EFFECTS OF LIGHT MANIPULATION THROUGH DIFFERENT PHOTO-SELECTIVE NET COLOURS AND LEDs ON LETTUCE (*LACTUCA SATIVA L.*) AND CABBAGE AS ESTIMATED BY CHLOROPHYLL FLUORESCENCE PARAMETERS, MACRO- AND MICRO- ELEMENT CONTENT AND PHYSICAL MEASUREMENTS

by
Willem Adriaan Gericke

Thesis presented in partial fulfilment of the requirements for the degree Master of Science in Agronomy at the University of Stellenbosch. This thesis has also been presented at the North-West University in terms of a joint agreement.

UNIVERSITEIT
iYUNIVESITHI
STELLENBOSCH
UNIVERSITY

Supervisor: Dr Marcellous Le Roux
Faculty of AgriSciences
Department of Agronomy

and

Dr Misha de Beer-Venter
Centre for Water Sciences and Management
North-West University
Potchefstroom

December 2018

Declaration

By submitting this thesis/dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that the reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in entirety or in part submitted it for obtaining any qualification.

December 2018

Abstract

Light quantity and quality ₚlays a fundamental role in seed germination, plant growth and development. Plants grown in high radiation regions can experience light stress, resulting in photoinhibition when the photosynthetic capacity of the light harvesting complexes in the photosystems are over-exposed. Shade nets are a cost-effective way to reduce high light quantities and to create a more desirable growing environment. Black shade nets are used widely for vegetable seedling and mature crop production in South Africa, due to its cost effectiveness and availability. Although, recent studies have indicated that coloured shade nets can increase plant responses and physiological processes like seed germination, plant architecture, circadian rhythms as well as plant growth and development. Chlorophyll fluorescence is a non-destructive method used to determine the amount and type of plant stress when a plant is exposed to sub-optimal growing conditions. Unfortunately, no literature could be found to indicate specific chlorophyll fluorescence parameter values, for lettuce and cabbage seedlings and mature crops, grown under different coloured shade nets in high solar radiation environments, and low radiation LEDs.

The objective of this study was two-fold. Firstly, to determine the chlorophyll fluorescence parameters for lettuce and cabbage seedlings and mature plants produced under different coloured shade nets with high solar radiation, and the leaf macro- and micro-element composition of lettuce and cabbage seedlings and mature plants, and physical analyses of mature cabbage plants. Secondly, to determine chlorophyll fluorescence parameters, and leaf macro- and micro-element composition of lettuce seedlings, under different colour combination low radiation LEDs.

The research was conducted by means of three replicates of five different coloured shade nets – with the black net as the control. 'Grand Slam' and 'Islandia' lettuce, and 'Conquistador' and 'Sapphire' cabbage cultivars were grown under each high solar radiation coloured net. The second trial entailed where 'Robinson', 'Grand Slam', 'Lolla Rossa' and 'Multi Red' lettuce seedlings were grown under high radiation under the same coloured shade nets and were compared to different LED combinations of low radiation. An analysis of variance (ANOVA) was used for data analysis, and Fisher's least significant differences were used to determine the mean data comparisons.

Dark pigmented lettuce had higher RC/ABS values than green lettuce, and these values differed statistically per cultivar. The RC/ABS values did not differ between the B+DR and B+FR LEDs, although they were significantly lower than under the coloured shade nets. The PI_{total} values decreased after head formation and this indicates decreasing P_N values. All chlorophyll fluorescence parameters were greatly influenced by plant age. The OJIP transient curve is indicative of P levels in lettuce. The largest P uptake differences between lettuce cultivars were under Photon Red and white nets - while the blue nets produced the least variance for P uptake in different lettuce cultivars. All macro- and micro-element uptake for lettuce seedlings was significantly higher under the low radiation B+DR LEDs than different coloured shade nets under high solar radiation. The B+DR LEDs vastly increased the uptake of Cu, followed by Na, Zn, Ca

Cabbages grown under white nets averaged 8.16 kg, and were 86% heavier than cabbages produced under black nets. Cabbage leaf length and width values were significantly higher under white nets. Also, it produced the lowest N, P and K leaf levels under the Photon Red and white nets, while cabbages under the black nets had the shortest and narrowest leaves - but the highest N, P and K values. The blue nets once again produced the smallest variance regarding the uptake ratio of N, P and K for the different cabbage cultivars.

Key words:

Chlorophyll a fluorescence, Coloured shade nets, LEDs, Seedlings, Macro- and Micro-element.

Opsomming

Lig kwaliteit en kwantiteit speel 'n deurslaggewende rol in saad ontkieming, plantgroei en ontwikkeling. Dit kan ook fotoinhibisie meebring wanneer die fotosintetiese kapasiteit van die lig absorberende komplekse in die fotosisteme oorbelig word, indien lig kwantiteit nie verminder word nie. Plantgroei reaksies en fisiologiese prosesse van 'n plant word beïnvloed deur lig-kwantiteit en kwaliteit te manipuleer, om sodoende 'n gunstiger groei omgewing te skep. Chlorofil fluoresensie is 'n nie-vernietigende metode wat gebruik word om die hoeveelheid en tipe stres te bepaal waaraan 'n plant blootgestel word in sub-optimale groei omstandighede. Skadunette word in die algemeen gebruik om plant stres te verminder, deur die beskikbare sonlig te manipuleer na aanvaarbare vlakke. In Suid-Afrika word swart skadunette hoofsaaklik gebruik vir groentesaailing sowel as volwasse groente produksie, as gevolg van die koste-effektiwiteit en bekostigbaarheid daarvan. Verskeie gewasse wat onder verskillende gekleurde nette gegroei was, het veel beter presteer as onder swart nette. Geen literatuur kon bekom word om die Chlorofil fluoresensie parameter waardes aan te dui vir slaai en kool saailinge en volwasse gewasse wat onder gekleurde nette in hoë son radiasie gebiede gegroei word nie.

Die doel van die studie is twee-voudig. Eerstens, om die Chlorofil fluoresensie parameters vir slaai en kool saailinge en volwasse plante wat onder verskillende gekleurde nette onder hoë son radiasie geproduseer is, te bepaal. Dit sluit ook in die vastelling van blaar makro- en mikro- element komposisie van slaai- en koolsaailinge wat geproduseer is onder verskillende kleur kombinasies van lae radiasie LEDs.

Die navorsing was gedoen deur drie replikasies van vyf verskillende gekleurde nette – met die swart net as die kontrole. "Grand Slam" en 'Islandia' slaai en 'Conquistador' en 'Sapphire' kool kultivars was gegroei onder hoë son radiasie onder gekleurde nette, terwyl 'Robinson', 'Grand Slam', 'Lolla Rossa' en ''Multi Red' slaai saailinge onder verskillende kleur kombinasies LEDs gegroei is. 'n Analise van die variasie (ANOVA) was gebruik vir data analise, en Fisher's minste beduidende verskille was gebruik om die gemiddelde data te vergelyk.

Donker gepigmenteerde slaai het hoër RC/ABS waardes as groen slaai getoon, en hierdie waardes het statisties verskil per kultivar. Die RC/ABS waardes het nie van mekaar verskil onder B+DR en B+FR LEDs nie, en was noemenswaardig laer as onder al die gekleurde nette. Die PI_{total} waardes het afgeneem na kop formasie en dit dui op die afname van P_N waardes. Alle Chlorofil fluoresensie parameters was grootliks beinvloed deur plant ouderdom. Die OJIP oorgangskurwe is aanduidend vir die P vlakke in slaai. Die grootste P opname verskille tussen die verskillende slaai kultivars, was onder foton rooi en wit nette – terwyl blou nette die minste variasie in P opname tussen die verskillende kultivars getoon het. Alle makro- en mikro-element opname vir slaai saailinge was noemenswaardig hoër onder die lae radiasie B+DR LEDs in vergelyking met die verskillende gekleurde nette onder hoë son radiasie. Die B+DR LEDs het die opname van Cu noemenswaardig laat toeneem, asook in 'n mindere mate die opname van Na, Zn en Ca.

Kool het 'n gemiddelde gewig van 8.16 kg onder die wit nette geweeg, en was 86% swaarder as die kool wat onder die swart nette geproduseer is. Die blaar lengte en breedte waardes van volwasse kool was noemenswaardig hoër onder die wit nette, maar het die laagste N, P en K vlakke gehad, terwyl die kool onder swart nette die kortste en smalste blare met die hoogste N, P en K waardes gehad het. Die blou nette het die kleinste variasie in verband met die N, P en K opname verhoudings getoon vir die verskillende kool kultivars.

v

Acknowledgements

I would like to take this opportunity to thank the Lord for the wisdom and perseverance I received from Him, and to my wife, Anel, and children Daniella, Wian and Lisa, for their support and encouragement during the tough times - especially when we moved abroad. Thank you for our company, Bulbs & Blooms, for all the financial expenditure and erecting the trial facilities and the equipment necessary to conduct the studies. Thank you Dr Marcellous Le Roux and Dr Misha de Beer for their advice and support.

Table of Contents

Content

VIII

List of Tables

X

List of Figures

CHAPTER 1

General Introduction

1.1   GENERAL INTRODUCTION

It is predicted that the world population could reach 9.8 billion people by 2050. Hung *et al.* (2004) proved that people who regularly consume fruit and vegetables, have a reduced chance of heart diseases and strokes. Therefore, to ensure a healthy growing population, it is critical to continuously produce quality vegetable seedlings, which will enable a stable global vegetable supply. Increased urbanisation and increased pressure on natural resources contribute to the current drive to produce crops indoors (plant factories).

Light is one of the main factors driving photosynthesis, and a specific combination of light quantity and quality is critical for sustaining plant growth and development. The source of light can be solar radiation or artificial lighting. Solar radiation is influenced by cloud cover and the photosynthetic photon flux density (PPFD) can therefore be very erratic, while artificial lighting produces a constant PPFD. The position of the light sources relative to plant photosynthetic surfaces has a large effect on crop productivity (Bickford and Dunn, 1972). The incident light levels will be affected by a change in the distance between the point source and the energy intercepted by a leaf surface (Bickford and Dunn, 1972). Therefore, the importance is emphasised that solar radiation levels differs vastly between summer and winter in high and low latitude countries. Artificial lighting is use to alleviate the low solar radiation phenomenon during winter months in these countries, and special care must be taken to determine the distance between the crop and the source of artificial lighting, to produce maximum photosynthesis.

Light emitting diodes (LEDs) radiate low levels of heat, while metal halide (MH) and high pressure sodium (HPS) lamps radiate significantly more heat (Nelson and Bugbee, 2015). Consequently, LEDs can be much closer to plant leaves, to produce the same amount of photosynthetic photon flux (PPF) at the photosynthetic surface. Plant pigments can efficiently absorb red wavelengths of 600 to 700 nm and blue

wavelengths of 400 to 500 nm (Sager and McFarlane, 1997) - the ideal range for photosynthetic activity. Leaf thickness and chloroplasts per cell are determined by the level of blue light rather than the red:far red (R:FR) ratio (Schuerger *et al.*, 1997). The sunlight ratio of red:far red (R:FR) light is 0.6 relative units (RU) in the morning and afternoon, and peaks at 1.0 – 1.3 RU at noon (Holmes and Smith, 1977). Blue light influences phototropism (Blaauw and Blaauw-Jansen, 1970), and stomatal control (Schwartz and Zeiger, 1984), as well as stem elongation, water relations, and CO_2 exchange (Cosgrove, 1981).

Photosynthesis drives plant biomass production, and is directly influenced by the amount of photosynthetically active radiation (PAR) a plant receives. On the other hand, the light spectrum, in particular blue light and the R:FR ratio, determines vegetable quality (Demotes-Mainard *et al.*, 2016).

1.2 PROBLEM STATEMENT

Black shade nets, with a shade factor of 40-80% are the most commonly used nets for ornamental crops and nurseries (Shahak *et al.*, 2004). Their study has further indicated that crops grow vegetatively under red and yellow nets, while blue nets cause dwarfing. Grey nets resulted in short branched plants with smaller leaves when compared to the traditional black nets (Oren-Shamir *et al.*, 2001; Priel, 2001; Shahak *et al.*, 2002). Various studies have revealed that plant growth is influenced through light quantity and quality manipulation, as well as by the colour of shade nets and LEDs. However, very little information is available regarding the effect of different coloured shade nets with high solar radiation, and also of low LED radiation on chlorophyll fluorescence parameters, nutrient uptake, and physical measurement of lettuce and cabbage seedlings and mature plants. The study between shade nets under high solar radiation compared to low radiation LEDs will give new insight on lettuce and cabbage plant stress parameters, colour specific nutrient uptake and plant morphology.

1.3   AIM AND OBJECTIVES

The aim of this study was two-fold and comprised two different trials. The focus of the first trial was to determine the differences in chlorophyll fluorescence parameters for lettuce and cabbage seedlings: and mature plants grown with high solar radiation under different coloured shade nets, compared to black nets. Furthermore, to determine differences for physical measurements and leaf macro- and micro-element uptake under the same conditions. The aim of the second trial was to determine whether chlorophyll fluorescence parameters and leaf macro- and micro-element uptake differ between the low radiation LEDs and high solar radiation under different coloured shade nets, in comparison to black nets.

Specific objectives included:

Three replicates of five different coloured shade nets, where the black net was the control, and with minimal variances between the shade nets regarding the shading factor were used to grow lettuce and cabbage seedlings and mature plants with high solar radiation.

Four lettuce cultivars were grown under the same nets with high solar radiation, and also with low radiation B+R, B+FR and R+FR LEDs.

4

1.4   References

Bickford ED, Dunn S. 1972. *Lighting for plant growth*. The Kent State Univ. Press: Kent, OH.

Blaauw O, Blaauw-Jansen G. 1970. The phototropic responses of *Avena coleoptiles. Acta Botanica Neerlandia*. 19: 755-763.

Cosgrove DJ. 1981. Rapid suppression of growth by blue light. *Plant Physiology*. 67: c584-590.

Demotes-Mainard S, Pérona T, Corotb A, Bertheloota J, Le Gourrierecb J, Pelleschi-Travierb S, Crespelb L, Morela P, Huché-Théliera L, Boumazab R, Vianb A, Guérina V, Leducb N, Sakr S. 2016. Plant responses to red and far-red lights, applications in horticulture. *Environmental and Experimental Botany*. 121: 4-21.

Holmes MG, Smith H. 1977. Function of phytochrome in natural environment 1. Characterization of daylight for studies in photomorphogenesis and photoperiodism. *Photochemistry and Photobiology*. 25: 533-538.

Hung HC, Joshipura KJ, Jiang R, Hu FB, Hunter D, Smith-Warner SA, Colditz GA, Rosner B, Spiegelman D, Willet WC. 2004. Fruit and vegetable intake and risk of major chronic disease. *Journal of the National Cancer Institute*. 96(21): 1577-1584.

Nelson JA, Bugbee. 2015. Analysis of Environmental Effects on Leaf Temperature under Sunlight, High Pressure Sodium and Light Emitting Diodes. *Public Library of Science*. 10(10): 1-13.

Oren-Shamir M, Gussakovsky EE, Spiegel E, Nissim-Levi A, Ratner K, Ovadia R, Giller YE, Shahak Y. 2001. Coloured shade nets can improve the yield and quality of green decorative branches of *Pittosporum variegatum. Journal of Horticultural Science and Biotechnology*. 76: 353-361.

Priel A. 2001. Coloured nets can replace chemical growth regulators. *FlowerTECH*. 4: 12-13.

Sager JC, McFarlane JC. 1997. Radiation. In: Langhans RW, Tibbitts TW (eds). *Plant growth chamber handbook*. Iowa State Univ. Press: North Central Region Research Publication No. 340, Iowa Agriculture and Home Economics Experiment Station Special Report no. 99, 1-29, Ames, IA.

Schuerger AC, Brown CS, Stryjewski EC. 1997. Anatomical features of pepper plants (*Capsicum annuum* L.) grown under red light-emitting diodes supplemented with blue or far-red light. *Ann. Bot*. (Lond.) 79: 273–282.

Schwartz A, Zeiger E. 1984. Metabolic energy for stomatal opening: Roles of photophosphorylation and oxidative phosphorylation. *Planta*. 161: 129-136.

Shahak Y, Lahav T, Spiegel E, Philosoph-Hadas S, Meir S, Orenstein H, Gussakovsky EE, Ratner K, Giller Y, Shapchisky S, Zur N, Rosenberger I, Gal Z, Ganelevin R. 2002. Growing Aralia and Monstera under colored shade nets. *Olam Poreah*. 13: 60-62.

Shahak Y, Gussakovsky EE, Gal E, Ganelevin R. 2004. ColorNets: Crop Protection and Light-Quality Manipulation in One Technology. *Acta Horticulturae*. 659: 143-151.

Chapter 2

Literature Review

2.1   INTRODUCTION

Vegetable growers have the difficult task of producing a consistent supply of high quality vegetables, this is due to changing climatic conditions which directly induce either biotic or abiotic stress (Cramer *et* al., 2011; Prasad and Chakravorty, 2015). Abiotic stress like water availability, fluctuating temperatures and the combination of varying light quantity and quality, is likely to be increased by global warming (Cramer *et* al., 2011; Ilić *et al.*, 2012). To counteract these changing climatic situations, different coloured shade nets are currently used to alter light quality and quantity (Meena and Meena, 2016), increase plant yield and phytochemical composition, increase crop yield and quality (Demotes-Mainard *et al.*, 2016), protect crops from hail and wind (Teitel *et al.*, 2008), heat and drought (Meena et al., 2014), and excessive solar radiation (Ilić *et al.*, 2011). Photosynthetically active radiation (PAR) under a net is determined by the PAR that passes through the net pores and the PAR scattered downwards from the net threads. The former is directly proportional to net porosity and the latter is net-colour dependant (Al-Helal and Abdel-Ghany, 2010).

Although black nets with a shading factor of 40-80% are considered the norm for many crops, they result in lower yields than nets of other colours (Shahak *et al.*, 2004). A 40% blue net increased the blue:red (B:R) ratio by 30% and simultaneously decreased the red:far red (R:FR) ratio by 10% compared to a 20% white net (Bastias *et al.*, 2012). Shahak *et al.* (2004) states that blue nets absorb light in the UV, red and far red regions and transmit light in the blue-green region (400-540 nm) - while red nets transmit light from 590 nm and up. They further found that blue nets increased the B:R ratio sharply, while maintaining the R:FR ratio. The increased B:R ratio from these nets resulted in a 10-fold increase in scattered light. Scattered light will penetrate deeper into plant canopies than light under 30% black nets (Shahak *et al.*, 2004). Red and yellow nets, in general stimulate vegetative growth, while blue nets produce compacted plants, and grey nets result in plants with shorter branches and smaller leaves. The blue, yellow and red nets can either increase or decrease the blue, yellow and red spectral bands of transmitted light. The pearl net (white) can

absorb light in the ultra-violet (UV_{A+B}) range, and has a high light-scattering capability (Shahak, 2008; Goren *et al.*, 2011; Alkalai-Tuvia *et al.*, 2014).

Cabbage (*Brassica oleracea*) and lettuce (*Lactuca sativa* L.) are consumed globally on a daily basis by many communities. A high intake of cabbage is associated with better memory and cognitive functioning (Nooyens *et al.*, 2011). The same authors further stated that information-processing speed was two times slower for people with a low cabbage intake compared to people with a high cabbage intake. Many vegetables and fruits contain high levels of antioxidants like ß-carotene, vitamins C and E, and polyphenols - and when consumed by humans decrease the vulnerability to oxidative stress that occurs with ageing (Joseph *et al.*, 2009).

Similarly, lettuce is consumed mainly raw as a leafy vegetable. It has high nutritional values of vitamins A, C and E, as well as minerals like calcium and iron, which are essential for preventing diseases and promoting health (Caldwell, 2003). According to Caldwell and Britz (2006), carotenoids in leafy green vegetables can reduce the incidence of cataracts and macular degeneration. Carotenoid pigments and chlorophyll synthesis may be cultivar specific, and sensitive to changing plant growth conditions (Kimura and Rodriguez-Amaya, 2003).

Seedling nurseries play a pivotal role, as they are responsible for supplying a variety of seedlings on a continual basis to farmers. To maintain healthy, disease-, pathogen- and virus-free seedlings, various production aspects must be considered. This includes excellent quality seed, disease free growing medium with the optimal chemical and physical properties, precise irrigation management with clean irrigation water, measured fertilisation and careful climate control - including all aspects of light management and lighting.

To ensure stable production of vegetables, top quality seedlings are needed. Plug seedlings are suitable for use with automatic seedling transplanters (Fujiwara *et al.*, 1999). However, the root area and water-holding capacity (WHC) in the plug is limited, and seedling density is high. These conditions create stress conditions in the form of limited rooting area and overshadowing between the plants as they grow (Sato *et al.*, 2003). Plant hormones such as phytochromes, orchestrate plant responses such as excessive stem elongation - resulting in reduced seedling uniformity (Fukushima *et al.*, 2014).

2.2   SEEDLING PRODUCTION

For optimal quality seedling production, the chemical and physical properties of the growing substrate are vital. Irrigation and fertilisation practices and environmental conditions such as moisture, temperature, nutrient supply and light, have a profound influence on growth, development, yield and quality (Zou *et al*., 2009). The growing substrate is a vital parameter, and influences the WHC, air filled porosity (AFP) - as well as water- and fertiliser availability to plants. It also provides anchorage for plant roots, and determines the cation exchange capacity (CEC) and the gas exchange abilities between the rhizosphere and atmosphere (Nelson, 1991; Argo and Bierbaum 1997). A huge demand arose for cost-effective soil-less growing substrates, due to soil-borne diseases, the bulkiness of the soils used and the vast amount of storage space needed. Many South African growers use a pine bark based growing medium, while vermiculite, perlite and bark is more popular in the USA (Michelle *et al*., 1990; Bunt 1988; Nelson 1991). Currently, growers worldwide are increasingly using coir, peat and rockwool (Xiong *et al*., 2017). Peat and rockwool is extensively used as a cultivation substrate due to its desirable physiochemical and biological properties which stimulate plant growth (Schmilewski, 2008; Krucker *et al*., 2010). Mineral retention, availability and movement is in the root-zone is related to substrate particle size, nutrient and water holding capacities, as well as cation exchange capacity (Ao *et al*., 2008; Urrestarazu *et al*., 2008; Carmona *et al*., 2012; Asaduzzaman *et al*., 2013).

According to Altland *et al*. (2008), nitrates and phosphates leach easily from nursery container substrates, thus posing an environmental threat through contamination of groundwater and surface water. They further state that the ideal pH for soil-less substrates range between 5.5 - 6.4, and in all likelihood, a lower substrate pH than 5.0 could lead to higher nitrogen (N) and phosphorous (P) retention, resulting in increased N and P availability for plants. Argo and Bierbaum (1997) concluded that calcium (Ca) availability is not reduced by a low pH below 5.0, but this low pH indicates that Ca sources applied to the growing medium may be low. This contradicts the results of Altland *et al*. (2008), which are that higher Ca availability was realised when Douglas fir bark was amended with sulphur (S), thus lowering the pH beyond its native pH.

2.3   LIGHT AND PLANT GROWTH- AND DEVELOPMENT

For the optimal functioning of plants, the spectral quality (wavelength), quantity (photon flux) and duration (photoperiod) of light is important (Kempen, 2012). Whereas photoperiod describes light as a factor of time available for photosynthesis, the spectral quality is important, not only in terms of quantity of light per wavelength, but also the ratio between different wavelengths. The quantity reveals the amount of light available to plants as photons, per unit time on a unit area, expressed in *µmol m⁻² s⁻¹* (Kempen, 2012). When photon absorption energy levels exceed the plant's photon absorption capacity, it results in photo-oxidative damage of plant cell components due to the formation of reactive oxygen species (ROS) (Zou *et al.*, 2009).

2.3.1 Light Quality

Ultra Violet (UV) radiation consists of UVC (100-290 nm), UVB (290-320 nm) and UVA (320-390 nm). The range of visible solar radiation (Figures 2.1–2.2) varies between 380 nm (blue) and 780 nm (red) (Foshi and Kumar, 2003), and infrared is divided in near infrared NIR (780 nm - 3 µm), and far infrared FIR (3 µm - 50 µm). Plants react to three spectral ranges of light through photosynthesis, phototropism and photomorphogenesis (Tamulaitis *et al.*, 2005), and use this light radiation as photosynthetically active radiation (PAR) in the process of photosynthesis - mainly in the blue, red, and near infrared wavelengths. Solar radiation within the PAR range is absorbed by photosynthetic pigments, mostly in light-harvesting antenna complexes situated in the thylakoid membranes (Blankenship, 2014; Hall and Rao, 1999; Lawlor, 2001). Recent studies indicate that the nutritional quality of vegetables can be improved through specific light quality (Lin *et al.*, 2013).

According to Savvides *et al.* (2011) light quality severely influenced leaf hydraulic conductance (K_{leaf}), and stomatal conductance (g_s), during the development of cucumber leaves. The K_{leaf} and g_s values were at least three times higher under a combination blue (420 nm) and red (640 nm) and blue light emitting diode (LED) lights, than under red LED alone. Leaf stomatal conductance is structurally influenced by light quality, via the effect of epidermal cell size on stomatal density.

Through the absence of blue light (BL), water supply and demand, and leaf photosynthetic capabilities, were compromised. The leaves grown under red light were extremely vulnerable to water stress, and Savvides *et al.* (2011) further stated that the net leaf photosynthesis (A_n) was the lowest in red LED-grown cucumber leaves and the highest in cucumber leaves grown under red and blue LEDs. When Silver birch (*Betula pendula*) shoots were exposed to a short light treatment (<5 hrs), it resulted in the highest K_{leaf} value under blue light, and the lowest under red light (Sellin *et al.*, 2011). This correlates well with the fact that changes in conductance of extra-xylem component affects K_{leaf} values through light quality (Voicu *et al.*, 2008; Sellin *et al.*, 2011) and light quantity (Scoffoni *et al.*, 2008).

According to Hogewoning *et al.* (2010), leaf mass per area and maximal photosynthetic capacity can be increased under low light intensities, by adding or increasing blue light in the blue/red light ratio. Spectral light that contains blue light rather than mono-chromatic red light, increased palisade and spongy mesophyll in leaves, as well as the thickness of secondary xylem in stems (Schuerger *et al.*, 1997). Research has further shown that light quality affects photosynthesis (Kim *et al.*, 2004), and also biological processes such as germination and flowering (Taiz and Zeiger, 2002).

Photosystem II (PSII) and I (PSI) play a vital role in photosynthetic electron transport (ET). Light quality is paramount for the functioning of PSII, and the influence of red light has proven to be harmful for photosynthesis (Hogewoning *et al.*, 2010; Murata *et al.*, 2007). Yan-xiu *et al.* (2015) proved that photoinhibition in PSII is caused by red LED light, and can be alleviated by adding blue LED light. After photoinhibition in PSII started, a repair process was initiated that, depended on the rate of repair (Tikkanen *et al.*, 2014).
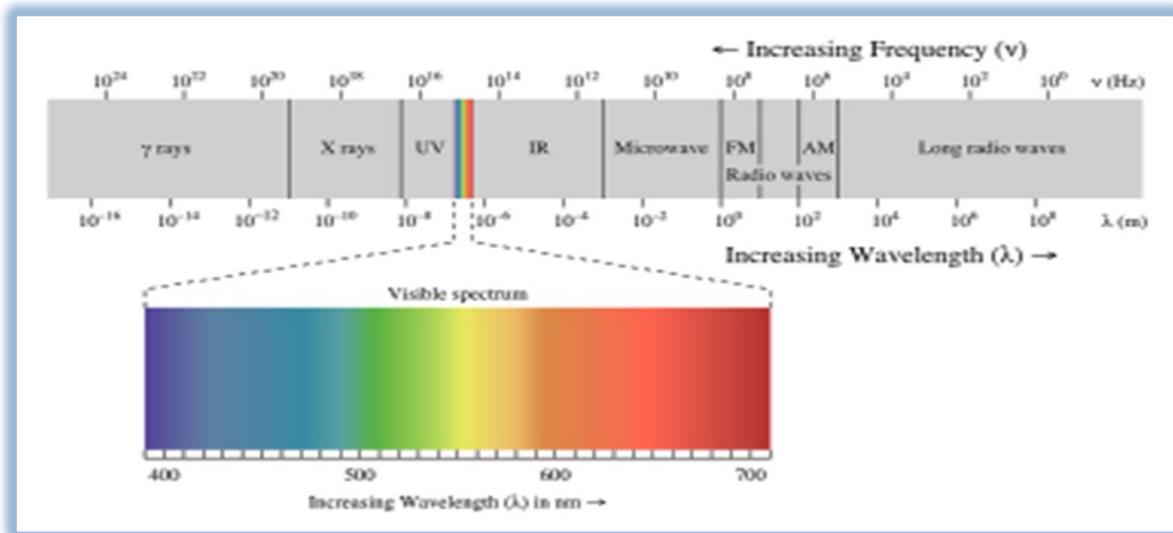
Figure 2.1: Visible light highlighted in the electromagnetic spectrum.
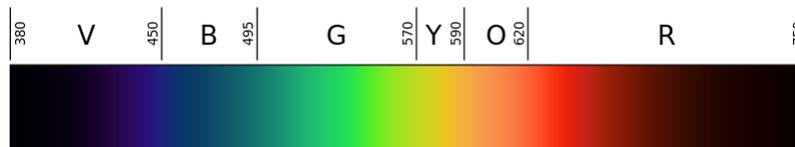
Figure 2.2: Colour of visible light expressed per wavelength.

2.3.1.1 Light photoreceptors

Plants can monitor the light environment and perceive signals that modulate growth and development by several photoreceptors: phytochromes (phy), cryptochromes (cry), phototropins (phot1 and phot2) and unidentified UV-B photoreceptors (Casal, 2000). Plant development is regulated by these photoreceptors (Smith, 2000). Whitelam and Halliday (2007) state that cryptochromes and phototropins are specifically blue light-sensitive, whereas phytochromes are more sensitive to red than to blue light.

2.3.1.2 Phytochromes

Phytochromes are red and far-red light plant photoreceptors that regulate various light responses such as seed germination, seedling photomorphogenesis, and shade avoidance (Keunhwa *et al*., 2011). According to Smith (2000), phytochromes are

photochromic photoreceptors and exist in two photoconvertible forms - P_r (phytochrome red) and P_{fr} (phytochrome far-red). Phytochrome red is biologically inactive and is converted to P_{fr}, the active form, upon absorption of red photons (Nagatani, 2010; Quail, 2010). Blue light can reverse this phenomenon by regulating cryptochromes and phototropins, and might be involved with DNA or DNA-binding protein interaction (Christie and Briggs, 2001). Phytochromes' peak absorption is between 665 nm and 730 nm. This activates a photoconversion of P_r and P_{fr}, due to a band absorption overlapping, and radiation below 700nm. There are five phytochromes ranging from phytochrome A (phyA) to phytochrome E (phyE), and these phytochromes are chromoproteins. These phytochromes have distinct and overlapping physiological functions, and work synergistically as well as antagonistically (Valverde *et al*., 2004). According to Casal (2000), these antagonism and synergism reactions are between phyA and phyB. The biochemical reactions are rapid, although the morphological ones are slower. An individual phytochrome's mechanism of action is the selective expression of target genes, and the rapid and reversible operation to modulate cellular ionic balances (Shacklock *et al*., 1992). Nearly all phases of plant development are regulated by phytochromes - for example seed germination, which needs low levels of red light and is determined and regulated by phyA. Furthermore, the exposures to deep shade, a condition where the R:FR ratio is very low, is only detected by the photoreceptor phyA (Yanovsky *et al*., 1995). Phytochrome B is the photoreceptor that senses the ratio of R:FR (Ballare *et al*., 1991a), and has a role in all stages of the plant life cycle: germination, establishment, architecture, and flowering. Phytochromes D and E are involved in the architecture of plants (Smith, 2000). Phytochrome D mutation reduces leaf area, and mediates petiole extension and hypocotyl growth (Devlin *et al*., 1999).

