REPUBLIC OF TURKEY
ERCIYES UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF AGRICULTURAL SCIENCES AND TECHNOLOGIES

NITROGEN METABOLISM AND ACCUMULATION IN SPINACH (*Spinacia oleracea* L.) UNDER VARIANT IRRADIANCE AND TEMPERATURES

Prepared by:
Abdul-Quddus Bayuko ABUBAKAR

Supervised by:
Asst. Prof. Dr. Abdullah ULAŞ
Prof. Dr. Halit YETİŞİR

PhD Thesis

November 2022
KAYSERI

REPUBLIC OF TURKEY
ERCIYES UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF AGRICULTURAL SCIENCES AND TECHNOLOGIES

NITROGEN METABOLISM AND ACCUMULATION IN SPINACH (*Spinacia oleracea* L.) UNDER VARIANT IRRADIANCE AND TEMPERATURES

(PhD Thesis)

Prepared by:
Abdul-Quddus Bayuko ABUBAKAR

Supervised by:
Asst. Prof. Dr. Abdullah ULAŞ
Prof. Dr. Halit YETİŞİR

November 2022
KAYSERI

COMPLIANCE WITH SCIENTIFIC ETHICS

I declare that all information in this study has been obtained following academic and ethical rules. At the same time, as required by these rules and conduct, I declare that I have fully cited and referenced all materials and results that are not novel to this work.

Abdul-Quddus Bayuko ABUBAKAR

COMPLIANCE WITH GUIDE

This PhD thesis on the topic **"Nitrogen Metabolism and Accumulation in Spinach (*Spinacia oleracea* L.) under Variant Irradiance and Temperatures"** has been prepared per Erciyes University's Graduate Thesis Proposal and Preparation Directive.

Prepared by:
Abdul-Quddus Bayuko ABUBAKAR

Supervised by:
Asst. Prof. Dr. Abdullah ULAŞ

Prof. Dr. Halit YETİŞİR

Head of Department

Assoc. Prof. Dr. Özhan ŞİMŞEK

ACKNOWLEDGEMENT

To Allah Almighty be thanks and glory for His mercies, guidance, protection and sustenance during my study period. My profound gratitude goes to my supervisors: Asst. Prof. Dr. Abdullah ULAŞ and Prof. Dr. Halit YETIŞIR for their guidance, contributions and constructive criticisms that led to the success of this work. Not forgetting Yusuf Cem YÜCEL, Abdul Aziz Karim, Mohammed Bello ADAM Fatima KARATAŞ, Haruna MUBARAK, Fatima TANVEER MIANO, Ayşe DÖŞEYİCİ; who supported me during my laboratory works. My heartfelt gratitude goes to my brothers, Osman, Mubarik and Musah for their selfless support and love that kept me moving without fear. Last but not the least, I would like to sincerely acknowledge Seyyid Burhaneddin Hizmet vakfi, Nihat KÜÇÜKTAŞ and all those whose names could not be mentioned financial support which made my stay and study in Turkey a successful one. Finally, I wish to thank all my friends, the Ghanaian students in Kayseri, especially M.D. Tahiru A. KANTOSHI, Osman IDDRIS, Ali Ustaz ADAM and all those who aided me in one way or the other throughout my study. May the Almighty Allah bless you all.

Abdul-Quddus Bayuko ABUBAKAR

November 2022, KAYSERI

NITROGEN METABOLISM AND ACCUMULATION IN SPINACH (*Spinacia oleracea* L.) UNDER VARIANT IRRADIANCE AND TEMPERATURES

Abdul-Quddus Bayuko ABUBAKAR

Erciyes University, Graduate School of Natural and Applied Sciences
PhD Thesis, November 2022
Supervisors: Asst. Prof. Dr. Abdullah ULAŞ
Prof. Dr. Halit YETİŞİR

ABSTRACT

The eminent threat of climate change, urbanisation, land pollution, the ever-increasing population, and the resultant rise in demand for fresh crop produce all year round has led to crop cultivation in enclosed environments. However, the nutritional value and quality of the crops produced under controlled environments could be compromised if the ideal environmental conditions are not provided. This study aimed to investigate how different light-temperature combinations influence nitrogen, especially nitrate accumulation and metabolism in spinach. Spinach plants were grown under optimum light and optimum temperature (OLOT), low light and optimum temperature (LLOT), optimum light and low temperature (OLLT), low light and low temperature (LLLT) conditions and treated with two nitrogen (N) doses (low-N 0.3mM, high-N 3mM). Results showed that significant differences existed among spinach varieties in their response to the various light-temperature combinations and N treatments. The average biomass production and total N uptake of spinach plants grown under the various conditions were higher in OLOT > LLOT > OLLT > LLLT in descending order, irrespective of variety and N dose. Nitrate content was averagely higher in plants grown under low light conditions regardless of variety and temperature. The findings indicate that growing spinach under low light intensities results in the accumulation of higher amounts of nitrates even if temperatures are kept high. The findings also revealed that low temperatures cause retarded growth but have less effect on nitrate accumulation provided light intensity is optimum. Further research is recommended to identify the ideal light-temperature combinations for spinach production in controlled environments.

