024).
- PROCROP PROFITABLE, SUSTAINABLE CROPPING (PROCROP), 2009. Maize growth & development. 1st ed. Coleambally: NSW Department of Primary Industries. https://www.dpi.nsw.gov.au/data/assets/pdf_file/0007/516184/Procrop-maize-growth-and-development.pdf (Accessed 01/08/2022).
- RIZZI, E., BALCONI, C., BOSIO, D., NEMBRINI, L., MORSELLI, A. & MOTTO, M., 1996. Accumulation and partitioning of nitrogen among plant parts in the high and low protein strains of maize. *Maydica.* 41: 325-332.

- ROSS, R.B., JASON, H.W., MATIAS, I.R. & FRED, E., 2013. Modern corn hybrids' nutrient uptake patterns. *Better Crops*. 97: 357-363.
- RUIZ DIAZ, D., 2021. Plant analysis for testing nutrient levels in corn. *Agronomy eUpdates*. Issue 860.
- SATTELMACHER, B., HORST, W.J. & BECKER, H.C., 1994. Factors that contribute to genetic variation for nutrient efficiency of crop plants. *J. Plant Nutr. Soil Sci.* 157: 215–224.
- SCHIMEL, D. & BENNETT, J., 2004. "Nitrogen mineralization: Challenges and uncertainties in the context of global climate change." *Plant and Soil*. 266(1): 87-102.
- SHAH, J., WAHOCHO, N., LAGHARI, G., LAGHARI, A., BANBHAN, G.M., TALPUR, K., AHMED, T., WAHOCHO, S. & LASHARI, A., 2016. Role of Nitrogen for Plant Growth and Development: A review. *AENSI*. 10(9): 209-218.
- SIVAGURU, M., PIKE, S., GASSMANN, W. & BASKIN, T. I., 2003. Aluminum Rapidly Depolymerizes Cortical Microtubules and Depolarizes the Plasma Membrane: Evidence that these Responses are Mediated by a Glutamate Receptor. *Plant Cell Physiol*. 44(7), 667-675.
- SMITH, K., 2020. Omnia GREENSULF™: More than just an effective nitrogen source. [Online]. Retrieved from <https://www.proagri.co.za/omnia-greensulf-more-than-just-an-effective-nitrogen-source/?lang=en> (Accessed 03/08/2022).
- SMITH, S.E. & READ, D.J., 2008. Mycorrhizal Symbiosis. 3rd ed. Academic Press.
- STRACHAN, S.D. & JESCHKE, M., 2018. Water and Nutrient Uptake During the Corn Growing Season. [Online]. Retrieved from <https://www.pioneer.com/us/agronomy/water-nutrient-uptake-corn.html> (Accessed 11/08/2022).
- STREETER, J.G., 2007. "Nodulation and nitrogen fixation in leguminous plants: Significance and future prospects." *Biol. Fertil. Soils*. 43(1): 133-145.
- TEZARA, W., MARTÍNEZ, P. & FERNIE, A.R., 2011. Effects of nitrogen availability on photosynthesis and chlorophyll content in plants. *Plant Physiol*. 157(4): 1212-1224.
- The Bureau for Food and Agricultural Policy (BFAP), 2015. Adding value in the South African maize value chain. https://www.sagis.org.za/BFAP_2015.pdf.
- VANDERHOEF, L. N., 1985. "Nitrate assimilation in higher plants." *Ann. Rev. Plant Physiol*. 36(1): 99-117.
- VOS, J., PUTTEN, P.V.D. & BIRCH, C., 2005. Effect of nitrogen supply on leaf appearance, leaf growth, leaf nitrogen economy and photosynthetic capacity in maize (*Zea mays* L.). *Field Crops Res*. 93(1): 64-73.

- YU, N., REN, B., ZHAO, B., LIU, P. & ZHANG, J., 2021. Leaf-nitrogen status affects grain yield formation through modification of spike differentiation in maize. *Field Crops Res.* 271: 108238. DOI: 10.1016/j.fcr.2021.108238.
- ZAHO, X., TONG, C., PANG, X., WANG, Z., GUO, Y., DU, F. & WU, R., 2011. Functional mapping of ontogeny in flowering plants. *Brief. Bioinformatics.* 13(3): 317-328.
- ZAYED, O., HEWEDY, O.A., ABDELMOTELEB, A., ALI, M., YOUSSEF, M. S., ROUMIA, A. F., SEYMOUR, D. & YUAN, C., 2023. Nitrogen Journey in Plants: From Uptake to Metabolism, Stress Response, and Microbe Interaction. *Biomolecules.* 13(10): 1443. DOI: 10.3390/biom13101443.

Chapter 3

Materials and Methods

3.1 Experimental site

Two field experiments were conducted on a farm during the 2022/23 and 2023/24 cropping seasons. The field is located in the northeastern Free State, in the Kransfontein district, between Bethlehem and Warden. It is geographically situated at coordinates 28°04'13" S 28°34'24" E and an altitude of 1698 m above sea level. The rainfall and temperature data were obtained from a DFM weather station located at the experimental site during the two seasons and are summarized in Table 3.1. There was a considerable difference between the rain data for the 2022/23 and 2023/24 seasons. The 2022/23 season recorded 328 mm more rainfall from October to April compared to the 2023/24 season. The chemical and physical properties of the soil are summarized in Table 3.2.

Table 3.1 Rainfall and Temperatures for the 2022/23 and 2023/24 seasons for Kransfontein

Season		Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Total
2022/23	Rainfall (mm)	0	123	203	114	61	173	18	32	63	0	787
	Avg. Temp (°C)	16	19	17	19	20	19	18	15	12	8	
2023/24	Rainfall (mm)	3	62	53	122	57	19	30	53	0	12	411
	Avg. Temp (°C)	15	17	20	20	20	21	21	16	15	9	

Table 3.2 Physical and chemical soil properties of experimental site Kransfontein

Physical properties	Season	
	2022/23	2023/24
Sand (%)	74	74
Clay (%)	18	18
Loam (%)	8	8
Bulk Density (kg m ⁻³)	1153	1153
Chemical properties		
pH (KCL)	4.5	4.3
Ex Acid	0.10	0.23
Acid Saturation (%)	3.1	7
S (mg kg ⁻¹)	9	11
P (Bray 1) (mg kg ⁻¹)	30	35
K (mg kg ⁻¹)	266	312
K (% of CEC)	21	24
Ca (mg kg ⁻¹)	366	340
Ca (% of CEC)	57	52
Mg (mg kg ⁻¹)	72	63
Mg (% of CEC)	18	16
Na (mg kg ⁻¹)	3	5
Na (% of CEC)	0	1
CEC (cmolc kg ⁻¹)	3.2	3.3
Ca / Mg	3.1	3.3
Mg / K	0.9	0.6
(Ca + Mg) / K	4	3

**Soil sample taken at a depth of 0 to 200 mm*

3.2 Experimental design and treatments

Two experiments, one with a non-prolific cultivar (single-headed – DKC 73-72 BT) and the other a prolific (multi-headed - DKC 72-70) cultivar, were planted on a sandy loam soil (18% clay). Each experiment was designed as a randomized complete block design with factorial combinations consisting of four replications (Table 3.3). Treatments consisted of two different nitrogen sources, viz. Limestone Ammonium Nitrate (LAN(28)) and Greensulf(35), three nitrogen topdressing application rates viz. 0, 20, and 40 kg N ha⁻¹ (these applications were made with a hand fertilizer spreader representing an aircraft or drone application since the plants were too tall to be cleared by a tractor), and at three different growth stages viz. V4, V9

to 10, and a combination of V4 & V9 to 10, replicated four (4) times (Table 3.3). Therefore, each trial consisted of 72 plots measuring 5.46 m wide (6 rows, 0.91 m apart) and 10 m long.

3.3 Reason for growth stages and nitrogen sources used

The four R principles are crucial for crop production, particularly in terms of crop nutrition, given the high input costs in the agricultural industry. The principles include the right source of nutrients, at the right rate, at the right time, and the right place. If a farmer can get the timing right for this strategy, it will lead to optimum yield. Still, it will also change according to the environmental conditions of that specific planting season (Jensen, 2013). In this experiment, two nitrogen sources, namely Greensulf and Limestone ammonium nitrate (LAN), were used to establish the right type of fertilizer source. The motivation for these two fertilizers used as sources was to determine if the 5% of sulphur in Green Sulphur influenced the yield and quality compared to LAN, which does not contain any sulphur. These fertilizer sources were applied as a top dressing at 0, 20, and 40 kg ha⁻¹ rates of nitrogen to determine the most efficient rate. These top dressings were applied at two different growth stages (V4 and V9-10) and a combination where the amount of fertilizer was split in half for each growth stage to determine the right time for applying nitrogen top dressing on maize in the north-eastern Free State. According to Jones (2017), maize nitrogen use from growth stages V3 to V6 amounts to 12 to 25 kg N ha⁻¹, and between growth stages V12 to R1, 100 to 140 kg N ha⁻¹. This is why growth stages V4 and V9-10 were used to determine if the nitrogen supplied at an early growth stage was efficient in supporting further growth or if a later application would be more efficient in carrying the maize plant to a physiologically mature growth stage.

3.4 Agronomic practices

The previous crop planted on the experiment site was maize (*Zea mays* L.). The experiment was conducted under dryland conditions over two cropping seasons from 2022/23 to 2023/24. The experimental site was ripped, disked, and levelled for seedbed preparation before planting. The farmer applied three tonnes of dolomitic lime broadcast over the field in August 2022. During the rip process, NH₃ (82%) gas was applied to the soil at a rate of 56 kg nitrogen per hectare using the NH₃ applicator. Fertilizer rates were determined according to the soil analysis (Table 3.1) and an estimated yield potential of seven (7) tonnes of maize per hectare. Thus, 334 kg ha⁻¹ 15:8:4(27)+5% Ca+4% S was applied, which amounted to an application of N, P, K, Ca, and S of 50.1 kg N ha⁻¹, 26.72 P ha⁻¹, 13.36 kg K ha⁻¹, 16.7 kg Ca ha⁻¹ and 13.36 kg S ha⁻¹. Planting dates were 05 December 2022 for the 2022/23 cropping season and 09 November 2023 for the 2023/24 cropping season. The planting process was done with a 6-row planter at a planting density of 32 000 plants per hectare. With planting pre-emergence herbicides consisting of Eptam super at 1.8 L ha⁻¹, Galago at 0.2 L ha⁻¹, Baseline 915 at 0.8 L

ha⁻¹, Cheetah at 0.5 L ha⁻¹ and Lambda at 0.1 L ha⁻¹. The field was sprayed with Roundup Baseline 960 at 0.5 L ha⁻¹, Galago at 0.1 L ha⁻¹, Cheetah 1.2 L ha⁻¹, Lambda at 0.1 L ha⁻¹, Extreme B at 2.0 L ha⁻¹ with a combination of Aroxy 250 SC 400 ml ha⁻¹ six weeks after emergence.

Each plot was manually harvested and threshed by hand into polypropylene bags at harvest. Moisture content was determined, and the yield was adjusted to 12.5% moisture content.

Table 3.3 Representation of the experimental layout

Block 1	Block 2	Block 3	Block 4
S1 R1 GS1	S2 R2 GS1	S1 R1 GS3	S2 R1 GS1
S1 R2 GS1	S1 R1 GS2	S1 R2 GS3	S2 R2 GS1
S1 R3 GS1	S2 R1 GS1	S1 R3 GS3	S2 R3 GS1
S1 R1 GS2	S2 R3 GS2	S2 R1 GS3	S2 R1 GS2
S1 R2 GS2	S1 R3 GS3	S2 R2 GS3	S2 R2 GS2
S1 R3 GS2	S2 R3 GS1	S2 R3 GS3	S2 R3 GS2
S1 R1 GS3	S2 R2 GS2	S1 R1 GS1	S2 R1 GS3
S1 R2 GS3	S1 R3 GS2	S1 R2 GS1	S2 R2 GS3
S1 R3 GS3	S1 R2 GS2	S1 R3 GS1	S2 R3 GS3
S2 R1 GS1	S2 R1 GS3	S2 R1 GS2	S1 R1 GS1
S2 R2 GS1	S2 R2 GS3	S2 R2 GS2	S1 R2 GS1
S2 R3 GS1	S1 R2 GS3	S2 R3 GS2	S1 R3 GS1
S2 R1 GS2	S1 R1 GS3	S2 R1 GS1	S1 R1 GS2
S2 R2 GS2	S2 R3 GS3	S2 R2 GS1	S1 R2 GS2
S2 R3 GS2	S1 R2 GS1	S2 R3 GS1	S1 R3 GS2
S2 R1 GS3	S1 R1 GS1	S1 R1 GS2	S1 R1 GS3
S2 R2 GS3	S1 R3 GS1	S1 R2 GS2	S1 R2 GS3
S2 R3 GS3	S2 R1 GS2	S1 R3 GS2	S1 R3 GS3

S1 (Source) – Greensulf (35), S2 - LAN (28)

R1 (Application rate) – 106 kg N ha⁻¹, R2 – 126 kg N ha⁻¹, R3 – 146 kg N ha⁻¹

GS1 (Growth stage) – V4, GS2 – V9- V10, GS3 – V4 & V9- V10

3.5 Data collection and measurements

The first two and last two plants of each row and the outer two rows of the six rows of plots were not used for measurements because they were used as border rows.

3.5.1 Leaf samples and preharvest measurements

The leaf samples were taken of the uppermost developed leaf from six different plants (two leaves on the side rows of the four rows used and four leaves on the middle two rows) for samples from V5 to R2, and ten whole plants were sampled for V4 from each block and put in brown paper bags. Only six plant leaves were used due to the time required to pick all 144 plot samples before 9:00 AM. Leaf samples were taken before the top dressing application, 14 days after the top dressing and R2 growth stage. The uppermost developed leaf was used for the leaf samples, except for R2 leaf samples, where the ear leaf was used. The samples were sent to Omnia's laboratory for complete nutrient analysis. Only R2 growth stage analysis will be discussed.

All the cobs per plant were counted at R4 growth stage on all four of the middle row's plants and were noted as first-, second-, and sprout cobs per row and then converted to total cobs plant⁻¹.

3.5.2 Yield and Yield Components

For the harvesting of the cobs, ten (10) plants were harvested and divided into three polypropylene bags, first, second, and sprout cobs for all the plots. The number of rows per head and the number of seeds in a row were counted for the harvested cobs. All the cobs were threshed into 50 kg bags, which were weighed, calculated, and converted to tonnes per hectare for all plots. After weighing, the moisture and hectolitre were tested with a Dickey-John hand moisture meter, and the yield was adjusted to 12.5% moisture. 1000 kernels were counted, and the weight was measured for each plot.

The nitrogen use efficiency (NUE) and precipitation use efficiency (PUE) was calculated using the following equations:

$$\text{NUE} = \text{Total yield (kg ha}^{-1}\text{)} \div \text{total kg N applied ha}^{-1}$$

$$\text{PUE} = \text{Total yield (kg ha}^{-1}\text{)} \div \text{total rainfall (mm)}$$

3.5.3 Grain quality

After the moisture was measured, two samples were taken, one of which was sent to Omnia's laboratory for a complete nutrient analysis of the grain to calculate the grain's nitrogen use efficiency (NUE). The second sample was sent to the University of the Free State (UFS) for analysis of starch index, milling index, protein content, fat content, and ash content using a near-infrared reflectance spectrometer (NIR).

3.5.5 Return on investment

Return on investment was done after harvest, and all nitrogen sources and application costs were calculated per hectare. The V4 growth stage was calculated for the application with a tractor and fertilizer spreader. The V9 growth stage was calculated for the application with an aeroplane from the Bethlehem crop sprayer's airport.

$$\text{ROI} = (\text{income}) - (\text{fertilizer cost} + \text{application cost} + \text{Farmers total input costs})$$

Income is the total yield per hectare multiplied by the 100-day average SAFEX yellow maize price per ton.

Fertilizer cost is the cost of the additional nitrogen applied per hectare.

Application costs include using a tractor and/or airplane to apply the additional nitrogen.

Farmers' total input costs per hectare include the seed cost, preplant ammonia gas application, planting fertilizer, lime, pesticide, fungicide, herbicide, crop insurance, and rent on the production loan.

3.6 Statistical analysis

Data was analysed using the statistical program NCSS 11.0.24, and the differences between the means were compared using Tukey's least significance differences test at 5% level of significance ($\text{LSD}_{T<0.05}$).

References

- JENSEN, T.L., 2013. 4Rs an Important Aspect of Successful Farming, Regardless of Regulation. [Online]. Retrieved from <https://nutrientstewardship.org/4r-news/4rs-an-important-aspect-of-successful-farming-regardless-of-regulation/> (Accessed 01/08/2022).
- JONES, D., 2017. Top Dressing Fertilizer For Maize: Why Timing Is Key. [Online]. Retrieved from <https://croppnuts.com/top-dressing-fertilizer-for-maize/> (Accessed 01/08/2022).

Chapter 4

The influence of nitrogen source, application rate, and growth stage on the leaf nutrient content of maize in the north-eastern Free State

4.1 Introduction

The leaves of the maize plant are a vital organ for light absorption and photosynthesis (Strable & Nelissen, 2021). The leaf area, the height of the maize plant, and the number of leaves affect the amount of energy the plant requires for growth and development. It is also a vital storage organ for nutrients to be later translocated to the reproductive organs (Lambert *et al.*, 2013). Nitrogen is essential in growth as it is crucial in chlorophyll synthesis, photosynthesis, DNA and RNA synthesis, protein synthesis, and enzymatic activity of maize (Liu *et al.*, 2022; Ruben, 2024). The role of nitrogen is well documented and considered the most "influential" macronutrient for growth and grain yield. Nitrogen fertility management is challenging because of nitrification, denitrification, volatilization, and leaching occurring throughout the season (Bruns & Ebelhar, 2006; Rahmawati, 2017).

Nitrogen has a significant effect on the macro and micronutrients in the leaf. Nitrogen can potentially increase nutrient uptake in general by stimulating root development and enlarging plant canopies, thereby increasing photosynthesis and transpiration of the plant (Miner *et al.*, 2018). Nitrogen sources have a positive influence on the stover N content, but could also have a decreasing effect on the stover P content. More specifically, the nitrogen source, calcium ammonium nitrate (CAN), produced the highest N and P content compared to nitrogen sources such as ammonium nitrate (AN), ammonium sulphate (AS), and urea (Biswas & Ma, 2016). This highlights the importance of N sources.

Bojtor *et al.* (2022) observed no influence of nitrogen source on leaf nutrient content but found an increase in nitrogen (N), sulphur (S), magnesium (Mg), calcium (Ca), iron (Fe), and copper (Cu) content at nitrogen application rates of 120 and 300 kg N ha⁻¹. Bruns & Ebelhar (2006) reported that increased nitrogen rates positively influenced the content of Mn, Zn, and Cu in ear leaves. Simultaneously, these authors found that the content of N, potassium (K), Mg, Fe, manganese (Mn), Zn, and Cu increased in immature ear samples when nitrogen rates were increased. No significant differences in P or Ca content were reported in developing maize ears. Davies *et al.* (2020) reported that N uptake by plants was higher when total nitrogen was applied at planting than when it was used in split applications later in the growing season.

This study aims to evaluate the effects of different nitrogen sources, application rates, and timing of application at various growth stages on the macro- and micronutrient content of two maize cultivars (non-prolific and highly prolific) through leaf analysis at the R2 growth stage (kernel blister stage) over two consecutive cropping seasons.

4.2 Materials and Methods

The materials and methods used were described in Chapter 3.

4.3 Results and Discussion

Analysis of variance indicated that data from the 2022/23 and 2023/24 cropping seasons were not homogeneous. This is ascribed to climatic differences between the cropping seasons (Table 3.1, Chapter 3). Therefore, the two cropping seasons were analysed separately and will be discussed accordingly.

A summary of the analysis of variance evaluating the effects of nitrogen source (NS), topdressing application rate (AR), and growth stage (GS) on the production of a non-prolific maize cultivar and a prolific maize cultivar is presented in Tables 4.1 and 4.2, respectively. Nitrogen source significantly influenced all the elements analysed for the 2022/23 and 2023/24 growing seasons, except Mn. The amount of nitrogen applied significantly affected the tested elements, but not as much as the source. Growth stages also significantly affected the tested elements, but only in the 2023/24 season, except for Fe and N:K ratio.

Table 4.1 Summary of analysis of variance indicating the effect of treatment factors on R2 leave samples for a non-prolific maize cultivar

Treatments	N		S		P		K		Ca		Mg		Cu		Mn		Zn		Fe		B		N:S		N:K		Fe:Mn		
	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	
Nitrogen source (NS)	*	ns	*	*	*	ns	*	*	ns	*	*	ns	*	*	ns	ns	*	ns	*	*	*	ns	*	*	ns	*	*	ns	ns
Application rate (AR)	*	ns	*	ns	*	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns
Growth stage (GS)	ns	ns	ns	*	ns	*	ns	*	ns	ns	ns	ns	*	ns	ns	*	ns	ns	*	*	ns	ns	*	*	ns	*	ns	ns	ns
NS X AR	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
NS X GS	ns	ns	ns	*	ns	*	ns	*	ns	ns	ns	ns	ns	*	ns	*	ns	ns	*	ns	ns	ns	ns	ns	*	ns	*	ns	*
AR X GS	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	*	ns	ns	*	ns	ns	ns	ns	ns
NS X AR X GS	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	*	*	ns	ns	ns	ns	ns
CV%	4.99	4.99	7.18	11.10	7.23	9.26	5.91	8.33	11.23	12.47	12.51	10.85	14.00	15.94	51.10	32.41	9.79	10.81	58.78	10.43	18.99	15.62	5.08	9.16	7.58	9.40	86.51	30.14	

ns – not significant; *P≤ 0.05; CV – coefficient of variance

Table 4.2 Summary of analysis of variance indicating the effect of treatment factors on R2 leave samples for a prolific maize cultivar

Treatments	N		S		P		K		Ca		Mg		Cu		Mn		Zn		Fe		B		N:S		N: K		Fe: Mn		
	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	
Nitrogen source (NS)	ns	ns	ns	*	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	*	ns	*	ns	ns	ns	ns
Application rate (AR)	*	ns	ns	ns	ns	ns	ns	ns	*	ns	*	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Growth stage (GS)	ns	*	ns	*	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	*	ns	*	ns	ns	ns
NS X AR	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
NS X GS	ns	ns	ns	*	ns	*	ns	*	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	*	ns	ns	ns
AR X GS	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns
NS X AR X GS	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV%	6.07	8.59	8.37	7.72	7.72	6.86	5.52	7.17	14.44	12.97	12.5	13.31	11.34	14.72	33.72	25.4	10.5	14.25	18.64	17.03	53.22	97.55	5.54	10.77	9.91	12.78	33.39	28.91	

ns – not significant; *P≤ 0.05; CV – coefficient of variance

4.3.1 Leave analysis

In this chapter, the influence of nitrogen source, application rate and growth stage on macro and micronutrient content at the R2 growth stage will be discussed.

Why use leaf analysis?

Leaf analysis measures the amount of nutrients a plant has absorbed from the soil, providing an alternative method for determining a crop's nutrient status. Nutrient concentration can be compared to critical or optimal range norms developed from fertilizer response trials for plants at different physiological stages of development and can be used for interpretation and identify the most limiting elements that can impact growth (Cornforth & Steele, 1981; Campbell & Plank, 2000b). It can also assist in determining nutrient deficiencies in the plant during the growing season before they become visible, thereby preventing unnecessary yield losses (Jones, 2023).

4.3.1.1 Macronutrient leaf concentration

4.3.1.1.1 N content

Non-prolific

Season 2022/23

The nitrogen source and application rate significantly affected leaf N content. No significant differences in N content were found as a result of the main effects and their interactions during the 2023/24 season (Table 4.1).

The maize plants treated with the Greensulf resulted in significantly higher N content (2.97%), which was 2.73% higher than that of plants fertilized with the LAN (2.89%) (Figure 4.1). Plants treated with a nitrogen topdressing application rate of 40 kg ha⁻¹ resulted in the highest leaf N content (3.03%), which was significantly higher than the plants treated with 0 (2.86%) and 20 (2.91%) kg N ha⁻¹ (Figure 4.2).

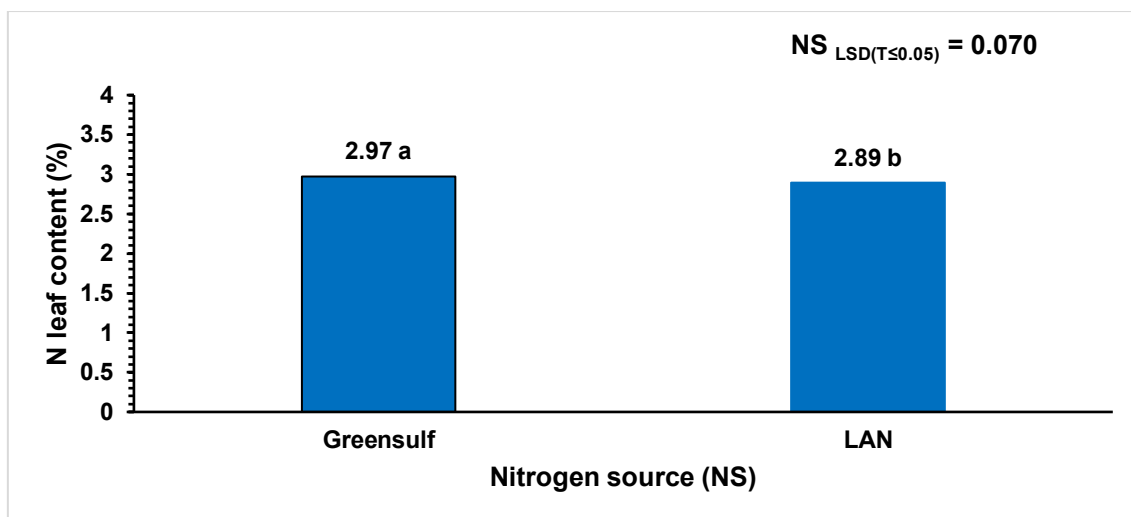


Figure 4.1 Effect of nitrogen source on the non-prolific cultivar's leaf N content (%) in the 2022/23 season. Values with different letters differ significantly.

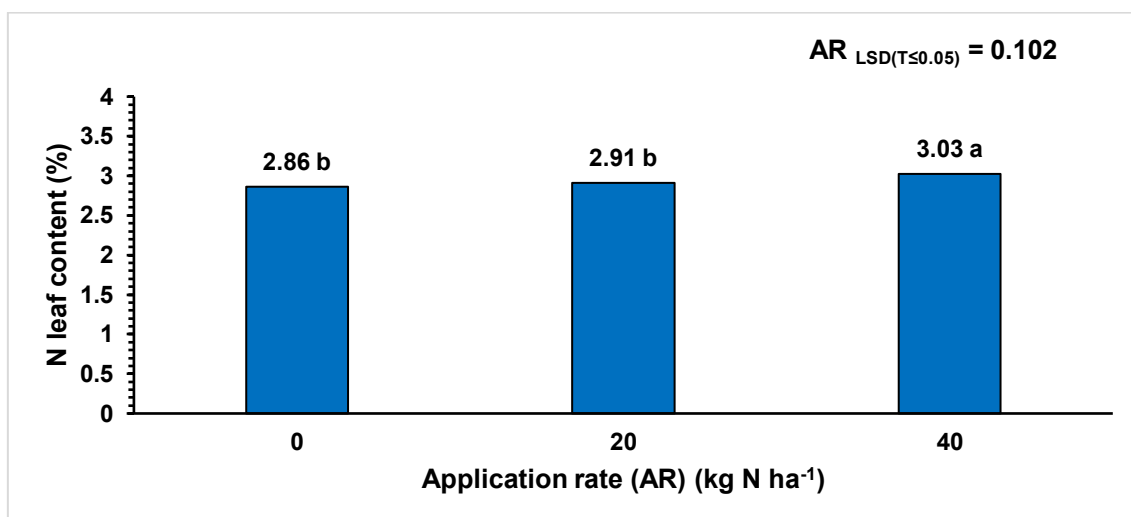


Figure 4.2 Effect of application rate on the non-prolific cultivar's leaf N content (%) in the 2022/23 season. Values with different letters differ significantly.

Prolific

Season 2022/23

The nitrogen content of the leaves was significantly influenced by the nitrogen application rate only (Table 4.2). Plants that received a top dressing of 40 kg N ha⁻¹ produced the highest leaf N content (3.18%), which was significantly higher than that of the control plants (0 kg N ha⁻¹) (3.04%). The plants that received a top dressing of 20 kg N ha⁻¹ (3.07%) did not differ significantly from the other treatments' N content (Figure 4.3).

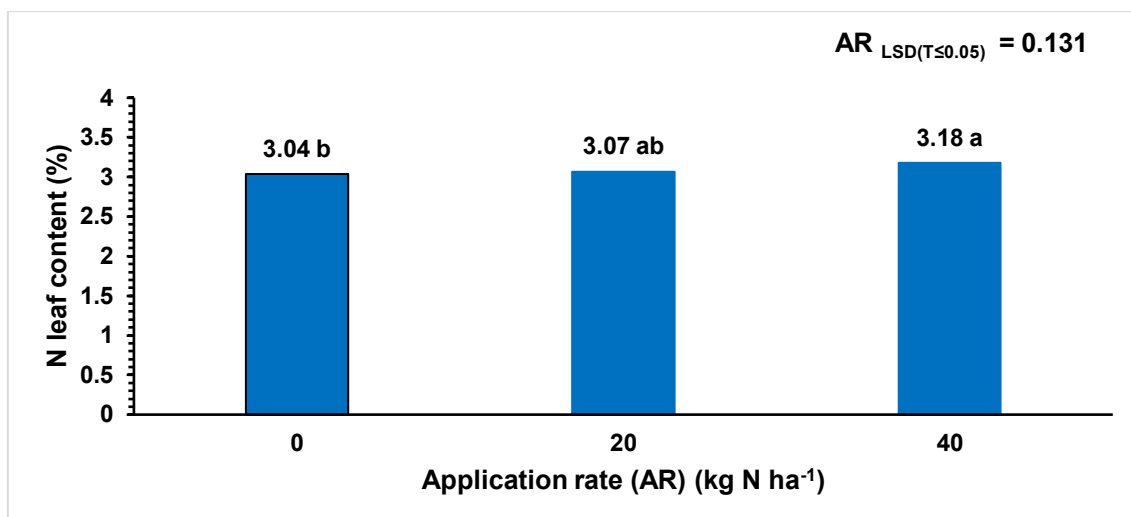


Figure 4.3 Effect of application rate on the prolific cultivar's leaf N content (%) in the 2022/23 season. Values with different letters differ significantly.

Season 2023/24

Leaf N content was significantly influenced by the growth stage at which the topdressing was applied (Table 4.2).

When nitrogen was applied at the V4 stage, the leaf N content of plants was lower (3.10%) compared to an application at the V9 stage (3.40%) and that of a split application at V4 & V9 (3.29%). Maize plants fertilized at V9 resulted in a significantly higher (9.23%) leaf N content than plants that received the topdressing at V4, with no other significant differences (Figure 4.4).

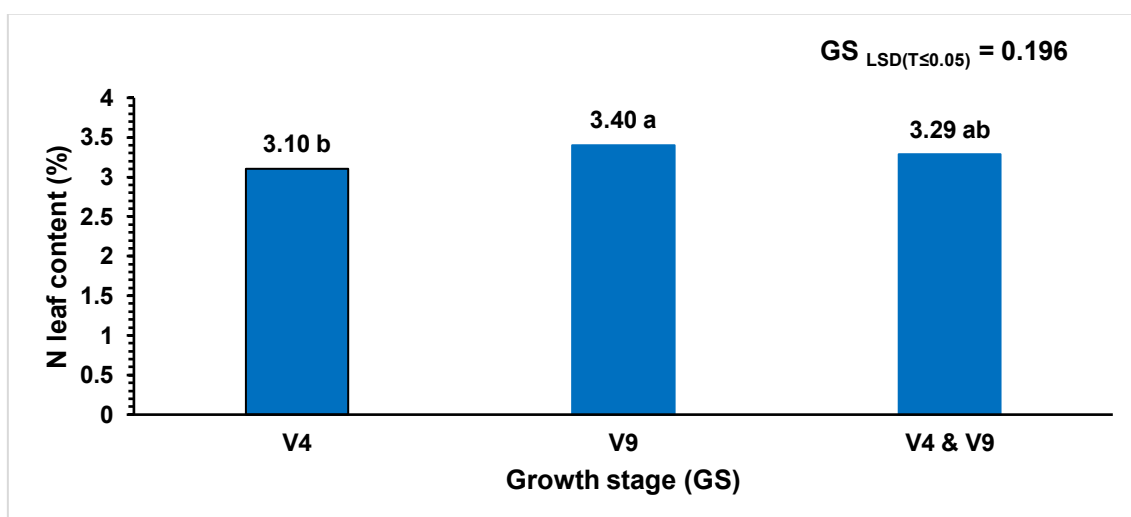


Figure 4.4 Effect of growth stage on the prolific cultivar's leaf N content (%) in the 2022/23 season. Values with different letters differ significantly.

Zhang *et al.* (2022) reported that nitrogen accumulation in the whole plant was significantly higher when treated with NO_3^- (sodium nitrate fertilizer) under hydroponic conditions compared to the NH_4^+ nitrogen source (ammonium sulphate fertilizer). Similarly, Biswas & Ma (2016) found that the N content of plants was affected by the nitrogen source used. They observed that CAN and AN produced a higher leaf N content than plants treated with AS or urea as nitrogen sources. These findings align with the results of this study, indicating that nitrogen sources have a significant impact on plant N content. Additionally, Bruns & Ebelhar (2006) reported that the N content of maize leaves at the R2 growth stage increased with increasing N levels up to 224 kg N ha^{-1} , after which there was a decrease in leaf N content. Ray *et al.* (2020) found similar results to those of Bruns & Ebelhar (2006), with leaf N content increasing with increased nitrogen rates up to 250 kg N ha^{-1} , followed by a decrease. Ostmeyer *et al.* (2023) observed that grain sorghum produced significantly higher leaf N content when nitrogen was applied in three split applications (at planting, GS3 - panicle initiation, and GS5 - booting) compared to plants only fertilized with nitrogen at planting, with no significant effects on plants fertilized at planting and at the GS3 stage. Although this research was conducted on grain sorghum, in this study, the same effect was found with the prolific maize cultivar, where leaf N content was significantly higher when fertilized at V9 and with no significant difference when nitrogen was applied in a split application at V4 & V9.

4.3.1.1.2 S content

Non-prolific

Season 2022/23

The S leaf content was significantly affected by the nitrogen source and application rate (Table 4.1). When Greensulf (0.19%) was applied, the plants produced significantly higher S levels in the leaves compared to plants that received LAN (0.17%) (Figure 4.5).

Maize fertilized at a topdressing nitrogen rate of 40 kg ha^{-1} resulted in significantly higher leaf S content (0.19%) than plants fertilized at a nitrogen application rate of 0 kg ha^{-1} (0.17%), with no other significant difference between treatments (Figure 4.6).

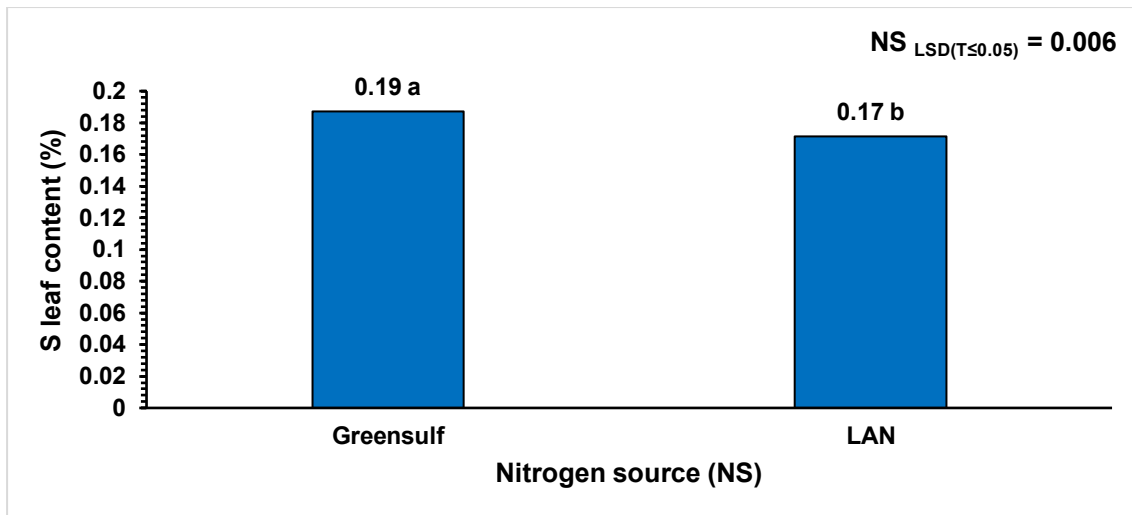


Figure 4.5 Effect of nitrogen source on the non-prolific cultivar's leaf S content (%) in the 2022/23 season. Values with different letters differ significantly.

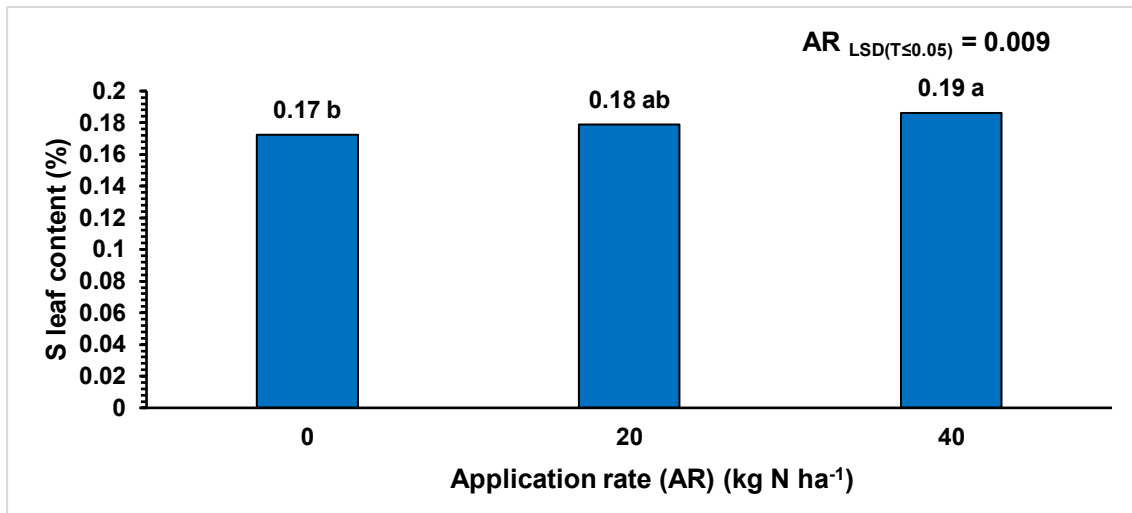


Figure 4.6 Effect of application rate on the non-prolific cultivar's leaf S content (%) in the 2022/23 season. Values with different letters differ significantly.

Season 2023/24

The nitrogen source, growth stage, and the interaction between the nitrogen source and growth stage significantly influenced the leaf S content (Table 4.1).

The LAN nitrogen source applied to plants in a split application at V4 & V9 resulted in significantly lower leaf S content than the other treatments (Table 4.3).

Table 4.3 S leaf content (%) as influenced by the interaction between nitrogen source and growth stage applied on a non-prolific cultivar for the 2023/24 season

Growth stages (GS)	Sources (NS)		Average
	Greensulf	LAN	
V4	0.16 ^a	0.17 ^a	0.17
V9	0.17 ^a	0.16 ^a	0.16
V4 & V9	0.16 ^a	0.12 ^b	0.14
Average	0.16	0.15	

$NS_{LSD (T \leq 0.05)} = 0.01$

$GS_{LSD (T \leq 0.05)} = 0.012$

$NS \times GS_{LSD (T \leq 0.05)} = 0.021$

Values with different letters differ significantly.

Prolific

Season 2023/24

Leaf S content was significantly influenced by nitrogen source, growth stage applied, the interaction between nitrogen source and growth stage applied, and the interaction between application rate and growth stage applied. No significant differences were observed in the 2022/23 season (Table 4.2).

LAN applied to plants at V4 resulted in significantly higher leaf S content (0.20%) than LAN applied at V9 (0.17%), V4 & V9 (0.14%), and Greensulf, which was applied at V4 (0.18%). In general, the S content of plants fertilized with LAN later in the growing season was low (Table 4.4).

Table 4.4 Leaf S content (%) as influenced by the interaction between nitrogen sources and growth stage applied on a prolific cultivar for the 2023/24 season

Growth stages (GS)	Sources (NS)		Average
	Greensulf	LAN	
V4	0.18 ^{bc}	0.20 ^a	0.19
V9	0.19 ^{ab}	0.17 ^c	0.18
V4 & V9	0.19 ^{ab}	0.14 ^d	0.17
Average	0.19	0.17	

$NS_{LSD (T \leq 0.05)} = 0.00655$

$GS_{LSD (T \leq 0.05)} = 0.00964$

$NS \times GS_{LSD (T \leq 0.05)} = 0.01671$

Values with different letters differ significantly.

An application rate of 20 kg N ha⁻¹ at V9 on plants resulted in the highest leaf S content (0.191%). It was significantly higher than that of plants receiving an application rate of 0 (0.156%) and 20 (0.169%) kg N ha⁻¹ in a split application at V4 & V9 (Table 4.5).

Table 4.5 Leaf S content (%) as influenced by the interaction between application rate and growth stage applied on a prolific cultivar for the 2023/24 season

Application rate (AR) (kg N ha ⁻¹)	Growth stage (GS)			Average
	V4	V9	V4 & V9	
0	0.19 ^{ab}	0.19 ^{ab}	0.16 ^c	0.18
20	0.19 ^{ab}	0.19 ^a	0.17 ^{bc}	0.18
40	0.19 ^{ab}	0.17 ^{abc}	0.17 ^{abc}	0.18
Average	0.19	0.18	0.17	

AR $LSD (T \leq 0.05) = ns$

GS $LSD (T \leq 0.05) = 0.010$

AR x GS $LSD (T \leq 0.05) = 0.022$

Values with different letters differ significantly.