2.3.1.3   Cryptochromes

Cryptochromes are photoreceptors that, consists of flavoproteins similar in sequence to photolyases, with the ability to sense and respond to blue light (390 to 500 nm), and ultraviolet-A light (320 to 390 nm) (Yang *et al*., 2017). Deoxyribonucleic acid (DNA) damage is the result of UV-B radiation, and is repaired by the photolyases of the flavoproteins (Cashmore *et al*., 1999). This process mediates the transference of

an electron (e-) from the excited state of the flavin to the pyrimidine dimer, and then isomerises to yield the original pyrimidine and return the e- to the flavin (Sancar, 1994). Although no net change occurs in the oxidation state of the reactants, light-dependant redox reactions are involved (Cashmore *et al.*, 1999). Phototropism, photomorphogenesis, stomatal opening, de-etiolation and leaf photosynthetic functioning are all processes in which blue light is involved with cryptochromes (Whitelam and Halliday, 2007). Etiolation is the process were the hypocotyls of dicot plants elongate in the dark. This happens when P_{fr} is depleted and the hypocotyls become insensitive to Gibberellic Acid (GA) and then elongate. As soon as the young plant comes into contact with light, de-etiolation of the hypocotyls starts, and cotyledons become photosynthetically active. Cryptochromes mediate a variety of light responses - including circadian rhythms (Christie and Briggs, 2001) and the production of anthocyanins and carotenoids in plants and fungi (Cashmore *et al.*, 1999)

Cryptochromes are categorised as cryptochromes 1 (cry1) and 2 (cry2) (Casal, 2000; Lin and Shalitin, 2003). Although cryptochrome 2 levels are not affected by red light, they are reduced with an increasing radiance of blue light, which is not the case for cryptochrome 1 (Casal, 2000). Cry2 and cry1 of the *Arabidopsis* cryptochrome family, are homologous with one another, and responsible for the production of anthocyanin, cotyledon expansion and hypocotyl shortening (Ahmad *et al.*, 1998). Hogewoning *et al.* (2010) state that as little as 7% blue light is sufficient to prevent dysfunctional photosynthesis in plants, and that it can be considered as being a qualitatively blue light effect. This corresponds with the photosynthetic capacity (A_{max}) of leaves grown under 7% blue light (BL), which was twice as high as leaves grown under 0% BL, and continued to rise up to 50% BL. However, at 100 % BL the A_{max} was lower, but photosynthetic functioning was normal (Hogewoning *et al.*, 2010).

2.3.1.4   Phototropins

Phototropism is the mechanism where a plant orientates itself, to maximise photosynthesis according to light incidence. Non-phototropic hypocotyl 1 (NPH1) is an encoded protein and reacts as a phototropic receptor (Christie and Briggs, 2001).

Due to this process, the NPH1 protein was named phototropin (Christie *et al.*, 1999). Currently, phototropins are known as phot1 and phot2 - (and no longer NPH1 and NPL1), and are responsible for a subsidiary role in transcription regulation of BL (Goh, 2009). Both phot1 and phot2 have an N-terminal photosensory, which is comprised of two different light oxygen voltage (LOV) domains: LOV1 and LOV2, and has a C-terminal Ser/Thr kinase domain (Demarsy and Frankhauser, 2009). In the dark, both LOV1 and LOV2 bind with flavinmononucleotide (FMN) in a noncovalent form. This is changed to covalent bindings of the FMN chromophore to an invariant cystein residue within both LOV domains, as soon as BL triggers the reaction. This is subsequently followed by a protein conformational change and altered kinase activity. LOV1 and LOV2 are responsible for phototropism and chloroplast movement, which enhances photosynthesis under low light growing conditions.

Chloroplast movement is accomplished after three chain reactions have taken place: (1) receptors perceive the light signal; (2) signals are transmitted as chemical messages by the signal transducer; and where (3) the effector systems respond to the signals (Takemiya *et al.*, 2005).

Phototropism is mediated, and the first positive curvature is experienced when a pulse of red light is given two hours before phytochromes perceive unilateral blue light (Janoudi, 1992). It is well documented by Janoudi *et al.* (1997) that even with no red light pulse, the first positive curvature is reduced in the phytochrome A and B double mutant, with the response to unilateral blue light.

2.3.2 Light Quantity

Plants use light as photosynthetically active radiation (PAR), although it is expressed as photosynthetic photon flux (PPF). This PPF determines the amount of useable light energy available to the plant, and is generally known as light intensity. Plants need adequate light quantity levels for plant growth and development, and an increase in light intensity could result in the synthesise of high levels of anti-oxidants. This will lead to disorders in the development and appearance of tomato fruit (Dorais

et al., 2001), such as sunscald and uneven ripening which are a consequence of excessive light on the fruit (Adegoroye and Jolliffe, 1987).

Walters (2005) state that in low-light conditions high levels of chlorophyll (chl) a/b – receptors in light-harvesting complexes (LHC) are found, especially those associated with photosystem II (PSII), whereas in high-light conditions the levels of photosystems, ATP synthase, Calvin Cycle enzymes, and cytochrome b6/*f* increase. Excess light leads to a decrease in photosynthetic efficiency, also known as photoinhibition (Powles, 1984). Under low light intensity most of the absorbed light will be used for photosynthesis, while under high light intensity only part of the absorbed light will be used (Long *et al.*, 1995). Changes in photosystem stoichiometry will optimise light use, and an increase in photosynthetic capacity will reduce photoinhibition (Walters, 2005). Photomorphogenesis of plants is also influenced greatly by light quantity, and there is evidence (Ballare *et al.*, 1991b) indicating that PAR is a controlling factor. Light-grown plants display different responses in stem elongation and growth under two levels of PAR with no reduction in BL (blue light) and the R:FR ratio (Ballare *et al.*, 1991b).

2.3.2.1   Photosynthesis

Photosynthesis is the process were chlorophyll, carotenoids and other photosynthetic pigment molecules in the photosynthetic light harvesting antenna molecules, absorb light energy as photons and convert light energy, water and carbon dioxide (CO_2) into carbohydrates and oxygen.

Antenna complexes harvest sunlight via energy transfer steps within and between the complexes. Reaction centres use the available energy as the primary driving force in photosynthesis's primary dark reactions (Zinth *et al.*, 1996).

This process consists of two stages - the first of which is the light-harvesting stage, where light-dependent reactions capture light energy and synthesise energy storing molecules ATP and NADPH. These storing molecules are then used to capture and reduce CO_2 during the second stage (Taiz and Zeiger, 2002).

2.3.2.2   Chlorophyll fluorescence

Light energy is absorbed by chlorophyll molecules, and can undergo one of three processes. It can be used to drive photosynthesis, be dissipated as heat, or be re-emitted as light – chlorophyll fluorescence (Maxwell and Johnson, 2000). Measuring the chlorophyll fluorescence characteristics of plants can thus unlock valuable information regarding the photosynthetic efficiency of plants (Björkman and Demmig, 1987).

The Kautsky effect is known as the characteristic changes in the intensity of *chlorophyll a* fluorescence, when a dark-adapted leaf is illuminated (Kautsky and Hirch, 1931). During photosynthesis, *chlorophyll a* fluorescence measures the energy of absorbed light quanta, which were not used during photosynthesis or emitted as heat (Kalaji *et al*., 2004). Chlorophyll fluorescence depicts three curves - OJ, JI and IP - and the Kautsky transient Chlorophyll (Chl) a fluorescence is used to study the effect of different environmental stresses on photosynthesis (Allakhverdiev and Murata, 2004). This is one of the main methods used to determine the functioning of Photosystem II (PSII), as well as its reaction to growing conditions and changes in the environment (Kalaji *et al*., 2004). Thus, the JIP-test is used to distinguish the responses of the photosynthetic apparatus to different stresses. It is based on the theory of energy flow in thylakoid membranes, and thus enables us to understand the relationship between the biophysical side of photosynthesis and various fluorescence parameters (Strasser and Akoyunoglou, 1981).

Fluorescence intensity has a minimum value (F_o) when leaves are in the dark adapted state, as the e- acceptor of PSII is in the open state or oxidised state. With high-light illumination the O-J transition phase is activated, exciting all pigment molecules within 2 milliseconds (ms). The thermal phases are slow, as the J-I and I-P phases rise to a maximum fluorescence at P or (F_m) and are reached within 1 second (Misra *et al*., 2012). The difference between the maximum fluorescence (F_m) and minimum fluorescence (F_o) is known as the variable florescence (F_v). Values of 0.78 to 0.84 are indicative of healthy plants (Björkman and Demmig, 1987).

Performance Index total can be expressed through the following multi-parametric expression:

$$PI_{abs(total)} = \frac{Y_{RC}}{1-Y_{RC}} \cdot \frac{\varphi_{PO}}{1-\varphi_{PO}} \cdot \frac{\psi_{EO}}{1-\psi_{EO}} \cdot \frac{\delta_{RO}}{1-\delta_{RO}}$$

The equation consists of four main categories.

The first is the concentration of reaction centre chlorophyll per total chlorophyll, and is described as e- absorption of light energy (ABS), and thus Y/(1-Y) is expressed as RC/ABS.

This is followed by the performance of light reaction, which is the trapping of excitation energy (TR), expressed as $[\varphi P_o/(1 - \varphi P_o)] = TR_o/DI_o = K_p/K_n = F_m-F_o/F_o = F_v/F_o$.

The dark reaction performance is the conversion of excitation energy to e- transport (ET), and is expressed as $ET_o/(TR_o - ET_o) = (1-V_j)/V_j$

The reduction of end acceptors is expressed as $(F_m-V_i)/(F_m-V_j) = \delta R_o/(1 - \delta R_o) = (F_m-F30ms)/(F_m-F2ms)$.

2.3.2.3   Photosynthetic pigments

Photosynthetic pigments are present in light-harvesting complexes (LHCIIb), and thus the solar energy is absorbed by the LHCIIb and transferred to photosystem II (PSII) reaction centres for photosynthesis (Xiao *et al.*, 2011). These pigments consist mainly of chlorophyll, carotenoid and anthocyanin, and each pigment absorbs PAR light in different wave-lengths. Plant morphogenesis is also wave length-specific, and processes such as apical shooting, pigment synthesis and healthy plant development are stimulated between 730 and 735 nm (Tamulaitis *et al.*, 2005). According to Whitelam and Halliday (2007), blue light is a determining factor affecting plant photomorphogenesis. Plants use different mechanisms to protect against the ever-changing light quality and quantity in the environment that cause photodamage. These protective mechanisms enable the dissipation of excess light. The most effective method is through non-photochemical quenching (NPQ), which is referred to as energy-dependent quenching (qE) and can develop and relax within

17

seconds (Horton *et al.*, 1996). Carotenoids have antioxidant properties, which can protect plants from photodamage (Xiao *et al.*, 2011).

2.3.2.4   Chlorophyll

Chlorophyll consists of a central magnesium atom surrounded by a light absorbing ring and a long phytol tail, which anchors the molecule to a membrane. Chlorophyll is divided into *chl a* and *chl b*. Chlorophyll's main function is to absorb light energy and transfer it into the photosynthetic apparatus (Demmig-Adams and Adams, 1996).
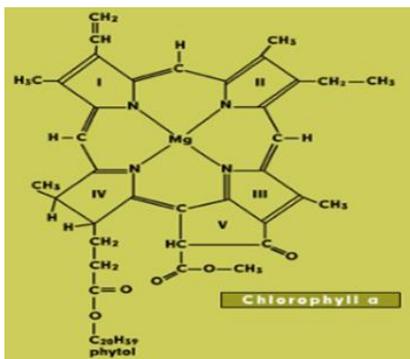
Figure 2.3: Chlorophyll a   **Figure 2.4:** Chlorophyll b
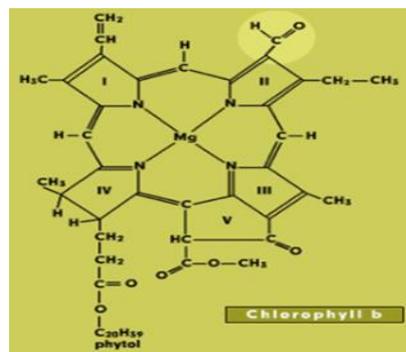
Chlorophyll a (Figure 2.3) absorbs most energy from wavelengths in the violet-blue and orange-red spectrum, and is used in oxygenetic photosynthesis. It also transfers resonance energy in the antenna complex ending in the reaction centre, where chlorophyll P700 (PSI), and chlorophyll P680 (PSII) are situated (Papageorgiou and Govindjee, 2004). *Chlorophyll a-* and *b* is synthesised at 662 nm and 642 nm respectively, while phototropic processes function between 400 and 500 nm. *Chlorophyll b* (Figure 2.4) mainly absorbs blue light energy in the longer wavelengths of blue light (Figure 2.5) - thus increasing the blue wavelengths. It absorbs light in lower light intensities, while *chl a* absorbs light with higher light intensities (Lange *et al.*, 1981).

18
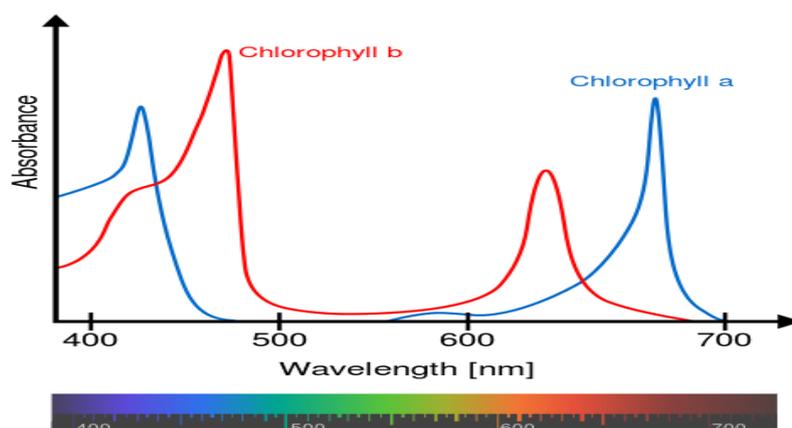
Figure 5: Absorbance spectra of chlorophyll a and b

2.3.2.5   Carotenoids

Carotenoids are found in the chloroplasts and chromoplasts of plants, and their main roles are to absorb blue light energy for photosynthesis and to protect chlorophyll from photodamage (Armstrong and Hearst, 1996). Non-photochemical quenching (NPQ) is a mechanism in plants to deal with high light intensity, and is referred to as energy-dependent quenching (qE) (Xiao *et al.*, 2011). According to Xiao and co-workers (2011), the antioxidant patterns of carotenoids change due to their binding to the LHCIIb. These carotenoids, which bind to LHCII, can protect the photosystem from photodamage through an energy transfer mechanism.

Xanthophylls are carotenoids that contain oxygen atoms and are known as lutein and zeaxanthin (Demmig-Adams and Adams, 1996). Lutein is the most abundant xanthophyll in higher plants and bonds to L1 and L2, where the occupancy is essential for protein folding and quenching of triplet chlorophyll (3Chl*) (Formaggio *et al.*, 2001). According to Dall'Osto *et al.* (2006), lutein can bind at site L1 of the major LHCII complex and of other LHC proteins of plants, thus quenching harmful 3Chl* - and in doing so preventing ROS formation.

Carotenes are carotenoids that comprise of hydrocarbons with no oxygen, and are known as α-carotene, ß-carotene and lycopene. These carotenoids can contribute energy to the photosynthetic system. They can avoid photodamage to this system,

which is achieved when incident light energy exceeds the need for photosynthesis and is dissipated (Demmig-Adams and Adams, 1996). Lutein and ß-carotene is known for its lung cancer curing abilities (Gallicchio *et al.*, 2008). According to Ohashi-Kaneko *et al.* (2007), spinach grown under blue fluorescent lamps had a higher carotenoid concentration than spinach grown under white fluorescent lamps, where both lamps had a PPFD 300 *µmol m^{-2} s^{-1}*.

2.4   SUPPLEMENTAL LIGHTING IN PROTECTED AGRICULTURE

Crops are grown globally, each with their own variations in geographical and climatic conditions. Some countries have limiting factors such as water quantity and quality, availability of fertile soils, and adequate light in the form of light quality, quantity and photoperiod. The latter factors forced agrarians to devise a new concept known as artificial lighting. Horticultural crops were first grown under incandescent bulbs, and more recently High Pressure Sodium (HPS) and Metal Halide (MH) lamps were used. Although these lamps have better light quantity and quality aspects compared to incandescent lamps - they are expensive, radiate a lot of heat, and have limited light quantity and quality characteristics. High Pressure Sodium (HPS) lamps portray light in the yellow to red spectrum, and although the blue light component can be altered, it has physical limitations in the red region (Tamulaitis *et al.*, 2005). Thus, fluorescent tubes followed later and were commercially used for the production of seedlings and tissue culture (Economou and Read, 1987). There was still a demand for a more energy-efficient light source, which radiated less heat and portrayed spectral qualities closer to natural sunlight. The light-emitting diode (LED) was developed and is currently the focus for horticultural research.

2.4.1 Light Emitting Diode (LED)

Light emitting diodes (LEDs) produce a narrow bandwidth of light (NBL) and specific wavelengths (Hoenecke *et al.*, 1992), with low radiant heat output and high light levels (Tamulaitis *et al.*, 2005). LEDs have the advantage that wavelengths, and plant growth can be manipulated using different coloured filters - thus enabling the possible spectrum to range from ultra violet (UV) to near infrared (IR). Krames *et al.*

(1999) further state that LEDs have a possible efficiency of up to 100%, with no physical limitations. These factors enable scientists to manipulate the spectral ranges that plants use for photosynthesis, -tropism and –morphogenesis.

The growth of lettuce and radish under LED illumination with a dominating wavelength of 640 nm, and supplemented by 455, 660, and 735 nm, outperformed the same crops grown under HPS lamp regarding photosynthesis and plant morphology characteristics (Tamulaitis *et al.*, 2005). This experiment further showed that by altering far red (735 nm) light from daytime to night time, the daytime photosynthetic processes were broken down and plant development was almost completely inhibited (Tamulaitis *et al.*, 2005). Extreme morphological changes in radish were prominent when small amounts of nocturnal far red light (735 nm) was applied. Soluble sugar levels in radish seedlings can be increased with the illumination of red LEDs (Zhang *et al.*, 2009)

According to Miyashita *et al.* (1995), potato plantlets cultivated under white fluorescent lamps and red LEDs showed no significant differences in leaf area and dry weight, when the red photon flux was increased from 630 to 690 nm. However, chlorophyll concentrations and shoot length of the potato plantlets increased with the same increasing red photon flux density. The amount of blue light in a primary light source is correlated to anatomical changes in leaf and stem tissue of the pepper plant (Schuerger *et al.*, 1997).

Brazaitytè *et al.* (2009) investigated the after-effect of different coloured LEDs used to cultivate tomato seedlings. Their study revealed that tomato seedlings under yellow light LEDs, in combination with the main LEDs with a bandwidth of 447, 638, 669 and 731 nm, yielded unripened tomatoes eight weeks after transplanting, and produced a lower total tomato yield compared with tomato seedlings cultivated under the same main LEDs. Furthermore, tomato seedlings grown under the main LEDs with supplemental 520 nm light, produced plants slightly taller and with one more leaf than transplants grown under the main LEDs supplemented with orange (662nm) light - which had a negative effect on the height of plants and the number of leaves formed.

Lettuce grown under monochromatic red LED light of 125 µmol m^{-2} s^{-1} showed signs of abnormal growth with winding leaves and had a higher rate of stem elongation

than lettuce grown under blue LED light of 170 $\mu mol\ m^{-2}\ s^{-1}$, as well as red and blue LED light with the same PPF (Yanagi *et al.*, 1996). According to Chen *et al.* (2015), the root and shoot dry weight of hydroponically grown lettuce was significantly higher under red and white LEDs compared to white LEDs only. There was also a significant increase in lettuce growth with an increase in the ratio of red:white LEDs. Lettuce grown under deep red (660 nm), deep blue (455 nm) and far red (740 nm) LEDs resulted in significant wider leaves, larger leaf area index (LAI), fresh weight and dry weight compared to the same lettuce cultivars grown under red (640 nm) and deep blue (455 nm) LEDs, red (640 nm) and blue (460 nm) LEDs, deep red (660 nm) and deep blue (455 nm) LEDs and HPS lamps as the control (Pinho *et al.*, 2017). They further state that the significant morphological differences were due to the influence of far red LED, and this is confirmed by Zhen and van Iersel (2017) that stated that the preferential excitation of PSI is increased through FR light.

2.5   SYNOPSIS

The overall objective of this study was to determine the effects of light quality on the growth, development and function of seedlings and mature plants of lettuce and cabbage crops. To achieve this, a range of trials were conducted using different coloured shade screens and LEDs during the seedling stage, as well as the active growth stage (from transplant to harvest). The physical parameters, leaf chemical concentration of the macro- and micro-elements, and the chlorophyll fluorescence parameters were determined. The chlorophyll fluorescence was used to determine the Performance Index both during the seedling and active growth stages.

2.6   References

Adegoroye AS, Jolliffe PA. 1987. Some inhibitory effects of radiation stress on tomato fruit ripening. *Journal of the Science of Food and Agriculture.* 39: 297-302.

Ahmad M, Jarillo Y, Cashmore AR. 1998. *Journal of Plant Cell.* 10: 197-207.

Al-Helal IM, Abdel-Ghany AM. 2010. Responses of plastic shading nets to global and diffuse PAR optical properties and evaluation. *Wageningen Journal of Life Sciences.* 57: 125-132.

Alkalai-Tuvia S, Goren A, Perzelan Y, Weinberg T, Fallik E. 2014. The influence of colored shade nets on pepper quality after harvest−a possible mode-of-action. *Agriculture and Forestry.* 60: 7-18.

Allakhverdiev SI, Murata N. 2004. Environmental stress inhibits the synthesis *de novo* of proteins involved in the photodamage-repair cycle of photosystem II in *Synechocysti*s sp PCC 6803. *Biochimica Biophysica Acta.* 1657: 23-32.

Altland JE, Buamscha MG, Horneck DA. 2008. Substrate pH Affects Nutrient Availability in Fertilized Douglas Fir Bark Substrates. *Journal of the American Society for Horticultural Science.* 43: 2171-2178.

Ao Y, Sun M, Li Y. 2008. Effects of organic substrates on available elemental contents in nutrient solution. *Bio Resource Technology.* 82: 241 - 245.

Argo WR, Bierbaum JA. 1997. The effect of root media on root zone P, Ca and Mg management In containers with impatiens. *Journal of the American Society for Horticultural Science.* 122: 275-284.

Armstrong GA, Hearst JE. 1996. Carotenoids 2: Genetics and molecular biology of carotenoid pigment biosynthesis. *Journal of the Federation of American Societies for Experimental Biology.* 10(2): 228.

Asaduzzaman M, Kobayashi Y, Mondal MF, Ban T, Matsubara H, Adachi F. 2013. Growing carrots hydroponically using perlite substrates. *Scientia Horticulturae.* 159: 113 - 121.

Ballare CL, Casal JJ, Kendrick RE. 1991a. Responses of wild-type and long hypocotyl mutant cucumber seedlings to natural and simulated shade light. *Journal of Photochemistry and Photobiology.* 54: 819-826.

Ballare CL, Scopel AL, Sanchez RA. 1991b. Photocontrol of stem elongation in plant neighbourhoods: Effects of photon fluence rate under natural conditions of radiation. *Plant, Cell and Environment.* 14: 57-65.

Bastias RM, Manfrini L, Grappadelli LC. 2012. Exploring the potential use of photo selective nets for fruit growth and regulation in apple. *Chilean Journal of Agricultural Research.* 72(2): 224-231.

Björkman O, Demmig B. 1987. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. *Planta.* 170: 489-504.

Blankenship RE. 2014. *Molecular Mechanics of Photosynthesis.* 2nd ed. Wiley: Chichester, UK

Brazaitytė A, Duchovskis P, Urbonavičiūtė A, Samuolienė G, Jankauskienė J, Kazėnas V, Kasiulevičiütė-Bonakėrė A, Bliznikas Z, Novičkovas A, Breivė K, Žukauskas A. 2009. After-effect of light-emitting diodes lighting on tomato growth and yield in greenhouse. *Scientific works of the Lithuanian Institute of Horticulture and Lithuanian University of Agriculture. Sonininkyste ir Darzininkyste.* 28: 1

Bunt AC. 1988. Media and mixes for container grown plants. Unwin Hyman: London.

Caldwell CR. 2003. Alkylperoxyl radical scavenging activity of red leaf lettuce (*Lactuca sativa* L.) phenolics. *Journal of Agricultural and Food Chemistry.* 51: 4589-4595.

Caldwell CR, Britz SJ. 2006. Effect of supplemental ultra violet radiation on the carotenoid and chlorophyll composition of greenhouse-grown leaf lettuce (*Lactuca sativa L.*) cultivars. *Journal of Food Composition and Analysis.* 19: 637-644.

Carmona E, Moreno MT, Avilés M, Ordovás J. 2012. Use of grape marc compost as substrate for vegetable seedlings. *Scientia Horticulturae.* 137: 69 - 74.

Casal JJ. 2000. Phytochromes, Cryptochromes, Phototropins: Photoreceptor interactions in plants. *Journal of Photochemistry and Photobiology.* 71(1): 1-11.

Cashmore ARJA, Jarillo Y, Wu J, Liu D. 1999. Cryptochromes: blue light receptors for plants and animals. *Journal of Science.* 284: 760-765.

Chen R, Liu H, Song S, Sun G, Chen R. 2015. Effects of Light Quality on Growth and Quality of Lettuces in Hydroponics. *Journal of the Science of Food and Agriculture.* 12: 154-156.

Christie JM, Briggs WR, 2001. Blue Light Sensing in Higher Plants. *Journal of Biological Chemistry.* 276: 11457-11460.

Christie JM, Salomon M, Nozue K, Wada M, Briggs WR. 1999. *Proceedings of the National Academy of Sciences of the United States of America.* 96: 8779-8783.

Cramer GR, Urano K, Delrot S, Pezzotti M, Shinozaki K. 2011. Effects of abiotic stress on plants: a systems biology perspective. *Bio Med Central Plant Biology.* 11: 163 -177.

Dall'Osto L, Lico C, Alric J, Giuliano G, Havaux M, Bassi R. 2006. Lutein is needed for efficient chlorophyll triplet quenching in the major LHCII antenna complex of higher plants and effective photoprotection in vivo under strong light. *Journal of BioMedical Plant Biology.* 6:32.

Demarsy E, Frankhauser C. 2009. Higher plants use LOV to perceive blue light. Journal of Current Opinion in Plant Biology. 12: 69-74.

Demmig-Adams B, Adams WW. 1996. The role of xanthophyll cycle carotenoids in the protection of photosynthesis. *Trends in Plant Science.* 1: 21-26.

Demotes-Mainard S, Péron T, Corot A, Bertheloot J, Gourrierec J, Le Travier S, Sakr S. 2016. Plant responses to red and far-red lights, applications in horticulture. *Environmental and Experimental Botany.* 121: 4-21.

Devlin PF, Robson PRH, Patel SR, Goosey L, Sharrock RA, Whitelam GC. 1999. Phytochrome D acts in the shade-avoidance syndrome in *Arabidopsis* by controlling elongation growth and flowering time. *Journal of the American Society of Plant Physiologists.* 119: 909-915.

Dorais M, Papadopoulos AP, Gosselin A. 2001. Greenhouse tomato fruit quality: the influence of environmental and cultural factors. *Horticultural Reviews.* 26: 239-319.

Economou AS, Read PE. 1987. Light treatment to improve efficiency of in vitro propagation systems. *Journal of the American Society for Horticultural Science.* 22: 751-754.

Formaggio E, Cinque G, Bassi R. 2001. Functional architecture of the major light harvesting Complex from Higher Plants. *Journal of Molecular Biology.* 314: 1157-1166.

Foshi AW, Kumar A. 2003. What can we Learn from the Electromagnetic Spectrum? *Resonance.* 8: 8-25.

Fujiwara TK, Isobe S, Limoto M. 1999. Effects of controlled atmosphere and low light irradiation using red light emitting diodes during low temperature storage on the visual quality of grafted tomato plug seedlings. *Environmental Control in Biology.* 37: 185-190.

Fukushima T, Sato K, Ohi T, Cho M. 2014. Growth characteristics of cabbage plug seedlings due to mutual shading among neighbouring seedlings. *Biosystem Engineering.* 121: 77-84.

Gallicchio L, Boyd K, Matanoski G, Tao XG, Chen L, Lam TK, Shiels M, Hammond E, Robinson KA, Caulfield LE, Herman JG, Guallar E, Alberg AJ. 2008. Carotenoids and the risk of developing cancer: A systematic review. *Journal of Clinical Biochemistry and Nutrition.* 88: 372-383.

Goh C. 2009. Phototropins and chloroplast activity in plant blue light signalling. *Plant Signalling and Behaviour.* 4(8): 693-695.

Goren A, Alkalai-Tuvia S, Perzelan Z, Fallik E. 2011. Photoselective shade nets reduce postharvest decay development in pepper fruits. *Advances in Horticultural Science.* 25(1): 26-31.

Hall DO, Rao KK. 1999. *Photosynthesis.* 6th ed. Cambridge University Press: Cambridge

Hoenecke ME, Bula RJ, Tibbits TW. 1992. Importance of blue photo levels for lettuce seedlings grown under red light emitting diodes. *Journal of the American Society for Horticultural Science.* 27: 427-430.

Hogewoning SW, Trouwborst G, Maljaars H, Poorter H, Van Leperen W. Harbinson J. 2010. Blue light dose-response of leaf photosynthesis, morphology, and chemical composition of *Cucumis sativus* grown under different combinations of red and blue light. *Journal of Experimental Botany.* 61: 3107-3117.

Horton P, Ruban AV, Walters RG. 1996. Regulation of light harvesting in green plants. *Annual Review of Plant Physiology and Plant Molecular Biology.* 47: 655-684.

Ilić ZS, Milenković L, Durovka M, Kapoulas N. 2011. The effect of color shade nets on the greenhouse climate and pepper yield. In: Symposium Proceeding 46th Croatian and 6th International Symposium Agriculture. Opatija. pp. 529-533.

Ilić ZS, Milenković L, Stanojević L, Cvetković D, Fallik E. 2012. Effects of the modification of light intensity by color shade nets on yield and quality of tomato fruits. *Scientia Horticulturae.* 139: 90-95.

Janoudi AK, Konjevic R, Whitelam GC, Gordon W, Poff KL. 1997. Both phytochrome A and phytochrome B are required for the normal expression of phototropism in *Arabidopsis thaliana* seedlings. *Journal of the American Society of Plant Physiologists.* 101: 278-282.

Janoudi AK, Poff KL. 1992. Action spectrum for enhancement of phototropism by *Arabidopsis thaliana* seedlings. *Journal of Photochemistry and Photobiology* 56: 655-659.

Joseph JA, Shukitt-Hale B, Wills LM. 2009. Grape juice, berries, and walnuts affect brain ageing and behaviour. *Journal of Nutrition.* 139: 1813-1817.

Kalaji MH, Woejko E, Loboda T. Pietkiewicz S, Wyszynski Z. 2004. Chlorophyll a fluorescence: A convenient tool for photosynthetic performance evaluation of barley plants grown under different nitrogen rates. *Zesz. Probl. Post. Nauk Roln.* 496: 375-83.

Kautsky H, Hirsch H. 1931. Neue Versuche zur Kohlensaureassimilation. *Naturwissenschaften.* 19: 96.

Kempen E. 2012. Greenhouse production techniques – Agronomy 312. Unpublished class notes. University of Stellenbosch.

