# The effects of irrigation and nitrogen on productivity, marketable yield and $^1$H-NMR based metabolic profiling of African nightshade *(Solanum retroflexum)*

By

MASEMOLA MAKHUTSE CLIVE

Submitted in accordance with the requirements for the degree
Of

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UNIVERSITY OF SOUTH AFRICA

SUPERVISOR: PROFESSOR GERHARD PRINSLOO

CO-SUPERVISOR: DR MELAKE FESSEHAZION

# STUDENT DECLARATION

**The effects of irrigation and nitrogen on productivity, marketable yield and $^1$H-NMR based metabolic profiling of African nightshade *(Solanum retroflexum)*.**

I, Masemola M.C, student number: 54922488 declare that:

(i) The research reported in this dissertation, except where otherwise indicated, is the result of my own endeavours in the College of Agriculture and Environmental Sciences, Department of Agriculture and Animal Health, UNIVERSITY OF SOUTH AFRICA, with the assistance of the Agricultural Research Council: Vegetable and Ornamental Plant Campus, Crop Science department, Indigenous Research Team;

(ii) This dissertation has not been submitted for any degrees or examination at any other university;

(iii) This dissertation does not contain data, figures or writing, unless specifically acknowledged, copied from other researchers; and

(iv) Where I have produced a publication of which I am an author or co-author, I have indicated which part of the publication was contributed by me.

Signed at…PRETORIA………..on the..14…. day

of……OCTOBER…………… 2018.

# DECLARATION BY SUPERVISORS

We hereby declare that we acted as supervisors for this MSc student:

**Student's Full Name:** Masemola Makhutse Clive
**Student number:** 54922488

**Dissertation Title: The effects of irrigation and nitrogen on productivity, marketable yield and $^{1}$H-NMR based metabolic profiling of African nightshade *(Solanum retroflexum)*.**

Regular consultation took place between the student and us throughout the investigation. We advised the student to the best of our ability and approved the final document for submission to the College of Agriculture and Environmental Sciences, Department of Agriculture and Animal Health, for examination by the university appointed examiners.

**SUPERVISOR:** Dr Gerhard Prinsloo……………………………Date……………………..

**CO-SUPERVISOR:** Dr Melake Fessehazion …………………… Date……………………..

# DECLARATION BY EDITOR

We hereby declare that we edited the work for this MSc student:

**Student's Full Name:** Masemola Makhutse Clive

**Student number:** 54922488

**Dissertation Title: The effects of irrigation and nitrogen on productivity, marketable yield and $^{1}$H-NMR based metabolic profiling of African nightshade *(Solanum retroflexum)*.**

Signed………… MASEMOLA M.C ……………………………

## COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCES

## DECLARATION 1 - PLAGIARISM

I, Masemola Makhutse Clive, student number, 54922488, declare that **The effects of irrigation and nitrogen on productivity, marketable yield and $^{1}$H-NMR based metabolic profiling of African nightshade *(Solanum retroflexum)*.** (Title of Dissertation) is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references. I further declare that I have not previously submitted this work, or part of it, for examination at Unisa for another qualification or at any other higher education institution.

Signed………MASEMOLA M.C………………………………

# COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCES

## DECLARATION 2- PUBLICATION

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part of research presented in this thesis (include publications in preparation, submitted, *in press,* published and details of the contributions to the experimental work and writing of each publication).

### Publication 1

**Effect of irrigation and nitrogen supply on canopy development, marketable yield and free proline content in African nightshade (*Solanum retroflexum*).**

M.C. Masemola [1], R. Laurie [1.*] G. Prinsloo [2], Fessehazion M.K[1], S.L & C. P Du Plooy [1]

[a] Agricultural Research Council- Vegetable and Ornamental Plants, Private Bag X293, South Africa, Pretoria, 0001

Private Bag X293, Pretoria, South Africa, 0001 Tel. +27 12 808 8000, Fax +27 12 808 6326

[b] College of Agriculture and Environmental Sciences, University of South Africa, Private Bag X6, South Africa, Johannesburg, 1710

Correspondence: *R. N. Laurie, Email: Prinsg@unisa.ac.za

Targeted Journal: Canadian Journal of Agricultural Science.

Status: Currently under review at the ARC: Publication review committee.

### Publication 2

**To determine the effect of irrigation, nitrogen management on $^{1}$H-NMR based metabolomic profiling, compound annotation and nutritional water productivity of African nightshade (*Solanum retroflexum*).**

M.C. Masemola[a,b], G. Prinsloo [b], M.K Fessehazion [a], H.T Araya [a] and C. P Du Plooy[a]

[a] Agricultural Research Council- Vegetable and Ornamental Plants, Private Bag X293, South Africa, Pretoria, 0001

Private Bag X293, Pretoria, South Africa, 0001 Tel. +27 12 808 8000, Fax +27 12 808 6326

[b] College of Agriculture and Environmental Sciences, University of South Africa, Private Bag X6, South Africa, Johannesburg, 1710

Correspondence: *M.C. Masemola MasemolaMC@hotmail.com

Status: Manuscript in preparation.

# CONFERENCE CONTRIBUTIONS

## Poster 1

Exhibited one poster during an exhibition by the GCARD in April 2016, at ARC - VOP. Agricultural Research Council-Vegetable Ornamental Plant Campus, Council for Science.

**ARC:VOP campus, CSIR and DST curbing malnutrition through cost effective irrigation strategies & management practices.**

[a]Agricultural Research Council - Roodeplaat, Vegetable and Ornamental Plant, Pretoria, South Africa.

[b]College of Agriculture and Environmental Sciences, University of South Africa, Johannesburg, South Africa.

Contact M.C. Masemola MasemolaMC[illegible]otmail.com , ARC, Roodeplaat-Vegetable and Plant and Ornamental Campus

## Presentation1

**Biomass yield and chemical analysis of African nightshade *(Solanum retroflexum)* as affected by nitrogen management.**

M.C Masemola, G. Prinsloo M.K Fessehazion & CP Du Plooy

Department of Agriculture and Animal Health, University of South Africa, Private
Bag X6, Florida, 1710, South Africa

Annual conference of the South African Association of Botanists. University of Free State, Bloemfontein, 1-15 January 2016

## Presentation 2

Agricultural Research Council: VOP campus Indigenous Research Forum

**$^1$H-NMR based metabolomic profiling and compound annotation of African nightshade (*Solanum retroflexum*) to determine the effect of irrigation, nitrogen management and shoot harvests**

[a]Agricultural Research Council - Roodeplaat, Vegetable and Ornamental Plant, Pretoria, South Africa.

[b]College of Agriculture and Environmental Sciences, University of South Africa, Johannesburg, South Africa.

Contact M.C. Masemola MasemolaMC✉otmail.com , ARC, Roodeplaat-Vegetable and Plant and Ornamental Campus.

## Workshops 1

Attended a workshop on biostatistics and NMR workshop at UNISA presented by Dr Gerhard Prinsloo & Professor Vervoort Jacques from Wageningen University in the Netherlands presented at the University of Free State 10-15 January 2016. Attended a workshop in Chemometrics at UNISA 07-11 March 2016.

## Workshops 2

Coping with Drought - Adaptations to water stress in the face of climate change, held between 18 and 19 April 2016, at Blandford Manor in Fourways, Johannesburg.

## PROJECT TEAM

### ARC-Institute for Ornamental and Vegetable Crops Campus

Mr M.C Masemola
Dr R.N Laurie
Dr M.K Fessehazion
Dr C.P du Plooy

### University of South Africa, Johannesburg, South Africa. College of Agriculture and Environmental Sciences

Dr. G Prinsloo

# EXECUTIVE SUMMARY

South Africa is currently facing three agricultural cross-related challenges, namely an ever-increasing human population growth, irrigation water scarcity and nutritional insecurity, while micronutrients of importance are magnesium (Mg), zinc (Zn) and iron (Fe) which are required for malnutrition alleviation, especially in resource poor households. Fresh produced vegetables and fruits are required to be increased and are being cultivated under environmental constraints such as climate change (flooding and droughts). In South Africa, poor and unemployed people mostly live in rural communities, informal urban settlements and on farms, with women, children and the elderly being the most affected group vulnerable to malnutrition, due to lack of capital and agricultural inputs. Therefore, the Food Agriculture Organization (2010), World Health Organization (1999) and the national agricultural industry including private sector needs to re-visit policy on sustainable projects based on curbing micronutrient deficiency, especially in sub-Saharan Africa (sSA). African leafy vegetables (ALV's) such as African nightshade *Solanum retroflexum* Dunal is highly nutritious in Mg, Fe and Zn and is considered mild drought tolerant, when compared to exotic leafy vegetables such as Swiss chard and cabbage. Meanwhile, metabolite profiling with $^1$H-Nuclear Magnetic Resonance (NMR) analysis is a proven analytical technique for chemical analysis, enabling us to detect diverse groups of secondary metabolites and abundant primary metabolites in various plant species. This research therefore provides a better understanding of the relationship between management practices, such as irrigation and nitrogen (N) application on the effect on the marketable yield, nutritional water productivity and the metabolite profile of *S. retroflexum*.

## Project aim and objectives

The main aim of the project was to provide a scientific quantification on the effects of irrigation and N application on nutritional water productivity, nutritional content (Mg, Fe and Zn), marketable yield and $^1$H-NMR based metabolic profile of African nightshade (*S. retroflexum*) under a rainshelter.

## Methodology

The experiment was conducted under a rain-shelter at the Agricultural Research Council-Vegetable and Ornamental Plants (ARC-VOP campus). The rain-shelter experimental design was a randomized complete block design, while the treatment design were a 4 x 3 factorial with two treatments, namely N and irrigation treatments, replicated three times. Nitrogen was applied as pre-plant application and three equal top dressings after each shoot harvest. Soil moisture was monitored on a weekly basis using a neutron probe, to determine the soil water content at each soil depth. The 33%FC treatment (irrigated to 33% of field capacity) was referred to as the stressed irrigation control, while the 66%FC treatment (irrigated to 66% of field capacity) was regarded as the moderate irrigation treatment and 100%FC treatment (irrigated to 100% field capacity) was the optimum irrigation treatment, as described by Beletse et al. (2012). Leaf samples were collected at three-week intervals and were done early in the morning, as recommended by Van Averbeke et al. (2007), placed in a cooler box to prevent them from wilting, and then dried in an oven. Dried leaf samples were analysed for Mg, Fe and Zn content. After oven drying of the leaf samples, they were grinded through a 1 mm sieve and prepared using a direct extraction method for metabolomic analysis. A 600 MHz NMR spectrometer with a proton frequency of 599.74 was utilized for an untargeted metabolomic approach for metabolic compound profiling. Water productivity (WP) was calculated as the yield of total dry raw edible biomass per unit of irrigation water applied. Nutritional water productivity (NWP) was calculated from WP x nutrient content of the crops. A two-way analysis of variance (ANOVA) was conducted to evaluate the main effects of the individual factors (N and irrigation levels) and their interactions in terms of biomass, irrigation use efficiency (IUE) and NWP of African nightshade (*S. retroflexum*).

## Summary and conclusions

Main findings of the study indicate that *S. retroflexum* is highly nutritious in Fe and Zn, which play a significant role in malnutrition alleviation and metabolic compounds that might assist the crop to survive drought conditions have been annotated. The highest nutrient contents for Fe and Zn were obtained from the moderately and optimum irrigation treatment, whereas Mg was not affected by the N or irrigation rate. Although, the less irrigated treatments (I33%FC) resulted in lower yields caused by water stress, it lead to high evapotranspirative demand, resulting to be the main causes of poor performance of *S. retroflexum* in the stressed treatments as compared to the well irrigated treatment (I66 and I100%FC), illustrating that increased N and water application lead to increased marketable yields.

$^1$H-NMR based untargeted metabolomic profiling was conducted, with orthogonal partial least square discriminatory analysis (OPLS-DA) performed using $^1$H-NMR spectra data. The analysis revealed clear separation between I33%FC and I66% FC. There was no separation of the samples regarding the N treatments, which is quite indicative of the small effect of N on the metabolite content of the respective treatments. Chlorogenic acid, proline, sucrose and trigonelline were responsible and associated with separation in the irrigation treatments. *Solanum retroflexum*, therefore can be ideal for cropping systems by smallholder and commercial farmers in water scarce areas such as South Africa.

## Recommendations for further research

The best management practice in a production system through adaptive agronomic mechanisms and the promotion of *S. retroflexum* with low water and nitrogen requirements for smallholder farmer’s gardens can improve the nutritional status of rural communities. These results will be utilized to generate farmer guidelines and manuals on subsistence and commercial production of *S. retroflexum*.

Further research work is required on water-driven and carbon-driven biomass production based crop models to simulate marketable yield under different agro-climatic zones, which could therefore be utilized for drought screening and simulation of marketable yield of different African nightshade species with different N and irrigation application rates for future cultivation purposes and commercialization of this crop.

# ABSTRACT

Efficient agronomic practices are vital for achieving sustainable management of water resources and N for producing highly nutritious leafy vegetables to curb malnutrition and poverty. The importance of proper N and irrigation of sustainable crop production is well recognised in literature, although irrigation and N application rate guidelines for ALV's might not be sufficient for advisory purposes, especially for smallholder and commercial farmers. The limited access is attributed by factors such as the lack of commercialisation as a result of limited agronomic information describing optimum management options for *S. retroflexum*. Availability of such information would contribute to successful commercialisation of this crop. The primary objective of this study was to establish optimum agronomic management practices for *S. retroflexum* for smallholder farmer as well as commercial production in South Africa.

This project consists of three main components with the overall objective to evaluate agronomic management practices of irrigation and N application on the marketable yield, nutritional water productivity and $^{1}$H-NMR metabolic profiling under a rainshelter. The results suggested that *S. retroflexum* responded positively to N application rates until an optimum marketable yield was obtained at 150 kg N·ha$^{-1}$ with I66%FC, followed by the I100%FC with 150 kg N·ha$^{-1}$,while, the lowest biomass was recorded in the I33%FC irrespective of the N application rate. This also indicates of how *S. retroflexum* is affected by insufficient irrigation, even at sufficient N application rates. N and irrigation are key factors limiting plant survival and growth and low applications has adverse effects on the marketable yield of *S. retroflexum*. Dry matter production increased with the higher N application and a linear increase was observed with N application having a significant effect on the dry matter production.

Maximum irrigation use efficiency (IUE), was obtained in the moderately irrigated treatment (I66%FC) and the 150 kg N·ha$^{\mathbf{-1}}$, followed by the I100%FC and 150 kg N·ha$^{\mathbf{-1}}$. The stressed irrigation and N treatments showed sustainably low irrigation use efficiency as compared to the well irrigated treatment. This suggests the competitive capacity of *S. retroflexum* roots to draw water from deeper parts of the soil profile, during stressed conditions to maintain the turgor pressure, indicating that production of *S. retroflexum* is possible in arid areas where

water could be a limiting input, but might not be profitable for farmers. However, the biomass yields obtained under the less irrigated soil water conditions may lack the quality needed to market the produce.

The NWP for Zn and Fe showed significant differences among the irrigation and N treatments. Although the NWP of Mg in *S. retroflexum* was neither influenced by irrigation nor N application, with no statistical differences between the irrigation and N application observed. Among the different irrigation and N treatments evaluated, I33%FC obtained the lowest NWP irrespective of the N application rate, followed by I100%FC. The NWP increased linearly with an increase in N application. Optimum Fe NWP was obtained with 150 kg N ha$^{-1}$, but declined significantly at application 225 kg N ha$^{-1}$ in I33%FC and I100%FC. Maximum Fe NWP was obtained with I66%FC with 150 kg N ha$^{-1}$. The NWP Fe for *S. retroflexum* in this study, are acceptable when compared to those obtained in literature. Therefore a significant interaction between N and irrigation application exist, with respect to Fe and Zn availability of *S. retroflexum*, which could be ideal for Fe and Zn malnutrition alleviation in resource poor households.

$^{1}$H-NMR based metabolomic profiling was utilized for compound annotation as effected by irrigation and N. Chlorogenic acid, proline, sucrose and trigonelline were associated with separation in the irrigation treatments. Since no separation of the irrigation or N treatments was observed with the PCA, an OPLS-DA plot was constructed. A pairwise comparison of the I33%FC and I100%FC samples was done, which provided better separation between the clusters for the first harvest. Even better separations were observed with a pairwise OPLS-DA analysis of the I33%FC and I100%FC samples for the second harvest. Primary metabolites such as sucrose, and proline and secondary metabolites trigonelline and chlorogenic acid were responsible for grouping of the stressed irrigation treatment as compared to the well irrigated treatment. Main findings of the study suggest that *S. retroflexum* is highly nutritious in Fe, Zn and Mg, which might play a significant role in malnutrition alleviation. *Solanum retroflexum* requires sufficient soil water content, for achieving high nutrient yield and nutritional water productivity.

The results clearly illustrate that the perception that *S. retroflexum* grow well on low soil fertility mostly practised in rural and smallholder farming is incorrect and misleading. Moreover, *S. retroflexum* can be an ideal cropping system for smallholder and commercial farmers in water scarce areas such as South Africa, although marketable yield is severely affected.