Keywords: Environmental stress, Light intensity, Temperature, Nitrate accumulation, *Spinacia oleracea*, Hydroponics

ISPANAK'TA (*Spinacia oleracea* L.) DEĞİŞKEN IŞINMA VE SICAKLIKLAR ALTINDA AZOT METABOLİZMASI VE BİRİKİMİ

Abdul-Quddus Bayuko ABUBAKAR

Erciyes Üniversitesi, Fen Bilimleri Enstitüsü
Doktora Tezi, Kasım 2022
Danışmanlar: Dr. Öğr. Üyesi Abdullah ULAŞ
Prof. Dr. Halit YETİŞİR

ÖZET

İklim değişikliği, kentleşme, toprak kirliliği ve sürekli artan nüfus tehdidi ile tüm yıl boyunca taze ürün talebindeki artış, kapalı ortamlarda mahsul ekimine yol açmıştır. Ancak, ideal çevre koşulları sağlanmadığında kontrollü ortamlarda üretilen mahsullerin besin değeri ve kalitesi tehlikeye girebilir. Bu çalışma, farklı sıcaklık ve ışık kombinasyonlarının ıspanakta nitrojeni, özellikle nitrat birikimini ve metabolizmasını nasıl etkilediğini araştırmayı amaçlamıştır. Ispanak bitkileri, optimum ışık ve optimum sıcaklık (OLOT), düşük ışık ve optimum sıcaklık (LLOT), optimum ışık ve düşük sıcaklık (OLLT), düşük ışık ve düşük sıcaklık (LLLT) koşullarında büyütülmüş ve iki azot (N) dozu ile muamele edilmiştir. (düşük-N 0.3mM, yüksek-N 3mM). Sonuçlar, çeşitli ışık-sıcaklık kombinasyonlarına ve N uygulamalarına tepkilerinde ıspanak çeşitleri arasında önemli farklılıklar olduğunu göstermiştir. Çeşitli koşullar altında yetiştirilen ıspanak bitkilerinin ortalama biyokütle üretimi ve toplam N alımı, çeşit ve N dozundan bağımsız olarak azalan sırada OLOT > LLOT > OLLT > LLLT'de daha yüksekti. Nitrat içeriği, çeşit ve sıcaklıktan bağımsız olarak düşük ışık koşullarında yetiştirilen bitkilerde ortalama olarak daha yüksekti. Bulgular, düşük ışık yoğunluklarında ıspanak yetiştirmenin, sıcaklıklar yüksek tutulsa bile daha yüksek miktarlarda nitrat birikmesine neden olduğunu gösteriyor. Bulgular ayrıca düşük sıcaklıkların büyümeyi geciktirdiğini, ancak ışık yoğunluğunun optimum olması koşuluyla nitrat birikimi üzerinde daha az etkiye sahip olduğunu ortaya koydu. Kontrollü ortamlarda ıspanak üretimi için ideal ışık-sıcaklık kombinasyonlarını belirlemek için daha fazla araştırma yapılması önerilir.

Anahtar Kelimeler: Çevresel stres, Işık yoğunluğu, Sıcaklık, Nitrat birikimi, *Spinacia oleracea,* su kültürü

TABLE OF CONTENTS

NITROGEN METABOLISM AND ACCUMULATION IN SPINACH (*Spinacia oleracea* L.) UNDER VARIANT IRRADIANCE AND TEMPERATURES

COMPLIANCE WITH SCIENTIFIC ETHICS ii
COMPLIANCE WITH GUIDE iii
ACCEPTANCE AND APPROVAL iv
ACKNOWLEDGEMENT v
ABSTRACT vi
ÖZET vii
TABLE OF CONTENTS viii
ABBREVIATIONS xi
LIST OF TABLES xii
LIST OF FIGURES xiii
INTRODUCTION 1

CHAPTER ONE

LITERATURE REVIEW

1.1 Spinach Production and Importance 6
1.2 Environmental Effects on Spinach Growth 7
1.2.1 Light Effect on Spinach Growth and Development 8
1.2.2 Light Effect on Spinach Nitrogen Uptake 9
1.2.3 Light Effect on Nitrogen Metabolism and Nitrate Accumulation 10
1.2.4 Temperature effect on Spinach Growth and Development 11
1.2.5 Temperature Effect on N Uptake and Metabolism 13
1.2.6 Genotypic Difference in Spinach 13

CHAPTER TWO

MATERIALS AND METHODS

2.1. Experimental Design and Treatments 15
2.2 Plant Materials 16
2.3. Experimental Setups 17
2.4 The Nutrient Solution 19