Keunhwa K, Jieun S, Sang-Hee L, Hee-Seok K, Juliun N, Giltsu C. 2011. Phytochromes inhibit hypocotyls negative gravitropism by regulating the development of endodermal amyloplast through phytochrome-interacting factors. *Proceedings of*

the National Academy of Sciences of the United States of America. 108(4): 1729-1734.

Kim SJ, Hahn EJ, Heo J-W, Pack KY. 2004. Effects of LEDs on net photosynthetic rate, growth and leaf stomata of chrysanthemum plantlets in vitro. *Journal of the American Society for Horticultural Science.* 101: 143-151.

Kimura M, Rodriguez-Amaya DB. 2003. Carotenoid composition of hydroponic leafy vegetables. *Journal of Agricultural and Food Chemistry.* 51: 2603 -2607.

Krames MR, Ochiai-Holcomb M, Höfler GE, Carter-Coman C, Chen EI, Tan I-H, Grillot P, Gardner NF, Chui HC, Huang J-W, Stockman SA, Kish FA, Craford MG, Tan TS, Kocot CP, Hueschen M, Posselt J, Loh B, Sasser G, Collins D. 1999. High-power truncated-inverted-pyramid $(Al_xGa_{1-x})_{0.5}In_{0.5}P/GaP$ light-emitting diodes exhibiting >50% external quantum efficiency. *Journal of Applied Physics Letters.* 75: 2365-2367.

Krucker M, Hummel RL, Cogger C. 2010. Chrysanthemum production in composted and noncomposted organic waste substrates fertilized with nitrogen at two rates using surface and subirrigation. *International Society for Horticultural Science.* 45: 1695 - 1701.

Lange L, Noble P, Osmond C, Ziegler H. 1981. Responses to Physical Environment 12A. *Physiological Plant Ecology I.* 67: 259.

Lawlor DW. 2001. *Photosynthesis.* 3rd ed. BIOS Scientific: Oxford

Lin K-H, Huang M-Y, Huang W-D, Hsu M-H, Yang Z-W, Yang C-M. 2013. The effects of red, blue, and white light-emitting diodes on the growth, development, and edible quality of hydroponically grown lettuce (*Lactuca sativa L. var. capitata). Scientia Horticulturae.* 150: 86-91.

Lin C, Shalitin D. 2003. Cryptochrome structure and signal transduction. *Plant Biology.* 54: 469-496.

Long SP, Humphries S, Falkowski PG. 1995. Photoinhibition of photosynthesis in nature. *Annual Review of Plant Physiology and Plant Molecular Biology.* 45: 633-662.

Maxwell K, Johnson GN. 2000. Chlorophyll fluorescence - a practical guide. *Journal of Experimental Botany.* 345: 659-668.

Meena, RK, Vashisth A, Singh R, Singh B, Manjaih KM. 2014. Study on change in microenvironment under different colour shade nets and its impact on yield of spinach (Spinacia oleracea L.). *Agricultural Meteorology.* 16: 104–111.

Meena R, Andhale RP, Meena RK. 2016. Effects of Shade Net Colours, Its Intensity and Fertilizer Levels on Growth and Yield of Beetroot (*Beta vulgaris* L.). *Journal of Pure and Applied Microbiology.* 10(2): 1553-1558.

Michelle J, Van S, Claire ES. 1990. Bark preparation and composting, pp. 5-30. In: Michelle et al. (eds), *Preparation and utilization of pine bark as a growing medium for plants.* Natal Witness Printers: Pietermaritzburg

Misra AN, Misra M, Singh R. 2012. Chlorophyll Fluorescence in Plant Biology, pp. 171-192. In: Misra AN (ed.), *Biophysics.* Intech.

Miyashita Y, Kitaya Y, Kozai T. 1995. Effects of red and far-red light on the growth and morphology plantlets in vitro: Using light emitting diode as a light source for micropropagation. *International Society for Horticultural Science.* 393:189-194.

Murata N, Takahashi S, Nishiyama Y, Allakhverdiev SI. 2007. Photoinhibition of photosystem II under environmental stress. *Biochimica et Biophysica Acta Bioenergetics.* 1767: 414-421.

Nagatani A. 2010. Phytochrome: Structural basis for its functions. *Current Opinion in Plant Biology*. 13: 565-570.

Nelson PN. 1991. *Greenhouse operations and management.* 4th ed. Prentice Hall: Englewood Cliffs, NJ.

Nooyens ACJ, Bueno-de-Mesquita B, van Boxtel MPJ, van Gelder BM, Verhagen H, Verschuren WMM. 2011. Fruit and vegetable intake and cognitive decline in middle-aged men and women: The Doetinchem Cohort Study. *British Journal of Nutrition.* 106: 752-761.

Ohashi-Kaneko K, Takase M, Kon N, Fujiwara K, Kurata K. 2007. Effect of light quality on growth and vegetable quality in leaf lettuce, spinach and komatsuna. *Environment Control Biology.* 45: 189-198.

Papageorgiou G, Govindjee. 2004. *Chlorophyll a fluorescence – a signature of photosynthesis. Springer.* Dordrecht.

Pinho P, Jokinen K, Halonen L. 2017. The influence of the LED light spectrum on the growth and nutrient uptake of hydroponically grown lettuce. *The Society of Light and Lighting.* 49: 866-881.

Powles SB. 1984. Photoinhibition of photosynthesis induced by visible light. *Annual Review of Plant Physiology.* 35: 15-44.

Prasad BVG, Chakravorty S. 2015. Effects of climate change on vegetable cultivation – a review. *Nature Environment and Pollution Technology*. 14(4): 923-929.

Quail PH. 2010. Phytochromes. *Current Opinion in Plant Biology*. 20: R503-R504.

Sancar A. 1994. Structure and function of DNA photolyase. *Journal of Biochemistry*. 33: 2-9.

Sato F, Yoshioka H, Fujiwara T, Higashio H, Urakami A, Tokuda S. 2003. Effects of the age of cabbage plug seedlings on initial growth and carbohydrate partitioning after transplanting. *Journal of the Japanese Society for Horticultural Science.* 72: 440-445 [in Japanese with English abstract].

Savvides A, Fanourakis D, Van Leperen W. 2011. Co-ordination of hydraulic and stomatal conductances across light qualities in cucumber leaves. *Journal of Experimental Botany.* 63(3): 1135-1143.

Schuerger AC, Brown CS, Stryjewski EC. 1997. Anatomical features of pepper plants (*Capsicum annuum* L.) grown under red light-emitting diodes supplemented with blue or far-red light. *Annals of Botany.* 79: 273-282.

Schmilewski G. 2008. The role of peat in assuring the quality of growing media. *Mires Peat*. 3: 1 - 8.

Scoffoni C, Pou A, Aasamaa K, Sack L. 2008. The rapid light response of leaf hydraulic conductance: New evidence from two experimental methods. *Plant, Cell & Environment.* 31: 1803-1812.

Sellin A, Sack L, Oˇunapuu E, Karusion A. 2011. Impact of light quality on leaf and shoot hydraulic properties: A case study in silver birch *(Betula pendula). Plant, Cell & Environment.* 34: 1079-1087.

Shacklock PS, Read ND, Trewavas AJ. 1992. Cytosolic free calcium mediates red light-induced photomorphogenesis. *Nature.* 358: 753-755.

Shahak Y. 2008. Photo-selective netting for improved performance of horticultural crops: A review of ornamental and vegetable studies in Israel. *Acta Horticulturae.* 770: 161-168.

Shahak Y, Gussakovsky EE, Gal E, Ganelevin R. 2004. ColorNets: Crop Protection and Light-Quality Manipulation in One Technology. *Acta Horticulturae*. 659: 143-151.

Smith H. 2000. Phytochromes and light signal perception by plants – an emerging synthesis. *Journal of Nature.* 407: 585-591.

Strasser RJ, Akoyunoglou G. 1981. Structure and Molecular Organisation of the Photosynthetic Apparatus. *Balaban International Science Services, Philadelphia, Pennsylvania.* pp 727-737.

Taiz L, Zeiger E. 2002. *Plant physiology*, 3rd ed. Sinauer Associates: Sunderland, MA.

Takemiya A, Inoue M, Doi T, Kinoshita T, Shimazaki K. 2005. Phototropins promote plant growth in response to blue light in low light environments. *Plant, Cell & Environment.* 17: 1120-1127.

Tamulaitis G, Duchovskis P, Bliznikas Z, Breive K, Ulinskaite R, Brazaitytè A, Novičkovas A, Žukauskas A. 2005. High-power light-emitting diode based facility for plant cultivation. *Journal of Physics.* 38: 3182-3187.

Teitel M, Lirion O, Haim Y, Seginer I. 2008. Flow through inclined and concertina shaped screens. *Acta Horticulture.* 801: 99-106.

Tikkanen M, Mekala NR, Aro E-M. 2014. Photosystem II photoinhibition-repair cycle protects photosystem I from irreversible damage. *Biochimica et Biophysica Acta-Bioenergetics.* 1837: 201-2015.

Urrestarazu M, Guillén C, Mazuela PC, Carrasco G. 2008. Wetting agent effect on physical properties of new and reused rockwool and coconut coir waste. *Scientiae Horticulturae.* 116: 104 - 108.

Valverde F, Mouradov A, Soppe W, Ravenscroft D, Samach A, Coupland G. 2004. Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. *Science.* 303: 1003-1006.

Voicu MC, Zwiazek JJ, Tyree MT. 2008. Light response of hydraulic conductance in bur oak (*Quercus macrocarpa*) leaves. *Tree Physiology.* 28: 1007-1015.

Walters RG. 2005. Towards understanding of photosynthetic acclimation. *Journal of Experimental Botany.* 56(411): 435-447.

Whitelam GC, Halliday KJ. 2007. *Light and plant development.* Blackwell Publishing: Oxford.

Xiao FG, Shen L, Ji HF. 2011. On photoprotective mechanisms of carotenoids in light harvesting complex. Biochemical and Biophysical Research Communications. 414: 1-4.

Xiong J, Tian Y, Wang J, Liu W, Chen Q. 2017.Comparison of Coconut Coir, Rockwool, and Peat Cultivations for Tomato Production: Nutrient Balance, Growth and Fruit Quality. *Frontiers in Plant Science.* 8(1327): 1 - 9.

Yanagi T, Okamoto K, Takita S. 1996. Effects of blue, red, and blue/red lights on two different PPF levels on growth and morphogenesis of lettuce plants. *International Society for Horticultural Science.* 440: 117-122.

Yang Z, Liu B, Su J, Liao J, Lin C, Oka Y. 2017. Cryptochromes Orchestrate Transcription Regulation of Diverse Blue Light Responses in Plants. *Photochemistry and Photobiology.* 93: 112-127.

Yanovsky MJ, Casal JJ, Whitelam GC. 1995. Phytochrome A, phytochrome B and HY4 are involved in hypocotyl growth responses to natural radiation in *Arabidopsis*: Weak de-etiolation of the phyA mutant under dense canopies. *Plant Cell & Environment.* 18: 788-794.

Yan-xiu M, Xiao-zhuo W, Li-hong G, Qing-yun C, Mei Q. 2015. Blue light is more essential than red light for maintaining the activities of photosystem II and I and

28

photosynthetic electron transport capacity in cucumber leaves. *Journal of Integrative Agriculture*. 10: 2095-3119.

Zhang H, Xu ZG, Cui J, Guo YS, Gu AS. 2009. Effects of different spectra on growth and nutrition quality of radish seedlings. *China Vegetables.* 10: 28-32.

Zhen S, van Iersel MW. 2017. Far-red light is needed for the efficient photochemistry and photosynthesis. *Journal of Plant Physiology*. 209: 115-122.

Zinth W, Arlt T, Wachtveilt J. 1996. The primary processes of bacterial photosynthesis ultra fast reactions for the optimum use of light energy. *Journal of Physics and Chemistry*. 100: 192-196.

Zou J, Rogers WE, Siemann E. 2009. Plasticity of *Sapium sebiferum* seedling growth to light and water resources: Inter- and intraspecific comparisons. *Basic and Applied Ecology*. 10(2009): 79 - 88.

Chapter 3

Light spectral alteration through different coloured shade nets modifies crop response of cabbage (*Brassica* spp), but not lettuce (*Lactuca sativa*)

Willem Gericke,[1] Misha de Beer[2] and Marcellous Le Roux[1*]

[1] *Department of Agronomy, University of Stellenbosch, Stellenbosch, South Africa.*
[2] *Centre for Water Sciences and Management, North West University, Potchefstroom, South Africa*

[*] *Corresponding author, e-mail:* mrl@sun.ac.za

3.1   Abstract

Lettuce and cabbage growth and development are influenced by fluctuating photosynthetically active radiation (PAR) in terms of altering light quantity and quality levels. Different coloured shade nets are used to protect these vegetables against high solar radiation levels. The aim of this trial was to determine the effect of these shade nets - with nearly the same light quantity abilities - on the growth and development of lettuce and cabbage seedlings. Seedlings were grown under 20% black net, 20% black and white, 20% blue, 30% Photon Red, and 30% white net. Chemical analyses, physical measurements and chlorophyll fluorescence parameters were used to determine differences in lettuce and cabbage seedlings and mature crops, grown under these shade nets. Time (in weeks) was the most influential factor affecting chlorophyll fluorescence parameters for both lettuce and cabbage in the seedling and maturing phases. The physical parameters in lettuce differed between cultivars, while net colour influenced the potassium (K) and sodium (Na) leaf content, and physical parameters, such as fresh biomass, and leaf length and width in cabbage. In general, the heaviest cabbages were harvested from the white nets, while the lightest were harvested from the black nets. Light quality manipulation can therefore have a significant effect on seedling and crop vigour.

Keywords: chemical analysis, chlorophyll fluorescence, physical parameters, seedlings, shade nets

3.2   Introduction

Spectral light is a determining factor in plant growth and development, and has a direct influence on assimilate and photosynthate production in plants. Cloud cover

alters light quantity and quality, and low clouds can significantly increase the R:FR ratio (Reinhardt *et al.*, 2010). Bastias *et al.* (2012) demonstrated that light spectrum can also be altered through different colour photo-selective nets. Their studies indicated that a 40% blue net increased the blue:red (B:R) ratio by 30% and reduced the red:far red (R:FR) ratio by 10% compared to a 20% white net. The same results were found under blue coloured nets where the B:R ratio was increased, while the R:FR ratio was not significantly changed (Shahak *et al.*, 2004). Red light is known to reduce electron transport (ET) from PSII to PSI, while blue light has the opposite effect on ET (Yan-xiu *et al.*, 2015). The light saturation point of lettuce is 500-520 $\mu mol\ m^{-2}\ s^{-1}$, which is lower than that of other vegetables (Glenn *et al.*, 1984; Li and Gong, 2002). Lettuce grown at a light quantity of ≥ 800 $\mu mol\ m^{-2}\ s^{-1}$, is more prone to photoinhibtion than lettuce cultivated at lower light quantities (Fu *et al.*, 2012).

The use of shade netting has become very popular in areas with high temperatures (Kittas *et al.*, 2009) and in particular lettuce growers commonly use different forms of shading to reduce high light intensities (Hu *et al.*, 1999). Other advantages of shade nets are sheltering from wind and hail, as well as protection from birds and insect transmitted diseases (Teitel *et al.*, 20080. Shade nets can be erected freestanding, or in conjunction with polyethylene multispan greenhouses (Shahak *et al.*, 2004). Plants that are grown under moderate climatic conditions are exposed to ambient temperature fluctuations, which include high and low temperatures (McKersie and Leshem, 1994). This type of thermal stress can induce changes in the membrane fluidity and saturation of fatty acids, which could affect photosynthetic ET efficiency (Kalaji *et al.*, 2004). Low temperatures severely affect the functioning of the photosynthetic system (Öquist *et al.*, 1987), increase the probability of photoinhibition (Goodde and Bornman, 2004), and decrease photosynthetic e⁻ transportation (Savitch *et al.*, 1997). According to Strauss *et al.* (2006), the PI_{abs} values were lower for soybeans exposed to seven consecutive nights of low temperatures than non-exposed soybeans. On the other hand, plants grown under high temperatures have distinct increases in membrane permeability, and have limited ET in PSII subunits. This is due to the presence of the K-stage (OJIP fluorescence), which indicates damage to the PSII subunits (Kalaji *et al.*, 2017). Leafy lettuce (*Lactuca sativa var. crispa* L.) grown under a far red filter had a lighter

green colour, tasted less bitter, and had a higher Ca content, resulting in less tipburn (Kleemann, 2004).

Plant photosynthetic pigments have limited absorption of all the energy meant for photosynthesis during high light conditions, which compromises photosynthetic reactions (photoinhibition), or in extreme cases damages the photosynthetic apparatus (Coleman *et al.*, 1988; Prasil *et al.*, 1992; Weng *et al.*, 2005). Plants can also trigger preventative mechanisms to protect themselves from light stress, photoinhibition, and injuries sustained through excessive solar radiation (Mulkey and Pearcy, 1992; Feild *et al.*, 2001; Hoch *et al.*, 2003). Moreover, once the damage to chloroplasts is repaired after high light intensity, they can withstand high photosynthetically active radiation (PAR) (Kozaki and Takeba, 1996; Baena-Gonzalez *et al.*, 1999; Govindjee, 2002). Tomato plants grown under high light intensities can synthesise high levels of anti-oxidants which can lead to disorders in the development and appearance of tomato fruit (Dorais *et al.*, 2001), as well as sunscald formation (Adegoroye and Jolliffe, 1987). Therefore, the Russians used a 35% shade netting during a hot Siberian summer with high light intensities and that resulted in the largest tomato crop, although it did not fully eradicate sunscald on the tomato fruit (El-Gizawy *et al.*, 1992).

Chlorophyll fluorescence is used to determine the fast chlorophyll fluorescence measurements that depict a typical 'OJIP' curve, with F_o as the origin. The O-J transition is a rapid ET process and is completed in less than two milliseconds, and is subsequently followed by the slow J-I (thermo-sensitive) and I-P (thermal) phases (Misra *et al.*, 2012). Maximum fluorescence F_m is achieved within one second, and indicates closed PSII centres (Schansker *et al.*, 2005). Light energy is absorbed by chlorophyll in photosynthetic systems, and can be used in the following three processes, which are all in competition with one another: a) as the driver for photosynthesis or a photochemistry process, b) being dissipated as heat or c) being re-emitted as fluorescence. The last mentioned, i.e. chlorophyll fluorescence, enables us to determine the heat dissipation and photochemical efficiency of plants (Misra *et al.*, 2012). Noomnarm and Clegg (2009) reported that molecules are excited from the ground electronic singlet state (S_0) to the electronic excited singlet state (S_1), with the absorption of a photon within $< 10\text{-}15 \text{ s}^1$, and is emitted as radiant energy after a time lapse. Higher molecular energy levels (S_2 to S_n) can be reached

and eventually the molecules will return to the (S_o) state through a process called fluorescence emission. Changes in the energy levels must equal the energy of the emitted photon (Misra *et al.*, 2012).

The purpose of this study was to compare differences between chlorophyll fluorescence parameters, physical measurements and chemical macro- and micro-elements of lettuce and cabbage seedlings, and mature crops grown under different coloured shade nets with minimal variance in solar light quantity.

3.3   Material and Methods

3.3.1 Location

Trial one was carried out from 16 April 2014 to 25 August 2014 on the farm Willemsheim, which is situated in the Buffelspoort area, North West Province, South Africa (25°48'30.5"S, 27°29'3.7"E). The farm is situated on the southern slopes of the Magalies Mountain, and is thus north facing and has a gradient of six percent. All irrigation water is gravity-fed from the mountain and filtered with a Netafim 120 micrometer disc filter. The mountain water's total dissolved solids (TDS) are 22 ppm and have no available bi-carbonate.

3.3.2 Plant material and experimental set-up

Fresh pelletised lettuce and cabbage seeds were sown in new polystyrene seedling trays of 200 cavities per tray, with dimensions of 70 cm x 35 cm x 7 cm. The growing medium used was a mixture of 60% coir and 40% Klasmann TS 1 fine peat. The coir was buffered with a 1% $CaNO_3$ solution, and no buffering was done for the Klasmann TS 1 fine peat.

The growing medium was pre-enriched (Table 3.1) with Nitrosol at 2 $L.m^{-3}$ and Scotts Osmocote Start controlled release (6-week period) 12+11+17+2MgO fertiliser, at 1 $kg.m^{-3}$. Micro-organisms were obtained from Cosmoroot and were mixed into the medium at a concentration of 10 $gr.m^{-3}$. The growing medium had a final EC of 0.8 $mS.cm^{-1}$ and a pH of 5.8. The lettuce varieties used were 'Robinson' Nickerson Zwaan and 'Grand Slam' from (Starke Ayres™), while the cabbage varieties were 'Conquistador' (Sakata™) and 'Sapphire' (Starke Ayres™). The lettuce and cabbage

varieties were selected as they were appropriate for the specific season, and were favoured among farmers.

Table 3.1: Nutrient composition of fertilisers, Nitrosol and Osmocote (%), and for Cosmoroot (ppm) and micro-nutrient compositions of products applied to the lettuce and cabbage seedlings.

	N	P	K	Other	
Nitrosol	8	3	6		
Osmocote	12	11	17	$2M_gO$	
Cosmoroot	70 (ppm)	205 (ppm)	50 (ppm)	L-Amino acid 30 (ppm)	Humic substance 155 (ppm)

The seeds were germinated in a germination room at 20°C, with humidity of 90%. Once germinated, one seedling tray of 200 seedlings per variety of lettuce and cabbage, was placed under each of the five different coloured shade nets at a height of 20 cm above the ground, or 2.3 m underneath the nets. Each colour net treatment was replicated three times, and had the same number of seedling trays. The seedlings were fertigated in each trial plot via micro-irrigation, using a Tank A and B system in conjunction with a double Dosatron D8R dosing system. Tank A consisted of 60 kg of calcium-nitrate and 50 kg of potassium-nitrate per 1000 L of stock solution. Dosatron A was set at a 1% injection rate, whilst Dosatron B was set at 1.4% with 30 kg magnesium-sulphate, 6 kg mono-potassium-phosphate, 7 kg potassium-sulphate, and 2 kg Microplex per 1000 L stock solution. Fertigation commenced on visual inspection, and the seedlings were fertigated until water started leaching out of the trays. Irrigation commenced at the same time, and the seedlings received the same volume.

Five-week-old lettuce and cabbage seedlings were transplanted into the soil under the same trial plots. No pre-plant fertiliser was added to the soil, and irrigation commenced simultaneously for all the colour nets, with the same nutrient

composition and injection rate as for the seedlings. Lettuce plants reached maturity at 15 weeks and cabbage at 21 weeks.

3.4   Net Structures

Twenty percent black shade net is considered the norm in seedling production, and was used as the control in the experiment. A HPS lamp was used as a constant light source (1000 $\mu mol\ m^{-2}\ s^{-1}$), to determine which coloured nets portrayed light quantity similar to the control. This method was used rather than sunlight, in order to eliminate the possibility of variation in sunlight quantity. Each net was placed individually over a frame under the HPS lamp, with a fixed intensity of 1000 $\mu mol\ m^{-2}\ s^{-1}$. The light intensities of the different coloured shade nets were measured individually, and the lamp's light intensity was also measured in-between readings. Light quantity readings were taken with a light meter (Model MQ-200, Apogee Instruments, Logan, UT). The coloured nets with the light quantity most similar to a 20% black net (control 780 $\mu mol\ m^{-2}\ s^{-1}$) were: 20% black and white (760 $\mu mol\ m^{-2}\ s^{-1}$), 20% blue (760 $\mu mol\ m^{-2}\ s^{-1}$), 30% Photon Red (760 $\mu mol\ m^{-2}\ s^{-1}$), and 30% white (740 $\mu mol\ m^{-2}\ s^{-1}$). Photon Red 30% with a light quantity of (760 $\mu mol\ m^{-2}\ s^{-1}$), was used instead of Photon Red 20% (790 $\mu mol\ m^{-2}\ s^{-1}$), as its light quantity was similar to 20% black and white, and 20% blue. Shade net percentage represents the material used in constructing the net per unit area, and does not represent the shade percentage of a specific net. Thus, a 20% shade net would consist of one-fifth of the net area under fibres, while with a 50% shade net the area would represent half the area under fibres. The net construction percentage does not indicate shade percentage. Each trial plot was replicated three times and had dimensions of 3 m x 2.5 m x 2.5 m - with 3 m spacing between plots to avoid overshadowing. There were no differences between the control and the different coloured nets regarding their inside light quantities. Light quantity readings were taken outside and inside for each net on clear sky days, and chlorophyll fluorescence readings were taken simultaneously.

3.5   Experiment 1

3.5.1 Measurements and analysis: Chlorophyll fluorescence

Once the seed germinated, an equal number of seedlings were placed under different coloured nets. One seedling tray per variety and four different varieties (two Iceberg lettuce: Grand Slam and Robinson and two cabbage Conquistador and Sapphire) were used per coloured net. All the seedling trays were placed on pallets, at a height of 20 cm above the ground. A total of 10, five-week-old lettuce and cabbage seedlings, per variety, were transplanted into the soil under the same colour trial plots, which equated to a density of 5.33 plants m^{-2}. Although this approach ensured enough plant material in terms of *inter alia* protection against disease and, irrigation failure, the cabbage planting density was 77% higher than normal open land production density, but it had the same density as lettuce open land production.

Chlorophyll fluorescence readings were taken at weeks 3, 4 and 5 for lettuce and cabbage in the seedling phase. In the maturing phase readings were taken at weeks 9, 11, 13 and 15 for lettuce, and weeks 9, 11, 13, 15, 17, 19 and 21 for cabbage. Measurements commenced after the plants were dark-adapted for 1 hour after dusk. Fully expanded, middle upper leaves were selected and measured. The data were captured with Handy PEA software, analysed and quantified with the 'Biolyzer' software according to Strasser *et al.* (2000), and then transferred to Excel 2010.

Table 3.2: Description of chlorophyll fluorescence parameters

RC/ABS	Reaction centre per electron absorption of light energy
PHI$_o$/(1-PHI$_o$)	Trapping of excitation energy
PSI$_o$/(1-PSI$_o$)	Conversion of excitation energy to electron transport
δ/1-δ	Reduction of end acceptors
PI$_{abs}$	Performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors
PI$_{total}$	Performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors

Non-destructive fast chlorophyll fluorescence was used in the dark adapted state to determine the functioning of RC/ABS, $PHI_o/(1-PHI_o)$, $PS1_o/(1-PSI_o)$, $\delta/1-\delta$, PI_{abs} and PI_{total} (as described in Table 3.2), for lettuce and cabbage in the seedling phase and maturing phase - using a HANDY-PEA Fluorimeter, (Hansatech Instruments Ltd., Pentney, King's Lynn, Norfolk, England). The light quantity was determined with a light meter (Model MQ-200, Apogee Instruments, Logan, UT).

3.5.2 Physical and chemical analyses

Leaf macro- (N, P, K, Ca, Mg) and micro- (Na, Mn, Fe, Cu, Zn and B) element analyses were done using the dry ashing extraction method, and physical measurements were done at week 15 for lettuce and at week 21 for cabbage. The physical measurements included: root length (mm), root mass (g), stem width (mm), wet head (g) and stem mass (g), and total mass (g). Plants were then dried in an oven for 48 hours at 70°C, and the wet:dry ratios were determined. The chemical analysis was expressed as a percentage of dried leaf weight, and micro element analysis as $mg.kg^{-1}$.

3.6   Statistical analysis

An analysis of variance (ANOVA) was used for data analysis. Mean comparisons of data were determined with Fisher's least significant difference ($p < 0.05$), using Statistica 13 software (StatSoft, Tulsa, OK, USA). The ANOVA test of variance was used to test for interaction between coloured net, cultivar, weeks, coloured nets and cultivar, coloured nets and weeks, cultivar and weeks, coloured nets and cultivars and weeks for RC/ABS, $PHI_o/(1-PHI_o)$, $PS1_o/(1-PSI_o)$, $\delta/(1-\delta)$, PI_{abs} and PI_{total}.

3.7   Results and Discussion
3.7.1 Lettuce seedlings Chlorophyll fluorescence

The age of the plant (in weeks) is the factor affecting all the chlorophyll parameters for lettuce and cabbage seedlings the most, with highly significant values ($p < 0.001$; Table 3.3). According to Tables 3.3 and 3.4, the lettuce seedling phase (week three

to five) had a highly significant value ($p < 0.001$) - indicating an influence between electron absorption of light energy (RC/ABS) and plant age. The RC/ABS values decreased as the plants matured. Minimal variances in maximum, minimum and average temperatures and RH, were observed under the different coloured photo-selective nets (see Table 3.5). The highest temperature was recorded under the blue net. This concurs with Mortensen and Strømme (1987), who recorded the highest temperature under a blue light, with high PAR levels. There were no measurable differences in light quantity between the different coloured photo-selective nets. The average light quantity for weeks three, four, and five was 785, 664, and 804 $\mu mol\ m^{-2}\ s^{-1}$, respectively, inside the nets, while it was 1061, 919, and 1084 $\mu mol\ m^{-2}\ s^{-1}$ outside the nets. According to Fu *et al.* (2012), the probable cause for the sharp decrease in RC/ABS value from week 3 to 4 and 5 is due to the high light quantity experienced in week 3 and again in week 5.

Table 3.3: Significant (*) and highly significant (**) *p* - values for individual chlorophyll fluorescence parameters of lettuce in the seedling phase for weeks 3-5.

	RC/ABS		$PHI_0/(1-PHI_o)$		$PSI_o/(1/PSI_o)$		$\delta/(1-\delta)$		PI_{abs}		PI_{total}	
Lettuce week 3-5	*F*	P	*F*	P	*F*	P	*F*	P	*F*	P	*F*	P
Cultivar	0.83	0.385	8.50	0.015*	6.76	0.026*	4.09	0.071	1.71	0.220	4.66	0.056
Colour Nets	1.35	0.317	0.30	0.869	0.03	0.998	1.19	0.373	0.57	0.692	1.67	0.233
Weeks	52.20	*p* < 0.001**	21.31	*p* < 0.001**	27.65	*p* < 0.001**	66.07	*p* < 0.001**	50.19	*p* < 0.001**	82.67	*p* < 0.001**
Cultivar Colour Nets	1.24	0.354	1.27	0.335	1.07	0.422	0.95	0.476	1.17	0.381	1.69	0.229
Cultivar Weeks	0.55	0.583	1.44	0.261	1.80	0.192	1.88	0.178	1.74	0.200	0.17	0.849
Colour Nets Weeks	0.74	0.656	0.86	0.567	1.06	0.429	0.89	0.543	0.74	0.655	0.58	0.781
Cultivar* Colour Nets *Weeks	0.93	0.517	2.66	0.036*	2.21	0.072	1.50	0.220	1.38	0.265	0.39	0.911

*$p < 0.05$, **$p < 0.001$ at $p = 0.005$

Table 3.4: Individual chlorophyll fluorescence parameters RC/ABS, PHIo/(1-PHIo), PSIo/(1-PSIo), and δ/(1-δ), expressed in relative mean units for lettuce in the seedling phase for weeks 3-5. Highly significant PI_{abs} values are the product of the first three parameters. Highly significant PI_{total} values are the product of the first four parameters. Significant differences between means within a parameter are indicated with different superscript letters.