# ACKNOWLEDGEMENTS

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I express my sincerest gratitude to the Crop Science Research Team Manager, Dr. C. P. Du Plooy and Dr. Robert Laurie Eco-physiologist and Biotechnologist from the Crop Science Department for the time that they set aside to visit the trial and to review some of the paper and the great ideas they shared with me during my research meetings. I would also like to acknowledge Ms Liesl Morey from the ARC-Biometry Unit for the statistical analysis. It was a pleasure working with the members of the Agricultural Research Council (VOP Campus) in particular the following colleagues: Hlamarisa Baloyi, Israel Nechipale and Derick Mosehla and Small Mashabela for their technical assistance during data collection. We also acknowledge the CSIR Bioscience Department for allowing me to use the $^1$H-NMR equipment.

My sincere gratitude goes to Dr Hintsa Araya, Senior Research and Research Team Leader, for African Vegetables, for his feedback with regard to statistical input and scientific editing of my research work, especially Chapter 4 Nutritional water productivity of African nightshade.

# DEDICATION

Being a product of many hands in all walks of life, I dedicate this dissertation to my family and the colleagues of goodwill, who have always desired the best from me.

# LIST OF ABBREVIATIONS

| | |
|---|---|
| ALV | African leafy vegetable |
| ANOVA | analysis of variance |
| ARC-VOP | Agricultural Research Council – Vegetable and Ornamental Plants campus |
| AWS | Automatic weather station |
| Ca | Calcium |
| $CaCN_2$ | Calcium cyanamide |
| $Co\ (NH_2)_2$ | Urea |
| CC | Canopy cover |
| Cci | $CO_2$ concentration |
| $C_4$ | Carbon $_4$ plant |
| $C_3$ | Carbon $_3$ plant |
| DAT | Days after transplanting |
| Dp | Deep percolation |
| Eta | Actual evapotranspiration |
| $ET_0$ | Reference evapotranspiration |
| FAO | Food and Agricultural Organization |
| FC | Field capacity |
| Fe | Iron |
| FNS | Food and nutrition security |
| GDD | Growth degree days |
| H | Leaf harvesting |
| HI | Harvesting index |
| Ha | Hectare |
| I | Irrigation |

| | |
|---|---|
| ISCW | Institute of Soil, Climate and Water |
| IWMI | International Water Management Institute |
| IUE | Irrigation use efficiency |
| K | Potassium |
| $KNO_3$ | Potassium nitrate |
| Ks | Saturated hydraulic conductivity |
| LAI | Leaf area index |
| LSD | Least Significant Difference |
| (LAN) ($NH_4NO_3 + CaCO_3$) | Limestone ammonium nitrate |
| masl | Metres above sea level |
| Mg | Magnesium |
| MHz | Megahertz |
| MVDA | Multivariate data analysis |
| NC | Nutritional content |
| NS | African nightshade |
| NFI | Nutritional food insecurity |
| NFS | Nutritional food security |
| NWP | Nutritional water productivity |
| $(NH_4)_2SO_4$ | Ammonium sulphate |
| $NH_4NO_3$, | Ammonium nitrate |
| ($NH_4NO_3$ $(NH_4)_2SO_4$) | Ammonium nitrate sulphate |
| $NH_4^+$ | Ammonium |
| $NO_3^-$ | Nitrate |
| R | Run-off |
| SWC | Soil water content |
| SA | South Africa |
| sSA | sub-Saharan Africa |

| | |
|---|---|
| Tr | Transpiration |
| TVC’s | Traditional vegetable crops |
| VPD | Vapour pressure deficit |
| OPLS-DA | Orthogonal partial least square discriminatory analysis |
| Org. C | Organic carbon |
| P | Phosphorus |
| *p* | Statistical probability |
| PCA | Principal component analysis |
| pH | Potential hydrogen |
| Pr | Rainfall |
| PAW | Plant available water |
| % | Percentage |
| $^1$H-NMR | Proton nuclear magnetic resonance |
| W | Water |
| ΔW | Change in SWC |
| WP | Water productivity |
| Y | Yield |
| Zn | Zinc |

# GLOSSARY OF CORE TERMINOLOGIES

| | |
|---|---|
| C4 photosynthesis | A mode of photosynthesis where CO2 is first incorporated into a 4-carbon compound and the PEP carboxylase enzyme plays a major role in the uptake of CO2. C4 plants have a high water use efficiency than C3 plants because they keep their internal CO2 concentration relatively low and therefore stomata is not open at all times, thus losing less water compared to C3 plants. Example: Amaranth (Annandale et al. 2012). |
| Evapotranspiration (ETa) | A combination of two processes- evaporation and transpiration, which occurs simultaneously. Evaporation refers to the physical process of water vaporisation into gaseous phase from the soil surface, whereas, transpiration is a biophysical process where water is transported from the plant root zone through its cells and stomata into the atmosphere (Wegerich & Warner, 2010). |
| Irrigation use efficiency (WUE) | Water use efficiency can be defined in agronomy as the ratio of crop yield (usually economic yield) to water used to produce the yield (Bluemling et al. 2007). |
| Crop water use | Is the water actually used by the crop. Crop water use is influenced by available water in the soil, crop species, and growth stage (Kaisi & Broner, 2009). |
| Crop water requirements | The amount of water that is needed by a crop to satisfy ET demand by the atmosphere throughout the growing period of the crop, in a specific location with its own climatic conditions (Allen et al. 1988). |
| Field capacity (FC) | It's the amount of water that is held in soil particles after it has been fully wetted (saturated) and allowed to drain freely for a few days (Kort, 2010). It corresponds to a soil water potential ($\Psi_m$) of about -10 J/kg. |
| Permanent wilting point (PWP) | Soil water content at which indicator plants growing in the soil will wilt and fail to recover even if placed in a saturated (100% relative humidity) atmosphere for 12 hours. It is estimated at -1.5 MPa matric potential |

| | |
|---|---|
| | (Dekker, 2003). |
| Water holding capacity | It is the amount of water that a soil can store and is available for plant water use. It is held between field capacity and permanent wilting point (USDA, 1998). |
| Water productivity (WP) | Crop water productivity can either be expressed as either fresh or dry plant mass per unit of ETa (Molden et al. 2003). However, in this study the term water productivity will be used, meaning physical crop water productivity, expressed as fresh plant mass per unit of ETa. |
| Nutritional water productivity: | Refers to the ratio between nutritional content of a product per unit water used (Renault & Wallender, 2000). |
| Micronutrients | Micronutrients are those nutrients required by human beings in relatively small quantities. They are vitamins and minerals, and are required in milligram and microgram amounts. Examples of essential micronutrients are Iron (Fe), Zinc( Zn) and Magnesium (Mg). |
| Nutritional content (NC) | The concentration of micro-nutrients (, Fe, and Zn) in raw edible yield. |
| $^1$H Nuclear Magnetic Resonance (NMR) | Proton NMR is appreciated by natural product chemist, academia and industry as a non-destructive information-rich method. NMR is basically grounded on physical concepts of electricity, magnetism and classical mechanics and is further extended with quantum mechanical treatments (Gerothanassis et al. 2002). |
| Principal component analysis (PCA) | It is an unsupervised clustering method used for reduction of complex data. PCA is used to describe variance in a set of multivariate data in terms of a set of underlying orthogonal variables and orthogonal variability representing metabolite concentrations expressed as a particular linear combination of the principal components (PCA),Choi, (2006); Lindon *et al.* (2001). |

| | |
|---|---|
| Orthogonal partial least square discriminatory analysis (OPLS-DA) | This analysis, is a supervised pattern recognition method of which the ultimate purpose is to discriminate systematic variation in the X-matrix into parts, in which one component is linearly related to the Y-matrix (Maree and Viljoen, 2011), followed by the contribution plots, with different peaks. |

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# CHAPTER 1: General Introduction

## 1.1 Introduction

The *Solanum nigrum* L. complex, commonly known as black nightshade, contains about 30 different species, most of which originate from South America (Poczai et al. 2010). African nightshade species are erect, annual or biannual herbaceous plants that can attain a height of 75 cm (Jansen van Rensburg et al., 2007). While, Schippers, (2002) described the African nightshade *Solanum retroflexum* Dunal plant as an erect spreading, pubescent herb up to 70 cm tall and different rhomboidal to ovate-lanceolate leaves 4 to 4.8 cm long and 3 to 4 cm broad. Poczai & Hyvonen, (2011) states that several nightshade species are utilised as food (leaves, stems and seeds) and medicinal plants in developing countries, in Africa particularly. The dietary diversity for South African children at national level was shown to be low, which makes it vital for consumption of ALV's such as nightshade which might assist in combating malnutrition and poverty in households (Steyn et al., 2006).

The productivity of *S. retroflexum* is classified as one of the most important African leafy vegetables (ALV's), in terms of its nutrient content and water productivity, which is directly affected by soil fertility condition and water availability. In the Southern African region, African nightshade is usually grown in gardens around rural homesteads by resource poor farmers in farm lands where the soils are characterised by low nutrient availability. Laker, (2007) states that South Africa is a country faced with water unavailability. A similar report was provided by Water Wheel, (2007) which also stated that South Africa is considered as the 30th driest country in the world with an annual average rainfall of less than 500 mm, which is significantly lower than the world annual average of 860 mm (Department of Water Affairs and Forestry, 2002). Climate change and the latest increase on the demand for households water due to the increase in human population and irrigation water, has generated much debate on improving water use efficiency in ALV's. Importance of irrigation management for adequate plant growth and development together with the fact that efficient nutrition plays a significant role in the health of human are well established in literature, consequently, the need to optimize water and N, also maximizing their output, becomes germane. Agriculture utilizes 75% of available water (FAO, 2005), and that is expected to increase due to impact of climate change and urbanization.

It is envisaged that by 2025, there will be an extra 2 billion people in the world and 80% of the predicted population is projected to come from developing countries such as South Africa (Molden & de Fraiture, 2004). Hence, the need to improve land productivity in-order to meet the increasing demands for vitamins and minerals, to compensate for the human population becomes imperative. Research has shown that *S. retroflexum* is one of the most common ALV's consumed in the rural communities of South Africa. The green leaves, stem and ripe black seeds are consumed as food and utilized for medicinal purposes. The green seeds are, however, poisonous for human consumption (Jansen van Rensburg et al., 2007). Its importance as a highly nutritional leafy vegetable both in terms of nutritional quality and quantity has been widely recommended (Modi, et al., 2006). Research on the response of *S. retroflexum* to deficit and variable N application had been carried out previously (Beletse et al. 2012; Van Averbeke et al. 2012). Scientific information provided by these groups of scientists suggested that studying irrigation and N independently, may not be feasible practically because N is mobile and its mobility in the soil is markedly affected by selection of irrigation strategies. In many arid parts of South Africa, crop production is mainly subjected to environment limitations such as water deficit (Slabbert & Van den Heever, 2007).

Jansen van Rensburg et al., (2007), suggested that predominance of malnutrition is mostly common in rural areas due to un-affordability of exotic vegetables serving as essential sources of vital nutrients and vitamins providing nutritional value in the diets of South African rural communities. There is a need for farmers to cultivate ALV's which can serve as a food source with high nutrient content and at the same time contributing to the alleviation of malnutrition. In order to better reintroduce this crop into farming communities, the use of cost effective production methods need to be developed, such as precise management of irrigation water together with fertilizers application, without compromising their marketable yield and nutritional value. The production of *S. retroflexum* by smallholder farmers and homestead gardeners is limited due to certain draw-backs associated with water and soil fertility constraints. In order to obtain optimum yield and high quality indigenous vegetables, adequate water and nutrients supply to growing plants and soil are required, contrary to the initial ideology and or past reports inclined with the opinion that these indigenous vegetables thrives under poor or low soil fertility condition, even under low water supply during growth and development (Van Averbeke et al., 2007).

In recent years, there is an increased awareness to cultivate indigenous vegetables by smallholder farmers and homestead gardeners (Oloefse & Van Averbeke, 2012). The hypothesis of this study is that the growth rate and optimum yield of *S. retroflexum* is dependent on soil nutrient availability and specific water supply. Research based on the metabolic profile of ALV's has not been well documented, as reports or information on investigating the chemical constituents is rare or scarce in literature on ALV's studies.

## 1.2 Research justification

In South Africa, there are a number of indigenous or indigenised ALV's which are common among the rural communities. However, the production of these vegetable crops by smallholder farmers and homestead gardeners is limited. In order to produce high yield and good nutritional quality of ALV's, water and nutrients are required, not as initially postulated that ALV's could be grown in soils with little or no input of nutrient sources. For a long time, South African researchers and policy makers have ignored ALV's but there have been changes during the past two decades. Most ALV's have increasingly received research attention and at the policy level, the value of these plants has also being well recognised and well emphasised (Department of Agriculture Forestry Fisheries, 2002). The promotion of neglected and underutilised crops such as *S. retroflexum* with a view to reintroduce them as an alternative source of food in cultivated agriculture will largely depend on availability of reliable and sound scientific agronomic practices and irrigation-use efficiency. With the climate changing at rapid pace in arid and semi-arid regions, increased municipal and industrial demands for water are necessitating major changes in irrigation management, scheduling and application in order to increase the efficiency of water use that is allocated for agricultural utilization.

Dakora & Keya (1997) indicated that poor soil fertility is a primary constraint in African crop production and these scientists emphasised the effect of this phenomenon on crop biomass yield together with the crops' nutritional content. N fertilization and irrigation are primary factors for normal growth and development in crop production. There is a need for in-depth research, aimed at determining the nutrient content and nutritional water productivity of *S.*

*retroflexum.* Dennis & Nell, (2002) indicated that the irrigation sector uses approximately 50% of available water and about 1.3 million hectares is under irrigation in South Africa, which is 7.2% of the total arable land and farmers using groundwater for irrigation is currently subjected to a water resource management charge of 0.54 c/m$^3$. Agricultural production under irrigation in South Africa uses groundwater, which irrigates 24% of the irrigable area, while surface water irrigates 76% of irrigable areas in South Africa, which make water productivity a national concern (Earth Trends, 2003). This makes it vital to improve crop water productivity, since good quality water is a precious scarce natural resource. The International Water Management Institute, (2007), described South Africa as a country that does not have sufficient water resources to meet their agricultural, domestic, industrial and environmental needs by the year 2025. Van Halsema & Vincent (2012) stated that the notion and concept of water productivity is defined as the ratio (or unit) WP= [product]/ [water used]. The concept also attributes a specific value of the productivity of water that can be optimized.

## 1.3 Theories and concepts

**Field capacity** (FC): the amount of water that is held in soil after it has been fully wetted (saturated) and allowed to drain freely for a few days (Kort, 2010). It corresponds to a soil water potential ($\Psi_m$) of about -10 J/kg.

**Permanent wilting point (PWP):** soil water content at which indicator plants growing in the soil will wilt and fail to recover even if placed in a saturated (100% relative humidity) atmosphere for 12 hours. It is estimated at -1.5 MPa matric potential (Dekker, 2003).

**Plant available water**: is the amount of water that a soil can store and is available for plant water use. It is held between field capacity and permanent wilting point (United States Department of Agriculture, 1998).

Accuracy of evapotranspirative ETa is dependent on accurate measurement or estimation of variables on the equation below. The amount of net irrigation that was required by a crop was determined by calculating Crop Water Requirement of *S. retroflexum* (CWR). Crop water use or evapotranspiration of varying treatments will be estimated using the soil water balance equation according to Annandale (1999):

$$ET = I - R - Dr - \Delta Q \text{............Equation 1}$$

PAW: is the plant available water, ETa: actual evapotranspiration in mm, I is irrigation in mm, P is rainfall amount in mm**,** $\Delta$ W is change in soil storage in mm, Cr is capillary rise in mm, Dp is deep percolation in mm, $\Delta$S change in sub-surface in and out- flow in mm, and R is runoff amount in mm (Figure 2).The change in soil moisture is determined using a neutron probe (NP) at minimum of weekly intervals (Figure 2). At planting, the soil is at field capacity content of the soil, thus plant available water (PAW) is determined from the neutron probe reading. To estimate deep percolation accurately and precisely, the access tubes are installed deep to determine any potential deep percolation to a depth of 1.1m.