2.4. Data Collection....20
2.4.2. Biomass (Fresh and Dry)....20
2.4.3. Leaf Number and Total Leaf Area....20
2.4.4. Root Dry Weight....21
2.4.5. Root Length....21
2.4.6. Nitrate Reductase Enzyme Activity....21
2.4.7. Leaf Chlorophyll....23
2.4.8. Photosynthesis....24
2.4.9. Nitrate Content Measurement....24
2.4.10. Total Nitrogen Determination....25
2.5. Data Analysis....26

CHAPTER THREE

RESULTS

3.1. Experiment One (Screening Experiment) Results....27
3.1.1. Shoot Fresh Weight, Leaf Area and Leaf Number....27
3.1.2. Shoot and Root Dry Weights and Root-Shoot Ratio....28
Shoot Dry Weight....28
Root Dry Weight....29
Root-Shoot Ratio....29
3.1.3. Root Length of spinach plants as affected by different N doses....31
3.1.4. Nitrogen Concentration, Total N-Uptake and Nitrate Concentration....32
Nitrogen Concentration....32
N-Uptake....32
Nitrate concentration....32
3.1.5. Nitrate Reductase Enzyme Activity Rate....33
3.1.6. Total Chlorophyll and Net Photosynthetic Rate....34
3.1.7. Conclusion....36
3.2. Results of LLOT, OLLT and LLLT Experiments. 37

3.2.1. Shoot Fresh weight 37
3.2.2. Leaf Area 39
3.2.3. Leaf Number 41
3.2.4. Shoot Dry Weight 43
3.2.5. Root Dry Weight 45
3.2.6. Root: Shoot Ratio 46
3.2.7. Root Length 48
3.2.8. Nitrogen Concentration 49
3.2.9. Nitrogen Uptake 51
3.2.10. Nitrate concentration 53
3.2.11. Nitrate Reductase Enzyme Activity 55
3.2.12. Chlorophyll Concentration 57
3.2.13. Net Photosynthetic Rate 57
3.2.14. Correlations 60

CHAPTER FOUR

DISCUSSION

4.1 Temperature Effect on Spinach Biomass Production 65
4.2 Light Effect on Spinach Biomass Production 67
4.3 Temperature and Light Effect on N Uptake and N Concentration 69
4.4 Temperature and Light Effect on Nitrate Content of Spinach 70
4.5 Genotypic Difference in Biomass Production and Nitrate Accumulation 72
4.6 Conclusions and Recommendations 74

REFERENCES 76
LIST OF APPENDIXES 86
Appendix 1: ANOVA Tables 86
CURRICULUM VITAE 96

ABBREVIATIONS

ATP :Adenosin triphosphate

Chl_{a+b} : Total chlorophyl

CRD : Complete randomised design

DW : Dry weight (shoot)

H_2SO_4 : Sulphuric acid

HN : High nitrogen

K : Potassium

KNO_3 : Potassium nitrate

LLLT : Low light low temperature

LLOT : Low light optimum temperature

LN : Low nitrogen

LSD : Least significant difference

Mg : Magnesium

N : Nitrogen

NaOH : Sodium hidroxide

NEED : N-(1-naptyl) ethylenediamine

NO_2^- : Nitrite

NO_3^- : Nitrate

NRA : Nitrate reductase activity

NUE : Nitrogen use efficiency

NUpE : Nitrogen uptake efficiency

OD : Optical density

OLLT : Optimum light low temperature

OLOT : Optimum light and temperature

RDW : Root dry weight

SA-H_2SO_4: Sulphosalicylic acid

LIST OF TABLES

Table 2.1: Experimental layout 16

Table 2.2. Plant materials 17

Table 2.3: Experimental Setup 19

Table 2.4: Composition of nutrient solution 20

Table 3.1: Shoot Fresh Weight, Leaf Area and Leaf Number 28

Table 3.2: Nitrogen Concentration, Total N-Uptake and Nitrate Concentration 33

Table 3.3. OLOT Experiment Results Correlation Table. 61

Table 3.4. LLOT Experiment Results Correlation Table. 62

Table 3.5. OLLT Experiment Results Correlation Table. 63

Table 3.6. LLLT Experiment Results Correlation Table. 64

LIST OF FIGURES

Figure 1.1: Components of environmental stress and stress response by plants 3
Figure 2.1: Spinach seedling nursery and transplanting onto nutrient solution.....16
Figure 2.2: Nitrite standard curve 23
Figure 2.3: Nitrate standard curve.....25
Figure 3.1: Shoot dry weight.....29
Figure 3.2: Root dry weight 30
Figure 3.3: Root-shoot ratios.....30
Figure 3.4: Root length 31
Figure 3.5: Leaf nitrate reductase activity rate.....34
Figure 3.6: Total chlorophyll content.....35
Figure 3.7: Photosynthetic rate 35
Figure 3.8: Shoot fresh weight.....38
Figure 3.9: Leaf area 40
Figure 3.10: Leaf number.....42
Figure 3.17: Shoot dry weight.....44
Figure 3.12: Root dry weight 46
Figure 3.13: Root-shoot ratios 47
Figure 3.14: Root length 49
Figure 3.15: Nitrogen concentration 50
Figure 3.16: Nitrogen uptake 52
Figure 3.17: Nitrate concentration 54
Figure 3.18: Leaf nitrate reductase activity rate.....56
Figure 3.19: Total chlorophyll content.....58
Figure 3.20: Photosynthetic rate 59

INTRODUCTION

Under variant environmental conditions, all living organisms (in this case plants) adopt different mechanisms to survive. These mechanisms could be a modification in their phenotypic/morphologic and physiologic characteristics. These modifications influence how plants react/respond to various biotic (weeds, insects, pathogens, etc.) and abiotic environmental factors (temperature, light, moisture, etc.), which in turn have an impact on plant growth, resource use efficiency, yield, and quality, though this may vary between plants species or among varieties of same species.