In summary, the non-prolific cultivar showed a significant increase (11.11% higher) in leaf S content when the Greensulf source was applied compared to the LAN source. A 40 kg N ha⁻¹ application rate resulted in the highest leaf S content for the non-prolific cultivar during the 2022/23 season. In the 2023/24 season, applying LAN at V4 & V9 resulted in significantly lower leaf S content in plants compared to other treatments. The prolific cultivar responded best to a LAN application at V4, resulting in the highest leaf S content (0.20%). An application rate of 20 kg N ha⁻¹ at V9 resulted in plants' highest leaf S content in the 2023/24 season.

Coetzee *et al.* (2017) found a significant response to nitrogen sources on plant nutrient concentrations. They reported that plants fertilized with a urea nitrogen source produced a higher leaf S content (1.94 g kg⁻¹) than those fertilized with LAN (1.74 g kg⁻¹) as nitrogen source. These findings emphasize the importance of selecting the correct N source.

Ciampitti *et al.* (2013) reported that leaf S content was affected by nitrogen application rate, with an increasing effect in S content from 112 (0.17 g m⁻²) to 224 kg N ha⁻¹ (0.20 g m⁻²) compared to the control of 0 kg N ha⁻¹ (0.10 g m⁻²). Parsley *et al.* (2019) observed that a nitrogen rate higher than 0 kg ha⁻¹ led to an increase in maize stover S content up to 90 kg ha⁻¹, with no significant differences between the nitrogen rates. In this study, it was also found that nitrogen application rates affected the leaf S content of maize plants, with a nitrogen topdressing of 40 kg ha⁻¹ for the non-prolific cultivar (0.19%) and 20 kg ha⁻¹ for the prolific cultivar (0.182%), resulting in the highest leaf S content.

4.3.1.1.3 P content

Non-prolific

Season 2022/23

Only nitrogen source and application rate significantly affected the leaf P content (Table 4.1).

The plants fertilized with the Greensulf showed a significantly higher leaf P content (0.28%) compared to plants fertilized with LAN (0.26%) (Figure 4.7). Plants that received an application rate of 40 kg N ha⁻¹ (0.28%) produced a significantly higher leaf P content than those with a 0 kg N ha⁻¹ (0.26%) application rate, with no significant differences compared to 20 kg N ha⁻¹ (0.27%) treated plants (Figure 4.8).

Season 2023/24

The leaf P content was significantly influenced by the growth stage at which the topdressing was applied and the interaction of the nitrogen source and growth stage (Table 4.1).

The LAN source applied to plants in a split application at V4 & V9 had a significantly lower leaf P content than the rest of the growth stage applications. Greensulf applied at V9 and LAN applied at V4, resulted in the highest leaf P content (0.24%) for both sources on treated plants (Table 4.6).

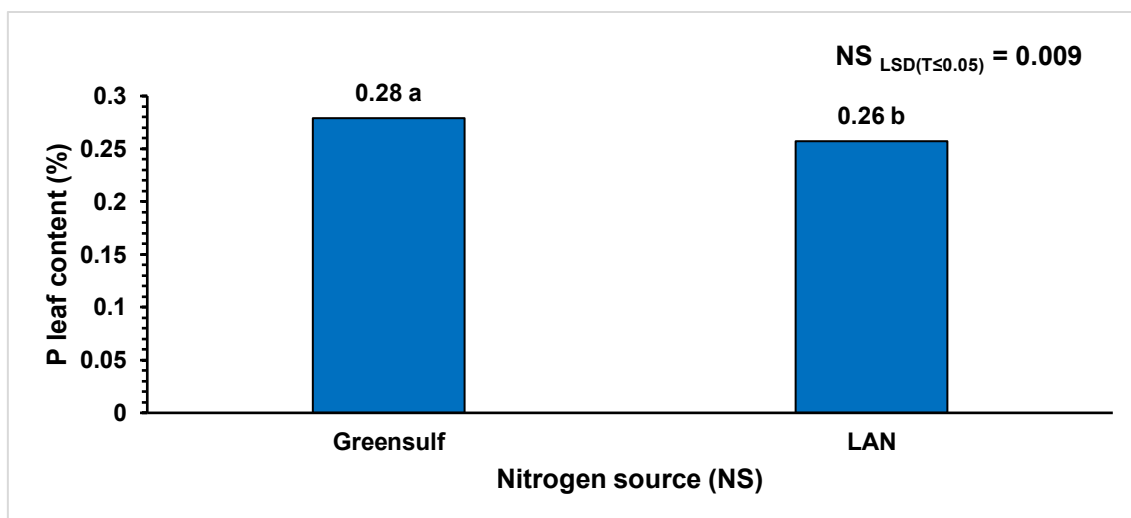


Figure 4.7 Effect of nitrogen source on the non-prolific cultivar's leaf P content (%) in the 2022/23 season. Values with different letters differ significantly.

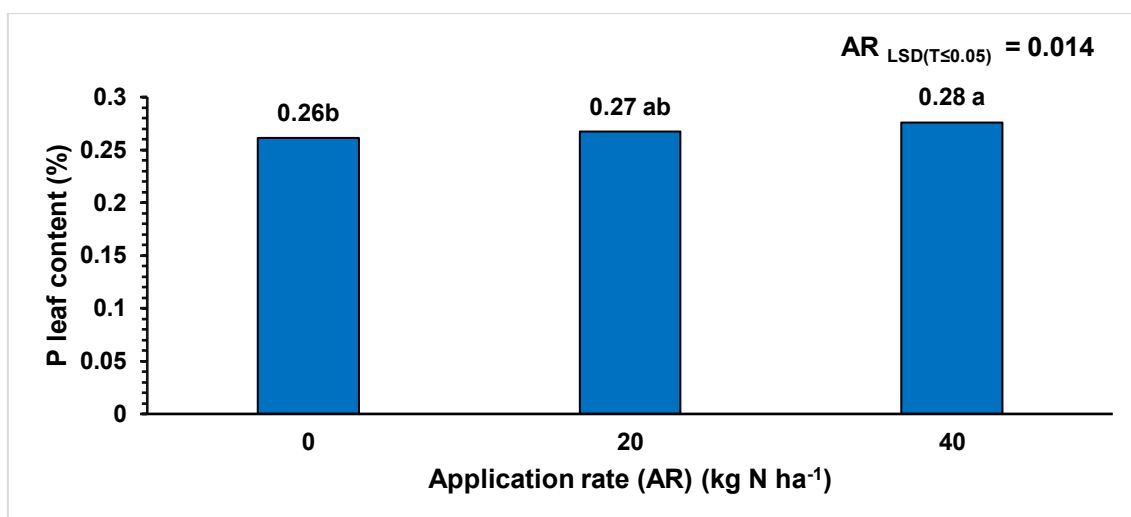


Figure 4.8 Effect of nitrogen application rate on the non-prolific cultivar's leaf P content (%) in the 2022/23 season. Values with different letters differ significantly.

Table 4.6 Leaf P content (%) as influenced by the interaction between nitrogen sources and growth stage applied on a non-prolific cultivar for the 2023/24 season

Growth stages (GS)	Sources (NS)		Average
	Greensulf	LAN	
V4	0.23 ^a	0.24 ^a	0.24
V9	0.24 ^a	0.23 ^a	0.24
V4 & V9	0.23 ^a	0.20 ^b	0.22
Average	0.23	0.22	

NS $LSD(T \leq 0.05) = ns$

GS $LSD(T \leq 0.05) = 0.015$

NS x GS $LSD(T \leq 0.05) = 0.026$

Values with different letters differ significantly.

Prolific

Season 2023/24

The leaf P content was significantly affected by the interaction between the nitrogen source and growth stage (Table 4.2). The maize plants that received LAN at the V4 stage (0.26%) and Greensulf at the V9 stage (0.25%) produced a significantly higher leaf P content (0.25-0.26%) than plants that received LAN in a split application at the V4 & V9 stages (0.23%). There was no other significant difference between treatment combinations (Table 4.7).

Table 4.7 Leaf P content (%) as influenced by the interaction between nitrogen sources and growth stage applied on a prolific cultivar for the 2023/24 season

Growth stages (GS)	Sources (NS)		Average
	Greensulf	LAN	
V4	0.24 ^b	0.26 ^a	0.25
V9	0.25 ^{ab}	0.24 ^b	0.25
V4 & V9	0.25 ^{ab}	0.23 ^{bc}	0.24
Average	0.24	0.24	

NS $LSD (T \leq 0.05) = ns$

GS $LSD (T \leq 0.05) = ns$

NS x GS $LSD (T \leq 0.05) = 0.014$

Values with different letters differ significantly.

Ewais *et al.* (2009) observed that nitrogen sources influenced maize leaf P content when combining ammonium sulphate (AS) and compost. This produced the highest leaf P content (0.236%) compared to using AS (0.226%) or compost (0.201%). Siam *et al.* (2008) reported that ammonia gas-treated plants had a higher leaf P content (0.288%) than plants treated with urea (0.196%) and AS (0.209%). This study agrees with the observations of these authors, where it was found that the non-prolific cultivar plants fertilized with Greensulf nitrogen source produced a 7.41% higher leaf P content, than plants fertilized with LAN, as well as some of the interactions between nitrogen source and growth stage on the prolific cultivar.

Burns & Ebelhar (2006) reported that nitrogen application rates influenced ear leaf P content, with the highest P content at an application rate of 224 kg N ha⁻¹ (2.0 mg g⁻¹) and the lowest P content at a rate of 134 kg N ha⁻¹ (1.8 mg g⁻¹), but with no significant differences between nitrogen application rates. Liu *et al.* (2022) also reported an increase in leaf P content with increasing nitrogen rates from 100 (0.163 mg plant⁻¹) to 200 (0.174 mg plant⁻¹) kg ha⁻¹, followed by a decrease at a rate of 300 kg ha⁻¹ (0.165 mg plant⁻¹). This study also resulted in increasing leaf P content as nitrogen rates increased, but with significant effects, where a nitrogen topdressing of 40 kg ha⁻¹ (total N of 146 kg ha⁻¹) with 0.28% P content was significantly higher than no nitrogen topdressing (total N of 106 kg ha⁻¹) with 0.26% P content.

4.3.1.1.4 K content

Non-prolific

Season 2022/23

Only the nitrogen source significantly affected the K leaf content (Table 4.1). The plants fertilized with the Greensulf produced a significantly higher leaf K content (1.79%) compared to the plants fertilized with the LAN source (1.71%) (Figure 4.9).

Season 2023/24

Nitrogen source, growth stage, and the interaction between the nitrogen source and growth stage significantly influenced the leaf K content (Table 4.1). The LAN source applied to plants in a split application at V4 & V9 resulted in a significantly lower leaf K content than plants subjected to all other treatment combinations (Table 4.8).

Prolific

Season 2023/24

The nitrogen source, growth stage, interaction between nitrogen source and application rate, as well as the interaction between nitrogen source and growth stage, significantly influenced the plants' leaf K content.

The plants fertilized with Greensulf as a topdressing at 0, 20, and 40 kg N ha⁻¹ resulted in significantly higher leaf K content (1.61%, 1.64%, and 1.68%) compared to plants fertilized with LAN at a rate of 40 kg N ha⁻¹ (1.47%). No other significant differences in K content were found (Table 4.9).

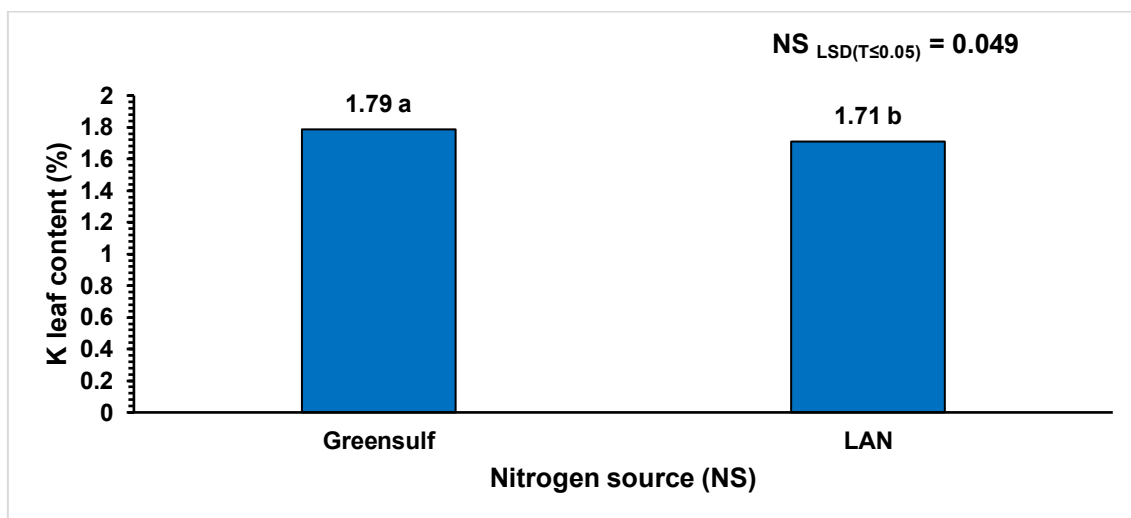


Figure 4.9 Effect of nitrogen source on the non-prolific cultivar's leaf K content (%) in the 2022/23 season. Values with different letters differ significantly.

Table 4.8 Leaf K content (%) as influenced by the interaction between nitrogen sources and growth stage applied on a non-prolific cultivar for the 2023/24 season

Growth stages (GS)	Sources (NS)		Average
	Greensulf	LAN	
V4	1.90 ^a	1.87 ^a	1.89
V9	1.84 ^a	1.87 ^a	1.85
V4 & V9	1.91 ^a	1.61 ^b	1.76
Average	1.88	1.78	

NS $LSD (T \leq 0.05) = 0.07249$

GS $LSD (T \leq 0.05) = 0.107$

NS x GS $LSD (T \leq 0.05) = 0.185$

Values with different letters differ significantly.

Table 4.9 Leaf K content (%) as influenced by the interaction between nitrogen sources and application rate on a prolific cultivar for the 2023/24 season

Application rate (AR) (kg N ha ⁻¹)	Sources (NS)		Average
	Greensulf	LAN	
0	1.61 ^a	1.58 ^{ab}	1.59
20	1.64 ^a	1.55 ^{ab}	1.60
40	1.68 ^a	1.47 ^b	1.57
Average	1.64	1.53	

NS $LSD (T \leq 0.05) = 0.05397$

AR $LSD (T \leq 0.05) = ns$

NS x AR $LSD (T \leq 0.05) = 0.138$

Values with different letters differ significantly.

Plants fertilized with Greensulf at V4, V9, V4 & V9 and LAN at V4 (1.67%, 1.61%, 1.65% and 1.70%) resulted in significantly greater leaf K content, than LAN in a split application at V4 & V9 (1.36%). Furthermore, plants fertilized with LAN at V4 had a significantly higher leaf K content than plants that received LAN at V9 (1.54%) (Table 4.10).

This study clearly shows that the nitrogen source influenced the leaf K content, with plants receiving Greensulf producing a 4.57% greater leaf K content than those receiving LAN for the non-prolific cultivar. The prolific cultivar also exhibited improved K content when using Greensulf as a nitrogen source. The conclusion that different N sources affect the K content of plant organs is supported by Siam *et al.* (2008), who reported that plants receiving ammonia gas as a nitrogen source resulted in a higher leaf K content (2.075%) than those that received urea (1.835%) or AS (1.965%) as nitrogen source. Ewais *et al.* (2009) also reported that nitrogen source influenced leaf K content, which was higher when AS and compost were

applied in a combination (0.350%) to plants than to plants that only received AS (0.327%) or compost (0.311%) separately.

Table 4.10 Leaf K content (%) as influenced by the interaction between nitrogen sources and growth stage applied on a prolific cultivar for the 2023/24 season

Growth stages (GS)	Sources (NS)		Average
	Greensulf	LAN	
V4	1.67 ^{ab}	1.70 ^a	1.68
V9	1.61 ^{ab}	1.54 ^b	1.58
V4 & V9	1.65 ^{ab}	1.36 ^c	1.50
Average	1.64	1.53	

NS $LSD (T \leq 0.05) = 0.05397$

GS $LSD (T \leq 0.05) = 0.079$

NS x GS $LSD (T \leq 0.05) = 0.097$

Values with different letters differ significantly.

Bruns & Ebelhar (2006) reported that nitrogen rate influenced leaf K content, with increasing leaf K content as nitrogen rates increased from 134 kg ha⁻¹ (16.8 mg g⁻¹) to 269 kg ha⁻¹ (17.3 mg g⁻¹), followed by a decrease when 314 kg ha⁻¹ (16.7 mg g⁻¹) was reached. Liu *et al.* (2022) discovered that leaf K content increased with increased nitrogen rates from 100 (1.54 mg plant⁻¹) to 200 kg ha⁻¹ (1.67 mg plant⁻¹), but a further increase led to a decrease when 300 kg ha⁻¹ (1.61 mg plant⁻¹) was applied. This study also found that nitrogen rates affected the leaf K content of the prolific cultivar.

4.3.1.1.5 Ca content

Non-prolific

Season 2022/23

Only the interaction between nitrogen source and application rate significantly influenced the leaf Ca content (Table 4.1).

Notably, although the analysis of variance indicated a significant interaction between nitrogen source and application rate for Ca content, no significant differences were found following the Tukey least significant difference test calculation. This indicates a strong tendency towards significance (Table 4.11).

Season 2023/24

The Ca leaf content was significantly influenced by the nitrogen source used (Table 4.1). The plants that received Greensulf (1.64%) had a significantly higher (7.2% increase) leaf Ca content compared to plants that received LAN as a nitrogen source (1.53%) (Figure 4.10).

Table 4.11 Leaf Ca content (%) as influenced by the interaction between nitrogen sources and application rate on a non-prolific cultivar for the 2022/23 season

Application rate (AR) (kg N ha ⁻¹)	Sources (NS)		Average
	Greensulf	LAN	
0	0.48 ^a	0.44 ^a	0.46
20	0.44 ^a	0.46 ^a	0.45
40	0.46 ^a	0.45 ^a	0.46
Average	0.46	0.45	

$NS_{LSD(T \leq 0.05)} = ns$

$AR_{LSD(T \leq 0.05)} = ns$

$NS \times AR_{LSD(T \leq 0.05)} = 0.062$

Values with different letters differ significantly.

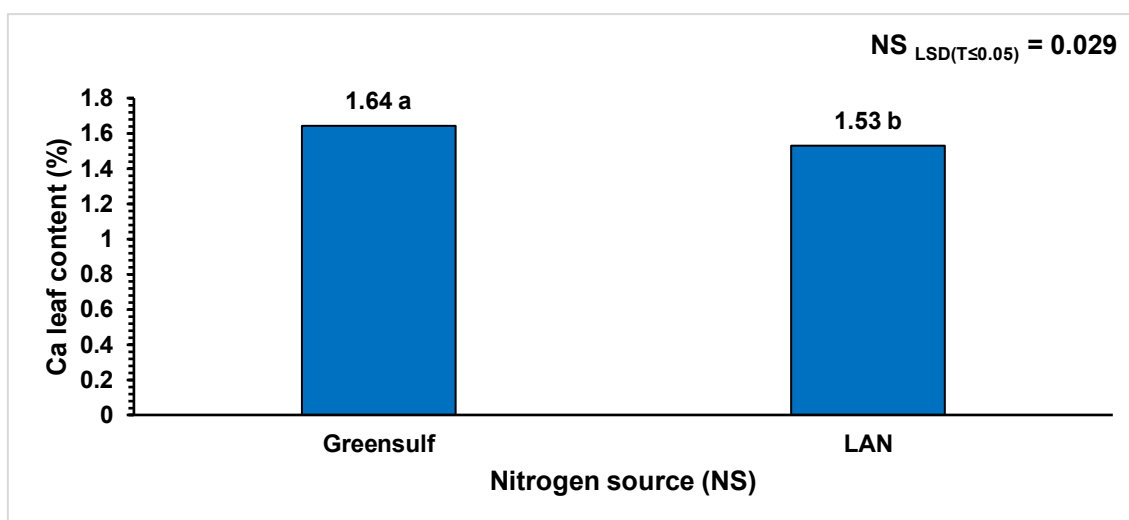


Figure 4.10 Effect of nitrogen source on the non-prolific cultivar's leaf Ca content (%) in the 2022/23 season. Values with different letters differ significantly.

Prolific

Season 2022/23

Only the application rate significantly affected the Ca leaf content of the plants (Table 4.2). An application rate of 0 (0.41%) and 40 (0.41%) kg N ha⁻¹ resulted in a significantly higher leaf Ca content than plants that received an application rate of 20 kg N ha⁻¹ (0.37%) (Figure 4.11).

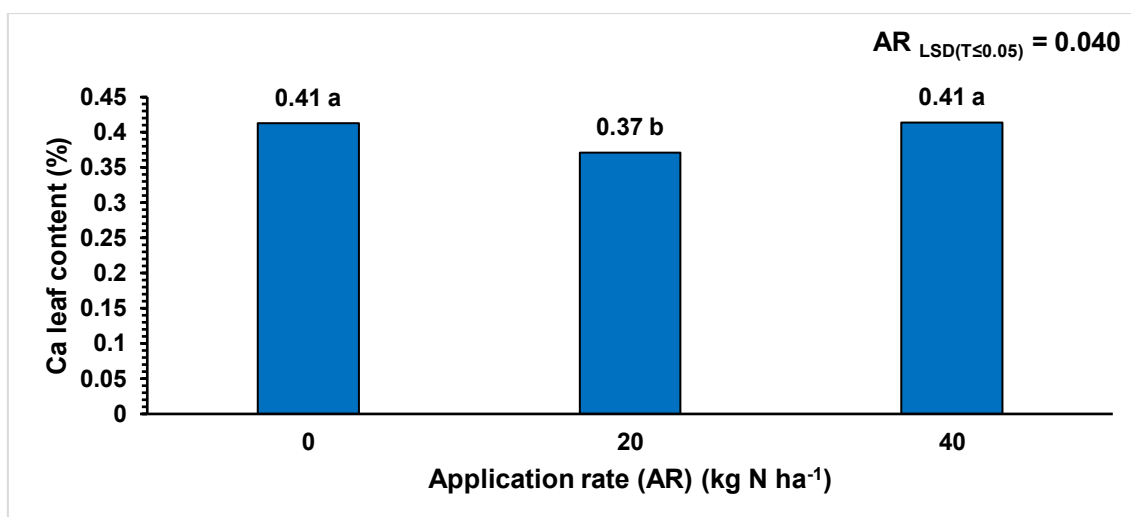


Figure 4.11 Effect of application rate on the prolific cultivar's leaf Ca content (%) in the 2022/23 season. Values with different letters differ significantly.

Season 2023/24

Leaf Ca content was only significantly influenced by the interaction between the nitrogen source and growth stage (Table 4.2). Plants fertilized with Greensulf at V9 and LAN in a split application at V4 & V9 resulted in a significantly higher leaf Ca content (0.51%) than those fertilized with Greensulf at V4 (0.45%) (Table 4.12).

Table 4.12 Leaf Ca content (%) as influenced by the interaction between nitrogen sources and growth stage applied on a prolific cultivar for the 2023/24 season

Growth stages (GS)	Sources (NS)		Average
	Greensulf	LAN	
V4	0.45 ^b	0.48 ^{ab}	0.47
V9	0.51 ^a	0.46 ^{ab}	0.49
V4 & V9	0.47 ^{ab}	0.51 ^a	0.49
Average	0.48	0.48	

NS $LSD(T \leq 0.05) = ns$

GS $LSD(T \leq 0.05) = ns$

NS x GS $LSD(T \leq 0.05) = 0.053$

Values with different letters differ significantly.

Overall, the non-prolific cultivar appeared to produce the highest leaf Ca content in the Greensulf control plants in the 2022/23 season. In the 2023/24 season, plants fertilized with Greensulf produced 6.94% higher Ca content in the leaves than plants fertilized with LAN. The prolific cultivar produced the highest leaf Ca content when plants were fertilized at a rate of 0 kg N ha⁻¹ (0.41%). When Greensulf was applied at V9 and LAN in a split application at V4 & V9, plants produced the highest leaf Ca content.

Nitrogen source had an effect on the leaf Ca content, and it was found that plants treated with Greensulf produced a higher Ca content compared to those treated with LAN. Once more, nitrogen sources affected nutrient content in plant tissue. This concurs with the findings of Coetzee *et al.* (2017), where plants fertilized with LAN yielded a higher Ca content than those fertilized with urea.

Bruns & Ebelhar (2006) did not find any significant differences in leaf Ca content at different nitrogen rates from 134 to 314 kg ha⁻¹, all producing 2.8 mg g⁻¹, except for an application rate of 179 kg ha⁻¹, which resulted in 2.9 mg g⁻¹ of Ca in the ear leaves of plants. This finding aligns with the results found for the non-prolific cultivar, with no significant effects between nitrogen rates on the leaf Ca content of plants. Liu *et al.* (2022) reported that nitrogen application rates significantly influenced the Ca content in maize sap. When plants were fertilized with 100 kg N ha⁻¹ (0.32 mg plant⁻¹), a significantly higher Ca content was found than that of plants fertilized with 200 kg N ha⁻¹ (0.3 mg plant⁻¹).

4.3.1.1.6 Mg content

Non-prolific

Season 2022/23

Only the nitrogen source significantly influenced the leaf Mg content (Table 4.1). The application of Greensulf (0.16%) to plants resulted in a significantly higher leaf Mg content than the application of LAN (0.15%) (Figure 4.12).

Prolific

Season 2022/23

Only the application rate significantly affected the Mg leaf content (Table 4.2). A topdressing application of 40 kg N ha⁻¹ (0.144%) resulted in a significantly higher leaf Mg content than a 20 kg N ha⁻¹ (0.131%) application on plants. No significant effects were observed between the 0 kg N ha⁻¹ (0.141%) application and the other treatments (Figure 4.13).

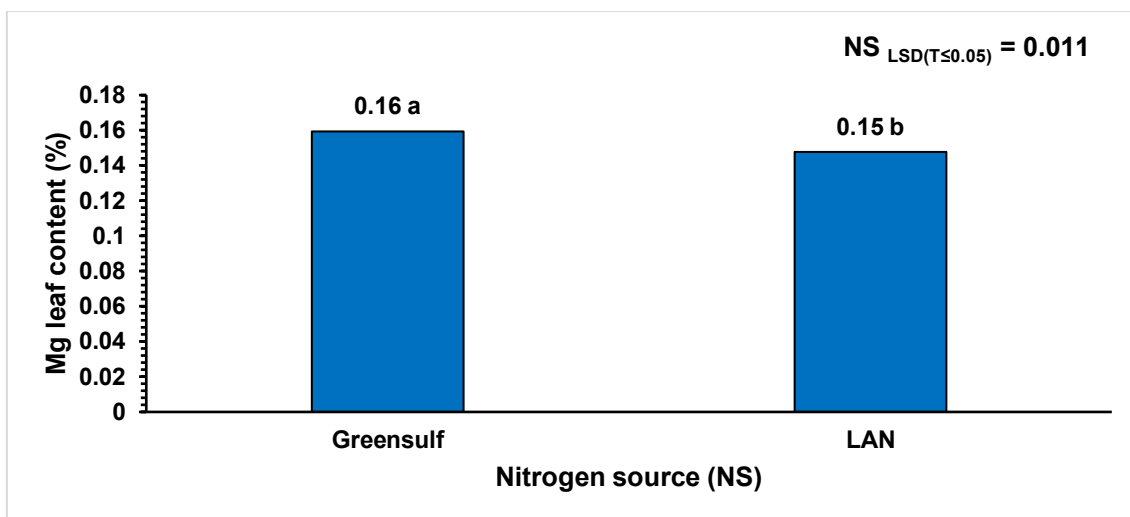


Figure 4.12 Effect of nitrogen source on the non-prolific cultivar's leaf Mg content (%) in the 2022/23 season. Values with different letters differ significantly.

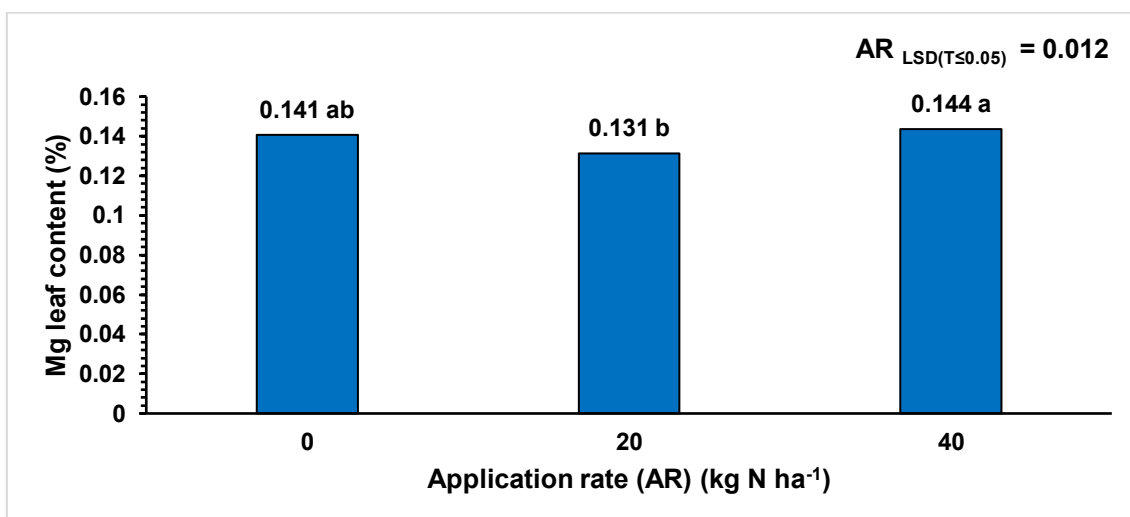


Figure 4.13 Effect of application rate on the prolific cultivar's leaf Mg content (%) in the 2022/23 season. Values with different letters differ significantly.

The non-prolific cultivar fertilized with Greensulf had the highest leaf Mg content (0.16%). Coetzee *et al.* (2017) reported that the Mg concentration in maize plants was greater when urea was used as a nitrogen source than when LAN was used, indicating that the nitrogen source may affect the Mg uptake of maize plants. The prolific cultivar produced the highest leaf Mg content when a topdressing application rate of 40 kg N ha⁻¹ (total of 146 kg N ha⁻¹) (0.144%) was applied to plants. Liu *et al.* (2022) also reported that nitrogen application rates influenced the leaf Mg content. The authors found that the highest Mg content was found in plants that were fertilized at a rate of 200 kg N ha⁻¹ (0.167 mg plant⁻¹) and that those fertilized at 100 kg ha⁻¹ (0.154 mg plant⁻¹) were significantly lower. Burns & Ebelhar (2006) reported no differences between plants treated at different nitrogen application rates (134, 179, 224, 269,

and 314 kg N ha⁻¹), with all producing 1.6 mg kg⁻¹ Mg content in the ear leaves, which is in contrast with the results of this study. Having said that, no significant difference in Mg content was observed between a topdressing application rate of 0 and 40 kg ha⁻¹, which concurs with the findings of Bruns & Ebelhar (2006).

4.3.1.2 Micronutrient leaf concentration

4.3.1.2.1 Cu content

Non-prolific

Season 2022/23

The leaf Cu content was significantly affected by the nitrogen source, application rate, and growth stage (Table 4.1).

Plants fertilized with LAN (11.72 mg kg⁻¹) resulted in a significantly higher leaf Cu content than plants that received Greensulf (8.83 mg kg⁻¹) as a nitrogen source (Figure 4.14). Applying 40 kg N ha⁻¹ (10.96 mg kg⁻¹) to plants resulted in a significantly higher leaf Cu content than applying 0 kg N ha⁻¹ (9.58 mg kg⁻¹). Furthermore, no significant differences were found between plants applied with a 20 kg N ha⁻¹ (10.29 mg kg⁻¹) and the other treatments (Figure 4.15). This is a near-linear increase in Cu content with incremental topdressing increases.

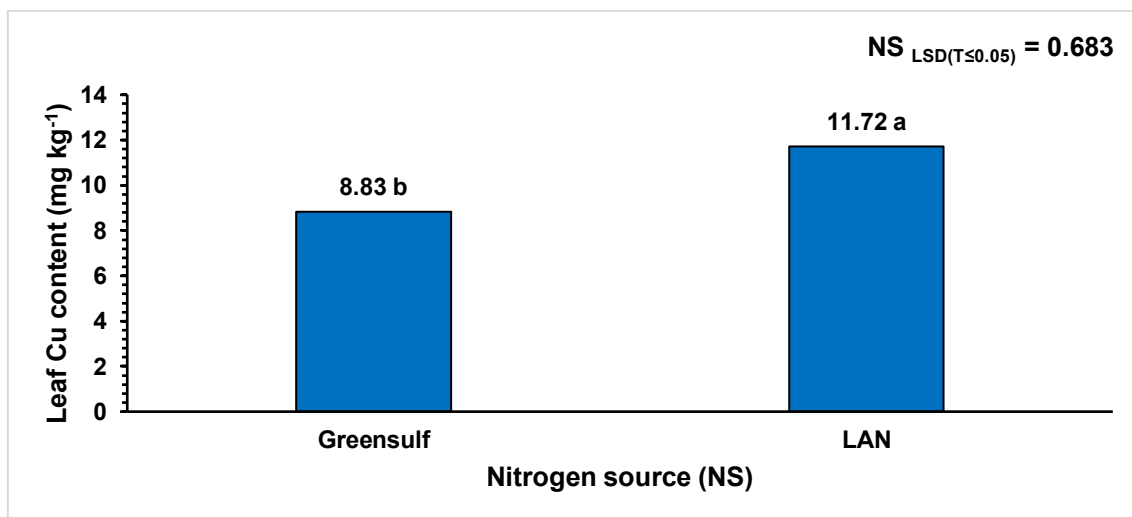


Figure 4.14 Effect of nitrogen source on the non-prolific cultivar's leaf Cu content (mg kg⁻¹) in the 2022/23 season. Values with different letters differ significantly.

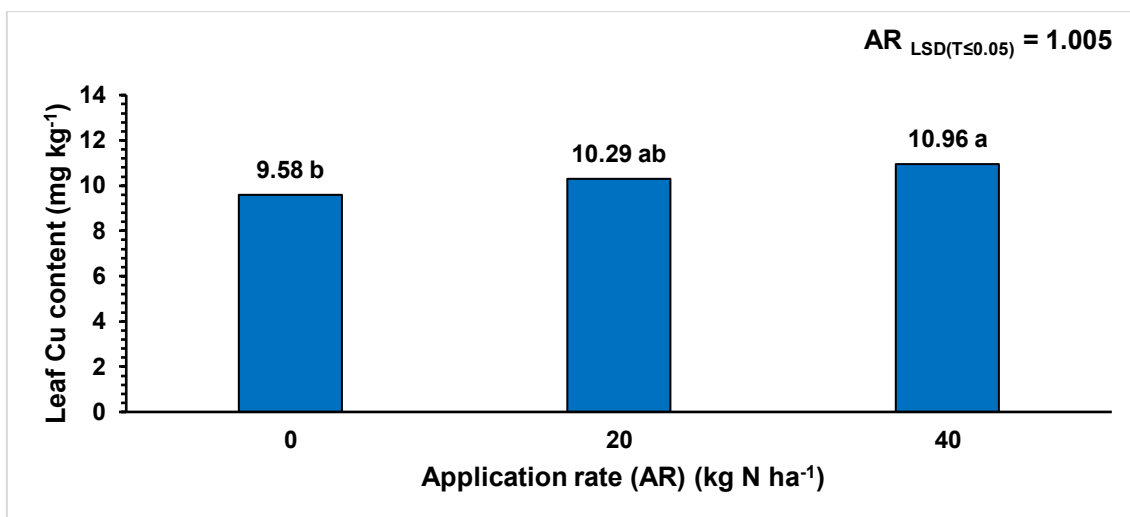


Figure 4.15 Effect of application rate on the non-prolific cultivar's leaf Cu content (mg kg⁻¹) in the 2022/23 season. Values with different letters differ significantly.

Notably, although the analysis of variance indicated a significant interaction effect between nitrogen source and application rate for Cu content, no significant differences were found following the Tukey least significant difference test calculation. This indicates a strong tendency towards significance (Figure 4.16).

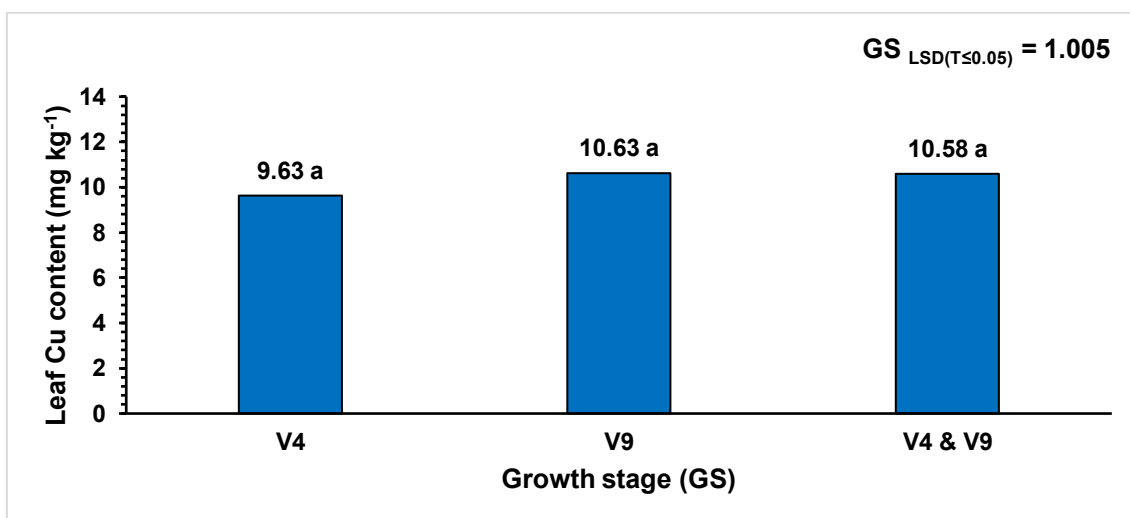


Figure 4.16 Effect of growth stage on the non-prolific cultivar's leaf Cu content (mg kg⁻¹) in the 2022/23 season. Values with different letters differ significantly.

Season 2023/24

Leaf Cu content was significantly influenced by the nitrogen source and the interaction between the nitrogen source and the growth stage applied (Table 4.1).

When Greensulf was applied to plants at V9 (13.83 mg kg⁻¹), in a split application at V4 & V9 (13.42 mg kg⁻¹), and when LAN was applied at V4 (13.08 mg kg⁻¹), it resulted in a significantly higher leaf Cu content than that of plants fertilized with LAN in a split application at V4 & V9 (10.17 mg kg⁻¹). There were no further significant differences between treatment means (Table 4.13).

Table 4.13 Leaf Cu content (mg kg⁻¹) as influenced by the interaction between nitrogen sources and growth stage applied on a non-prolific cultivar for the 2023/24 season

Growth stages (GS)	Sources (NS)		Average
	Greensulf	LAN	
V4	12.58 ^{ab}	13.08 ^a	12.83
V9	13.83 ^a	12.17 ^{ab}	13.00
V4 & V9	13.42 ^a	10.17 ^b	11.79
Average	13.28	11.81	

NS $LSD (T \leq 0.05) = 0.949$

GS $LSD (T \leq 0.05) = ns$

NS x GS $LSD (T \leq 0.05) = 2.420$

Values with different letters differ significantly.

Prolific

Only the nitrogen application rate significantly affected the leaf Cu content in the 2022/23 season. No significant differences were observed in the main effects or interactions during the 2023/24 season (Table 4.2).

When the plants were fertilized with a topdressing of 40 kg N ha⁻¹, it resulted in significantly higher leaf Cu content (11.50 mg kg⁻¹) than plants fertilized at a rate of 0 and 20 kg N ha⁻¹ (10.58 and 10.63 mg kg⁻¹) (Figure 4.17).

To summarise, the LAN source produced a significantly higher leaf Cu content of 28.13% than plants receiving the Greensulf source on the non-prolific cultivar in the 2022/23 season. A 40 kg N ha⁻¹ (10.96 mg kg⁻¹) application rate produced the highest leaf Cu content in treated plants. Applying a nitrogen source at V9 (10.63 mg kg⁻¹) on plants yielded the highest leaf Cu content. In general, when LAN was applied at V4 and Greensulf at V9 or at both V4 & V9, it increased the Cu nutrient content.

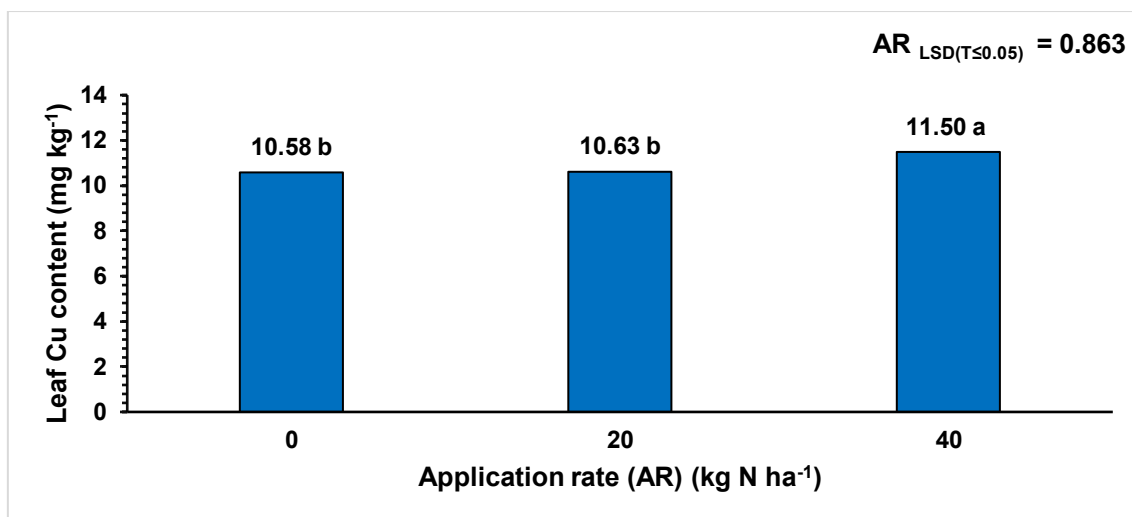


Figure 4.17 Effect of application rate on the prolific cultivar's leaf Cu content (mg kg⁻¹) in the 2022/23 season. Values with different letters differ significantly.