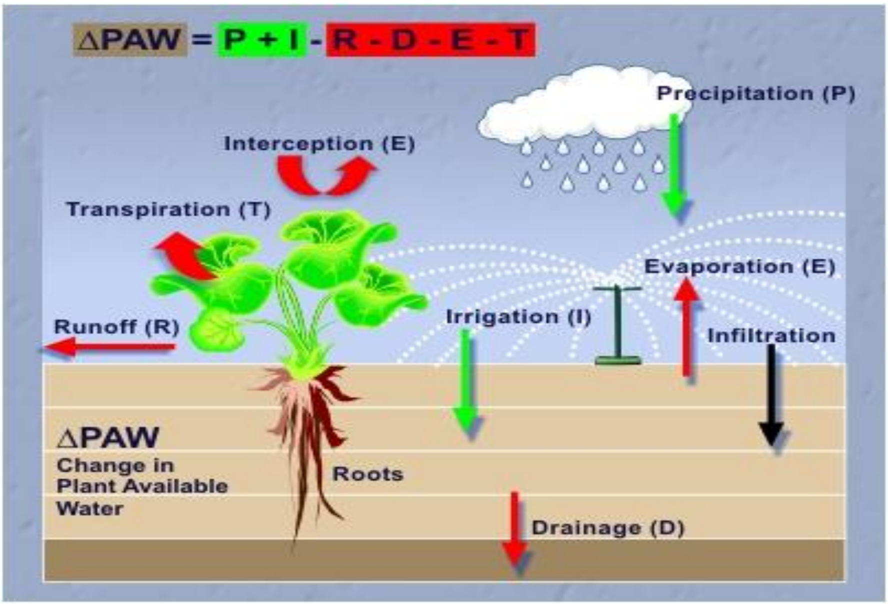

**Figure 1:** Schematic presentation of the soil water balance (Annandale *et al*., 2005)

Cr can be assumed to be negligible (drip irrigation system was utilized for irrigation) as the water table was deep below the soil surface. A black plastic was installed (1.5m deep) between the rows to prevent water from moving from plot to plot. P was excluded in the water balance because the experiment was conducted under a rainfall shelter. Sinclair et al. (1983) estimated crop productivity as a value for the whole cropping season, i.e. actual yield (Ya) divided by water use or actual irrigation water applied (ETa) as follows:

Average Productivity $\frac{\text{Marketable yield}}{\text{Irrigation applied}}$……………….Equation 2

Scientific published literature on NWP in South Africa is limited on *S. retroflexum*. This would suggest that there is much scope for future research on this important topic. It is also emphasised that therein an existing knowledge gap that requires to be addressed by the current study and that there is currently limited information on the response of *S. retroflexum* to the variable application rates of N and irrigation on biomass yield in South Africa. Wishart, (2007), stated that $^1$H-NMR spectroscopy can be used for the identification and quantification of chemicals from complex mixtures and Tyagi et al., (2010), described metabolites as the small chemical components in every cell. Metabolomics measures the major traits responsible for food quality, taste and nutrition determined by the unique chemical fingerprint. Therefore, metabolomic profiling was employed to gain information on system biology and cell physiology of *S. retroflexum*.

## 1.4 Objectives

-To determine a nitrogen response curve of *S. retroflexum* at different irrigation levels

-To determine if there is an interactive effect of water and nitrogen application on growth, marketable yield and nutritional quality

-To annotate metabolites of *S. retroflexum* as affected by nitrogen and irrigation level

## 1.5 Dissertation layout

This dissertation is divided into six chapters. The first chapter constitute the general introduction, which highlights the effect of multi-related problems that affects South Africa, such as malnutrition, nutritional water productivity and metabolomics. It also emphasises the need to improve water-use efficiency, particularly in South Africa. Chapter 2 provides a general literature review of production of *S. retroflexum*, description and taxonomy of *S. retroflexum*, malnutrition trends locally, internationally and consumption of African leafy vegetables, nutritional water productivity and availability of N in the soil. Chapter 3 discusses marketable yield of nightshade as affected by N and water management. Chapter 4 investigates the nutritional water productivity as affected by N and water management, micronutrients, Zn, Fe and Mg. $^1$H-NMR based metabolic profiling analysis as a result of nitrogen and irrigation management is discusses in Chapter 5. The general discussion and recommendations for implementation and future research are provided in chapter 6.

# CHAPTER 2: Literature review

## 2.1 Description and taxonomy of *S. retroflexum* Dun.

The Solanaceae family, to which the genus *Solanum* L belongs, is a cosmopolitan family containing many essential vegetables and fruits such as potatoes, tomatoes and chillies. It is composed of approximately 90 genera and between 2000 and 3000 species; this family is widely distributed throughout tropical and temperate regions of the world. The *Solanum* species, is commonly known as the black garden or common nightshade. *Solanum retroflexum* (figure 2) is one of the largest and most variable species group of the genus (Schippers, 2002).

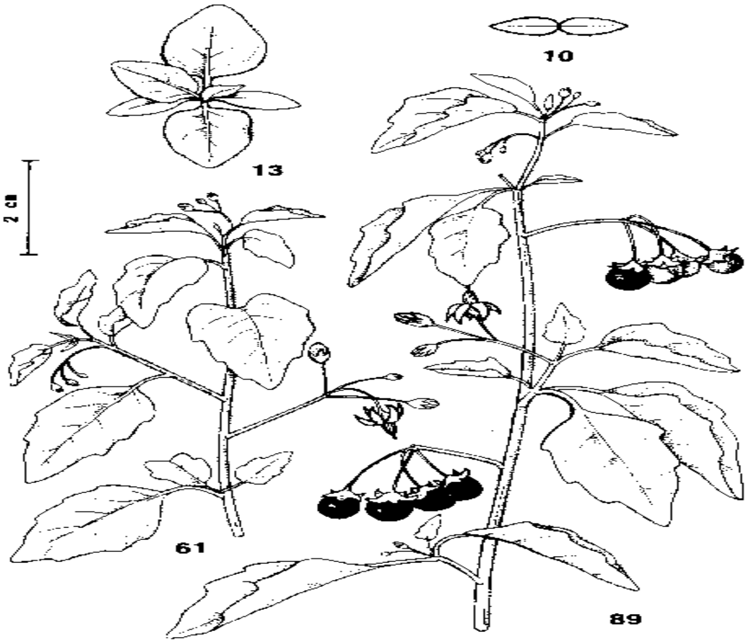

**Figure 2:** Monographic scale of S. *retroflexum* (Meier, 2001).

Jansen van Rensburg et al., *(*2007) indicated that *Solanum retroflexum* grow mainly in fairly humid environments with an average rainfall of 500 mm per annum. *Solanum retroflexum* plants are said to be tolerant to temperatures ranging between 15$^{o}$C and 35$^{o}$C. Grubben et al. (2004) indicated that poor germination in nightshade seed was caused by inadequate removal of sugar and germination inhibitors present in the fruit during the extraction of the seed. When *S. retroflexum* is grown during late winter, maximum growth and biomass production are obtained when the plants are exposed to full sunlight (Jansen van Rensburg et al., 2007).

## 2.2 Malnutrition trends in the South African and global scenes

Malnutrition and poverty continues to remain a global and continental problem. Bourne et al., (2002) stated that globally iron, vitamin A, iodine and zinc deficiencies are the most prevalent form of micronutrient deficiency. The FAO, (2010) estimated that globally, a total of 925 million people were undernourished, while Wenhold & Faber, (2008), stated that acute under-nutrition causes wasting and chronic under-nutrition results in stunting. Traditional societies have always exploited edible wild plants to provide adequate levels of nutrition (Newman, 1975) and edible wild leafy vegetables play an important role in African agricultural and nutritional systems (Keller et al., 1969).

Faber & Wenhold (2007) indicated that the most important micronutrient deficiencies globally are iron, vitamin A, iodine and zinc. Furthermore, 64% of children between 1 – 9 years of age are experiencing stunted growth and are underweight due to malnutrition. For every human, the prime need is to have adequate access to highly nutritious and healthy food on a daily basis. Nutritional deficiencies in energy and protein, as well as iodine, iron, zinc and vitamin A can contribute to poor growth of children, as stated by the WHO (1999).

Haskell et al., (2004) reported that despite the uncertainty about the bio-availability of ß-carotene in dark green leafy vegetables, controlled studies have demonstrated that consumption of cooked green leafy vegetables improve the vitamin A status of children, men and pregnant women. Micronutrient malnutrition relates to insufficient dietary quality and affects among others immune system responses and labour productivity and mental development in both children and adults (Faber & Wenhold, 2007). According to the WHO (1995), approximately 80% of dietary intake of vitamin A comes from plant food (vegetables and fruits). In 2000, the WHO estimated that more than 3.7 million deaths were attributed to underweight (mostly due to malnutrition) in children, and those deficiencies in iron, zinc, or vitamin A caused an additional 750,000 to 850,000 deaths (FAO, 2004).

## 2.3 Consumption of African leafy vegetables

In South Africa, people obtain leafy vegetables in different ways. Most commonly, they are harvested from the wild, or from crop lands, where they grow as weeds, but some leafy vegetable species are obtained exclusively through cultivation (Jansen van Rensburg et al., 2007). Wehmeyer & Rose, (1983) identified more than 100 different species in South Africa being used as leafy vegetables. Consumption of ALV's is particularly associated with a significant reduction of chronic disease that has a negative effect on our daily lifestyle. Consumption of fruits and vegetable intake needs to be increased in South African children as the consumption of fruits and vegetables is low due to low access and low availability (Steyn et al. 2006). Parsons (1993) suggested that the iKung people, who have lived in Southern Africa for at least 120 000 years, relied heavily on gathering of plants from the wild for their survival. The role of ALV's in the consumption patterns of South African households is highly dependent on factors such as poverty status, degree of urbanization, distance to fresh produce market and time of the year (Vorster et al., 2002).

The leafy vegetable dish may be prepared from a single species or from a combination of different species (Faber et al., 2007) while cooking methods described by Van Averbeke et al., (2007), may vary from thorough boiling, which may include the replacement of the first cooking water with fresh in the case of bitter-tasting species, such as *S. retroflexum*, to steaming involving the use of very small quantities of water and short cooking times. Consumption of green leafy vegetables is one of the measures that can enhance dietary diversification. Latham, (1997) stated that *S. retroflexum* is known to be higher in micronutrients, such as ß-carotene, vitamin C, protein, calcium and iron, than pale green leaves of vegetables.

## 2.4 Nitrogen Management

### 2.4.1 Different nitrogen sources used in crop production

Mengel & Kirkby, (2001) indicated that different N fertilizers are applied as pre-plant or top dressed after crop emergence during the growing season. Major N fertilizers include ammonium sulphate ($(NH_4)_2SO_4$), ammonium nitrate ($NH_4NO_3$), limestone ammonium nitrate (LAN) ($NH_4NO_3$ + $CaCO_3$), ammonium nitrate sulphate ($NH_4NO_3$ $(NH_4)_2SO_4$), potassium nitrate ($KNO_3$), urea (Co $(NH_2)_2$), calcium cyanamide ($CaCN_2$) and anhydrous ammonia ($NH_3$). N has a vital role in growth of plants, that is, its use as the main component in the synthesis of amino acids, protein, nucleic acids and compounds of secondary plant metabolism such as the alkaloids. Furthermore, shortage of N in the soil has detrimental effects on crop yield (Fertilizer Society of Southern Africa, 2007).

### 2.4.2 Importance of improved nitrogen use efficiency

The fertilizer requirements of crops have been researched extensively in South Africa (Farina et al., 1992). Although on AVL's there still exist knowledge gaps on nutrient uptake and fertilizer recommendation for the production of ALV's as compared to exotic commercial vegetables. In South Africa horticultural and fruit crops account for only 20 percent of total fertilizer use, their total value of production of R10 000 million is nearly 80 percent that of field crops (FAO, 2005). Drury et al., (1999) highlighted that in cooler temperate climate, $NO_3$ losses through the tile drainages have approached 26 kg N $ha^{-1}$ or 23% of the total N applied. In general, loss of N can only occur when mineral N ($NH_4$ and $NO_3$) are present in excess of plant need (Raun & Johnson, 1999). This creates a thrust needed to improve N use efficiency, required to improve N fertilizer use efficiency to ensure an excellent uptake by the plant and achieve a good economic yield of high nutritional value of *S. retroflexum*.

Andow et al. (2009), suggested that the importance of improving the nutritional composition of food for health purposes and environmental sustainability could be reached by reducing unnecessary fertilizer application, chemicals, water, and fuel while minimizing reductions in yield. Locascio et al. (1984) suggested all major marketable quality attributes in horticultural crops, including visual quality and nutritional contents, are directly influenced by N availability.

### 2.4.3 Nitrogen availability in the soil

Plants only use about 50% of the applied N which implies a significant loss in revenue and energy (Newbould, 1989). While N use in Europe is now decreasing, N usage in North America is still relatively on the rise (Burt et al. 2009a). Loss of N and phosphorus (P) through leaching due to excessive application in agriculture is widely considered the main cause of eutrophication in fresh and salt water supplies throughout the world (Erhart et al., 2007). Several practices have recently been implemented to attempt to reduce ground water pollution by N, through practices such as split applications (Drake et al., 2002).

In fertilizer products, N is present as ammonium ($NH_4^+$), ammonia ($NH_3$), nitrate ($NO_3^-$) and amide in urea with nitrate ($NO_3^-$) the most dominant form in most agricultural soils (Mengel & Kirkby, 2001). This is attributed to the fact that under aerobic conditions $NH_4^+$ is quickly converted to $NO_3^-$ through nitrification. However, this is not always the case, under anaerobic conditions the amount of $NH_4^+$ is higher than $NO_3^-$ because aerobic bacteria such as nitrobacter utilize oxygen from $NO_3^-$ resulting in denitrification. These two forms $NH_4^+$ and $NO_3^-$ have different behaviors on soil; $NH_4^+$ tends to lower soil pH whereas $NO_3^-$ increases it (FSSA, 2007). Conversely, uptake of $NH_4^+$ is best in a neutral medium and depressed as the pH falls, yet $NO_3^-$ uptake is more rapid at low pH levels (Mengel & Kirkby, 2001). Nitrate is absorbed into the roots by proton cotransport which explains its higher rate at lower pH.

### 2.4.4 Loses of nitrogen in the soil

Loss of N is a major problem affecting farmers because of costs. The FSSA (2007) suggested that nitrates ($NO_3^-$) are predominately lost through denitrification and leaching. Ammonium leaches at a lesser extent, because it partially adsorbs on soil colloids and like $K^+$ it tends to get locked up on 2:1 clay soils. Urea has the highest N percentage, however its major problem is volatilization. Volatilization of urea (and ammonia) is affected by fertilizer placement, soil temperature, moisture, wind, precipitation and soil pH. Granular N fertilizer placement through broadcasting method results in more volatilization as compared to banding. Banding urea at 2.5 cm depth can conserve urea and this could be improved when there is good soil moisture (Mengel &Kirkby, 2001).

It is recommended that irrigation be supplied shortly after fertilization because water dissolves the fertilizer, subsequently moving it below the soil surface (Clain et al., 2007). Increased wind velocity (from 1.7 to 3.4 $ms^{-1}$) was found to reduce ammonia loss from 19 to 7.5%, likely due to drying of the soil surface (Bouwmeester et al., 1983). High soil pH and high soil temperature increase urea volatilization. Bouwmeester et al., (1983) revealed that high organic matter increased volatilization and attributed it to the fact that organic matter has lots of microorganisms which produce urease, an enzyme that increase hydrolysis of urea. Good farming practices therefore need to be implemented to alleviate serious losses.

There are different types of N fertilizers with different available N available for production purposes (FSSA: 2007), integrated crop production systems (Ganeshamurthy, 2004; Peck et al., 2006), fertigation (FSSA: 2007), best management practices (Alva et al., 2006) and N foliar applications (Reay et al., 1998). Not all of the above mentioned N application methods could reduce the loses of the total amount of N applied and all the applications methods were effective in limiting underground water source pollution by increasing plant N use efficiency.

### 2.4.5 Nitrogen availability in leafy vegetables

Of all the N forms, $NO_3^-$ is taken up the fastest and in greater amounts in most plants. Nitrate is absorbed by roots and translocated to the aerial parts unaltered. This results in a faster uptake and in summer when temperatures are relatively high absorption is even quicker (Mengel & Kirkby, 2001). Ammonium ($NH_4^+$) uptake is slower due to the fact that it has to be assimilated in the roots prior to translocation in the form of amino acids to the shoots (FSSA, 2007). With urea it can either be converted into $NH_4^+$ first or directly absorbed. However, urea taken up directly is slowly translocated as compared to $NO_3^-$ or $NH_4^+$. Transportation of these N forms occurs through the xylem ($NO_3^-$) and phloem (amino acid forms). Factors which determine the rate of uptake include the availability of ions in the nutrient medium, physiological need of the plant and type of N source (Mengel and Kirkby, 2001). Barker & Bryson, (2007) stated that the general function on the photosynthetic capacity of plants is dependent upon the availability of sufficient N for optimum yield realization. N is also an essential part of protein development and chloroplast structure.

In vegetable crops, N application is vital for vegetative reproduction and in most cases N deficiencies would have negative implications on vegetative growth, often to the detriment of root development (Mengel et al. 2001). Fertilizer application is an extremely vital aspect in determining horticultural crop yield, quality and nutritional content (Martinez-Ballestra et al., 2008). N deficiency symptoms have been well characterized for all common horticultural crops (FSSA, 2007). In green leafy vegetable crops, such as cabbage and lettuce, nitrate ($NO^-_3$) accumulation was likely to become a significant problem when grown in soil conditions with high N availability and low light (Demsar et al., 2004). Head-forming leafy vegetables, such as cabbage and broccoli can form split heads under high N (Locascio et al., 1984).

## 2.5 Water Management

### 2.5.1 Irrigation use

Sinclair et al. (1983) defined irrigation-use efficiency as the ratio of biomass production, expressed as, total crop biomass or grain yield, to water consumed expressed as transpiration or total water input to the system. Renault & Wallender, (2000) defined the difference in water use between $C_3$ and $C_4$ plants and the consequences on total amount of water use by the crop during biomass production. Improved water productivity, which produces more crops per drop (van Dam et al., 2003) can be achieved through improved soil crop management.