Biotic stress-causing agents induce stress on their host by infesting it with diseases and depriving it of its nutrients, which sometimes leads to the death of the plant. Many insect species e.g. chewing insects destroy the leaves of plants and thereby reducing their photosynthetic capacity and may lead to significant production losses (Verma et al., 2013) if left uncontrolled. Abiotic stress-causing agents such as moisture stress (drought or flood), extreme temperatures (cold, frost, and heat), salinity, and fertility (deficiency or toxicity) dictate the physical growing environment of the plant and are key determinants to growth, development, yield, and quality of plants (Gull et al., 2019).

Plants are immobile by nature and thus have no option but to respond to various environmental conditions (Gull et al., 2019) by modifying their internal and external structures to withstand them or otherwise will simply succumb! These environmental

factors are collectively termed “stress”. Stress is a term described as any external force or factor that has a negative impact on a plant (Taiz & Zeiger, 2002), adversely affecting its growth, development and/or productivity (Gull et al., 2019). Plant stress is generally triggered by abrupt changes in the environment (Verma et al., 2013), causing a wide range of plant responses, including changes in gene expression, cellular metabolism, growth rates, crop output, etc. (Gull et al., 2019).

It must however be noted that an environment under which one plant may be stressful may not necessarily be a stressful one for another plant. Many plants have the ability to withstand certain stress and are thus classified as stress-resistant or tolerant. Stress resistance is the ability of the organism to adapt or acclimatise to stress (Hopkins & Huner, 2008). Adaptation or acclimatisation to stress is the product of coordinated processes happening at all organisational levels of the plant, from the anatomical and morphological to the cellular, biochemical, and molecular (Taiz & Zeiger, 2002).

In this present study, the focus will be on the abiotic stress factors; particularly, how variant temperatures and light intensities affect spinach growth, development, nutrient accumulation (especially nitrate), especially under controlled growth environments (greenhouses). Vegetable production under enclosed environments is increasingly being adopted globally due to unpredictable climatic conditions, reduced land area for cultivation, intensive and longer cropping times on field conditions, all year-round demand for fresh produce, higher yields, and quality. Production in greenhouse has several advantages over outdoor production in terms of quality, as the products are not prone to sudden environmental changes. There is also a high market value for greenhouse produce due to their ‘nice’ visual quality than field produce and hence a reason for the huge investments into building greenhouses (Bot, 2004). Initially, greenhouses were

mostly used to provide a near-perfect growing conditions for exotic plants, especially during the cold seasons, and were later used in the production of vegetables when environmental conditions are suboptimal (Gruda, 2005).

It must, however, be mentioned that the nutritional value/quality of the vegetables produced under controlled environments may be compromised with the reason being that it is very difficult, if not impossible, to create a near-natural environment of light and heating, especially in a large-scale production. In many greenhouse conditions, somehow due to improper construction or maintenance, either temperature, light, or humidity is poorly maintained (either low or high). Under such poor conditions, the plant's morphological and physiological development is affected.