Barunawati *et al.* (2013) observed no significant differences in leaf Cu content on wheat flag leaves when ammonium nitrate and urea were used as nitrogen sources. Therefore, the N source does not matter. This finding contrasts with the results of this study, where Greensulf and LAN sources had a significant effect on maize leaf content. Barunawati *et al.* (2013) also did not find significant effects on flag leaf Cu content on the application timing (total nitrogen at planting or anthesis (EC65) stage). These findings are in contrast to the results of this study, where it was observed that the nitrogen source and growth stage had a significant effect on leaf Cu content. However, this study was done on maize plants, not wheat, which could affect the results. Wierzbowska *et al.* (2021) reported that different nitrogen sources significantly influenced the Cu content in maize straw, which concurred with the findings of this research. These authors found that plants fertilized with ammonium nitrate (AN)/urea (5.06 mg kg⁻¹) and UAN/urea (5.01 mg kg⁻¹) sources were significantly higher in Cu content than those fertilized with UAN before planting & UAN at V6 (4.39 mg kg⁻¹), UAN+S before planting & UAN+Mg at V6 (4.65 mg kg⁻¹), UAN+P before planting & UAN+P at V6 (4.42 mg kg⁻¹), UAN+P before planting & UAN+S at V6 (4.65 mg kg⁻¹) and UAN+P before planting & UAN+Mg at V6 (3.93 mg kg⁻¹) sources in straw Cu content. This research agrees with the results found on the reaction of the non-prolific cultivar to nitrogen sources. Losak *et al.* (2011) found no effect of nitrogen rates on leaf Cu content when nitrogen was applied at 0, 120, and 240 kg ha⁻¹ (split into 120 kg before planting and 120 kg at V5). These results contrast with results found in this study, where an application of 146 kg N ha⁻¹ (40 kg N ha⁻¹ topdressing) on plants resulted in a significantly higher leaf Cu content than the other treatments. Liu *et al.* (2022) results agree with this study's results, which found those plants fertilized with an application rate of 300 kg

N ha⁻¹ produced a higher leaf Cu content (0.071 µg plant⁻¹) than those treated with a 100 (0.069 µg plant⁻¹) and 200 (0.063 µg plant⁻¹) kg N ha⁻¹.

4.3.1.2.2 Mn content

Only the non-prolific cultivar was significantly affected by the growth stage and the interaction between the nitrogen source and growth stage during the 2023/24 season (Table 4.1). Plants treated with Greensulf at V4 (188.67 mg kg⁻¹) showed a significantly higher leaf Mn content compared to those treated with a split application at V4 & V9 (107.92 mg kg⁻¹), with no significant differences among the other treatments (Table 4.14).

Wierzbowska *et al.* (2021) reported that Mn content was significantly higher in plant straw when it was fertilized with the nitrogen source ammonium nitrate/urea (105 mg kg⁻¹) and the lowest when fertilized with UAN+S/UAN+Mg (25.77 mg kg⁻¹). They concluded that nitrogen fertilization generally increased the straw Mn content of plants and that different solid nitrogen fertilizers influenced the straw Mn content. Siam *et al.* (2008) reported that plants fertilized with ammonium sulphate produced a higher Mn content (60 mg kg⁻¹) than those fertilized with ammonium gas (56.75 mg kg⁻¹) and urea (53.75 mg kg⁻¹). These findings align with this study, which also observed that different nitrogen sources affect leaf Mn content, although their research tested plant straw rather than leaves at the R2 growth stage.

Table 4.14 Leaf Mn content (mg kg⁻¹) as influenced by the interaction between nitrogen sources and growth stage applied on a non-prolific cultivar for the 2023/24 season

Growth stages (GS)	Sources (NS)		Average
	Greensulf	LAN	
V4	188.67 ^a	148.50 ^{ab}	168.58
V9	135.08 ^{ab}	143.83 ^{ab}	139.46
V4 & V9	107.92 ^b	161.08 ^{ab}	134.50
Average	143.89	151.14	

NS LSD ($T \leq 0.05$) = ns

GS LSD ($T \leq 0.05$) = 33.39

NS x GS LSD ($T \leq 0.05$) = 57.87

Values with different letters differ significantly.

4.3.1.2.3 Zn content

Non-prolific

Season 2022/23

The leaf Zn content was significantly affected by the nitrogen source and application rate (Table 4.1). The plants that received Greensulf (37.08 mg kg⁻¹) as a nitrogen source resulted

in a significantly higher leaf Zn content than those that received LAN (33.81 mg kg⁻¹) (Figure 4.18).

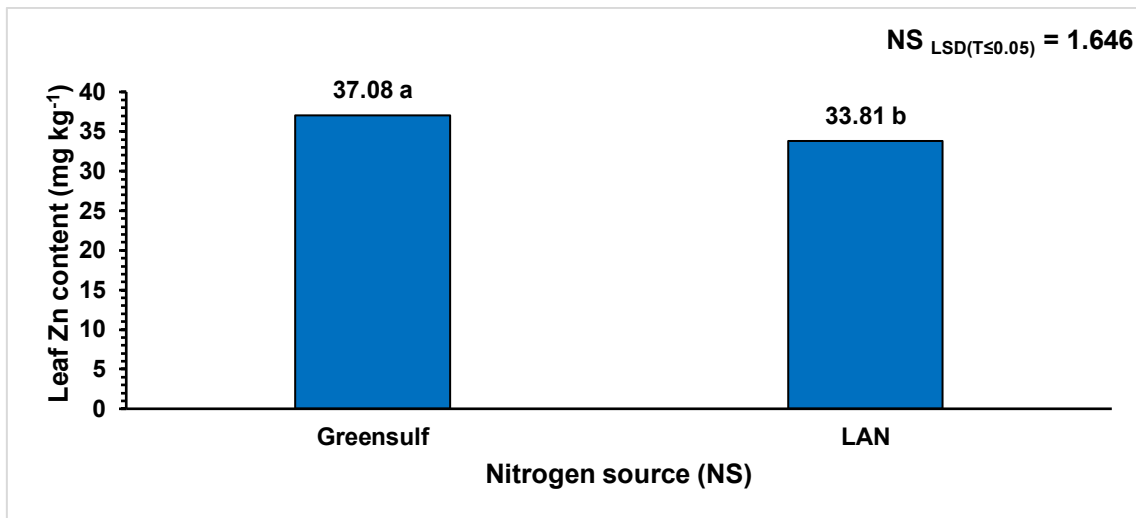


Figure 4.18 Effect of nitrogen source on the non-prolific cultivar's leaf Zn content (mg kg⁻¹) in the 2022/23 season. Values with different letters differ significantly.

A 40 kg N ha⁻¹ topdressing on maize plants resulted in a significantly higher leaf Zn content (36.92 mg kg⁻¹) than that of the 0 kg N ha⁻¹ (34.25 mg kg⁻¹), with no significant differences between a topdressing of 20 kg N ha⁻¹ (35.17 mg kg⁻¹) and the aforementioned topdressing applications (Figure 4.19). Once more, a near-linear increase in Zn content was observed, along with an increase in N topdressing application rates.

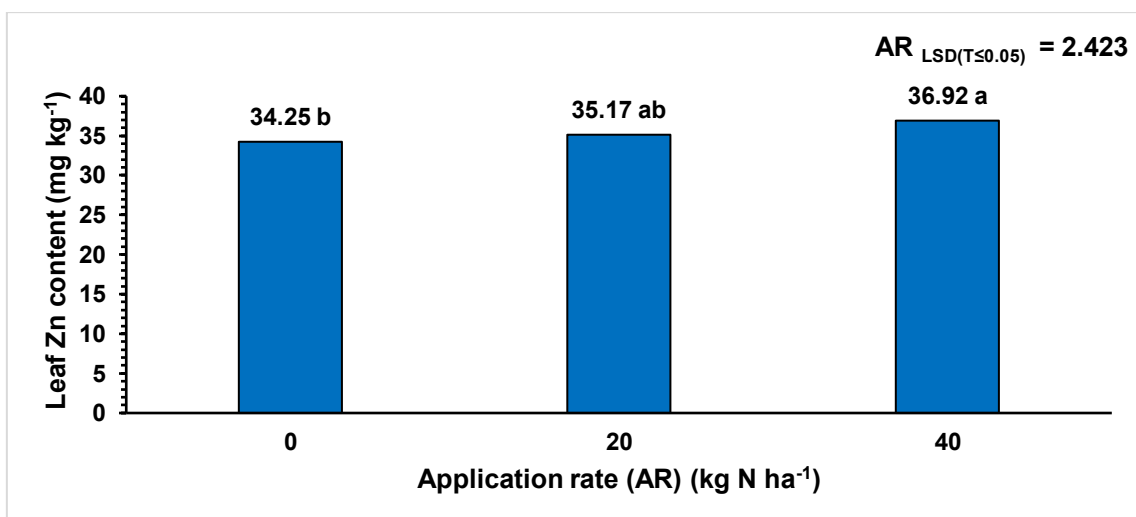


Figure 4.19 Effect of application rate on the non-prolific cultivar's leaf Zn content (mg kg⁻¹) in the 2022/23 season. Values with different letters differ significantly.

It was reported by Wierzbowska *et al.* (2021) that different nitrogen sources affected the Zn content of maize straw. They found that plants fertilized with ammonium nitrate/urea (26.85 mg kg⁻¹) had a significantly higher Zn straw content than the control (13.53 mg kg⁻¹) and UAN+S/UAN+Mg (16.06 mg kg⁻¹). Siam *et al.* (2008) also reported that ammonium sulphate produced the highest Zn content (60 mg kg⁻¹), followed by ammonium gas (56.75 mg kg⁻¹) and urea (53.75 mg kg⁻¹) in decreasing order. These observations support the result found in this study, where plants fertilized with Greensulf produced a 9.23% higher Zn leaf content than plants fertilized with LAN.

Siam *et al.* (2008) also reported that the ear leaf, Zn content decreased with an increasing nitrogen rate, with the control (0 kg N ha⁻¹) producing the highest Zn content (30 mg kg⁻¹) followed by 100 (26.3 mg kg⁻¹), 120 (22.7 mg kg⁻¹) and 140 (18.33 mg kg⁻¹) kg N ha⁻¹ in decreasing order. This finding is in contrast to the results found by Burns & Ebelhar (2006), who reported that leaf Zn content increased with a rate of 134 (22.0 mg kg⁻¹) to 269 (27.1 mg kg⁻¹) kg ha⁻¹ with a decrease with a further increase in nitrogen rates (314 kg ha⁻¹ with 26.1 mg kg⁻¹). The results obtained by Burns & Ebelhar (2006) agree with those found in this study, which showed an increase in leaf Zn content as nitrogen rates increased.

Season 2023/24

There were no significant differences under the main factors, only in the three-way interaction, which will not be discussed (Table 4.1).

4.3.1.2.4 Fe content

Non-prolific

Season 2022/23

The Fe content in the leaf was significantly influenced by the nitrogen source, growth stage, the interaction between nitrogen source and growth stage, and the interaction between application rate and growth stage (Table 4.1). When plants received LAN in a split application at V4 & V9, their leaf Fe content (196.5 mg kg⁻¹) tested significantly higher than the other treatment combinations (Table 4.15).

Table 4.15 Leaf Fe content (mg kg⁻¹) as influenced by the interaction between nitrogen sources and growth stage applied on a non-prolific cultivar for the 2022/23 season

Growth stages (GS)	Sources (NS)		Average
	Greensulf	LAN	
V4	79.25 ^b	90.75 ^b	85.00
V9	92.83 ^b	93.50 ^b	93.17
V4 & V9	84.83 ^b	196.50 ^a	140.67
Average	85.64	126.92	

NS $LSD (T \leq 0.05) = 29.64$

GS $LSD (T \leq 0.05) = 43.63$

NS x GS $LSD (T \leq 0.05) = 75.61$

Values with different letters differ significantly.

An application rate of 40 kg N ha⁻¹ in a split application at V4 & V9 (207.88 mg kg⁻¹) treated plants resulted in significantly greater leaf Fe content than the other treatment combinations, except for a 0 kg N ha⁻¹ at V4 & V9 (120.38 mg kg⁻¹) (Table 4.16).

Table 4.16 Leaf Fe content (mg kg⁻¹) as influenced by the interaction between application rate and growth stage applied on a prolific cultivar for the 2022/23 season

Application rate (AR) (kg N ha ⁻¹)	Growth stage (GS)			Average
	V4	V9	V4 & V9	
0	86.13 ^b	98.00 ^b	120.38 ^{ab}	101.50
20	79.50 ^b	96.50 ^b	93.75 ^b	89.92
40	89.38 ^b	85.00 ^b	207.88 ^a	127.42
Average	85.00	93.17	140.67	

AR $LSD (T \leq 0.05) = ns$

GS $LSD (T \leq 0.05) = 43.63$

AR x GS $LSD (T \leq 0.05) = 101.2$

Values with different letters differ significantly.

Season 2023/24

The nitrogen source and growth stage significantly influenced the leaf Fe content (Table 4.1). The plants fertilized with Greensulf (82.67 mg kg⁻¹) produced a significantly higher (5.33%) leaf Fe content than plants fertilized with LAN (78.22 mg kg⁻¹) (Figure 4.20). Plants that received nitrogen topdressing at V4 (85.46 mg kg⁻¹) resulted in significantly higher leaf Fe content than those in a split application at V4 & V9 (75.25 mg kg⁻¹), with no significant difference between plants top dressed at V9 (80.63 mg kg⁻¹) and the other growth stages (Figure 4.21).

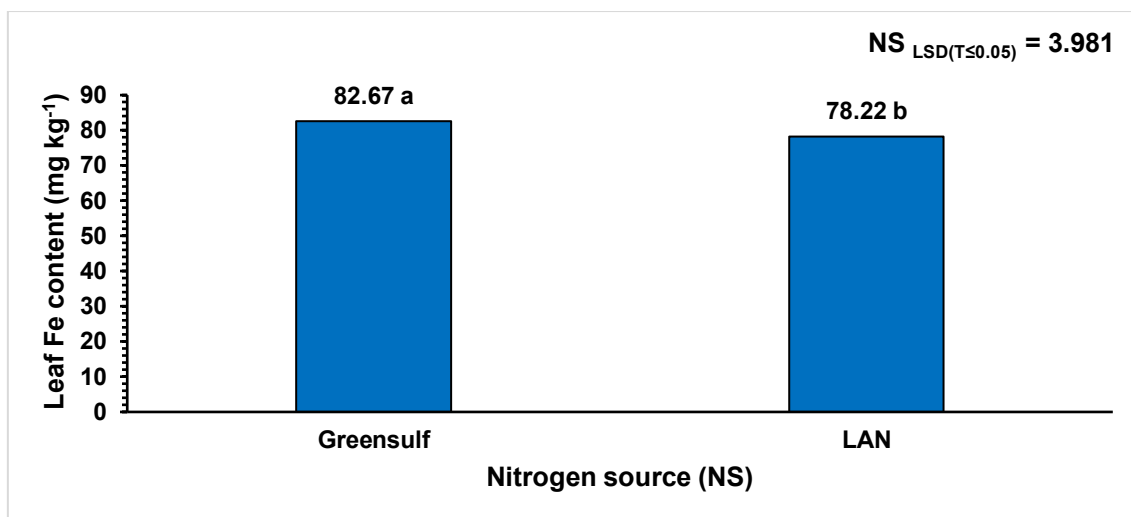


Figure 4.20 Effect of nitrogen source on the non-prolific cultivar's leaf Fe content (mg kg^{-1}) in the 2023/24 season. Values with different letters differ significantly.

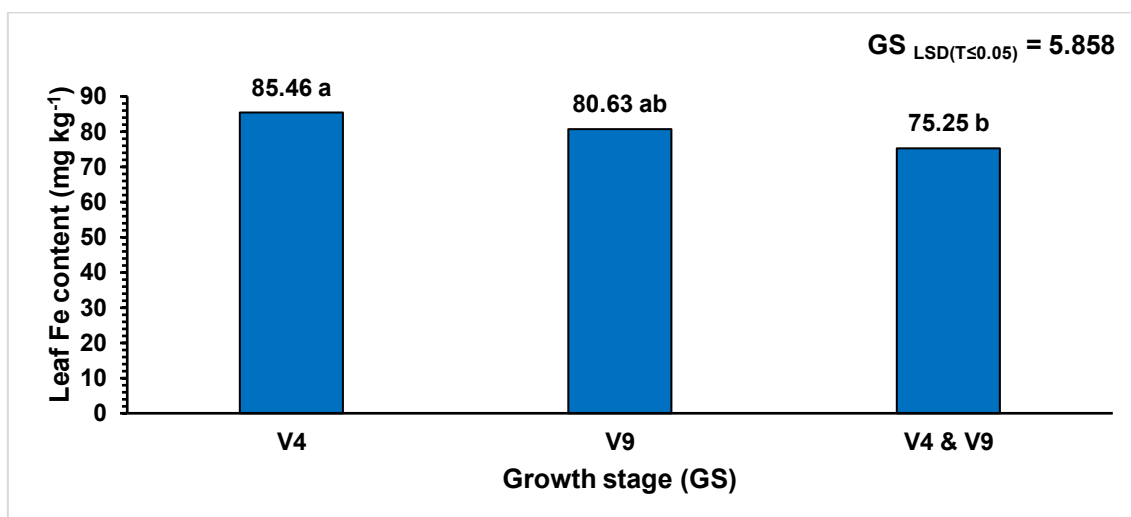


Figure 4.21 Effect of growth stage on the non-prolific cultivar's leaf Fe content (mg kg^{-1}) in the 2023/24 season. Values with different letters differ significantly.

Wierzbowska *et al.* (2021) reported that plants fertilized with ammonium nitrate/urea resulted in the highest straw Fe content ($168.81 \text{ mg kg}^{-1}$), and plants fertilized with UAN+P/UAN+Mg resulted in the lowest straw Fe content (72.88 mg kg^{-1}). Siam *et al.* (2008) found that neither the nitrogen source nor the nitrogen rate affected the leaf Fe content of fertilised plants. They reported that plants that did not receive any nitrogen had the highest leaf Fe content compared to plants that did receive nitrogen. This finding contrasts with the results of Wierzboska *et al.* (2021) and this study. Barunawati *et al.* (2013) observed differences in leaf Fe content in wheat flag leaves when ammonium nitrate and urea were used as nitrogen sources, and when nitrogen was applied at planting or anthesis. However, they found no significant differences resulting from these treatments. Although this was conducted on wheat, the results may still

be relevant to maize, as both are members of the grass family and are cereal grains. This observation, however, contrasts with the results of this study. The nitrogen source and growth stage applied significantly affected the leaf Fe content.

4.3.1.2.5 B content

Non-prolific

Nitrogen source and the interaction between application rate and growth stage significantly affected the leaf B content in the 2022/23 season. No significant effects were found as a result of the main effects or their interactions on the leaf B content in the 2023/24 season (Table 4.1).

The plants that received Greensulf as a nitrogen source had a significantly higher leaf B content (16.81 mg kg^{-1}) than those of plants that received LAN (13.97 mg kg^{-1}) as a nitrogen source (Figure 4.22).

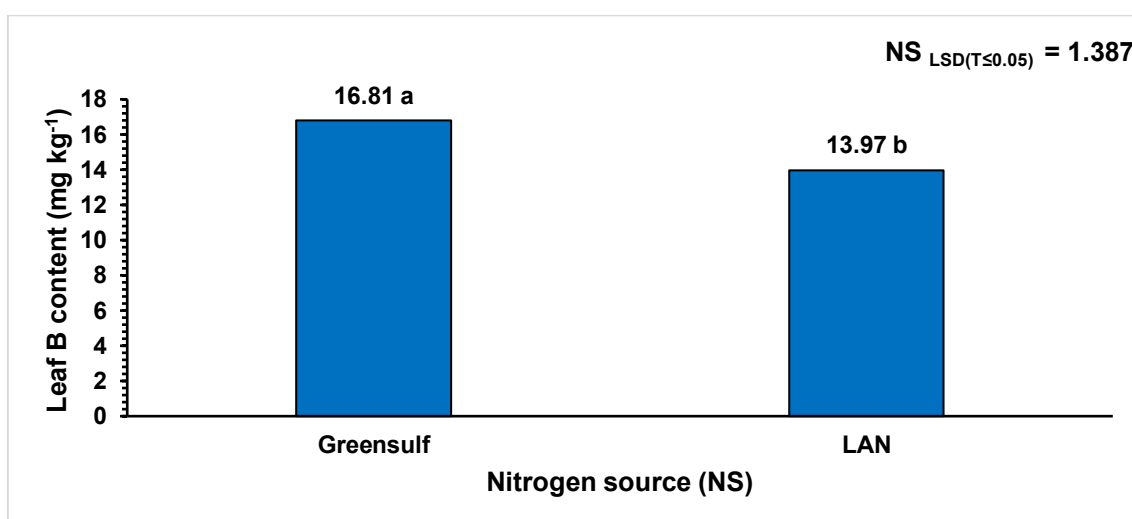


Figure 4.22 Effect of nitrogen source on the non-prolific cultivar's leaf B content (mg kg^{-1}) in the 2022/23 season. Values with different letters differ significantly.

It is noteworthy that although the analysis of variance indicated a significant interaction effect between application rate and growth stage for B content, no significant differences were found following the Tukey least significant difference test calculation. This suggests a strong tendency towards significance. The highest leaf B content was produced with plants fertilized with a topdressing rate of 40 kg N ha^{-1} (15.67 mg kg^{-1}) and when nitrogen was applied on the V4 growth stage (16.08 mg kg^{-1}) (Table 4.17).

Prolific

The leaf B content was significantly influenced by nitrogen source, growth stage, and the interaction between application rate and growth stage in the 2022/23 season. There were no significant effects in the 2023/24 season on the leaf B content (Table 4.2). The leaf B content of plants was significantly higher (12.25 mg kg⁻¹) when Greensulf was used compared to LAN (7.00 mg kg⁻¹) in the 2022/23 season (Figure 4.23).

Table 4.17 Leaf B content (mg kg⁻¹) as influenced by the interaction between application rate and growth stage applied on a non-prolific cultivar for the 2022/23 season

Application rate (AR) (kg N ha ⁻¹)	Growth stage (GS)			Average
	V4	V9	V4 & V9	
0	15.38 ^a	17.00 ^a	14.38 ^a	15.58
20	14.75 ^a	15.50 ^a	14.50 ^a	14.92
40	18.13 ^a	13.63 ^a	15.25 ^a	15.67
Average	16.08	15.38	14.71	

$AR_{LSD(T \leq 0.05)} = ns$

$GS_{LSD(T \leq 0.05)} = ns$

$AR \times GS_{LSD(T \leq 0.05)} = 4.736$

Values with different letters differ significantly.

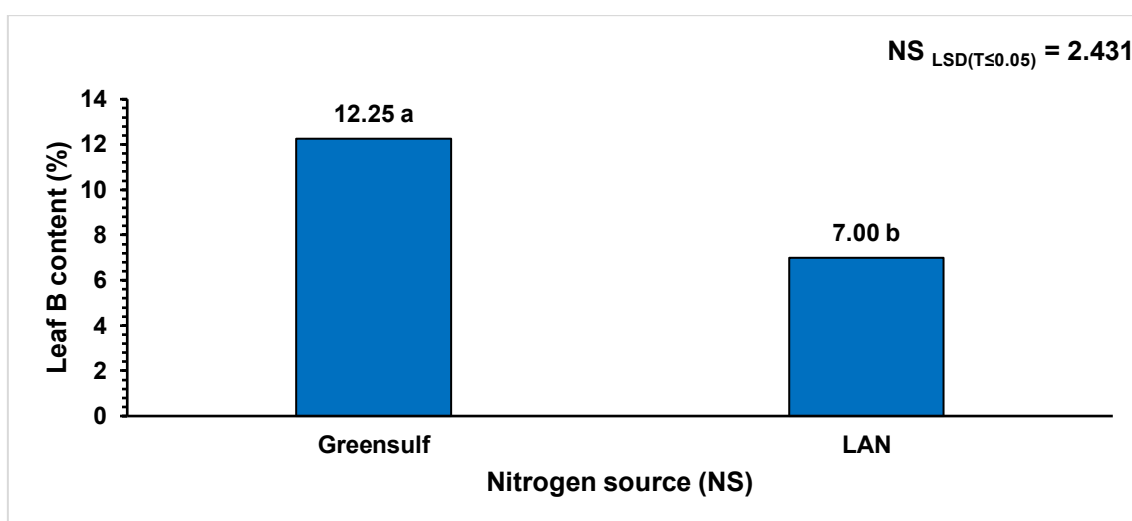


Figure 4.23 Effect of nitrogen source on the prolific cultivar's leaf B content (mg kg⁻¹) in the 2022/23 season. Values with different letters differ significantly.

Plants fertilized at a rate of 40 kg N ha⁻¹ at the V9 growth stage produced a significantly higher leaf B content (15.25 mg kg⁻¹) than those fertilized at 40 kg N ha⁻¹ at V4 (6.63 mg kg⁻¹) and 20 kg N ha⁻¹ as a split application at V4 & V9 (6.63 mg kg⁻¹), with no further significant differences in leaf B content of the remaining treatment combinations (Table 4.18).

Table 4.18 Leaf B content (mg kg⁻¹) as influenced by the interaction between application rate and growth stage applied on a prolific cultivar for the 2022/23 season

Application rate (AR) (kg N ha ⁻¹)	Growth stage (GS)			Average
	V4	V9	V4 & V9	
0	11.75 ^{ab}	8.63 ^{ab}	9.00 ^{ab}	9.79
20	8.13 ^{ab}	12.75 ^{ab}	6.63 ^b	9.17
40	6.63 ^b	15.25 ^a	7.88 ^{ab}	9.92
Average	8.83	12.21	7.83	

AR $LSD_{(T \leq 0.05)} = ns$

GS $LSD_{(T \leq 0.05)} = 3.578$

AR x GS $LSD_{(T \leq 0.05)} = 8.303$

Values with different letters differ significantly.

Both non-prolific and prolific cultivar plants had significantly higher leaf B content when treated with Greensulf as a nitrogen source. The highest leaf B content was obtained at a nitrogen rate of 40 kg ha⁻¹ for both cultivars, but at different growth stages (V4 for non-prolific and V9 for prolific cultivars).

Coetzee *et al.* (2017) reported that no significant effect was found on different nitrogen sources (urea and LAN) on the concentration of maize plant B. This finding contradicts the results found in this study. Wierzbowska *et al.* (2021) reported that the nitrogen source applied to maize plants significantly affected the straw B content. They noted that plants fertilized with ammonium nitrate/urea produced significantly higher straw B content (4.87 mg kg⁻¹) than plants fertilized with UAN+P/UAN+Mg (4.02 mg kg⁻¹). These results are consistent with those found in this study, indicating that nitrogen sources have a significant effect on the leaf B content of plants. Hamnér *et al.* (2017) found a positive correlation coefficient between the nitrogen rate applied and wheat leaf B content at stem elongation (ZS37) (0.904), flowering (ZS65) (0.992), and grain (0.045). Although it was conducted on wheat, a similar effect could also be observed in this study, where leaf B content was influenced by the nitrogen application rate and growth stage applied.

4.3.1.3 Nutrient leaf ratio

4.3.1.3.1 N:S ratio

Non-prolific

Season 2022/23

The leaf N:S ratio was significantly affected by the nitrogen source and the interactions between the nitrogen source and the application rate and growth stage (a three-factor interaction will not be discussed) (Table 4.1). The plants that were fertilized with LAN resulted

in a significantly higher leaf N:S ratio (16.97:1) than those fertilized with Greensulf (15.93:1) (Figure 4.24).

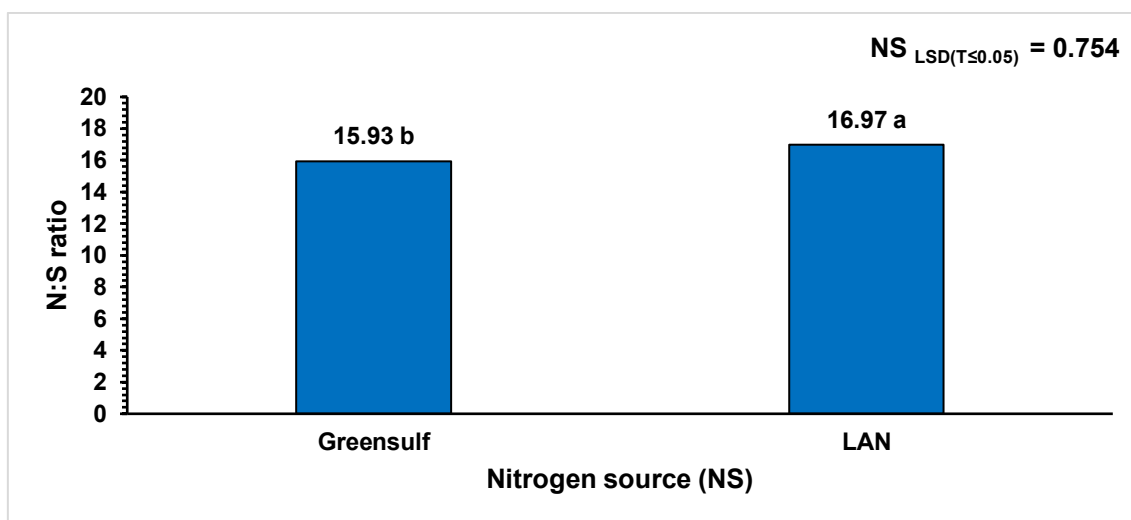


Figure 4.24 Effect of nitrogen source on the non-prolific cultivar's leaf N:S ratio in the 2022/23 season. Values with different letters differ significantly.

Season 2023/24

The leaf N:S ratio was significantly influenced by nitrogen source, growth stage, the interaction between nitrogen source and growth stage, and the interaction between application rate and growth stage (Table 4.1).

Applying LAN as a nitrogen source to plants in a split application at the V4 & V9 growth stages resulted in a significantly higher leaf N:S ratio (22.70:1) than that of plants in all other interaction combinations (Table 4.19). The 0 kg N ha⁻¹ at V4 & V9 treated plants (20.79:1) were significantly higher in leaf N:S ratio than the rest of the interaction combinations, except for an application rate of 20 (19.21:1) and 40 (18.92:1) kg N ha⁻¹ applied in a split application at V4 & V9. The 20 and 40 kg N ha⁻¹ treatments applied in a split application at V4 & V9 were significantly higher in leaf N:S ratio than all other interaction treatments except for an application rate of 40 kg N ha⁻¹ at V9 (17.83:1) (Table 4.20).

Prolific

There were no significant differences between the main effects or their interactions in the 2022/23 season on the leaf N:S ratio. The nitrogen source, growth stage, and the interaction between nitrogen source and growth stage significantly influence the leaf N:S ratio in the 2023/24 season (Table 4.2).

The plants fertilized with LAN in a split application at V4 & V9 delivered significantly the highest leaf N:S ratio (23.89:1) than all interaction combinations. Plants treated with LAN only at the V9 growth stage were significantly higher than most other treatment combinations, except for Greensulf applied at V9, with no further significant differences found (Table 4.21).

Table 4.19 Leaf N:S ratio as influenced by the interaction between nitrogen sources and growth stage applied on a non-prolific cultivar for the 2023/24 season

Growth stages (GS)	Nitrogen source (NS)		Average
	Greensulf	LAN	
V4	16.56 ^b	15.36 ^b	15.96
V9	16.27 ^b	16.66 ^b	16.46
V4 & V9	16.58 ^b	22.70 ^a	19.64
Average	16.47	18.24	

$NS_{LSD (T \leq 0.05)} = 0.7544$

$GS_{LSD (T \leq 0.05)} = 1.110$

$NS \times GS_{LSD (T \leq 0.05)} = 1.924$

Values with different letters differ significantly.

Table 4.20 Leaf N:S ratio as influenced by the interaction between application rate and growth stage applied on a non-prolific cultivar for the 2023/24 season

Application rate (AR) (kg N ha ⁻¹)	Growth stage (GS)			Average
	V4	V9	V4 & V9	
0	16.14 ^c	15.95 ^c	20.79 ^a	17.63
20	15.42 ^c	15.61 ^c	19.21 ^{ab}	16.75
40	16.32 ^c	17.83 ^{bc}	18.92 ^{ab}	17.69
Average	15.96	16.46	19.64	

$AR_{LSD (T \leq 0.05)} = ns$

$GS_{LSD (T \leq 0.05)} = 1.110$

$AR \times GS_{LSD (T \leq 0.05)} = 2.577$

Values with different letters differ significantly.

Table 4.21 Leaf N:S ratio as influenced by the interaction between nitrogen sources and growth stage applied on a prolific cultivar for the 2023/24 season

Growth stages (GS)	Nitrogen source (NS)		Average
	Greensulf	LAN	
V4	17.22 ^c	15.96 ^c	16.59
V9	17.72 ^{bc}	19.77 ^b	18.74
V4 & V9	17.13 ^c	23.89 ^a	20.51
Average	17.36	19.87	

$NS_{LSD (T \leq 0.05)} = 0.952$

$GS_{LSD (T \leq 0.05)} = 1.40$

$NS \times GS_{LSD (T \leq 0.05)} = 2.427$

Values with different letters differ significantly.

The non-prolific cultivar plants fertilized with LAN led to a 6.32% higher leaf N:S ratio than those fertilized with Greensulf in the 2022/23 season. The highest leaf N:S ratio was obtained

with plants fertilized with LAN source in a split application at V4 & V9 (22.70:1) and with 0 kg N ha⁻¹ in a split application at V4 & V9 (20.79:1) in the 2023/24 season. The prolific plants fertilized with LAN source in a split application at V4 & V9 (23.89) produced the highest leaf N:S ratio in the 2023/24 season.

Li *et al.* (2019) reported that different nitrogen and sulphur fertilizer sources at various application rates influence the N:S ratio of different maize plant parts. They noted that leaf N, cob S content, and N:S ratio had a strong relationship with the grain yield produced by maize plants and found that an N:S ratio of between 16.3 and 19.6 is a sufficient range. The findings of this study aligns with those of previous studies, which also demonstrated that nitrogen sources have an impact on the N:S ratio of maize plants. When considering the sufficiency ranges for the N:S ratio, as referenced by Campbell and Plank (2000b), it is noted that S becomes limiting when the N:S ratio is 18 or higher. Under these conditions, the Greensulf source provides an optimal N:S ratio compared to the LAN source.

4.3.1.3.2 N:K ratio

Non-prolific

Season 2022/23

The leaf N:K ratio was significantly influenced by application rate and growth stage (Table 4.1). Plants that were fertilized at a 40 kg N ha⁻¹ resulted in a significantly higher leaf N:K ratio (1.72:1) than plants fertilized at a 0 kg N ha⁻¹ rate (1.63:1), with no significant differences between plants fertilized at a 20 kg N ha⁻¹ (1.71:1) and the other rates (Figure 4.25).

Once again, the analysis of variance revealed a significant difference in the N:K ratio due to the growth stage. However, no significant differences were found following the Tukey least significant difference test calculation. This indicates a strong tendency towards significance (Figure 4.26). The highest N:K ratio was obtained with nitrogen application at V9 (1.74).

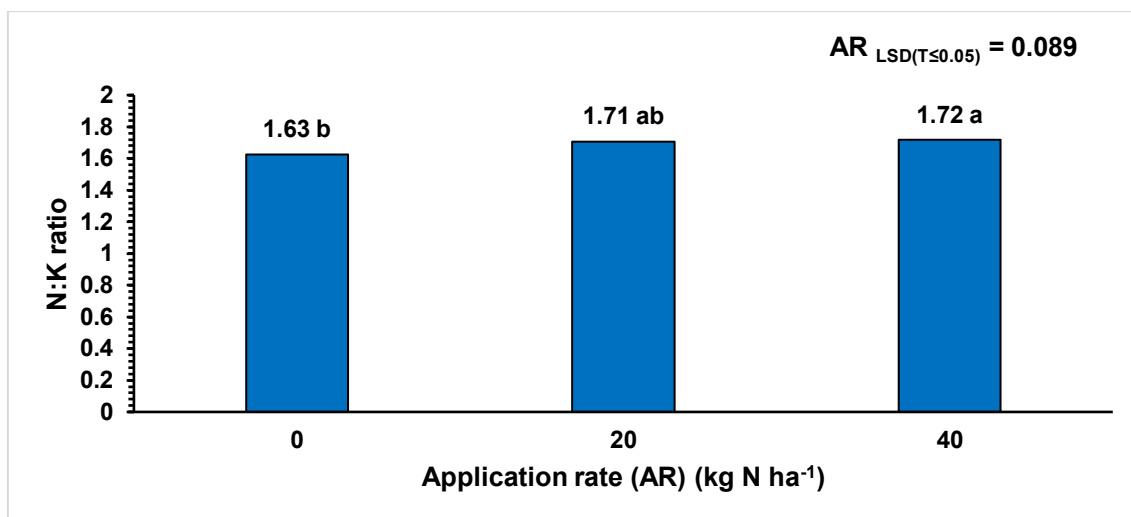


Figure 4.25 Effect of application rate on the non-prolific cultivar's leaf N:K ratio in the 2022/23 season. Values with different letters differ significantly.

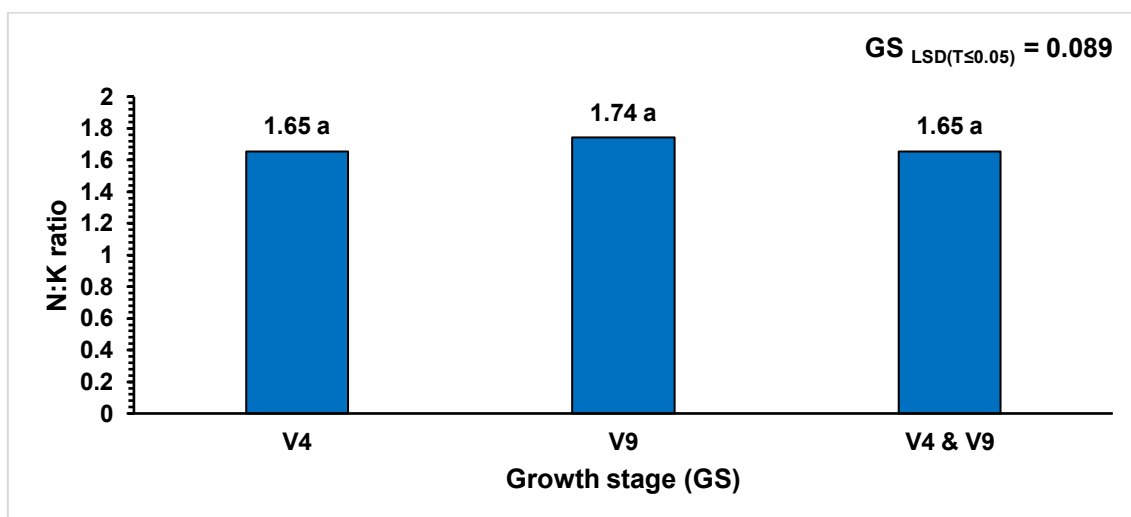


Figure 4.26 Effect of growth stage on the non-prolific cultivar's leaf N:K ratio in the 2022/23 season. Values with different letters differ significantly.

Season 2023/24

Nitrogen source and the interaction between the nitrogen source and growth stage significantly influenced the leaf N:K ratio (Table 4.1). When LAN was used as a nitrogen fertilizer on plants in a split application at V4 & V9 (1.64:1), the leaf N:K ratio was significantly higher than all other interaction combinations (Table 4.22).

Table 4.22 Leaf N:K ratio as influenced by the interaction between nitrogen sources and growth stage applied on a non-prolific cultivar for the 2023/24 season

Growth stages (GS)	Nitrogen sources (NS)		Average
	Greensulf	LAN	
V4	1.42 ^b	1.42 ^b	1.42
V9	1.48 ^b	1.44 ^b	1.46
V4 & V9	1.39 ^b	1.64 ^a	1.52
Average	1.43	1.50	

$NS_{LSD (T \leq 0.05)} = 0.0653$

$GS_{LSD (T \leq 0.05)} = ns$

$NS \times GS_{LSD (T \leq 0.05)} = 0.167$

Values with different letters differ significantly.

Prolific

There were no significant differences in the maize leaves' N:K ratio due to any of the main effects or treatment combinations in the 2022/23 season.

The leaf N:K ratio was significantly affected by the nitrogen source, growth stage, and the interaction between the nitrogen source and growth stage in the 2023/24 season (Table 4.2). When nitrogen was applied to plants at V9 and in a split application at V4 & V9, it resulted in a significantly higher leaf N:K ratio than when LAN and Greensulf were applied at V4. No further significant differences were observed (Table 4.23).

Table 4.23 Leaf N:K ratio as influenced by the interaction of nitrogen sources and growth stage applied of a prolific cultivar for the 2023/24 season

Growth stages (GS)	Nitrogen sources (NS)		Average
	Greensulf	LAN	
V4	1.84 ^c	1.87 ^c	1.85
V9	2.14 ^{bc}	2.22 ^{ab}	2.18
V4 & V9	1.96 ^{bc}	2.49 ^a	2.23
Average	1.98	2.19	

$NS_{LSD (T \leq 0.05)} = 0.1266$

$GS_{LSD (T \leq 0.05)} = 0.186$

$NS \times GS_{LSD (T \leq 0.05)} = 0.3228$

Values with different letters differ significantly.

Yan *et al.* (2022) found that maize plants fertilized with 175 kg N ha⁻¹ resulted in a higher leaf N:K ratio (1.05:1) than plants fertilized at 0 kg N ha⁻¹ (0.85:1). This finding relates to the results of this study, which found that nitrogen rate influenced the leaf N:K ratio. Szule *et al.* (2017) reported that there was no significant effect of nitrogen source on the dry matter N:K ratio of maize when plants were fertilized with ammonium nitrate (1.08:1) and Canwil nitro-chalk

(1.08:1) nitrogen sources. This finding contrasts with the results found in this study, where the nitrogen source affected the leaf N:K ratio.