### 2.5.2 Deficit Irrigation practice as a management strategy

The ultimate goal of scheduled irrigations is to apply sufficient amount at different growth stages of the crop based on sound agronomic principles to minimize input and maximize output, without compromising biomass yield and nutritional content. Trout et al., (2009), stated that past research studies have shown that the yield with deficit irrigation were usually less than the reduction in irrigation water applied, that implied that the marginal productivity of irrigation water applied tend to be low when application is near full irrigation. Soil water deficit affect and reduces radiation by plants and consequently less biomass is produced and it is also well documented in literature (Delfine et al., 2000). Sinclair & Ludlow, (1985) stated that the water balance of plants could be determined by difference between transpiration and root uptake. Water deficit often induce plant water stress, which normally result in an adverse effect on crop growth together with quality, and this could result in yield reduction (Wang et al., 2003).

Jones, (1992) stated that field conditions under reduced water supply affect the turgor pressure and growth of plants as it is dependent on the rate of water supply. Declining plant water status under increased soil water deficit has negative implications on various physiological processes in the plant and plant productivity as a result (Lawlor, 2002). Under extremely severe soil water stress there is stomatal closure and a further decline in the plant water status could lead to death of the plants (Sinclair & Ludlow, 1986).

## 2.6 Knowledge Gaps

Insufficient information exists on the interactive effect of irrigation water management and soil fertility by variable N applied on the nutritional value of *S. retroflexum*. N availability and uptake by the plant, is influenced by variable soil water regime, since N moves in the soil through diffusion and mass flow (FSSA, 2007). Van Averbeke et al. (2007) indicated that research results documenting the response of *S. retroflexum* to variable application rates of fertilisers is limited in South Africa. Jansen van Rensburg et al., (2004) indicated that ALV's have the reputation of requiring less plant nutrition than their exotic counterparts such as Swiss chard for optimum growth and development. Soil fertility is one of the vital components in the production of ALV's and this research will therefore test methodologies to produce *S. retroflexum* using different N application rates and different irrigation levels, as they constitute agricultural inputs in sustainable agricultural systems. These will benefit resource poor smallholders, equipping them with reliable sound scientific information on how to increase the yield and income per unit production area. Even though indigenous and indigenised green leafy vegetables are often described as drought-tolerant crops, availability of water is a key issue in most areas in Africa, including most of South Africa. Due to low rainfall patterns and very high potential evapotranspiration (due to high temperatures and low relative humidities), more than 80% of South Africa is classified as hyper-arid to semi-arid (Bennie & Hensley, 2000).

Edmonds & Chweya, (1997), indicated that the yield potential of *S. nigrum* depends on a number of factors, including number of harvests and agro-ecological conditions, but under favourable conditions leaf yields of 20 t $ha^{-1}$ can be achieved. Nutrient availability in the soil and irrigation as management practice determines the extent of the yield potential of a crop.

Jansen van Rensburg et al., (2007) indicated that under favourable conditions, cumulative fresh leaf yields of 20 $t \cdot ha^{-1}$ could be achieved but Chabalala & Van Averbeke (2011) recorded fresh leaf yields as high as 81.8 $t \cdot ha^{-1}$ from three shoot harvests. The limited information available suggests that the fresh leaf yield of *S. retroflexum* is subject to considerable variability. Relatively low fresh yields were obtained from *S. retroflexum* of 1.5-3.0 t $ha^{-1}$ (Moald, 1998) which is significantly lower when compared to potential yield of 20-30 t $ha^{-1}$ obtained by Edmonds & Chweya, (1997). Moald, (1998) stated that the main reason for poor and low yields were poor agronomic practices, which include lack of precise fertilizer use and irrigation requirements. Consequently, this results in an urgent need for optimizing water and fertilization for good economical marketable yield of good quality. Oloefse & Van Averbeke, (2012), recommended that the water and plant nutrient requirement of *S. retroflexum* and their response to variables of these factors, awaits systematic scientific enquiry.

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# CHAPTER 3

## Marketable yield of African nightshade *(Solanum retroflexum)* as affected by different application rates of nitrogen and irrigation

### Abstract

There is a need to produce highly nutritious vegetables with less fertiliser and irrigation water. This research project was aimed at investigating potential improvements in marketable yield of African nightshade. A completely randomized block design trial was conducted in January 2015 under a rainshelter at ARC:VOP campus Roodeplaat, Pretoria (Republic of South Africa). African nightshade was irrigated with three different irrigation treatments (100% FC, 66% FC, and 33% FC) and received four nitrogen (N) treatments 0 kg N·ha$^{-1}$,75 kg N·ha$^{-1}$,150 kg N·ha$^{-1}$ and 225 kg N·ha$^{-1}$. Agronomic efficiency was increased at I66%FC with 150 kg N·ha$^{-1}$, while N application rate at 225 kg N·ha$^{-1}$ resulted in a sharp decline on marketable biomass yield, irrespective of irrigation rate, suggesting that it has negative effect on production of African nightshade to increase N above 150 kg N·ha$^{-1}$. The biomass production in the N control treatment was substantially lower than the treatments that received N fertilizer application at planting and as topdressing. This is an indication that *S. retroflexum* production is susceptible to low N fertility.

***Keywords***: Soil fertility; water; African leafy vegetables

### 3.1. Introduction

For sustainable leafy vegetable production, availability of adequate nutrients and water supply are the most vital inputs that determine the yield that could be achieved (Edmonds & Chweya, 1997). In the sub-Saharan Africa the extent of production of vegetables including ALV's is hampered by declining soil fertility levels and a reduction of rainfall (Van Averbeke & Oloefse, 2012). Subsequently, there is a requirement for supplemental fertilizers and irrigation water in order to achieve maximum marketable yield. Despite the latest fertilizer and irrigation application equipment and guidelines in commercial exotic vegetables, there are knowledge gaps in smaller holder farming systems of indigenous vegetable production such as *S. retroflexum*. Importance of N in plant production has being well documented in literature. Gregory (1988) stated that crops vary in the way they respond to N availability in the soil, since they differ in distribution and density of their root systems. Adequate N availability promotes optimum vegetative growth and the development of deep green colour in the leaves (FSSA, 2007). Efficient utilisation of irrigation water in the agricultural sector has been critical for decades as water is becoming an expensive and scarce natural resource. This is due to fierce competition between agriculture and other industries, including households. Achieving maximum crop yield of leafy vegetables depends on full evapotranspiration requirements of the crop being met (Costa et al., 2007). Masinde et al. (2006) stated that soil water deficit have been observed to cause a significant reduction in shoot expansion and canopy cover.

*Solanum retroflexum* is one of the most common ALV's used by rural communities of South Africa. There is limited availability of this crop in the commercial markets, due to unavailability of sound scientific agronomic information describing cultural management practices. Maunder & Meaker (2007) stated that ALV's such as *S. retroflexum* are perceived to require lower water and nutrients than the exotic leafy vegetable counterparts including Swiss chard. The response of *S. retroflexum* to deficit irrigation and N fertilisation were studied by Beletse et al., (2012) and Van Averbeke et al., (2007) respectively. Studying irrigation and N independently, may not be practical because N is mobile and its movement in the soil is markedly affected by water and other management practices.

Positive interactive effect of N availability and irrigation on plant growth is well documented (Tisadale et al., 1985). Consequently, to achieve optimum marketable yield, nutritional and water requirements of crops should be met (Van Averbeke, 2012). *Solanum retroflexum*'s ability to extract N from the soil and utilising it for optimal biomass production depends on both the quantity of N and irrigation water applied throughout the growth season of the crop, since N depend on irrigation for uptake and translocation through the xylem. This chapter is aimed at determining the *S. retroflexum* N requirement and its response to variable water application rates on fresh marketable yield. Monaghan et al. (2007) stated that despite the availability of sound scientific guidelines, water and N use efficiency are still generally not well documented especially in ALV's. Moreover, understanding the integrated water and fertiliser management of *S. retroflexum* will assist and will contribute to its successful commercialisation. The objective of the study was therefore, to determine the response of *S. retroflexum* to different irrigation and nitrogen levels.

## 3.2 Material and Methods

### 3.2.1 Site description

The trial was conducted under a rainshelter in from August 2014 to January 2015 at the Agricultural Research Council, Vegetable and Ornamental Plant, Roodeplaat experimental campus (ARC-VOP) (25° 35' S, $28^{\circ}$ 38' E, 1200 m.a.s.l) which is about 36 km north of Pretoria. The site is located in the summer rainfall (October-March) agro climatic zone of South Africa, with an average rainfall of about 500 mm per year. January is the month with the highest average maximum temperature ($30^0$ C), whilst July is the month with the lowest minimum temperature ($1.5^0$ C).

Soil samples were collected at the beginning of the season from the top 0.9 m for determining soil chemical (fertility) and physical properties. The fertility status of the soil is presented in Table 1. Since there is no well-established fertiliser recommendation for *S. retroflexum*, fertilisation was done based on Swiss chard recommendations. N was applied depending on specific treatment, while 50 kg $ha^{-1}$ potassium chloride (50% K) and 50 kg $ha^{-1}$ superphosphate (11% P) were applied during planting.

**Table 1**: Soil chemical properties of the site at different soil depths.

| Soil depth | P | K | Ca | Mg | pH | Org.C | Total N | N-$NO_3^-$ | N-$NH_4^+$ |
|---|---|---|---|---|---|---|---|---|---|
| **(cm)** | **(mg/kg)** | **(mg/kg)** | **(mg/kg)** | **(mg/kg)** | **(water)** | **%** | **%** | **(mg/kg)** | **(mg/kg)** |
| 0-30 | 19.6 | 340 | 782 | 268 | 7.13 | 0.64 | 0.033 | 3.16 | 2.62 |
| 30-60 | 8.8 | 285 | 731 | 279 | 7.46 | 0.42 | 0.024 | 1.34 | 2.3 |
| 06-90 | 2.7 | 429 | 725 | 342 | 7.68 | 0.3 | 0.021 | 1.61 | 2.55 |

The physical properties of the soil including textural classes and hydraulic properties are presented in Table 2. According to the South African Soil Classification Working Group, (1991) the soil at the study site is classified as Hutton soil with a sandy clay loam texture up to a depth of 0.9 m.

**Table 2:** Soil physical properties for trial site up to 0.9 m

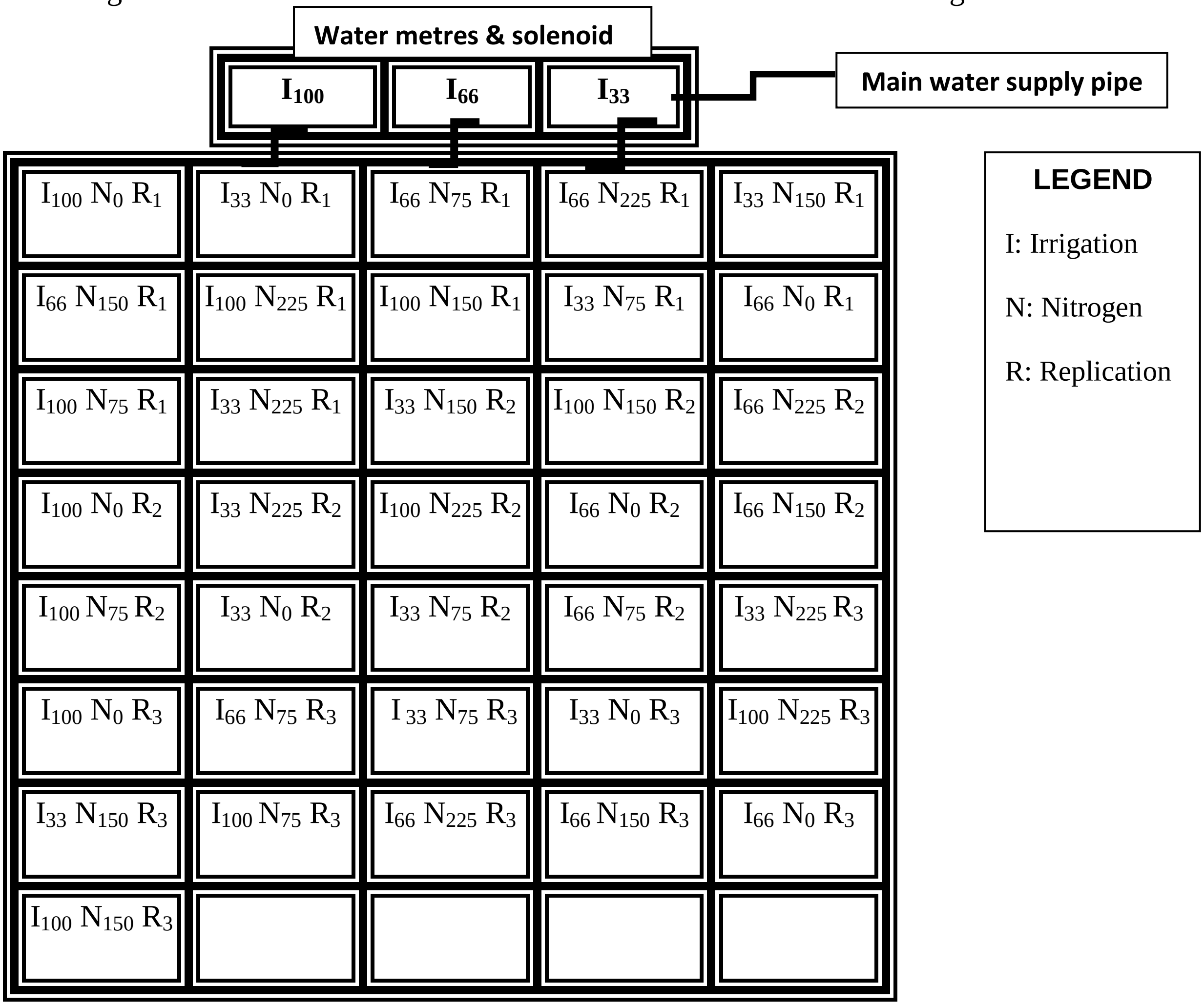
| | **Textural class** | | | | **Hydraulic Properties** | | | |
|---|---|---|---|---|---|---|---|---|
| **Depth** | **Sand** | **Silt** | **Clay** | **Texture** | **PWP** | **FC** | **Ksat** | **BD** |
| (m) | (%) | (%) | (%) | | ($m^{-3}m^{-3}$) | ($m^{-3}\,m^{-3}$) | (mm $day^{-1}$) | (g $cm^{-3}$) |
| 0 – 0.9 | 56 | 7.6 | 36 | SiCLL | 0.23.1 | 0.39.1 | 122.4 | 1.5 |

### 3.2.2 Seedling preparation

Seedlings were prepared in seedling trays using Hygromix® Hygrotech South Africa and vermiculite® as growing media and fertigated with multi-feed® Hygrotech South Africa nutrient solution to supply nutrients for early growth and development. Seedlings were thinned at the 3rd leaf stage and a single healthy seedling without any physical defect was retained in each tray cubicle. Seedlings were transplanted after four weeks.

### 3.2.3 Irrigation system, layout and application

A controlled pressure-compensated drip irrigation system (Netafim, Cape Town, South Africa) comprising of an electrical powered pump, filters and polyethylene drip tape, with discharge rate of 2 L per hour at 150 kPa was installed. The amount of water applied to each treatment was recorded using water meters. A CPN 503 DR Hydroprobe was used to monitor the soil water content at soil depths of 0.20, 0.40, 0.60, 0.80 and 1.00m, before and after each irrigation cycle. Prior commencement of the trial, wet and dry spot calibration measurements were done to determine their respective corresponding volumetric soil water contents in order to obtain a best-fit regression equation, for the site-specific condition (Shenkut et al., 2013). The regression equation was used to develop an excel spreadsheet for conversion of neutron probe reading count per second (CPS) to corresponding volumetric soil water content reading. Soil water deficit measurements were carried out before each irrigation event.



| | | | | |
|---|---|---|---|---|
| $I_{100}$ $N_0$ $R_1$ | $I_{33}$ $N_0$ $R_1$ | $I_{66}$ $N_{75}$ $R_1$ | $I_{66}$ $N_{225}$ $R_1$ | $I_{33}$ $N_{150}$ $R_1$ |
| $I_{66}$ $N_{150}$ $R_1$ | $I_{100}$ $N_{225}$ $R_1$ | $I_{100}$ $N_{150}$ $R_1$ | $I_{33}$ $N_{75}$ $R_1$ | $I_{66}$ $N_0$ $R_1$ |
| $I_{100}$ $N_{75}$ $R_1$ | $I_{33}$ $N_{225}$ $R_1$ | $I_{33}$ $N_{150}$ $R_2$ | $I_{100}$ $N_{150}$ $R_2$ | $I_{66}$ $N_{225}$ $R_2$ |
| $I_{100}$ $N_0$ $R_2$ | $I_{33}$ $N_{225}$ $R_2$ | $I_{100}$ $N_{225}$ $R_2$ | $I_{66}$ $N_0$ $R_2$ | $I_{66}$ $N_{150}$ $R_2$ |
| $I_{100}$ $N_{75}$ $R_2$ | $I_{33}$ $N_0$ $R_2$ | $I_{33}$ $N_{75}$ $R_2$ | $I_{66}$ $N_{75}$ $R_2$ | $I_{33}$ $N_{225}$ $R_3$ |
| $I_{100}$ $N_0$ $R_3$ | $I_{66}$ $N_{75}$ $R_3$ | $I_{33}$ $N_{75}$ $R_3$ | $I_{33}$ $N_0$ $R_3$ | $I_{100}$ $N_{225}$ $R_3$ |
| $I_{33}$ $N_{150}$ $R_3$ | $I_{100}$ $N_{75}$ $R_3$ | $I_{66}$ $N_{225}$ $R_3$ | $I_{66}$ $N_{150}$ $R_3$ | $I_{66}$ $N_0$ $R_3$ |
| $I_{100}$ $N_{150}$ $R_3$ | | | | |

**Figure 3:** Irrigation system layout and treatment experimental design.

### 3.2.4 Experimental treatments and irrigation management

Plot sizes of 4.16 $m^2$ with a border spacing of 0.5 m between plots were used. Plastic sheeting was inserted to a depth of 2 m to limit the movement of water between plots. A factorial trial of four fixed N application rates namely: $N_0$ (0 kg $N \cdot ha^{-1}$), $N_{75}$ (75 kg $N \cdot ha^{-1}$), $N_{150}$ (150 kg $N \cdot ha^{-1}$) and $N_{225}$ (225 kg $N \cdot ha^{-1}$) and three water levels namely I100, I66 and I33 were assigned in a complete randomised design with three replications. Irrigation treatments were applied on a weekly basis as follows:

I100- irrigated to field capacity weekly filling the soil profile in full
I66 - irrigated 66% of I100
I33 - irrigated 33% of I100

Irrigation was applied, depending on the measured average soil water deficit per irrigation treatment to refill the soil profile to field capacity, as suggested by Beletse et al. (2012).