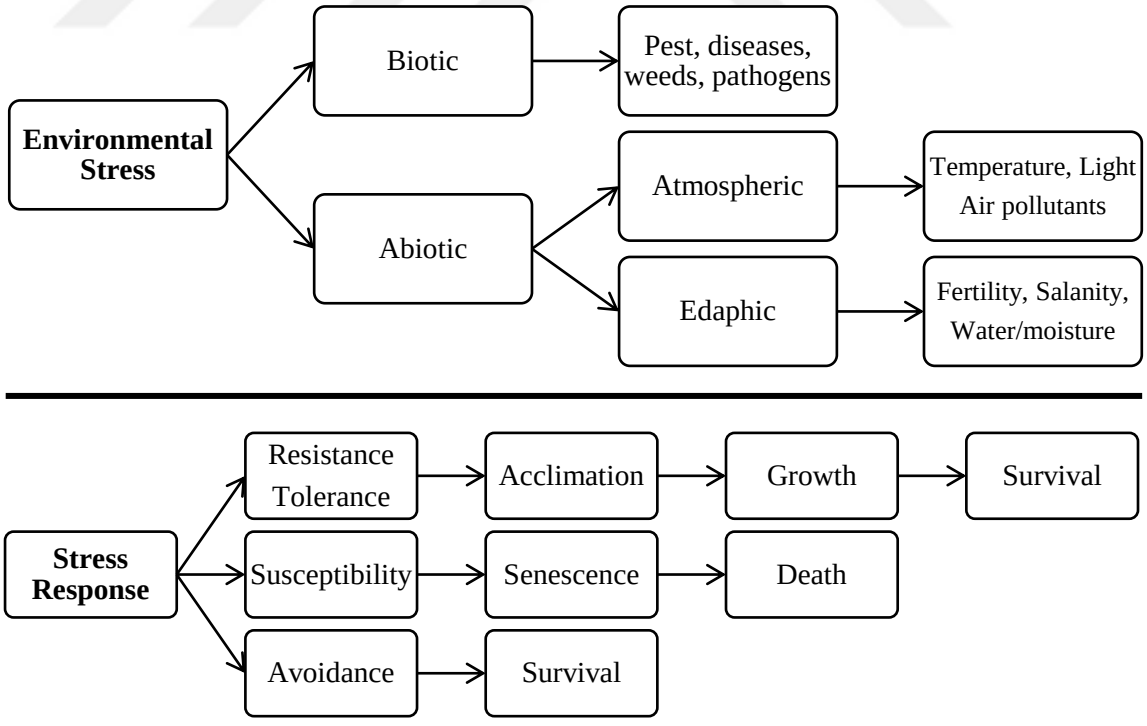


Figure 1.1: Components of environmental stress and stress response by plants

Many researchers have reported on the impact of suboptimal environmental conditions on vegetable crops (Cantliffe, 1973; Corré & Breimer, 1979; Boese & Huner, 1990; Nieuwhof, 1994; Anjana et al., 2007; Lillo, 2008; Boczulak et al., 2014; Zhou et al., 2019; Xu et al., 2021). Beevers & Hageman (1969) reported the influence of light and temperature on plant's nitrate uptake, nitrate translocation, and the activity of nitrate assimilating enzymes. Under poor lightening conditions and low temperatures, such as in winter, various leafy vegetables accumulate high amounts of nitrate in their shoots (Corré & Breimer, 1979) if the nitrate supply is unlimited (Steingröver et al., 1982).

Spinach is one of the most important and nutritious vegetables eaten raw or cooked and has attracted and continues to attract lots of interest day-in-day out in human nutrition because of its excellent profile of vitamins, minerals, antioxidants and therapeutic effects. However, just like other leafy vegetables, spinach accumulates lots of nitrogen in the form of nitrate (NO_3^-), particularly with the supply of large amounts of NO_3^- and under poor environmental conditions. Spinach, a cool-season crop accumulates nitrates in its leaves when light conditions are poor and temperature is low (Corré & Breimer, 1979) in the presence of excess NO_3^- in the growth medium.

However, with there are many known adverse health effects that come with ingesting products (such as vegetables) or drinking water with high amounts of nitrates. In the digestive tract, NO_3^- is converted to nitrite (NO_2^-), which oxidizes haemoglobin, forming a substance called methaemoglobin. This is responsible for a potentially lethal disorder called methemoglobinemia, also known as "blue baby syndrome". There has been concerns that over-fertilization with NO_3^- fertilisers may be a major cause of the disorder. In addition, NO_2^- may lead to the formation of nitrosamines, which are carcinogenic (Ryder, 1979).

One possible solution to these health risks that may arise from consuming spinach with high nitrate contents is to produce them in ideal conditions where optimum yields could be obtained but also with low nitrate contents. It is thus necessary to investigate and identify the potential factors that may be directly or indirectly influencing nitrate accumulation in spinach.

With this in mind, we setup a series of hydroponic experiments in a controlled growth chamber with the aim of investigating how different light-temperature combinations influence nitrogen metabolism and nitrate accumulation in spinach. The study also sought to examine the effect of genotype on nitrate accumulation in spinach. And to make recommendations on what conditions are most appropriate for spinach productions as well as which varieties are better adapted to what conditions.

CHAPTER ONE

LITERATURE REVIEW

1.1. Spinach Production and Importance

Spinach (*Spinacia oleracea* L.) is a member of the *Chenopodiaceae* (Goosefoot family). Spinach is a low growing fleshly leaf annual that forms a heavy rosette of broad, crinkly tender leaves. Spinach cultivation began in Persia, modern-day Iran, around 400 AD. Spinach was introduced to Spain in 1100 AD by the Arabs and by 1400 AD it had spread to across Europe and later to the Americas in the 19th century (Ryder, 1979).

Spinach is a cool climate crop and can germinate at minimum temperature of 2°C minimum and a maximum of 30°C though the optimum range is between 7 to 24°C. Young plants can withstand temperatures as low as -9 °C though their growth rate would be significantly retarded. Best crop growth occurs at 15 to 20°C with a minimum temperature of 10°C and a maximum of 32°C (Ryder, 1979).