4.3.1.3.3 Fe:Mn ratio

Non-prolific

Season 2022/23

Only the nitrogen source significantly influenced the leaf Fe:Mn ratio (Table 4.1). The leaf Fe:Mn ratio of plants fertilized with LAN (1.05) was significantly higher than plants that received Greensulf (0.60) (Figure 4.27).

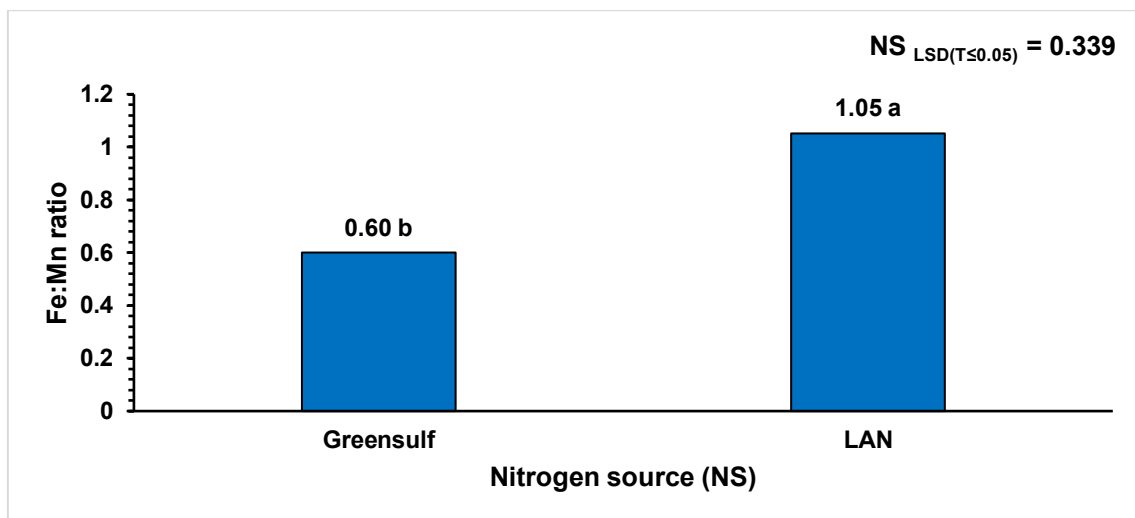


Figure 4.27 Effect of nitrogen source on the non-prolific cultivar's leaf Fe:Mn ratio in the 2022/23 season. Values with different letters differ significantly.

Season 2023/24

The leaf Fe:Mn ratio was only significantly affected by the interaction between the nitrogen source and growth stage (Table 4.1). The plants fertilized with Greensulf in a split application at V4 & V9 (0.80:1) resulted in a significantly higher leaf Fe:Mn ratio than plants that were fertilized with Greensulf at V4 (0.49:1) and with LAN in a split application at V4 & V9 (0.53:1), with no further significant differences between the interaction combinations (Table 4.24).

Table 4.24 Leaf Fe:Mn ratio as influenced by the interaction between nitrogen sources and growth stage applied on a non-prolific cultivar for the 2023/24 season

Growth stages (GS)	Nitrogen sources (NS)		Average
	Greensulf	LAN	
V4	0.49 ^b	0.62 ^{ab}	0.56
V9	0.66 ^{ab}	0.58 ^{ab}	0.62
V4 & V9	0.80 ^a	0.53 ^b	0.66
Average	0.65	0.58	

NS $LSD (T \leq 0.05) = ns$

GS $LSD (T \leq 0.05) = ns$

NS x GS $LSD (T \leq 0.05) = 0.2234$

Values with different letters differ significantly.

In summary, plants fertilized with LAN produced a 75% higher leaf Fe: Mn ratio than plants treated with Greensulf in the 2022/23 season. In the 2023/24 season, plants fertilized with Greensulf source in a split application at V4 & V9 resulted in the highest leaf Fe:Mn ratio of 0.80:1. Campbell & Plank (2000a) reported that a sufficiency range for Fe:Mn should be greater than or equal to 1. This finding shows that only LAN are in the sufficient range (1.05:1), meaning plants fertilized with LAN are not limited to Fe or Mn.

Conclusion

All treatment factors, *viz.* nitrogen source, application rate, and growth stage, affected the leaf concentration of nutrients. The nutrient concentration within the leaves of the plants varied with the application of Greensulf and LAN. Although Greensulf was significantly more efficient in the uptake of most nutrients, with some exceptions, LAN was more efficient regarding nutrient ratios (especially N:S, N:K, and Fe:Mn ratios). Leaf nutrient content increased with nitrogen application rate and was the highest at 40 kg N ha⁻¹. Most of the nutrient uptake was more efficient when nitrogen topdressing was applied as a split application (V4 & V9), resulting in increased leaf nutrient content. Applying Greensulf at V9 and LAN in a split application at V4 & V9 appears to yield the best results. However, the non-prolific and prolific cultivars differed in the timing of nitrogen application, with some nutrients being taken up in the leaves. Significant variations in results can primarily be attributed to two extremely different cropping seasons. These results were obtained under field conditions in two distinct growing seasons; therefore, it is essential to verify them in further studies under field conditions.

References

- BARUNAWATI, N., HETTWER GIEHL, R. F., BAUER, B. & VON WIRÉN, N., 2013. The influence of inorganic nitrogen fertilizer forms on micronutrient retranslocation and accumulation in grains of winter wheat. *Front Plant Sci.* 4: 47650.
- BISWAS, D.K & MA, B., 2016. Effect of nitrogen rate and fertilizer nitrogen source on physiology, yield, grain quality, and nitrogen use efficiency in corn. *Can. J. Plant Sci.* 96(3):392-403.
- BOJTOR, C., NASIR MOUSAVI, S. M., ILLÉS, Á., GOLZARDI, F., SZÉLES, A., SZABÓ, A., NAGY, J. & MARTON, C. L., 2022. Nutrient Composition Analysis of Maize Hybrids Affected by Different Nitrogen Fertilisation Systems. *Plants.* 11(12):1-13.
- BRUNS, H.A. & EBELHAR, M.W., 2006. Nutrient Uptake of Maize Affected by Nitrogen and Potassium Fertility in a Humid Subtropical Environment. *Commun. Soil Sci. Plant Anal.* 37(2): 275-293.
- CAMPBELL, C.R. & PLANK, C.O., 2000 a. Reference sufficiency ranges – field crops. In: C.R. Campbell (eds). Reference sufficiency ranges for plant analysis in the southern region of the United States. North Carolina. pp 1-134.
- CAMPBELL, O.R. & PLANK, C.O., 2000 b. Reference sufficiency ranges for plant analysis in the southern region of the United States. Southern Cooperative Series Bulletin. 394.
- CIAMPITTI, I.A., CAMBERATO, J.J., MURRELL, S.T. & VYN, T.J., 2013. Maize Nutrient Accumulation and Partitioning in Response to Plant Density and Nitrogen Rate: I. Macronutrients. *Agron. J.* 3(105): 783-795
- COETZEE, P., CERONIO, G.G. & DU PREEZ, C.C., 2017. Effect of phosphorus and nitrogen sources on essential nutrient concentration and uptake by maize (*Zea mays* L.) during early growth and development. *S. Afr. J. Plant Soil.* 34(1): 55-64.
- CORNFORTH, I.S. & STEELE, K.W., 1981. Interpretation of maize leaf analyses in New Zealand. *New Zeal. J. Exp. Agr.* 9(1): 91-96.
- DAVIES, B., COULTER, J. A. & PAGLIARI, P. H., 2020. Timing and rate of nitrogen fertilization influence maize yield and nitrogen use efficiency. *PLoS ONE.* 15(5): 1-19.
- EWAIS, M.A., ABD EL-LATIF, A.M. & SAYED, D.A., 2009. Influence of mineral and nitrogen fertilization on maize yield and its components. *J. Agric. Sci. Mansoura Univ.* 34(6): 7079-7090.
- HAMNÉR, K., WEIH, M., ERIKSSON, J. & KIRCHMANN, H., 2017. Influence of nitrogen supply on macro- and micronutrient accumulation during growth of winter wheat. *Field Crops Res.* 213: 118-129.
- JONES, D., 2023. Leaf testing to make an impact [Online]. Retrieved from <https://cropnuts.com/leaf-testing-to-make-an-impact/> (Accessed 27/09/2024).

- LAMBERT, R.J., MANSFIELD, B. & MUMM, R.H., 2013. Effect of leaf area on maize productivity. *Maydica*. 59: 58-63.
- LI, N., YANG, Y., WANG, L., ZHOU, C., JING, J., SUN, X. & TIAN, X., 2019. Combined effects of nitrogen and sulfur fertilization on maize growth, physiological traits, N and S uptake, and their diagnosis. *Field Crops Res*. 242: 107593.
- LIU, X., ZHANG, L., YU, Y., QIAN, C., LI, C., WEI, S., LI, C. & GU, W., 2022. Nitrogen and Chemical Control Management Improve Yield and Quality in High-Density Planting of Maize by Promoting Root-Bleeding Sap and Nutrient Absorption. *Front. Plant Sci*. 13: 754232.
- LOSAK, T., HLUSEK, J., MARTINEC. J., JANDAK, J., SZOSTKOVA, M., FILIPCİK, R., MANASEK, J., PROKES, K., PETERKA, J., VARGA, L., DUCSAY, L., OROSZ, F. & MARTENSSON, A., 2011. Nitrogen fertilization does not affect micronutrient uptake in grain maize (*Zea mays* L.). *Acta Agriculturae Scandinavica, Section B — Soil & Plant Sci*. 61(6): 543-550.
- MINER, G. L., DELGADO, J. A., IPPOLITO, J. A., BARBARICK, K. A., STEWART, C. E., MANTER, D. K., DEL GROSSO, S. J., HALVORSON, A. D., FLOYD, B. A. & D'ADAMO, R. E., 2018. Influence of long-term nitrogen fertilization on crop and soil micronutrients in a no-till maize cropping system. *Field Crops Res*. 228: 170-182.
- OSTMEYER, T. J., IMPA, S. M., BEAN, S. R., DHILLON, R., HAYES, C., RITCHIE, G., ASEBEDO, A. R., EMENDACK, Y., & KRISHNA JAGADISH, S. V., 2023. Impact of in-season split application of nitrogen on intra-panicle grain dynamics, grain quality, and vegetative indices that govern nitrogen use efficiency in sorghum. *JPNSS*. 186(6), 647-660.
- PARSLEY, H.R., CAIRNS, J.E., CAMBERATO, J.J. & VYN, T.J., 2019. Nitrogen fertilizer rate increases plant uptake and soil availability of essential nutrients in continuous maize production in Kenya and Zimbabwe. *Nutr Cycl Agroecosyst*. 115(3): 373-389.
- RAHMAWATI, R., 2017. Effect of nitrogen fertilizer on growth and yield of maize composite variety lamuru. *ATJ*. 2(2): 36-41.
- RAY, K., BANERJEE, H., DUTTA, S., SARKAR, S., MURRELL, T. S., SINGH, V. K. & MAJUMDAR, K., 2020. Macronutrient Management Effects on Nutrient Accumulation, Partitioning, Remobilization, and Yield of Hybrid Maize Cultivars. *Front. Plant Sci*. 11: 535999.
- RUBEN, D., 2024. The Role of Nitrogen in Optimal Maize Growth [Online]. Retrieved from <https://nutrinorm.co.uk/fertilisation/nitrogen-optimal-maize-growth/> (Accessed 26/09/2024).

- SIAM, H.S., ABD-EL-KADER, M.G. & EL-ALIA, H.I., 2008. Yield and Yield Components of Maize as Affected by Different Sources and Application Rates of Nitrogen Fertilizer. *Res. J. Agric. & Biol. Sci.* 4(5): 399-412.
- STRABLE, J. & NELISSEN, H., 2021. The dynamics of maize leaf development: Patterned to grow while growing a pattern. *Curr. Opin. Plant Biol.* 63: 102038.
- SZULE, P., MICHALSKI, H.W. & WILCZEWSKA, W., 2017. The dynamics of accumulation of n, p, k and mg in the initial period of growth as a reaction of maize cultivars (*Zea mays* L.) On the method of the application of a nitrogen fertilizer. *FEB.* 26(12): 7189-7198.
- WIERZBOWSKA, J., SIENKIEICZ, S. & SWIATLY, A., 2021. Content of micronutrients in grain and straw of common maize fertilized with urea-ammonium nitrate solution with added P, Mg or S. *J. Elem.* 26(4): 807-827.
- YAN, S., WU, Y., FAN, J., ZHANG, F., GUO, J., ZHENG, J., WU, L. & LU, J., 2022. Quantifying nutrient stoichiometry and radiation use efficiency of two maize cultivars under various water and fertilizer management practices in northwest China. *Agric Water Manag.* 271: 107772.
- ZHANG, H., SHEN, R. & ZHAO, X., 2022. Nitrogen Source Preference in Maize at Seedling Stage Is Mainly Dependent on Growth Medium pH. *Agr.* 12(9): 2149.

Chapter 5

The influence of nitrogen source, application rate, and growth stage on the yield components and yield of non-prolific and prolific maize cultivars in the north-eastern Free State

5.1 Introduction

The production of maize depends on several factors, including, but not limited to, climate conditions, input costs, equipment, and agronomic practices. Sustainability in maize production depends on responsible agronomic practices. Fertilization, one of the agronomic practices, specifically fertilizer application practices, would include selection of the right product (source), applying it at the right time (growth stage), using the fertilizer at the correct rate, and applying it at the right place (method). These practices will ensure i) protection of the natural resources (environment and soil), ii) increased biological productivity, and iii) financial sustainability (Diko & Jun, 2020).

The suboptimal application of nitrogen is an important limiting factor. Timely and effective application of nitrogen is vital for maize's vegetative and reproductive growth. Maize requires large quantities (23 kg N tonne⁻¹ biomass) of nitrogen throughout the growing season. This is to sustain biomass accumulation, promote protein synthesis, and promote grain yield. Maize cultivars react differently in productivity and response to nitrogen fertilization (Siam *et al.*, 2008; Joshi *et al.*, 2014). Insufficient nitrogen during the vegetative phases directly affects root growth, stem elongation, and the uptake of other nutrients (Rawal *et al.*, 2023).

Siam *et al.* (2008) observed that different nitrogen sources significantly increased biomass, cob weight, cob grain weight, 100-kernel weight, straw (residue), and grain yield of maize plants compared to the control. These authors also observed significant differences between nitrogen sources and ammonia gas, which produced significantly higher plant yields than those obtained by applying ammonium sulphate or urea. Ammonium sulphate produced significantly higher dry mass, fresh mass, and plant height than the urea source. Siam *et al.* (2008) concluded that urea was prone to volatilization, resulting in more nitrogen losses than other fertilizers.

Nitrogen rate and application timing are essential management practices to reduce nitrogen losses, maximize uptake, and be more economical (Mosisa *et al.*, 2022). Studies on increasing

maize yield through different methods of nitrogen fertilization found that grain yield was significantly increased with nitrogen application rates. It was found that nitrogen rates up to 140 kg ha⁻¹ resulted in an increase in stem diameter, cob length, cob leaf area, cob diameter, kernels per row, rows per cob, 100 kernel weight, and grain yield with no further increase as nitrogen rates was increased (El-Douby *et al.*, 2001; Siam *et al.*, 2008). Joshi *et al.* (2014) reported that a nitrogen application of 100 kg ha⁻¹ was found to result in significantly higher nitrogen concentrations in the grain yield than a nitrogen application of 50 and 75 kg ha⁻¹. The maize plant utilises approximately 8% of its total nitrogen requirement within the first 25 days after emergence. During this period, it is critical not to avoid a nitrogen deficiency. This could result in fewer kernels per row on the maize cob. If nitrogen is deficient during the mentioned period, yields can be dramatically reduced. Therefore, it is important to plant with a fertilizer that contains nitrogen to provide the plant with sufficient nitrogen during the early growing stages.

The total nitrogen requirement for the season should not be applied all at once, which could lead to leaching losses. Thus, nitrogen application throughout the season should be applied in several increments to reduce nitrogen leaching losses and increase nitrogen use efficiency, especially on low clay content soils (Joshi *et al.*, 2014). Researchers reported that site-specific nitrogen application timing according to the plant's demand produced the highest nitrogen use efficiency and optimized maize yields (Mosisa *et al.*, 2022). According to Alemu *et al.* (2014), splitting the nitrogen amount into three applications —one-third at planting, one-third at vegetative growth, and one-third at tasselling—is recommended for optimal maize production. Joshi *et al.* (2014) reported that nitrogen application split into three equal applications, one at planting, one at 25 days after planting, and one at 45 days after planting, resulted in the highest yields.

Therefore, this study aims to evaluate the effects of different nitrogen sources, application rates, and timing of growth stage application on the yield components and yield of two maize cultivars (non-prolific and highly prolific) over two cropping seasons.

5.2 Materials and Methods

The materials and methods used were described in Chapter 3.

5.3 Results and Discussion

Analysis of variance indicated that data from the 2022/23 and 2023/24 cropping seasons were not homogeneous. This is attributed to the climatic differences between the cropping seasons,

primarily in terms of rainfall and temperature (Table 3.1). Therefore, the two cropping seasons were analysed separately and will be discussed accordingly.

A summary of the analysis of variance evaluating the effect of nitrogen source (NS), topdressing application rate (AR), and growth stage (GS) on the production of a non-prolific maize cultivar is summarised in Table 5.1 and that of a prolific cultivar is summarised in Table 5.2. The nitrogen source significantly influenced the number of cobs per plant, grain mass per cob, grain yield, 1000-kernel weight, and precipitation use efficiency in the non-prolific cultivar. Topdressing application rate significantly affected the grain per plant, 1000 kernel weight, and nitrogen efficiency. The growth stage only significantly influenced grain mass per cob and 1000 kernel weight. Kernels per plant and 1000-kernel weight were significantly affected by the interaction between nitrogen source and topdressing application rate. The interaction of nitrogen source and growth stage on topdressing significantly affected the number of cobs per plant and grain mass per cob. Hectolitre mass was insignificant for all the main effects and interactions thereof for the non-prolific cultivar.

For the prolific cultivar (Table 5.2), the nitrogen source had a significant effect on 1000-kernel weight as a main effect. The topdressing application rate significantly influenced thousand-kernel weight, grain yield, nitrogen efficiency, and precipitation use efficiency. The interaction of nitrogen source and topdressing application rate significantly influenced hectolitre mass. The interaction between the nitrogen source and the growth stage of topdressing applied significantly affected the number of cobs per plant and kernels per plant.

5.3.1 Yield components and yield of a non-prolific and a prolific cultivar

The cobs per plant, grain per plant, grain per cob, kernels per plant, 1000-kernel weight, grain yield, nitrogen efficiency, precipitation use efficiency, and return on investment will be discussed.

Table 5.1 Summary of analysis of variance indicating the effect of treatment factors on yield components and yield of a non-prolific maize cultivar

Treatments	Yield components and yield																			
	Cobs plant ⁻¹		Kernels plant ⁻¹		Grain mass plant ⁻¹ (g)		Grain mass cob ⁻¹ (g)		Hectolitre mass (g)		1000-kernel weight (g)		Grain yield (tonnes ha ⁻¹)		Nitrogen use efficiency (kg grain kg ⁻¹ N)		Precipitation use efficiency (kg grain ha ⁻¹ mm ⁻¹ rain)		Return on Investment (ROI)	
	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24
Nitrogen source (NS)	ns	*	ns	ns	ns	ns	ns	*	ns	ns	*	ns	*	ns	ns	ns	*	ns	ns	ns
Application rate (AR)	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	*	ns	ns	ns	*	*	ns	ns	ns	*
Growth stage (GS)	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	*	ns
NS X AR	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
NS X GS	*	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
AR X GS	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
NS X AR X GS	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV%	7.63	16.06	12.77	22.68	4.62	11.31	7.48	24.46	2.86	99	4.81	11.05	6.00	13.97	11.26	16.77	6.00	13.97	72.09	261

ns – not significant

*P ≤ 0.05

CV – coefficient of variance

Table 5.2 Summary of analysis of variance indicating the effect of treatment factors on yield components and yield of a prolific maize cultivar

Treatments	Yield components and yield																					
	Cobs plant ⁻¹		Kernal plant ⁻¹		Grain mass plant ⁻¹ (g)		Grain mass cob ⁻¹ (g)		Hectolitre mass (g)		1000-kernel weight (g)		Grain yield (tonnes ha ⁻¹)		Nitrogen efficiency (kg grain kg ⁻¹ N)		Precipitation use efficiency (kg grain ha ⁻¹ mm ⁻¹ rain)		Return on Investment (ROI)			
	2022/23	2023/24	2022/23	2022/23	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24
Nitrogen source (NS)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Application rate (AR)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	*	ns	*	*	*	ns	ns	ns	ns	*
Growth stage (GS)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
NS X AR	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
NS X GS	*	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
AR X GS	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
NS X AR X GS	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV%	4.71	15.33	4.56	16.37	7.28	9.33	8.6	18.27	2.6	3.62	4.89	7.97	6.8	13.02	12.06	16.31	6.85	13.02	11.2	83		

ns – not significant

*P ≤ 0.05

CV – coefficient of variance

5.3.1.1 Number of cobs per plant

Grain yield is a function that depends on the characteristics of the cobs per plant and the number of kernels per cob. A well-developed maize cob can produce up to 1000 kernels if no stress occurs during the growing season (Makanza *et al.*, 2018). Assefa & Mekonnen (2019) found a positive correlation between the number of cobs per plant and grain yield ($r = 0.86$). They also found a positive correlation between the number of cobs per plant and the number of kernels per cob ($r = 0.63$), as well as a correlation of $r = 0.61$ between the number of cobs per plant and the hundred kernel weight. For these reasons, this parameter was measured during this trial.

Non-prolific

Season 2022/23

The number of cobs per plant was not significantly affected by the nitrogen source (NS), topdressing application rate (AR), or the growth stage (GS) at which the topdressing was applied. However, the interaction effect of nitrogen source (NS) and growth stage (GS) significantly influenced the number of cobs per plant (Table 5.1).

Greensulf was applied as a split application (V4 & V9), resulting in the greatest number of cobs per plant (1.42). This was significantly greater than the number of cobs (1.28) obtained with Greensulf applied at V9 only. All other application combinations did not differ significantly from each other (Table 5.3).

Table 5.3 Number of cobs per plant as influenced by the interaction between nitrogen source and growth stage on the non-prolific cultivar for the 2022/23 season

Growth stage (GS)	Nitrogen Source (NS)		GS average
	Greensulf	LAN	
V4	1.32 ^{ab}	1.34 ^{ab}	1.33
V9	1.28 ^b	1.36 ^{ab}	1.32
V4 & V9	1.42 ^a	1.31 ^{ab}	1.37
Average	1.34	1.34	

NS $LSD (T \leq 0.05) = ns$

GS $LSD (T \leq 0.05) = ns$

NS x GS $LSD (T \leq 0.05) = 0.124$

Values with different letters differ significantly.

Season 2023/24

For the 2023/24 growing season, only nitrogen sources significantly influenced the number of cobs per plant (Table 5.1).

Significantly more cobs per plant (1.47) were found when fertilized with LAN than with Greensulf (1.31). The difference in the number of cobs per plant when fertilized with Greensulf and LAN was 11.51% (Figure 5.1).

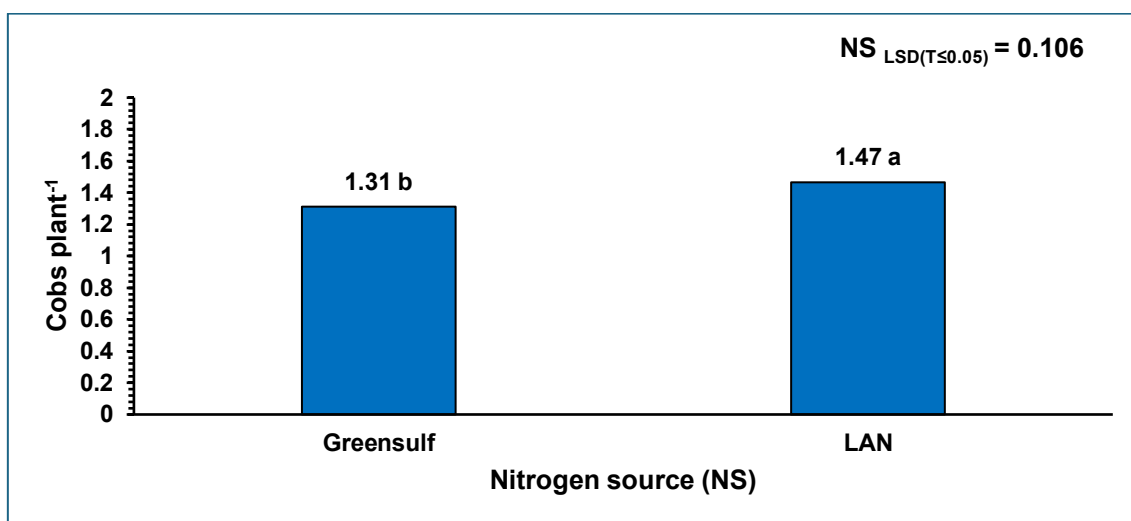


Figure 5.1 Effect of nitrogen source on the number of cobs per plant of the non-prolific cultivar in the 2023/24 season. Values with different letters differ significantly.

Prolific

Season 2022/23

A similar trend was observed between the prolific cultivar and the non-prolific cultivar during the 2022/23 season. The number of cobs per plant was once more significantly affected by the interaction effect of nitrogen source and growth stage (Table 5.2).

However, a shift in the application time was observed. Greensulf applied to maize at V9 (1.98) resulted in a significantly higher number of cobs per plant than Greensulf, which was applied at V4 (1.86). This was the only significant difference observed. All other treatment combinations did not differ significantly from either the greatest or lowest number of cobs per plant (Table 5.4).

Table 5.4 Number of cobs per plant as influenced by the interaction between nitrogen sources and growth stages on the prolific cultivar for the 2022/23 season

Growth stage (GS)	Nitrogen sources (NS)		GS average
	Greensulf	LAN	
V4	1.86 ^b	1.96 ^{ab}	1.91
V9	1.98 ^a	1.89 ^{ab}	1.94
V4 & V9	1.97 ^{ab}	1.94 ^{ab}	1.96
Average	1.94	1.93	

NS $LSD (T \leq 0.05) = ns$

GS $LSD (T \leq 0.05) = ns$

NS x GS $LSD (T \leq 0.05) = 0.1101$

Values with different letters differ significantly.

Season 2023/24

No significant difference in the number of cobs per plant was obtained during this season for any main effects or interactions (Table 5.2).

In general, results for both non-prolific and prolific cultivars over the seasons revealed that when Greensulf was applied, the crop showed some response. This response, however, was inconsistent across the two seasons. Therefore, a clear indication of which N-source would be beneficial concerning the number of cobs per plant or when to be applied (at what stage) was not found.

The results of this study align with the findings reported by Kh *et al.* (2010), Ali *et al.* (2015), and Amunullah *et al.* (2016). These authors reported that different nitrogen sources affect the number of cobs per plant. A study by Siam *et al.* (2008) found that applying nitrogen in three equal amounts at planting, 20 days after planting, and 40 days after planting produced the greatest number of cobs per plant. This finding supports the idea that applying nitrogen at different growth stages affects the number of cobs produced by plants. Ali *et al.* (2015) and Mosisa *et al.* (2022) reported that higher application rates of nitrogen split into three applications over the vegetative growing season of maize lead to an increase in the number of cobs per plant. Although not significant, this was also observed in this study, especially for the 2022/23 season (results not shown).

5.3.1.2. Kernels per plant

This is an important parameter in determining the grain (g) produced by the plant and the grain yield. Maize grain yield is primarily correlated with kernel formation, which is highly sensitive

to nutrient deficiencies and environmental conditions during the plant's tasselling and silking phases (Otegui & Bonhomme, 1998). Nitrogen limitation during the maize growing season can reduce leaf area and radiation interception, resulting in slower plant growth and fewer kernels per plant (Ruiz *et al.*, 2022).

Non-prolific

Season 2022/23

During the 2022/23 season, there were no significant differences in the number of kernels per plant as influenced by the main effects or interactions thereof (Table 5.1). Although no significant differences were found, a decrease in kernels per plant was observed with Greensulf as the rate of nitrogen was increased. In contrast, LAN resulted in the opposite, with an increase in the number of kernels per plant. Greensulf produced the highest kernel count per plant with a split application of 20 kg N ha⁻¹ at V4 & V9 (769.34), and LAN produced the most kernels per plant with an application rate of 40 kg N ha⁻¹ at V4 (741.46) (Table 5.5).

Table 5.5 Kernels per plant as influenced by nitrogen source, application rate and growth stage (time of fertilizer applied for the 2022/23 season of the non-prolific cultivar)

Nitrogen source (NS)	Application rate (AR) (kg N ha ⁻¹)	Growth stages (GS)			AR average
		V4	V9	V4 & V9	
Greensulf	0	704.85	678.71	765.43	716.33
	20	702.46	653.59	769.34	708.46
	40	661.79	668.24	765.38	698.47
GS Greensulf average		689.70	666.85	766.71	
LAN	0	630.22	641.48	669.91	647.20
	20	719.50	726.89	660.50	702.30
	40	741.46	688.19	708.27	712.64
GS LAN average		697.06	685.52	679.56	
GS average		693.38	676.18	723.14	

NS *LSD* ($T \leq 0.05$) = **ns**

AP *LSD* ($T \leq 0.05$) = **ns**

GS *LSD* ($T \leq 0.05$) = **ns**

NS x AR *LSD* ($T \leq 0.05$) = **ns**

NS x GS *LSD* ($T \leq 0.05$) = **ns**

AR x GS *LSD* ($T \leq 0.05$) = **ns**

NS x AR x GS *LSD* ($T \leq 0.05$) = **ns**

Values with different letters differ significantly.

Season 2023/24

Kernels per plant were significantly affected by the interaction of the nitrogen source and the nitrogen application rate only (Table 5.1).

The number of kernels per plant decreased as the amount of nitrogen applied increased for both nitrogen sources, except for the LAN application rate of 40 kg ha⁻¹ (553.31), which was 63.31 kernels more than the LAN application rate of 0 kg ha⁻¹. (Table 5.6). Plants fertilized with LAN produced significantly more kernels per plant at an application rate of 40 kg ha⁻¹ (553.31) than Greensulf at an application rate of 40 kg ha⁻¹ (398.10), but did not differ significantly from the other treatments. The average application rate showed a decrease in the number of kernels per plant with increasing nitrogen application rates, but no significant differences were observed. The average number of kernels per plant was higher for the LAN source than for the Greensulf source, but the difference was not statistically significant. In general, the results were inconclusive.

Table 5.6 Kernels per plant as influenced by the interaction between nitrogen sources and application rate applied of the non-prolific cultivar for the 2023/24 season of the non-prolific cultivar

Application rate (AR) (kg N ha ⁻¹)	Sources (NS)		AR average
	Greensulf	LAN	
0	517.74 ^{abc}	490.00 ^{abc}	503.87
20	493.34 ^{abc}	467.82 ^{abc}	480.58
40	398.10 ^b	553.31 ^a	475.71
Average	281.84	302.23	

NS $LSD (T \leq 0.05) = ns$

AR $LSD (T \leq 0.05) = ns$

NS x AR $LSD (T \leq 0.05) = 133.6$

Values with different letters differ significantly.

Prolific

Season 2022/23

Kernels per plant were not significantly affected by the main factors, including nitrogen source, nitrogen application rate, or growth stage. However, a significant difference in kernels per plant was obtained with the interaction effect of nitrogen source and growth stage (Table 5.2).

There were no significant differences in the number of kernels for Greensulf and LAN as nitrogen sources applied at the different growth stages. However, a significant difference was observed within Greensulf. Greensulf applied at V9 (1068.61) produced significantly more kernels per plant compared to Greensulf applied at V4 (1010.39). Still, those mentioned above did not differ significantly from the number of kernels per plant applied as a split application

(V4 & V9) (1059.81), or the number of kernels when LAN was applied at V4 (1056.10), V9 (1042.90), as well as V4 & V9 (1051.54) (Table 5.7).

Table 5.7 Kernels per plant as influenced by the interaction between nitrogen source and growth stages on the prolific cultivar for the 2022/23 season

Growth stages (GS)	Nitrogen source (NS)		GS average
	Greensulf	LAN	
V4	1010.39 ^b	1056.10 ^{ab}	1033.25
V9	1068.61 ^a	1017.18 ^{ab}	1042.90
V4 & V9	1059.81 ^{ab}	1043.26 ^{ab}	1051.54
Average	1046.27	1038.85	

NS $LSD (T \leq 0.05) = ns$

GS $LSD (T \leq 0.05) = ns$

NS x GS $LSD (T \leq 0.05) = 57.51$

Values with different letters differ significantly.

Season 2023/24

No significant differences in the number of kernels per plant were found for any of the main factors nor the interaction effects thereof (Table 5.2). The two nitrogen sources, Greensulf (789.88) and LAN (799.49), produced the highest number of kernels per plant when applied as a split application at V4 & V9 (Table 5.8). The reason for this phenomena is unknown. No other trends were observed.

During the 2022/23 and 2023/24 seasons of the non-prolific and prolific cultivars, several trends and significant differences in the number of kernels per plant were observed, influenced by the main factors and their interactions. It appears that the application rate may affect the non-prolific cultivar more than the nitrogen source. According to the results, the prolific cultivar is more sensitive to the timing of nitrogen application than to the nitrogen source used.

Kh *et al.* (2010) results concur with the results of kernels per plant. These authors reported a significant difference in plant response to the nitrogen sources used, as well as significant differences in nitrogen application rates. Gheith *et al.* (2022) reported that the highest nitrogen application rate yielded the greatest number of kernels, which is consistent with the results found in LAN (40 kg ha⁻¹) (Table 5.5). Assefa & Mekonnen (2019) reported an increase in kernels per plant with increasing nitrogen rates up to 69 kg ha⁻¹ (640.9). A further increase in nitrogen rate, up to 115 kg ha⁻¹ (583.9), decreased the number of kernels produced per plant. This result concurs with the kernels per plant results found in the 2023/24 season, with an application rate of 0 kg ha⁻¹ (106 kg total N ha⁻¹), the application rate of 20 kg ha⁻¹ (126 kg total N ha⁻¹), and application rate of 40 kg ha⁻¹ (146 kg total N ha⁻¹).

Table 5.8 Kernels per plant as influenced by the interaction between nitrogen sources, application rate applied, and growth stage of application on the prolific cultivar for the 2023/24 season

Nitrogen source (NS)	Application rate (AR) (kg N ha ⁻¹)	Growth stages (GS)			AR average
		V4	V9	V4 & V9	
Greensulf	0	802.52	783.25	723.99	769.92
	20	749.67	813.99	794.45	786.04
	40	737.25	737.91	851.22	775.46
GS Greensulf average		763.14	778.38	789.88	
LAN	0	770.97	710.45	828.88	770.10
	20	767.39	790.70	743.55	767.22
	40	739.26	810.11	826.05	791.80
GS LAN average		759.21	770.42	799.49	
GS average		761.17	774.40	794.69	

NS $LSD (T \leq 0.05) = ns$

AP $LSD (T \leq 0.05) = ns$

GS $LSD (T \leq 0.05) = ns$

NS x AR $LSD (T \leq 0.05) = ns$

NS x GS $LSD (T \leq 0.05) = ns$

AR x GS $LSD (T \leq 0.05) = ns$

NS x AR x GS $LSD (T \leq 0.05) = ns$

Values with different letters differ significantly

5.3.1.3 Grain mass per plant

The importance of grain mass per plant cannot be overemphasized regarding grain yield. The higher the grain mass per plant, the higher the grain yield. This parameter is also very responsive to nitrogen fertilization. Nitrogen application at an appropriate rate increases the effective grain filling rate and duration, increasing grain weight (Grupo, 2022; Yue *et al.*, 2022).

Non-prolific

Season 2022/23

Only the main effect of nitrogen application rate significantly impacted grain mass per plant (Table 5.1).

With increasing nitrogen application rate, there was an increase in the grain mass per plant. The only significant difference (3.31%) in grain mass per plant was between an application rate of 0 kg ha⁻¹ (319.93 g) and that of 40 kg ha⁻¹ (330.70 g) (Figure 5.2).

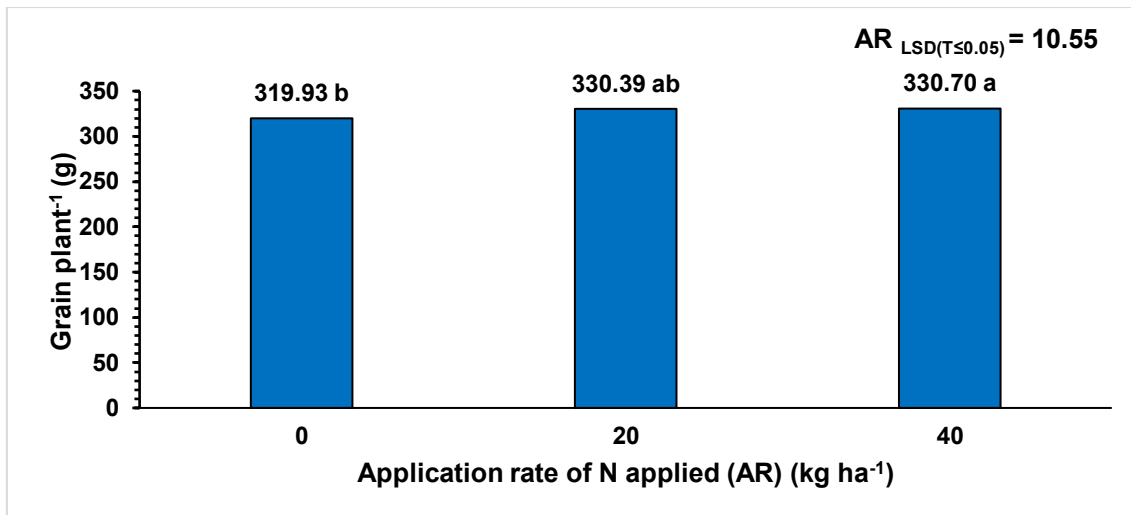


Figure 5.2 Effect of nitrogen amount applied on grain mass per plant (g) for 2022/23 season for the non-prolific cultivar. Values with different letters differ significantly.

Season 2023/24

No significant differences in grain mass per plant were observed for any of the main factors or their interactions (Table 5.1). Evaluation of the results (Table 5.9) revealed that the only tendency was that a single topdressing application at V4 resulted in a greater grain mass per plant than at V9 or a split topdressing application at V4 & V9.

Prolific cultivar

Seasons 2023 and 2024

No significant differences in grain mass per plant were observed as a result of the main factors (nitrogen source, topdressing application rate, or application at different growth stages) or their interaction, for either season (Table 5.2). Evaluation of the result showed no trend in the data (Data not shown). This might be attributed to the significant difference between seasons in rainfall, rainfall distribution, and temperature (Table 3.1, Chapter 3).

The nitrogen application rate appears to be the only beneficial effect that significantly increased the grain mass per plant for the non-prolific cultivar. The timing of nitrogen source application was found to increase grain mass per plant of the non-prolific cultivar when applied at the optimal time (V9 for Greensulf and V4 for LAN), although the increase was not significant (Table 5.9).

Table 5.9 Grain mass per plant (g) as influenced by the interaction of nitrogen sources, application rate, and growth stage applied for the 2023/24 season of the non-prolific cultivar

Nitrogen source (NS)	Application rate (AR) (kg N ha ⁻¹)	Growth stages (GS)			AR average
		V4	V9	V4 & V9	
Greensulf	0	138.48	165.90	136.81	147.06
	20	154.13	141.37	132.70	142.73
	40	151.27	137.40	134.78	141.15
GS Greensulf average		147.96	148.22	134.76	
LAN	0	145.83	135.09	138.98	139.97
	20	137.39	134.83	135.07	135.76
	40	147.16	147.92	151.34	148.81
GS LAN average		143.46	139.28	141.79	
GS average		145.71	143.75	138.28	

NS $LSD (T \leq 0.05) = ns$

AP $LSD (T \leq 0.05) = ns$

GS $LSD (T \leq 0.05) = ns$

NS x AR $LSD (T \leq 0.05) = ns$

NS x GS $LSD (T \leq 0.05) = ns$

AR x GS $LSD (T \leq 0.05) = ns$

NS x AR x GS $LSD (T \leq 0.05) = ns$

Values with different letters differ significantly

Siam *et al.* (2008) reported that increasing nitrogen rates significantly increased the grain weight of the plant, with a maximum of up to 140 kg N ha⁻¹. Yue *et al.* (2022) also found that nitrogen rate significantly altered the grain weight of maize plants; however, they did not report any significant effects on the timing of application or the interaction between nitrogen rate and timing of application. Although they found a trend that a nitrogen application rate of 300 kg N ha⁻¹, split into three applications, one-third at planting, one-third at V6, and one-third at V11, provided the highest grain weight per plant, it was not significantly higher than the rest of the other treatments. According to Mosisa *et al.* (2022), nitrogen application timing significantly affected the grain produced per plant. They reported that an application rate of 92 kg N ha⁻¹, split into three applications of one-fourth at planting, two-fourth at vegetative stages (Six-to-eight leaf stage), and one-fourth at the tasselling stage, produced the highest grain weight per plant. This reasoning is consistent with the results found for the non-prolific and prolific cultivars.

5.3.1.4 Grain mass per cob

This parameter is one of the most important agronomic factors affecting the grain per plant and the grain yield of maize. Baduwal *et al.* (2022) found a substantial positive association between ear weight and grain yield. These authors also reported a strong link between ear weight and kernels per row, ear length, ear diameter, and 1000-kernel weight. Bășa *et al.* (2016) reported that maize cob mass responds strongly to nitrogen application and greatly influences maize grain yield.

Non-prolific

Season 2022/23

The grain mass per cob was influenced by the main effect of applying topdressing at different growth stages and the interaction between the nitrogen source and growth stage (Table 5.1).