Weeks	RC/ABS	$PHI_o/(1\text{-}PHI_o)$	$PSI_o(1\text{-}PSI_o)$	$δ/(1\text{-}δ)$	PI_{abs}	PI_{total}
3	9.32[a]	4.63[b]	1.12[a]	0.89[a]	48.33[a]	43.01[a]
4	8.13[b]	4.75[a]	1.14[a]	0.76[b]	44.02[b]	33.46[b]
5	7.56[c]	4.46[c]	0.97[b]	0.78[b]	32.37[c]	25.51[c]

These results corroborate the findings of previous studies, the effects of which were attributed to the plant's photosynthetic pigments capacity to absorb energy - resulting in photoinhibition under prevailing high light conditions (Coleman *et al.*, 1988; Prasil *et al.*, 1992; Weng *et al.*, 2005). In extreme situations a photo-synthetic apparatus could be irreversibly damaged, for example light in excess of 800 *$μmol\ m^{-2}\ s^{-1}$* (Fu *et al.*, 2012). From these results one can conclude that lettuce seedlings were highly sensitive to excess PAR, as it indicates they could not repair the incurred damage to their chloroplasts (Kozaki and Takeba, 1996; Baena-Gonzalez *et al.*, 1999; Govindjee, 2002). Alternatively, the decreasing RC/ABS values can also be due to the influence of low minimum temperatures, which were prominent (Table 3.5), and which impeded the functioning of the photosynthetic system (Öquist *et al.*, 1987). Low temperatures are associated with increased incidences of photoinhibition (Goodde and Bornman, 2004).

Table 3.5: Illustrates the maximum, minimum and average temperatures and relative humidity per net colour for the lettuce and cabbage seedlings from week one to five.

	Black		Black & White		Photon Red		Blue		White	
	T °C	RH%	T °C	RH %	T °C	RH %	T °C	RH %	T °C	RH %
Max	40.8	100	41.0	100	41.3	100	41.6	100	41.5	100
Min	3.8	6.0	3.6	5.9	3.9	6.5	3.5	6.1	3.4	7.0
Ave	17.1	67.1	17.3	66.1	17.3	65.5	17.3	67.5	17.7	66.8

The trapping of excitation energy $PHI_o/(1\text{-}PHI_o)$ values varied significantly from each other for week 3-5 (Table 3.4). The ($PHI_o/(1\text{-}PHI_o)$) values increased from week 3 to

4, followed by a severe decrease from week 4 to 5 (Table 3.4). The increasing $PHI_o/(1-PHI_o)$ values from week 3 to 4 may have been due to the decreasing light intensity in the same time period from week 3 to 4. The light intensity increased sharply from week 4 to 5 - with marked declines $PHI_o/(1-PHI_o)$ for the same period. This concurs with Faseela and Puthur (2017), where rice seedlings were germinated and grown for nine days at 300 $\mu mol\ m^{-2}\ s^{-1}$ and then exposed to 2000 $\mu mol\ m^{-2}\ s^{-1}$ of high light for 8 hours, and showed severe decreasing $PHI_o/(1-PHI_o)$ and $PSI_o/(1-PSI_o)$ values. Plants have a higher photosynthetic efficiency under low light conditions compared to high light conditions. This is due to the formation of smaller reaction centres under high light conditions (Long *et al.*, 1994). Thus, the lettuce seedlings have probably trapped the excited electrons more efficiently, due to larger reaction centres that developed with decreasing light quantity from week 3 to 4, enabling a higher proficiency for $PHI_o/(1-PHI_o)$ in week 4 (Long *et al.*, 1994). The $PHI_o/(1-PHI_o)$ value consequently decreased in week 5 due to higher radiation - resulting in smaller reaction centres and less effective energy absorbed ions.

The conversion of excitation energy to electron transport ($PSI_o/(1-PSI_o)$) varied significantly over time (Table 3.4), with no significant increase from weeks 3 to week 4, followed by a steep decrease from week 4 to 5. The combined effect of the higher potential $PHI_o/(1-PHI_o)$ value for week 4 and the lower RC/ABS for the same time resulted in a slightly more efficient $PSI_o/(1-PSI_o)$ quantum yield of ET from week 3 to 4; the decreasing light quantity from week 3 to 4 was responsible for this. The increasing light quantity from week 4 to 5 was the drive toward a lower RC/ABS value (Fu *et al.*, 2012). Heat stress could also have deactivated PSII RA_A (Oukkaroum *et al.*, 2009) - judging from F_K values at 0.3 ms (Figure 3.1). These combined effects resulted in stress conditions and led to deterioration in the efficiency of ET (He *et al.*, 1996), and resulted in a potential non-photochemical quenching (NPQ) situation. The combined effect of lower RC/ABS and $PHI_o/(1-PHI_o)$ values for week 4 to 5, resulted in a lower $PSI_o/(1-PSI_o)$ value.

The reduction of end acceptors ($\delta/(1-\delta)$), indicates the quantum yield of reduction of end electron acceptors at the PSI acceptor side. The $\delta/(1-\delta)$ decreased significantly from week 3 to 4, followed by a non-significant increase from week 4 to 5. Week 3 differed significantly from week 4 and 5 (Table 3.4). The $\delta/(1-\delta)$ values were all <1 for week 3 to 5 - with a marked decline in week 4 and a slightly higher value in week

5. The efficiency of transporting electrons between PSII and PSI deteriorated. The mechanisms protecting the photosynthetic apparatus were activated in pigment antennae, when the plants were exposed to high amounts of PAR. This action led to a slower ET rate, and the partial degradation of the key protein D1 (Hendrich, 1995; Horton *et al.*, 1996; Baroli and Melis, 1998).

The PI_{abs} showed a gradual decrease from week 3 to 5. Week 3 had a mean relative value of around 49 relative units (RU), followed by a mean relative value of nearly 45 in week 4, and an even lower mean relative value of 33 for week 5 (Table 3.4). This decrease in PI_{abs} values can be ascribed to the minimum and maximum temperatures the plants were exposed to. Soybean plants exposed to 7 consecutive nights of low temperatures resulted in lower PI_{abs} values than the control (Strauss *et al.*, 2006). These low temperatures triggered the plants to adapt, and they regulated their maximum photosynthetic ability (Adams *et al.*, 2001) by increasing thermal energy dissipation (Demmig-Adams *et al.*, 1996) and reducing the formation of reactive oxygen species (ROS) (Morgan-Kiss *et al.*, 2006). The lowered PI_{abs} values are due to the combined interaction of the afore-mentioned chlorophyll fluorescence parameters. Another reason for the weekly decreasing PI_{abs} values is linked to decreasing P values (Ripley *et al.*, 2004), which coincided with the decreasing controlled-release period of six weeks for the Osmocote fertiliser, which can be reduced by high temperatures.

The PI_{total} values were lower compared to the PI_{abs} values for week 3 to 5 (Table 3.4). This is due to smaller $\delta/(1-\delta)$ values than 1, from weeks 3 to 5, with the lowest value in week 4 – which negatively influenced the PI_{total} values. This resulted in the gradually declining PI_{total} values (Table 3.4). According to Živčák *et al.* (2014), the PI_{abs} and PI_{total} values indicate changing nitrogen (N) levels in wheat. They further state that the PI_{total} values are more sensitive in terms of determining fluctuating N levels than the insensitive F_v/F_m values. Seemingly, lettuce seedlings were showing signs of N depletion over time (Table 3.4). This is corroborated by the high *F* value (Table 3.4), which indicates that the PI_{total} value is influenced over time, and coincides with the fact that Osmocote controlled-release fertiliser is released over a six-week period.
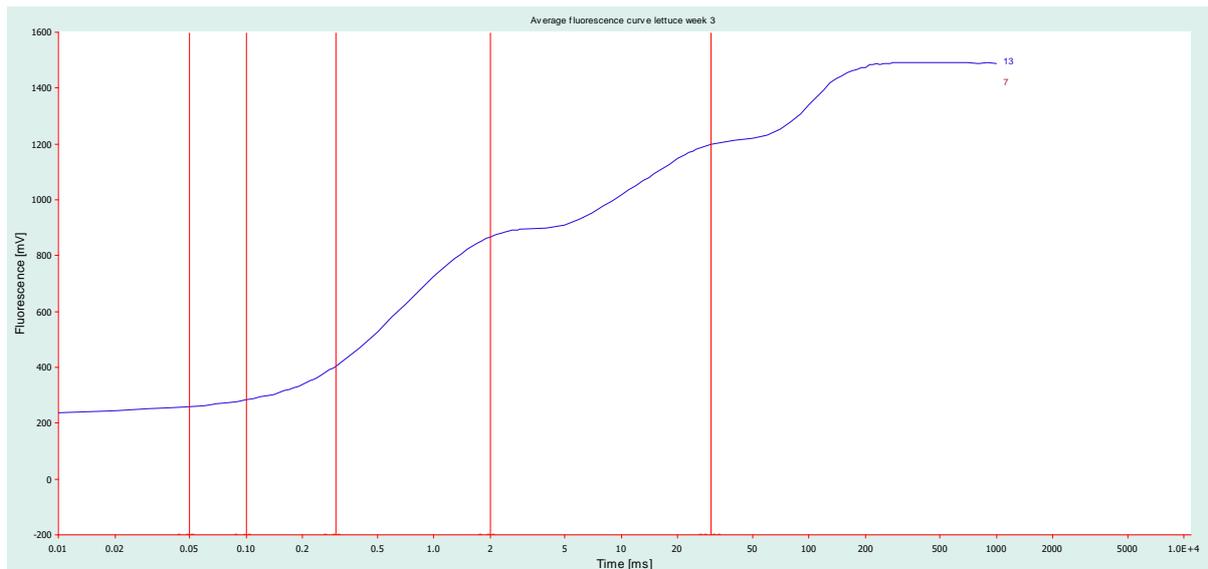
Figure 3.1: The OJIP fluorescence curve for the averaged value of the different lettuce cultivars in week five. F_o is the minimal fluorescence when all PSII RC's are open, F_J is the relative variable fluorescence at 2ms and indicates the number of closed RC's relative to the number of RC's that could be closed, F_I is the relative variable fluorescence at 30ms, F_M is the maximum fluorescence when all PSII RC's are closed. F_K commences at 0.3ms, and indicates heat stress.

3.7.2 Mature lettuce plants: Chlorophyll fluorescence

The plant age (in weeks), once again had the most pronounced influence on all chlorophyll fluorescence parameters for lettuce in the maturing phase from week 9 to 15. The average light quantity for weeks 9, 11, 13 and 15 was 822, 743, 925 and 982 *µmol m^{-2} s^{-1}*, respectively, inside the nets, and 1130, 985, 1269 and 1374 *µmol m^{-2} s^{-1}* outside the nets (Table 3.8). Light stress normally coincides with other types of stresses. Numerous studies have demonstrated the negative effect it has on photosynthesis, although the influences have not yet been fully elucidated (Kalaji *et al.*, 2017). Furthermore, the energy fluxes within PSII still need to be elucidated (Kalaji *et al.*, 2017). The RC/ABS values increased progressively from week 9 to 15, and a highly significant interaction occurred from weeks 9 to 11 and 13 to 15 (Table 3.7). The minimum, maximum and average temperatures (Table 3.6) during the maturing phase were lower than during the seedling phase. This correlates with the lower F_K values at 0.3 ms in the OJIP curves from week 15 (Figure 3.4). The lower F_K values in Figure 3.4 indicate that the plants experienced less heat stress, which coincides with increasing RC/ABS, trapping, and ET values (Kalaji and Loboda,

42

unpublished data). Photosystem II antennae size of barley grown under different light intensities correlated with their corresponding chlorophyll fluorescence indices (Chernev *et al.*, 2006). Light quantity increased from week 11 to week 13 and 15 by 24.5% and 32% respectively. This forced the plants to have smaller antennae - thus reducing the possibility of photoinhibition (Tanaka and Tanaka, 2000), and resulted in higher RC/ABS values.

Table 3.6: The maximum-, minimum- and average temperatures and relative humidity (RH) per net colour for the lettuce in the maturing phase from week 9 to 15.

	Black		Black & White		Photon Red		Blue		White	
	Temp (°C)	RH (%)	Temp (°C)	RH (%)	Temp (°C)	RH (%)	Temp (°C)	RH (%)	Temp (°C)	RH (%)
Max	35.4	100	35.5	100	35.8	100	36.1	100	36.0	100
Min	2.0	10.2	2.0	9.8	2.1	10.3	1.8	9.9	1.8	10.5
Ave	15.3	50.8	15.4	50.4	15.5	50.1	15.5	50.9	15.6	50.5

The $PHI_o/(1-PHI_o)$ increased slightly from week 9-11, while the $PSI_o/(1-PSI_o)$ significantly increased for the same time-frame (Table 3.7). The increased ET value coincided with decreasing light quantity, and totally contradicts the findings of Bailey *et al.* (2001). These authors state that ET is increased with higher light quantities. A possible explanation for the higher ET value could be due to a lower leaf temperature in week 11 (Salvucci and Crafts-Brandner, 2004). There was a significant increase for $PHI_o/(1-PHI_o)$ from week 11-13, while a gradual and significant decrease for $PSI_o/(1-PSI_o)$ occurred in the same time-frame. For week 13-15, the $PHI_o/(1-PHI_o)$ decrease was gradual, while the $PSI_o/(1-PSI_o)$ increase was significant. This might have been due to an increasing source:sink ratio, which enabled a higher net photosynthesis (P_N) - resulting in a higher sink demand, and initiated head formation (Yan *et al.*, 2011).

The $δ/(1-δ)$ values followed a decrease – increase - decrease trend for week 9-15 (Table 3.7). The first decrease - increase curve occurred from week 9-13 and could be linked to a decrease in light quantity for week 11. According to Bailey *et al.*

43

(2001), an increase in light quantity will lead to an increase in ET, which explains the higher $\delta/(1-\delta)$ values for week 13 compared to week 11. Light quantity and quality can be altered by cloud cover, and low cloud cover significantly increased the R:FR ratio (Reinhardt *et al.*, 2010). Red light is shown to severely lower ET from PSII donor side up to PSI (Yan-xiu *et al.*, 2015). This action can explain the increased light quantity for week 15 with a decrease in $\delta/(1-\delta)$ values.

The PI_{abs} parameter is a highly sensitive indicator of the physiological condition of plants (van Heerden *et al.*, 2007). There is also a very high correlation between the decreasing PI_{abs} values and decreasing CO_2 assimilations. The PI_{total} values were all lower than the PI_{abs} values - due to the influence of the $\delta/(1-\delta)$ values. When the $\delta/(1-\delta)$ values are bigger than 1, the PI_{total} values will be larger than the PI_{abs} values and vice-versa. The PI_{abs} value for week 9 differed significantly from weeks 11, 13 and 15. In addition, although the value from week 11 did not differ from week 13, there was a difference in week 15. The lower value for PI_{total} compared with PI_{abs} for week 11, might have been due to the lower $\delta/(1-\delta)$ value, which negatively affected the higher $PSI_o/(1-PSI_o)$ value for the same week to create a more gradual gradient for the same time. The same effect is visible in week 15 (see Figure 3.3). The findings indicate that the PI_{total} value will decrease as the lettuce is in the final stage of maturity, and is ready to harvest. The PI_{total} values for week 9 differ significantly from weeks 11, 13 and 15. The PI_{abs} percentage increases were 21.5%, 25.7% and 37.8%, respectively, from week 9 to 11, 13, and 15 - while PI_{total} increases were 16.0%, 28.6% and 25.3% for the same time-span.

44
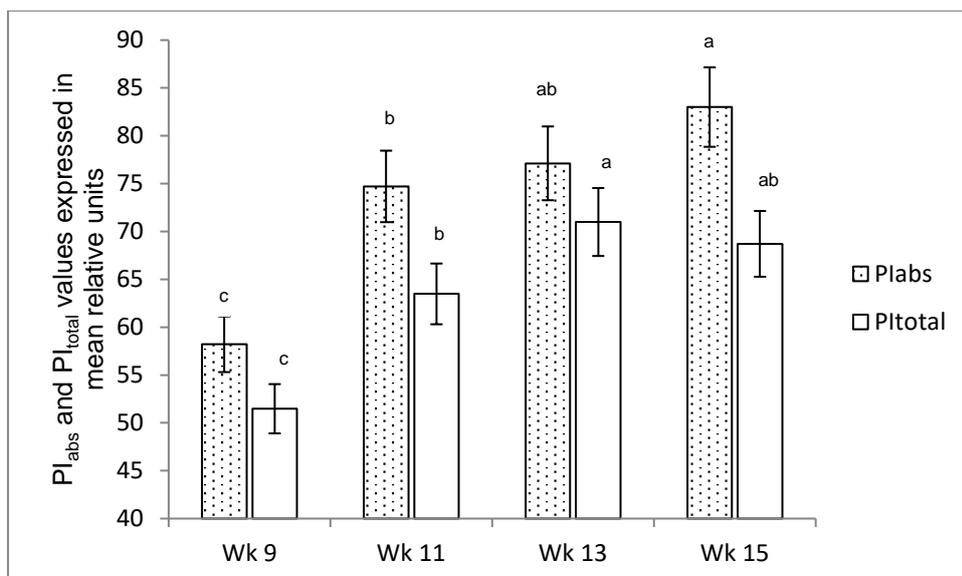
Figure 3.2: The average PI$_{abs}$ and PI$_{total}$ values expressed in mean relative units of two lettuce cultivars in the maturing phase for weeks 9-15, with differences between means (n=7; $p < 0.001$). Error bars indicate upper and lower 95% confidence levels.

Table 3.7: Individual chlorophyll fluorescence parameters RC/ABS, PHIo/(1-PHIo), PSIo/(1-PSIo) and δ/(1-δ) expressed in relative mean units for lettuce in the maturing phase for weeks 9-15. The first three parameters RC/ABS, PHIo/(1-PHIo), and PSIo/(1-PSIo) account for PI$_{abs}$ value, while δ/(1-δ) is included for PI$_{total}$ values. Significant differences between means (n=7) within a parameter are indicated with different superscript letters.

Weeks	RC/ABS	PHIo/(1-PHIo)	PSIo(1-PSIo)	δ/(1-δ)	PIabs	PItotal
9	10.35[c]	4.69[b]	1.20[c]	0.88[a]	58.22[c]	51.23[c]
11	10.78[b]	4.72[b]	1.39[a]	0.84[b]	70.73[b]	59.42[b]
13	11.28[b]	4.88[a]	1.33[b]	0.90[a]	73.21[ab]	65.89[a]
15	11.90[a]	4.85[a]	1.39[a]	0.80[b]	80.22[a]	64.17[ab]

Table 3.8: The combined light quantity averages under different coloured nets for inside and outside values measured in *µmol m^{-2} s^{-1}* for lettuce in the maturing phase weeks 9-15.

Weeks	9	11	13	15
Light quantity outside µmol m^{-2} s^{-1}	1130	985	1269	1374
Light quantity outside µmol m^{-2} s^{-1}	822	743	925	982

Table 3.9: Significant (*) and highly significant (**) *p* - values of macro and micro element chemical analysis, and physical measurements for lettuce in the maturing phase in week 15.

Lettuce week 15	P		Wet : Dry ratio		Total wet mass		Wet head mass		Wet root mass		Stem width	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Cultivar	0.00	0.966	35.41	P < 0.001**	33.99	P < 0.001**	33.66	P < 0.001**	51.87	P < 0.001**	30.64	P < 0.001**
Colour nets	1.04	0.435	0.44	0.777	1.63	0.240	1.62	0.244	3.60	0.046*	1.43	0.294
Cultivar* Colour nets	3.49	0.049*	3.44	0.052	1.10	0.406	1.12	0.401	2.96	0.075	2.47	0.112

*$p < 0.05$, **$p < 0.001$ at $p = 0.005$
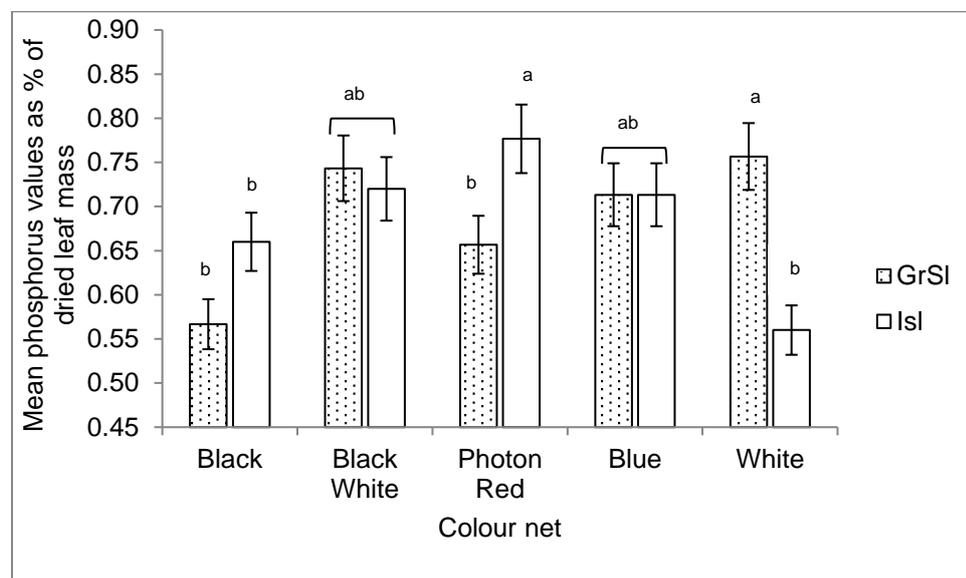
Figure 3.3: Mean phosphorus levels measured expressed as a % of dried leaf mass of two lettuce cultivars per coloured nets in week 15, with differences between the means (n=7; *p* < 0.05). Error bars indicate upper and lower values with 95% confidence levels.

46
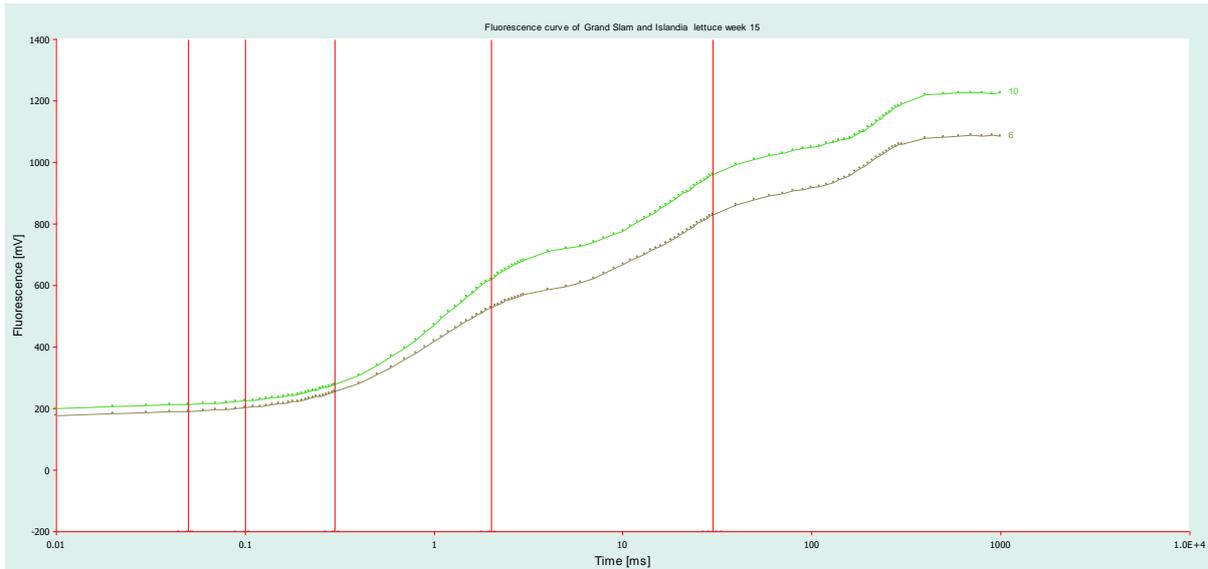
Figure 3.4: OJIP transient curve of averaged Grand Slam (green), and Islandia (grey) values under blue net for week 15. The blue net showed no significant difference for OJIP transient relative units expressing P values for Islandia and Grand Slam lettuce week 15 at I value on 30 ms, where $VI = (F_I – F_O) / (F_M – F_O)$ relative variable fluorescence at the I - step F_K at 0.3ms is normal.
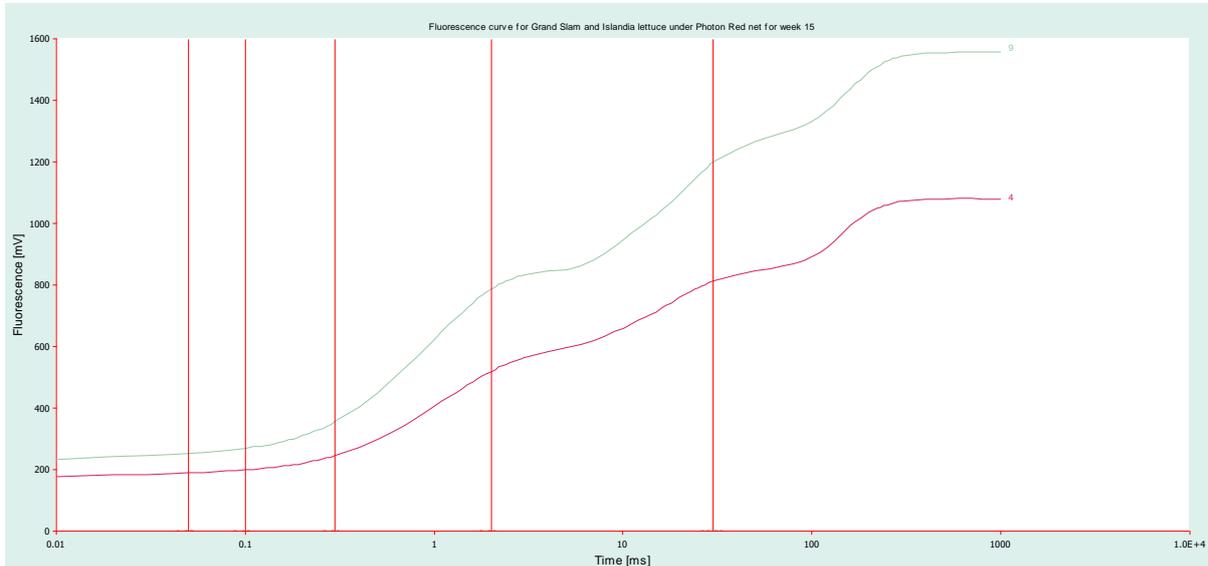
Figure 3.5: OJIP transient curve of averaged Islandia (blue), and Grand Slam (red) values under Photon Red net for week 15. The Photon Red net showed significant differences for OJIP transient relative units expressing P values between Islandia and Grand Slam lettuce week 15 at the I value on 30 ms. Islandia had a higher F_K at 0.3 ms than Grand Slam indicating limited electron transport and partial damage to the oxygen evolving complex.
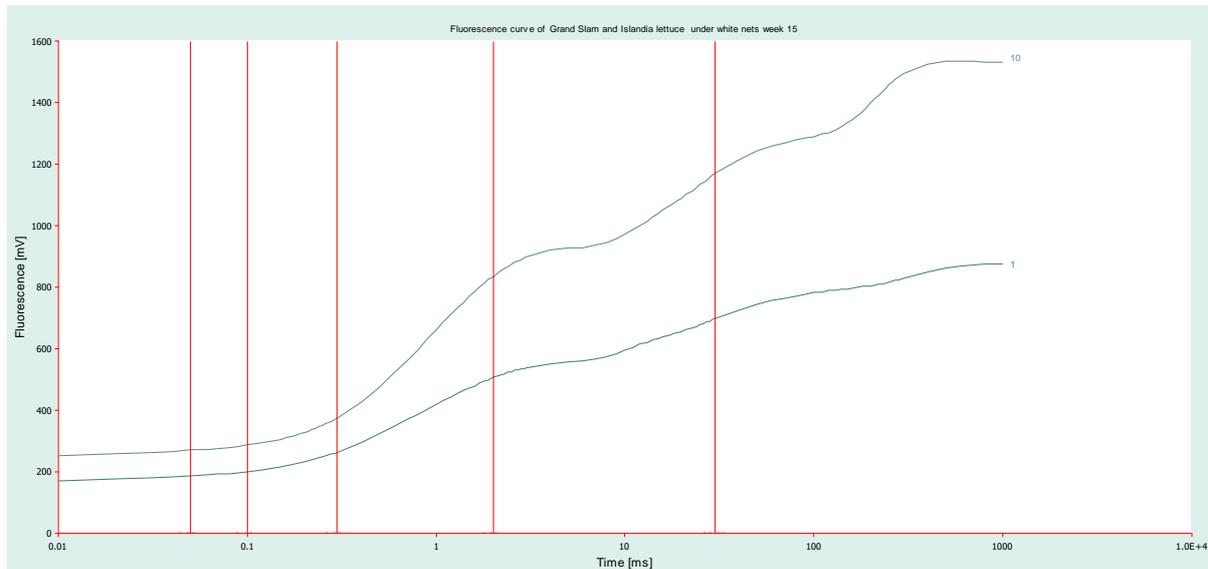
Figure 3.6: OJIP transient curve of averaged Grand Slam (blue) and Islandia (green) values under white net for week 15. The white net showed a significant difference for OJIP transient relative units expressing P values between Islandia and Grand Slam lettuce week 15. The low *I* value at 30ms for Islandia indicates low leaf P levels, and corresponds with the P leaf analysis values in Figure 3.3.

3.7.3 Macro- and micro-element analysis of mature lettuce

Phosphorus is a very important element and affects the NADP regeneration (Marschner 1995), starch synthesis and transportation of sugars across the chloroplast membrane, ATP production, and energy metabolism (Rao *et al.*, 1990). A plant's photosynthetic performance is affected by the amount of P supplied, and an increase in plant biomass is due to higher P levels (Ripley *et al.*, 2004). The ET rate, ribulose bisphosphate (RuBP) and regeneration of the CO_2 acceptor are all negatively influenced by P deficiencies (Rao *et al.*, 1990). Of all the macro- and micro-elements that were analysed and statistically compared to one another, P was the only element with significantly different values. Although there were no interactions between cultivars and P levels in the lettuce in week 15, there was a significant interaction between the cultivars and colour of nets for P (Figure 3.3; Table 3.9). The differences of the P values (% of dried leaf mass), between the two

cultivars under the blue net were minuscule (Figure 3.3). This coincided with the small variances in the OJIP transient curves (Figure 3.4), where Grand Slam had slightly higher values than Islandia.

As depicted in Figure 3.6, the I value at 30ms on the OJIP fluorescence curve for Islandia under the white net is nearly linear, and this corresponds with the lower leaf P (Figure 3.3). Furthermore, the same OJIP graph tendency occurred for both cultivars in Figures 3.4, 3.5 and 3.6 and corresponded with the P leaf analysis. There was no significant difference between Grand Slam under the white net and Islandia under the Photon Red net, and vice versa (Figures 3.5 and 3.6). These results correspond with findings from previous studies where the *I* step in the OJIP fluorescence curve of P-deficient plants straightened and eventually dissipated (Frydenvang *et al.*, 2015; Ripley *et al.*, 2004). Furthermore, photoreceptors such as cryptochromes (cry 1 and 2) can sense and respond to blue light at 390-500 nm (Cashmore *et al.*, 1999), and cryptochrome 2 is reduced with an increase in blue light (Casal, 2000). It was shown that red light does not affect Cry 2, which emphasises that enhanced blue light can potentially alter and affect the P level in the lettuce leaves (Casal, 2000; Lin and Shalitin, 2003). This clearly indicates that the combination of cultivar and net colour has a detrimental influence on P levels in mature lettuce leaves. Thus, a blue net has the potential to reduce Cry 2 levels in lettuce, and this can be directly linked to and is probably responsible for, the slightly lower but more uniform levels of P in lettuce leaves. This is, at best, speculative - and direct evidence needs to elucidate this proposed effect.

3.8   Cabbage

3.8.1 Cabbage seedlings: Chlorophyll fluorescence

There was a highly significant interaction ($p < 0.001$) between weeks and the RC/ABS in week 5, as well as between the $PHI_o/(1-PHI_o)$ process in weeks 3-4 to 5 for cabbage in the seedling phase (Table 3.10 - 3.11). Both these parameters depict the same curve (Table 3.11) - with a rise from week 3 to 4, followed by a steeper decline from week 4 to 5. This is in line with the average light quantity for week 3, 4 and 5, with light quantities of 785, 664 and 804 *$\mu mol\ m^{-2}\ s^{-1}$*, respectively, inside the

nets. The increased RC/ABS for week 4 is due to the reduced light quantity, and stimulated larger reaction centres (Long *et al.*, 2004), which corresponds with low light conditions and high levels of chlorophyll a/b associated in PSII (Walters *et al.*, 2005).