Data at FAOSTAT indicates that the total volume of spinach production has increased from 38 million tonnes in 2010 to 59.5 million tonnes in 2020 (56.58% increase). China, USA, Kenya, Turkey, and Japan, in descending order, are the first five major producers for the 2020 data year (FAOSTAT, 2020).

The increase in global production is mainly due to increasing awareness of the numerous health benefits of consuming spinach. Spinach has an excellent profile of vitamins, minerals, and antioxidants. It is one of the most important and nutritious vegetables eaten raw or cooked. It provides a very good amount of vitamin B6, riboflavin, folate, niacin, soluble dietary fibre, omega 3-fatty acid and minerals.

Spinach has many therapeutic uses in addition to its food value. One of the important minerals in spinach is iron; which in the body has many important biochemical roles, including oxygen binding in haemoglobin (Geissler & Powers, 2009) and thus prevents from some diseases such as anaemia and osteoporosis resulting from iron deficiency (Oulai et al., 2014). Spinach can be used for; the treatment of gastrointestinal disorders, blood generating, growth stimulation in children, appetite stimulation and convalescent support (Miano, 2016). Spinach is rich in carotenoid lutein, which plays an important role in reducing age-related vision loss resulting from macular degeneration and cataracts (Miano, 2016).

1.2. Environmental Effects on Spinach Growth

With the increasing all year-round demand for fresh vegetables such as spinach, farmers are resorting to cultivating in the greenhouse where the environment can be maintained to a near optimum for the growth and development of a crop in question. Greenhouses allow for vegetable production outside of the normal growing season. While the producer may benefit economically, the product quality can be less desirable (Gruda, 2005). This is because, it is very difficult, if not impossible, to create a near natural environment using artificial means, either due to inadequate knowledge, improper construction, or lack of proper maintenance. In effect, the quantity and quality of crops produced is reduced.

1.2.1. Light Effect on Spinach Growth and Development

One of the main requirements for healthy growth and development of plants is light. Plants are categorised in two groups: heliophytes (plants adapted to high light intensities) and sciophytes (plants adapted to low light intensities) (Sinha, 2004). In fact, the distribution of plants from north to south is to a greater extend limited by light intensity and duration. A high light intensity loving desert plant e.g., cowpea will only grow long vines and may not fruit at all if grown under shady conditions. The opposite can be said to be true for plants adapted to low light intensities such as lycopodium, which may not grow well under higher light exposures. Other plants can tolerate either condition but not without modifications in their morphological and physiological features. Linking this to cultivated plants, one can say that crop yield as well as quality will be significantly affected, if a crop is not cultivated under appropriate light conditions.

Plant growth, development and physiology is significantly affected by the quality of light (Ohashi-Kaneko et al., 2007). According to Hanyu & Shoji (2000), controlling light quality in controlled environmental conditions could improve crop yield and quality. Increase in growth and dry matter accumulation was achieved by supplemental natural light with artificial light (Grimstad, 1987; Hanyu & Shoji, 2000; Hikosaka et al., 2017). This increase in growth and dry matter accumulation according to Gruda (2005) is as a result of increased photosynthetic rates at optimal light intensities. Suboptimal yields of greenhouse vegetables are usually attributed to lower daily light intensities. At low light intensities, a plant's ability to photosynthesise is significantly weakened, and hence, the amount of dry matter they could accumulate is reduced (Yoneda & Suzuki, 1998). In spinach, elongation may occur under low light intensities resulting in less desired products (Hanyu & Shoji, 2002). Gaudreau et al. (1994) observed that supplemental light

resulted in a more than 270% increase in biomass production of lettuce grown under controlled conditions compared to natural light only. They also reported that the production circle was shortened by 30% with supplemental light compared to natural light.

1.2.2. Light Effect on Spinach Nitrogen Uptake

Light intensity does not only affect the rate of plant development but also affects nutrient uptake and metabolism. Light quality, intensity, and photoperiod are thought to affect plant nutrient uptake and use, with several research findings reporting on correlations between light availability and nutrient absorption (Sakuraba & Yanagisawa, 2018; Cui et al., 2019; Lin et al., 2020). Nutrient absorption is influenced by light in several ways, especially with light as the only energy source for photosynthesis (F. Wang et al., 2019). According to Lillo (2008), light signals control the absorption, transport, and assimilation of nitrogen into organic molecules. Investigating the nutritional content of lettuce grown during summer and spring under greenhouse conditions, Fallovo et al. (2009) reported an increase in nitrogen (N), potassium (K), and magnesium (Mg) uptakes under strong natural radiation. Their results also revealed that N, K, and Mg uptake in lettuce plants grown in summer was significantly increased by 4%, 20%, and 15%, respectively than in plants grown in spring. This is because, under low light intensities for example, the rate of gas exchange in many plants is reduced and as such, the demand as well as the energy required for the uptake, translocation and assimilation of nutrients is reduced. And when energy supply to the roots is low, nutrient absorption is generally reduced (Corre, 1983).