Greensulf applied at growth stage V4 yielded the significantly highest grain mass per cob (284.95 g), but did not differ significantly from Greensulf applied at V9 (263.29 g). Both Greensulf and LAN, when applied as a split application at V4 & V9, resulted in the least grain mass per cob (Table 5.10).

Table 5.10 Grain per cob (g) as influenced by the interaction between nitrogen source and growth stages on the non-prolific cultivar in the 2022/23 season

Growth stages (GS)	Nitrogen source (NS)		GS average
	Greensulf	LAN	
V4	284.95 ^{ab}	247.23 ^{bc}	266.09 ^{ab}
V9	263.29 ^{ab}	241.66 ^{bc}	252.48 ^{bc}
V4 & V9	231.26 ^c	234.44 ^c	232.85 ^c
Average	155.90	144.67	

$NS_{LSD (T \leq 0.05)} = ns$

$GS_{LSD (T \leq 0.05)} = 12.85$

$NS \times GS_{LSD (T \leq 0.05)} = 22.28$

Values with different letters differ significantly

Season 2023/24

The grain mass per cob was significantly influenced by the main effect of the nitrogen source only (Table 5.1). The nitrogen source Greensulf resulted in a significantly higher grain mass (14.59%) per cob (114.38 g) than the nitrogen source LAN (98.83 g) (Figure 5.3).

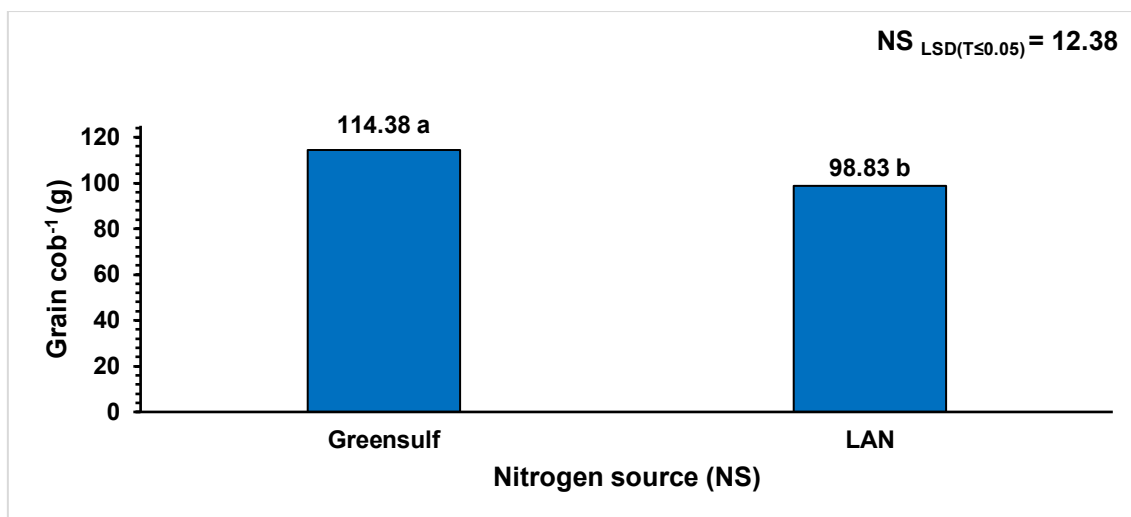


Figure 5.3 Effect of nitrogen sources on grain per cob (g) of the non-prolific cultivar during the 2023/24 season. Values with different letters differ significantly.

Prolific

Seasons 2022/23 & 2023/24

The grain mass per plant for the 2022/23 and 2023/24 seasons was not significantly affected by the main effects of nitrogen source, nitrogen application rate, growth stage, or their interactions (Table 5.2).

In the 2022/23 cropping season, the grain mass per cob was significantly influenced by the interaction between the nitrogen source and the growth stage of application, and only by the nitrogen source in the 2023/24 season for the non-prolific cultivar. The significant variation in results for nitrogen source made it uncertain whether the nitrogen source affected the grain yield per plant of the prolific cultivar.

Siam *et al.* (2008) and Kh *et al.* (2010) reported that nitrogen sources significantly affected ear weight, which supports the results from the 2023/24 season. Băşa *et al.* (2016) and Mosisa *et al.* (2022) reported that increasing nitrogen rates led to an increase in ear weight up to the highest rate (120 and 115 kg N ha⁻¹), further supporting the trend of the prolific cultivar. Mosisa *et al.* (2022) also reported significantly higher ear mass when nitrogen was applied in three split applications: 25% at planting, 50% at the vegetative stage (six- to eight-leaf stage), and 25% at the tasselling stage. Gheith *et al.* (2022) found that a split nitrogen application of 50% at first and 50% at the second irrigation resulted in significantly higher ear weight than the other treatments. These authors' results concur to some extent with the finding in this study in that the timing of application (growth stage) influenced the grain per cob for both cultivars.

5.3.1.5 Hectolitre mass

The hectolitre mass parameter is reported to be important for maize grain quality, especially the milling index. Low hectolitre mass often has a lower percentage of hard endosperm, producing a lower yield of prime and larger grits when milled. Correlation between hectolitre mass and grain density ($r = 0.94$) and hectolitre mass and grain hardness ($r = 0.72$), and this hectolitre mass is used as an indicator of the quality of the grain (Engelbrecht, 2008). Barary *et al.* (2015) found that hectolitre mass had a significant positive correlation between grain yield ($r = 0.929$), 400-grain weight ($r = 0.780$), number of rows per ear ($r = 0.597$), and number of kernels per row ($r = 0.677$).

Non-prolific

Seasons 2022/23 and 2023/24

The variance of analysis revealed that the non-prolific cultivar's hectolitre mass was not affected by the main factors (nitrogen source, application rate, and growth stage) nor the interaction for the 2022/23 or 2023/24 growing seasons (Table 5.1).

Prolific cultivar

Season 2022/23

Only the interaction between application rate (AR) and nitrogen source (NS) influenced the hectolitre mass (Table 5.2).

Greensulf had a slightly greater influence on hectolitre mass (76.02 kg hl^{-1}) than the LAN (75.9 kg hl^{-1}) did, except for LAN at a rate of 40 kg N ha^{-1} , which resulted in a 2.1% higher hectolitre mass than Greensulf at a rate of 40 kg N ha^{-1} (Table 5.11). It is noteworthy to highlight that although the analysis of variance indicated a significant interaction effect between nitrogen source and application rate for hectolitre mass, no significant differences were found following the Tukey least significant difference test calculation. This suggests a strong tendency towards significance. The Tukey test is strict in separating means.

Table 5.11 Hectoliter mass (kg hl⁻¹) as influenced by the interaction between nitrogen source and nitrogen rates on the prolific cultivar in the 2023/24 season

Application rate (AR) (kg ha ⁻¹)	Nitrogen sources (NS)		AR average
	Greensulf	LAN	
0	76.78 ^a	75.79 ^a	76.29
20	76.38 ^a	75.44 ^a	75.91
40	74.89 ^a	76.48 ^a	75.69
Average	76.02	75.90	

NS *LSD* (*T*≤0.05) = *ns*

AR *LSD* (*T*≤0.05) = *ns*

NS x AR *LSD* (*T*≤0.05) = 2.392

Values with different letters differ significantly

Season 2023/24

No significant differences in hectolitre mass were obtained as a result of any of the main factors' effect or the interaction effect thereof (Table 5.2). The data (Table 5.12) was inspected and revealed no tendencies.

Table 5.12 Hectolitre mass (kg hl⁻¹) as influenced by the interaction of nitrogen source, application rate and growth stage applied on the prolific cultivar in the 2023/24 season

Nitrogen source (NS)	Application rate (AR) (kg N ha ⁻¹)	Growth stages (GS)			AR average
		V4	V9	V4 & V9	
Greensulf	0	76.65	76.40	77.26	76.78
	20	75.50	76.83	76.80	76.38
	40	75.10	75.28	74.30	74.89
GS Greensulf average		75.75	76.17	76.13	
LAN	0	75.45	75.50	76.43	75.79
	20	76.05	76.00	73.88	75.31
	40	76.00	75.53	77.93	76.48
GS LAN average		75.83	75.68	76.08	
GS average		75.79	75.92	76.10	

NS *LSD* (*T*≤0.05) = *ns*

AR *LSD* (*T*≤0.05) = *ns*

GS *LSD* (*T*≤0.05) = *ns*

NS x AR *LSD* (*T*≤0.05) = *ns*

NS x GS *LSD* (*T*≤0.05) = *ns*

AR x GS *LSD* (*T*≤0.05) = *ns*

NS x AR x GS *LSD* (*T*≤0.05) = *ns*

Values with different letters differ significantly

A study by Mircea *et al.* (2023) and Sapucy *et al.* (2020) also found that the hectolitre mass of maize did not increase as nitrogen levels increased but was significantly affected by the maize hybrids and climate conditions. Barary *et al.* (2015) also reported that the hectolitre mass differed significantly among maize hybrids. Plavsic *et al.* (2007) found that hectolitre mass was

significantly influenced by irrigation rate, but no significant effects were found on account of nitrogen application rate during the trial period. Sample impurities influence hectolitre mass; when removed from the sample, hectolitre mass increases (Engelbrecht, 2008). For this study, the effects of nitrogen source, application rate, and growth stage were inconclusive.

5.3.1.6 1000-kernel weight

Thousand kernel weight, which indicates grain growth and plumpness, is a common indicator of grain yield (Adhikari *et al.*, 2021). Iqbal *et al.* (2015) reported that the 1000-kernel weight and the number of kernels per cob are essential factors determining grain yield. A study conducted by Deivasigamani and Swaminathan (2018) on “Assessment of seed test weight in major field crops” concluded that grain weight is an indicator of grain quality and bulk density in cereal crops, and can be influenced by various factors, including genetic traits, environmental influences, and nutrient availability.

Non-prolific

Season 2022/23

The 1000 kernel weight was significantly influenced by all three main factors (nitrogen source, application rate, and growth stage), as well as the interaction between nitrogen source and application rate (Table 5.1).

Application of nitrogen at different growth stages showed significant effects on the 1000-kernel weight (Figure 5.4). The application of nitrogen at the V9 growth stage resulted in a significantly higher 1000 kernel weight (393.34 g) than the nitrogen split application at the V4 & V9 growth stages (378.88 g). Application at the V4 growth stage (389.91 g) showed no significant differences from the other growth stages.

The interaction of nitrogen source and application rate showed that Greensulf at 0 kg ha⁻¹ (394.42 g), Greensulf at 40 kg ha⁻¹ (397.37 g) and LAN at 40 kg ha⁻¹ (395.84 g) differed significantly from LAN at 0 kg ha⁻¹ (366.99 g) (Table 5.13). The application of Greensulf and LAN at a rate of 20 kg ha⁻¹ resulted in no differences for 1000-kernel weight. Inspection of the data indicated that Greensulf averaged a higher 1000-kernel weight (392.74 g) than the LAN source (382.01 g). Furthermore, an application rate of 40 kg ha⁻¹ averaged a higher 1000 kernel weight (396.61 g) than the other application rates.

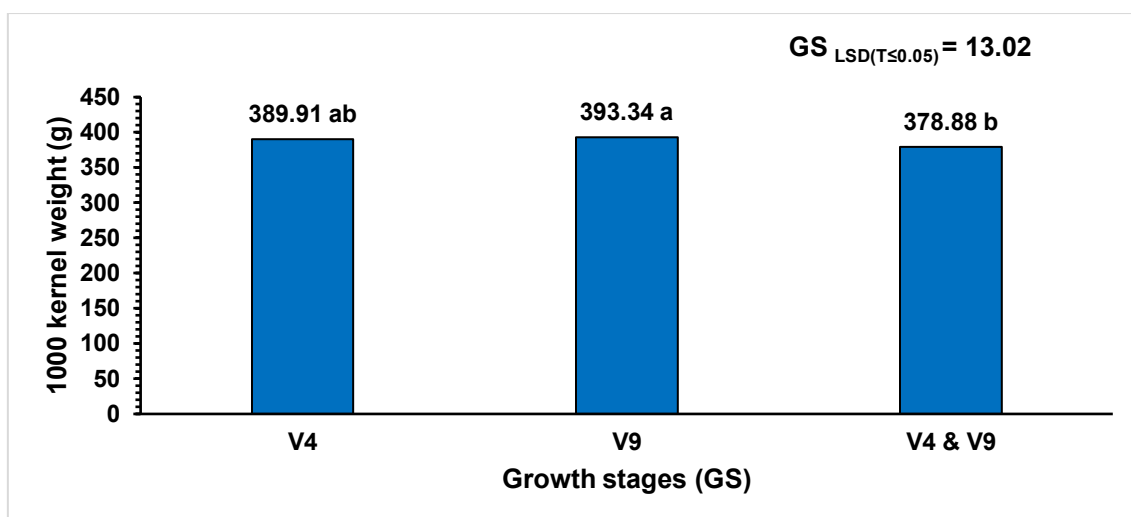


Figure 5.4 1000-kernel weight as influenced by growth stage applied for the non-prolific cultivar in the 2022/23 season. Values with different letters differ significantly.

Table 5.13 1000 kernel weight as influenced by the interaction between nitrogen source and application rate on the non-prolific cultivar in the 2022/23 season

Application rate (AR) (kg N ha ⁻¹)	Nitrogen source (NS)		AR average
	Greensulf	LAN	
0	394.42 ^a	366.99 ^b	380.71
20	386.44 ^{ab}	383.21 ^{ab}	384.83
40	397.37 ^a	395.84 ^a	396.61
Average	392.74	382.01	

NS $LSD(T \leq 0.05) = 8.849$

AR $LSD(T \leq 0.05) = 13.06$

NS x AR $LSD(T \leq 0.05) = 22.56$

Values with different letters differ significantly

Season 2023/24

No significant differences in 1000-kernel mass were found for the main factors or their interaction effects. (Table 5.1). Inspection of the data revealed that the 1000 kernel weight was the greatest for Greensulf and LAN applied to V4 (Table 5.14).

Table 5.14 1000-kernel weight as influenced by the interaction of nitrogen source, application rate and growth stage applied for the non-prolific cultivar in the 2023/24 season

Nitrogen source (NS)	Application rate (AR) (kg N ha ⁻¹)	Growth stages (GS)			AR average
		V4	V9	V4 & V9	
Greensulf	0	195.52	208.60	186.49	196.87
	20	206.26	190.77	182.50	193.18
	40	199.41	184.60	181.22	188.41
GS Greensulf average		200.40	194.66	183.40	
LAN	0	202.83	177.94	191.46	190.74
	20	187.19	181.98	183.85	184.34
	40	201.07	194.07	198.68	197.94
GS LAN average		197.03	184.66	191.33	
GS average		198.71	189.66	187.37	

NS $LSD (T \leq 0.05) = ns$

AR $LSD (T \leq 0.05) = ns$

GS $LSD (T \leq 0.05) = ns$

NS x AR $LSD (T \leq 0.05) = ns$

NS x GS $LSD (T \leq 0.05) = ns$

AR x GS $LSD (T \leq 0.05) = ns$

NS x AR x GS $LSD (T \leq 0.05) = ns$

Values with different letters differ significantly

Prolific

Season 2022/23

Only the nitrogen source and application rate significantly affected 1000-kernel weight (Table 5.2).

Maize fertilized with LAN (280.96 g) produced a significantly higher 1000 kernel weight (2.88%) than that fertilized with Greensulf source (273.09 g) (Figure 5.5). Both nitrogen applications (20 and 40 kg N ha⁻¹) resulted in significantly higher 1000-kernel weights than the control (269.47 g), with no significant differences between the two higher application rates (Figure 5.6).

Season 2023/24

No significant differences in 1000-kernel weight were observed due to the main factors or their interactions (Table 5.2). Inspection of the data revealed no trends (Data not shown).

An overview of the non-prolific and prolific cultivars for the 2022/23 and 2023/24 seasons revealed that the 1000 kernel weight of both cultivars was influenced by the nitrogen source used, but was only significant for the 2022/23 season. The growth stage in the 2022/23 season

had a significant impact on the non-prolific cultivar. Both cultivars were significantly influenced by nitrogen application rate in the 2022/23 season.

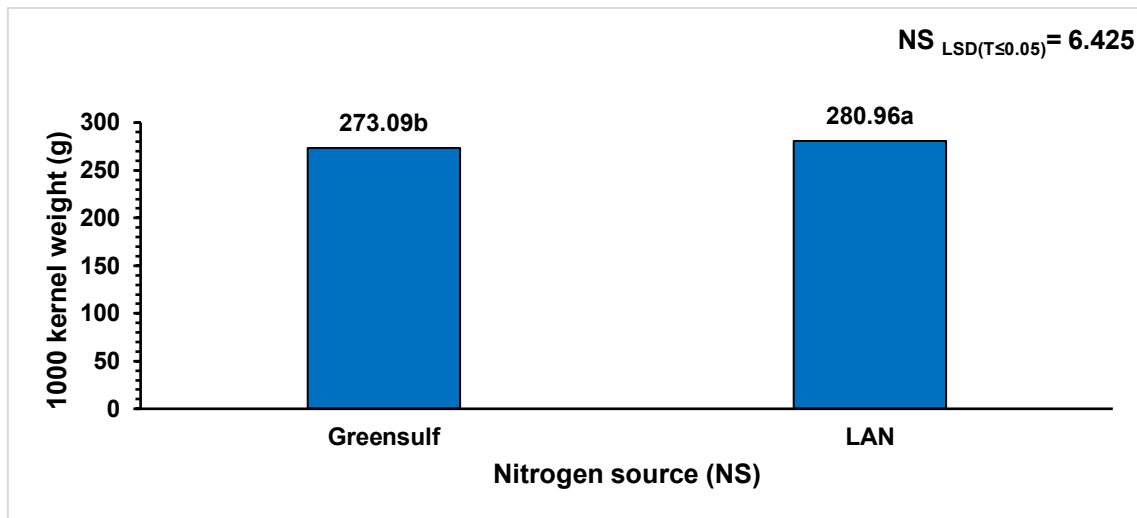


Figure 5.5 1000-kernel weight as influenced by nitrogen source of the prolific cultivar for the 2022/23 season. Values with different letters differ significantly.

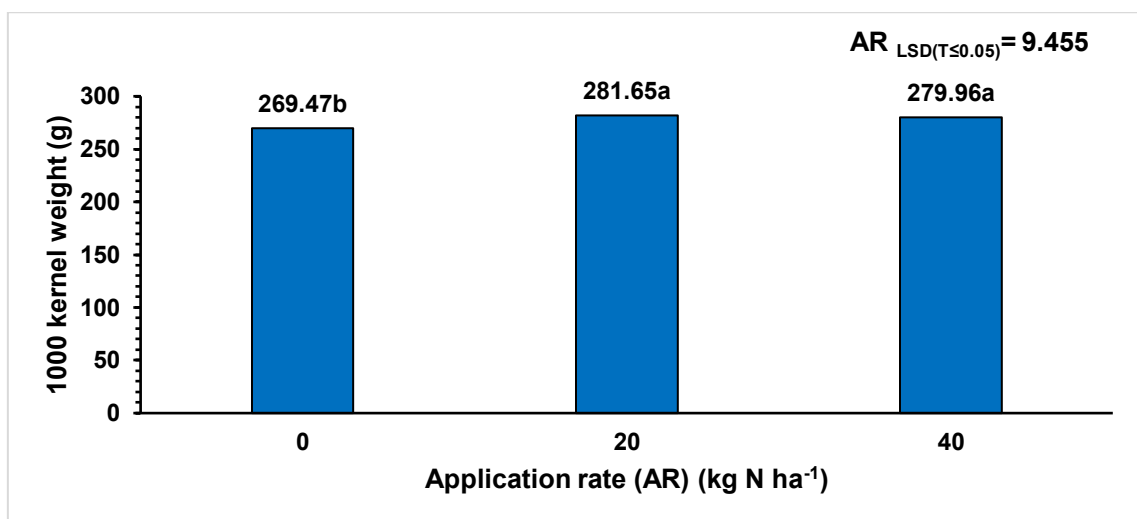


Figure 5.6 1000-kernel weight as influenced by nitrogen application rate of the prolific cultivar for the 2022/23 season. Values with different letters differ significantly.

Mosisa *et al.* (2022) reported that applying 150 kg N ha⁻¹ in three equal applications resulted in the highest 1000-kernel weight. Gheith *et al.* (2022) found significant differences in 100-kernel weight depending on nitrogen application rate and application time. The results showed that the weight of 100 kernels increased as the rate of nitrogen increased and when the nitrogen was applied into two equal amounts and applied at different growth stages, compared to applying the total amount of nitrogen at planting. Hundred kernel weight increased as nitrogen rates were increased, which is consistent with the results from the LAN nitrogen

source (Galindo *et al.*, 2019). Hokmalipour *et al.* (2010) and Mergener *et al.* (2022) found that the 1000-kernel weight was significantly affected by nitrogen application rate, with grain weight being higher at increased nitrogen application rates. The results reported by these authors are consistent with the findings of this research, which shows that the application rates influenced both the cultivars and the timing of nitrogen application (growth stage) in the non-prolific cultivar. Drulis *et al.* (2022) found that nitrogen sources and nitrogen application rates significantly influenced the 1000-kernel weight of maize, which agrees with the results found for the non-prolific and prolific cultivars in both cropping seasons.

5.3.1.7 Grain yield

Grain yield is a key component in increasing food production to meet the demands of a growing global population (Baltes *et al.*, 2017). Nitrogen is essential for increasing yield and grain quality (Rawal *et al.*, 2023). It is also an important component for calculating the nitrogen use efficiency of the crop. For these reasons, it is an important parameter to measure when conducting nitrogen studies on maize.

Non-prolific

Season 2022/23

Grain yield was significantly affected by nitrogen source only (Table 5.1). Maize fertilized with Greensulf produced a significantly higher grain yield (9.91 tonne ha⁻¹) than LAN (9.63 tonne ha⁻¹) did, which was 2.87% more (Figure 5.7).

Season 2023/24

Grain yield was not significantly affected by any main factors or interactions. (Table 5.1). Evaluation of the results summarised in Table 5.15 revealed no clear or definite trend. Unexpectedly, the yields for both Greensulf and LAN fertilised maize decreased with an increase in application rate. Maize fertilized with Greensulf and LAN produced the highest yield when applied at the V9 growth stage.

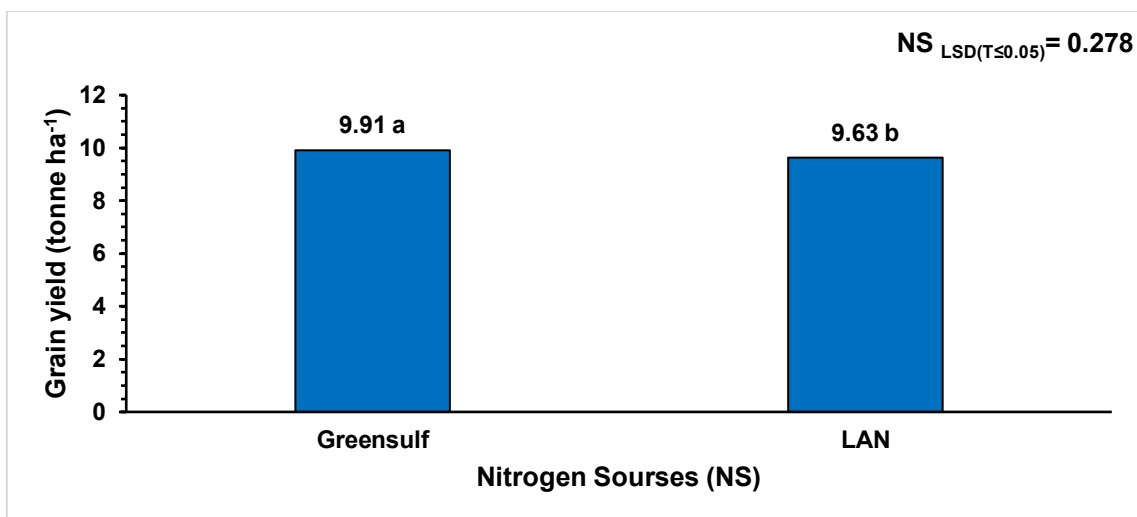


Figure 5.7 Effect of nitrogen sources on grain yield for the 2022/23 season. Values with different letters differ significantly.

Table 5.15 Grain yield (tonne ha⁻¹) as influenced by the interaction of nitrogen source, application rate and growth stage applied of the non-prolific cultivar for the 2023/24 season

Nitrogen source (NS)	Application rate (AR) (kg N ha ⁻¹)	Growth stages (GS)			AR average
		V4	V9	V4 & V9	
Greensulf	0	3.40	4.01	3.33	3.58
	20	3.74	3.59	3.07	3.46
	40	3.71	3.32	3.26	3.43
GS Greensulf average		3.62	3.64	3.22	
LAN	0	3.47	3.65	3.08	3.40
	20	3.24	3.33	3.21	3.26
	40	3.24	3.39	3.61	3.41
GS LAN average		3.32	3.45	3.30	
GS average		3.47	3.55	3.26	

NS LSD (T≤0.05) = ns

AR LSD (T≤0.05) = ns

GS LSD (T≤0.05) = ns

NS x AR LSD (T≤0.05) = ns

NS x GS LSD (T≤0.05) = ns

AR x GS LSD (T≤0.05) = ns

NS x AR x GS LSD (T≤0.05) = ns

Values with different letters differ significantly

Prolific

Season 2022/23

Only the main factor, nitrogen application rate, significantly influenced grain yield (Table 5.2).

Maize fertilized with 20 kg N ha⁻¹ (10.9 tonne ha⁻¹) resulted in significantly greater grain yield than maize that received 0 kg N ha (10.28 tonne ha⁻¹ – control), with no significant difference between the yields obtained by maize fertilized at the aforementioned application rates and the yield of maize fertilized with 40 kg N ha⁻¹ (Figure 5.8).

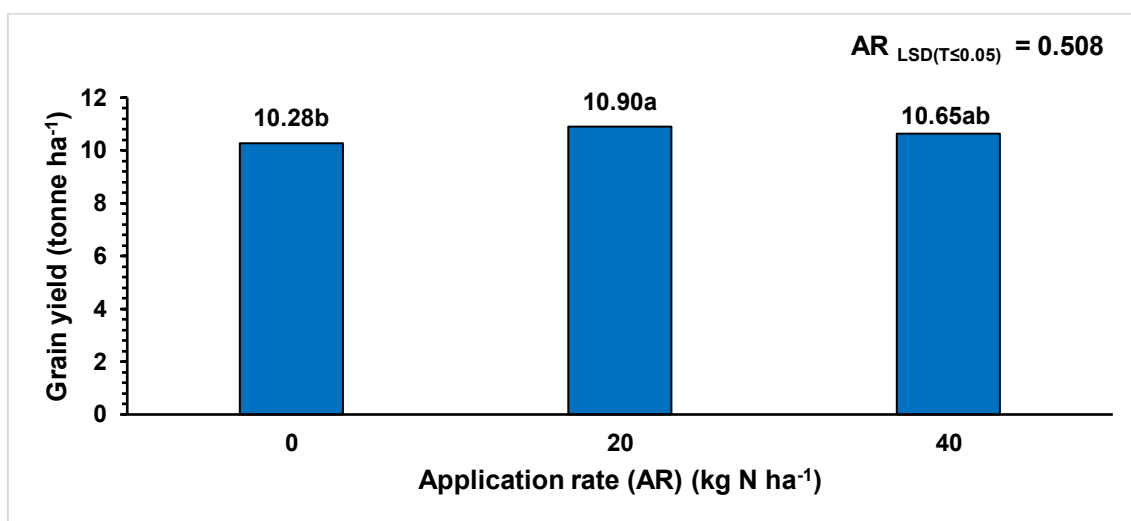


Figure 5.8 Grain yield (tonne ha⁻¹) as influenced by nitrogen application rate for the prolific cultivar in the 2022/23 season. Values with different letters differ significantly.

Season 2023/24

There was no significant influence of the main effects or the interaction thereof on the grain yield of maize (Table 5.2).

A summary of the results is presented in Table 5.16. These results correspond to the results obtained for the non-prolific cultivar in the same season, where a decrease in yield was observed with an increase in application rate. Furthermore, for both Greensulf and LAN, the highest yields were obtained when applied to V9.

Table 5.16 Grain yield (tonne ha⁻¹) as influenced by the interaction of nitrogen source, application rate and growth stage applied of the prolific cultivar for the 2023/24 season.

Nitrogen source (NS)	Application rate (AR) (kg N ha ⁻¹)	Growth stages (GS)			AR average
		V4	V9	V4 & V9	
Greensulf	0	4.22	4.50	4.41	4.38
	20	4.20	4.08	4.09	4.12
	40	4.08	4.07	4.09	4.08
GS Greensulf average		4.17	4.22	4.20	
LAN	0	4.65	4.49	4.26	4.47
	20	4.14	4.44	4.58	4.39
	40	4.48	4.38	4.26	4.37
GS LAN average		4.42	4.44	4.37	
GS average		4.29	4.33	4.28	

NS $LSD (T \leq 0.05) = ns$

AR $LSD (T \leq 0.05) = ns$

GS $LSD (T \leq 0.05) = ns$

NS x AR $LSD (T \leq 0.05) = ns$

NS x GS $LSD (T \leq 0.05) = ns$

AR x GS $LSD (T \leq 0.05) = ns$

NS x AR x GS $LSD (T \leq 0.05) = ns$

Values with different letters differ significantly

An overview of the results showed that the grain yield of the non-prolific cultivar when using Greensulf as a nitrogen source in the 2022/23 season was significantly higher than when using the LAN source. The 2022/23 season results showed that grain yield increased with increasing additional nitrogen application, whereas the 2023/24 season showed the opposite results, with a decrease in grain yield. The nitrogen application rate significantly influenced the prolific cultivar in the 2022/23 season, and such trends also occurred for both cultivars in the 2023/24 season. Application at V9 resulted in the highest grain yield for both nitrogen sources, regardless of the cultivar planted.

A study by Ullah *et al.* (2024) on the influence of different nitrogen and sulfur sources on maize yield reported that nitrogen fertilizers with additional sulfur performed better overall than the other nitrogen sources. This concurs with the findings of this study (Figure 5.7) for Greensulf, which contains 4% sulfur in the 2022/23 season. Conversely, Yue *et al.* (2022) also reported that nitrogen application rates, application time, and interaction did not significantly affect the grain yield. This finding also aligns with the results of this study during the 2023/24 growing season. Ochieng *et al.* (2023) showed that applying the right nitrogen source can influence crop yield, while excessive nitrogen application rates can lead to nutrient losses (leaching and volatilization) or even reduce crop yield. These findings (Ochieng *et al.* 2023) largely concur

with the results for the 2022/23 season (Figure 5.8), where Greensulf produced 280 kg grain ha⁻¹ more than LAN at the same application rates and time.

5.3.1.8 Nitrogen use efficiency

Nitrogen use efficiency is a crucial element that enhances maize production. Conversely, the actual volatilization of nitrogen loss can contribute to air pollution if nitrogen loss is not managed efficiently, and nitrogen loss through runoff and leaching can lead to surface and groundwater pollution. This can be overcome by using split nitrogen applications (with varying application rates) instead of a single heavy dose of nitrogen and identifying the optimal time (growth stage) when the plant needs it most (Dawar *et al.*, 2024). Optimizing nitrogen efficiency in maize production is crucial for increasing grain yield, optimizing economic return, and reducing nitrogen pollution (Hokmaliquour *et al.*, 2010).

Non-prolific

Seasons 2022/23 & 2023/24

The nitrogen use efficiency was only significantly influenced by the main factor, application rate (Table 5.1).

Nitrogen use efficiency of the topdressings was reduced as the nitrogen application rate increased from 0 kg ha⁻¹ to 20 kg ha⁻¹ N and again to 40 kg ha⁻¹ N (Figures 5.9 & 5.10). An application rate of 0 kg ha⁻¹ yielded significantly higher nitrogen efficiency than 20 kg ha⁻¹ and 40 kg ha⁻¹, with no significant differences in nitrogen use efficiency between the last two application rates. However, the nitrogen use efficiency of the 2023/24 season was nearly one-third of that of the 2022/23 growing season. This can be attributed solely to the change in climatic characteristics between the two seasons.

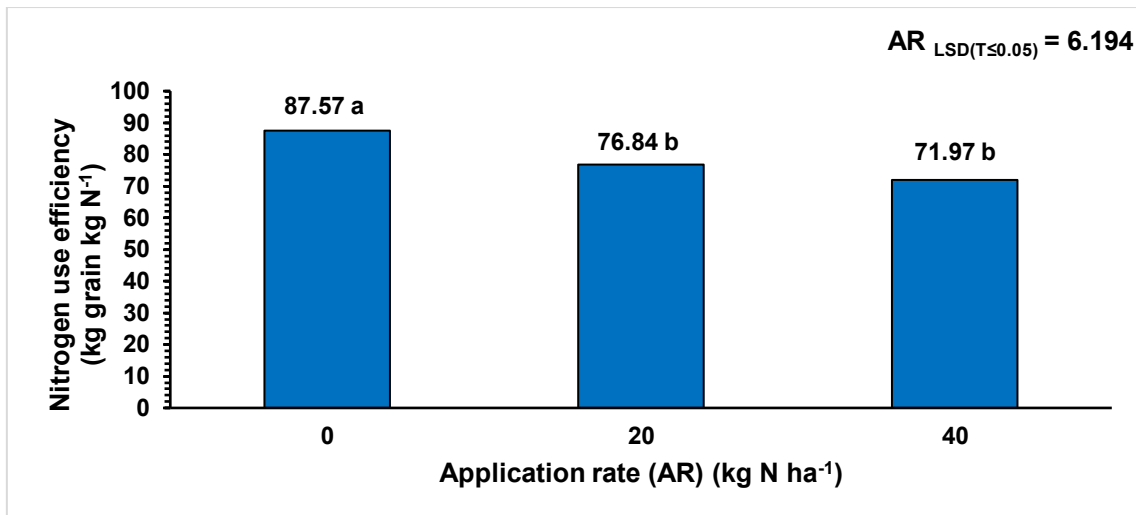


Figure 5.9 Effect of nitrogen application rate on the nitrogen use efficiency of a non-prolific cultivar for the 2022/23 season. Values with different letters differ significantly.

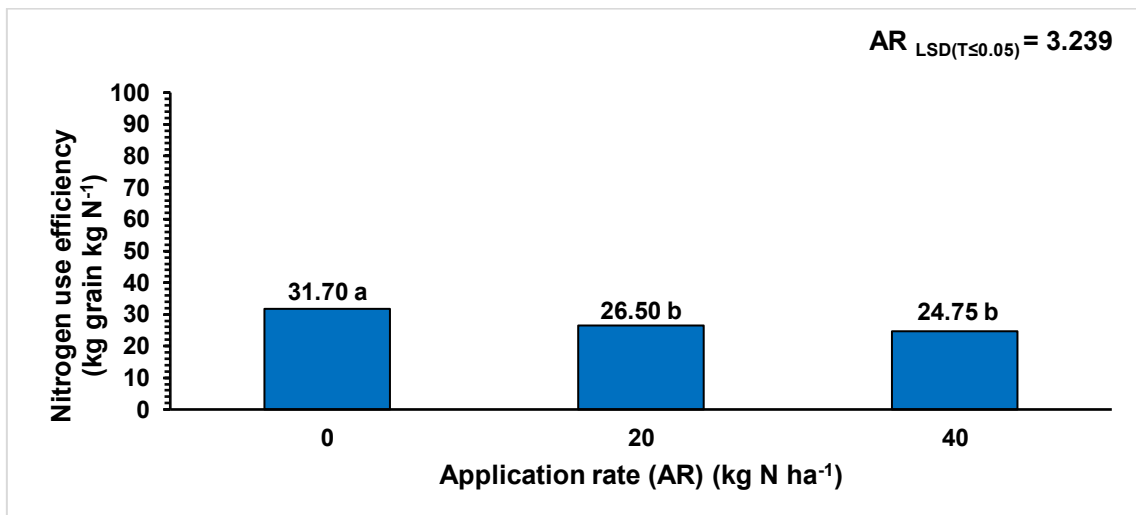


Figure 5.10 Effect of nitrogen amount applied on the nitrogen use efficiency of a non-prolific cultivar for the 2023/24 season. Values with different letters differ significantly.

Prolific

Seasons 2022/23 & 2023/24

The nitrogen use efficiency was only significantly influenced by the nitrogen application rate. (Table 5.2). The trend was nearly the same as that of the non-prolific cultivar (Figures 5.11 & 5.12).

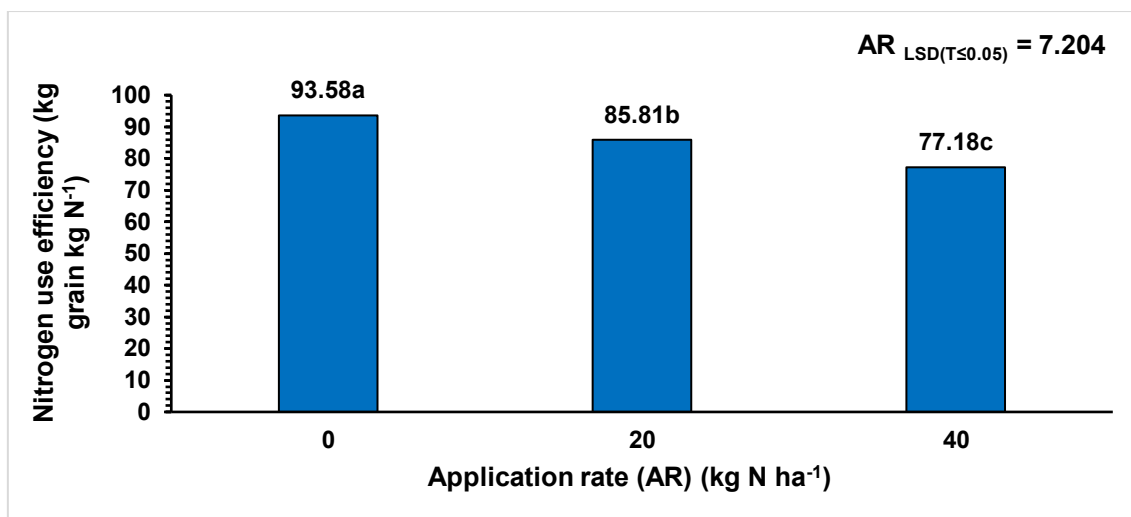


Figure 5.11 Effect of nitrogen amount applied on nitrogen use efficiency on a prolific cultivar for season 2022/23. Values with different letters differ significantly.

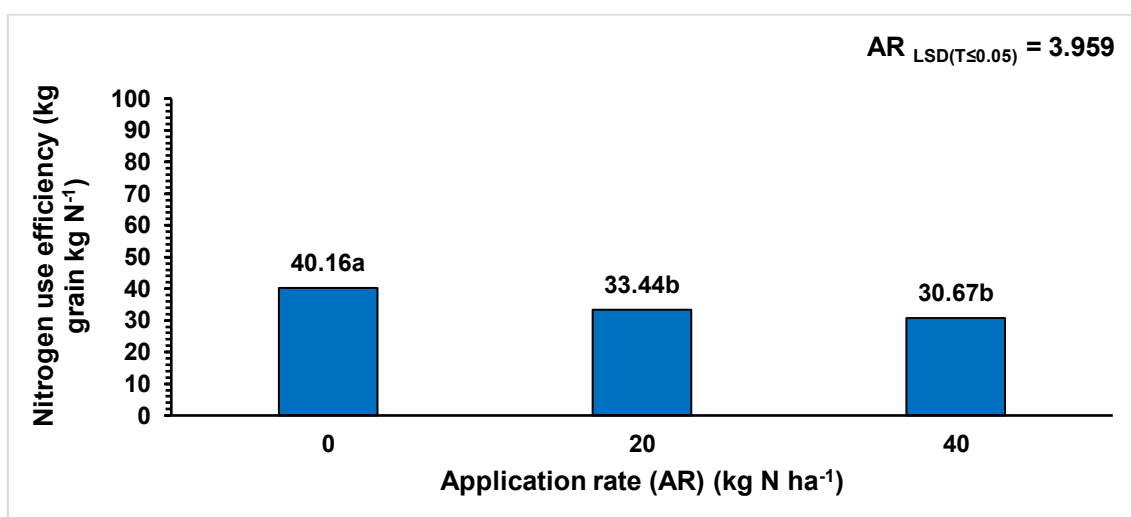


Figure 5.12 Effect of nitrogen amount on nitrogen use efficiency on a prolific cultivar for season 2023/24. Values with different letters differ significantly.

Hokmalipour *et al.* (2010) and Ochieng *et al.* (2021) reported that nitrogen use efficiency decreased with an increase in nitrogen application rate, which agrees with the results of this study. Mosisa *et al.* (2022) showed an increase in nitrogen agronomic use (NAE) (kg grain per kg N⁻¹) for N applications from 23 to 92 kg N ha⁻¹, with 92 kg N ha⁻¹ as the optimum. Beyond this rate, e.g., at 115 kg N ha⁻¹, the NAE began to decline again. This finding relates to the results of the 2023/24 season, where the control of 0 kg N ha⁻¹ application rate (106 kg N in total) resulted in the highest nitrogen efficiency versus the 20 (126 kg N in total) and 40 (146 kg N in total) kg N ha⁻¹ application rates. Galindo *et al.* (2019) reported that the nitrogen source (Urea) had a linear decrease in kg grain per kg N as the nitrogen rates increased.

5.3.1.9 Precipitation use efficiency

According to Meng *et al.* (2013), the exploitable yield gap is the difference between farmers' actual and potential yields, given favourable combinations of soil, climate, and crop management practices. Water productivity is one of the world's yield gap constraints, especially under rain-fed conditions. Global water productivity (crop water efficiency) averages approximately $13 \text{ kg ha}^{-1} \text{ mm}^{-1}$ for dryland maize cultivation, representing about 50% of the global water production potential for maize (Rattalino *et al.*, 2018). This approach can be used as a benchmark for effective management practices in on-farm use of available water across field cohorts.

Non-prolific

Season 2022/23

Significant impacts on precipitation use efficiency (PUE) were found only for nitrogen sources of the 2022/23 season (Table 5.1). No significant effects were found during the 2023/24 season (Figure 5.13).