The lower light intensity also resulted in higher $PHI_o/(1-PHI_o)$ values (Long *et al.*, 2004). The reduced RC/ABS from week 4 to 5 is associated with a light quantity increase of 21%, thus resulting in smaller chlorophyll molecules (Long *et al.*, 1994). These high light conditions realised a massive photon influx into PSII RC (Horton *et al.*, 1996). The consequence was that the electron (e-) acceptor side of PSII experienced photoinhibition, and this resulted in a reduction of ET (Baroli and Melis, 1998). Simultaneously, the leaf areas (LA) were enlarged and light quantity was lowered. This is considered a common adaptation to lower irradiance (Marler *et al.*, 1994) in order to intercept more light radiation (Yang *et al.*, 2014). The restriction of the seedling leaf space forced the leaves into a more upright position, thus orientating them more parallel to solar radiation, and this consequently formed a photo-protection strategy (Larbi *et al.*, 2015). The enlarged LA from week 4 to 5, coincides with an increased biomass production, and is directly linked to higher light interception (Monteith, 1977) for week 5.

The PI_{total} values declined significantly for each week from a mean relative value of 110 RU in week 3 to 86 RU in week 4, followed by 60 RU in week 5 (Table 3.11). This shows a strong resemblance to the readings done on the lettuce seedlings for the same time-frame. According to the PI_{total} values of both cabbage and lettuce seedlings, N demands were more than the nutrients supplied (Živčák *et al.*, 2014). PI_{total} values were not mentioned for K-deficient rice, although it showed a lower ET rate and higher NPQ values (Jia *et al.*, 2008). A significantly lower ET rate value was also observed for week 5, indicating a low K supply. This is backed by the six-week time-frame of the Osmocote controlled release fertiliser.

Table 3.10: Highly significant differences for individual chlorophyll fluorescence parameters RC/ABS, PHIo/(1-PHIo), PSIo/(1-PSIo) and δ/(1-δ) expressed in relative mean units for cabbage in the seedling phase for weeks 3-5. Significant differences between means within a parameter are indicated as $p < 0.05$ (*) and highly significant values $p < 0.001$ (**) at 95% confidence levels.

Cabbage week 3-5	RC/ABS		$PHI_o/(1\text{-}PHI_o)$		$PSI_o/(1\text{-}PSI_o)$		$\delta\text{-}(1\text{-}\delta)$		PI_{abs}		PI_{total}	
	F	P	*F*	P	*F*	P	*F*	P	*F*	P	*F*	P
Cultivar	0.23	0.644	0.51	0.491	0.00	0.935	4.12	0.070	0.17	0.692	1.53	0.245
Colour nets	1.23	0.360	1.75	0.216	1.60	0.249	0.63	0.653	1.09	0.411	1.87	0.193
Weeks	32.31	P < 0.001**	41.32	P < 0.001**	90.08	P < 0.001**	26.27	P < 0.001**	72.61	P < 0.001**	55.48	P < 0.001**
Cultivar Colour nets	1.07	0.421	0.91	0.495	0.32	0.860	0.21	0.928	0.73	0.592	0.88	0.511
Cultivar* Weeks	2.65	0.957	0.43	0.656	0.65	0.531	4.51	0.024*	0.75	0.485	3.25	0.060
Colour nets Weeks	2.35	0.058	1.90	0.118	1.73	0.151	1.44	0.240	1.66	0.171	3.26	0.296
Cultivar Colour nets Weeks	0.73	0.668	0.98	0.482	0.95	0.498	1.48	0.225	1.14	0.381	1.31	0.627

Table 3.11: Chlorophyll fluorescence parameters for cabbage in the seedling phase for weeks 3-5, with significant differences between means within a parameter indicated by different superscript letters.

Weeks	RC/ABS	PHIo/(1-PHIo)	PSIo(1-PSIo)	δ/(1-δ)	PIabs	PItotal
3	12.15[a]	4.28[b]	1.90[a]	1.11[a]	98.80[a]	109.67[a]
4	12.37[a]	4.45[a]	1.86[a]	0.80[c]	102.39[a]	84.64[b]
5	10.53[b]	3.88[c]	1.36[b]	0.99[b]	55.56[b]	62.69[c]

3.8.2 Mature cabbage plants: Chlorophyll fluorescence

All chlorophyll fluorescence parameters for cabbage from week 9 to 21 (Tables 3.12 and 3.13) were affected as growth and development occurred. The RC/ABS from week 9 to week 11 did not differ and increased significantly for week 13 - due to an increased light quantity, thus forming smaller reaction centres (Long *et al*., 2004).

The severe drop in RC/ABS for week 17 was apparently due to the sharp decrease in light quantity, and coincides with an increase in δ/(1-δ) and PI_{total} values from week 15 to 17 (Table 3.13). These results concur with Albert *et al.* (2010), who also measured lower RC/ABS values with increased δ/(1-δ) and PI_{total} values in Arctic plants at high altitude under high ultra violet (UV) B radiation. The PI_{total} had higher values than the PI_{abs}, mainly because the δ/(1-δ) (Table 3.13) was highly effective in transporting e^- from PSII to PSI (Albert *et al.*, 2010). The smallest difference between the PI_{total} and PI_{abs} value occurred for week 11. This might have been due to the lowest δ/(1-δ) value for week 11, and can be the result of a lower light quantity (743 *$μmol\ m^{-2}\ s^{-1}$*) and colder temperatures (Table 3.6; Zhang and Scheller, 2004). This combination of 3°C and 100 *$μmol\ m^{-2}\ s^{-1}$* showed a 30% decrease in reduction of end acceptor values (Zhang and Scheller, 2004).

Significant interaction was observed between cultivar and $PHI_o/(1-PHI_o)$ where Conquistador had the highest mean relative value, and Sapphire the lowest (Table 3.12). The significant interaction between cultivar and δ/(1-δ) resulted in Conquistador with the lowest mean relative value, and Sapphire with the highest mean relative value (Table 3.12). Significant interaction also occurred between colour nets and PI_{total} (Table 3.12), where the black and white nets had the lowest values, followed by black and Photon Red nets with similar values. The PI_{total} value of the blue net was much higher than for the Photon Red net. This is due to the effect of δ/(1-δ) values have on the PI_{total} values, where the B:R light ratio is increased under blue nets (Basile *et al.*, 2012) and δ/(1-δ) is increased under blue light (Yan-xiu *et al.*, 2015). The white net had PI_{total} values that were slightly higher than values for the blue net. This correlates with the fact that PAR is significantly enhanced under white nets and enables higher P_N. This correlates with photos taken during the growing phase, where both cabbage cultivars grew very erratically under black and white net, while the same cultivars performed better under the Photon Red net and grew optimally under the white nets.

Figure 3.7: Cabbage and lettuce under a black and white combination net at 4 weeks after transplant.

Figure 3.8: Cabbage and lettuce under Photon Red nets at 4 weeks after transplant.
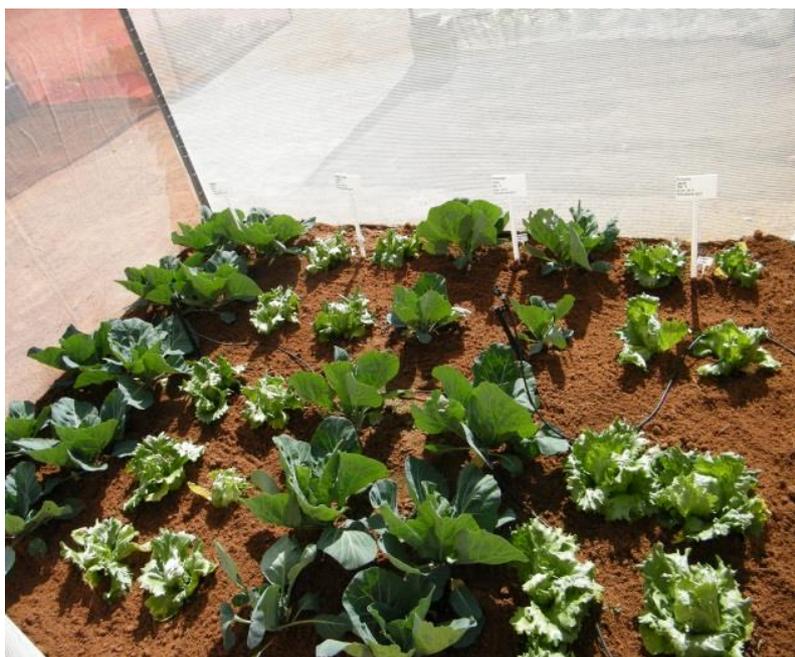
Figure 3.9: Cabbage and lettuce under white net at 4 weeks after transplant.

Table 3.12: Highly significant differences for individual chlorophyll fluorescence parameters RC/ABS, PHIo/(1-PHIo), PSIo/(1-PSIo) and δ/(1-δ), expressed in relative mean units for cabbage in the maturing phase for weeks 9-21. Significant differences between means within a parameter are indicated as $p < 0.05$ (*) and highly significant values $p < 0.001$ (**) at 95% confidence levels.

	10RC/ABS		$PHI_o/(1-PHI_o)$		$PSI_o/(1-PSI_o)$		δ-(1-δ)		PI_{abs}		PI_{total}	
Cabbage Week 9-21	*F*	P	*F*	P	*F*	P	*F*	P	*F*	P	*F*	P
Cultivar	4.70	0.055	9.64	0.011*	0.36	0.561	13.90	0.004*	0.81	0.388	2.19	0.170
Colour nets	2.34	0.126	1.45	0.289	3.05	0.697	1.80	0.205	2.96	0.074	3.93	0.036*
Weeks	138.48	$p < 0.001$**	155.79	$p < 0.001$**	416.17	$p < 0.001$**	42.85	$p < 0.001$**	314.37	$p < 0.001$**	171.85	$p < 0.001$**
Cultivar* Colour nets	0.67	0.630	0.85	0.528	0.60	0.669	0.35	0.841	0.69	0.614	0.64	0.644
Cultivar* Weeks	0.43	0.855	0.93	0.484	0.50	0.804	0.51	0.799	0.34	0.912	0.95	0.465
Colour nets* Weeks	1.12	0.350	0.66	0.866	0.57	0.933	1.25	0.237	0.56	0.941	0.68	0.847
Cultivar* Colour nets* Weeks	0.99	0.494	1.02	0.453	1.23	0.258	1.67	0.056	1.25	0.242	1.28	0.220

Table 3.13: Highly significant differences for individual chlorophyll fluorescence parameters RC/ABS, PHIo/(1-PHIo), PSIo/(1-PSIo) and δ/(1-δ), expressed in relative mean units for cabbage in the maturing phase for weeks 9-21. Significant differences between means within a parameter are indicated with different superscript letters.

Week	RC/ABS	PHIo/(1-PHIo)	PSIo/(1-PSIo)	δ/(1-δ)	PIabs	PItotal
9	14.33[d]	4.55[f]	2.43[f]	1.47[a]	158.36[f]	233.42[f]
11	14.22[d]	4.95[e]	3.09[e]	1.24[c]	217.71[e]	270.81[e]
13	16.54[a]	5.19[c]	3.47[d]	1.47[a]	297.95[cd]	438.88[c]
15	16.37[ab]	5.05[d]	3.52[d]	1.41[b]	290.62[d]	408.61[d]
17	14.07[e]	5.46[b]	5.19[a]	1.43[b]	398.30[b]	568.77[a]
19	16.03[b]	5.09[d]	3.75[c]	1.42[b]	305.79[c]	433.92[c]
21	15.64[c]	5.59[a]	4.77[b]	1.23[c]	417.16[a]	514.77[b]

3.8.3 Mature cabbage plants: Macro- and micro-elemental analyses

Macro- (N, P, K, Ca, Mg), and micro-element (Na, Mn, Fe, Cu, Zn and B) analyses, as well as physical measurements were done in week 21 on the whole cabbage heads. The averaged N, P, and K values of the 2 cultivars (Conquistador and Sapphire) were used - due to no significant differences between the 2 cultivars per net colour (Figure 3.10). Varying N, P and K levels were observed for the averaged cabbage cultivar heads under the different coloured nets (Figure 3.10). There were significant interactions between the black net, Photon Red net and white net treatments (Figure 3.10). The cabbage leaf analysis with the largest values for N, P and K, were all under the black net, and varied significantly from the lowest values under the Photon Red and white nets. Black nets reduce the light quantity, but do not alter the spectrum of light (Shahak *et al.*, 2008), red and pearl nets spectrally modify light, while blue nets increase light scattering and the ratio of B:R (Shahak *et al.*, 2004). Cryptochrome 2 was reduced with an increase in blue light (Casal, 2000), and was therefore probably responsible for the higher uniformity of the N:P:K ratio under the blue nets.

Water and mineral element uptake is determined through root efficiency, and the scattered light under red nets can increase the R:FR ratio (Demotes-Mainard *et al.*, 2016). This action is beneficial for root hair density - thus decreasing the negative

effect of FR light on root hair density production (Demotes-Mainard *et al.*, 2016). It also promotes mycorrhiza formation, which particularly affects N and P absorption. The findings of Demotes-Mainard *et al.* (2016) contradict the lower N and P values measured under the Photon Red net in this study (Figure 3.10). The lower N, P and K values under the Photon Red net and white net (Figure 3.10), are probably due to the increased leaf length and width compared to the reduced leaf length and width under the black net (Figure 3.11). This action could possibly explain the correlation of varying N, P, and K values per leaf length and width and per net colour (Figures 3.10 and 3.11).

The second largest value for N was under black and white, and P and K values were marginally higher under the blue net compared to the black and white net. The mean N, P and K values under the Photon Red net differ significantly from the black net, and are almost the same as the minimum mean values for N, P and K under the black and white net. The N and K values were slightly lower under the white net, than under the Photon Red net, while the P value was higher under the white net compared to under the Photon Red net. There were no significant differences under the Photon Red nets and the white nets regarding N, P and K.
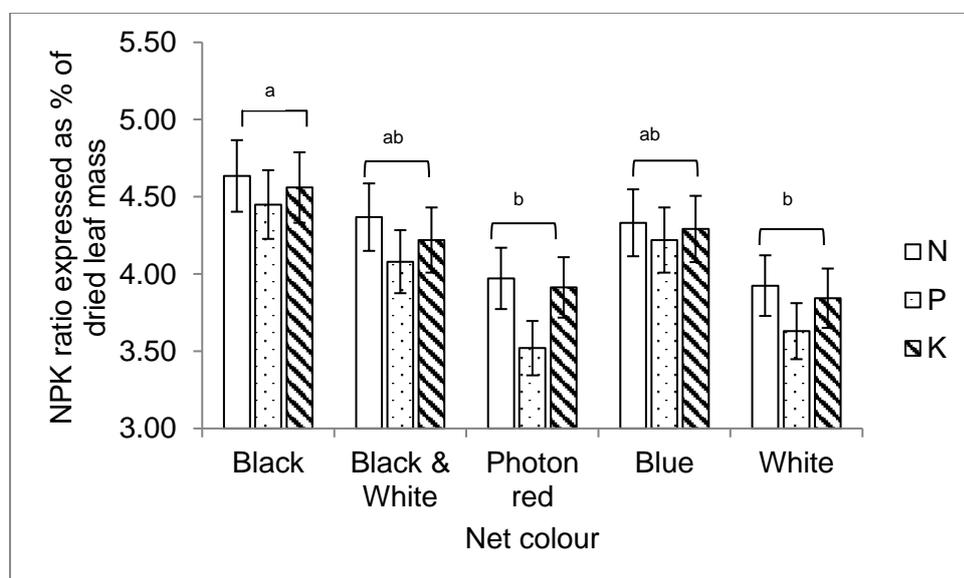
Figure 3.10: N, P and K levels between the two cabbage cultivars (Conquistador and Sapphire) for each colour net. Significant values ($p < 0.05$) for N, P and K are indicated with different superscripts. Mean P values for all the coloured nets are multiplied by a factor of 10 for easier comparison between N and K. The mean averaged N, P and K values for both cabbage cultivars are plotted on the same

graph to illustrate the ratio of N, P and K as a % of dried leaf mass for cabbage per net colour. Error bars indicate upper and lower 95% confidence levels.

3.8.4 Mature cabbage plants: physical analysis

The physical measurements of the cabbage cultivars were significantly influenced by net colour (Table 3.14). The physiology and development of plants are severely influenced by blue and red light, where blue light stimulated hypocotyl reduction and increased biomass in lettuce, while hypocotyl elongation and leaf area expansion are stimulated by red light (Johkan *et al.*, 2010; McNellis and Deng, 1995). Significant interactions were seen between leaf length and net colour, leaf width and net colour (Figure 3.11), and between leaf length and net colour and cultivar. The significant interaction between leaf width and net colour (Figure 3.11) depicts similar graph trends as the significant interaction between leaf length and net colour. The averaged cabbage leaf length and width for both cultivars were 19% longer and 13% wider under Photon Red net than under the black net. This concurs with Nithiwatthn and Piyanath (2017), where lettuce leaf lengths and widths were larger under a 50% red net than under a green and black net. Our results also corroborate findings where LA were larger under red nets than black nets (Shahak *et al.* 2008; Meena *et al.*, 2014). Mortensen and Strømme (1987) proposed that lower plant dry weight of chrysanthemum, tomato and lettuce, is the result of a smaller leaf area. The Photon Red nets produced cabbage with rapidly expanding leaves, and had the largest variance for leaf length and width, while the smallest variance occurred under the blue nets. This concurs with Shahak *et al.* (2004), where red nets induced full scattered light with a lower B:R ratio, while under blue nets the scattered light increased 10-fold because of an increased B:R ratio. The white nets had the largest values for both leaf length and width, with a smaller variance than Photon Red nets. White nets reflect almost all the incident PAR over the whole PAR spectrum (Al-Helal and Abdel-Ghany, 2010). Thus, there is a probability that the white nets reflect the scattered light with a similar B:R ratio to the blue nets - due to similarities in the ratio of leaf length and width for blue and white nets (Figure 3.11). The deeper penetrating scattered light (Shahak *et al.*, 2004), with a possibly increased B:R ratio under white nets could explain the larger leaves, as blue light is more efficient than red light in

stimulating photosynthesis, and maintaining photosystem activity and photosynthetic ET capacity (Yan-xiu *et al.*, 2015).

Table 3.14: Interaction between individual physical parameters such as leaf length and width, and total wet head mass for net colour and also cultivar and net colour for cabbage in the maturing phase in weeks 21. Significant differences between means within a parameter are indicated as $p < 0.05$ (*) and highly significant values $p < 0.001$ (**) at 95% confidence levels.

	Leaf length (mm)		Leaf width (mm)		Total wet head and stem mass (gr)	
Cabbage at week 21	*F*	P	*F*	P	*F*	P
Cultivar	0.51	0.493	0.93	0.359	1.34	0.273
Net colour	5.59	0.013*	5.90	0.011*	13.07	P < 0.001**
Cultivar* Net colour	4.81	0.020*	2.74	0.089	1.55	0.261

*$p < 0.05$, **$p < 0.001$ at $p = 0.005$
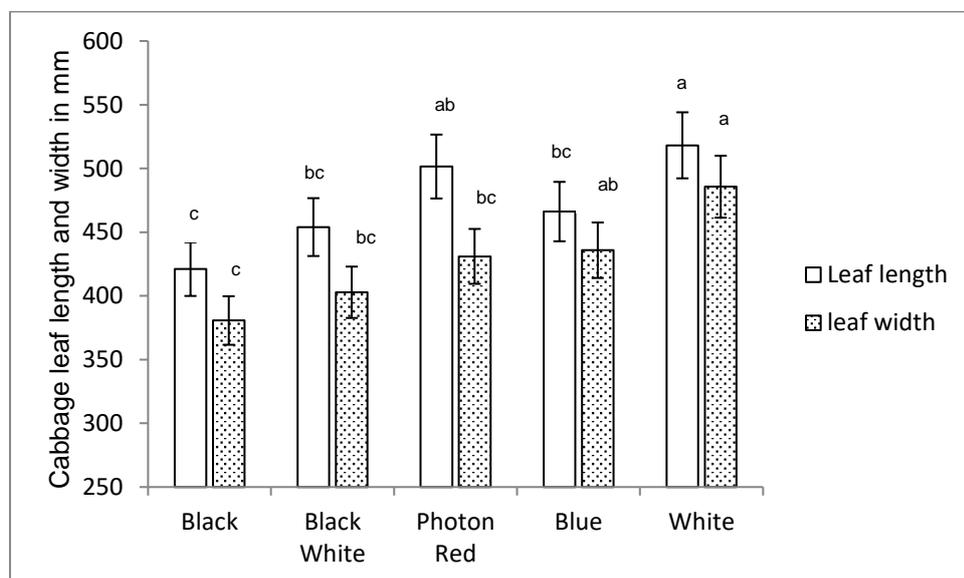
Figure 3.11: Averaged mean lengths and widths for two cabbage cultivars in the maturing phase in week 21 of growth, with significance ($p < 0.05$) depicted by different letters. Error bars indicate upper and lower values for 95% confidence levels. The statistical interaction is illustrated individually for leaf length and leaf width per colour net, and not between them.

3.8.5 Biomass accumulation

The mean average mass of both cabbage cultivars (Conquistador and Sapphire) under the Photon Red net was 29% higher than under the black net. Similar results were demonstrated for beetroot, with a 28% higher yield (t.Ha^{-1}) under a 35% red net versus a 35% black net (Meena and Meena 2016). The increase of the R:FR ratio under the Photon Red net stimulated a biomass increase, and corroborates the findings of Kasperbauer (1987).

The blue net produced a significant yield increase of 45% over the black net, and this may be attributed to black nets having a lower reflectance of PAR than blue nets (Al-Helal and Abdel-Ghany, 2010) - consequently leading to less light being scattered and a lower P_N value under the black net (Shahak et $al.$, 2004). The cabbage weight was significantly lower under the Photon Red net compared to the white net. Plant biomass of $Arabidopsis$ was lowered through the action of phyB under red light compared to white light (Demotes-Mainard et $al.$, 2016). The white net produced a mean averaged mass for both cultivars of 8.16 kg, with an 86% weight increase over the black net (Figure 3.12). Interestingly, the planting density under the different coloured nets was 51% higher than the conventional open land cabbage production, with an average head weight of 3.0 - 5.0 kg (Sakata and Starke Ayres). The average cabbage mass produced under the black net and black and white net did not differ significantly from one another.
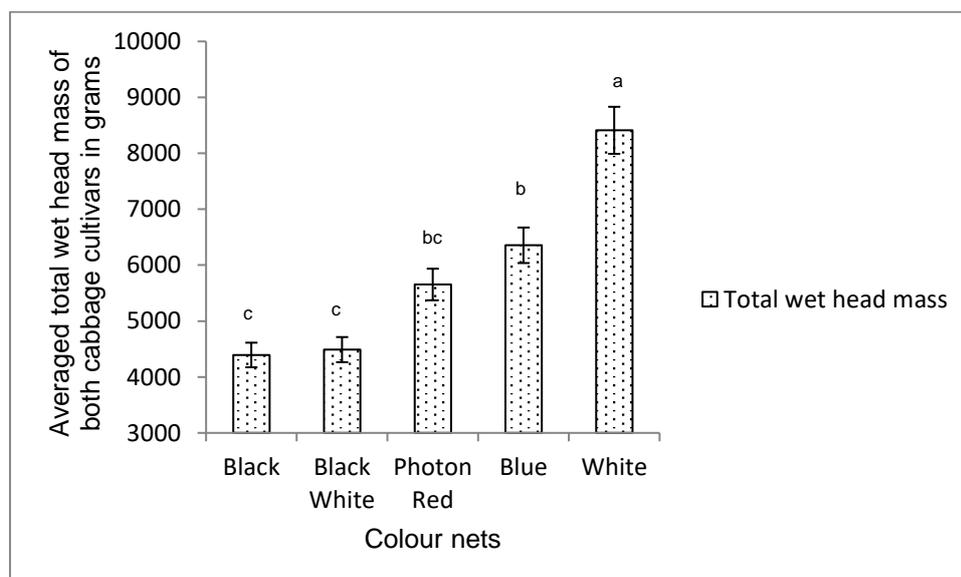
Figure 3.12: Mean total fresh head mass of heads for two cabbage cultivars (Conquistador and Sapphire) in the maturing phase in week 21, with significant ($p <$ 0.001) differences depicted by different letters. Error bars indicate upper and lower values for 95% confidence levels.

3.9   Conclusion

The growing time (in weeks) had the largest effect on chlorophyll fluorescence parameters in lettuce and cabbage in the seedling and maturing phase. The colour of the nets had no significant effect on any chlorophyll fluorescence parameters in the lettuce and cabbage seedlings or maturing lettuce. Phosphorous in mature lettuce leaves was the only element influenced by the interaction between cultivar and net colour. The blue net resulted in the most consistent P levels in mature lettuce, irrespective of cultivar, and was also responsible for the least variance in the ratio of N:P:K levels in both mature cabbage cultivar leaves. It can thus be concluded that lettuce and cabbage grown under blue nets will result in produce with more consistent nutritional values. The PI_{total} values and cabbage weight were significantly influenced by net colour. White nets increased the weight by 86 %, compared to the control (black net). Seemingly, no literature could be found to cross-reference with. Thus, cabbage production can be increased significantly under 30 % white nets, so reducing world food pressure. Physical cabbage measurements are net colour-specific, with the highest values recorded under white net for leaf length and width and total wet head mass. Future farmers can invest in either blue or white nets. The blue nets will result in minimal variance regarding nutritional value for lettuce and cabbage crops, while white nets will produce a significantly higher cabbage yield - but with lower nutritional value.

3.9   References

Adams WW, Demmig-Adams B, Rosenstiel TN, Ebbert V. 2001. Dependence of photosynthesis and energy dissipation activity upon growth form and light environment during the winter. *Photosynthesis Research.* 67: 51-62.

Adegoroye AS, Jolliffe PA. 1987. Some inhibitory effects of radiation stress on tomato fruit ripening. *Journal of the Science of Food and Agriculture.* 39: 297-302.

Albert KR, Mikkelsen TN, Ro-Poulsen H, Michelsen A, Arndal MF, Bredahl L, Håkansson KB, Boesgaard K, Schmidt NM. 2010. Improved UV-B screening capacity does not prevent negative effects of ambient UV irradiance on PSII performance in High Arctic plants. Results from a six year UV exclusion study. *Journal of Plant Physiology.* 167: 1542-1549.

Al-Helal IM, Abdel-Ghany AM. 2010. Response of plastic shading nets to global and diffuse PAR transfer: Optical properties and evaluation. *Wageningen Journal of Life Sciences.* 57: 125-132.

Baena-Gonzalez E, Barbato R, Aro E. 1999. Role of phosphorylation in the repair cycle and oligomeric structure of photosystem II. *Planta.* 208: 196-204.

Bailey S, Walters RG, Jansson S, Harton P. 2001. Acclimation of *Arabidopsis thaliana* to the light environment: The existence of separate low light and high light responses. *Planta.* 213: 74-801.

Baroli I, Melis A. 1998. Photoinhibitory damage is modulated by the rate of photosynthesis and by the photosystem II light-harvesting chlorophyll antennae size. *Planta.* 205: 288-296.

Basile B, Giaccone M, Cirillo C, Ritieni A, Graziani G, Shahak Y, Forlani M. 2012. Photo-selective hail nets affect fruit size and quality in Hayward kiwifruit. *Scientia Horticulturae.* 141: 91-97.

Bastias RM, Manfrini L, Grappadelli LC. 2012. Exploring the potential use of photo-selective nets for fruit growth and regulation in apple. *Chilean Journal of Agricultural Research.* 72(2): 224-231.

Casal JJ. 2000. Phytochromes, Cryptochromes, Phototropins: Photoreceptor interactions in plants. Journal of Photochemistry and Photobiology. 71(1): 1-11.

Cashmore ARJA, Jarillo Y, Wu J, Liu D. 1999. Cryptochromes: blue light receptors for plants and animals. Journal of Science. 284: 760-765.

Chernev P, Goltsev V, Zaharieva I, Strasser RJ. 2006. A highly restricted model approach quantifying structural and functional parameters of Photosystem II probed by the chlorophyll a fluorescence rise. *Ecological Engineering and Environmental Protection.* 2: 19-29.

Coleman LW, Rosen BH, Schwartzbach SD. 1998. Preferential loss of chloroplast proteins in nitrogen deficient euglema. *Plant and Cell Physiology.* 29(6): 1007-1014.

Demmig-Adams B, Adams WW, Barker DH, Logan BA, Bowling DR, Verhoeven AS. 1996. Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. *Physiologica Plantarm.* 98(2): 253-264.

Demotes-Mainard S, Péron T, Corot A, Bertheloot J, Le Gourrierec J, Pelleschi-Travier S, Crespel L, Morel P, Huché-Thélier L, Boumaza R, Vian A, Guérin V, Leduc N, Sakr S. 2016. Plant response to red and far-red lights, applications in horticulture. *Environmental and Experimental Botany.* 121: 4-21.

Dorais M, Papadopoulos AP, Gosselin A. 2001. Greenhouse tomato fruit quality. *Journal of American Society for Horticultural Science.* 26: 239-319.

El-Gizawy AM, Abdullah MMF, Goma HM, Mohamed SS. 1992. Effect of different shading levels on tomato plants 2. Yield and fruit quality. *International Society of Horticultural Science.* 323: 349-354.

Faseela P, Puthur JT. 2017. Chlorophyll *a* fluorescence changes in response to short and long term high light stress in rice seedlings. *Scientia Horticulturae.* 22: 30-33.

Feild TS, Lee DW, Holbrook NM. 2001. Why leaves turn red in autumn. The role of anthocyanins in senescing leaves of red-osier dogwood. *Plant Physiology.* 127(2): 566-574.

Frydenvang J, van Maarschalkerweerd M, Carstensen A, Mundus S, Schmidt SB, Pedas PR, Laursen KH, Schjoerring JK, Husted S. 2015. Sensitive Detection of Phosphorus Deficiency in Plants Using Chlorophyll *a* Fluorescence. *Plant Physiology* 169: 353-361.

Fu W, Li P, Wu Y. 2012. Effects of different light intensities on chlorophyll fluorescence characteristics and yield in lettuce. *Scientia Horticulturae* 135: 45-51.

Glenn EP, Cardran P, Thompson TL. 1984. Seasonal effects of shading on growth of greenhouse lettuce and spinach. *Scientia Horticulturae.* 24: 231-239.

Goodde D, Bornman JS. 2004. Regulation of photosynthesis in higher plants, pp. 49-51. In Archer MD, Barber J (eds), *Molecular to Global Photosynthesis* Imperial College Press: London.

Govindjee A. 2002. A role for a light-harvesting antennae complex of photosystem II in photoprotection. *The Plant Cell.* 14: 1663-1668.

He J, Chee CW, Goh CJ. 1996. 'Photoinhibition' of *Heliconia* under natural tropical conditions: The importance of leaf orientation for light interceptional leaf temperature. *Plant, Cell & Environment.* 19(11): 1238-1248.

Hendrich W. 1995. Response of the photosynthetic apparatus to the excess light intensity. *Acta Physiologica Plantarum.* 17: 153-165.

Hoch WA, Singsaas EL, Mccown BH, 2003. Resorption protection: Anthocyanins facilitate nutrient recovery in autumn by shielding leaves from potentially damaging light levels. *Plant Physiology.* 133(3): 1296-1305.

Horton P, Ruban AV, Walters RG. 1996. Regulation of light harvesting in green plants. *Annual Review of Plant Physiology.* 47: 655-684.