1.2.3. Light Effect on Nitrogen Metabolism and Nitrate Accumulation

Nitrogen metabolism is also partly affected by light intensity. Light intensity and nitrogen fertilisation are the main factors affecting nitrate concentration in vegetables (Cantliffe, 1973). Plant photosynthetic efficiency varies significantly with light intensity, resulting in a continuous change in carbohydrate (sugar) accumulation (Xu et al., 2021). Carbohydrates accumulation affects nitrate assimilation as the sugars and organic acids play a significant role in osmotic adjustment (Anjana et al., 2007)., However, the concentrations of nitrate, organic acids, and sugars change drastically with changing light intensities. Under high light intensities, there is enough production of sugars and organic acids to maintain osmotic pressure and electro-neutrality without the need for nitrates. On the other hand, due to the poor rate of photosynthesis at low light intensities, the synthesis of organic acids is reduced (Blom-zandstra & Lampe, 1985), resulting in less availability of organic compounds for storage in the vacuoles, hence, nitrate can be used as an alternative. To the plant, this would be more energy efficient as nitrate absorption, translocation, and storage in vacuoles requires less energy (ATP) than the synthesis of organic compounds (Lambers & Steingröver, 1978). This explains why nitrate content is usually high in vegetables cultivated under low light conditions.

According to Gruda (2005), crops such as lettuce and spinach, mostly accumulate nitrate when grown under low-light environments, such as in greenhouses and during winter conditions. The amount of nitrate in plants is reported to be inversely related to light intensity (Anjana et al., 2007). High light intensity is reported to have caused a reduction in nitrate accumulation (Blom-zandstra & Lampe, 1985; Ohashi-Kaneko et al., 2007; Van Der Boon et al., 1990). Growing lettuce under different light intensities but same nutrient

concentration, Fang, (2018) as cited in Xu et al. (2021) reported higher nitrate levels at low light intensity (≤250 μmol m^{-2} s^{-1}) than at high light intensity (≥250 μmol m^{-2} s^{-1}).

Light intensity and nitrate reductase enzyme activity in leaves are closely correlated. Nitrate reductase activity mostly follows a diurnal pattern where the activity is higher during light hours than dark hours leading to a reduction in nitrate concentrations during the light period. Nitrate concentrations may be many times higher in plants cultivated permanently under low light conditions (such as in greenhouses or during the winter) than in high light conditions (Marschner, 1995). Vegetables accumulate high amounts of nitrate in their shoots under poor lightening conditions (Corré & Breimer, 1979) especially if the nitrate supply is unlimited (Steingröver et al., 1982). This is especially clear in some plants in the *Chenopodiaceae* family such as spinach, which has a strong affinity for nitrate accumulation in its shoots as well as using nitrate in the vacuoles for osmoregulation. For these species, nitrate concentrations in the leaves can exceed 6000 mg/kg fresh weight, when grown under low light conditions (Wedler, 1980 as cited in Marschner, 1995).

1.2.4. Temperature effect on Spinach Growth and Development

One of the environmental factors that affects plant development is temperature. Plant growth, metabolism, reproduction, and distribution across regions is greatly influenced by temperature (Sinha, 2004). Plants neither like extreme higher nor lower temperatures as both conditions affect their development. The response of plants to various temperature extremes varies among plants species. While some plants may survive extreme temperatures such as chilling temperatures, others may not, as each plant has its unique set (upper and lower limits) of temperature requirements for growth and development, beyond which growth diminishes or stops. (Hopkins & Huner, 2008)

Vegetables are very sensitive to environmental fluctuations compared to field crops. The optimal temperature for appropriate growth and development in vegetables varies by species. Temperature variations in particular may result in abnormal growth and development of vegetables, ultimately reducing yield and quality (Pramanik et al., 2020). Vegetable growth under optimum temperatures and irradiation ranges is usually very effective and the opposite is true for vegetables grown during the cold season where temperatures are suboptimal couple with low irradiation (Gruda, 2005).

At temperatures below 10°C, many vegetables suffer chilling injury, which ultimately affects their growth, yield and product quality. The degree of injury/damage varies depending on whether the vegetable is a temperate or tropical. Temperate vegetables such as spinach usually could withstand minor/shorter freezing conditions. Tropical and subtropical vegetables are mostly susceptible to chilling and freezing injury, even with a small exposure to freezing temperatures (Pramanik et al., 2020). Spinach is well known for its adaptiveness to cooler temperatures. Even so, its growth and development is significantly retarded at temperatures below 10°C just as many other vegetables. Comparing the developmental ages of the second pair of spinach leaves, Boese & Huner (1990) reported that, it took 92 days for plants grown under 5°C to reach full leaf expansion compared to 32 days under 16°C. This is because of inhibition of cell division, enzyme deactivation, reduced water and mineral absorption, inhibition of photosynthesis etc at lower temperatures. (Sinha, 2004; Gruda, 2005; Zhou et al., 2019).