Uniform irrigation was applied during the first two weeks in-order to have a good stand, uniform canopy cover and avoid flowering. Inorganic N was divided into three topdressings and incorporated into the soil to minimise volatilisation and to reduce the extent of leaching (Drake et al., 2002). The N fertiliser was applied in the form of limestone ammonium nitrate (LAN-28% N) and 40% was applied prior to transplanting and 20% was applied after each shoot harvest.  The experiment received potassium chloride (50% K) at 50 kg K $ha^{-1}$ and single superphosphate (11% P) application at a rate of 50 kg P $ha^{-1}$ at planting using the basal application method according to the requirements of Turmeric (Haque et al. 2007).

### 3.2.5 Harvesting procedure

The first harvest commenced 8 weeks after seedling emergence while the rest of the harvests were conducted at approximately three weeks intervals. Van Averbeke et al. (2007) recommended the 8th true leaf, considered by farmers as the first marketable leaf, could be harvested when the plants had reached the 8-leaf. The harvestable parts of the plant, which are the leaves, were sampled early in the morning before 10:00 am to prevent the leaf from being subjected to wilting and physiological stress. *Solanum retroflexum* is a vegetable crop and its yield was reported in fresh mass basis for the purpose of agronomic and productivity quantification. Plant samples were harvested from an area of 1 $m^2$ within the plot and the same plants were consistently harvested throughout the trial to minimise experimental error and variation within the replicates. The fresh leaf samples were then oven dried at 65°C to constant mass to determine the dry mass.

### 3.2.6 Meteorological weather data

An automatic weather station (AWS) was used to measure the meteorological data including rainfall, temperature, solar radiation and relative humidity. Temperature was measured using CS-500 Vaisala thermometer, relative humidity and solar radiation using LI-200 Pyranometer; and rainfall using a tipping bucket Texas instrument inc. rain gauge (Campbell scientific Loga UT.USA). The site's weather details during the experimental period are presented in Table 3.

**Table 3:** Mean monthly maximum (T max) and minimum (T min) temperature (°C), relative humidity (RH, %), monthly average reference evapotranspiration ($ET_O$, mm day$^{-1}$), rainfall (mm) data for the 2014\15 cropping season.

| Month | Maximum Temperature (°C) | Minimum Temperature (°C) | Maximum Relative Humidity (%) | Minimum Relative Humidity (%) | Mean $ET_O$ (mm) |
|---|---|---|---|---|---|
| August | 24.6 | 5.6 | 73.7 | 19.2 | 3.3 |
| September | 29.1 | 9.8 | 71.1 | 14.7 | 4.8 |
| October | 29.2 | 12.4 | 73.8 | 21.3 | 5.3 |
| November | 27.2 | 14.8 | 85.2 | 37.3 | 4.4 |
| December | 28.2 | 16.8 | 88.9 | 43.3 | 4.8 |
| January | 29.7 | 16.9 | 88.3 | 39.1 | 5.2 |

### 3.4 Statistical data analysis

Biomass yield response to different applied N and irrigation rates, were subjected to analysis of variance (ANOVA). Bar graphs were utilised for visual observations between treatments. Statistical quantification of the data was done at ARC: Biometric division in Hatfield, Pretoria, South Africa.

## 3.5 Results and discussion

### 3.5.1 Fresh marketable yield

Marketable yield was significantly ($p<0.05$) influenced by treatment interactions between the amount of water and N fertiliser applied and responded positively to different N and water application rates with respect to I66 with 150 kg N·ha$^{-1}$ as illustrated in table 4. Expansive plant growth was significantly increased as the result of available soil water content for both well irrigated treatments. Moald, (1998) pointed out that low yields resulted from poor knowledge of precise fertiliser use and irrigation requirements during the growth season. There were significant differences ($p \leq 0.05$) between the irrigation treatments and of the N application rates between I66 and I100 when compared to I33 for N75, N150 and N225. There were however no significant differences between I66 and I100 for any level of N application. It is interesting to note that the lowest yield was obtained with the highest irrigation level with no N application. Results from this investigative research work concur with findings of Chabalala & Van Averbeke, (2011) which reported that higher fresh biomass yield were obtained with high N application. This confirms the ability of the crop to laterally occupy above ground, which is an indication of its competiveness for solar radiation interception when the plants are grown under well-irrigated conditions (Beletse et al. 2012).

**Table 4**: Marketable yield of African nightshade as effected by irrigation and N application. Irrigation: I100%FC= Irrigation 100% of field capacity, I166%FC= Irrigation I66% of field capacity, I133%FC= Irrigation 33% of field capacity.
Nitrogen: N0 =0 kg N·ha$^{-1}$, N75 kg = 75 N·ha$^{-1}$, N150 =150 kg N·ha$^{-1}$, N225 =225 kg N·ha$^{-1}$.

| | N application kg N·ha$^{-1}$ | | | |
|---|---|---|---|---|
| Irrigation | **N 0** | **N 75** | **N 150** | **N 225** |
| **I33%FC** | 9.97$^{b}$ | 12.56$^{c}$ | 16.59$^{de}$ | 14.99$^{d}$ |
| **I66%FC** | 10.59$^{b}$ | 17.04$^{d}$ | 26.08$^{g}$ | 19.01$^{ef}$ |
| I100%FC | 7.06$^{a}$ | 15.25$^{d}$ | 22.22$^{g}$ | 18.8$^{e}$ |
| **Mean** | 9.20$^{a}$ | 14.95$^{d}$ | 21.63$^{f}$ | 17.60$^{d}$ |

Table 4 illustrates that different N treatments were sensitive to the availability of water and N in the soil and resulted in a variation in marketable yield accumulation. Lower mean marketable yield was obtained under 225 kg N·ha$^{-1}$, compared to 150 kg N·ha$^{-1}$ which could have been caused by excessive use of N application leading to leaching and denitrification (Umar et al., 2007). The N control treatment performed poorly due to low N application and availability of N in the soil and resulted in low uptake. Marketable yield increased from 75 kg N·ha$^{-1}$ to the highest marketable yield which was recorded at 150 kg N·ha$^{-1}$ at I66. Treatment combination I33 and 0 kg N·ha$^{-1}$ resulted in low fresh biomass yield production (9.97 t ha$^{-1}$). Van der Werf & Nagel (1996); Lambers et al. (1998) stated that when crops planted from N unavailable soil condition to N available soil, the growth rate of the shoot is increased significantly.

Under moderate soil water stress, leaf expansion is inhibited relatively more than photosynthesis leading to carbon accumulation and increase assimilation which leads to higher marketable yield obtained (Sangakkara et al., 2001). Difference in the marketable yield between the adequately irrigated (I100%FC) and the severely limited irrigated treatments (I33%FC) could have been attributed to the differences in aerial canopy cover characteristics, since enough water was available in the soil profile to support the plant's photosynthetic requirements. This concurs with the result obtained by Jones, (1992), which suggested that in soil conditions where adequate irrigation was applied, there is little evapotranspiration loss from canopy cover and the soil due to reduced light penetration together with high relative humidity.

One of the drought avoidance mechanism's under soil water deficit conditions is the closure of the stomatal pores which prevents the decline in plant water status. The decrease in plant water under increasing soil water deficits had serve negative effects on plant productivity on the I33, as the plants were exposed to water stress. Sinclair & Ludlow, (1986) stated that under severe stress, stomatal closure and further decline in the plant water status which usually leads to eventual low biomass yield obtained. The decline in marketable yield obtained at I33, is a result of high evaporative demand which probably could be the main cause of poor biomass production of *S. retroflexum* which requires sufficient irrigation for optimum growth.

Water has been described as the single physiological factor upon which plant growth and development depends on, than most environmental variables (Kramer & Boyer, 1995). The response of *S. retroflexum* to different irrigation treatments varied strongly. Significant differences in the total marketable yield between irrigation I33 and I66 were observed in table 4. McGiffen et al., (1992) indicated that *S. retroflexum* is highly sensitive to water stress, experiencing more than 50% reduction of the height and biomass when irrigating to water holding capacity was done biweekly instead of weekly. For any crop including *S. retroflexum*, N use is a function of the release characteristics from the point of application to uptake together with its translocation and it's availability as well as other external environmental factors that could enhance or suppress growth adversely. This suggests that *S. retroflexum* is sensitive to irrigation and drought stress, as observed in table 4. Plant physiological processes are reduced after about two thirds of the extractable soil water within the crop root zone has been utilised (Turner, 2000).

In broad production terms, the trend in the biomass yield response is related to application rate of inorganic N. This probably indicates that N supply is a key nutrient for high marketable yield production. Ghannoum et al. (2001) stated that deficit irrigation results in a reduction of respiration and that could lead to a reduction in biomass yield. All N treatments responded positively to variable N application rates until an optimum rate was obtained at which biomass peaked at 150 kg N·ha$^{-1}$. The effect of irrigation is however very significant in I66, where the highest yield was obtained at 150 kg N·ha$^{-1}$, as illustrated in table 4. This also indicates how the plant is affected by insufficient irrigation, even at sufficient N application rates. N and irrigation are key factors limiting plant survival and growth and low applications has adverse effects on the marketable yield of *S. retroflexum*.

Table 4 illustrates the highest marketable yield was obtained under the I66 with a yield of 26.08 t·ha$^{-1}$, while I100 achieved a marketable yield of 22.22 t·ha$^{-1}$. *Solanum retroflexum* tended to respond positively to increase in N application rates from 0-150 kg N·ha$^{-1}$. The lowest fresh marketable yield of 7.06 t·ha$^{-1}$ was achieved with less N application, even at adequate irrigation levels, and this is in agreement with earlier research work by Van

Averbeke et al. (2012), which support the results and the same group of scientists suggested that there was a clear observation that AVL's including *S. retroflexum* required optimum fertilisation for normal development.

The perception that *S. retroflexum* could adapt well to low soil fertility conditions was invalid, irrespective of the irrigation treatment, the mean marketable yield from 75 kg N·ha$^{-1}$was statistically lower compared to 150 kg N·ha$^{-1}$. *Solanum retroflexum* requires optimum N fertiliser (150 kg N·ha$^{-1}$) application in-order to produce high fresh biomass yield. Linn & Doran, (1984) stated that under completely saturated soil conditions chemo-denitrification may occur, which results in the reduction of $NO^{-3}$ to $N_2O$ which is unavailable N for uptake by plants and it drains away from the crop root zone. The result concurs with lower marketable yield obtained at I100 with 150 and 225 kg N·ha$^{-1}$.

I100 achieved the highest marketable yield of 22.22 t·ha$^{-1}$which is lower than I66 and concurs with research results obtained by Beletse et al. (2012), which suggested that the reduction in marketable yield obtained in the I100 could be attributed to high frequency of irrigation applied to replenish the soil water deficit, which could have caused nutrient loss from the crop root zone. I33 had the lowest fresh biomass yield compared to the well irrigated treatments (I66 and I100), this implies that increased irrigation application lead to increased yield. Under well irrigated conditions, water becomes readily and freely available from soil and it is taken up by the plant to support photosynthetic requirements and the rate of stomatal conductance is optimum; thus, the balance between the rate of transpiration and uptake of soil water is met. Hence, we observe a significant difference between the irrigation treatments I33 and I66.

### 3.5.2 Dry marketable yield

The total dry marketable yield varied with irrigation treatments and N application rates. Arora & Mohan, (2001), stated that dry matter partitioning is preferentially to the roots as compared to the shoots as drought adaptation mechanism of low N availability in soils. The results in table 5 reveal that dry matter yield were significantly increased with increasing N application rates at 75 kg N·ha$^{-1}$ up to 150 kg N·ha$^{-1}$, although the highest dry matter was produced with 150 kg N·ha$^{-1}$with I66. No significant differences were observed between 75 kg N·ha$^{-1}$and 225 kg N·ha$^{-1}$with I66, as shown in table 5.

**Table 5**: Interaction effect of different N and irrigation application on total dry biomass (t ha$^{-1}$).

| Irrigation | 0 kg N ha$^{-1}$ | 75 kg N ha$^{-1}$ | 150 kg N ha$^{-1}$ | 225 kg N ha$^{-1}$ |
|---|---|---|---|---|
| **I33% FC** | $0.581^{eb}$ | $0.672^{bc}$ | $1.424^{e}$ | $0.672^{bc}$ |
| **I66% FC** | $0.864^{d}$ | $1.504^{e}$ | $2.829^{h}$ | $1.742^{ef}$ |
| **I100% FC** | $0.338^{a}$ | $0.956^{d}$ | $1.948^{g}$ | $0.986^{d}$ |
| **Mean** | $1.783^{c}$ | $3.13^{b}$ | $6.20^{a}$ | $3.37^{b}$ |

Treatment means followed by different superscripted letters which differ significantly at $p \leq 0.05$.

Irrigation: I100%FC= Irrigation 100% of field capacity, I166%FC= Irrigation I66% of field capacity, I133%FC= Irrigation 33% of field capacity.

Nitrogen: N0 =0 kg N·ha$^{-1}$, N75 kg = 75 N·ha$^{-1}$, N150 =150 kg N·ha$^{-1}$, N225 =225 kg N·ha$^{-1}$.

Biomass production in the N control treatment for I33 and I100 were substantially the lowest than the treatments that received N fertilizer application. N application resulted in the significant increase on the dry marketable yield as a result of increased leaf area and better absorption of improved photosynthetic capacity. The large decrease in biomass yield in the I33 irrigation treatment with the lowest N application suggesting that dry matter in this treatment was partitioned preferentially to the crop root system rather than the shoot system.

The results in table 5 concur with those obtained by McGiffen et al. (1992), who observed *S. retroflexum* species in literature to be susceptible to water stress, when experiencing more than 50% of soil moisture loss which results in a reduction of the biomass when irrigated to water holding capacity was applied biweekly instead of weekly.

Masinde et al. (2006) expressed that there was a general conclusion that *S. retroflexum* has limited osmotic adjustment capacity and adapt to drought mainly by regulating transpiration and it was achieved by reduction of leaf area leading to a decline in the transpiration, which is clearly illustrated in table 5. Dry matter production was reduced as a consequence of reduction of plant leaf area which is directly affected by low soil water content at I33. Increase in leaf temperature beyond certain thresholds leads to inactivation of photosynthetic enzymes, thereby inhibiting dry matter production (Lawlor, 2002). Saab & Sharp, (1989) pointed out that leaf expansion in a drying soil can be reduced before any measurable decline in leaf water status of most commercial crops.

Table 5, illustrates that dry matter leaf production was reduced by the stressed irrigation treatment after the plants had utilized about 60% of the transpirable water, subsequently the dry matter leaf production was reduced by low N and irrigation applied. The highest dry matter was obtained under I166 at 150 kg $N \cdot ha^{-1}$. Adequate irrigation is required for increased photosynthetic capacity and leaf area increase subsequently resulting in improved dry matter, as indicated by Masinde et al. (2006). Plants subjected to 75 and 225 kg $N \cdot ha^{-1}$ application showed significantly dry matter production, whereas the control 0 had the lowest total dry matter production, as illustrated in table 4.

## 3.6 Conclusion

The rainshelter experiment suggests that marketable yield production requires the addition of N to produce optimal and profitable marketable yield of *S. retroflexum*. The results clearly illustrated that the perception that *S. retroflexum* grow well on low soil fertility mostly practised in rural and smallholder farming is incorrect and misleading. The study revealed that *S. retroflexum* responded positively to variable N application rates. In the context of crop production, the effectiveness of N use is a vital attribute, defined as the extent of the expansion of the aerial canopy cover of the plant in relation to marketable yield production of *S. retroflexum* obtained when N is applied at the optimum application rate. Similarly, the best results were obtained with adequate irrigation at I66. The notion that medium water stress negatively affects crop stress indicators such as marketable yield is not accepted. Water stress at the I66 had a significant effect on the marketable yield of *S. retroflexum* as it performed better when compared to the other irrigation treatment I33 and I100. I33 had the lowest marketable yield of all irrigation levels tested.