Hu RG, Li PP, Mao HP. 1999. Experimental research on photosynthetic characteristics of greenhouse lettuce and optimization of environmental parameter. *Journal of Jiangsu University: Natural Science Edition.* 20: 1-3.

Jia Y, Yang X, Islam E, Feng Y. 2008. Effects of Potassium Deficiency on Chloroplast Ultrastructure and Chlorophyll Fluorescence in Inefficient and Efficient Genotypes of Rice. *Journal of Plant Nutrition.* 31(12): 2105-2118.

Johkan M, Shoji K, Goto F, Hashida S, Yoshihara T. 2010. Blue light-emitting diode light irradiation of seedlings improves seedling quality and growth after transplanting in red leaf lettuce. *HortScience.* 45, 1809-1814.

Kalaji MH, Goltsev VN, Zuk-Golaszewska K, Zivack M, Brestic M. 2017. *Chlorophyll Fluorescence: Understanding Crop Performance – Basics and Applications.* CRC Press, Taylor & Francis Group.

Kalaji MH, Woejko E, Loboda T. Pietkiewicz S, Wyszynski Z. 2004. Chlorophyll a fluorescence: A convenient tool for photosynthetic performance evaluation of barley plants grown under different nitrogen rates. *Zesz. Probl. Post. Nauk Roln.* 496: 375-83.

Kasperbauer MJ. 1987. Far-red light reflection from green leaves and effects on phytochrome-mediated assimilate partitioning under field conditions. *Plant Physiology*. 85: 350-354.

Kittas C, Rigakis N, Katsoulas N, Bartzanas T. 2009. Influence of shading screens on microclimate, growth and productivity of tomato. *International Society of Horticultural Science.* 807: 97-102.

Kozaki A, Takeba G.1996. Photorespiration protects C3 plants from photooxidation. *Nature*. 384: 557-560.

Kleemann M. 2004. Effect of photoselective plastics on the quality of lettuce. *International Society of Horticultural Science.* 633: 173-179.

Larbi A, Vázquez S, El-Jendoubi H, Msallem M, Abadía J, Abadía A, Morales F. 2015 Canopy light heterogeneity drives leaf anatomical, eco-physiological, and photosynthetic changes in olive trees grown in a high-density plantation. *Photosynthetic Research.* 123: 141-155.

Li Z, Gong SF. 2002. Vertical columa and system of columnar soilless culture (scsc) and its application to cultivation of lettuce. (*Capitata* L.) *Chinese Journal of Applied and Environmental Biology.* 8:142–147.

Lin KH, Huang MY, Huang WD, Hsu MH, Yang ZW, Yang CM. (2013). The effects of red, blue, and white light-emitting diodes on the growth, development, and edible quality of hydroponically grown lettuce (*Lactuca sativa L. var. capitata)*. *Scientia Horticulturae*. 150: 86-91.

Long SP, Ainsworth EA, Rogers A, Ort DR. 2004. Rising atmospheric carbon dioxide: plants FACE the future, *Annual Review of Plant Biology*. 55: 59 - 628.

Long SP, Humphries S, Falkowski PG. 1994. Photoinhibition and photosynthesis in nature *Annual Review of Plant Physiology.* 45(1): 633-662.

Marler TE, Schaffer B, Crane JH. 1994. Developmental light level affects growth, morphology, and leaf physiology of young carambola trees. *Journal of the American Society for Horticultural Science.* 119: 711-718.

Marschner H. 1995. In *Mineral Nutrition of Higher Plants*, pp. 131-135. Academic Press: London.

McKersie BD, Leshem YY. 1994. *Stress and Stress Coping in Cultivated Plants*. Dordrecht: Kluwer Academic.

McNellis TW, Deng XW. 1995. Light control of seedling morphogenetic pattern. *Plant Cell*. 7: 1749-1761.

Meena, RK, Vashisth A, Singh R, Singh B, Manjaih KM. 2014. Study on change in microenvironment under different colour shade nets and its impact on yield of spinach (Spinacia oleracea L.). *Agricultural Meteorology*. 16: 104–111.

Meena R, Andhale RP, Meena RK. 2016. Effects of Shade Net Colours, Its Intensity and Fertilizer Levels on Growth and Yield of Beetroot (*Beta vulgaris* L.). *Journal of Pure and Applied Microbiology*. 10(2): 1553-1558.

Misra AN, Misra M, Singh R. 2012. Chlorophyll Fluorescence in Plant Biology. In: Misra, AN (ed.), *Biophysics*. InTech.

Monteith JL, 1977. Climate and efficiency of crop production in Britain. *Philos Trans R Soc Lond B Biol Sci.* 281: 277-294.

Morgan-Kiss RM, Priscu JC, Pocock T, Gudynaite-Savitch L, Huner NP. 2006. Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. *Microbiology and Molecular Biology.* 70(1): 222-252.

63

Mortensen LM, Strømme E. 1987. Effects of light quality on some Greenhouse Crops. *Scientia Horticulturae.* 33: 27-36.

Mulkey SS, Pearcey RW. 1992. Interactions between acclimation and photoinhibition of photosynthesis of a tropical understorey herb *Alocasia macrorrhiza* during simulated canopy gap formation. *Functional Ecology.* 6: 719-729.

Nithiwatthn C, Piyanath P. 2017. The Influence of Color Shading Net on the Growing of Lettuce. *Applied Mechanics and Materials.* 866(1): 33-37.

Noomnarm U, Clegg R. 2009. Fluorescence lifetimes: Fundamentals and interpretations *Photosynthesis Research.* 101: 181-194.

Oukkaroum A, Schansker G, Strasser RJ. 2009. Drought stress effects on Photosystem I content and Photosystem II thermotolerance analysed using Chl *a* fluorescence kinetics in barley varieties differing in their drought tolerance. *Physiologica Plantarum.* 137(2): 188 - 199.

Öquist G, Greer D, Ögren E. 1987. Light stress at low temperature, pp. 67-87. In: Kyle J, Osmond CB, Arntzen CJ (eds), *Photoinhibition.* Elsevier: Amsterdam.

Prasil O, Adir N, Ohad I. 1992. Dynamics of photosystem II: Mechanism of photoinhibition and recovery processes, pp. 295-348. In: Barber J (ed.), *The photosystems: Structure, Function and Molecular Biology.* Elsevier: Amsterdam.

Rao IM, Fredeen AL, Terry N. 1990. Leaf phosphate status, photosynthesis, and carbon partitioning in sugar beet. III. Diurnal changes in carbon partitioning and carbon export. *Plant Physiology.* 92: 29-36.

Reinhardt K, Smith WK, Carter GA. 2010 Clouds and cloud immersion alter photosynthetic light quality in a temperate mountain cloud forest. *Botany.* 88: 462-470.

Ripley BS, Redfern SP, Dames J. 2004. Quantification of the photosynthetic performance of phosphorous-deficient *Sorghum* by means of chlorophyll-*a* fluorescence kinetics. *South African Journal of Science.* 100: 615-618.

Sakata and Starke Ayres. Information from official websites.

Salvucci ME, Crafts-Brandner SJ. 2004. Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. *Plant Physiology.* 120(2): 179-186.

Savitch L, Gray G, Huner NA. 1997. Feedback-limited photosynthesis and regulation of sucrose-starch accumulation during cold acclimation and low-temperature stress in a spring and winter wheat. *Planta.* 201(1): 18-26.

Schansker G, Toth SZ, Strasser RJ. 2005. Methylviologen and dibromothymoquinone treatments of pea leaves reveal the role of photosystem I in the Chl a fluorescence rise OJIP. *Biochimica Biophysica Acta.* 1706: 250-261.

Shahak Y, Gal E, Offir Y, Ben-Yakir D. 2008. Photoselective shade netting integrated with greenhouse technologies for improved performance of vegetable and ornamental crops. *International Society for Horticultural Science.* 797: 75-80.

Shahak Y, Gussahovsky EE, Gal E, Ganelevin R. 2004. ColorNets: Crop protection and light-quality manipulation in one technology. *International Society of Horticultural Science.* 659: 143–151.

Strasser RJ, Srivastava A, Tsimilli-Michael M. 2000. The fluorescence trancient as a tool to characterise and screen photosynthetic samples, pp. 445-453. In: Mohanty P, Yunus and Pathre (eds), *Probing photosynthesis: Mechanism, Regulation & Adaptation.* Taylor & Francis London.

Strauss A, Krüger G. Strasser R, Heerden PV. 2006. Ranking of dark chilling tolerance in soybean genotypes probed by the chlorophyll *a* fluorescence transient OJIP. *Environmental and Experimental Botany.* 56(2): 147-157.

Tanaka R, Tanak A. 2000. Chlorophyll *b* is not just an accessory pigment but a regulator of the photosynthetic antenna, pp. 240-245. In: *PORPHYRINS 9-4th International Porphyrin-Heme Symposium* Tokyo.

Teitel M, Lirion O, Haim Y, Seginer I. 2008. Flow through inclined and concertina shaped screens. *Acta Horticulture.* 801: 99-106.

Van Heerden PDR, Swanepoel JW, Krüger GHJ. 2007. Modulation of photosynthesis by drought in two desert scrub species exhibiting C3-mode CO_2 assimilation. *Environmental and Experimental Botany.* 61(2): 124-136.

Walters RG. 2005. Towards understanding of photosynthetic acclimation. *Journal of Experimental Botany.* 56: 435-447.

Weng XY, Xu HX, Jiang DA. 2005. Characteristics of gas exchange, chlorophyll fluorescence and expression of key enzymes in photosynthesis during leaf senescence in rice plants. *Journal of Integrative Plant Biology.* 47(5): 560-566.

Yan ST, Li XD, Li WD, Fan PG, Duan W, Li SH. 2011. Photosynthetic and chlorophyll fluorescence response to low sink demand of tubers and roots in *Dahlia pinnata* source leaves. *Biologia Plantarum.* 55(1): 83-89.

Yan-xiu M, Xiao-zhuo W, Li-hong G, Qing-yun C, Mei Q. 2015. Blue light is more essential than red light for maintaining the activities of photosystem II and I and photosynthetic electron transport capacity in cucumber leaves. *Journal of Integrative Agriculture*. 10: 2095-3119.

Yang SJ, Sun M, Zhang YJ, Cochard H, Cao KF. 2014. Strong leaf morphological, anatomical, and physiological responses of a subtropical woody bamboo (*Sinarundinaria nitida*) to contrasting light environments. *Plant Ecology.* 215: 97-109.

Zhang S, Scheller HV. 2004. Photoinhibition of Photosystem I at Chilling Temperature and Subsequent Recovery in Arabidopsis thaliana. *Plant Cell Physiology*. 45: 1595-1602.

Živčák M, Olšovská K, Slamka P, Galambošová J, Rataj V, Shao H, Brestič M. 2014. Application of chlorophyll fluorescence performance indices to assess the wheat photosynthetic functions influenced by nitrogen deficiency. *Plant Soil and Environment.* 60(5): 210-215.

Chapter 4

Physiological responses of lettuce (*Lactuca* sativa) seedling leaves exposed to different coloured LEDs and shade nets

Willem Gericke,[1] Misha de Beer[2] and Marcellous Le Roux[1][*]

[1] *Department of Agronomy, University of Stellenbosch, Stellenbosch, South Africa.*
[2] *Centre for Water Sciences and Management, North West University, Potchefstroom, South Africa*
[*]*Corresponding author, e-mail:* mrl@sun.ac.za

4.1   Abstract

Light quantity and quality can affect plant growth and development significantly. Lettuce (*Lactuca sativa*) seedlings are sensitive to high levels of radiation. To understand the phenomenon better, different colour combination LEDs with low radiation and different coloured nets exposed to high solar radiation were trialled. The influence of low Photosynthetically Active Radiation (PAR) LED light emitted with a 1:1 ratio of blue+deep red (58 *µmol m^{-2} s^{-1}*) and blue+far red (40 *µmol m^{-2} s^{-1}*) compared to the high solar PAR light (1195 *µmol m^{-2} s^{-1}*) for week 5, under different coloured shade nets, was used to evaluate chlorophyll fluorescence parameters for different lettuce cultivars. The chlorophyll fluorescence parameters under the LEDs were all significantly lower than under the different coloured nets. The chlorophyll fluorescence parameters measured were reaction centre per electron absorption of light energy (RC/ABS), trapping of excitation energy (PHIo/(1-PHIo)), conversion of excitation energy to electron transport (PSIo/(1-PSIo)), reduction of end electron acceptors (δ/(1-δ)), Performance Index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors (PI$_{abs}$), and potential for energy conservation from exciton to the reduction of PSI end electron acceptors (PI$_{total}$). Net colour and LEDs had highly significant influences on the different chlorophyll fluorescence parameters for the various cultivars. All the chlorophyll fluorescence parameters were significantly lower under the low radiation blue+deep red and blue+far red LEDs, than under the different high radiation coloured nets. The macro- and micro-element values such as phosphorous (P), calcium (Ca), magnesium (Mg), sodium (Na), manganese (Mn), copper (Cu), zinc (Zn) and boron (B), were significantly higher for all the cultivars under blue+deep red LEDs than all the coloured shade nets. The low radiation of 1:1 blue+deep red LEDs influenced the macro- and micro-element absorption - irrespective of lettuce cultivar.

Keywords: chemical analysis, chlorophyll fluorescence, LED, seedlings, shade nets

4.2   Introduction

Plant growth and development is fundamentally affected through light quantity and quality. Black shade nets reduce light quantity without altering light quality (Shahak, 2008), and the shading factor is nearly proportional to the net porosity (Appling, 2012). This phenomenon differs from coloured shade nets, as light quality remains unchanged when it passes through the holes of the coloured shade net - but becomes scattered and spectrally altered when it is reflected off the net fibres (Appling, 2012). Plant physiological responses are enhanced through spectral modification (Shahak *et al.*, 2008). These coloured shade nets have the ability to increase the light scattering by 50% or more (Ilić and Fallik, 2017), and alter the blue:red (B:R) and red:far red (R:FR) ratios (Stamps, 2009). Blue shade nets increase the B:R ratio - thus resulting in a 10-fold increase in scattered light penetrating deeper into plant canopies (Shahak *et al.*, 2004). Plants adapt and modify their biological cycles through different types of photoreceptors, such as phytochromes, cryptochromes and phototropins that perceive changes in light quality (Galvão and Frankhauser, 2015; Huché-Thélier *et al.*, 2016; Whitelam and Halliday, 2007). Phytochrome B senses the ratio of R:FR (Ballare *et al.*, 1991), while phytochrome A detects very low ratios of R:FR (Yanovsky *et al.*, 1995). Vegetative growth and foliage vigour are stimulated under red and yellow shade nets, while more compact plants are produced under blue nets. Grey nets absorb infra-red (IR) light and result in plants with enhanced branching and smaller leaves. Pearl coloured nets absorb ultra-violet (UV_{A+B}) light, and have the highest light- scattering capability (Shahak, 2008; Goren *et al.*, 2011; Kong *et al.*, 2013; Alkalai-Tuvia *et al.*, 2014).

Seedling growers in geographical areas with high solar radiation and temperatures are challenged to produce compact, vigorous, hardy seedlings that will withstand transplant shock under similar environmental conditions. Vegetable production is practised in open lands, under shade nets, in poly- and glass-greenhouses. The use of shade netting has become very popular in areas with high temperatures, and is also used for crop sheltering from wind, and protection from birds and insect-transmitted diseases (Teitel *et al.*, 2008). Furthermore, shade nets are used to protect agricultural crops against excessive thermal radiation, and in doing so, this improves the thermal climate (Kittas *et al.*, 2009). Shade nets, photo-selective screens and polyethylene and glass-clad greenhouses decrease light quantity and

alter light quality - thus influencing plant photosynthesis, -tropism and – morphogenesis. According to Nitz and Schnitzler (2004), UV-B (280-320 nm) is basically depleted under glass greenhouses, which correlates with Stewart *et al.* (2000), where field-grown tomatoes produced in South Africa and Spain had 4 to 5 times more flavonols than tomatoes produced in UK glasshouses. Care must be taken when choosing the type of shade net, as too much shading affects the distribution of photosynthates in cucumber fruit (Marcelis, 1993).

Natural solar radiation is often supplemented by a type of artificial lighting - such as high pressure sodium (HPS), metal halide (MH), and recently light-emitting diodes (LEDs), especially in high and low latitudes around the globe. This enables growers to generate a higher light quantity, while simultaneously improving light quality. The quantity reveals the amount of light available to plants as photons, per unit time on a unit area, expressed in *µmol m^{-2} s^{-1}* (Kempen, 2012). Light quality is used by plants through photosynthesis as photosynthetically active radiation (PAR) - mainly in the blue, red and near infra-red wavelengths. Light quality affects biological processes such as germination and flowering (Taiz and Zeiger, 2002) and photosynthesis (Kim *et al.*, 2004).

Light emitting diodes consist of a chip from a semiconductor material, which is infused with impurities to create a p-n junction - also known as the positive-negative junction. When an electron current passes through the semiconductor a monochrome light is emitted (Yeh and Chung, 2009). Monochromatic LEDs are one of the most energy efficient lighting sources, especially in the horticulture industry with controlled environments (Martineau *et al.*, 2012). Yang (2008) states that the combination of blue and red LEDs has a spectral absorption peak with an 80-90% light energy utilisation rate, and is beneficial for photosynthesis and morphogenesis. These LEDs can alter seedling morphology and physiology through a specific narrow band light wavelength (Lefsrud *et al.*, 2008). Microgreens illuminated with blue LEDs had significantly higher accumulations of macro- and micro-elements in comparison to when radiated with blue+red (B+R) LEDs.

Light-emitting diodes are the new focus of all artificial lighting-related research. This is due to the manufacturing possibilities of specific coloured LEDs and their associated narrow bandwidths. These narrow bandwidths provide scientists with the

possibilities for plant-specific research, thus promoting higher profits for growers. According to Hogewoning *et al.* (2010), maximal photosynthetic capacity and leaf mass per area can be increased under low light intensities, by increasing or adding blue light in the B:R light ratio. Blue light rather than red light has increased palisade and spongy mesophyll in leaves, and secondary xylem thickness in stems (Schuerger *et al.,* 1997). According to Savvides *et al.* (2011), the net leaf photosynthesis (An) of cucumber leaves cultivated under red LEDs was lower than their counterparts cultivated under a combination of red and blue LEDs, and were more vulnerable to water stress. Blue light fraction is the % of blue light (320 to 496 nm) per total light (320 to 700 nm) (Dougher and Bugbee, 2004). Lettuce is highly sensitive to a blue light fraction of 0 to 6%, and the response of stem elongation is determined by the amount of absolute blue light (Dougher and Bugbee, 2001). Furthermore, the cell division and expansion of lettuce leaves significantly increased with an increasing blue light fraction (Dougher and Bugbee, 2004). Blue light acts as a powerful signal to control stomatal operation - and is 20 times more effective than red light in opening stomata (Sharkey and Raschke, 1981; Shimazaki *et al.*, 2007). Blue LED light significantly increased macro- and micro-element absorption through stomatal opening and membrane transport activity in broccoli microgreens (Kopsell and Sams, 2013).

The focus of this trial was to determine the effects of the different colour combination LEDs with low radiation for lettuce seedlings. This was compared to the effects of different coloured nets radiated by natural high solar radiation. Parameters assessed included, chlorophyll fluorescence and chemical macro- and micro-element absorption.

4.3 Material and Methods

4.3.1 *Location*

The second trail was carried out from 4 August 2014 to 8 September 2014, on the farm Willemsheim, which is situated in the Buffelspoort area, North West Province, South Africa (25°48'30.5"S, 27°29'3.7"E). The farm is situated on the southern slopes of the Magalies Mountain, and is thus north facing and has a gradient of six percent. All irrigation water is gravity fed from the mountain and is filtered through a

Netafim 120 micrometer disc filter, and had 22 ppm dissolved solids with no available bi-carbonate.

4.3.2 *Plant material and experimental set-up*

Fresh pelletised Iceberg lettuce (Robinson from Nickerson Zwaan and Grand Slam from Starke Ayres) and fancy red lettuce (Multi Red and Soltero from Starke Ayres) seeds, were sown in new polystyrene seedling trays containing 200 cavities per tray and with dimensions of 70 cm x 35 cm x 7 cm. The growing medium used was a mixture of 60% coir and 40% Klasmann TS 1 fine peat. The coir was buffered with a 1% $CaNO_3$ solution, and no buffering was done for the Klasmann TS 1 fine.

This combination growing medium was pre-enriched with Nitrosol at 2 $L.m^{-3}$ and Scotts Osmocote Start controlled-release 12+11+17+2 MgO fertiliser at 1 $kg.m^{-3}$. Micro-organisms were obtained from Cosmoroot and mixed into the medium at a concentration of 10 $gr.m^{-3}$. The growing medium had a final EC of 0.8 $mS.cm^{-1}$, and a pH of 5.8.

Table 4.1: Nutrient composition of fertilisers, as % for Nitrosol, and Osmocote, and ppm for Cosmoroot and micro-nutrient compositions of products applied to the lettuce seedlings.

	N	**P**	**K**	**Other**	
Nitrosol	8	3	6	Minerals	Trace elements
Osmocote	12	11	17	$2M_gO$	Trace elements
Cosmoroot	70 (ppm)	205 (ppm)	50 (ppm)	L-Amino acid (ppm)	Humic substance 155 (ppm)

Seeds were germinated in a germination room at 20°C with 90% humidity. Once germinated, a single seedling tray with 160 seedlings, which comprised 4 lettuce cultivars of 40 seedlings each, was placed under each of the 5 different coloured shade nets on pallets 20 cm above the ground. Each coloured net was replicated three times. One seedling tray was also placed underneath each of the three different LED light combinations, such as 1:1 blue+deep red (B+DR), 1:1 blue+far red (B+FR) and 1:1 red+far red (R+FR) at a 50 cm height.

The seedlings were fertigated using a Tank A and B system in conjunction with a double Dosatron D8R dosing system set at 3 bar pressure, gravity fed. The fertigation water was evenly distributed to each trial plot through micro-irrigation. Tank A consisted of 60 kg calcium-nitrate and 50 kg potassium-nitrate per 1000 L of stock solution. Dosatron 1 was set at a 1% injection rate for tank A, while Dosatron 2 was set at 1.4% with 30 kg magnesium-sulphate, 6 kg mono-potassium-phosphate, 7 kg potassium-sulphate and 2 kg Microplex per 1000 L stock solution. Fertigation commenced after a visual inspection was done to determine the amount of water still available in the plug by compressing it. The seedlings were fertigated until water started leaching out of the trays - and all the seedling trays were fertigated simultaneously and received the same volume of water.

4.4   Net Structures

Twenty percent black shade net is considered the norm in seedling production, and was used as the control in the experiment. An HPS lamp (1000 $\mu mol\ m^{-2}\ s^{-1}$), was used as a constant light source to determine which coloured nets portrayed light quantities similar to the control. This method was used rather than sunlight to eliminate the possibility of variance in sunlight quantity.

One at a time, each net was placed over a frame under the HPS lamp, with intensity of 1000 $\mu mol\ m^{-2}\ s^{-1}$. The light intensities of the different shade nets were measured, as well as the light intensity of the lamp between readings. A light quantity meter (Model MQ-200, Apogee Instruments, Logan, UT) was placed on the same spot and height for each net reading. The coloured nets with the light quantity, as close as possible to 20% black net (control 780 $\mu mol\ m^{-2}\ s^{-1}$), were 20% black and white (760

$\mu mol\ m^{-2}\ s^{-1}$), 20% blue (760 $\mu mol\ m^{-2}\ s^{-1}$), 30% Photon Red (760 $\mu mol\ m^{-2}\ s^{-1}$) and 30% white (740 $\mu mol\ m^{-2}\ s^{-1}$). Although Photon Red 20% had a light intensity of 790 $\mu mol\ m^{-2}\ s^{-1}$, it was not used, and Photon Red 30% was used because it had the same light quantity as the control.

The trial plot dimensions were 3 m x 2.5 m x 2.5 m for each plot, with a 3 m spacing between them to avoid overshadowing. No light quantity differences were measured between the control and the other coloured nets regarding their inside light quantity during the trial. Light quantity readings were taken on clear sky days inside and outside for each net, and coincided with chlorophyll fluorescence readings taken in the dark on the same dates.

4.5   LED Structures and LED Combinations

The LED structures were erected inside a 10 m x 30 m polyethylene tunnel covered with 30% titanium nets on the outside, and with roll-up sides to reduce heat build-up inside the structure. The nine LED structures measuring 3 m x 3 m x 2.4 m each, were constructed from 100% blackout screens. Photosynthetically active radiation (PAR) light readings were taken inside the non-illuminated LED structures with a light meter (Model MQ200, Apogee Instruments, Logan, UT) - and further readings confirmed there was no interference from solar radiation inside the LED structures.

Variable light quantity research LEDs manufactured by Philips (Netherlands) were used on a 1:1 ratio for blue, red and far red colours. The light quantity of the research LEDs was maximised by means of a homemade potentiometer. Three sets of each colour combination of B+DR, B+FR and R+FR were used. The B+DR combination had a combined light quantity of 58 $\mu mol\ m^{-2}\ s^{-1}$, B+FR produced 40 $\mu mol\ m^{-2}\ s^{-1}$, and the R+FR was 14 $\mu mol\ m^{-2}\ s^{-1}$. The LEDs were activated by timers per solar sunrise and sunset. High light radiation under shade screens, and low LED light quantity values for the different light colour combinations were used to determine the chlorophyll fluorescence, and macro- and micro-element chemical analysis.

4.6   Experiment 2

4.6.1 *measurements and analysis: Chlorophyll fluorescence readings*

Non-destructive fast chlorophyll fluorescence was used to determine the functioning of RC/ABS, $PHI_o/(1-PHI_o)$, $PS1_o/(1-PSI_o)$, $\delta/(1-\delta)$, PI_{abs} and PI_{total} (as described in Table 4.2) in weeks 4 and 5 for lettuce in the seedling phase. The HANDY-PEA Fluorimeter, (Hansatech Instruments Ltd., Pentney, King's Lynn, Norfolk, England), was used for the measurements. These readings were taken 1 hour after dusk, when the plants were in a dark-adapted state. Fully expanded, middle upper leaves were chosen for the measurements. Chlorophyll fluorescence readings were taken from the seedlings grown under the different coloured shade nets with high solar radiation, as well as from the seedlings grown under low radiation B+DR and B+FR and R+FR LEDs. The seedlings, especially under the R+FR LEDs, became extremely elongated with thin stems and narrow leaves, and collapsed during chlorophyll fluorescence measurements. Thus very few of these chlorophyll fluorescence readings were measurable, and therefore they were excluded from the results. The data were captured with Handy PEA software and then analysed and quantified with 'Biolyzer' software (according to Strasser *et al.*, 2000) - and then transferred to Excel 2010. Only data with significant values ($p < 0.05$) and highly significant ($p < 0.001$) with 95% confidence levels are discussed in the results.

Table 4.2: Description of chlorophyll fluorescence parameters

RC/ABS	Chlorophyll concentration of reaction centre indicating electron absorption of light energy
$PHI_o/(1-PHI_o)$	Trapping of excitation energy
$PSI_o/(1-PSI_o)$	Conversion of excitation energy to electron transport
$\delta/1-\delta$	Reduction of end electron acceptors
PI_{abs}	Performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors
PI_{total}	Performance index (potential) for energy conservation from exciton to the reduction of PSI end electron acceptors

4.6.2 *Macro- and micro-element measurements*

The growing medium was rinsed off from the seedling samples, and the seedlings were dried in an oven at 70°C to a constant dry mass. Leaf macro- (P, K, Ca, Mg) and micro- (Na, Mn, Fe, Cu, Zn and B) elements were measured using the dry ashing extraction method for all the seedlings under the coloured nets and the (B+DR) LEDs. Nitrogen could not be determined for the seedlings from the (B+FR) LEDs, because too little plant material was available for analysis. Leaf macro-elements were expressed as a % of dried leaf mass, while micro-element analysis was expressed as mg.kg^{-1}.

4.7   Statistical Analysis

The analysis of variance (ANOVA) was used for data analyses. Mean comparisons of data were determined with Fisher's least significant difference ($p < 0.05$) using Statistica 13 software (StatSoft, Tulsa, OK, USA). The ANOVA test of variance was used to test for interaction between colour net, cultivar, weeks, colour nets and cultivar, colour nets and weeks, cultivar and weeks, colour nets and cultivars and weeks for RC/ABS, PHI$_o$/(1-PHI$_o$), PS1$_o$/(1-PSI$_o$), δ/(1-δ), PI$_{abs}$ and PI$_{total}$ - as well as the physical measurements and macro- and micro-elements.

4.8   Results and Discussion

Light quantity was measured inside and outside the different coloured nets in week 4 and 5. The average light quantity was used, due to insignificant differences measured in light quantity between the different coloured nets. The average outside light quantity in week 4 was 1225 *µmol m^{-2} s^{-1}*, while the average inside light quantity for all the net colours was 899 *µmol m^{-2} s^{-1}*. In week 5, the average outside light quantity reached 1660 *µmol m^{-2} s^{-1}*, and the average inside light quantity was 1195 *µmol m^{-2} s^{-1}*. During the same period, light quantity was measured under the different LED combinations, and the averaged readings were *58 µmol m^{-2} s^{-1}* for B+DR LEDs,

40 $\mu mol\ m^{-2}\ s^{-1}$ for B+FR LEDs, and 13 $\mu mol\ m^{-2}\ s^{-1}$ for R+FR LEDs. The seedlings grown under the different coloured nets were exposed to much higher photosynthetically active radiation (PAR) levels than their counterparts under the different LED combinations for the same period.

The chlorophyll fluorescence differed significantly between the cultivars except the δ/(1-δ), which was influenced the most by net colour and LEDs (Table 4.3). The four lettuce cultivars, Robinson and Grand Slam (i.e. green Iceberg cultivars), Multi Red (Dark Red Oak) and Soltero (Lolla Rossa), had the same graph curves for all the chlorophyll fluorescence parameters, except for the reduction of end electron acceptors δ/(1-δ) (Table 4.4).

All the seedlings of the different cultivars grown under the R+FR LEDs were extremely long, thin and weak, with poorly developed leaves, were extremely sensitive to water stress and had low photosynthetic capacity. This concurs with Yanagi *et al*. (1996), who found that lettuce grown under monochromatic red LEDs of 125 $\mu mol\ m^{-2}\ s^{-1}$ produced abnormal growth and, had a higher rate of stem elongation with winding leaves - which was not observed under blue LED light of 170 $\mu mol\ m^{-2}\ s^{-1}$ or B+R LEDs with the same PPF. A clear majority of seedlings under the R+FR LEDs broke when touched, making it impossible to gather enough chlorophyll fluorescence readings for statistical analysis. The few readings that were taken had very low PI$_{total}$ values. Seedlings grown under a combination of B+DR and B+FR LEDs had higher PI$_{total}$ values than under the R+FR LED combinations. This concurs with Hogewoning *et al*. (2010), who found that by increasing blue light in the blue:red ratio, the photosynthetic capacity will be increased.