Maize fertilized with Greensulf delivered $0.68 \text{ kg grain ha}^{-1} \text{ mm rain}^{-1}$ more than the LAN source during the 2022/23 season.

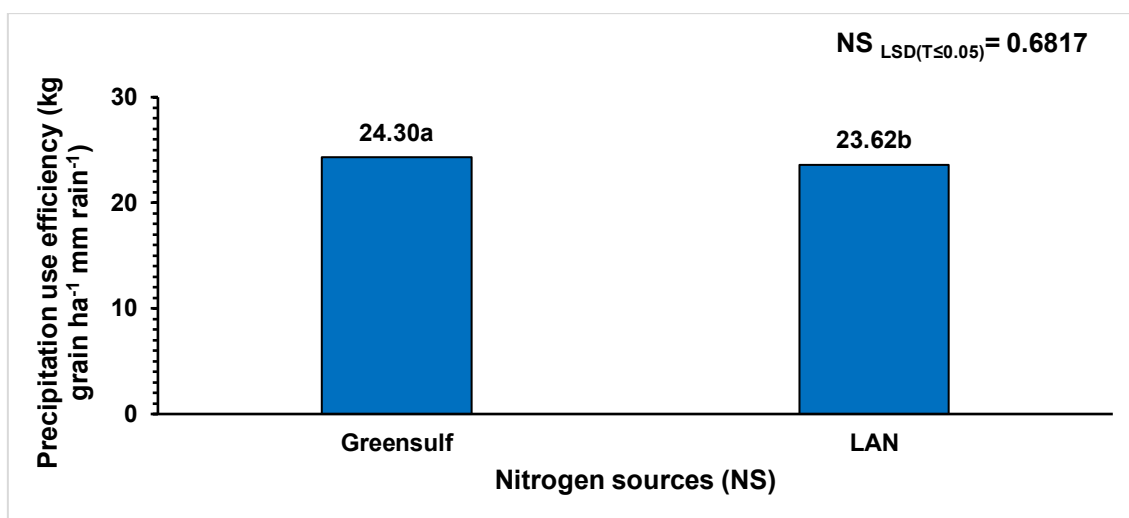


Figure 5.13 Effect of nitrogen sources on precipitation use efficiency of the non-prolific cultivar for the 2022/23 season. Values with different letters differ significantly.

Prolific

Season 2022/23

The water productivity of the prolific cultivar was only significantly influenced by the nitrogen application rate in the 2022/23 planting season (Table 5.2).

The PUE of the prolific cultivar was the highest at an application rate of 20 kg N ha⁻¹ (26.72 kg grain ha⁻¹ mm rain⁻¹), which was significantly higher than that at an application rate of 0 kg N ha⁻¹ (25.22 kg grain ha⁻¹ mm rain⁻¹) (Figure 5.14).

Ran *et al.* (2023) concluded that water stress had a greater influence on maize growth and grain yield than nitrogen stress. They also concluded that it is more important to prioritize soil water content to promote plants' nitrogen uptake because available water influences crop nitrogen availability. This finding was observed when comparing the two cropping seasons. The 2022/23 season was wetter than the 2023/24 season, which significantly affected nitrogen source and application rates. These findings could be why the amount of kg grain ha⁻¹ mm rain⁻¹ decreased for both cultivars in the 2023/24 season, as almost no rain fell in the R1 to R3 growth stages of the maize plants.

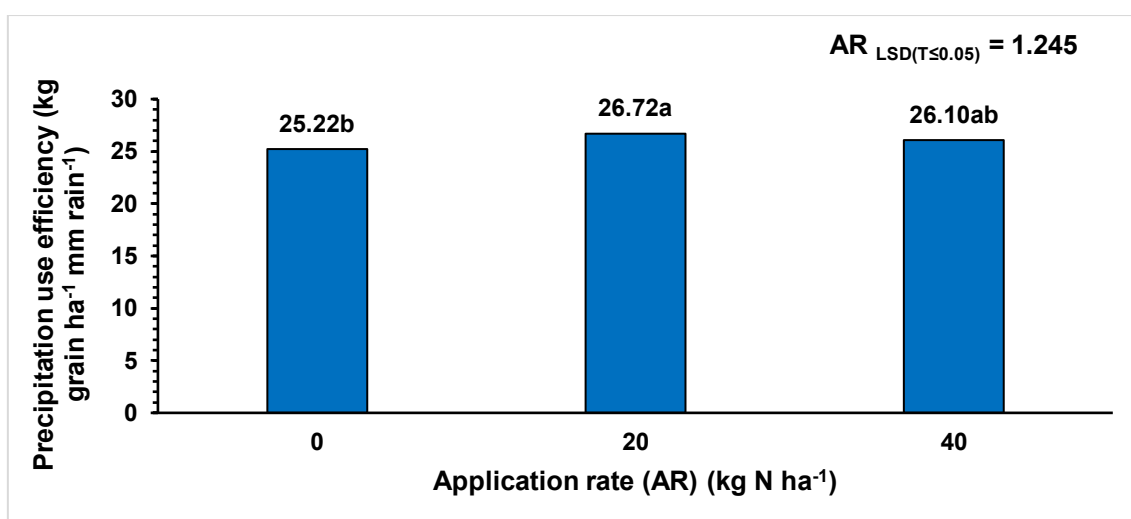


Figure 5.14 Effect of nitrogen application rate on precipitation use efficiency of the non-prolific cultivar for the 2022/23 season. Values with different letters differ significantly.

5.3.1.10 Return on investment

The return on investment is a basic indicator of profitability or loss, calculated as the cost to produce a crop per hectare, and is used to assess the profitability of the crop (Ghose & Franchetti, 2018). This is an important measurement to determine whether the additional grain yield is cost-effective in relation to the additional nitrogen input.

Non-prolific

Season 2022/23

None of the main effects (source, application rate, and growth stage) or interaction thereof had a significant impact on the return on investment (ROI) (Table 5.1). Some trends were observed for the interaction between nitrogen source and growth stage.

Maize fertilized with Greensulf at a rate of 0 kg N ha⁻¹ at V9 growth stage produced the highest ROI (R 26,452.06), which was R 1,373.28 ha⁻¹ more than that of maize fertilized with LAN applied at a rate of 20 kg N ha⁻¹ at V4 growth stage (R 25,078.78). A decreasing tendency in ROI was observed at each growth stage or combination of growth stages with increasing application rates of Greensulf (Figure 5.15).

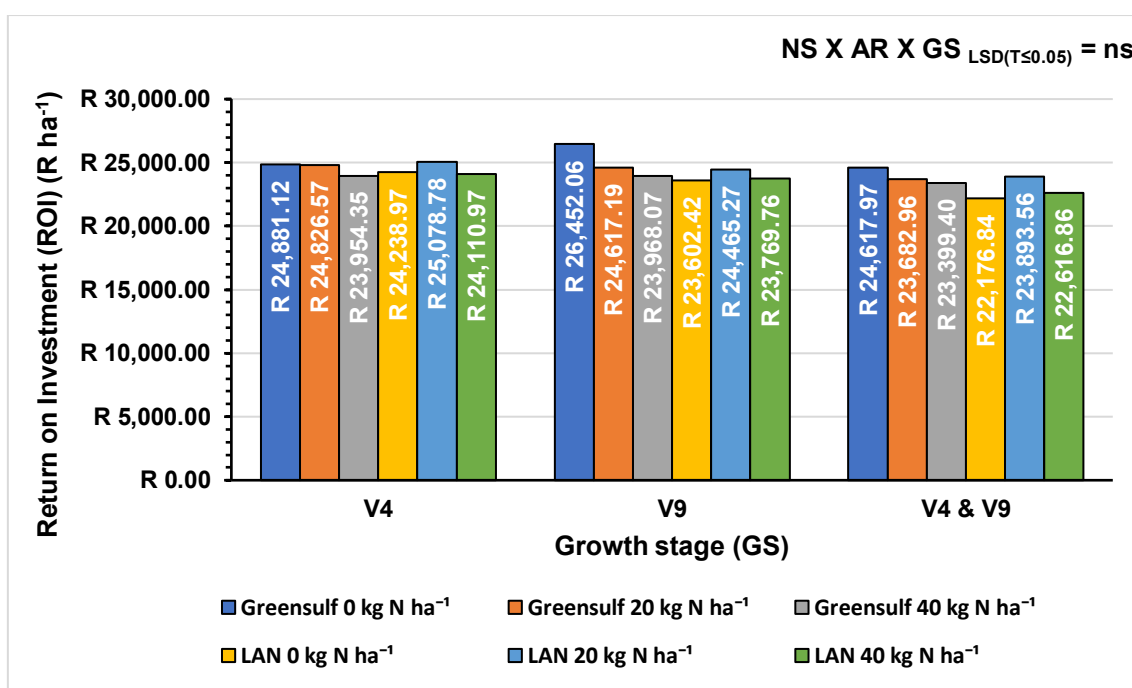


Figure 5.15 Effect of the interaction between nitrogen sources, application rate and growth stage applied on the ROI of the non-prolific cultivar for the 2022/23 season.

Season 2023/24

The ROI was significantly affected by the nitrogen application rate only (Figure 5.1). Maize that did not receive any topdressing (0 kg N ha⁻¹) resulted in a significantly larger ROI (R 166.84 ha⁻¹) than maize that was fertilized with 40 kg N ha⁻¹ (- R 1,456.97 ha⁻¹), with no significant differences between the rest of the application rates (Figure 5.16).

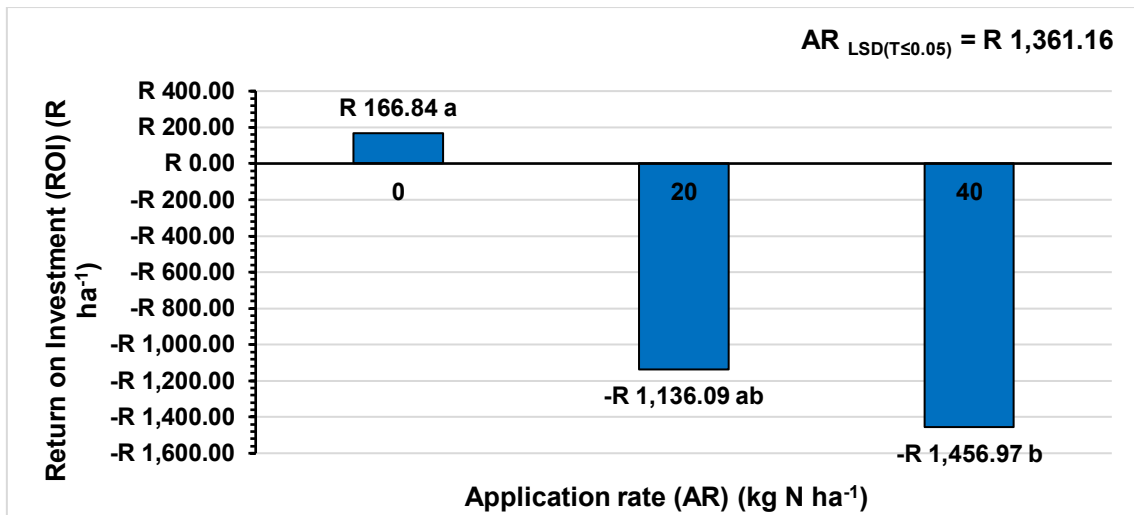


Figure 5.16 Effect of application rate on the ROI of the non-prolific cultivar. Values with different letters differ significantly.

Prolific

2022/23

The main effects (nitrogen source, application rate and growth stage) and their interactions did not significantly affect the ROI (Table 5.2). No trends were observed (Figure 5.17).

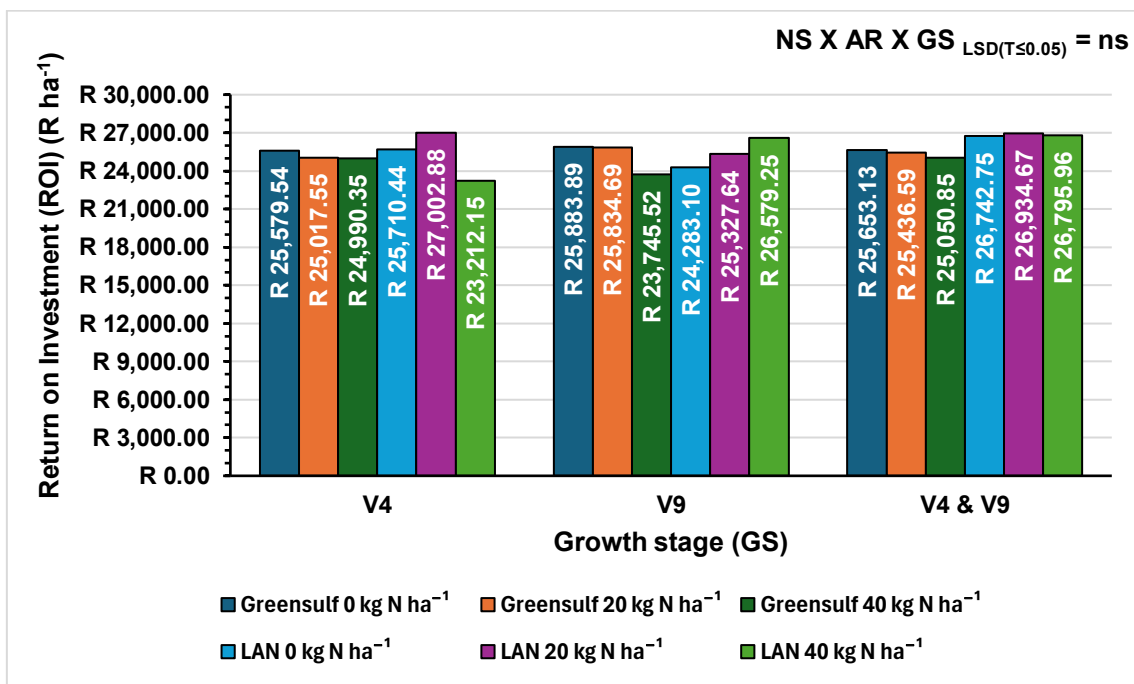


Figure 5.17 Effect of the interaction between nitrogen sources, application rate and growth stage applied on the ROI of the prolific cultivar for the 2022/23 season.

Season 2023/24

Application rate had a significant effect on the ROI of the prolific cultivar (Table 5.2).

The ROI of maize produced without receiving a topdressing (0 kg N ha⁻¹ application) (R 3,920.81 ha⁻¹) was significantly greater (220%) than that of maize receiving 40 kg N ha⁻¹ (R 1,781.41 ha⁻¹). The ROI of maize fertilized with 20 kg ha⁻¹ (R 2,447.19 ha⁻¹) did not differ significantly from that of maize receiving no N or 40 kg N ha⁻¹ (Figure 5.18).

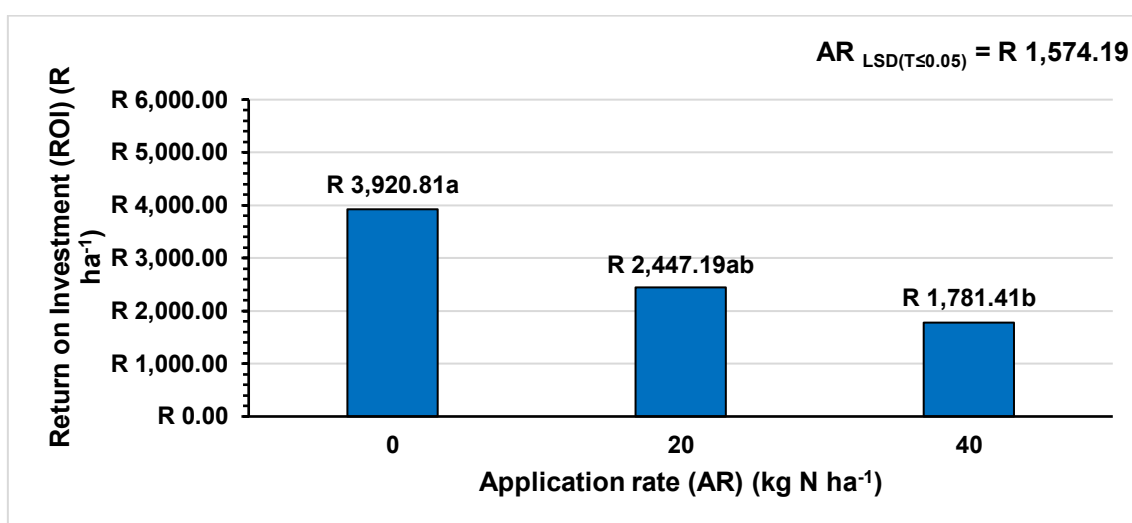


Figure 5.18 Effect of application rate on ROI for a prolific cultivar in 2023/24 season. Values with different letters differ significantly.

Both non-prolific and prolific cultivars were significantly affected by nitrogen application rate, resulting in a decrease in ROI as nitrogen rates were increased during the 2023/24 season. Trends were observed in the 2022/23 season, where the application rate and growth stage had an impact on the ROI of both cultivars.

Siam *et al.* (2008) found economic differences between nitrogen sources and different application rates. These authors concluded that application rates higher than 100 to 120 kg N ha⁻¹ were not viable due to higher costs and low returns depending on the source, except for Ammonia gas at a rate of 140 kg N ha⁻¹, which produced the best results. Essel *et al.* (2020) reported that a nitrogen application rate of 60 kg N ha⁻¹ resulted in an optimal net return from using nitrogen fertilizer, with further applications leading to a reduction in the net return and, thus, financial losses. Similar to Essel *et al.* (2020), Jansen *et al.* (2013) reported that excessive nitrogen fertilizer costs result in fertilizer costs being uneconomical in relation to the price of grain, resulting in a lower net return. This study also found that nitrogen sources and

rates influence ROI. Except for the LAN source of 20 kg N ha⁻¹ (total of 126 kg N ha⁻¹) for the prolific cultivar, 0 kg N ha (total of 106 kg N ha⁻¹) was more economically feasible than the higher application rates. Joshi *et al.* (2014) conducted a study on the effects of nitrogen sources, rates, and split applications on maize yield, concluding that split applications of nitrogen sources yielded a better economic return than applying the full amount at planting. Furthermore, a split application of 1/3 at planting, 1/3 at 25 days post-emergence, and 1/3 at 45 days post-emergence produced the highest economic return. This supports the study that application time has an impact on the ROI observed in both maize cultivars during the two seasons.

Conclusion

In conclusion, nitrogen source, application rate, and growth stage significantly affected the yield components and overall yield of both the non-prolific and prolific maize cultivars. The nitrogen source Greensulf substantially impacted several parameters, including grain mass per cob, 1000-kernel weight (non-prolific cultivar), grain yield, and precipitation use efficiency. On the other hand, the LAN nitrogen source notably influenced the number of cobs per plant and 1000-kernel weight (prolific cultivar). A nitrogen topdressing application of 20 kg ha⁻¹ had the most considerable effect on yield components and overall yield, except for return on investment (ROI) and nitrogen use efficiency (NUE), where the control treatment showed significantly higher results. The two cultivars responded differently to the nitrogen application under various conditions. The non-prolific cultivar performed better when nitrogen was applied at the V9 growth stage, while the prolific cultivar performed better with a split nitrogen application between the V4 & V9 stages. These factors should be considered separately for each cultivar due to the differences observed.

References

- ADHIKARI, K., BHANDARI, S., ARYAL, K., MAHATO, M. & SHRESTHA, J., 2021. Effect of different levels of nitrogen on growth and yield of hybrid maize (*Zea mays* L.) varieties. *J. Agric. Nat. Res.* 4(2): 48-62.
- ALEMU, D., YIRGA, C., & BEKELE, A., 2014. Situation and outlook of maize in Ethiopia. 1st ed. Addis Ababa: Ethiopian Institute of Agricultural Research. ISBN: 978-99944-53-97-9.
- ALI, S., DIN, S., ULLAH, O., SHAH, S., DIN, S., KHAN, T. & DIN, I., 2015. Yield and Yield Components of Maize Response To compost and Fertilizer-Nitrogen. *Food Sci. Qual. Manage.* 38: 39-44.
- AMUNULLAH, IQBAL, A., ALI, A., FAHAD, S. & PARMAR, B., 2016. Nitrogen Source and Rate Management Improve Maize Productivity of Smallholders under Semiarid Climates. *Front. Plant Sci.* (7): 1-9.
- ASSEFA, K. & MEKONNEN, Z., 2019. Effect of level and time of nitrogen fertilizer application on growth, yield and yield components of maize (*Zea mays* L.) at Arba Minch, Southern Ethiopia. *Afr. J. Agric. Res.* 14(33): 1785-1794.
- BADUWAL, P., CHAND, H., KAYASTHA, P., LAMICHHANE, P., PANDEY, B., KC, B., MAGAR, B.R., BHANDARI, J. & KHANAL, S., 2022. Correlation analysis of maize (*Zea mays* L.) genotypes: A review. *IJEAB.* 7(6): 153-157.
- BALTES, N.J., GIL-HUMANES, J & VOYTAS, D.F., 2017. *Progress in Molecular Biology and Translational Science*. In: D.P. Weeks & B. Yang (eds). Genome Engineering and Agriculture: Opportunities and Challenges. Academic Press. pp 1-26.
- BARARY, M., KORDI, S., RAFIE, M. & MEHRABI, A., 2015. Effect of Harvesting Time on Grain Yield, Yield Components, and Some Qualitative Properties of Four Maize Hybrids. *Int. J. Agric. Res.* 3(4): 1-7.
- BĂȘA, A.G., ION, V., DUMBRAVĂ, M., TEMOCICO, G., EPURE, L.I. & STEFAN, D., 2016. Grain Yield and Yield Components at Maize under Different Preceding Crops and Nitrogen Fertilization Conditions. *Agric. Agric. Sci. Procedia.* 10: 104-111.
- DAWAR, K., DAWAR, A., TARIQ, M., MAIN, I.A., MUHAMMAD, A., FARDI, L., KHAN, S., KHAN, K., FAHAD, S., DANISH, S., AL-GHAMDI, A.A., ELSHIKH, M.S. & TAHZEEB-UL-HASSAN, M., 2024. Enhancing nitrogen use efficiency and yield of maize (*Zea mays* L.) through Ammonia volatilization mitigation and nitrogen management approaches. *BMC Plant Biol.* 24(1): 74.
- DEIVASIGAMANI, S. & SWAMINATHAN, C., 2018. Evaluation of Seed Test Weight on Major Field Crops. *IJRSAS.* 4(1): 2454-6224.

- DIKO, A. & JUN, W., 2020. Influencing Factors of Maize production in South Africa: The case of Mpumalanga, Free State and North West Provinces. *Asian. J. Agric. Res.* 14(1): 25-34.
- DRULIS, P., KRIAUCIŪNIENĖ, Z. & LIAKAS, V., 2022. The Influence of Different Nitrogen Fertilizer Rates, Urease Inhibitors and Biological Preparations on Maize Grain Yield and Yield Structure Elements. *Agron.* 12(3):741.
- EL-DOUBY, K.A., ALI, E.A. & TOAIMA, S.E.A., 2001. Effect of nitrogen fertilizer defoliation and plant density on maize grain yield. *Egypt J. Agric. Res.*, 79(3): 965-981.
- ENGELBRECHT, M.L., 2008. Assessment of variance in measurement of hectolitre mass of wheat and maize, using equipment from different grain producing and exporting countries. M.Sc. Thesis, Stellenbosch University, pp 114.
- ESSEL, B., ABAIDOO, R.C., OPOKU, A. & EWUSI-MENSAH, N., 2020. Economically Optimal Rate for Nutrient Application to Maize in the Semi-deciduous Forest Zone of Ghana. *J Soil Sci Plant Nutr.* 20(4):1703-1713.
- GALINDO, F., TEIXEIRA FIHLO, M., BUZETTI, S., PAGLIARI, P., SANTINI, J., ALVES, C., MEGDA, M., NOGUEIRA, T., ANDREOTTI, T. & ARF, O., 2019. Maize Yield Response to Nitrogen Rates and Sources Associated with *Azospirillum brasilense*. *Agron. J.* 111(4): 1985-1997.
- GHEITH, E., EL-BADRY, O., FAID, S., ALI, H., SIDDIQUI, M., GHAREEB, R., EL-SHEIKH, M., JEBRIL, J., ABDELSALAM, N. & KANDIL, E., 2022. Maize (*Zea mays* L.) Productivity and Nitrogen Use Efficiency in Response to Nitrogen Application Levels and Time. *Front. Plant Sci.* 13: 941343.
- GHOSE, S. & FRANCHETTI, M.J., 2018. *Sustainable Food Waste-To-energy Systems*. In: T.A. Trabold & C.W. Babbitt (eds.). *Sustainable Food Waste-To-energy Systems*. Academic Press. pp: 203-229.
- GRUPO, D.F., 2022. The importance of nitrogen in maize crops. [online]. Retrieved from <https://www.dfgrupo.com/importancia-del-nitrogeno-en-el-cultivo-del-maiz/> (Accessed 2024-09-14).
- HOKMALIPOUR, S., SHIRI-E-JANGARD, M., DABANDI, M.H., PEYGHAMI-E-ASHENAEI, F., HASANZADEH, M., SEIEDI, M.N. & SHABANI, R., 2010. Comparison of Agronomical Nitrogen Use Efficiency in Three Cultivars of Corn as Affected by Nitrogen Fertilizer Levels. *World Appl. Sci. J.* 8(10): 1168-1174.
- IQBAL, M.A., AHMAD, Z., AFZAL, S. & AHMAD, M., 2015. Optimizing Nitrogen Level to Improve Growth and Grain Yield of Spring Planted Irrigated Maize (*Zea mays* L.). *J. Adv. Botany Zool.* 2(3): 1-4.
- JANSEN, J.A., WORTMANN C.S., STOCKTON M.C. & KAIZZI K.C., 2013. Maximizing net returns to financially constrained fertilizer use. *Agron J.* 105(3):573–578.

- JOSHI, A., GUPTA, J.K., CHOUDHARY, S.K., MUJALDE, S. & GRAG, M., 2014. Effect of Nitrogen Sources, doses and Split applications on yield and economics of maize (*Zea mays* L.) in the Malwa region of Madhya Pradesh (India). *J. Agric. Vet. Sci.* 7(6): 24-28.
- KH, N., EL-GIZAWY, N. & SALEM, H., 2010. Influence of Nitrogen Sources on Yield and its Components of Some Maize Varieties. *World J. Agric. Sci.* 6(2): 218-223.
- MAKANZA, R., ZAMAN-ALLAH, M., CAIRNS, J.E., EYRE, J., BURGUEÑO, J., PACHECO, A., DIEPENBROCK, C., TAREKEGNE, A., OLSEN, M. & PRASANNA, B.M., 2018. High-throughput method for ear phenotyping and kernel weight estimation in maize using ear digital imaging. *Plant Methods.* 14(1): 49.
- MENG, O., HOU, P., WU, L., CHEN, X., CUI, Z. & ZHANG, F., 2013. Understanding production potentials and yield gaps in intensive maize production in China. *Field Crops Res.* 143: 91-97.
- MERGENER, R., SANGOI, L. & COELHO, A., 2022. Productivity and profitability of maize as affected by nitrogen sources and rates. *Semina: Ciências Agrárias.* 43: 1457-1468.
- MIRCEA, V., AEDIN, C. & MARIN, D.I., 2023. Researches concerning the influence of the maize hybrid maturity and the irrigation regime on the thousand kernel weight and hectoliter mass. *A. Agron.* 66(1): 431-440.
- MOSISA, W., DECHASSA, N., KIBRET, K., ZELEKE, H. & BEKEKO, Z., 2022. Effects of timing and nitrogen fertilizer application rates on maize yield components and yield in eastern Ethiopia. *Agrosyst. Geosci. Environ.* (5): 1-14.
- OCHIENG, I., GITARI, H., MOCHOGE, B., REZAEI-CHIYANEH, E. & GWEYI-ONAYANGO, J.P., 2021. Optimizing Maize Yield, Nitrogen Efficacy and Grain Protein Content under Different N Forms and Rates. *J Soil Sci. Plant Nutr.* DOI: 10.1007/s42729-021-00486-0.
- OCHIENG, I., RANJA, S., SELEIMAN, M., PADHAN, S.R., CHEPTOEK, R., SOW, S., WASONGA, D. & GITARI, H., 2023. Increasing rainwater use efficiency, gross return, and grain protein of rain-fed maize under nitrate and urea nitrogen forms. *Not bot Horti Agrobo.* 51(3): 13293.
- OTEGUI, M.E. & BOHOMME, R., 1998. Grain yield components in maize: I. Ear growth and kernel set. *Field Crops Res.* 56(3): 247-256.
- PLAVSIC, H., JOSIPOVIC, M., ANDRIC, L., JAMBROVIC, A. & SOSTARIC, J., 2007. Influences of irrigation and n fertilization on maize (*Zea mays* L.) properties. *Cereal Res. Commun.* 35(2): 933–936.
- RAN, J., RAN, H., MA, L., JENNINGS, S.A., YU, T., DENG, X., YAO, N & HU, X., 2023. Quantifying water productivity and nitrogen uptake of maize under water and nitrogen stress in arid Northwest China. *Agric. Water Manag.* 285: 108370

- RATTALINO, E.J.I., GUILPART, N., SADRAS, V., CASSMAN, K.G., VAN ITTERSUM, M.K., SCHILS, R.L.M. & GRASSINI, P., 2018. Water productivity of rainfed maize and wheat: A local to global perspective. *Agric For Meteorol.* 259(15): 364-373.
- RAWAL, N., VISTA, S.P., KHADKA, D. & PANERU, P., 2024. Grain Yield, Nitrogen Accumulation, and Its Use Efficiency of Maize (*Zea mays* L.) as Influenced by Varying Nitrogen Rates. *Int. J. Res. Agron.* 1: 1-12.
- RUIZ, A., ARCHONOULIS, S.V. & BORRÁS, L., 2022. Kernel weight relevance in maize grain yield response to nitrogen fertilization. *Field Crops Res.* 286: 108631.
- SAPUCAY, M., COELHO, A.E., BRATTI, F., LOCATELLI, J.L., SANGOI, L., ALVADI, A., BALBINOT & J., ZUCARELI C., 2020. Nitrogen rates on the agronomic performance of second-crop corn single and intercropped with ruzi grass or showy rattlebox. *Pesqui. Agropecu. Trop.* 50. DOI: 10.1590/1983-40632020v5065525.
- SIAM, H.S., ABD-EL-KADER, M.G. & EL-ALIA, H.I., 2008. Yield and Yield Components of Maize as Affected by Different Sources and Application Rates of Nitrogen Fertilizer. *Res. J. agric. & Biol. Sci.* 4(5): 399-412.
- ULLAH, I., MUHAMMAD, D. & MUSARAT, M., 2024. Effect of Various Nitrogen and Sulfur Sources on Maize-Wheat Yield and N:S Uptakes Under Two Different Climatic Conditions. *Agric. Res.* DOI: 10.1007/s40003-024-00749-z.
- YUE, K., LI, L., XIE, J., LIU, Y., ANWAR, S. & FUNJOE, S.K., 2022. Nitrogen Supply Affects Yield and Grain Filling of Maize by Regulating Starch Metabolizing Enzyme Activities and Endogenous Hormone Contents. *Front. Plant Sci.* 12: 1-14.

Chapter 6

The influence of nitrogen source, application rate and growth stage on the grain quality and nutrient content of a non-prolific and prolific maize cultivar in the north-eastern Free State

6.1 Introduction

This chapter will evaluate the effect of nitrogen sources, topdressing application rates, and growth stages on the grain quality of maize.

Environmental conditions significantly impact the grain quality of maize, especially drought and heat stress. These abiotic stress factors influence the physiological and biological processes of the maize plant. Heat and drought stress during the grain-filling stage of maize can profoundly impact grain quality. High temperatures during the early vegetative stages of maize can lead to reduced starch production. Drought conditions also led to a reduction in starch content and grain size, but increased protein content (Lindiwe & Erick, 2024).

Nitrogen is regarded as an essential component for protein production, which dramatically affects the grain quality of maize. Achieving the right balance in nitrogen fertilizer rates is crucial for enhancing the quality of grain components. (Omar *et al.*, 2022; Lindiwe & Erick, 2024). A study by Biswas & Ma (2016) showed that grain quality was significantly affected by the nitrogen source and the nitrogen rate. The nitrogen source had a significant effect on grain quality, including grain moisture, kernel size, and density, with calcium ammonium nitrate (CAN) yielding the best results, followed by ammonium nitrate (AN) and ammonium sulphate (AS) during two growing seasons. Biswas & Ma (2016) also reported that CAN, as an N source significantly affected the N content in the kernel.

Biswas & Ma (2016) reported that the grain quality of maize increased when fertilized, and rates increased from 30 to 150 kg N ha⁻¹, independent of the nitrogen source used. Grain moisture and kernel density were found to be unresponsive to nitrogen rate, while kernel size and grain yield were significantly affected by nitrogen rate, according to Biswas & Ma (2016). Omar *et al.* (2022) reported that different nitrogen rates did not significantly affect grain quality, particularly in terms of grain moisture content, oil content, protein content, and starch content. However, they recommended a rate of 50 to 100 kg N ha⁻¹ for optimal grain yield. Amanullah

& Shah (2010) reported that increasing nitrogen rates (82 to 93 kg N ha⁻¹) as a single application increased grain protein, decreasing maize's oil and starch content.

Amanullah & Shah (2010) reported that when nitrogen application was split into an early vegetative and late reproductive stage, the protein, P, and K content in the grain increased. According to Subedi & Ma (2005), the application of nitrogen fertilizer at V8, and later reduced the grain yield and protein content, while early applications of nitrogen increased protein content but reduced the grain's starch and fat content. Lindiwe & Erick (2024) reported that the timing of nitrogen applications is vital for grain quality. These authors observed that nitrogen application split into two application times (at planting and the five-leaf stage) produced the highest protein, fat, and ash content.

This study evaluated the effects of nitrogen source, application rate, and growth stage (time of application) on the grain quality and nutrient content of a non-prolific and highly prolific maize cultivar over two cropping seasons.

6.2 Materials and Methods

The materials and methods used were discussed in Chapter 3.

6.3 Results and Discussion

Analysis of variance indicated that data from the 2022/23 and 2023/24 cropping seasons were not homogeneous, probably because of climatic differences between the cropping seasons (Table 3.1). Therefore, the two cropping seasons were analysed separately and will be discussed accordingly.

A summary of the analysis of variance evaluating the effect of nitrogen source (NS), topdressing application rate (AR), and growth stage (GS) on the grain quality of a non-prolific maize cultivar is summarised in Tables 6.1 and that of a prolific cultivar in Tables 6.2. The non-prolific cultivar protein content was significantly influenced by the nitrogen application rate for both cropping seasons and by the interaction of the nitrogen source and growth stage for the 2022/23 season only. The nitrogen rate applied for the 2022/23 season significantly influenced the fat content. The starch and fibre content, as well as the milling index, were significantly influenced by the topdressing application rate for the 2023/24 season. The nitrogen source significantly influenced the content of nitrogen (N) and manganese (Mn). The zinc (Zn) content was significantly influenced by the interaction between the nitrogen source and growth stage applied during the 2022/23 season. The Zn content was significantly

influenced by the nitrogen source used, and the iron (Fe) content was significantly influenced by the growth stage applied during the 2023/24 season.

The protein content of the prolific cultivars was significantly influenced by the nitrogen application rate, and the growth stage during the 2022/23 season. The topdressing application rate significantly influenced the fat content for both seasons, and the interaction between nitrogen source and application rate significantly affected the fat content for the 2022/23 season. The growth stage applied in the 2022/23 season significantly influenced the Mn content. In the 2023/24 season, N content was significantly influenced by the interaction between nitrogen source and application rate, as well as the interaction between application rate and growth stage. Phosphorus (P) content was significantly influenced by the interaction between the nitrogen source and growth stage, and the nitrogen source had a significant influence on B during the 2023/24 season.

6.3.1 Grain quality of a non-prolific and prolific cultivar

In this chapter the protein content, fat content, starch content, fiber content, milling index and grain nutrient content (N, P, Cu, Zn, Mn, B and Fe) will be discussed.

6.3.1.1 Protein content

Nitrogen is a crucial nutrient for plants, playing a vital role in various metabolic pathways essential to their growth and development. It is also involved in protein synthesis. Researchers reported that N improves grain quality and increases maize's protein and mineral content (Amanullah & Shah, 2010; Demari *et al.*, 2016; Omar *et al.*, 2022). Maize is the third-largest grain crop in the world and serves as an essential source of protein for both humans and animals (Motukuri, 2019). Protein plays a vital role in the growth, maintenance, and physiological functions of the bodies of animals and humans (Adhikari *et al.*, 2022), emphasizing the importance of grain quality.

Non-prolific

Season 2022/23

Protein was significantly influenced by application rate (AR) and the interaction of nitrogen source and application rate (NS x AR) (Table 6.1).

Table 6.1 Summary of analysis of variance indicating the effect of treatment factors on grain quality for a non-prolific maize cultivar

Treatments	Protein (%)		Fat (%)		Stach (%)		Fiber (%)		Milling Index	
	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24
Source (NS)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Application rate (AR)	*	*	*	ns	ns	*	ns	*	ns	*
Growth stage (GS)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
NS X AR	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
NS X GS	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
AR X GS	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
NS X AR X GS	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV%	4.57	2.76	9.83	9.41	0.85	0.97	2.95	2.26	7.52	6.89

ns – not significant

*P ≤ 0.05

CV – coefficient of variance

Table 6.2 Summary of analysis of variance indicating the effect of treatment factors on grain quality for prolific maize cultivar

Treatments	Protein (%)		Fat (%)		Stach (%)		Fiber (%)		Milling Index	
	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24	2022/23	2023/24
Source (NS)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Application rate (AR)	*	ns	*	*	ns	ns	ns	ns	ns	ns
Growth stage (GS)	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
NS X AR	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
NS X GS	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
AR X GS	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
NS X AR X GS	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV%	4.01	2.67	8.28	9.34	0.67	11.53	2.14	211.17	5.57	7.77

ns – not significant

*P ≤ 0.05

CV – coefficient of variance

Generally, the protein content decreased with an increase in application rate (Table 6.3). The protein content of maize plants that did not receive nitrogen (0 kg N ha⁻¹ - control) produced the highest protein content. This appearance was observed for both Greensulf and LAN. For both Greensulf (7.29%) and LAN (7.14%), the highest protein contents were obtained when no fertiliser was applied as a top dressing (Table 6.3). Except for the protein content of plants topdressed with 40 kg ha⁻¹ Greensulf, the protein contents of all other treatment combinations did not differ significantly.

Table 6.3 Protein content (%) as influenced by the interaction of nitrogen source and application rate for a non-prolific cultivar for the 2022/23 season

Application rate (AR) (kg N ha ⁻¹)	Nitrogen source (NS)		AR average
	Greensulf	LAN	
0	7.29 ^a	7.14 ^{ab}	7.22
20	7.11 ^{ab}	6.88 ^{bc}	7.00
40	6.71 ^c	6.99 ^{abc}	6.85
Average	7.04	7.00	

NS *LSD* (*T*≤0.05) = *ns*

AR *LSD* (*T*≤0.05) = **0.2241**

NS x AR *LSD* (*T*≤0.05) = **0.3884**

Values with different letters differ significantly.

Season 2023/24

Only application rate (AR) significantly affected the protein content of the grain (Table 6.1). The highest protein content was achieved with an application rate of 20 kg N ha⁻¹ (9.63%), which did not differ significantly from the protein content of maize that received no nitrogen (0 kg N ha⁻¹) (9.55%) (Figure 6.1). The protein content of both the treatments mentioned above was significantly greater than that (9.41%) of maize plants fertilized with 40 kg N ha⁻¹.

Prolific

Season 2022/23

The protein content was significantly influenced by the nitrogen application rate and growth stage (Table 6.2).

The plants that did not receive any nitrogen (0 kg N ha⁻¹) (6.99%) had a significantly higher grain protein content than the plants that received rates of 20 kg ha⁻¹ (6.63%) and 40 kg N ha⁻¹ (6.57%) (Figure 6.2).

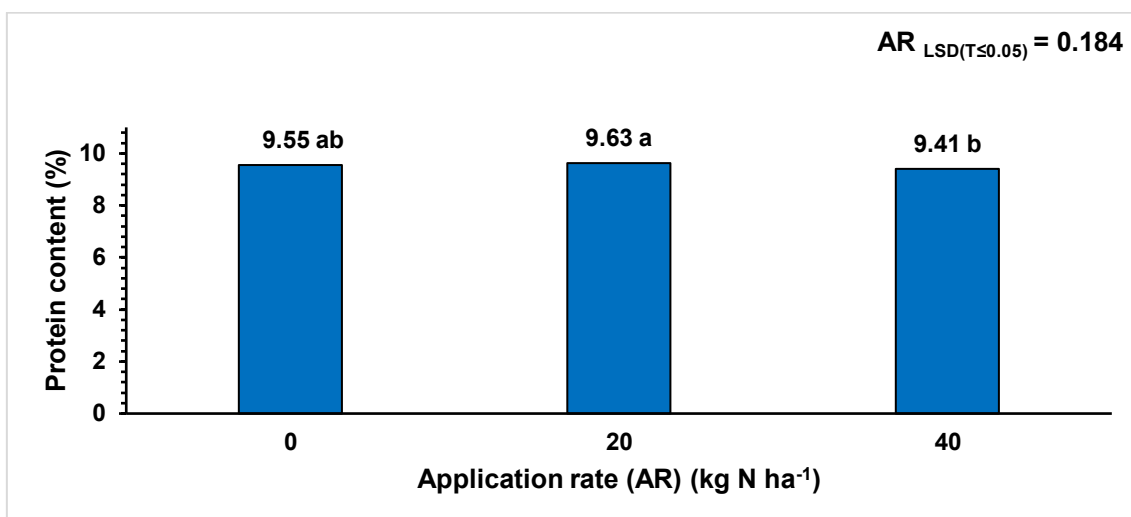


Figure 6.1 Protein content (%) as influenced by the nitrogen application rate of a non-prolific cultivar for the 2023/24 season. Values with different letters differ significantly.