## 3.7 Recommendations

The marketable yield results presented in Table 4, illustrated the imperfect conception that *S. retroflexum* is adapted to soil low in N fertiliser, which is normally practiced by resource poor farmers in smallholder farming system. From these results, we can conclude that soils with N in adequate quantities promote aerial vegetative growth and the development of deep green colour in the leaves which is associated with chlorophyll content of plants. *Solanum retroflexum* requires soluble and available plant nutrients for sustainable and profitable production. Good irrigation management practices are required to ensure that sufficient supply of soil moisture is sustained throughout the growth season of ALV's without compromising the marketable yield of *S. retroflexum*. N application to the crop is aimed at improving effectiveness in their growth and for optimum marketable yield. From this study it is concluded that 150 kg $N{\cdot}ha^{-1}$ should be applied. Adequate irrigation and efficient N management practices are required to improve the ratio of biomass production over transpiration, has been shown to be fairly constant for a given species in a particular climatic condition. I66 produced the highest fresh biomass yield and it's recommended that farmers manage their soil water very well in-order to achieve optimum yields.

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# CHAPTER 4

## Nutritional water productivity and irrigation use efficiency of African nightshade *(Solanum retroflexum)* as affected by nitrogen and water application

### Abstract

Soil fertility and irrigation application are key inputs for production of African nightshade (*S. retroflexum)* as it enhances the nutritional quality. An experiment was conducted as a factorial trial design with four fixed N application rates (0, 75, 150 & 225 kg N·ha$^{-1}$) and three irrigation treatments (33, 66 and 100% of field capacity) to determine the irrigation use efficiency (IUE) and the nutritional water productivity of African nightshade (*S. retroflexum*) under rainshelter conditions at ARC:VOP campus, Roodeplaat, Pretoria in South Africa. A high IUE was observed at I66%FC treatment, followed by I100%FC and lowest IUE at I33%FC, indicating that *S. retroflexum* requires sufficient irrigation. Deficit irrigation had an effect on mean nutritional water productivity (NWP) of iron (Fe) & zinc (Zn) although Magnesium (Mg) availability was not affected by neither N nor irrigation application. Moreover, the highest mean Fe was achieved at I66%FC (1787. 16 mg kg$^{-1}$) and second highest mean Fe was obtained at I33%FC (1434.16 mg kg$^{-1}$) and the lowest was achieved at 100%FC (1158.88 mg kg$^{-1}$) of dry material. The Zn content followed the similar trend of Fe at I66%FC achieved highest NWP with 150 kg N·ha$^{-1}$ suggesting that irrigation and N are crucial inputs required for optimum NWP. In conclusion, the overall nutritional yield results demonstrated a decreasing trend with a decrease in the water-deficit treatment, but could be ideal for cropping systems by smallholder and commercial farmers in water scarce areas.

***Keywords*** African leafy vegetables; malnutrition; deficit irrigation; micronutrients

## 4. 1 Introduction

Climate change and poor soil fertility form part of major setbacks for ensuring food security in the sub-Saharan Africa (Scherr, 2001), placing the substantial majority of the resource poor smallholder farmers in a vulnerable situation for optimum production of vegetables sustainably with irrigation and N stress, which are key limiting factors in crop production for normal growth and development in ALV's. Oelofse & van Averbeke, (2012) showed that ALV's have the potential of providing more than 50% of the recommended daily allowance for Fe, Zn, and Vitamin A, which are required for malnutrition and poverty alleviation in South Africa. It is further stated that ALV's are highly nutritious, and drought tolerant as compared to exotic vegetables. Consumption is presently hampered and highly variable across regions by a variety of factors such as lack of technical knowledge and information, lack of quality seeds, poor marketing, and negative cultural connotations of the "poor man's crops" which results in poor policy frameworks (Wenhold et al., 2012).

African nightshade (*S. retroflexum*) is one of the most common indigenous leafy vegetables utilized by rural communities of South Africa and its regarded as a $C_4$ crop, which means they have a lower intercellular $CO_2$ concentration (Cci), thus they produce more dry matter yield per unit water which improves their water use efficiency (Beletse et al., 2012). Thus, the crop fits well within the purpose towards alleviating the poverty and malnutrition, because it is suggested that they withstand harsh adverse environmental conditions such as drought, higher temperatures, and infertile marginal soils. Their exceptional drought adaptation and higher nutritional value in Mg, Fe and Zn, make them ideal crops for a water-constrained country such as South Africa (Oelofse & Averbeke, 2012). Mwai et al., (2007), stated that *S. retroflexum* play a vital role in bridging the nutritional and poverty gaps and it is reported to be a rich source of micro nutrients and the crop is also listed among the three top ALV's identified for promotion and improvement through intense research work. These provide opportunities to work on the nutritional water productivity and irrigation use efficiency of *S. retroflexum* in South Africa with different inputs, with the aim of producing more nutritious food with the same or less water, without compromising the quantity or quality.

Nutritional water productivity is calculated as water productivity multiplied by the nutrient content of crops (NWP = WP x nutrient content of crops). Its units are nutritional unit per volume of water used as described by Vincent & van Halsema (2012). Re-introducing the crop to South African farming systems requires knowledge of yield response to agronomic management practices. Mavengahama et al., (2013) stated that more controlled experiments need to be conducted in order to understand the effect of fertiliser application, and leaf harvesting on the nutritional composition of traditional leafy vegetables (TVC's). The application of N and irrigation are crucial in sustainable production. The study on the effect of management practices could be beneficial in enhancing highly nutritious marketable yield of *S. retroflexum*. Literature on the irrigation use efficiency and nutritional water productivity of *S. retroflexum* is, however, very limited, and therefore further research work was required to help alleviate malnutrition and poverty in sub-Saharan Africa. Thus, the research objective of the study was to quantify and document the effect of management practices of N and irrigation on the irrigation use efficiency and nutritional water productivity of *S. retroflexum.*

## 4.2 Material and methods

The experimental site was described in chapter 3, section 3.2.1. Experimental treatments and irrigation management are described in chapter 3, section 3.2.4

## 4.3 Irrigation use efficiency

Crop water use or evapotranspiration of varying treatments was estimated using the soil water balance equation according to Jovanovic & Annandale (1999):

$$Eta = I - R - P \ -Dr - \Delta Q$$

Where: I is irrigation, R is runoff, Dr is deep drainage below the rooting depth (0.6 m), and ΔQ represents soil water storage. All terms are expressed in mm. R was assumed to be negligible because a trench was dug between the plots and plastic sheeting was inserted vertically to a depth of 1, 5 m to limit the movement of water between plots. A positive ΔQ indicates a gain in soil water storage. ΔQ was estimated from soil water content measurements to be conducted using a neutron probe between two irrigation intervals to a depth of 1.0 m.

## 4.4 Nutritional water productivity

African nightshade (*S. retroflexum*) leaves were sampled at an interval of 21 days after transplanting to determine the effect of deficit irrigation and N application on the nutritional content of the crop. The different combinations treatments from different replicates were oven dried, grinded and mixed to make a composite leaf sample (as described in section 3.2.5). Standard leaf nutritional analysis were performed from composite samples from four shoot harvests, and subjected to statistical analyses to observe treatment effects on the nutritional water productivity. The leaf samples were sent to Agricultural Research Council-Soil Water Climate campus (Pretoria, South Africa) for standard nutritional content (NC) analysis of Mg, Fe and Zn. Nutritional water productivity (NWP) was calculated on a Microsoft excel spreadsheet according to the method of Renault (1999):

$$\text{Nutritional water productivity} = \frac{\text{Marketable yield}}{\text{Irrigation use}} \times \text{Nutritional content} \quad \text{…..Equation .2}$$

## 4.5 Statistical analysis

Data plant samples were harvested from an area of $1m^2$ within the plot and the same plants were consistently harvested throughout the trial to minimise experimental error and variation within the replicates. The statistical method used was described in chapter 3, section 3.7.

## 4.6 Results and discussion

Bruulsema et al. (2012) stated that by the year 2050, the human population will require a 70% increase in global agricultural production, moreover South Africa is experiencing increasing malnutrition, especially Fe and Zn deficiencies. In many parts of Africa, the production of *S. retroflexum* is subjected to climatic limitation of soil water deficit. Wang et al., (2003), suggested that soil water deficit resulting in plant stress, which has adverse effects on nutritional quality, can cause significant reduction in nutritional water productivity. Some ALV's are excellent rich sources of micronutrients (Odhav et al., 2007), but their availability are influenced by input factors such as water availability to the plant, (Masinde et al. 2006) and fertilizers use (Van Averbeke et al., 2012). Table 6 presents results of nutritional water productivity and irrigation use efficiency (kg ha $mm^{-1}$) of *S. retroflexum* from different irrigation and N treatments application rates within 400 mm crop root zone.

Table 6 illustrates that N and irrigation application had clear statistical effects on the *S. retroflexum* IUE and NWP, Maximum IUE and NWP obtained in the moderately irrigated treatment I66 with 150 kg N ha$^{-1}$. Results in table 5 also showed that the I33 irrigation treatment had the lowest IUE and the lowest NWP as compared to the I100, indicating the possibility that *S. retroflexum* requires sufficient agricultural production inputs to be produced under limited soil water conditions but might not be economical to farmers. I100 0-75 kg N·ha$^{-1}$ had achieved similar productivity as compared to I33.

**Table 6:** NWP and IUE of African nightshade (*S. retroflexum*) as effected by N and irrigation

Irrigation: I100%FC= Irrigation 100% of field capacity, I166%FC= Irrigation I66% of field capacity, I133%FC= Irrigation 33% of field capacity.

Nitrogen: N0 =0 kg N·ha$^{-1}$, N75 kg = 75 N·ha$^{-1}$, N150 =150 kg N·ha$^{-1}$, N225 =225 kg N·ha$^{-1}$.

| **Treatments** | **Nitrogen application rate** | **Total marketable yield** | **Total irrigation use** | **Irrigation use efficiency** | **Nutritional water productivity** | | |
|---|---|---|---|---|---|---|---|
| **%FC** | **kg N·ha$^{-1}$** | **(t ha$^{-1}$)** | **(mm)** | **(kg ha$^{-1}$ mm)** | **Fe mg.~~/~~kg$^{-1}$** | **Zn mg.~~/~~kg$^{-1}$** | **Mg mg.~~/~~kg$^{-1}$** |
| 33 | 0 | 9,97 | 274 | 36.39$^{d}$ | 521.59$^{f}$ | 150.47$^{e}$ | 30.06$^{c}$ |
| | 75 | 12,56 | 204 | 61.56$^{b}$ | 1724.71$^{de}$ | 509.82$^{d}$ | 36.34$^{bc}$ |
| | 150 | 16,59 | 267 | 62.13$^{b}$ | 2032.69$^{d}$ | 619.85$^{cd}$ | 39.88$^{b}$ |
| | 225 | 14,99 | 265 | 56.56$^{c}$ | 1453.59$^{e}$ | 620.75$^{cd}$ | 34.34$^{bc}$ |
| 66 | 0 | 10,59 | 385 | 27.50$^{d}$ | 1787.04$^{de}$ | 310.84$^{e}$ | 18.85$^{d}$ |
| | 75 | 17,04 | 304 | 56.05$^{c}$ | 3682.49$^{c}$ | 1343.80$^{bc}$ | 40.25$^{ab}$ |
| | 150 | 26,08 | 276 | 94.49$^{a}$ | 6469.42$^{a}$ | 4023.07$^{a}$ | 46.86$^{ab}$ |
| | 225 | 19,01 | 268 | 70.93$^{b}$ | 4825.60$^{b}$ | 1978.95$^{b}$ | 55.34$^{a}$ |
| 100 | 0 | 7,06 | 453 | 15.58$^{e}$ | 297.58$^{g}$ | 197.29$^{e}$ | 12.80$^{d}$ |
| | 75 | 15,25 | 362 | 42.13$^{cd}$ | 1254.07$^{e}$ | 984.44$^{c}$ | 29.92$^{c}$ |
| | 150 | 22,22 | 398 | 56.83$^{c}$ | 2252.36$^{d}$ | 1607.53$^{b}$ | 46.11$^{b}$ |
| | 225 | 18,8 | 567 | 33.04$^{d}$ | 831.51$^{f}$ | 625.34$^{cd}$ | 26.77$^{c}$ |

The irrigation use efficiency results in table 6 concur with those obtained by Beletse et al. (2012), who stated that *S. retroflexum* responded differently to different irrigation treatments. It was reported that less irrigated treatment (25% FC) resulted in lower biomass yield as comparable to the well irrigated treatments and recorded higher water productivity. In this study, IUE varied significant within the different N and irrigation applications, although maximum productivity was obtained where deficit irrigation I66 was applied. The results suggest that *S. retroflexum* responded differently to different irrigation treatments. Less irrigated treatments I33 resulted in lower yields compared to the well irrigated treatment I66 and I100, suggesting that increased water application leads to increased biomass yields.

NWP for Fe, Zn, and Mg were calculated and analyses of variance conducted. Results in table 6, indicate that there were statistically significant ($p$<0.05) interaction effects between the irrigation and N treatments, with regard to Fe and Zn, although there was less effect on irrigation and N on the Mg NWP on *S. retroflexum*. The results in table 6, of NWP Fe for *S. retroflexum* with stressed irrigated treatment (I33) ranged from 521.89 to 2032.89 mg kg$^{-1}$ with a mean of 1433.16 mg kg$^{-1}$, while the moderately irrigation treatment (I66%FC) Fe NWP ranged from 1787.04 mg kg$^{-1}$ to 6469.42 mg kg$^{-1}$.

The Fe NWP of I100%, ranged the from 297.58 to 2252.36 mg kg$^{-1}$ and a mean of 1158.88 mg kg$^{-1}$. This suggest that there was a significant increase in the Fe when irrigation was increased from I33 to the I100, the NWP increased linearly with an increase in N application, optimum Fe NWP was obtained with 150 kg N ha$^{\mathbf{-1}}$, but declined significantly at application 225 kg N·ha$^{-1}$ in both irrigation treatments. The maximum Fe NWP was however obtained with I66 with 150 kg N·ha$^{-1}$. The NWP Fe ranges for *S. retroflexum* in this study are acceptable when compared to those of spinach leaves (Bhattacharjee, 1997), influenced by input application especially N. Therefore a significant interaction between fertilizer and irrigation application exists, with respect to micro nutrient availability of *S. retroflexum*, which could be ideal for Fe malnutrition alleviation in resource poor households. Table 6 illustrates that I33 had the lowest NWP Zn of *S. retroflexum* which ranged from 150.47 to 620.75 mg kg$^{-1}$ and a mean of 661.28 mg kg$^{-1}$, the lowest N application resulted in the lowest

Zn content, while the NWP peaked with an increase N application rate, especially at 150 kg N·ha$^{-1}$ but declined sharply at 225 kg N·ha$^{-1}$.

The Zn NWP of I66% ranged from 310.84 to 4023.07 mg kg$^{-1}$ with a mean of 1914.17 mg kg$^{-1}$. This suggested that there was a significant increase in the Zn NWP when the irrigation applied from I33 to the I100, the NWP increased linearly with an increase in N application, optimum Zn NWP was obtained with 150 kg N ha$^{\mathbf{-1}}$, but declined significantly at application 225 kg N ha$^{\mathbf{-1}}$ in both I33 and I66, and the optimum NWP productivity was obtained in I66. This is very important for poverty and malnutrition, especially in women and children, as stated by Oelofse and Van Averbeke (2012). Mg was lower in all the irrigation and N treatments, applying less water had no negative effect on nutritional water productivity of *S. retroflexum*. The results depict that NWP was always higher on the I66with 150 kg N·ha$^{-1}$ treatment as compared to I33 and I100 (0 and 75 kg N·ha$^{-1}$.

## 4.7 Conclusion and Recommendations

The main aim of this study was to assess the irrigation use efficiency and nutritional water productivity for selected micronutrients (Fe and Zn and Mg). Main findings of the study indicate that *S. retroflexum* is highly nutritious in Fe, Zn and Mg, which play a significant role in malnutrition alleviation. The highest nutrient contents for Fe and Zn were obtained from the moderately irrigated treatment with 150 kg N·ha$^{-1}$. Although, the less irrigated treatments (I33) resulted in lower NWP caused by water stress lead to high evapotranspirative demand, and high air and soil temperatures, resulting to be the main causes of poor performance of the ALVs in the low water treatments compared to the well irrigated treatment (I66 and I100), suggesting that increased water application and N could lead to increased NWP. These results suggest that *S. retroflexum* is more efficient in utilizing water and N for Fe and Zn uptake. Moreover, *S. retroflexum* can be ideal for cropping systems by smallholder and commercial farmers in water scarce areas such as South Africa.

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# CHAPTER 5

## $^1$H-NMR based metabolomic profiling and compound annotation of African nightshade (*Solanum retroflexum*) to determine the effect of irrigation and nitrogen management

### Abstract

African nightshade (*Solanum retroflexum*) is utilized in various parts of South Africa, primarily as a source of food and for medicinal purposes. Currently there is no scientific relationship between the nutritional content of *Solanum retroflexum* and production practices such as irrigation and N application. The aim of the study was to investigate the effect of soil moisture content, N levels and shoot harvest on the phytochemical composition. The present study was done to evaluate the interactive effect of four fixed applied rates of N (0, 75, 150 225 kg N·ha$^{-1}$) and three irrigation treatments (I33%, I66% and I100% FC) applied through a drip irrigation system, performed under a rainshelter. $^1$H-NMR-based metabolomic profiling was conducted, while orthogonal partial least square discriminatory analysis (OPLS-DA) was performed on $^1$H-NMR spectral data. The analysis revealed a clear separation between I33 and I66% FC. There was no separation of the samples regarding the N treatments which is indicative of the small effect of N on the metabolite content of the respective treatments. Chlorogenic acid, proline, sucrose and trigonelline were responsible for the separation of the irrigation treatments and shoot harvests. The analysis resulted in annotation of metabolites, which are responsible for the difference between severely and moderately irrigation stress conditions of *S. retroflexum*.