There were highly significant differences between the coloured nets and the LED combinations regarding chlorophyll fluorescence parameters (Tables 4.4 and 4.5). Higher natural solar radiation was experienced under all the different coloured nets compared to the low LED lighting radiation. This ensured larger values for all chlorophyll fluorescence parameters under all the different coloured nets when compared to the chlorophyll fluorescence parameters under the different LED combinations. Striking resemblances were noted for each chlorophyll fluorescence parameter per different coloured net, as well as for the B+DR and B+FR LED combinations. The blue net had the highest overall values for each chlorophyll

fluorescence parameter of all the coloured nets - including the LED combinations (Table 4.5). The overall lowest readings for all the chlorophyll fluorescence parameters were achieved under the B+FR LED combination, except for the δ/(1-δ) value, which was the lowest under the B+DR LEDs

4.8.1   *Lettuce Chlorophyll fluorescence: per cultivar and light combination*

4.8.1.1   *RC/ABS*

Grand Slam and Robinson had the lowest RC/ABS values of the 4 cultivars, and didn't differ statistically from each other (Table 4.4). Soltero, which had a visually noticeable carotenoid content (partial red and green leaves) had a 37% higher RC/ABS value than Grand Slam, and a 33% higher RC/ABS value than Robinson (Table 4.4). Multi Red had completely dark red leaves due to the high carotenoid content, and the RC/ABS values were significantly higher than for all the other cultivars – 51% higher than Grand Slam, 46% higher than Robinson and 11% higher than Soltero (Table 4.4). The higher RC/ABS values are mainly due to the influence of carotenoids, and according to Armstrong and Hearst (1996) carotenoids are known to absorb blue light - and protect chlorophyll against photodamage. However, photosynthetic pigments are present in light-harvesting complexes (LHCIIb), and thus the solar energy is absorbed by the LHCIIb and transferred to photosystem II (PSII) reaction centres for photosynthesis (Xiao *et al.*, 2011). These pigments consist mainly of chlorophyll, carotenoid and anthocyanin and each pigment absorbs PAR light in different wave lengths. *Chlorophyll a* absorbs light with higher light intensities in the violet-blue and orange-red spectrum, while *chlorophyll b* absorbs blue light energy with a lower light intensity in the longer wavelengths of blue light (Lange *et al.*, 1981; Papageorgiou and Govindjee, 2004). According to Kimura and Rodriguez-Amaya (2003), carotenoid pigments and chlorophyll synthesis can be cultivar-specific, and can be sensitive to changing plant growth conditions. Their study correlates with ours due to the large *F*-value for interaction between RC/ABS and cultivars seen in Table 4.3. It is thus clear that carotenoids have a significant effect for specific cultivars in terms of realising higher RC/ABS values under high light conditions.

Although there were no significant differences for RC/ABS under the different coloured shade nets, the values did differ significantly from B+DR and B+FR LEDs (Table 4.5). The light quantities under the LEDs were much lower than under the coloured shade nets, and consequently resulted in significantly lower RC/ABS values. Furthermore, there were larger variances in RC/ABS values under the LEDs than under the different coloured nets. An explanation for this phenomenon is that all the lettuce cultivars under the various coloured nets potentially experienced photoinhibition due to the high light quantities. This complies with the fact that a plant's photosynthetic pigments have difficulty absorbing all the energy meant for photosynthesis during high light conditions - resulting in photosynthetic reactions (photoinhibition), or in extreme cases, damage to the photosynthetic apparatus (Coleman *et al.*, 1988; Prasil *et al.*, 1992; Weng *et al.*, 2005). Furthermore, the RC/ABS value indicates the chlorophyll concentration of the reaction centre. The degradation rate of chlorophyll is higher than the chlorophyll synthesis rate under high light quantities. This leads to a decreased chlorophyll concentration because of chloroplast formation inhibition. Therefore, shaded leaves have higher chlorophyll concentrations per unit of leaf weight than leaves grown in the sun (Gonçalves *et al.*, 2001; Fu *et al.*, 2012; Kosma *et al.*, 2013).

According to Ilić *et al.* (2017), their study revealed that lettuce cultivated under blue and black shade nets had higher total chlorophyll content than under any other coloured shade net (Ilić *et al.*, 2017). These findings corroborate the results of this study, where the highest RC/ABS value were measured under the blue nets. For this trial, the black and white nets had higher values than the black nets, and this can be due to the increased light scattering effect of the coloured net (Ilić and Fallik, 2017). Furthermore, the higher value of the black and white net versus the black net is possible because Ilić *et al.* (2017) did not specifically trial the black and white type nets.

Although the B+DR LEDs had a non-significant higher value than the B+FR LEDs, the probabilities are due to the following factors. A recent study has indicated that peppers grown in China with a light ratio of 8:1 versus 6:3 R:B LEDs, had a higher non-photochemical quenching (NPQ) value - resulting in a higher proportion of light being dissipated as heat. The peppers grown under a 6:3 R:B ratio had lower NPQ values, and thus more light could be used for photochemical reactions (Xue *et al.*,

2016). This correlates with the results of this study, where the RC/ABS values under the 1:1 B+DR LEDs and 1:1 B+FR LEDs, differed significantly from all the other colour nets (Table 4.5). This is due to the increased (B:R) ratio under blue nets (Basile *et al.*, 2012), where chlorophyll a absorbs light with higher light intensities - and photons in the violet-blue and orange-red spectrum (Lange *et al.*, 1981; Papageorgiou and Govindjee, 2004). Furthermore, the influence of red light is proven to be harmful for photosynthesis (Hogewoning *et al.*, 2010; Murata *et al.*, 2007).

Thus, light quantity has a larger influence on the RC/ABS values than light quality, as the RC/ABS values were lower under the LEDs than the coloured nets. This indicated that high light quantity induced lower RC/ABS values, and vice versa for lower light quantities.

4.8.1.2   *$PHI_o/(1-PHI_o)$*

There were no statistical differences between the $PHI_o/(1-PHI_o)$ values of Grand Slam and Robinson, although they differed significantly from Multi Red and Soltero. Multi Red had the highest $PHI_o/(1-PHI_o)$ value, followed by Soltero (Table 4.4). This suggests that darker coloured lettuce leaves (carotenoids) generate higher $PHI_o/(1-PHI_o)$ values, and this is cultivar specific. Although no statistical differences were measured for the $PHI_o(1-PHI_o)$ values, the highest $PHI_o(1-PHI_o)$ value was under the blue nets, while the lowest and second lowest $PHI_o(1-PHI_o)$ values were measured under the white and Photon Red nets respectively (Table 4.5). The probable reason for the reduced $PHI_o(1-PHI_o)$ value under the Photon Red net, is the increased R:FR ratio under a red net, while a blue net increases the B:FR ratio but not the R:FR ratio (Ilić and Fallik, 2017). The same phenomenon was visible under the white net, where the R:FR ratio is increased (Ilić and Fallik, 2017).

The $PHI_o(1-PHI_o)$ value was the lowest under the B+DR LED for all the coloured nets and LED combinations (Table 4.5). There were non-significant $PHI_o(1-PHI_o)$ differences between the B+DR and B+FR LED values, but both LED combinations had highly significant differences relative to all the different coloured nets. An interesting fact is that the lowest $PHI_o(1-PHI_o)$ values were measured under the

B+DR LEDs, and the second lowest $PHI_o(1-PHI_o)$ under the B+FR LED. This study revealed the lowest $PHI_o(1-PHI_o)$ values under the B+DR LEDs, while the B+FR LEDs produced higher readings. Therefore, it can be concluded that phytochrome B (phyB) plays a pivotal role in determining $PHI_o(1-PHI_o)$ values, as phytochrome B (phyB) detects the ratio of R:FR (Ballare *et al.*, 1991).

4.8.1.3   *$PSI_o/(1-PSI_o)$*

The $PSI_o/(1-PSI_o)$ is defined as the conversion of excitation energy to ET, and thus reflects how efficiently the excitation energy is transformed into ET. The $PSI_o/(1-PSI_o)$ values were once again significantly influenced by the cultivar. The $PSI_o/(1-PSI_o)$ values for Multi Red were 88% higher than for Grand Slam, 76% higher than Robinson, and 11% higher than Soltero (Table 4.3). There were no statistical differences between the $PSI_o/(1-PSI_o)$ values under the different colour nets. Red light is known to reduce the electron transport rate (ETR) from PSII donor side to PSI, while blue light increases the ETR from PSII donor side to PSI (Yan-xiu *et al.*, 2015). This coincides with the reduced values under the red net due to a reduced B:R ratio. The significantly higher $PSI_o/(1-PSI_o)$ values recorded under the nets versus the LEDs, are due to the subsequently higher recorded PAR levels. These results corroborate Bailey *et al.* (2001) who found that an increased light quantity resulted in an increased ETR.

The $PSI_o/(1-PSI_o)$ values were significantly lower under the B+DR and B+FR LEDs than under the coloured shade nets (Table 4.5), and this is due to the influence of light quantity. The $PSI_o/(1-PSI_o)$ values under the B+DR LEDs were slightly higher than the B+FR LEDs (Table 4.5). The same tendency was observed under the shade nets, where the blue nets had a higher value than the Photon Red nets. However, when the $PSI_o/(1-PSI_o)$ values of the B+FR and B+DR LEDs were divided by their respective light quantity per LED combination (40 *$\mu mol\ m^{-2}\ s^{-1}$* for B+FR, and 58 *$\mu mol\ m^{-2}\ s^{-1}$* for B+DR LEDs), the B+FR LEDs had a 38% higher value than the B+DR LEDs. Thus the B+FR LEDs were more efficient than the B+DR LEDs regarding ET per amount of PAR light.

The higher $PSI_o/(1-PSI_o)$ value under the B+FR LEDs emphasises the importance of FR light, and corroborates Myers and Graham (1963) who found that FR light is mostly absorbed by PSI, and the rate of e^- donation from PSII to PSI is determined by the excitation status of PSII. The electron transport rate is used to reflect the photosynthetic rate under a specific light quantity (Kramer *et al.*, 2004). This phenomenon is due to the interaction of phytochromes, cryptochromes and phototropins. Phytochromes are red and far-red light plant photoreceptors, and exist in two photoconvertible forms, P_r (phytochrome red) and P_{fr} (phytochrome far-red) (Keunhwa *et al.*, 2011; Smith, 2000). Phytochrome red is the biologically inactive form, and upon absorption of red photons, it is converted to P_{fr}, the active form (Nagatani, 2010; Quail, 2010). Blue light, on the other hand, alters the functioning of phytochromes through the functioning and regulating effect of cryptochromes and phototropins (Christie and Briggs, 2001).

4.8.1.4 δ/(1-δ)

In the OJIP chlorophyll fluorescence phases, the IP parallel represents the reduction of electron acceptors in and around PSI (Schansker *et al.*, 2005), and indicates how efficiently the end electron acceptors are reduced at the PSII donor side for electrons to be transported from PSII to PSI. Both cultivar and coloured nets had a highly significant interaction with chlorophyll fluorescence parameters (Tables 4.3 and 4.4). All the chlorophyll fluorescence parameters were influenced by the cultivars, except for the reduction of end electron acceptors δ/(1-δ) which was significantly different between the net colour and LEDs and weeks. Grand Slam, Robinson and Multi Red showed non-significant interaction between each other, with readings from 0.77 – 0.80 RU, but differed significantly from Multi Red with 0.67 RU. This indicated that the PSI of Multi Red was not effectively reduced as for the other three cultivars. Zhen and van Iersel (2017) proved that absorbed FR light increases the quantum efficiency of PSII, and is due to the preferential excitation of PSI by FR light, and the plastoquinone pool is faster re-oxidised. Therefore, PSI can readily accept e- from PSII - resulting in a higher δ/(1-δ) value. Soltero had the lowest δ/(1-δ) value of 0.67 RU, which indicated the opposite might have happened due to photoinhibition which damaged the phytochromes and resulted in less FR light being absorbed - and

consequently a lower δ/(1-δ) value. When light conditions are over-excited at PSII - (like blue and red light), the plastoquinone (PQ) pool or intermediate electron transporter between PSII and PSI becomes reduced. This happens when the electrons from PSII are being moved into the PQ pool faster than their ability to be absorbed (Allen, 2003). The primary electron acceptor Q_A of PSII thus prevents the transfer of electrons away from PSII. Therefore, PSII reaction centres cannot use the light for photochemistry, and are considered closed (Maxwell and Johnson, 2000). This suggests that Soltero's ability to donate electrons from PS_{II} to PS_I is less than that of the other cultivars.

The δ/(1-δ) values differed significantly between both combination LEDs and all the coloured nets. However, the δ/(1-δ) values did not differ statistically between the coloured nets (Table 4.5). A probable explanation is that blue nets are known to increase the B:R ratio (Basile *et al.*, 2012), and simultaneously a lower B:R ratio was achieved under the red net and, Photon Red net in this study. Cryptochromes are blue light sensitive (Whitelam and Halliday, 2007) and are known to mediate the production of anthocyanins and carotenoids in plants (Cashmore *et al.*, 1999). Red light does not affect Cryptochrome 2 (Cry 2) levels, although they are reduced with an increasing radiance of blue light, which is not the case for cryptochrome 1 (Cry 1) (Casal, 2000). Thus, the higher δ/(1-δ) value under the blue net was possibly due to a lower cryptochrome 2 level, as a result of the increased B:R ratio.

There was no significant difference between the different coloured LED combinations. The lower light quantities of the B+DR and B+FR LEDs, compared to the high light quantities under the coloured nets, resulted in significantly lower δ/(1-δ) values for the B+DR and B+FR LEDs compared to the coloured nets, and is probably caused by violet-blue and orange-red light spectrum energy - which is mainly absorbed by chlorophyll P680 in PSII (Papageorgiou and Govindjee, 2004). The B+FR LEDs produced a slightly higher δ/(1-δ) value compared to the B+DR LEDs. Interestingly however, the B+FR LEDs gave a value of 83 RU when the B+FR LED light quantity of 40 $μmol\ m^{-2}\ s^{-1}$ was divided by the corresponding δ/(1-δ) value of 0.48 RU. This value was much lower than the a value of 131 for the B+DR LEDs, and suggests that the B+FR LEDs are much more efficient in realising a higher δ/(1-δ) value than B+DR LEDs per $μmol\ m^{-2}\ s^{-1}$ of light. These results coincide with the fact that FR light increases the proportion of open PSII reaction centres, which

mediates through the preferential excitation of PSI (Evans, 1987; Hogewoning *et al.*, 2012), and the re-oxidised plastoquinones can accept electrons from the excited PSII reaction centres (Maxwell and Johnson, 2000). The reason that the $\delta/(1-\delta)$ value for B+FR and B+DR LEDs did not differ significantly, is probably because blue light can reverse the photoconvertible forms of phytochromes by regulating cryptochromes and phototropins, and the phytochromes might be involved with DNA or DNA- binding protein interaction (Christie and Briggs, 2001). This action probably leads to less FR light being absorbed in relation to the red LED light.

4.8.1.5 PI_{total}

Although, the PIabs and PItotal values did not differ significantly from each other per cultivar type (Table 4.4), coloured net or LED light combination (Table 4.5). The PItotal values were overall lower than the PIabs values for all the cultivars, coloured nets and LED light combinations. This is due to the interaction of the $\delta/(1-\delta)$ values being less than one. This indicates that light quantity plays a major role in determining PIabs and PItotal values.

Table 4.3: Chlorophyll fluorescence parameters with significant (*) and highly significant (**) values for lettuce in the seedling phase (week 5) under different colour nets and LED combinations.

	RC/ABS		$PHI_0/(1-PHI_0)$		$PSI_0/(1/PSI_0)$		$\delta/(1-\delta)$		PI_{abs}		PI_{total}	
Lettuce week 4-5 net & LED	*F*	P	*F*	P	*F*	P	*F*	P	*F*	P	*F*	P
Cultivar	298.19	$p < 0.001$**	100.45	$p < 0.001$**	434.87	$p < 0.001$**	14.49	$p < 0.001$**	445.84	$p < 0.001$**	110.71	$p < 0.001$**
Colour Nets and LED	83.42	$p < 0.001$**	33.73	$p < 0.001$**	20.19	$p < 0.001$**	40.81	$p < 0.001$**	43.77	$p < 0.001$**	23.48	$p < 0.001$**
Weeks	64.34	$p < 0.001$**	4.98	0.043*	14.46	0.002*	22.84	$p < 0.001$**	0.85	0.372	3.36	0.088
Cultivar* Colour Nets and LED	5.65	$p < 0.001$**	1.03	0.457	15.80	$p < 0.001$**	1.42	0.173	18.55	$p < 0.001$**	6.03	$p < 0.001$**
Cultivar* Weeks	4.69	0.007*	0.18	0.911	1.21	0.317	0.13	0.945	3.14	0.036*	1.60	0.204
Colour Nets and LED* Weeks	5.23	0.005*	1.89	0.151	1.25	0.338	7.43	0.001*	1.08	0.418	0.96	0.489
Cultivar Colour Nets and LED *Weeks	1.16	0.338	0.88	0.606	0.73	0.764	1.24	0.281	1.82	0.056	1.21	0.298

Table 4.4: Highly significant differences for individual chlorophyll fluorescence parameters RC/ABS, PHIo/(1-PHIo), PSIo/(1-PSIo) and δ/(1-δ), expressed in relative mean units per lettuce cultivar in the seedling phase for week 5. Significant differences between means within a parameter are indicated with different superscript letters.

	RC/ABS	$PHI_o/$ $(1-PHI_o)$	$PSI_o/$ $(1/PSI_o)$	$δ/(1-δ)$	PI_{abs}	PI_{total}
Grand Slam	8.13[c]	4.43[c]	1.12[c]	0.77[a]	40.34[c]	31.06[c]
Robinson	8.40[c]	4.49[c]	1.19[c]	0.79[a]	44.88[c]	35.46[c]
Multi Red	12.26[a]	5.30[a]	2.10[a]	0.80[a]	136.45[a]	109.16[a]
Soltero	11.17[b]	5.09[b]	1.89[b]	0.67[b]	107.46[b]	71.96[b]

Table 4.5: Chlorophyll fluorescence parameters RC/ABS, PHIo/(1-PHIo), PSIo/(1/PSIo), δ/(1-δ), PIabs, PItotal and coloured nets and LED combinations, expressed in mean relative units for lettuce in the seedling phase for week 5. Significant differences between means within a parameter are indicated with different superscript letters.

	RC/ABS	$PHI_o/(1-PHI_o)$	$PSI_o/(1/PSI_o)$	$δ/(1-δ)$	PI_{abs}	PI_{total}
Black	11.56[a]	5.69[a]	1.72[a]	0.88[a]	113.08[a]	99.41[a]
Black white	11.63[a]	5.74[a]	1.71[a]	0.92[a]	114.12[a]	105.23[a]
Photon Red	11.28[a]	5.66[a]	1.69[a]	0.85[a]	107.83[a]	91.73[a]
Blue	11.67[a]	5.81[a]	1.74[a]	0.93[a]	117.96[a]	109.38[a]
White	11.45[a]	5.65[a]	1.65[a]	0.87[a]	106.86[a]	92.86[a]
B+FR LED	5.79[b]	4.57[b]	1.24[b]	0.48[b]	32.81[b]	15.35[b]
B+DR LED	6.54[b]	4.50[b]	1.30[b]	0.44[b]	38.26[b]	17.67[b]

There were no significant differences for the PItotal values for Grand Slam and Robinson between the different coloured nets or B+FR LEDs and B+DR LEDs, and thus the PItotal differences between Robinson and Gram Slam are not portrayed in Figure 4.1.
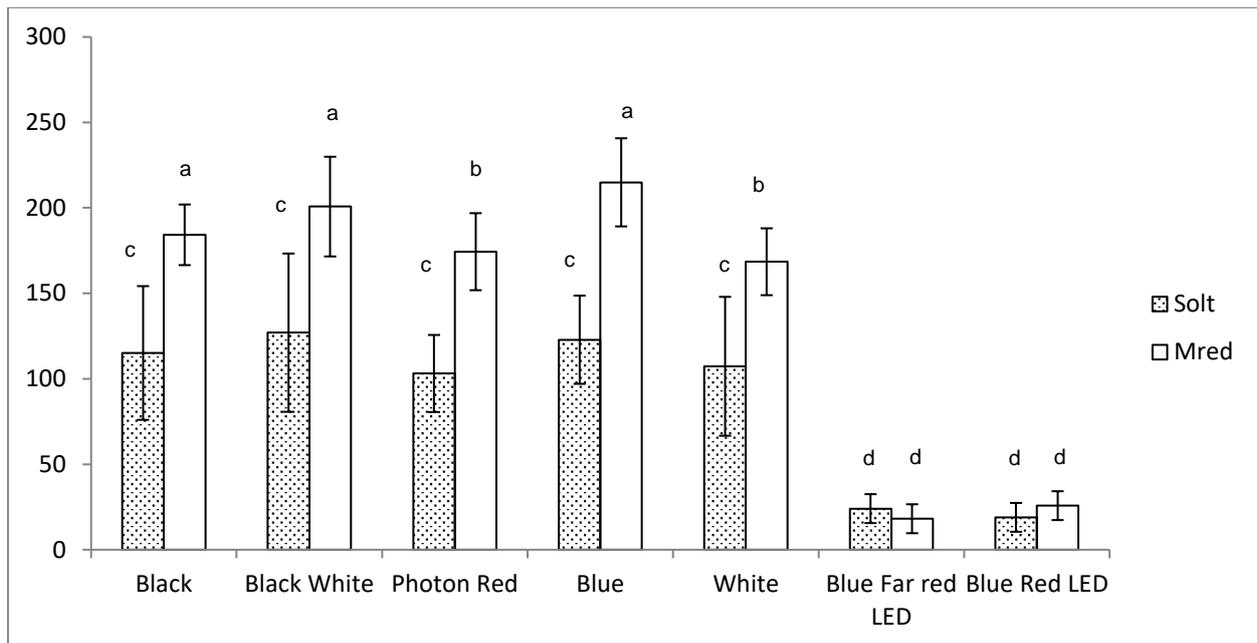
Figure 4.1: Average PI$_{total}$ values expressed in mean relative units for Soltero and Multi Red cultivars in the seedling phase, with significant ($p < 0.05$) differences a, b, c and d for PI$_{total}$ per black, black and white, Photon Red, blue and white net and B+FR and B+DR LED combinations. Error bars indicate upper and lower 95% confidence levels.

There were however, significant differences for Soltero and Multi Red between the coloured nets and the different LED combinations. Significant differences occurred for Soltero between the B+FR LEDs and all the coloured nets, as well as between the B+DR LEDs and all the different coloured nets. Interestingly, there was no significant difference for Soltero between the different LED combinations. The PI$_{total}$ values were about 5 times lower under the low radiation LEDs than under the coloured nets with high solar radiation. Multi Red showed a significant difference for the PI$_{total}$ values between the Photon Red net and black net, black and white net and blue nets. The PI$_{total}$ values for Multi Red under the white net did not differ significantly from under the Photon Red net, although significant differences were observed when compared to the black net, black and white net, and blue nets. The PI$_{total}$ values from Soltero and Multi Red did not differ from one another under the B+FR and B+DR LEDs. Soltero had lower PI$_{total}$ values than Multi Red under all the different coloured nets and B+DR LEDs, except for the B+FR LED combination which produced higher values for Soltero than for Multi Red. The most interesting fact is that the PI$_{total}$ values for Grand Slam, Robinson, Soltero and Multi Red, were

all non-significantly different from one another under the B+FR and B+DR LEDs. Therefore, the non-significant cultivar differences can be associated with the reduced light intensity from the LEDs which stimulated efficient photosynthesis, as NPQ did not realize. Another possible cause can be because of the LEDs narrow band and is more precise for chlorophyll *a* and *b* synthesis than the whole PAR spectrum.

The B+DR and B+FR LED colours significantly reduced the variance of PI_{total} values between Multi Red and Soltero cultivars. Significant variations in PI_{total} values were observed between Multi Red and Soltero cultivars under high solar radiation for all the different coloured nets. The PI_{total} values for Multi Red and Soltero did not differ significantly from one another under the B+DR and B+FR red LEDs, although there was a consistent significant difference for both cultivars under all the coloured nets. Therefore, it is clear that LED manipulation can be used to reduce variations in PI_{total} values for red leaf lettuce, and that light quantity, on the other hand, has a direct influence on PI_{total} values.

There was also highly significant interaction between the different cultivars and coloured nets for the RC/ABS, $PSI_o/(1-PSI_o)$, PI_{abs}, and PI_{total} values. This is due to the large influence the cultivar has on these specific chlorophyll fluorescence parameters - as indicated by the very large *F* values in Table 4.1.

4.9   Macro- and Micro-element Analysis.

The seedlings grown under the different coloured shade nets were all compact, had a well-developed root structure, and good leaf colouration. All four different seedling cultivars grown under the B+DR LEDs were overall shorter, better developed, with larger leaves, and had better developed root structure than the seedlings grown under the B+RF and R+FR LEDs. There were distinct visual differences in leaf colour between the green and red cultivars. Multi Red lettuce leaves were a darker red than Soltero, while Robinson and Grand Slam leaves were a dark green colour. All the seedlings under the R+FR LEDs were extremely elongated, with thin and fragile stems, and the leaves and root structures were undeveloped.

Grand Slam, Robinson, Soltero and Multi Red seedlings were a pale green colour, and there were no visual leaf colour differences between the green and red lettuce cultivars. The seedlings of all the cultivars grown under the B+FR LEDs were overall shorter with slightly larger leaves and had a more developed root system than seedlings under the R+FR LEDs - but to a lesser degree than under the B+DR LEDs. These physical appearances concur with Amoozgar *et al.* (2017), who found that lettuce plants had elongated and fragile stems and leaves. The seedlings under the R+FR LEDs, as well as under the B+FR LEDs, did not produce enough plant material for chemical macro- and micro-element analysis, and thus only data from the different coloured nets and B+DR LEDs were compared to one another. The seedling material gathered from the B+DR LEDs were limited, and thus all macro- and micro-elements were analysed except for N. Overall, macro- and micro-nutrient element leaf content was significantly higher under the B+DR LEDs than under any of the shade coloured nets.

Recent studies revealed that red LEDs can affect the metabolic pathways in plants which influences water absorption and increases the macro- and micro-element leaf content (Amoozgar *et al.* 2017). According to Chen *et al.* (2014), the uptake of Na, Fe, Mn, Cu and Mo was significantly increased in lettuce when the LED spectrum corresponded to either 450 and/or 660 nm, which enhanced the chlorophyll-b and -a functioning. Furthermore, lettuce grown under red rich LEDs showed an increase in N, P, K, Mg and S uptake; however, deep red LEDs (660 nm) showed superior nutrient uptake capabilities over red LEDs (640 nm) (Pinho *et al.*, 2017). This is because in relation to red (640 nm), deep red LEDs (660 nm) are in closer proximity to the peak absorption of chlorophyll-a (Pinho *et al.*, 2017). The nutrient uptake and the utilisation-associated genes are directly regulated by photoreceptor-mediated light signalling (Chen *et al.*, 2016; Huang *et al.*, 2015; Lee *et al.*, 2011). These physiological processes are under the control of the transcription factor ELONGATED HYPOCOTYL5 (HY5), which includes photosynthesis (Toledo-Ortiz *et al.*, 2014) and nutrient usage (Chen *et al.*, 2016; Huang *et al.*, 2015). Furthermore, HY5 accumulation is increased by red, far red, and blue light, and thus plays a pivotal role in nutrient uptake under various light conditions (Sakuraba and Yanagisawa, 2017). However, the mineral absorption and ion transporters are influenced by the R:FR light ratio, and are not yet documented (Demotes-Mainard *et*

al., 2016). This implies that nutrient uptake is likely to be stimulated through light signalling.

Highly significant interaction ($p < 0.001$) with large *F*-values were observed between the cultivar and net colour and LEDs, for Ca, Mg, Cu and Zn, and shows that the significant difference in uptake for the elements compared to other elements were driven through the combination of the cultivar, net colour and LEDs (Table 4.6). Highly significant interactions were also observed between the net colour and LEDs for all the macro- and micro- elements (Table 4.6) - except for K and Fe (Table 4.6). Highly significant interaction ($p < 0.001$) occurred between the cultivars and macro- and micro-elements for phosphorous (P), calcium (Ca), magnesium (Mg), manganese (Mn), copper (Cu) and zinc (Zn), while significant interaction ($p < 0.05$) occurred between the cultivar and potassium (K), sodium (Na), and iron (Fe) (Table 4.6). Boron (B) was the only element where the uptake was not influenced by the cultivar (Table 4.6). Within all the highly significant interactions for cultivar and net colour and LEDs, Grand Slam had the lowest values, followed by slightly higher values for Robinson, and larger values for Soltero. Multi Red had the highest values overall for all the macro- and micro-elements. There were no statistical differences for any of the macro- and micro-elements between the individual colour shade nets. Thus, the B+DR LEDs were only compared to the black net (control).

4.9.1 *Macro- and micro-element uptake*

The average phosphorous (P) value of all the cultivars under the B+DR LEDs was 2.1 times greater than the averaged values of all the cultivars under the coloured net (Figure 4.2). This corroborates Amoozgar *et al.* (2017) where the P value of lettuce grown under B+R LEDs was 2.5 times greater than when grown under greenhouse conditions.

The Ca value under the black net was 0.81%, and shows similarities with Amoozgar *et al.* (2017), where the Ca content for lettuce was 0.72 g 100g^{-1}, which can also be expressed as a %. Their study indicated that Ca uptake was 3.5 times higher under the B+R LEDs than under greenhouse conditions, while it was 2.75 times higher under the B+DR LEDs compared to the black net in this study. The lower Ca uptake

ratio under the B+DR LEDs is probably due to the lower B+DR LED light quantity of 58 $\mu mol\ m^{-2}\ s^{-1}$, compared to the 300 $\mu mol\ m^{-2}\ s^{-1}$ light quantity used Amoozgar *et al.* (2017). Blue light also triggers the opening of ion channels located on cell plasma membranes, increases Ca uptake into the cytosol, and influences the cryptochrome signalling process (Lin, 2002). Therefore, the increased B:R ratio of 50%:50% in this study - in comparison to the B:R ratio of 30%:70% of Amoozgar *et al.* (2017) - explains the higher Ca content when it is expressed per light quantity in $\mu mol\ m^{-2}\ s^{-1}$. Another plausible explanation for the higher macro- and micro-element uptake in this study is possibly due to the increased B:R ratio, and is confirmed by Kopsell and Sams (2013) who found that blue light LEDs significantly increased macro- and micro-element uptake in broccoli microgreens.

The Zn value was 5.89 times greater under the B+DR LEDs than the averaged value under the different coloured nets. This value is considerably higher than the findings of Amoozgar *et al.* (2017), where it was 1.28 times greater under the B+R LEDs than under greenhouse conditions. The higher Zn value under the B+DR LEDs versus the coloured nets in this trial, because blue LEDs (460 nm) had a greater effect on Zn uptake than deep blue LEDs (455 nm), and is because of the specific blue wavelength colour (Pinho *et al.*, 2017).

The element copper (Cu) showed the greatest difference in values between the black net and B+DR LEDs. According to Cheng and Allen (2001), the uptake of Cu by lettuce roots and shoots is driven by the ratio of the concentration of free Cu ions in a solution, which is linearly related to the concentration of hydrogen (H^+) ions in the solution. They further state that Ca reduces the uptake of Cu in lettuce roots. This contradicts our findings, as the Ca uptake under B+DR LEDs was 2.75 times greater than under the black net (Figure 4.2). Furthermore, the Cu uptake under the B+DR LEDs was 11.7 times higher than under the black net (Table 4.7 and Figure 4.2). The leaf Cu content under all the coloured nets was very low, and is possibly due to the influence of photoinhibition that was experienced under the high solar radiation. The LEDs on the other hand, portrayed low radiation light quantity with narrow band widths. This enabled specific light quality absorption, which resulted in significantly higher Cu content in lettuce leaves produced under B+DR LEDs. According to Marschner (1995), copper is required in the cytosol, endoplasmic reticulum (ER), mitochondrial inner membrane, chloroplast stroma, apoplast, and the

thylakoid membrane - and is essential for optimal plant functioning. Also, Cu aids structurally in certain metalloproteins, related to ET in chloroplasts, mitochondria and plant oxidative stress responses.

Table 4.6: The interaction between averaged macro and micro elements per cultivar, colour net and the combination of cultivar and colour net for lettuce seedlings in the seedling phase in week five.