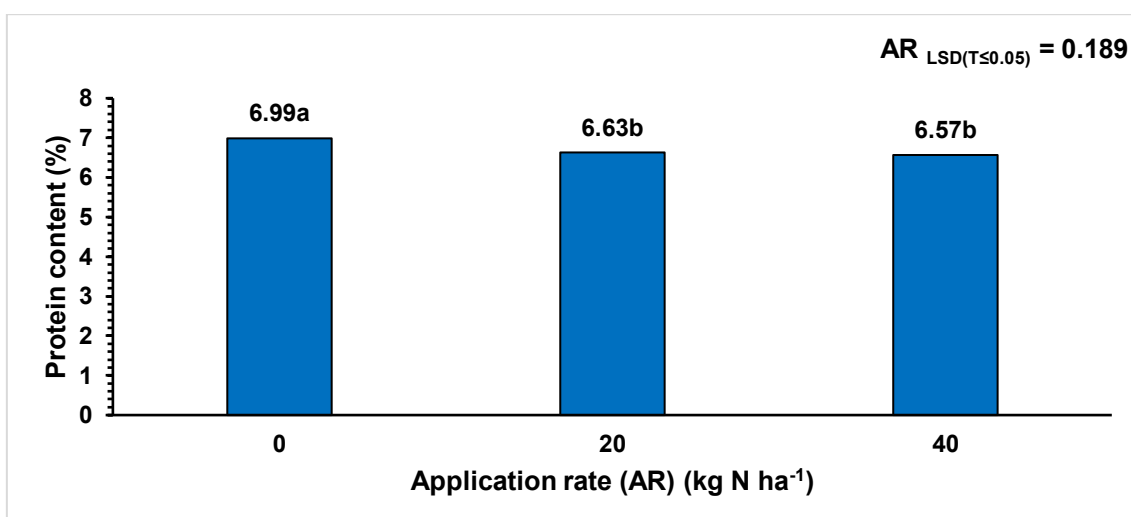


Figure 6.2 Protein content (%) as influenced by the nitrogen application rate of a prolific cultivar for the 2022/23 season. Values with different letters differ significantly.

When the topdressing was applied, the growth stage significantly influenced grain protein content, with the V9 growth stage (6.80%) being the optimal time for application. The V9 growth stage resulted in a significantly higher protein content than the plants fertilized at the V4 stage (6.61%). However, the protein contents did not differ significantly from the plants fertilized in a split application at V4 & V9 (6.78%).

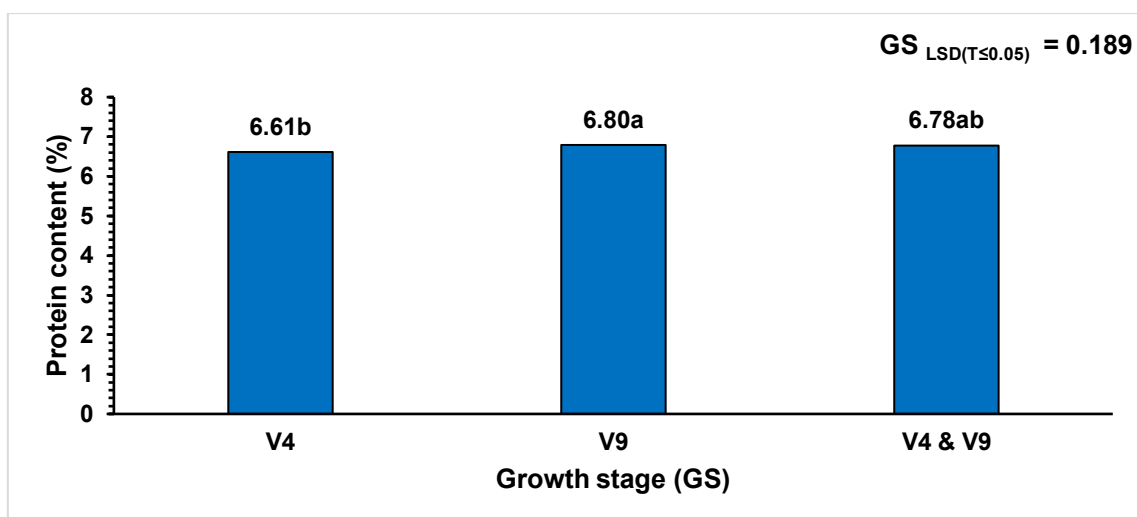


Figure 6.3 Protein content (%) as influenced by the growth stage for a prolific cultivar in the 2022/23 season. Values with different letters differ significantly.

Season 2023/24

Protein content was not significantly affected by the main treatments or their interaction effects (Table 6.2). The only observation, although not statistically significant, was that the protein content of maize grain increased when a split (V4 & V9) topdressing was applied compared to a single application at either the V4 or V9 growth stage.

Table 6.4 Protein content (%) as influenced by the nitrogen source, application rate and growth stage applied for the prolific cultivar in the 2023/24 season

Nitrogen source (NS)	Application rate (AR) (kg N ha ⁻¹)	Growth stages (GS)			AR average
		V4	V9	V4 & V9	
Greensulf	0	9.89	9.94	10.33	10.05
	20	10.14	10.25	10.02	10.14
	40	10.08	10.26	10.33	10.22
GS Greensulf average		10.04	10.15	10.23	
LAN	0	9.93	10.36	10.10	10.13
	20	10.34	10.23	10.33	10.30
	40	9.96	10.05	10.19	10.07
GS LAN average		10.08	10.21	10.21	
GS average		10.06	10.18	10.22	

NS_{LSD (T≤0.05)} = ns

AR_{LSD (T≤0.05)} = ns

GS_{LSD (T≤0.05)} = ns

NS x AR_{LSD (T≤0.05)} = ns

NS x GS_{LSD (T≤0.05)} = ns

AR x GS_{LSD (T≤0.05)} = ns

NS x AR x GS_{LSD (T≤0.05)} = ns

Values with different letters differ significantly.

The protein content of both seasons was significantly influenced by the rate of nitrogen applied to the non-prolific and prolific cultivars. The protein content decreased with an increase in application rate, except for maize plants fertilized with 20 kg ha⁻¹ nitrogen during the 2023/24 season for the non-prolific cultivar. In the 2022/23 season, significant differences were observed in the interaction between nitrogen source and application rate on grain protein content. This indicates that the grain of the control treatments had a higher protein content than the grain of the control treatments with application rates of 20 and 40 kg N ha⁻¹, regardless of the source for the non-prolific cultivar.

Idikut *et al.*, (2009) reported a negative correlation ($r = -0.710$) between grain protein and grain yield, indicating a decrease in protein content with higher maize yield. Ertiro *et al.* (2022) also observed a negative correlation between protein content and yield ($r = -0.23$). These findings could be attributed to the lower yields obtained in the 2023/24 season (Chapter 5). This could help explain why the grain protein content in the 2023/24 season was higher than that of the 2022/23 season. A study by Siam *et al.*, (2008) reported that the protein content of maize grain was significantly affected by the nitrogen source and the nitrogen application rate. These authors found that ammonia gas as a nitrogen source at 140 kg N ha⁻¹ produced the highest grain protein content compared to urea and ammonium sulphate sources at the same nitrogen rate. This finding agrees with the interaction between the nitrogen source and the application rate of the non-prolific cultivar.

According to Aakash *et al.* (2022) and Omar *et al.*, (2022), protein content was significantly affected by application rate, with the highest protein content achieved with the lower range of N application rates (100 kg ha⁻¹) and the lowest protein content at higher application rates (150 kg ha⁻¹). This concurs with the results obtained in this study.

Grain protein content is primarily determined by genetic makeup, but other factors, such as nitrogen application and climate, can also have significant influences (Ochieng *et al.*, 2023). Previous study has found that environmental factors can impact protein content by up to 89% (Martinez *et al.*, 2010). These findings could also be the reason for the results of the two seasons in which the control plants produced the highest protein content in the 2022/23 season and that there were no significant differences in grain protein content between the control and the additional nitrogen rates of the 2023/24 season. Amanullah and Shah (2010) showed an increase in kernel protein content when nitrogen was applied in split applications and not just at planting. The best protein content was achieved with 180 kg N ha⁻¹ applied in 6 separate applications from plant to the late reproductive growing stages (70 days after emergence), resulting in 6 g kg⁻¹ higher grain protein content than a fertilizer application

restricted to planting alone. These results concur with the results found in both seasons of the prolific cultivar.

6.3.1.2 Fat content

The quality of the oil extracted from maize grains depends on the grain's fat content (Bathla *et al.*, 2019). Fat is one of the main contents (protein, moisture, fats, and carbohydrates) tested in maize grain for quality and food safety purposes for consumers (Hamisu *et al.*, 2023). This is because it plays an essential role in animal and human nutrition. The fat in maize is an important ingredient in most animal rations for being an easily digestible energy source and essential fatty acid for animals. During oil synthesis, air temperature and nutrient management significantly impact the fatty acid-generating organs of plants (Stępień *et al.*, 2024). This finding motivates the importance of measuring the fat content of maize grain.

Non-prolific

Season 2022/23

Only the nitrogen application rate affected the fat content during this season (Table 6.1). A significantly increased nitrogen rate during the 2022/23 season increased the grain's fat content. Maize plants topdressed with 40 kg N ha⁻¹ (4.45%) resulted in a significantly higher fat content than control plants (4.12%). The fat content of plants fertilized with 20 kg N ha⁻¹ did not differ significantly from the fat content of either control plants or 40 kg ha⁻¹ topdressing (Figure 6.4).

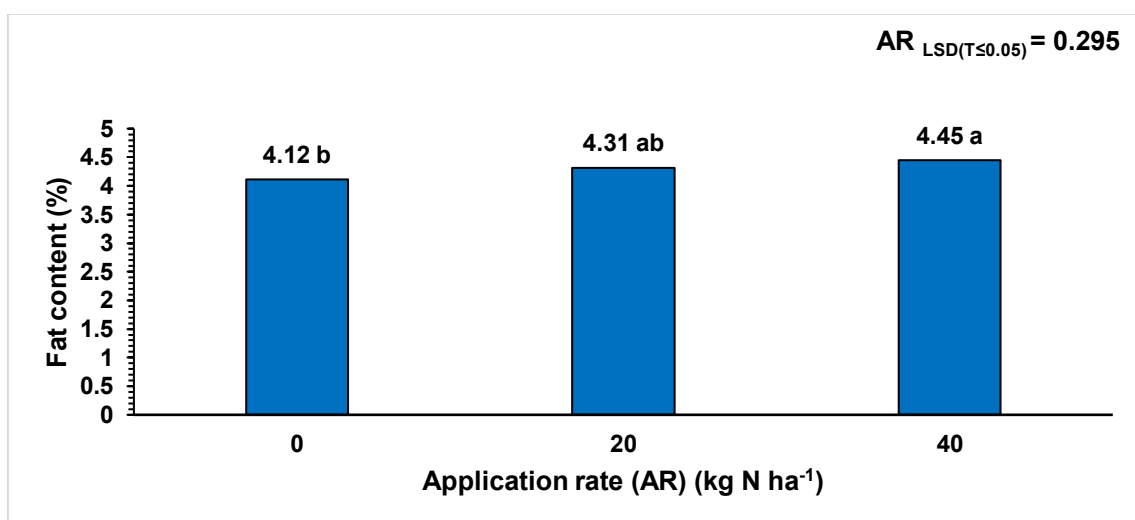


Figure 6.4 Fat content (%) as affected by application rate for the non-prolific cultivar in the 2022/23 season. Values with different letters differ significantly.

Season 2023/24

There was no significant effect on the grain fat content due to the main effects or their interactions (Table 6.1). Only trends were observed.

It appears that the plants fertilized with both nitrogen sources at a rate of 20 kg N ha⁻¹ produced the highest fat content of all the nitrogen rates. Furthermore, regardless of the source, plants that received the topdressing as a split application (V4 & V9) produced the highest fat content (Table 6.5).

Table 6.5 Fat content (%) as influenced by the nitrogen source, application rate and growth stage applied for the non-prolific cultivar in the 2023/24 season

Nitrogen source (NS)	Application rate (AR) (kg N ha ⁻¹)	Growth stages (GS)			AR average
		V4	V9	V4 & V9	
Greensulf	0	3.81	4.21	4.41	4.14
	20	4.13	4.45	4.13	4.24
	40	4.18	4.06	4.42	4.22
GS Greensulf average		4.04	4.24	4.32	
LAN	0	3.96	3.82	4.36	4.04
	20	4.48	4.19	4.51	4.39
	40	3.90	4.38	4.26	4.18
GS LAN average		4.11	4.13	4.38	
GS average		4.08	4.18	4.35	

NS $LSD (T \leq 0.05) = ns$

AR $LSD (T \leq 0.05) = ns$

GS $LSD (T \leq 0.05) = ns$

NS x AR $LSD (T \leq 0.05) = ns$

NS x GS $LSD (T \leq 0.05) = ns$

AR x GS $LSD (T \leq 0.05) = ns$

NS x AR x GS $LSD (T \leq 0.05) = ns$

Values with different letters differ significantly.

Prolific

Season 2022/23

The fat content of grain was significantly influenced by the interaction between nitrogen source and application rate (Table 6.2).

The LAN source-treated plants showed no significant differences between application rates. However, the fat content of the plants receiving LAN at 40 kg N ha⁻¹ (3.84%) was significantly lower than that of the plants receiving Greensulf at 20 kg N ha⁻¹ (4.27%). At 20 kg N ha⁻¹, the

Greensulf source, treated plants' fat content was significantly higher than that of the control plants (3.74%) (Table 6.6).

Table 6.6 Fat content (%) as affected by the interaction between the nitrogen source and application rate for the prolific cultivar in the 2022/23 season

Application rate (AR) (kg N ha ⁻¹)	Source (NS)		AR average
	Greensulf	LAN	
0	3.74 ^b	3.90 ^{ab}	3.82
20	4.27 ^a	3.95 ^{ab}	4.11
40	3.98 ^{ab}	3.84 ^b	3.91
Average	4.00	3.90	

NS $LSD (T \leq 0.05) = ns$

AR $LSD (T \leq 0.05) = 0.2282$

NS x AR $LSD (T \leq 0.05) = 0.3955$

Values with different letters differ significantly.

Season 2023/24

The prolific cultivar was only significantly influenced by the nitrogen application rate (Table 6.2). Plants that received a nitrogen topdressing application rate of 20 kg ha⁻¹ had a significantly higher fat content (3.96%) than those with a 0 kg N ha⁻¹ (3.70%), with no significant difference between the other treatments (Figure 6.5).

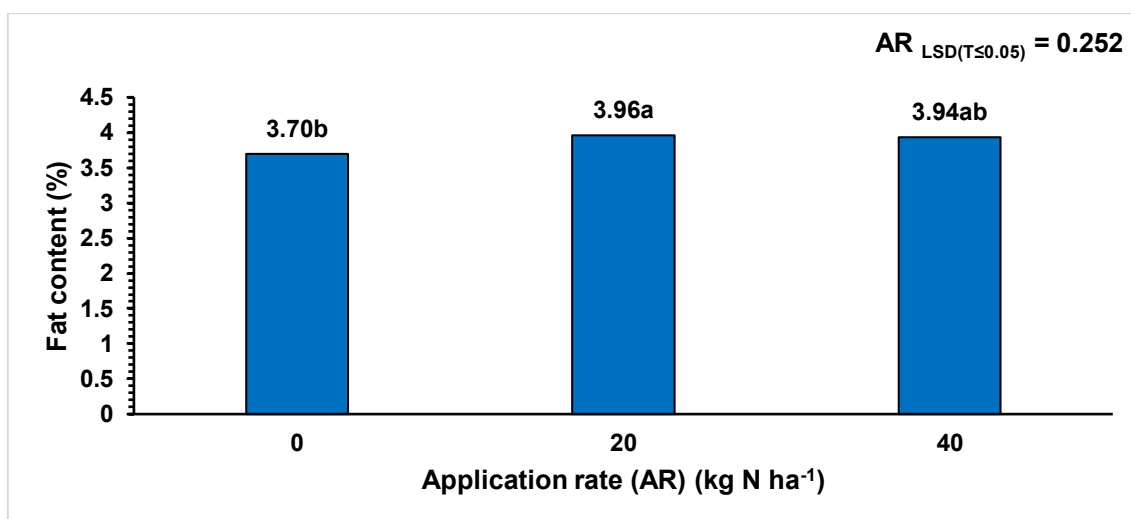


Figure 6.5 Fat content (%) as affected by application rate for the prolific cultivar in the 2023/24 season. Values with different letters differ significantly.

Shynkaruk and Lykhochvor (2021) reported that increasing fertilizer rates reduced grain fat content, with fat content being higher at 80 kg N ha⁻¹ than at applications of 120 and 160 kg N ha⁻¹. The results of the current study contradict previous research findings and indicate an

increase in fat content with higher nitrogen rates. The highest fat content was at a total nitrogen rate of 140 kg ha⁻¹ (40 kg N ha⁻¹ topdressing) for the non-prolific cultivar and 120 kg ha⁻¹ (20 kg N ha⁻¹ topdressing) for the prolific cultivar. Shehzad *et al.* (2012) and Liu *et al.* (2022) also reported that increasing the nitrogen rate increased kernel fat content, which agrees with the results found for both non-prolific and prolific cultivars in this study. Regarding nitrogen sources, Stępień *et al.* (2024) could not find significant differences in grain fat content between nitrogen sources and concluded that the unfavourable weather conditions could have influenced the results of their research. This finding contradicts the results on the prolific cultivar in the 2022/23 season of the current study, which had significant differences between nitrogen sources.

6.3.1.3 Starch content

This parameter is the first of three major nutritional components of maize grain (starch, protein, and fat), which is essential for human consumption (Hamisu *et al.*, 2023). Starch is also the major component of the kernel, comprising up to 72-73% of the kernel's weight. Maize accounts for approximately 80% of the world's starch extraction and is widely used in the food industry for products such as pasta, snacks, and thickeners (Paraginski *et al.*, 2014). It was reported that temperatures, planting dates, and planting conditions significantly affected starch content. Information regarding the response of starch to fertilization is scant (Kaplan *et al.*, 2019). This necessitates the evaluation of starch response to fertilisation practices.

Non-prolific

There were no significant differences in grain starch content for the 2022/23 season. In the 2023/24 season, the grain starch content was significantly influenced by the amount of nitrogen applied (Table 6.1).

The starch content was highest at an application of 40 kg ha⁻¹ (62.54%), followed by the control at 0 kg ha⁻¹ (62.42%), which were both significantly higher than that of plants fertilized with 20 kg ha⁻¹ (61.96%) (Figure 6.6).

Prolific

In the 2022/23 and 2023/24 seasons, there were no significant effects on the grain starch content or the main effects or the interactions thereof (Table 6.2).

Omar *et al.* (2022) found that the starch content increased significantly with nitrogen rates up to 100 kg N ha⁻¹ (72.43%); however, no further significant increases were achieved at rates above this level (150 kg N ha⁻¹, 72.43%). This confirms the results for the non-prolific cultivar,

where the plants treated with 0 kg N ha⁻¹ (106 kg ha⁻¹ total N) and 40 kg N ha⁻¹ (146 kg N ha⁻¹ total N) produced the highest starch content but without significant differences. Siam *et al.* (2008) found that increasing ammonia gas rates significantly increased grain starch content, with the highest starch content at 140 kg N ha⁻¹ (76.8%), compared to urea (68.2%) and ammonium sulphate (72.2%). Liu *et al.* (2022) found that higher nitrogen rates (100 and 200 kg N ha⁻¹) increased the starch content (71.69% and 73.18%) of maize kernels, which can also be found in this study's results.

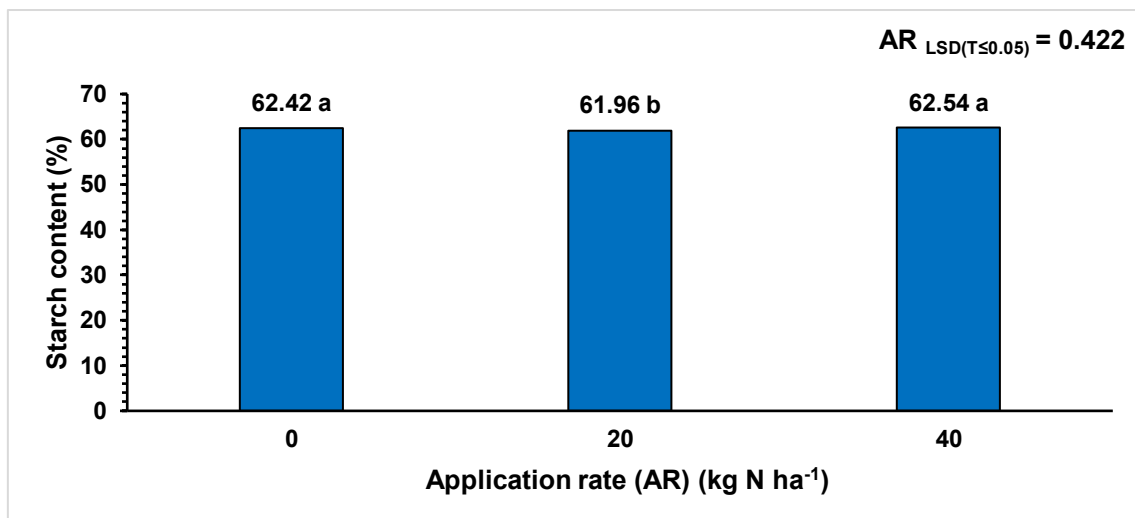


Figure 6.6 Starch content (%) as affected by application rate of the non-prolific cultivar in the 2023/24 season. Values with different letters differ significantly.

6.3.1.4 Fibre content

Grain fibre plays a significant role in grain nutrition. Research has shown that a high crude fibre content is associated with high nutritional composition in grain as well as high contents of crude fat, protein, and fibre (Kara *et al.*, 2022). Fibre is an important food product for human and animal consumption because it is rich in nutrition, easily digested and absorbed, and it has some health functions (Shungui, 2009). Kara *et al.* (2022) also found that factors such as weed control, irrigation, and fertilization could significantly impact the quality (crude fiber, crude ash, starch, Ca, Mg, and P) of maize forage.

Non-prolific

Season 2022/23

No significant differences were found as a result of the main effects or their interactions on grain fibre content.

Season 2023/24

The nitrogen application rate significantly influenced grain fibre content (Table 6.1). The nitrogen rates of 0 kg ha⁻¹ (2.54%) and 20 kg ha⁻¹ (2.56%) treated plants showed no significant differences in fibre content compared to the nitrogen rate of 40 kg ha⁻¹ (2.52%) treated plants. However, a topdressing application rate of 20 kg ha⁻¹ resulted in significantly higher grain fibre content than a rate of 40 kg ha⁻¹ (Figure 6.7).

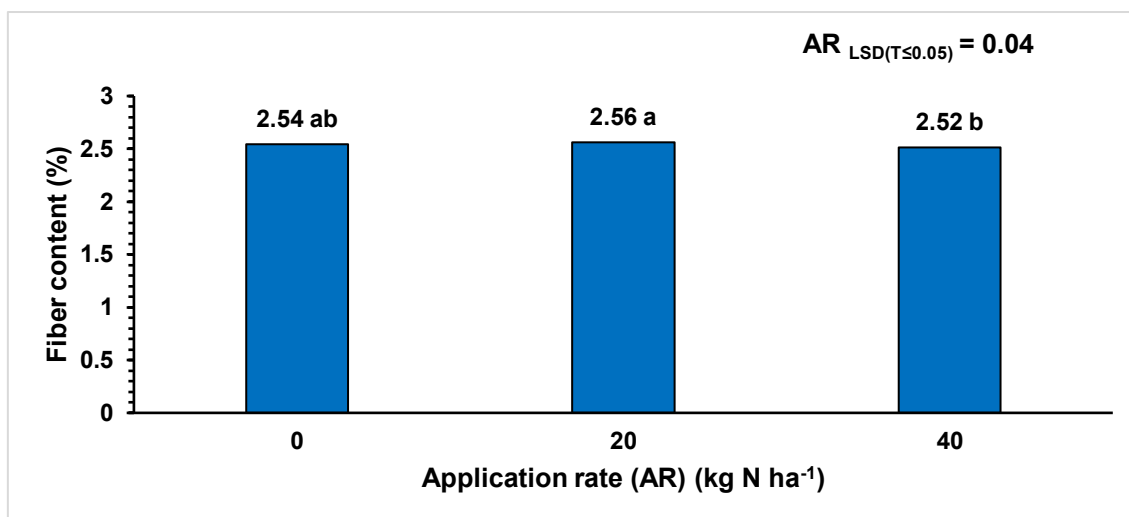


Figure 6.7 Fibre content (%) as affected by application rate for the 2023/24 season. Values with different letters differ significantly.

Prolific

There were no significant differences in the fibre content of the prolific cultivar in the 2022/23 and 2023/24 seasons due to the main effects or their interactions (Table 6.2).

Leite *et al.* (2021) and Bazitov *et al.* (2023) reported that the crude fibre content of grain was reduced with an increasing nitrogen rate. Almodares *et al.* (2009) also reported a decrease in crude fiber content when nitrogen rates were increased. These findings contradict the results found in this study for the non-prolific cultivar. Shehzad (2012) found that the crude fibre content of maize was increased with increasing nitrogen rates of up to 180 kg N ha⁻¹, which supports the findings in this study on the non-prolific cultivar.

6.3.1.5 Milling index (%)

According to the Southern African Grain Laboratory NPC (SAGL) (2017), the milling index indicates the milling ability and milling quality of maize kernels. The higher the milling index, the higher the extraction of high-grade products such as maize meal, samp, maize rice, and

maize grits manufactured from the kernel's endosperm. This makes it an important parameter to measure and determine what influence nitrogen fertilization practices have on the milling index.

Non-prolific

The grain milling index was not significantly affected in the 2022/23 season. In the 2023/24 season, the nitrogen application rate significantly impacted the grain milling index (Table 6.1).

No significant differences were observed in the milling index of plants that received no additional topdressing and that of plants that received either 20 or 40 kg N ha⁻¹, between the control plants (0 kg N ha⁻¹) (65.08%), plants fertilized with a nitrogen rate of 20 kg ha⁻¹ (64.78%) and a nitrogen rate of 40 kg ha⁻¹ (68.11%). A topdressing of 40 kg ha⁻¹ (68.11%) resulted in a significantly higher grain milling index than that of plants which received only 20 kg N ha⁻¹ (64.78%) as a topdressing (Figure 6.8).

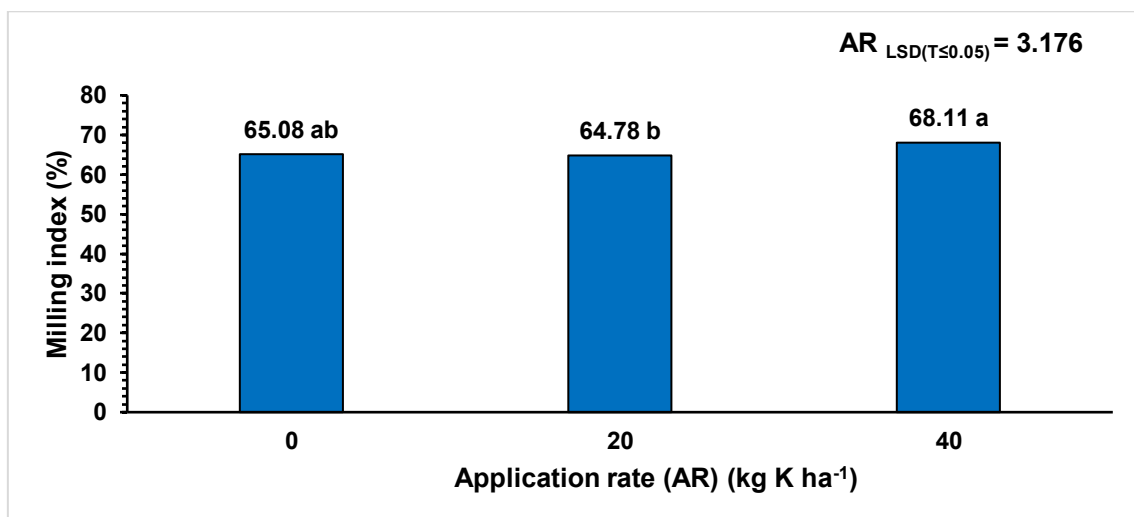


Figure 6.8 Milling index (%) as influenced by application rate for 2023/24 season. Values with different letters differ significantly.

Prolific

The milling index was not significantly influenced by the main effects of nitrogen source, application rate, and growth stage, or the interactions thereof, in the 2022/23 and 2023/24 seasons (Table 6.2).

Inspection of the data revealed that the milling index of plants that received no topdressing was generally higher than that of plants that received 20 or 40 kg N ha⁻¹ as a topdressing (Table 6.7).

Oikeh *et al.* (1998) reported that plants receiving a total nitrogen application of 30 to 60 kg ha⁻¹ produced a high-value dry milling quantity and concluded that cultivar and nitrogen rate influence milling quality. These results verify the finding that nitrogen application rate influences the milling index. No further supporting or contrasting results were found regarding the influence of nitrogen rate on the milling index which support the findings of this study.

Table 6.7 Milling index (%) as influenced by the nitrogen source, application rate, and growth stage applied for the prolific cultivar in the 2022/23 and 2023/24 season

2022/23 season					
Nitrogen source (NS)	Application rate (AR) (kg N ha ⁻¹)	Growth stages (GS)		AR average	
		V4	V9	V4 & V9	
Greensulf	0	71.56	72.13	71.08	71.59
	20	69.29	71.00	73.74	71.34
	40	65.75	69.39	71.58	68.90
GS Greensulf average		68.87	70.84	72.13	
LAN	0	72.30	71.81	68.53	70.88
	20	69.63	69.80	66.34	68.59
	40	66.39	71.75	69.39	69.18
GS LAN average		69.44	71.12	68.08	
GS average		69.15	70.98	70.11	
2023/24 Season					
Greensulf	0	60.51	59.51	60.66	60.23
	20	59.54	62.06	59.19	60.26
	40	63.73	57.86	61.41	61.00
GS Greensulf average		61.26	59.81	60.42	
LAN	0	62.51	62.24	66.29	63.68
	20	58.33	65.01	61.55	61.63
	40	60.60	57.85	58.50	58.98
GS LAN average		60.48	61.70	62.11	
GS Average		60.87	60.76	61.27	

NS *LSD* (*T*≤0.05) = *ns*

NS x AR *LSD* (*T*≤0.05) = *ns*

NS x AR x GS *LSD* (*T*≤0.05) = *ns*

AR *LSD* (*T*≤0.05) = *ns*

NS x GS *LSD* (*T*≤0.05) = *ns*

Values with different letters differ significantly.

GS *LSD* (*T*≤0.05) = *ns*

AR x GS *LSD* (*T*≤0.05) = *ns*

6.3.2 Grain nutrient content

The nutritional content of maize grain is essential information because it directly impacts the quality of the product consumed, whether as animal feed or for human use. Vital nutrients such as P, K, Mg, and Mn are essential for maize and are necessary for bone health, nerve function, and electrolyte balance. Therefore, this measurement is crucial for the consumer (Hamisu *et al.*, 2023). Interactions between nitrogen and macro- and micronutrients can lead to an increase (synergism) or decrease (antagonism) in uptake through plant metabolism and can be observed through these measurements (Maňásek *et al.*, 2012).

6.3.2.1 N content

Non-prolific

The N grain content was only significantly affected by the nitrogen source for the non-prolific cultivar in the 2022/23 season. No significant effects were found on the grain N content for the 2023/24 season (Table 6.12).

The grain N content was significantly higher (1.07%) when LAN was used compared to Greensulf (0.95%) in the 2022/23 season (Figure 6.9).

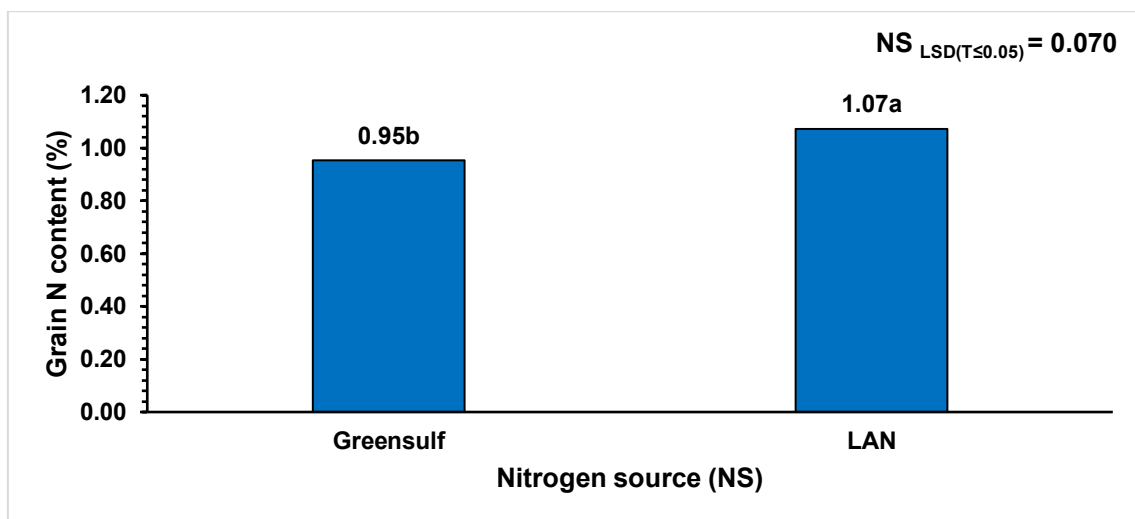


Figure 6.9 Grain N content (%) as affected by nitrogen source of a non-prolific cultivar for the 2022/23 season. Values with different letters differ significantly.

Grain nutrient summary of analysis

Table 6.8 Summary of analysis of variance indicating the effect of treatment factors on grain nutrients for a non-prolific maize cultivar

Treatments	S		N		P		K		Ca		Mg		Cu		Mn		Zn		Fe		B	
	2022/ 23	2023/ 24	2022/ 23	2023/ 24	2022/ 23	2023/ 24	2022/ 23	2023/ 24	2022/ 23	2023/ 24	2022/ 23	2023/ 24	2022/ 23	2023/ 24	2022/ 23	2023/ 24	2022/ 23	2023/ 24	2022/ 23	2023/ 24	2023/ 24	2023/ 24
Source (NS)	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	*	ns	ns	ns	ns
Application rate (AR)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Growth stage (GS)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns
NS X AR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
NS X GS	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
AR X GS	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
NS X AR X GS	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV%	19.97	8.52	20.55	14.26	13.01	10.53	20.13	10.32	19.89	31.73	10.67	10.19	22.27	28.53	10.33	19.44	8.29	12.38	19.17	19.56	17.36	9.07

ns – not significant

*P ≤ 0.05

CV – coefficient of variance

Table 6.9 Summary of analysis of variance indicating the effect of treatment factors on grain nutrients for a prolific maize cultivar

Treatments	S		N		P		K		Ca		Mg		Cu		Mn		Zn		Fe		B	
	2022/ 23	2023/ 24	2022/ 23	2023/ 24	2022/ 23	2023/ 24	2022/ 23	2023/ 24	2022/ 23	2023/ 24	2022/ 23	2023/ 24	2022/ 23	2023/ 24	2022/ 23	2023/ 24	2022/ 23	2023/ 24	2022/ 23	2023/ 24	2023/ 24	2023/ 24
Source (NS)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*
Application rate (AR)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Growth stage (GS)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
NS X AR	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
NS X GS	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
AR X GS	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
NS X AR X GS	ns	ns	ns	*	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
CV%	5.63	5.03	6.09	10.39	9.43	7.78	7.55	7.13	21.73	23.99	19.00	28.78	11.67	32.93	17.02	14.93	21.15	10.04	32.06	20.15	8.13	11.20

ns – not significant

*P ≤ 0.05

CV – coefficient of variance

Prolific

Season 2022/23

The grain N content had no significant effects on the main effects or interactions in the 2022/23 season (Table 6.9).

Season 2023/24

Grain N content was significantly influenced by the interactions between nitrogen source and application rate, the interaction between application rate and growth, as well as the nitrogen source, application rate and growth stage (the latter three-way interaction will not be discussed) (Table 6.9).

The plants that received Greensulf at 40 kg N ha⁻¹ topdressing had a significantly higher grain N content (0.91%) than the control plants (0.78%), with no further significant differences in N content observed among the remaining treatment interactions (Table 6.10).

Table 6.10 Grain N content (%) as influenced by the nitrogen source and application rate for the prolific cultivar in the 2023/24 season

Application rate (AR) (kg N ha ⁻¹)	Nitrogen source (NS)		AR average
	Greensulf	LAN	
0	0.78 ^b	0.84 ^{ab}	0.81
20	0.84 ^{ab}	0.83 ^{ab}	0.83
40	0.91 ^a	0.82 ^{ab}	0.87
Average	0.84	0.83	

$NS_{LSD (T \leq 0.05)} = ns$

$AR_{LSD (T \leq 0.05)} = ns$

$NS \times AR_{LSD (T \leq 0.05)} = 0.1050$

Values with different letters differ significantly.

Maize plants which received a nitrogen topdressing application rate of 40 kg ha⁻¹ as a split application at the V4 & V9 growth stages produced a significantly higher N grain content (0.93%) than the control plants of the V9 growth stage (0.76%), with no other significant differences in N for the remainder of treatment combinations (Table 6.11).

Table 6.11 Grain N content (%) as influenced by the nitrogen application rate and growth stage applied for the prolific cultivar in the 2023/24 season

Application rate (AR) (kg N ha ⁻¹)	Growth stage (GS)			AR average
	V4	V9	V4 & V9	
0	0.84 ^{ab}	0.76 ^b	0.82 ^{ab}	0.81
20	0.83 ^{ab}	0.86 ^{ab}	0.81 ^{ab}	0.83
40	0.85 ^{ab}	0.82 ^{ab}	0.93 ^a	0.87
Average	0.84	0.81	0.85	

$AR_{LSD (T \leq 0.05)} = ns$

$GS_{LSD (T \leq 0.05)} = ns$

$AR \times GS_{LSD (T \leq 0.05)} = 0.141$

Values with different letters differ significantly.

Biswas and Ma (2016) reported that the nitrogen sources used influenced the N content of kernels. These authors concluded that N content in grain was higher when plants were fertilized with CAN (calcium ammonium nitrate) than AN (ammonium nitrate) and urea sources. This finding correlates with the results from both cultivars of the current study, which showed that the nitrogen source influenced the kernel N content. The grain N content of the prolific cultivar increased with increasing nitrogen levels, a finding similar to those made by Mondal *et al.* (2023) and Rawal *et al.* (2024). These authors found that with high nitrogen levels (210 kg N ha⁻¹), the N content was higher than that of the control group with no nitrogen fertilizer applied. Siam *et al.* (2008) found that the interaction between nitrogen source and rate significantly affected the grain N content, with ammonium gas at 140 kg N ha⁻¹ resulting in the highest N content compared to the other nitrogen sources. This finding supports the results on the interaction between nitrogen sources (Greensulf and LAN) and application rates (106, 126, and 146 kg N ha⁻¹ total N) in the prolific cultivar.

6.3.2.2 P content

Non-prolific

No significant differences in the P content occurred as a result of the main effects (nitrogen source, application rate, and growth stage) or the interactions on the non-prolific cultivar in the 2022/23 or 2023/24 seasons (Table 6.8).

Prolific

The grain P content of the prolific cultivars was significantly affected only in the 2023/24 season by the interaction between the nitrogen source and growth stage (Table 6.9).

Inspection of the data revealed no significant differences in P content on account of the nitrogen source used. The only significant difference in P content was observed between single topdressing applications at V4 and V9 for Greensulf (Table 6.12).

Biswas and Ma (2016) reported that neither the nitrogen source nor the application rate influenced grain P content. This concurs with the results of this study, which found that the non-prolific cultivar showed no significant differences in grain P content across the two seasons. Siam *et al.* (2008) and Szule *et al.* (2017) reported differences between nitrogen sources, where ammonium nitrate resulted in a higher grain P content than the Canwil nitro-chalk source. Siam *et al.* (2008) reported that ammonia gas, as a nitrogen source, produced the highest grain P content, supporting the notion that nitrogen sources influence grain P content. Ciampitti *et al.* (2013) observed that nitrogen application at the early vegetative stage (V5) resulted in higher P content than applications made later in the vegetative stage (V10 to V15) or the reproductive stage (R1 to R3). Chen *et al.* (2016) found that grain P content was higher when nitrogen (180 kg N ha) was split into two applications: half applied before planting and the other half applied at the V6 growth stage. These results are consistent with those found for the prolific cultivar with nitrogen topdressing applications at the V4 stage.

Table 6.12 Grain P content (%) as influenced by the nitrogen source and growth stage applied for the prolific cultivar in the 2023/24 season

Growth stage (GS)	Nitrogen source (NS)		GS average
	Greensulf	LAN	
V4	0.39 ^a	0.37 ^{ab}	0.38
V9	0.35 ^b	0.38 ^{ab}	0.37
V4 & V9	0.37 ^{ab}	0.37 ^{ab}	0.37
Average	0.37	0.37	

NS_{LSD (T≤0.05)} = ns

GS_{LSD (T≤0.05)} = ns

NS x GS_{LSD (T≤0.05)} = 0.035

Values with different letters differ significantly.

6.3.2.3 Cu content (mg kg⁻¹)

Non-prolific

The Cu content in the grain of the non-prolific cultivar was not significantly influenced by the main effects of nitrogen source, application rate, and growth stage of the application, nor the interactions thereof in the 2022/23 and 2023/24 seasons (Table 6.8).

Prolific

The grain Cu content of the prolific cultivar was significantly influenced by the interaction between application rate and growth stage applied in the 2022/23 season, with no significant differences in the 2023/24 season (Table 6.9).

Although the P-value is 0.043, indicating significance, there were no significant differences between the means when the Tukey test was used to separate them. This indicates a strong tendency towards significance; however, the Tukey test is a stringent method for separating means, and as a result, no significant differences were detected (Table 6.13). The highest Cu content was produced with plants fertilized at a topdressing rate of 20 kg N ha⁻¹ (6.24 mg kg⁻¹) and when nitrogen was applied on the V9 growth stage (6.21 mg kg⁻¹).