***Keywords*****:** water stress, metabolomics, primary metabolites, secondary metabolites, phytochemical, $^1$H-NMR

## 5.1 Introduction

African nightshade (*S. retroflexum*) as an ALV forms an integral part of a collective of leafy vegetable species that has historically been part of the traditional foods of African rural communities (Oelofse & Van Averbeke, 2012). Sustainable agriculture requires continuous innovation in order to improve the management of natural resources, especially irrigation water and N. Pirzad et al. (2012), indicated that medicinal and phytochemical composition of plants is generally influenced by deficit water and duration of stress induced. Newbould (1989), stated that plants utilize nearly 50% of the applied N which implies a significant loss in revenue and N productivity. Deficit irrigation is an important strategy utilized to reduce agricultural water use in arid and semi-arid regions (Ayana, 2011). There are high demands for efficiency, increased environmental concerns and fertility management on environmental preservation for future generations. $^1$H-NMR is described as a technique for metabolomic profiling studies as it does not require extensive solvent sample preparation and can uniformly detect all compounds with $^1$H-NMR-measurable nuclei (Yang, 2006). Plant metabolomics requires an effective extraction method of metabolites from plant cells because of huge variation in chemicals diversity in plants (Hunter, 2008).

Thus, analytical techniques such as $^1$H-NMR based metabolomics could be crucial for successful identification and quantification of specific metabolites, which can be linked to human nutrition (Ramautar et al., 2006). Coumarins has been conjectured to be synthesised in response to water stress (White et al., 2008) in *Pelargonium sidoides*. A detailed systematic study on *Salvia miltiorrhiza* in response to water depletion was reported by Dai et al. (2010), who identified primary and secondary metabolites as a result of management practices. Early response to water stress supports new metabolic and structural capabilities mediated by altered gene expression, to improve plant functioning under stress (Chaves et al., 2002). Metabolite availability in samples are not biological-specific; hence, a multivariate analysis approach for a particular metabolite or metabolite class can be adopted regardless of the species being investigated (Goodacre et al., 2004). Cubero-Leon et al., (2014) suggested that one of the advantages of using $^1$H-NMR untargeted approaches is that unexpected changes in the metabolite profile of food may be detected without the need of an a-priori hypothesis.

Meanwhile, Capuano et al., (2013), mentioned that this technique would require the establishment of larger databases that take into account greater variability and must be based on well-designed experimental studies that include all sources of variation such as individual variability or fertilisation practice, geographical location and climate.

While, Rowena & Soga, (2007) reported that metabolite profiling involves identification and quantitation of a group of metabolites common to chemical class, $^1$H-NMR techniques have recently been linked and combined with multivariate data analysis to characterize metabolites and profile various plants (Kim et al. 2011). However, the quality control of vegetables was previously done via $^1$H-NMR based metabolomics approach (Frederich et al. 2011), who observed variation in compound availability as a result of agronomic practice. Principal component analysis (PCA) is an unsupervised clustering method used for reduction of data complexity (Lindon et al. 2001). Meanwhile, Choi, (2006) stated that PCA is used to describe variance in a set of multivariate data in terms of a set of underlying orthogonal variables and orthogonal variability representing metabolite concentrations expressed as a particular linear combination of the principal components.

Cultivation of *S. retroflexum* has been considered as a commercially viable means of improving the nutritional content, and identifying phytochemical composition as a result of N and deficit irrigation application is therefore important. Advancement of both medicinal and nutritional properties of *S. retroflexum* requires identifying, quantifying, analysing and translating the importance of the different chemical components of the crop, as well as the shift of these components as a result of agronomic management strategies imposed on the plants during the growth season. Thus, the relationship between chemical composition and, metabolic profiling and compound identification through high technology approaches to monitor changes between the different N and irrigation treatments under a rainshelter condition and site-specific management practices, with different shoot harvest in particular remains unstudied.

## 5.2 Materials and methods

### 5.2.1 Site and weather description

The experimental site and weather data was described in detail in chapter 3 section 3.2.1.

### 5.2.2 Experimental and design and irrigation management

The experimental design and irrigation water use during the crop growth seasons from planting to senescence were described in detail in chapter 3, section 3.2.4.

### 5.2.3 Sample collection

The sample collection and harvesting procedure were described in chapter 3 section 3.4.5.

### 5.2.4 Sample preparation and extraction method for general metabolomics

Leaf samples were grinded through a 1 mm sieve and were prepared using a direct extraction method, for an untargeted metabolomic approach for profiling of metabolites. A 600 MHz $^1$H-NMR spectrometer with a proton frequency of 599.74 was utilized to determine metabolomic profiling. The sample preparation, extraction and data processing were performed using methods described by Maree & Viljoen, (2012). Fifty milligrams of the dried leaf material was dissolved in 750 µl deuterated methanol and 750 µl potassium phosphate buffer ($KH_2PO_4$) with 0.1% TSP dissolved in deuterium water. The mixture was sonicated for 20 minutes and then centrifuged for 10 minutes at 13 000 rpm, room temperature, and 600 µl of the supernatant was transferred to standard 5 mm $^1$H-NMR tubes (Norell, Sigma-Aldrich). Gradient shimming was used to improve the magnetic field homogeneity prior to all acquisitions with 32 scans recorded. All spectra were Fourier- transformed, phase-corrected and baseline-corrected manually.

### 5.2.5 $^1$H-NMR data mining and processing

$^1$H-NMR spectra were processed using MestReNova 10.0 (Mestrelab Research). The regions containing the water (4.75-5.10 ppm) and methanol (3.28-3.33ppm) peaks were excluded, and remaining spectral regions divided into 0.04-ppm bins, and converted to ASCII format. The ASCII files were imported to Microsoft Excel 2010 for variable labelling according to treatments and ultimately transposed. Subsequently the transposed and treatment labelled excel files were imported to a Multivariate statistical projection method using SIMCA 13.0 (Umetrics, Umeå, Sweden). It was utilized to compare sets of spectra to identify clusters of similarities or differences between the different N and irrigation treatments, which help in classification of individual plant samples and their functions. Metabolites responsible for similarities and differences between classes were systematically investigated using loading and contribution plots that resulted from PCA and OPLS-DA plots (Krishnan et al. 2005).

## 5.3 Results

The PCA, which is an unsupervised analysis method and the score scatter plot show separation of samples, based on their chemical profiles. The second phase of OPLS-DA analysis, is a supervised pattern recognition method of which the ultimate purpose is to discriminate systematic variation in the X-matrix into parts, in which one component is linearly related to the Y-matrix (Maree & Viljoen, 2011), followed by the contribution plots, which identify NMR regions of importance for clustering. Chenomx database the Human Metabolome Database and previously published data were used in annotation of the metabolites important in the samples. Figure 4 presents the PCA analysis, showing no separation between the N and irrigation treatments. Performing analysis of the N treatments alone, also did not result in any separation.

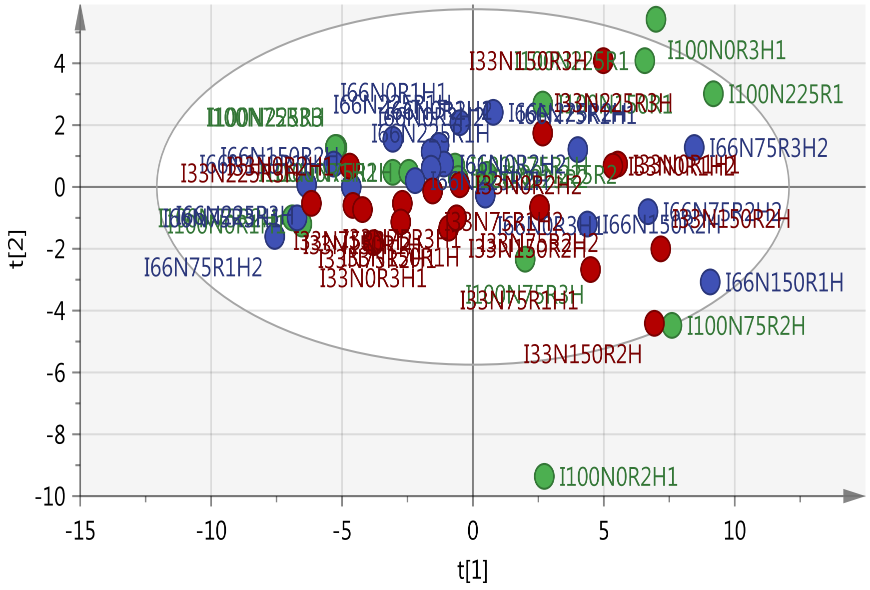
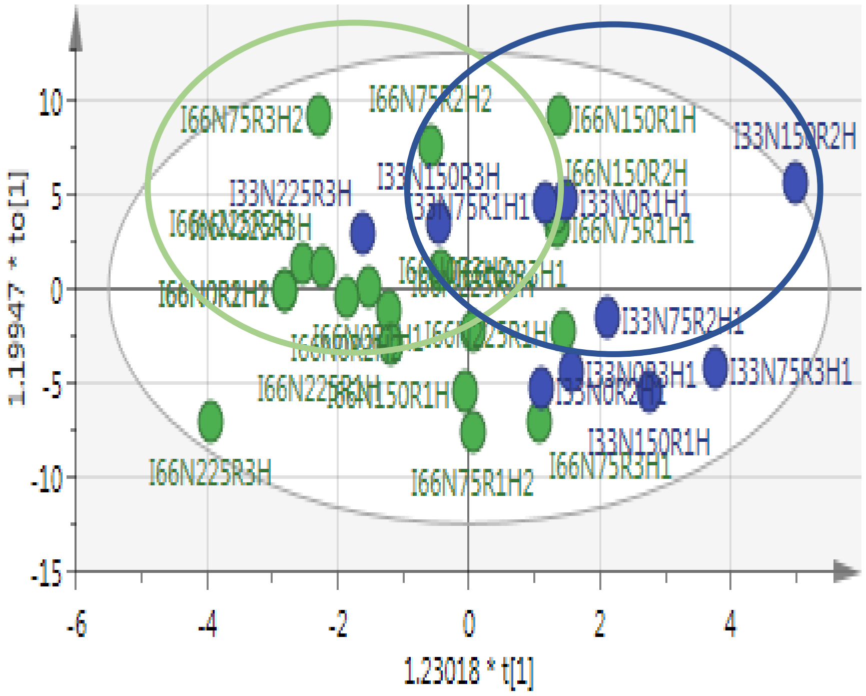


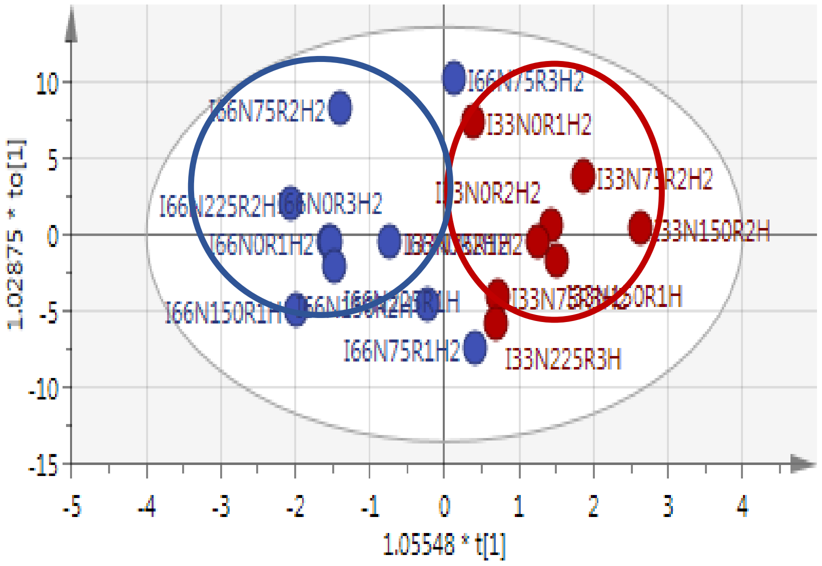
**Figure 4**: PCA analysis with 33% FC (green) with its respective N (0, 75, 150, 225 kg N·ha$^{-1}$), 66%FC (blue) with its respective N (0, 75, 150 225 kg N·ha$^{-1}$) and 100%FC (red) with its respective N (0, 75, 150, 225 kg N·ha$^{-1}$).

Since no separation of the treatments was observed with the PCA, an OPLS-DA plot was constructed. To simplify the analysis, a pairwise comparison of the I33 and I66 samples was done, which provided better separation between the clusters for the first harvest (Figure 5A) and second harvest (Figure 5B). Some overlap of treatment samples was still observed towards the centre of the ellipse. Better separation was observed for the second harvest (Figure 5B) with clear separation of the I33 and I66 treatments.



~~A~~

**Figure 5 A**: OPLS-DA of harvest 1, with ellipse representing Hoteling within 95% confidence. I66% FC (green) with its respective N (0, 75, 150 225 kg N·ha$^{-1}$) and I33%FC (blue) with its respective N (0, 75, 150 225 kg N·ha$^{-1}$).



~~B~~

**Figure 5 B**: OPLS-DA of harvest 2, with ellipse representing Hoteling within 95% confidence. I66% FC (blue) with its respective N (0, 75, 150 225 kg N·ha$^{-1}$) and I33%FC (red) with its respective N (0, 75, 150 225 kg N·ha$^{-1}$).

Even better separation was observed with a pairwise OPLS-DA analysis of the I33 and I100 samples for the first harvest (Figure 6A). Slightly more overlap of the samples was observed for the second harvest (Figure 6B).

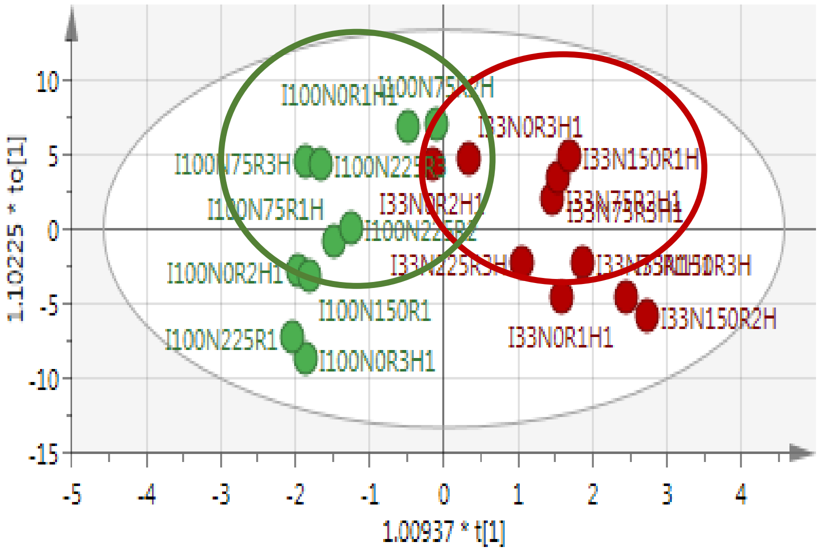


**Figure 6 A**: OPLS-DA of harvest 1, with the ellipse representing Hoteling within 95% confidence. I33%FC (red) with its respective N (0, 75, 150 225 kg N·ha$^{-1}$) and I100%FC (green) with its respective N (0, 75, 150 225 kg N·ha$^{-1}$).

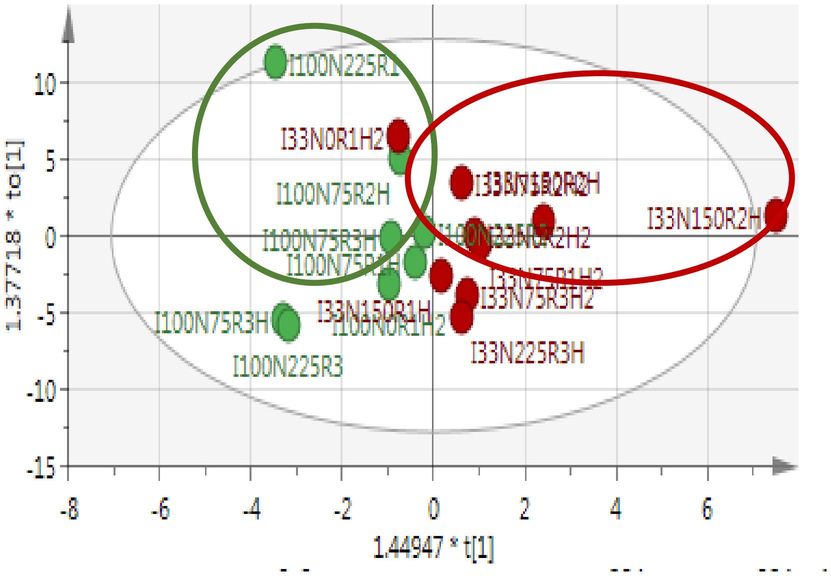


**Figure 6 B**: Harvest OPLSDA results of *Solanum retroflexum* on $^{1}$H-NMR spectra with the ellipse representing Hoteling within 95% confidence. I33%FC (red) with its respective N (0, 75, 150 225 kg N·ha$^{-1}$) and I100%FC (green) with its respective N (0, 75, 150 225 kg N·ha$^{-1}$).