Lettuce week 5 colour net and LED	Phosphorus		Potassium		Calcium		Magnesium		Sodium	
	F	P	F	P	F	P	F	P	F	P
Cultivar	34.20	$p < 0.001$**	4.84	0.006*	15.54	$p < 0.001$**	52.71	$p < 0.001$**	3.71	0.020*
Net colour and LED	86.63	$p < 0.001$**	6.26	0.004*	113.76	$p < 0.001$**	56.45	$p < 0.001$**	400.75	$p < 0.001$**
Cultivar *net colour and LED	1.90	0.058	1.36	0.219	3.33	0.002*	3.42	0.002*	1.24	0.291

Lettuce week 5 colour net and LED	Manganese		Iron		Copper		Zinc		Boron	
	F	P	F	P	F	P	F	P	F	P
Cultivar	12.13	$p < 0.001$**	5.81	0.002*	16.65	$p < 0.001$**	11.16	$p < 0.001$**	2.51	0.074
Net colour and LED	37.11	$p < 0.001$**	1.86	0.176	21.40	$p < 0.001$**	173.25	$p < 0.001$**	38.56	$p < 0.001$**
Cultivar *net colour and LED	1.16	0.344	1.79	0.076	2.83	0.005*	18.18	$p < 0.001$**	0.57	0.876

89

Table 4.7: Mean averages of the combined lettuce cultivars per colour net or LED combination for P, K, Ca and Mg as % of dried leaf, and Na, Mn, Fe, Cu, Zn and B in (mg.kg^{-1}) between the B+DR LEDs and all the colour nets for lettuce in the seedling phase in week 5. Significant differences between means within a parameter are indicated with different superscript letters.

	P	K	Ca	Mg	Na
Black	0.45^b	7.69^b	0.81^b	0.30^b	546.58^b
Black white	0.44^b	7.86^b	0.86^b	0.30^b	511.92^b
Photon Red	0.45^b	7.90^b	0.85^b	0.30^b	522.75^b
Blue	0.45^b	7.57^b	0.82^b	0.29^b	526.17^b
White	0.42^b	7.25^b	0.78^b	0.28^b	508.50^b
Blue:Red LED	0.93^a	8.80^a	2.23^a	0.55^a	2892.6^a

	Mn	Fe	Cu	Zn	B
Black	75.00^b	235.25^b	0.75^b	16.25^b	27.83^b
Black white	76.75^b	250.25^b	0.83^b	15.25^b	25.75^b
Photon Red	76.67^b	247.33^b	0.67^b	15.83^b	26.83^b
Blue	76.00^b	255.17^b	0.75^b	15.42^b	25.25^b
White	72.92^b	246.67^b	0.83^b	15.17^b	24.33^b
Blue:Red LED	161.16^a	467.67^a	8.75^a	95.33^a	76.33^a
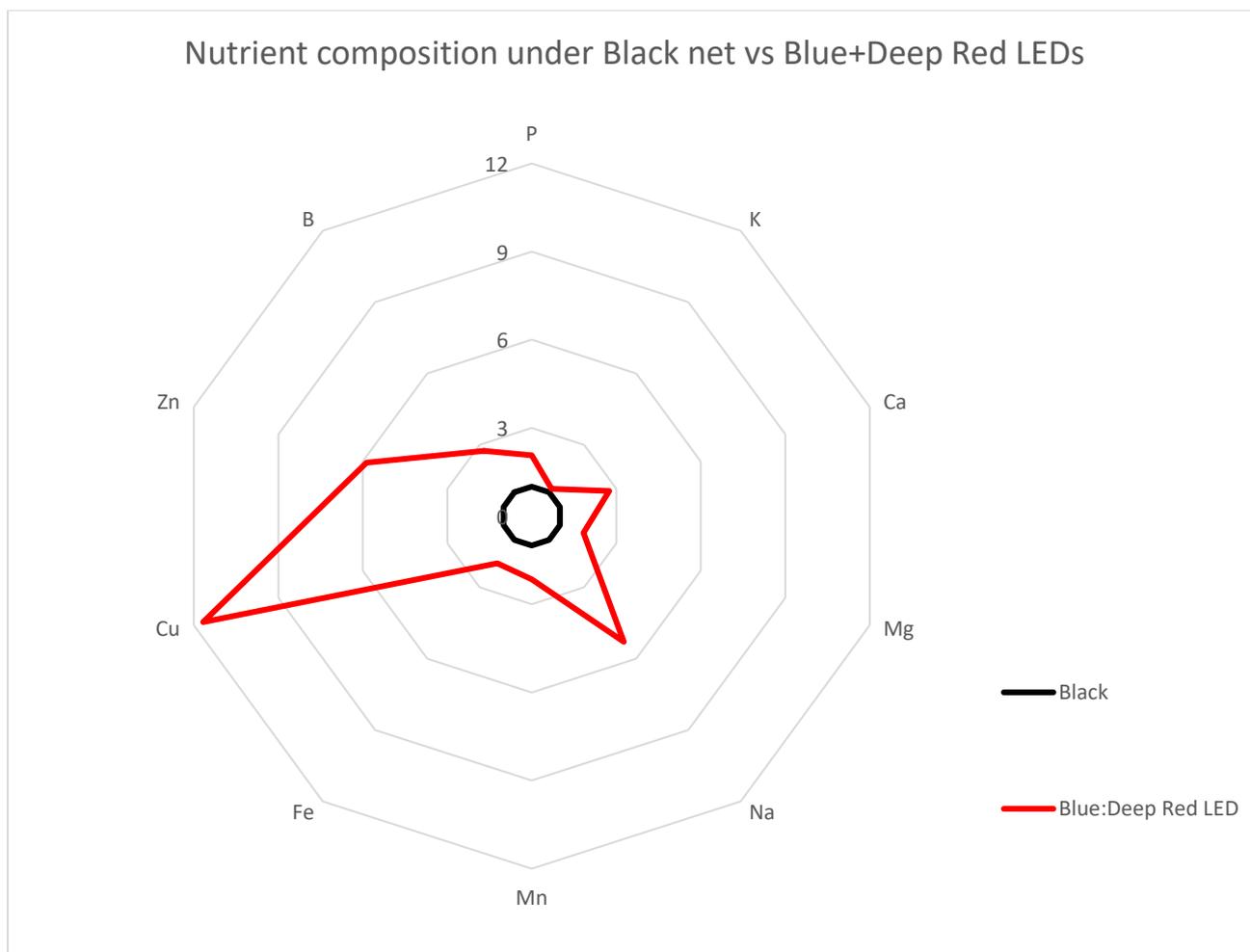
Figure 4.2: Averaged relative macro- and micro-nutrient uptake of Robinson, Grand Slam, Soltero and Multi Red lettuce under black nets and B+DR LEDs. The macro- and micro-nutrient values of the black net are normalised to 1.

4.10 Conclusion

All chlorophyll fluorescence parameters were greatly influenced by the cultivar - except the reduction of end electron acceptors which was more prone to the influences of the net colours and LEDs. The RC/ABS, $PHI_o/(1-PHI_o)$ and $PSI_o/(1-PSI_o)$, PI_{abs} and v PI_{total} values differed significantly per cultivar, and was the highest for the dark pigmented Multi Red lettuce cultivar followed by Soltero, Grand Slam

and Robinson. However, there were no significant differences between Grand Slam and Robinson for all the fluorescence parameters. The PI$_{total}$ values of all the cultivars were non-significantly different from one another under the B+FR and B+DR LEDs. This indicated that light quantity, as well as colour specific wavelength LEDs have a major influence on PI$_{total}$ values.

There were no significant differences between lettuce leaf macro- and micro-element composition under the different coloured nets. However, significant differences were observed for all the macro-and micro-elements between the B:DR LEDs and the coloured nets. The B:DR LEDs had the greatest effect on Cu, and leaf composition was 11.43 times higher, and indicated significant differences from the nets. Zinc was 5.89 times higher under the B:DR LEDs than under the coloured nets. The 1:1 ratio of B:DR LEDs resulted in higher Ca content value, and was 2.75 times higher under the B:DR LEDs than under the black nets. Magnesium was also 1.87 times higher under the B:DR LEDs than the black net. This emphasises the importance the importance on leaf macro- and micro element composition, as micro- element phytotoxicity can become a problem with these high values. There was a significant difference in the average potassium values of all the cultivars under the Photon Red net and the white net.

4.11   References

Alkalai-Tuvia S, Goren A, Perzelan Y, Weinberg T, Fallik E. 2014. The influence of coloured nets on pepper quality after harvest – a possible mode-of-action. *Agriculture and Forestry*. 60: 7-18.

Allen JF. 2003. State transitions -a question of balance. *Science.* 299: 1530-1532.

Amoozgar A, Mohammadi A, Sabzallan MR. 2017. Impact of light-emitting diode irradiation on photosynthesis, phytochemical composition and mineral element content of lettuce cv. Grizzly. *Journal of Photosynthetica.* 55 (1): 85-95.

Appling SM. 2012. Coloured Shade Cloth Affects the Growth of Basil, Cilantro, and Parsley. MSc thesis, Polytechnic Institute and State University, Blacksburg, Virginia.

Armstrong GA, Hearst JE. 1996. Carotenoids 2: Genetics and molecular biology of carotenoid pigment biosynthesis. *The FASEB Journal*. 10(2): 228.

Bailey S, Walters RG, Jansson S, Horton P. 2001. Acclimation of *Arabidopsis thaliana* to the light environment: The existence of separate low light and high light responses. *Planta*. 213: 794-801.

Ballare CL, Casal JJ, Kendrick RE. 1991. Responses of wild-type and long hypocotyl mutant cucumber seedlings to natural and simulated shade light. *Journal of Photochemistry and Photobiology*. 54: 819-826.

Basile B, Giaccone M, Cirillo C, Ritieni A, Graziani G, Shahak Y, Forlani M. 2012. Photo-selective hail nets affect fruit size and quality in Hayward kiwifruit. *Scientia Horticulturae*. 141: 91-97.

Casal JJ. 2000. Phytochromes, Cryptochromes, Phototropins: Photoreceptor interactions in plants. *Journal of Photochemistry and Photobiology*. 71(1): 1-11.

Cashmore ARJA, Jarillo Y, Wu J, Liu D. 1999. Cryptochromes: blue light receptors for plants and animals. *Journal of Science*. 284: 760-765.

Chen XL, Guo WZ, Xue XZ, Morewane MB. 2014. Effects of LED Spectrum Combinations on the Absorption of Mineral Elements of Hydroponic Lettuce. *Spectroscopy and Spectral Analysis*. 34(5): 1394-397.

Chen X, Yao Q, Gao X, Jiang C, Harberd NP, Fu X. 2016. Shoot-to-root mobile transcription factor HY5 coordinates plant carbon and nitrogen acquisition. *Current Biology*. 26: 640-646.

Cheng T, Allen H. 2001. Prediction of uptake of copper from solution by lettuce (*Lactuca sativa* Romance). *Environmental Toxicology and Chemistry*. 20(11): 2544-2551.

Christie JM, Briggs WR. 2001. Blue Light Sensing in Higher Plants. *Journal of Biological Chemistry*. 276: 11457-11460.

Coleman LW, Rosen BH, Schwartzbach SD. 1998.Preferential loss of chloroplast proteins in nitrogen deficient euglema. *Plant and Cell Physiology*. 29(6): 1007-1014.

Demotes-Mainard S, Péron T, Corot A, Bertheloot J, Gourrierec J, Le Travier S, Sakr S. 2016. Plant responses to red and far-red lights, applications in horticulture. *Environmental and Experimental Botany*. 121: 4-21.

Dougher TAO, Bugbee B. 2001. Differences in the Response of Wheat, Soybean and Lettuce to Reduced Blue Radiation. *Journal of Photochemistry and Photobiology*. 73(2): 199-207.

Dougher TAO, Bugbee B. 2004. Long-Term Blue Light Effects on the Histology of Lettuce and Soybean Leaves and Stems. *Journal of the American Society for Horticultural Science*. 129(4): 467-472.

Evans JR. 1987. The dependence of quantum yield on wavelength and growth irradiance. *Australian Journal of Plant Physiology*. 14: 69-79.

Fu W, Li P, Wu Y. 2012. Effects of different light intensities on chlorophyll fluorescence characteristics and yield in lettuce. *Scientia Horticulturae*. 135: 45-51.

Galvão VC, Fankhauser C. 2015. Sensing the light environment in plants: photoreceptors and early signalling steps. *Current Opinion in Neurobiology*. 34: 46-53.

Gonçalves JFDC, Marenco RA, Vieira G. 2001. Concentration of photosynthetic pigments and chlorophyll fluorescence of mahogany and tonka bean under two light environments. *Revista Brasileira de Fisiologia Vegetal*. 13: 149-157.

Goren A, Alkalai-Tuvia S, Perzelan Y, Aharon Z, Fallik E. 2011. Photoselective shade nets reduce postharvest decay development in pepper fruits. *Advances in Horticultural Science*. 25: 26-31.

Hogewoning SW, Trouwborst G, Maljaars H, Poorter H, Van Leperen W. Harbinson J. 2010. Blue light dose-response of leaf photosynthesis, morphology, and chemical composition of *Cucumis sativus* grown under different combinations of red and blue light. *Journal of Experimental Botany*. 61: 3107-3117.

Hogewoning SW, Wientjes E, Douwstra P, Trouwborst G, van Ieperen W, Croce R, Harbinson J. 2012. Photosynthetic quantum yield dynamics: from photosystems to leaves. *Plant Cell*. 24: 1921-1935.

Huang L, Zhang H, Zhang H, Deng XW, Wei N. 2015. *HY5* regulates nitrite reductase 1 (*NIR1*) and ammonium transporter1;2 (*AMT1;2*) in *Arabidopsis* seedlings. *Journal of Plant Sciences*. 238: 330-339.

Huché-Thélier L, Crespel L, Le Gourrierec J, Morel P, Sakr S, Leduc N. 2016. Light signalling and plant responses to blue light and UV radiation – perspectives for applications in horticulture. *Environmental and Experimental Botany*. 121: 22-38.

Ilić ZS, Fallik E. 2017. Light quality manipulation improves vegetable quality at harvest and postharvest: A review. *Environmental and Experimental Botany*. 139: 79-90.

Ilić ZS, Milenković L, Šunić L, Fallik E. 2017. Effect of shading by colour nets on plant development, yield and fruit quality of sweet pepper grown under plastic tunnels and open field. *Zemdirbyste-Agric*. 104: 53-62.

Kempen E. 2012. Greenhouse production techniques – Agronomy 312. Unpublished class notes. University of Stellenbosch.

Keunhwa K, Jieun S, Sang-Hee L, Hee-Seok K, Juliun N, Giltsu C. 2011. Phytochromes inhibit hypocotyls negative gravitropism by regulating the development of endodermal amyloplast through phytochrome-interacting factors. *Proceedings of the National Academy of Sciences of the United States of America.* 108(4): 1729-1734.

Kim SJ, Hahn EJ, Heo J-W, Pack KY. 2004. Effects of LEDs on net photosynthetic rate, growth and leaf stomata of chrysanthemum plantlets in vitro. *Journal of American Society for Horticultural Science*. 101: 143-151.

Kimura M, Rodriguez-Amaya DB. 2003. Carotenoid composition of hydroponic leafy vegetables. *Journal of Agricultural and Food Chemistry*. 51: 2603 -2607.

Kittas C, Rigakis N, Katsoulas N, Bartzanas T. 2009. Influence of shading screens on microclimate, growth and productivity of tomato. *International Society for Horticultural Science*. 807: 97-102.

Kong Y, Avraham L, Perzelan Y, Alkalai-Tuvia S, Ratner K, Shahak Y, Fallik E. 2013. Pearl netting affects postharvest fruit quality in "Vergasa" sweet pepper via light

environment manipulation. *International Society for Horticultural Science*. 150: 290-298.

Kopsell DA, Sams CE. 2013. Increases in Shoot Tissue Pigments, glucosinolates, and mineral Elements in Sprouting Broccoli after Exposure to Short-duration Blue Light from Light Emitting Diodes. *Journal of the American Society for Horticultural Science*. 138(1): 31-37.

Kosma C, Triantafyllidis V, Papasavvas A, Salahas G, Patakas A. 2013. Yield and nutritional quality of greenhouse lettuce as affected by shading and cultivation season. *Emirates Journal of Food and Agriculture*. 25: 974-979.

Kramer DM, Johnson G, Kiirats O, Edwards GE. 2004. New fluorescence parameters for the determination of QA redox state and excitation energy fluxes. *Photosynthesis Research*. 79: 209-218

Lange L, Noble P, Osmond C, Ziegler H. 1981. Responses to Physical Environment 12A. *Physiological Plant Ecology1*. 67: 259.

Lee BR, Koprivova A, Kopriva S. 2011. The key enzyme of sulfate assimilation, adenosine 5'-phophosulfate reductase, is regulated by HY5 in Arabidopsis. *The Plant Journal*. 67: 1042-1054.

Lefsrud MG, Kopsell DA, Sams CE. 2008. Irradiance from distinct wavelength light-emitting diode affect secondary metabolites in kale. *American Society for Horticultural Science*. 43: 2243-2244.

Lin C. 2002. Blue light receptors and signal transduction. *The Plant Cell*. 14: S207-225.

Marcelis LFM. 1993. Fruit growth and biomass allocation to the fruits in cucumber. 2. Effect of irradiance. *International Society for Horticultural Science*. 54: 123-139.

Marschner H. 1995. Mineral nutrition of higher plants. Academic Press: London

Martineau V, Lefsrud MG, Tahera Naznin M, Kopsell DA. 2012. Comparison of supplemental greenhouse lighting from light emitting diode and high pressure sodium light treatments for hydroponic growth of Boston Lettuce. *American Society for Horticultural Science*. 47: 477-482.

Maxwell K, Johnson GN. 2000. Chlorophyll fluorescence - a practical guide. *Journal of Experimental Botany.* 51: 659-668.

Murata N, Takahashi S, Nishiyama Y, Allakhverdiev SI. 2007. Photoinhibition of photosystem II under environmental stress. *Biochimica et Biophysica Acta Bioenergetics.* 1767: 414-421.

Myers J, Graham JR. 1963. Enhancement in Chlorella. *Journal of Plant Physiology*. 38: 105-116.

Nagatani A. 2010. Phytochrome: structural basis for its functions. *Current Opinion in Plant Biology*. 13: 565-570.

Nitz GM, Schnitzler WH. 2004. Effect of PAR and UV-B radiation on the quality and quantity of the essential oil in sweet basil (*Ocimum basilicum* L.). *International Society for Horticultural Science*. 659: 375-381.

Papageorgiou G, Govindjee. 2004. Chlorophyll a fluorescence – *a signature of photosynthesis. Springer*. Dordrecht.

Pinho P, Jokinen K, Halonen L. 2017. The influence of the LED light spectrum on the growth and nutrient uptake of hydroponically grown lettuce. *The Society of Light and Lighting*. 49: 866-881.

Prasil O, Adir N, Ohad I. 1992. Dynamics of photosystem II: Mechanism of photoinhibition and recovery processes, pp. 295-348. In: Barber J (ed.), *The photosystems: Structure, Function and Molecular Biology*. Elsevier: Amsterdam.

Quail PH. 2010. Phytochromes. *Current Opinion in Plant Biology.* 20: R503-R504.

Sakuraba Y, Yanagisawa S. 2017. Light signalling-induced regulation of nutrient acquisition and utilisation in plants. *Seminars in Cell & Developmental Biology*.

Savvides A, Fanourakis D, Van Leperen W. 2011. Co-ordination of hydraulic and stomatal conductances across light qualities in cucumber leaves. *Journal of Experimental Botany.* 63(3): 1135-1143.

Schansker G, Toth SZ, Strasser RJ. 2005. Dark-recovery of the chl *a* fluorescence transient (OJIP) after adaptation: the qT-component of non-photochemical quenching is related to an activated photosystem I acceptor side. *Biochimica et Biophysica Acta.* 1757: 787-797.

Schuerger AC, Brown CS, Stryjewski EC. 1997. Anatomical features of pepper plants (*Capsicum annuum* L.) grown under red light-emitting diodes supplemented with blue or far-red light. *Ann. Bot.* (Lond.) 79: 273–282.

Shahak Y. 2008. Photo-selective netting for improved performance of horticultural crops: a review of ornamental and vegetable studies in Israel. *International Society for Horticultural Science.* 770: 161-168.

Shahak Y, Gal E, Offir Y, Ben-Yakir D. 2008. Photoselective shade netting integrated with greenhouse technologies for improved performance of vegetable and ornamental crops. *International Society for Horticultural Science.* 797: 75-80.

Shahak Y, Gussahovsky EE, Gal E, Ganelevin R. 2004. ColorNets: Crop protection and light-quality manipulation in one technology. *International Society for Horticultural Science.* 659: 143-151.

Sharkey TD, Raschke K. 1981. Separation and measurement of direct and indirect effects of light on stomata. *Journal of Plant Physiology.* 68: 33-40.

Shimazaki K, Doi M, Assman SM, Kinoshita T. 2007. Light regulation of stomatal movement. *Annual Review of Plant Biology.* 58: 219-247.

Smith H. 2000. Phytochromes and light signal perception by plants – an emerging synthesis. *Journal of Nature.* 407: 585-591.

Stamps RH. 2009. Use of coloured shade netting in horticulture. *International Society for Horticultural Science.* 44: 239-241.

Stewart A, Bozonnet S, Mullen W, Jenkins GI. 2000. Occurrence of flavonols in tomatoes and tomato based products. *Journal of Agricultural Food Chemistry.* 48: 2663-2669.

Strasser RJ, Srivastava A, Tsimilli-Michael M. 2000. The fluorescence trancient as a tool to characterise and screen photosynthetic samples, pp.445-453. In: Mohanty P, Yunus, Pathre (eds), *Probing photosynthesis: Mechanism, regulation & adaptation.* London: Taylor & Francis.

Taiz L, Zeiger E. 2002. Plant physiology, 3rd ed. Sinauer Associates: Sunderland, MA.

Teitel M, Lirion O, Haim Y, Seigner I. 2008. Flow through inclined and concertina shaped screens. *International Society for Horticultural Science.* 801: 99-106.

Toledo-Ortiz G, Johansson H, Lee KP, Bou-Torrent J, Stewart K, Steel G, Rodriguez-Conception M, Halliday KJ. 2014. The HY5-PIF regulatory module coordinates light and temperature control of photosynthetic gene transcription. *PLOS Genetics* 10: e1004416.

Weng XY, Xu HX, Jiang DA. 2005. Characteristics of gas exchange, chlorophyll fluorescence and expression of key enzymes in photosynthesis during leaf senescence in rice plants. *Journal of Integrative Plant Biology.* 47(5): 560-566.

Whitelam GC, Halliday KJ. 2007. *Light and plant development.* Blackwell Publishing: Oxford.

96

Yanagi T, Okamoto K, Takita S. 1996. Effects of blue, red, and blue/red lights on two different PPF levels on growth and morphogenesis of lettuce plants. *International Society for Horticultural Science.* 440: 117-122.

Yang QC. 2008. Application and prospect of Light Emitting Diode (LED) in agriculture and bio-industry. *Journal of Agricultural Science and Technology.* 6: 42-47.

Yanovsky MJ, Casal JJ, Whitelam GC. 1995. Phytochrome A, phytochrome B and HY4 are involved in hypocotyl growth responses to natural radiation in *Arabidopsis*: Weak de-etiolation of the phyA mutant under dense canopies. *Plant Cell & Environment.* 18: 788-794.

Yan-xiu M, Xiao-zhuo W, Li-hong G, Qing-yun C, Mei Q. 2015. Blue light is more essential than red light for maintaining the activities of photosystem II and I and photosynthetic electron transport capacity in cucumber leaves. *Journal of Integrative Agriculture*. 10: 2095-3119.

Yeh N, Chung JP. 2009. High-brightness LEDs - energy efficient lighting sources and their potential in indoor plant cultivation. *Journal of Renewed Sustainable Energy Revelation.* 13: 2175-2180.

Xiao FG, Shen L, Ji HF. 2011. On photoprotective mechanisms of carotenoids in light harvesting complex. Biochemical and Biophysical Research Communications. 414: 1-4.

Xue L, Lu W, Guyue H, Xiao CW, Yu Z, Guo XS, Zhichao F. 2016. Effects of light emitting diode on the winter growth of greenhouse plants in the Yangtze River Delta of China. *Botanical Studies.* 57(2): 1-8.

Zhen S, van Iersel MW. 2017. Far-red light is needed for the efficient photochemistry and photosynthesis. *Journal of Plant Physiology*. 209: 115-122.

Chapter 5

Conclusions and Recommendations

5.1 Conclusions

Dark red pigmented lettuce had higher RC/ABS values than green lettuce. This correlates with the findings of Armstrong and Hearst (1996), who found that carotenoids absorb blue light and protect chlorophyll against photodamage. The highest RC/ABS values for lettuce seedlings were measured under the blue nets, and simultaneously B+DR LEDs had higher values than the B+FR LEDs.

The RC/ABS of the lettuce seedlings also decreased with plant age, and was probably due to the limited space as the seedlings grew, which constrained the leaves from fully absorbing solar radiation. Light quantity is influential in the conversion of excitation energy to ET. Therefore, the reduction of end electron acceptors is reduced by high light quantity - resulting in a reduced ET rate. The chlorophyll fluorescence parameters showed similarities for the lettuce and cabbage seedlings with regard to these parameters. The chlorophyll fluorescence parameters of mature lettuce were mostly influenced by plant age. The PI_{total} values decreased in physiologically matured plants - especially after head formation which led to reduced photosynthesis.

The phosphorus (P) uptake for lettuce was the only element that was significantly influenced under different coloured shade nets. The uptake of P was nearly identical for the 2 lettuce cultivars under the blue nets, while it differed significantly under Photon Red and white nets. The flatter chlorophyll fluorescence OJIP transient curves per lettuce cultivar and net colour corresponded to the lower P uptake, and vice versa. Thus, the OJIP transient curves are indicative of P values in lettuce leaves. The uptake of N, P, and K did not differ between the cabbage cultivars per specific net colour; however, the averaged uptake of both cultivars differed significantly between the net colours. The uptake of N, P and K was the highest under the black net and the lowest under the Photon Red and white nets. The different uptakes concur with the findings of Shahak (2008) where black nets reduce light quantity and had no influence on light quality, while red and pearl coloured nets spectrally modify light - with pearl nets being the most effective (Shahak *et al.*, 2004).

The blue nets stimulated a higher N, P, and K uptake and less variance between the N:P:K ratio, than under the Photon Red and white nets. Blue nets increase the B:R ratio (Shahak *et al.*, 2004), and cryptochrome 2 (cry 2) is reduced with an increase in blue light (Casa, 2000). Therefore, it can be concluded that cry 2 influences the uptake and ratio of N:P:K in cabbage under blue nets.

The black nets produced the shortest cabbage leaf length and width, while the white nets produced the longest and widest leaves. Photon Red nets had the largest variance between leaf length and width, while under blue and white nets the opposite was measured. This indicates that an increase in light scattering due to blue and white nets, increased cabbage leaf length and width, and the N and K uptake was the lowest under the white nets. The average mass for the 2 cabbage cultivars grown under a white net 8.16 kg, and was 86% higher than their counterparts grown under black nets. The higher cabbage mass cultivated under the white nets were because of higher reflectance associated with white nets. This increase in light had a positive effect on PI_{total} values resulting in a higher photosynthetic rate. This new discovery can help producers increase food production globally in high solar radiation areas. Blue nets will produce less tonnes per ha than white nets - but with a more uniform ratio of N:P:K nutrient uptake. This can potentially lead to a higher nutritional value, but needs to be validated.

The lettuce grown under B+FR LEDs had a higher $\delta/(1-\delta)$ value than under the B+DR LEDs. This is because FR is preferentially absorbed by PSI (Zhen and van Iersel, 2017) - thus increasing the rate of e^- being accepted at PSI. The afore-mentioned fact is corroborated where the Photon Red nets had a lower $\delta/(1-\delta)$ value than the blue net, and this is due to the increased R:FR ratio under red nets (Ilić and Fallik, 2017) and possibly under Photon Red nets but needs to be validated. The PI_{total} value for Soltero was higher under the B+FR LEDs than under the B+DR LEDs. This shows than PI_{total} values can be increased by far red light, especially in lighter coloured lettuce.

All the macro- and micro -element leaf values were significantly higher under the low radiation B+DR LEDs than any of the coloured nets under high solar radiation. Our findings differ from previous studies where some macro- and micro-elements were

higher under B+R and B+DR LEDs, when compared to other artificial lighting systems. The reason for the significantly higher macro- and micro-element values in our study relative to the other studies could be due to the 1:1 ratio of B:DR LEDs or the lower radiation used. The uptake of Cu followed by Zn and Na was influenced the most by the B+DR LEDs. This concur with Chen *et al.* (2014), where Cu and Na uptake was significantly increased in lettuce when LEDs were used with a light spectrum of 450 nm and 660 nm, and indicates that Cu and Na uptake is wavelength colour specific.

5.2 Recommendations

The chlorophyll fluorescence parameters for the lettuce and cabbage seedlings decreased per week. This decrease correlates with the seedlings which showed signs of competition for space, and coincides with the period when the slow release fertiliser was released. Further research under shade nets using chlorophyll fluorescence parameters can be used to determine the optimal space required for lettuce and cabbage seedling development – whilst measuring the actual nutrient concentrations in the root zone and trialling different slow-release fertilisers over time.

Blue light is 20 times more effective in regulating stomatal opening than red light (Sharkey and Raschke, 1981; Shimazaki *et al.*, 2007). It is clear from this study that the uptake ratio of N:P:K varied the least under the blue nets, while the opposite was true for the uptake under the Photon Red nets. Therefore, it is recommended to investigate different N, P, K uptake values per stomatal activity, net colour and the influence of different R:B and R:FR ratios, under nets illuminated by high solar radiation. Further studies are necessary to determine whether different hues of blue colour net and/or the shade net % affect the efficiency of chlorophyll a and/or b regarding N, P and K uptake.

In this study, a reversed correlation indicated a possible relationship between net colour and cabbage leaf length and width for N, P and K uptake. A study is needed to determine the different N, P and K uptake values in vegetables, and how the uptake is triggered through light scattering manipulation in conjunction with different ratios of B:R and R:FR under white, blue and red nets. A study is also needed to

determine whether positive macro- and micro-element uptake is possible, through specific ratio and colour LEDs in unfavourable pH ranges in different soilless substrates.

5.3   References

Armstrong GA, Hearst JE. 1996. Carotenoids 2: Genetics and molecular biology of carotenoid pigment biosynthesis. *Journal of the Federation of American Societies for Experimental Biology.* 10(2): 228.

Casal JJ. 2000. Phytochromes, Cryptochromes, Phototropins: Photoreceptor interactions in plants. *Journal of Photochemistry and Photobiology.* 71(1): 1-11.

Chen XL, Guo WZ, Xue XZ, Morewane MB. 2014. Effects of LED Spectrum Combinations on the Absorption of Mineral Elements of Hydroponic Lettuce. *Spectroscopy and Spectral Analysis.* 34(5): 1394-397.

Ilić ZS, Fallik E. 2017. Light quality manipulation improves vegetable quality at harvest and postharvest: A review. *Environmental and Experimental Botany.* 139: 79-90.

Shahak Y. 2008. Photo-selective netting for improved performance of horticultural crops: A review of ornamental and vegetable studies in Israel. *Acta Horticulturae.* 770: 161-168.

Shahak Y, Gussakovsky EE, Gal E, Ganelevin R. 2004. ColorNets: Crop Protection and Light-Quality Manipulation in One Technology. *Acta Horticulturae.* 659: 143-151.

Sharkey TD, Raschke K. 1981. Separation and measurement of direct and indirect effects of light on stomata. *Journal of Plant Physiology.* 68: 33-40.

Shimazaki K, Doi M, Assman SM, Kinoshita T. 2007. Light regulation of stomatal movement. *Annual Review of Plant Biology.* 58: 219-247.

Zhen S, van Iersel MW. 2017. Far-red light is needed for the efficient photochemistry and photosynthesis. *Journal of Plant Physiology.* 209: 115-122.