Table 6.13 Grain Cu content (mg kg⁻¹) as influenced by the nitrogen application rate and growth stage applied for the prolific cultivar in the 2022/23 season

Application rate (AR) (kg N ha ⁻¹)	Growth stage (GS)			AR average
	V4	V9	V4 & V9	
0	6.38 ^a	6.21 ^a	5.71 ^a	6.10
20	6.46 ^a	6.07 ^a	6.18 ^a	6.24
40	5.69 ^a	6.34 ^a	6.51 ^a	6.18
Average	6.18	6.21	6.13	

AR $LSD (T \leq 0.05) = ns$

GS $LSD (T \leq 0.05) = ns$

AR x GS $LSD (T \leq 0.05) = 0.953$

Values with different letters differ significantly.

Bruns and Ebelhar (2006) and Liu *et al.* (2022) also found that Cu content in grain tends to increase slightly as nitrogen rates increase, but not significantly. These authors also concluded that this was more likely due to the increase in grain weight due to the additional nitrogen supply. Chen *et al.* (2016) reported that the timing of nitrogen application (before planting and at V6) influenced the Cu content of the grain, which supports the finding that the growth stage at which the application is made influences the Cu content of the grain.

6.3.2.4 Mn content (mg kg⁻¹)

Non-prolific

The Mn content of the non-prolific cultivar was significantly influenced by the nitrogen source in the 2022/23 season, but there were no significant effects in the 2023/24 season (Table 6.8).

The use of the Greensulf as a nitrogen source on maize plants resulted in a significantly higher (5.4%) Mn content in the grain (7.59 mg kg⁻¹) than that of the LAN nitrogen source (7.20 mg kg⁻¹) (Figure 6.10).

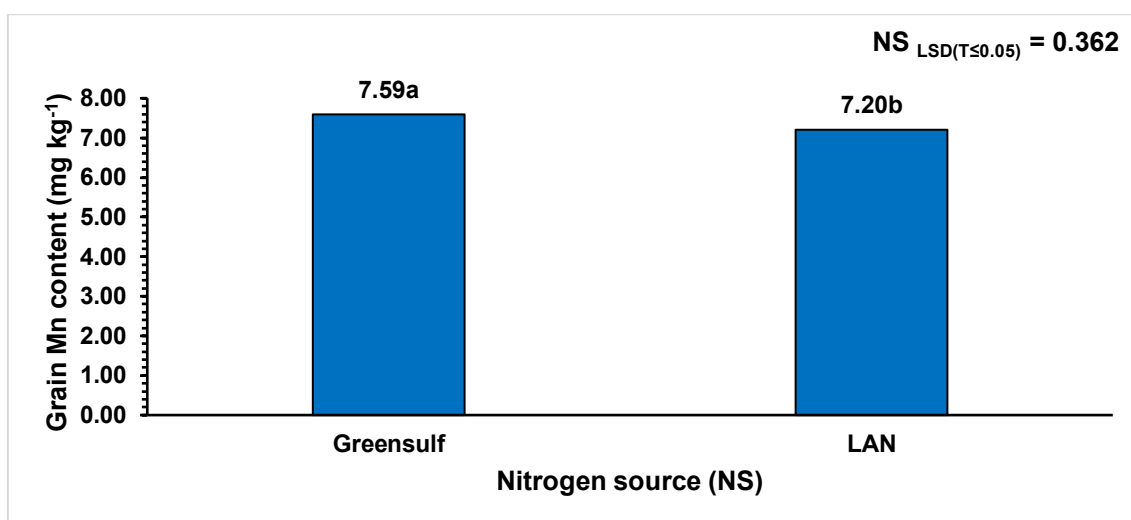


Figure 6.10 Effect of nitrogen source on the grain Mn content (mg kg⁻¹) for a non-prolific cultivar for the 2022/23 season. Values with different letters differ significantly.

Prolific

In the 2022/23 season, the grain Mn content of the prolific cultivar was significantly influenced by the growth stage at which nitrogen was applied (Table 6.9). No significant effects were observed in the 2023/24 season for the main effects or their interactions.

Season 2022/23

Plants that received a nitrogen topdressing application at the V9 growth stage had a significantly higher grain Mn content (9.21 mg kg⁻¹) than that of plants receiving the topdressing at the V4 growth stage (8.00 mg kg⁻¹). The plants fertilized in a split application showed no significant differences from the other growth stages (Figure 6.11).

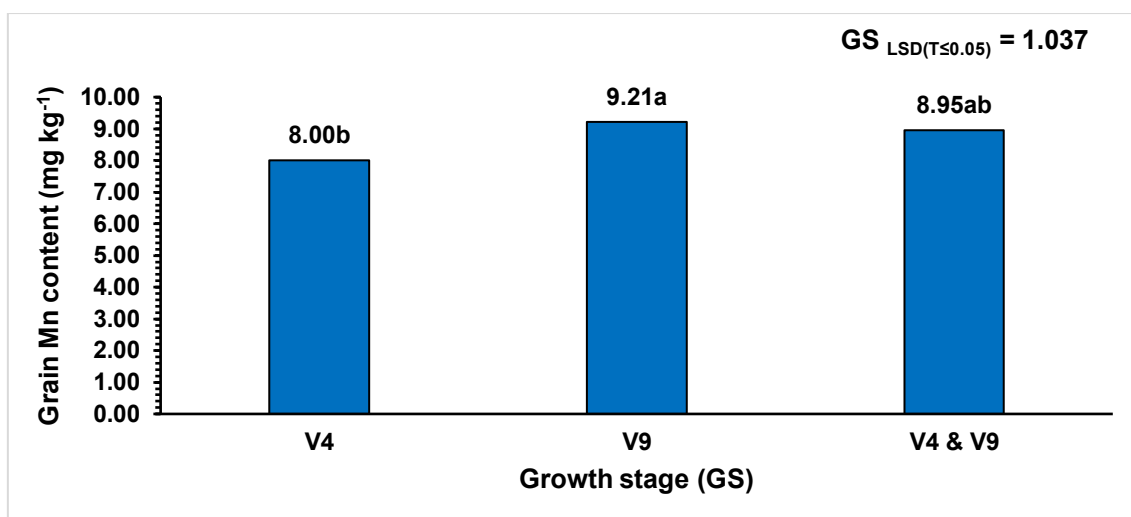


Figure 6.11 Effect of growth stage nitrogen applied on the grain Mn content (mg kg⁻¹) for a prolific cultivar in the 2022/23 season. Values with different letters differ significantly.

Both cultivars' non-prolific and prolific were significantly influenced by one of the main effects of the nitrogen source and growth stage applied. The nitrogen source significantly influenced the non-prolific cultivar, leading to a 0.39 mg kg⁻¹ higher Mn content in the grain. The growth stage of application significantly influenced the prolific cultivar, with an application at the V9 growth stage producing the highest Mn content in the grain.

Siam *et al.* (2008) reported that nitrogen sources had influenced grain Mn content, with ammonium sulphate being the most effective source for increasing Mn content compared to urea and ammonium gas sources. Wierzboska *et al.* (2021) found that the Mn content of grain was the highest when fertilized with UAN before planting and UAN+Mg topdressing (V4 to V6) than the other nitrogen sources used. These studies are consistent with the results for the non-prolific cultivar in the 2022/23 season, which indicated significant differences due to the nitrogen source used. Chen *et al.* (2016) reported that the timing of nitrogen application significantly influenced Mn content in grain when applied before planting and at the V9 growth stage, which concurs with the results of this study, indicating that application time influences grain Mn content.

6.3.2.5 Zn content (mg kg⁻¹)

Only the non-prolific cultivar showed a significant difference in grain Zn content. This cultivar was significantly influenced by the interaction between the nitrogen source and growth stage in the 2022/23 season and by the nitrogen source used in the 2023/24 season (Table 6.8).

Season 2022/23

No significant differences in the Zn content of plants fertilized with Greensulf applied at different stages of growth were obtained. LAN applied as a split application at growth stages V4 & V9 (24.05 mg kg⁻¹) accumulated the highest zinc content in the grain compared to applying LAN at V4 (21.37 mg kg⁻¹) or V9 (21.50 mg kg⁻¹) only (Table 6.14).

Table 6.14 Grain Zn content (mg kg⁻¹) as influenced by the nitrogen source and growth stage applied for the non-prolific cultivar in the 2022/23 season

Growth stage (GS)	Nitrogen source (NS)		GS average
	Greensulf	LAN	
V4	23.04 ^{ab}	21.37 ^b	22.21
V9	22.37 ^{ab}	21.50 ^b	21.94
V4 & V9	22.37 ^{ab}	24.05 ^a	23.21
Average	22.60	22.31	

NS_{LSD (T≤0.05)} = ns

GS_{LSD (T≤0.05)} = ns

NS x GS_{LSD (T≤0.05)} = 2.251

Values with different letters differ significantly.

In the 2023/24 season, plants fertilized with LAN (29.28 mg kg⁻¹) accumulated a significantly higher zinc content (9.55% more) compared to plants fertilized with Greensulf (26.61 mg kg⁻¹) (Figure 6.12).

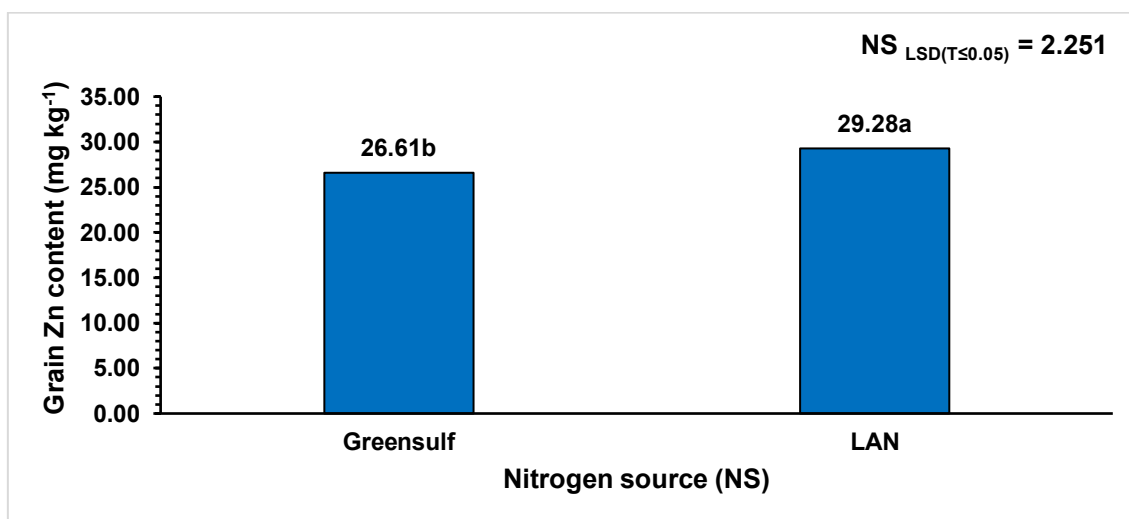


Figure 6.12 Effect of nitrogen source on the grain Zn content (mg kg⁻¹) for a non-prolific cultivar in the 2023/24 season. Values with different letters differ significantly.

According to Siam *et al.* (2008), ammonium sulphate was the most effective nitrogen source for increasing Zn content in grain compared to other nitrogen sources. Sarwar *et al.* (2012) found that nitrogen source significantly influenced the Zn content of maize grain and that the highest Zn content was obtained when urea was combined with organic nitrogen (75% urea and 25% organic ratio). This confirms and concurs with the findings of this study, which indicate that the nitrogen source influences grain Zn content. Unfortunately, these findings do not indicate the most effective source. Chen *et al.* (2016) reported that grain Zn content increased when nitrogen was applied to plants before planting and at the V6 stage. Zhao *et al.* (2022) reported that a split application of nitrogen during the vegetative and reproductive growth stages had a greater impact on grain Zn content than a single application at planting. The results of the authors above are consistent with the results found in this study for the non-prolific cultivar.

6.3.2.6 Fe content (mg kg⁻¹)

The Fe content was significantly influenced by the growth stage only for the non-prolific cultivar in the 2023/24 season, with no significant effects observed for the prolific cultivar in either season (Tables 6.8 and 6.9).

Non-prolific

Season 2023/24

Although the P-value is 0.0284, indicating significance, there were no significant differences between the means of the treatments (Figure 6.13). All that can be concluded is that plants fertilized in a split nitrogen application at V4 & V9 produced the highest Fe content in the grain, regardless of the source and the application rate, and that growth stage strongly tends towards significant differences.

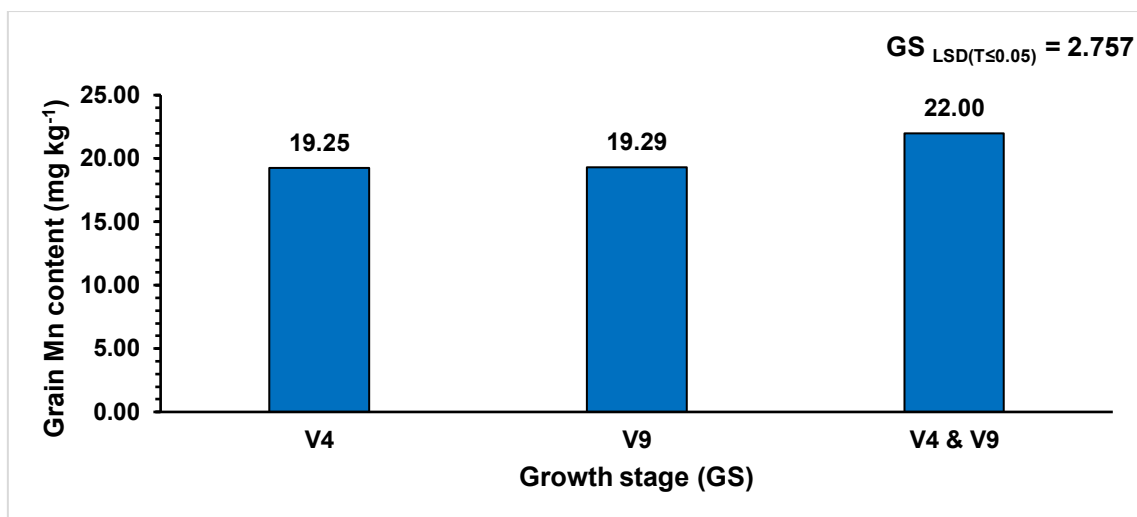


Figure 6.13 Effect of growth stage nitrogen applied on the grain Fe content (mg kg⁻¹) for a non-prolific cultivar for the 2023/24 season. Values with different letters differ significantly.

Zhao *et al.* (2022) found that Fe content in maize grain was higher when total nitrogen was applied at planting and the lowest when the nitrogen was split into three applications (at planting, early vegetative, and reproductive growth stage). Losak *et al.* (2011) found similar results to Zhao *et al.* (2022), with a higher Fe content, achieved through a single nitrogen application. This result contradicts the results of this study with the highest Fe grain content with a nitrogen topdressing split application at V4 & V9.

6.3.2.7 B content (mg kg⁻¹)

The prolific cultivar was the only cultivar that showed significant differences in grain B content with different nitrogen sources in the 2023/24 season (Tables 6.8 and 6.9).

Maize plants fertilized with Greensulf resulted in a significantly higher (5.95% higher) grain B content (13.36 mg kg⁻¹) than plants fertilized with LAN (12.61 mg kg⁻¹) (Figure 6.14). Wierzbowska *et al.* (2021) reported differences in boron grain content when using different nitrogen sources, with UAN+S/UAN+Mg (3.01 mg kg⁻¹) producing significantly higher B content than other (UAN/urea, UAN/UAN, UAN+P/UAN+P, UAN+P/UAN+S and UAN+P/UAN+Mg) nitrogen sources. These results are consistent with the findings of this study, which state that the nitrogen source affects the grain B content of maize.

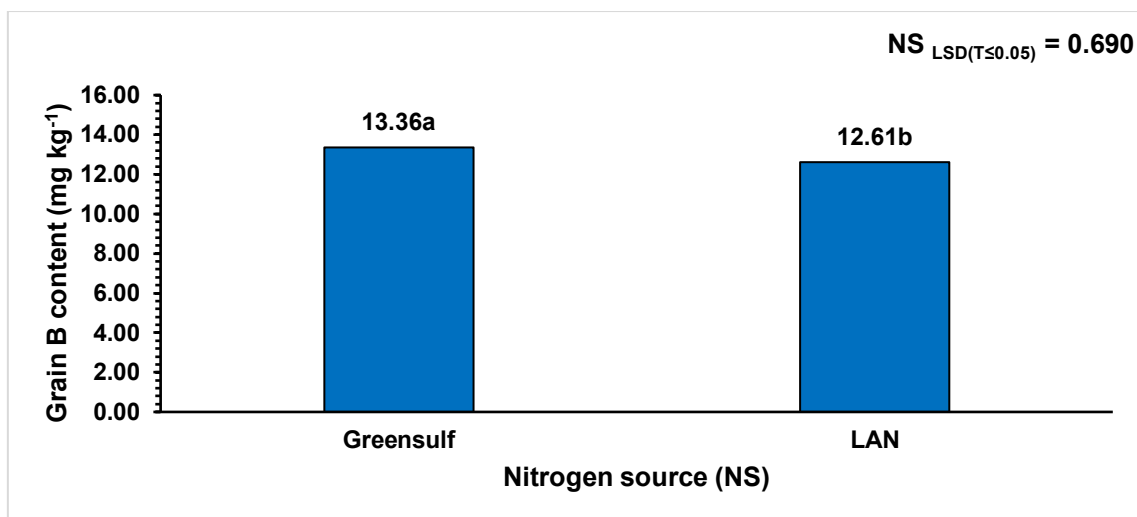


Figure 6.14 Effect of nitrogen source on the grain B content (mg kg⁻¹) for a prolific cultivar in the 2023/24 season. Values with different letters differ significantly.

Conclusion

In conclusion, the main effects of nitrogen source, topdressing application rate, and growth stage significantly affected maize grain quality and nutrient content. The interaction between the nitrogen source and the application rate significantly influenced the grain's protein, fat, N, and Zn contents, and the interaction between the nitrogen source and the growth stage applied significantly influenced the grain's N, P, and Zn contents. The nitrogen source had a significant effect on the grain Mn and B content. As a main effect, the application rate significantly affected the grain's starch, fibre content, and milling index. The growth stage significantly influenced grain protein and Mn content. In this study, nitrogen impacted grain quality and some grain nutrients. Unfortunately, the results show no strong tendencies, and it is inconclusive to recommend a specific source at a specific rate and time of application (growth stage). This is mainly attributed to an extreme climatic variation in the season.

References

- AAKASH, THANKUR, N.S., SINGH, M.K., BHAYAL, I., MEENA, K., CHOUDHARY, S.K., KUMAWAT, N., SHIGH, R.K., SINGH, U.P., SINGH, S.K., SANODIYA, P., KUMAR, A. & SINGH, A.K., 2022. Sustainability in Rainfed Maize (*Zea mays* L.) Production Using Choice of Corn Variety and Nitrogen Scheduling. *Sustainability*. 14: 3116.
- ADHIKARI, S., SCHOP, M., DE BOER, I.J.M. & HUPPERTZ, T., 2022. Protein Quality in Perspective: A Review of Protein Quality Metrics and Their Applications. *Nutr.* 14(5):947.
- ALMODARES, A., JAFARINIA, M. & HADI, M.R., 2009. The Effects of Nitrogen Fertilizer on Chemical Compositions in Corn and Sweet Sorghum. *Am-Euras. J. Agric. & Environ. Sci.* 6(4): 441-446.
- AMANULLAH, P. & SHAH, P., 2010. Timing and rate of nitrogen application influence grain quality and yield in maize planted at high and low densities. *J. Sci. Food Agric.* 90:21-29.
- BATHLA S, JAIDKA M AND KAUR R., 2020. Nutritive Value. Maize - Production and Use. IntechOpen. [Online]. Retrieved from <http://dx.doi.org/10.5772/intechopen.88963> (Accessed 23/09/2024).
- BAZITOV, R., MIHAYLOVA, M. & GANCHEV, G., 2023. Influence of nitrogen fertilization on the energy value of maize grain in non-ruminants. *Agric. Sci. Technol.* 15(2): 46-50.
- BISWAS, D.K & MA, B., 2016. Effect of nitrogen rate and fertilizer nitrogen source on physiology, yield, grain quality, and nitrogen use efficiency in corn. *Can. J. Plant Sci.* 96(3):392-403.
- BRUNS, H.A. & EBELHAR, M.W., 2006. Nutrient Uptake of Maize Affected by Nitrogen and Potassium Fertility in a Humid Subtropical Environment. *Commun. Soil Plant Sci. Anal.* 37:275-293.
- CHEN, Q., MU, X., CHEN, F., YUAN, L. & MI, G., 2016. Dynamic change of mineral nutrient content in different plant organs during the grain filling stage in maize grown under contrasting nitrogen supply. *Eur. J. Agron.* 80: 137-153.
- CIAMPITTI, I. A., CAMBERATO, J. J., MURRELL, S. T. & VYN, T. J., 2013. Maize Nutrient Accumulation and Partitioning in Response to Plant Density and Nitrogen Rate: I. Macronutrients. *Agron. J.* 105(3): 783-795.
- DEMARI, G.H., CARVALHO, I.R., NARDINO, M., SZAREKSKI, V.J., DELLAGOSTIN, S.M., CORAZZA DA ROSA, T., FOLLMANN, D.N., MONTEIRO, M.A., BASSO, C.J., PEDÓ, T., AMONDE, T.Z. & ZIMMER, P.D., 2016. Importance Of Nitrogen in Maize Production. *Int. J. Curr. Res.* 8(8): 36629-36634.

- ERTIRO, B. T., DAS, B., KOSGEI, T., TESFAYE, A. T., LABUSCHAGNE, M. T., WORKU, M., OLSEN, M. S., CHAIKAM, V. & GOWDA, M., 2022. Relationship between Grain Yield and Quality Traits under Optimum and Low-Nitrogen Stress Environments in Tropical Maize. *Agron*, 12(2), 438.
- HAMISU, A., MUHAMMAD, I., ULLAH, S. & DANLADI, G., 2023. Comparative study on the nutritional value of local maize variety (Hakorin Hajjiya) and improved varieties of maize (sammaz14 and golden strawberry). *Int. J. Botany Stud.* 8(6): 1-9.
- IDIKUT, L., ATALAY, A.I., KARA, S.N. & KAMALAK, A., 2009. Effect of Hybrid on Starch, Protein and Yields of Maize Grain. *J. Anim. Vet. Adv.* 8(10): 1945-1947.
- KAPLAN, M., KARAMAN, K., KARDES, Y. M. & KALE, H., 2019. Phytic acid content and starch properties of maize (*Zea mays* L.): Effects of irrigation process and nitrogen fertilizer. *Food Chem.* 283: 375-380.
- KARA, E., ILERI, O., ERKOVAN, S., SÜRMEEN, M., ERKOVAN, H.I. & KOÇ, A., 2022. Crude Fiber, Ether Extract, and Some Mineral Contents of the Corn Silage Grown at Different Weed Densities. *Turkjrf.* 3(1): 25-29.
- LEITE, R.G., CARDOSO, A.D., FONSECA, N.V.B., SILVA, M.L.C., TEDESCHI, L.O., DELEVATTI, L.M., RUGGIERI, A.C. & REIS, R.A., 2021. Effects of nitrogen fertilization on protein and carbohydrate fractions of Marandu palisadegrass. *Sci Rep.* 11: 14786.
- LINDIWE, M. & ERICK, S., 2024. Maize Grain Quality in Response to the Timing of Nitrogen Fertilizer Application and Environmental Variation. *Asian J. Plant Sci.* 23(1): 22-34.
- LIU, X., ZHANG, L., YU, Y., QIAN, C., LI, C., WEI, S., LI, C. & GU, W., 2022. Nitrogen and Chemical Control Management Improve Yield and Quality in High-Density Planting of Maize by Promoting Root-Bleeding Sap and Nutrient Absorption. *Front Plant Sci.* 13: 754232.
- LOSAK, T., HLUSEK, J., MARTINEC, J., SZOSTKOVA, M., FILIPCIK, R., MANASEK, J., PROKES, K., PETERKA, J., VARGA, L., DUCSAY, L., OROSZ, F. & MARTENSSON, A., 2011. Nitrogen fertilization does not affect micronutrient uptake in grain maize (*Zea mays* L.). *Acta Agric. Scand.- B Soil Plant Sci.* 61(6): 543-550.
- MAŇÁSEK, J., LOŠÁK, T., PROKEŠ, K., HLUŠEK, J., VÍTEŽOVÁ, M., ŠKARPA, P. & FILIPČÍK, R., 2012. Effect of nitrogen and potassium fertilization on micronutrient content in grain maize (*Zea mays* L.). *Acta Univ. Agric. Silvic. Mendel. Brun.* 61: 123-128.
- MARTINEZ, M.F., ARELOVICH, H.M. & WEHRHAHNE, L.N., 2010. Grain yield, nutrient content and lipid profile of oat genotypes grown in a semiarid environment. *Field Crops Res.* 116: 92-100.

- MONDAL, S., KUMAR, R., MISHARA, J.S., DASS, A., KUMAR, S., VIJAY, K.V., KUMARI, M., KHAN, S.R. & SINGH, V.K., 2023. Grain nitrogen content and productivity of rice and maize under variable doses of fertilizer nitrogen. *Heliyon*. 9(6):17321.
- MOTUKURI, S.R.K, 2019. Maize - Production and Use. Quality Protein Maize: An Alternative Food to Mitigate Protein Deficiency in Developing Countries. *IntechOpen*. pp: 1-12.
- OCHIENG, I., RANJA, S., SELEIMAN, M., PADHAN, S.R., CHEPTOEK, R., SOW, S., WASONGA, D. & GITARI, H., 2023. Increasing rainwater use efficiency, gross return, and grain protein of rain-fed maize under nitrate and urea nitrogen forms. *Not Bot Horti Agrobo*. 51(3): 13293.
- OIKEH, S.O., KLING, J.G. & OKORUWA, A.E., 1998. Nitrogen fertilizer management effects on maize grain quality in the West African moist Savanna. *Crop Sci*, 38: 1056-1061.
- OMAR, S., GHANI, R.A., KHAEM, H., SGHAIER, A.H. & JOLÁNKAI, M., 2022. The effect of nitrogen fertilisation on yield and quality of maize (*Zea mays* L.). *Acta Alimentaria*. 51(2):249-258.
- PARAGINSKI, R.T., VANIER, N.L., MOOMAND, K., DE OLIVEIRA, M., ZAVAREZE, E.R., SILVA, R.M., FERREIRA, C.D. & ELIAS, M.C., 2014. Characteristics of starch isolated from maize as a function of grain storage temperature. *Carbohydr. Polym*. 102: 88-94.
- RAWAL, N., VISTA, S., KHADKA, D. & PANERU, P., 2024. Grain Yield, Nitrogen Accumulation, and Its Use Efficiency of Maize (*Zea mays* L.) as Influenced by Varying Nitrogen Rates. *Int. J. Agron*. (1): 1-12. DOI: 10.1155/2024/4104123.
- SARWAR, M., JILANI, G., RAFIQUE, E., AKHTAR, M.E. & CHAUNDHRY, A.N., 2012. Impact of Integrated Nutrient Management on Yield and Nutrient Uptake by Maize under Rain-Fed Conditions. *Pak. J. Nutr*. 11(1): 27-33.
- SHEHZAD, M.A., MAQSOOD, M., BHATTI, M.A., AHMAD, W. & SHAHID, M.R., 2012. Effects of nitrogen fertilization rate and harvest time on maize (*Zea mays* L.) fodder yield and its quality attributes. *Asian J. Pharm. Biol. Res*. 2(1): 19-26.
- SHUNGUI, X.U., 2009. Raw fiber food and preparation method thereof. [Online]. Retrieved from <https://typeset.io/papers/raw-fiber-food-and-preparation-method-thereof-e24zhvujwu> (Accessed 25/09/2024).
- SHYNKARUK, L. & LYKHOCHVOR, V., 2021. Influence of fertilization and foliar feeding on maize grain qualitative indicators. *Ukr. J. Ecol*. 11(6):113-116.
- SIAM, H.S., ABD-EL-KADER, M.G. & EL-ALIA, H.I., 2008. Yield and Yield Components of Maize as Affected by Different Sources and Application Rates of Nitrogen Fertilizer. *Res. J. Agric. & Biol. Sci*. 4(5):399-412.
- Southern African Grain Laboratory NPC (SAGL), 2017. South African Maize Crop Quality Report 2016/2017 season. Pretoria: SAGL Associations.

- STĘPIEŃ, A., WOJTKOWIAK, K. & KOLANKOWSKA, E., 2024. Corn Grain Fatty Acid Contents in Response to Organic Fertilisers from Meat Industry Waste. *Sustain.* 16(3): 952.
- SUBEDI, K.D. & MA, B.L., 2005. Nitrogen uptake and partitioning in stay-green and leafy maize hybrids. *Crop Sci.*, 45: 740-747.
- SZULE, P., MICHALSKI, T., WALIGORA, H. & WILCZEWSKA, W., 2017. The dynamics of accumulation of N, P, K and Mg in the initial period of growth as a reaction of maize cultivars (*Zea mays* L.) On the method of the application of a nitrogen fertilizer. *Fresenius Environ. Bull.* 26(12): 7189-7198.
- WIERZBOWSKA, J., SIENKIEWICZ, S. & ŚWIATŁY, A., 2021. Content of micronutrients in grain and straw of common maize fertilized with urea-ammonium nitrate solution with added P, Mg or S. *J. Elem.* 26(4): 807-827.
- ZHAO, Q., CAO, W., CHEN, X., STOMPH, T. J. & ZOU, C., 2022. Global analysis of nitrogen fertilization effects on grain zinc and iron of major cereal crops. *Glob. Food Secur.* 33: 100631.

Chapter 7

Summary, conclusion and recommendations

7.1 Introduction

The largest cultivated and most important grain crop in South Africa is maize, followed by sunflower, wheat, and soybeans. Maize is mainly cultivated for human and animal consumption in the Southern Africa Customs Union (SACU) (Prospectus on the South African Maize Industry (PSAMI), 2021). Grain yield, biomass, number of kernel rows, number of kernels per row, and ear length are just a few of the yield-determining components of maize that are affected by nitrogen, which is widely acknowledged as a major yield-determining factor (Adhikari *et al.*, 2016). Increasing nitrogen fertilization to enhance yields has become standard practice in the north-eastern Free State. However, there is limited research on the most recent genetic material from this region regarding the source, application rate, and growth stage of different maize cultivars.

This study aimed to determine which nitrogen source, applied at what rate and at what growth stage, will affect maize response in the north-eastern Free State. The study aimed to determine the effect of nitrogen source and topdressing application rate, applied at specific growth stages, on: (i) leaf nutrient content, (ii) grain yield and yield components, and (iii) grain quality and return on investment.”

7.2 Leaf analysis

Regarding Chapter 4, the study evaluated the effects of nitrogen source, application rate, and growth stage on leaf nutrient content. Parameters included N, K, P, S, Mg, Cu, Zn, Fe, and B, and the N:S, N:K and Fe:Mn ratios of a non-prolific and prolific maize cultivar.

The study revealed that the application of Greensulf as a nitrogen source significantly impacted the non-prolific cultivar, with a leaf nitrogen content 2.73% higher than the LAN source. A topdressing application rate of 40 kg N ha⁻¹ produced the highest leaf nitrogen content in the 2022/23 season. The prolific cultivar also showed similar results, with the optimal application time for nitrogen application being at V9. The nitrogen source applied significantly affected the non-prolific cultivar, with plants receiving Greensulf producing 7.41% higher leaf P content than those treated with LAN. In the 2023/24 season, applying Greensulf at V9 and LAN at V4 resulted in the highest leaf P content for both sources (0.24%). The

prolific cultivar was significantly influenced by the interaction between the nitrogen source and the growth stage applied, resulting in a growth stage similar to that of the non-prolific cultivar. In the 2022/23 season, the non-prolific cultivar fertilized with Greensulf produced 4.57% higher leaf K content compared to plants fertilized with LAN. However, the increase was only significant compared to plants that received LAN at 40 kg N ha⁻¹. Additionally, plants that received the LAN at V4 produced the highest leaf K content, but this difference was only significant compared to the LAN at V9 and in a split application at V4 & V9.

The control plants of the non-prolific cultivar produced the highest leaf Ca content in the 2022/23 season. The non-prolific cultivar showed a significant increase (11.11% higher) in leaf S content when Greensulf was applied compared to plants receiving LAN. A 40 kg N ha⁻¹ application rate resulted in the highest leaf S content for the non-prolific cultivar during the 2022/23 season. However, applying LAN at V4 & V9 led to a significantly lower leaf S content in plants compared to other treatments. The prolific cultivar responded best to a LAN application at V4 (0.20%), resulting in the highest leaf S content. A 20 kg N ha⁻¹ application rate at V9 resulted in the highest leaf S content in the 2023/24 season. The nitrogen source had a significant impact on the leaf Mg content of the plants. Greensulf (0.16%) resulted in a higher leaf Mg content than LAN (0.15%). The application rate also affected Mg leaf content. A 40 kg N ha⁻¹ (0.144%) application resulted in higher leaf Mg content than a 20 kg N ha⁻¹ (0.131%) application. No significant effects were observed between the 0 kg N ha⁻¹ (0.141%) application and other treatments. The LAN source produced a significantly higher leaf Cu content (28.13%) than plants receiving the Greensulf on the non-prolific cultivar in the 2022/23 season. A 40 kg N ha⁻¹ application rate resulted in the treated plants' highest leaf Cu content. Both non-prolific and prolific cultivar plants had a significantly higher leaf B content when treated with Greensulf as a nitrogen source.

In the 2022/23 season, non-prolific cultivar plants fertilized with LAN showed a 6.32% higher leaf N:S ratio than those fertilized with Greensulf. The highest leaf N:S ratio was achieved with plants fertilized with LAN in a split application at V4 & V9 (22.70:1) and 0 kg N ha⁻¹ in a split application at V4 & V9 (20.79:1). Prolific plants fertilized with LAN in a split application at V4 & V9 (23.89:1) also produced the highest leaf N:S ratio. Non-prolific cultivar plants produced the highest leaf N: K ratio when fertilized at a rate of 40 kg N ha⁻¹ (1.72:1) and when the nitrogen was applied at the V9 growth stage (1.74:1). Plants fertilized with LAN nitrogen source produced a 54.55% higher leaf Fe: Mn ratio than those treated with Greensulf source in the 2022/23 season.

7.3 Yield components and yield

In Chapter 5, the impact of nitrogen source, application rate, growth stage, and interaction effects on the yield components and yield of a non-prolific and a prolific cultivar was assessed. The non-prolific cultivar showed the highest number of cobs per plant, with Greensulf producing the most when applied in a split application at V4 & V9. The prolific cultivar also showed a response when fertilized with Greensulf but was not consistent over the two seasons.

The number of kernels per plant for the non-prolific cultivar was significantly influenced by the interaction between nitrogen source and application rate, with LAN applied at 40 kg N ha⁻¹ producing 32.63% more kernels per plant than Greensulf at a rate of 40 kg N ha⁻¹. When Greensulf was applied at V9, it produced significantly more kernels (58.22 more kernels) than when it was applied at V4 on the prolific cultivar. The optimal nitrogen application rate was 40 kg ha⁻¹, producing 330.70 g of grain mass per plant, with no significant differences on the prolific cultivar. Application of Greensulf at V4 produced the highest grain mass per cob for the non-prolific cultivar in the 2022/23 season, and the Greensulf source produced 14.59% more grain mass per cob than the LAN source in the 2023/24 season. A nitrogen application rate of 40 kg ha⁻¹ for the non-prolific (397.37 g) and 20 kg ha⁻¹ for the prolific cultivar (281.65 g) produced the highest 1000-kernel weight. Application of nitrogen at V9 resulted in the highest 1000-kernel weight for the non-prolific cultivar, and the prolific cultivar fertilized with LAN source produced 7.78 g more per 1000 kernels. Hectolitre mass was not significantly affected by the main effects of the non-prolific or the prolific cultivar, possibly due to maize hybrids and climatic conditions.

Nitrogen source significantly impacted the grain yield of the non-prolific cultivar, with Greensulf resulting in 280 kg ha⁻¹ more grain than LAN in the 2022/23 season. The same trend was observed in the 2023/24 season, with slightly greater grain yield (1.77%) for Greensulf fertilized plants of the non-prolific cultivar. The nitrogen application rate also significantly affected the grain yield of prolific cultivars, although there were no significant effects in the 2023/24 season. The highest grain yield (10.90 tonne ha⁻¹) was achieved with a topdressing application rate of 20 kg ha⁻¹. Nitrogen use efficiency decreased with increasing nitrogen rates, while the nitrogen source only affected precipitation use efficiency (PUE). Non-prolific cultivar fertilised with Greensulf produced 2.84% more kg of grain per mm of rain than those fertilised with LAN. The prolific cultivar showed the highest PUE (26.72 kg mm⁻¹) with an application rate of 20 kg N ha⁻¹. A trend towards a decline in PUE was noted in the 2023/24 season, possibly due to climatic conditions. ROI was significantly influenced by the nitrogen application

rate in the 2023/24 season for both non-prolific and prolific cultivars, with a decrease in ROI with increasing nitrogen rates.

7.4 Grain quality and nutrient content

Regarding Chapter 6, it was found that the nitrogen application rate for maize plants significantly influenced the protein content and fat content during the 2022/23 season. The protein content decreased with an increase in application rate, except for the non-prolific cultivar fertilized with 20 kg N ha⁻¹. The interaction between nitrogen source and application rate significantly influenced the grain protein content, with control treatments having a higher protein content. The fat content of maize plants was also significantly influenced by the nitrogen application rate, with plants top-dressed with 40 kg N ha⁻¹ having a higher fat content than that of control plants. The prolific cultivar was only significantly influenced by the nitrogen application rate, with plants receiving a nitrogen application rate of 20 kg ha⁻¹ resulting in a significantly higher fat content. The highest starch content was observed at 40 kg ha⁻¹ (62.54%), followed by 0 kg ha⁻¹ (62.42%) and 20 kg ha⁻¹ (61.96%). The nitrogen application rate significantly impacted grain fibre content, with a topdressing application rate of 20 kg ha⁻¹ resulting in substantially higher grain fibre content. The milling index was not significantly influenced by the nitrogen source, application rate, or growth stage applied; however, the milling index of plants without topdressing was generally higher.

Plants receiving LAN were found to have a higher grain nitrogen content than Greensulf-treated plants (0.95%). Greensulf-treated plants had a higher grain nitrogen content (0.91%) than control plants (0.78%), while maize plants with a 40 kg N ha⁻¹ nitrogen split application rate at the V4 & V9 growth stages had a higher grain nitrogen content (0.93%). The study revealed no significant differences in P content as influenced by the main factors, namely nitrogen source, application rate, and growth stage, on a non-prolific cultivar in the 2022/23 or 2023/24 seasons. However, the interaction between nitrogen source and growth stage in the 2023/24 season significantly affected the grain P content of a prolific cultivar.

The highest Cu content was produced with plants fertilized at a topdressing rate of 20 kg N ha⁻¹ and nitrogen applied at the V9 growth stage. The nitrogen source and growth stage significantly influenced the Mn content of maize plants in the 2022/23 and 2023/24 seasons. Greensulf significantly increased the Mn content in the non-prolific cultivar by 5.4% compared to the LAN nitrogen source. Growth stage also significantly influenced the Mn content of the prolific cultivar. The study concluded that plants fertilized with a split nitrogen application at V4 & V9 produced the highest Fe content in the grain, regardless of the source and application

rate. In the 2023/24 season, maize plants fertilized with Greensulf showed significantly higher grain B content (5.95% higher) than those fertilized with LAN (12.61 mg kg⁻¹).

7.5 Conclusion and Recommendations

In conclusion, the study demonstrated the significant impact of nitrogen source, application rate, and growth stage on the leaf nutrient content, yield component, and grain yield of maize plants in both the 2022/23 and 2023/24 seasons. The non-prolific cultivar exhibited notable increases in leaf N, P, S, Mg, and Cu content when treated with Greensulf, whereas LAN application resulted in higher leaf Ca content. The prolific cultivar was also influenced by the nitrogen source and growth stage, with LAN application at V4 producing the highest leaf S content and LAN leading to higher leaf Cu and B contents. Furthermore, the study revealed that Greensulf applications resulted in higher grain N content and yield compared to LAN, while LAN application at a rate of 20 kg ha⁻¹ produced the grain with the highest fat and starch content. The findings also highlighted the intricate interactions between nitrogen source, application rate, and growth stage on yield components. Greensulf showed superior cob production for the non-prolific cultivar and LAN, resulting in a higher 1000-kernel weight. Overall, the findings emphasized the complex interactions between nitrogen source, application rate, and growth stage that affect yield components. Greensulf proved particularly effective for cob production in the non-prolific cultivar, resulting in a higher 1000-kernel weight. This research stresses the importance of selecting specific nitrogen sources, application rates, and growth stages to optimize the nutritional content and yield of maize crops, offering valuable insights for agricultural practices and future research initiatives.

Based on the results from trials with a non-prolific and a prolific cultivar in the north-eastern Free State, the following recommendations are made:

- Use Greensulf nitrogen fertilizer for improved leaf and grain nutrient content for both non-prolific and prolific cultivars.
- The non-prolific cultivar responded best with an additional 40 kg N ha⁻¹ (for a total of 146 kg N ha⁻¹), while the prolific cultivar performed best with an additional 20 kg N ha⁻¹ (for a total of 126 kg N ha⁻¹).
- Apply nitrogen topdressing for the non-prolific cultivar at the V9 growth stage and use a split application at V4 & V9 for the prolific cultivar.
- A 106 kg N ha⁻¹ fertilization rate yields the most cost-effective maize production for both cultivars.

References

- ADHIKARI, P., BARAL, B.R. & SHRESTHA, J., 2016. Maize response to time of nitrogen application and planting seasons. *JMRD*. 2(1): 83-93.
- PROSPECTUS ON THE SOUTH AFRICAN MAIZE INDUSTRY (PSAMI), 2021. Description on South African Maize supplied by PSAMI. Pretoria: The Maize Trust.