Data sets exposed to OPLS-DA analysis illustrated a good separation between the I33 and I100 with their respective N application rates, with the two shoot harvests. A contribution plot was constructed to determine the $^{1}$H-NMR regions that were responsible for the clustering (Figure 7). The bars above the line represent $^{1}$H-NMR regions that were positively associated with the I33 and the bars below the line associated with the I100. Chenomx, the Human Metabolome Database and previously published data were used to annotate the compounds in the samples.

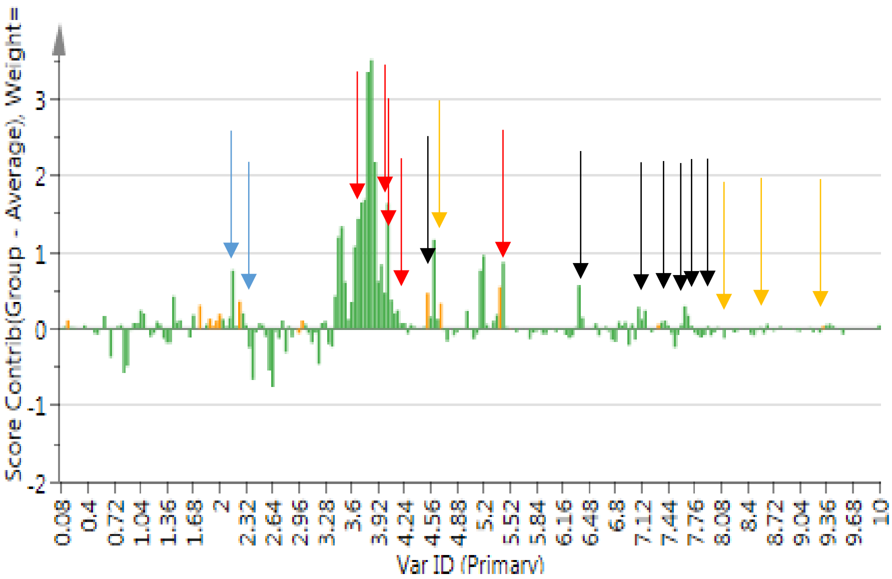


**Figure 7:** Contribution plot illustrating $^{1}$H-NMR regions, which had an effect on the separation of the irrigation, N and shoot harvests for I100%FC and I33%FC (harvest 1). Red = sucrose, blue = proline, black = chlorogenic acid and yellow = trigonelline.

The $^{1}$H-NMR regions for the annotated compounds were compared with the values found in literature, the human metabolome database, and with the database Chenomx (table 7). All the compounds were positively associated with the I33 treatments and represent an increase or production of these compounds as a result of this treatment.

**Table 7**: Annotated compounds from the $^1$H-NMR analysis and human metabolome database.

| Treatment combination | Metabolite | Human metabolome database NMR peaks (ppm) | Actual NMR peaks from the study (ppm) |
|---|---|---|---|
| I33%FC | Sucrose | 5.40 | 5.39 |
| | | 4.21 | 4.16 |
| | | 4.04 | 4.02 |
| | | 3.89 | 3.84 |
| | | 3.87 | 3.82 |
| | | 3.75 | 3.79 |
| | | 3.55 | 3.55 |
| I33%FC | Chlorogenic acid | 7.65 | 7.61 |
| | | 7.19 | 7.13 |
| | | 7.12 | 7.05 |
| | | 6.94 | 6.88 |
| | | 6.39 | 6.36 |
| | | 4.25 | 4.26 |
| | | 3.88 | 2.12 |
| I33%FC | Proline | 4.12 | 4.18 |
| | | 3.41 | 3.41 |
| | | 3.33 | 3.34 |
| | | 2.34 | 2.39 |
| | | 2.06 | 2.01 |
| I33%FC | Trigonelline | 9.13 | 9.14 |
| | | 8.85 | 8.82 |
| | | 8.82 | 8.81 |
| | | 8.08 | 8.38 |
| | | 4.43 | 4.42 |

Overlaying the $^1$H-NMR profiles of the I33 and I100 treatments shows clearly the change in peak height (Figure 8).The height of the peak represents the concentration of the compound in the extract and a higher peak therefore indicates an increased concentration.

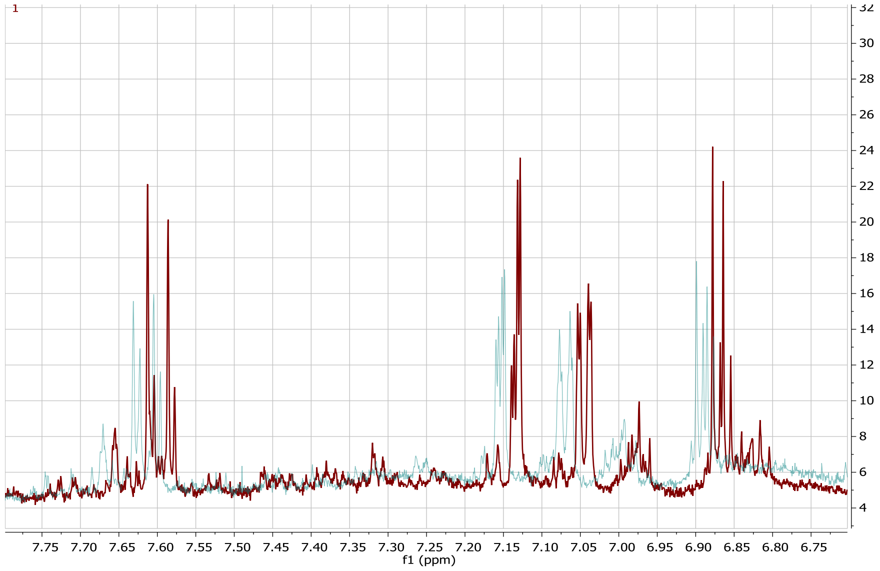


**Figure 8**: H-NMR spectrum of I33%FC (red) and I100%FC (blue) showing the increase in peak height.

Figures 9-12: Represent the structures of the chlorogenic acid, trehalose, proline and trigonelline which were annotated.

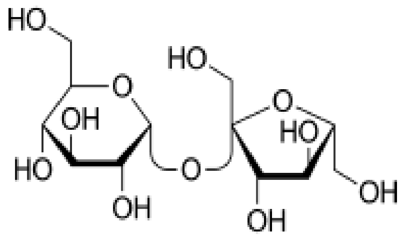


**Figure 9**: Sucrose

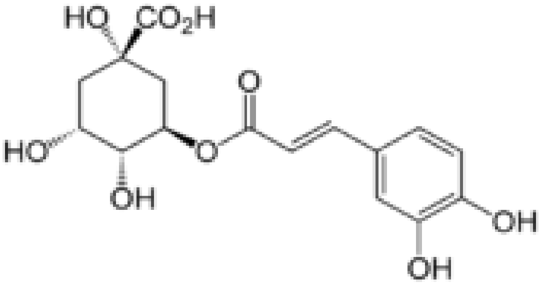


**Figure 10**: Chlorogenic

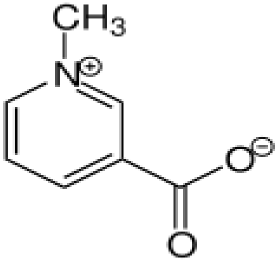


**Figure 11**: Trigonelline

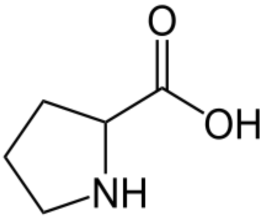


**Figure 12**: Proline

**5.4 Discussion**

Wang et al., (2007), described PCA as a best-known technique for exploratory multivariate analysis for data visualization. Kumar (2015), stated that chemical fingerprinting involves the collection of spectral data for extracts in standardized conditions and ignores the problem of making individual assignments of peaks in the complex $^1$H-NMR spectra. Jahangir et al. (2008), used $^1$H-NMR to identify between 16 and 20 compounds representative for food quality metabolomics. On the contrary, an untargeted metabolomics approach focuses on the detection of as many groups of metabolites as possible to obtain fingerprints without necessarily identifying or quantifying specific compounds (Monton & Soga, 2007).

$^1$H-NMR based metabolomics is an emerging technique based on large-scale qualitative and quantitative measurements, and the chemical identification of metabolites is fundamentally important for extraction of biological context from the data (Bedair & Sumner, 2008). An investigation on the agronomic effect of different application rates of irrigation, N management was done using $^1$H-NMR to profile metabolites and using the Chenomx software programme and the human metabolome database for compound annotation of shoot harvests of *S. retroflexum*. The PCA analysis showed no separation for the treatment combination of N and irrigation. However, when the data sets were transformed to the supervised method of data analysis (OPLSA-DA), there were grouping of irrigation treatments with their respective N application, which suggest that large variation between the irrigation treatments. However stressed conditions lead to significant increased synthesis in various compounds including secondary metabolic compounds as was also reported by Lea et al., (2007).

Metabolomics have been used in various studies to identify compounds differentiating treatments. In a comparative study between non-irrigated and irrigated soya beans in response to irrigation on the concentration of trigonelline, Choi et al., (2003) observed that trigonelline concentrations ranged from 364 to 555 μg $g^{-1}$(d.m.) under irrigation, and from 404 to 570 μg $g^{-1}$(d.m.) under non-irrigation. In this study, similar results were obtained with trigonelline that showed an increase under water stress treatments (I33).

Vegetables synthesize and accumulate phytochemicals that are often important in signalling and stress protection against drought or abiotic stress (Ashraf, 2007; (Mafakheri et al., 2010). Chalker-Scott & Fnchigami, (1989) stated that nutrient stress also has a marked effect on phenolic levels in plant tissues. Chlorogenic acid is a naturally occurring phenolic acid in human diet and various pharmacological activities inherent to medicinal plants has been attributed to their phenolic composition. Verbruggen & Hermans, (2008) observed significant accumulation of free proline content with a variety of plant species as an adaptive response mechanism to environmental adverse conditions to avoid severe water loss and wilting. It has been reported that under deficit soil water conditions the free proline content is prone to increase as a physiological response, which leads to a reduction of cellular water (Alhasnawi et al. 2014). Proline and chlorogenic acid was also annotated in this study, and it was increased in the stressed water treatment (I33) in African nightshade (*S. retroflexum*) which confirms the reports of various authors in previous studies, (Verbruggen & Hermans, 2008).

Biological activities of *S. retroflexum* are unknown although it has been utilized for centuries as medicine and a nutritious food source (Oelofse & Van Averbeke, 2012). ALV’s are reported to have medicinal properties (Jansen van Rensburg 2007), which could assist with ensuring wellbeing in humans with alleviation of illness. Compounds such as trigonelline and chlorogenic acid have proven medicinal uses such as for diabetes and central nervous system. This is the first report of annotation of these compounds in *S. retroflexum* and explains some of the medicinal uses of the plant.

## 5.5 Conclusion

Agronomic management strategies of N and irrigation are required to assist farmers and agronomists to make sound scientific sound decisions to significantly improve yield, but also take into consideration the phytochemical concentrations and profiles in vegetable crops. $^1$H-NMR-based metabolomics has been successfully applied to identify the metabolomic changes in *S. retroflexum* with different N and irrigation application rates. $^1$H-NMR profiling was done to illustrate the variations and separation of the three irrigation treatments and N treatments into clusters, however there were no good separation regarding the fertiliser treatments. Primary metabolites such as sucrose, and proline, which are known stress compounds, were increased in the I33 treatment. The secondary metabolites trigonelline and chlorogenic acid were also increased in the water stressed treatment, and is often associated with stress conditions in plants to protect them in adverse conditions.

## 5.6 References

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# CHAPTER 6

## 6.1 Conclusions and recommendations

In Southern Africa, nutritional food insecurities, climate change and poor soil fertility are a major problem. This has the likely potential to further exacerbate nutritional securities particularly of the poor and vulnerable groups in rural areas. In order to effectively alleviate and combat malnutrition, commercial and emerging farmers have to promote the consumption of African leafy vegetables, such as *S. retroflexum*, as part of their dietary requirement because they are highly nutritious in Fe, Zn and Mg, through improved agronomic, sound scientific cultivation and ensuring optimum resource use efficiency methods. The study was conducted to evaluate the interactive effects of four fixed applied rates of N (0, 75, 150 and 225 kg N·ha$^{-1}$) and three irrigation treatments (I33, I66 and I100) on nutritional water productivity. $^{1}$H-NMR based metabolomic profiling was performed to annotate compounds associated with water stress in *S. retroflexum*.

The rainshelter experiment suggests that marketable production required the addition of N to produce optimal and profitable marketable yield of *S. retroflexum*. The results clearly showed that the perception that *S. retroflexum* grow well on low soil fertility mostly practised in rural and smallholder farming is incorrect and misleading. In this study, the best marketable yield of 26.08 t ha$^{-1}$ was obtained at I66 with 150 kg N·ha$^{-1}$. N application to *S. retroflexum* is aimed at improving effectiveness in their growth and for optimum fresh marketable yield. From an agronomic perspective this includes ensuring the availability of the N, which address of N deficiencies which could hamper plant growth, whilst avoiding excessiveness, which could have negative effects on plants.

Good irrigation management practices are required to ensure that sufficient supply of soil moisture is sustained throughout the growth season of ALV's without compromising the marketable yield of *S. retroflexum*. This study also disproves the perception that *S. retroflexum* grows well with no or minimal irrigation application as was used in this study (I33). Adequate irrigation and efficient N management practices are required to improve the ratio of biomass production over transpiration, which has been shown to be constant for a given species in a particular climatic condition. The best irrigation regime from the three irrigation treatments, were I66 as I33 and I100 achieved lower marketable yields.

The nutritional water productivity results suggested that there was a reduction in the iron content at I100 when compared to the rest of the irrigation treatments. The ranges of dietary iron content of *S. retroflexum* varies from 43.95 to 115.19 mg kg$^{-1}$ with a mean of 77.78 mg kg$^{-1}$ with I100 irrigation, while the moderately stressed irrigation treatment I66 had the highest iron availability with a range of 153.38 to 186.37 mg kg$^{-1}$ and a mean of 179.37 mg kg$^{-1}$. I33 had the second highest iron content edible portion for *S. retroflexum*, with a range of 175.41 to 126.04 mg kg$^{-1}$ and a mean of 147.41 mg kg$^{-1}$. This was achieved at the lowest N application resulting in the highest iron content and the iron content decreased with an increase in N application rate. There is a significant interaction between fertilizer and irrigation application, with respect to micro nutrient availability of *S. retroflexum*. We can practise deficit irrigation on *S. retroflexum* vegetable if water resources become scarce in the near future and diets of most vulnerable group will not suffer much, although it has significant effects on available micronutrients which are required.

$^1$H-NMR-based metabolomics has been successfully applied to study the metabolomic changes in *S. retroflexum* with different N and irrigation application rates. $^1$ H-NMR profiling was used to illustrate the variations and separation of the three irrigation treatments and N treatments into clusters, however there were no good separation regarding the fertiliser treatments. Primary metabolites such as sucrose, and proline and secondary metabolites chlorogenic acid and trigonelline were annotated as the compounds responsible for clustering of the stressed irrigation samples. Proline is known as a signalling metabolic compound for plants under stress conditions (Verbruggen & Hermans, 2008).

Globally, 80% of the indigenous population in developing countries relies upon traditional medicine and medicinal plants as primary healthcare. Gershenzon (1984) indicated that plants respond to abiotic stresses by inducing various phytochemicals with health-promoting qualities in the human diet, including chlorogenic acid. Trigonelline has been shown to contain antibacterial, antiviral, and anti-tumor activities, and it has been shown to reduce diabetic auditory neuropathy and platelet aggregation (Mahady 2001; Zhou & Zhou, 2012).

The presence of these compounds in *S. retroflexum* therefore supports to the use of *S. retroflexum* for medicinal purposes.

The findings of the study rejects the notion that *S. retroflexum* is drought tolerant and can withstand adverse climatic conditions when compared to a commercial vegetable crop like kale, Swiss chard and mustard spinach. Moreover, with low input application cultivation practices such as (with low irrigation and fertiliser inputs, especially N), water nutritional productivity of *S. retroflexum* decline, but with addition of water and fertiliser it can be improved. Under low application cultivation practices, marketable yield and nutritional productivity decreased significantly, suggesting that water and soil fertility stresses have a major influence on the productivity of *S. retroflexum*. The presence of the annotated primary metabolites namely proline and sucrose, support previous studies showing proline to be an indicator of stress in plants. Secondary metabolites such as trigonelline and chlorogenic acid are linked to medicinal properties and support the use of this plant for nutritional as well as medicinal purposes. Different *S. retroflexum* species should be commercialized in South Africa, since they are highly nutritious and can be used in food-processing to add value and promote the production of the crop in rural and smallholder farmers in South Africa.

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