While low temperatures cause chilling and freezing injury, temperatures above 40°C also cause desiccation in many vegetables. Persistent high temperatures cause morpho-anatomical changes in vegetables, thereby affecting product quantity and quality (Pramanik et al., 2020). High temperatures also affect other processes such as flowering,

pollen viability, germination, etc. Vegetable growth and development is rapid under higher temperatures which results to improper organ development (Wheeler et al., 2000).

1.2.5. Temperature Effect on N Uptake and Metabolism

Temperature light and moisture are major environmental factors that influence nutrient uptake by plants. According to Anjana et al. (2007), environmental, physiological, and nutritional factors are the main factors responsible for nitrate accumulation in plants. Vegetables cultivated under high temperature conditions usually have higher nitrate contents (Corré & Breimer, 1979; Van Der Boon et al., 1990; Nieuwhof, 1995; Rouphael et al., 2012). This is due to reduced photosynthetic activity and increased respiration, forcing the plant to absorb much nitrate to compensate for the lack of organic sugars to maintain osmoticum. Also, at higher temperatures, nitrate reductase enzyme activity decreases resulting in nitrate accumulation (Corré & Breimer, 1979; Laurie & Stewart, 1993). Santamaria et al. (2001) observed an interaction between temperature, light intensity, and available nitrogen on nitrate accumulation in rocket salad (*Eruca sativa*). They observed that, under low light intensity, nitrate accumulation increased with increased temperature, whereas under high light intensity, the observed increase in nitrate content was mainly due to excess nitrogen supply. This suggests that, regulating nitrate supply while cultivating under optimum light intensities will help reduce the nitrate content of leafy vegetables.

1.2.6. Genotypic Difference in Spinach

Individuals of a same specie may differ from one another either morphologically or physiologically. The exhibited differences are expression of traits acquired over time through evolution, as they try to adapt the variant environmental conditions. The

expression of these traits also has an impact on the plant's growth, yield and products. Yield, taste, nutritional properties and quality etc. are affected and thereby making the plant's produces either desirable or less desirable. These variations may be among a same species or across species and is referred to as genotypic variation. According to Fageria (1989), plant genetic variability is the heritable trait of a crop species or variety that differs in growth or production when cultivated under the same conditions as other species or cultivars of the same species. It is crucial to identify potential sources of genetic diversity in order to equip breeders with the necessary alternatives to deal with changing environmental factors, production methods, mineral and phytonutrient levels, and other factors like the emergence of new pathogens or diseases (Prohens & Nuez, 2008). Genotypic differences in growth, dry matter partitioning and accumulation of various elements and compounds in vegetables in response to different growing environments and fertilisers have been reported (Barker et al. 1971; Huang et al. 2010; Tang et al. 2013; Wang et al. 2018). Genotypic differences in dry matter accumulation and nitrogen use efficiency (NUE) among spinach genotypes grown under same nitrogen conditions have been reported (Chan-Navarrete et al., 2014; Giordano et al., 2021; Stagnari et al., 2007).

On nitrate accumulation, many researchers have reported on genotypic differences in spinach (Barker et al., 1971; Maynard & Barker, 1979; Tang et al., 2013; Wang et al., 2018). These differences are due to the differential abilities in nutrient acquisition, translocation and assimilation of individual genotypes and their interaction with the environment (Baligar et al., 2001). This indicates that, different varieties are better suited for some environments than others (Baresel et al., 2008). Identifying which variety performs better under a certain condition than others is thus necessary to help mitigate the potential negative effects on growth, yield and quality while ensuring resource use efficiency.

CHAPTER TWO

MATERIALS AND METHODS

The study was conducted in the Plant Physiology Laboratory's controlled growth chamber of the Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Erciyes University between 2019 and 2021. With the study's aimed of investigating how different light-temperature combinations influence nitrogen metabolism, accumulation and yield in vegetables, it is prudent to eliminate as much as possible all potential factors that could affect the results other than temperature and light. Hence, a controlled growth chamber was the most ideal place to carry out the study.

2.1. Experimental Design and Treatments

A total of four separate experiments were conducted. For all the experiments of the study, treatments were laid out randomly in a Completely Randomised Design (CRD) with three replications as illustrated in Table 2.3. Seeds were germinated in peat media and hardened until the second leaf of fully developed (Figure 2.1), after which three seedlings of each variety were then transferred into an eight-litre plastic container with modified Hoagland's nutrient solution (see section 2.4) and grown for seven weeks (35 days). Photoperiod was maintained at 10 hours of light and 14 hours of darkness. Relative humidity of the growth chamber was 55%. Two nitrogen levels were applied: Low Nitrogen (LN) and High Nitrogen (HN) (Table 2.1).

Table 2.1: Experimental layout. V= Variety, R=Replication

High N	V_1R_1	V_2R_1	V_3R_1	V_4R_1	……	V_1R_2	V_2R_2	V_3R_2	V_4R_2	……	V_xR_3
Low N	V_1R_1	V_2R_1	V_3R_1	V_4R_1	……	V_1R_2	V_2R_2	V_3R_2	V_4R_2	……	V_xR_3

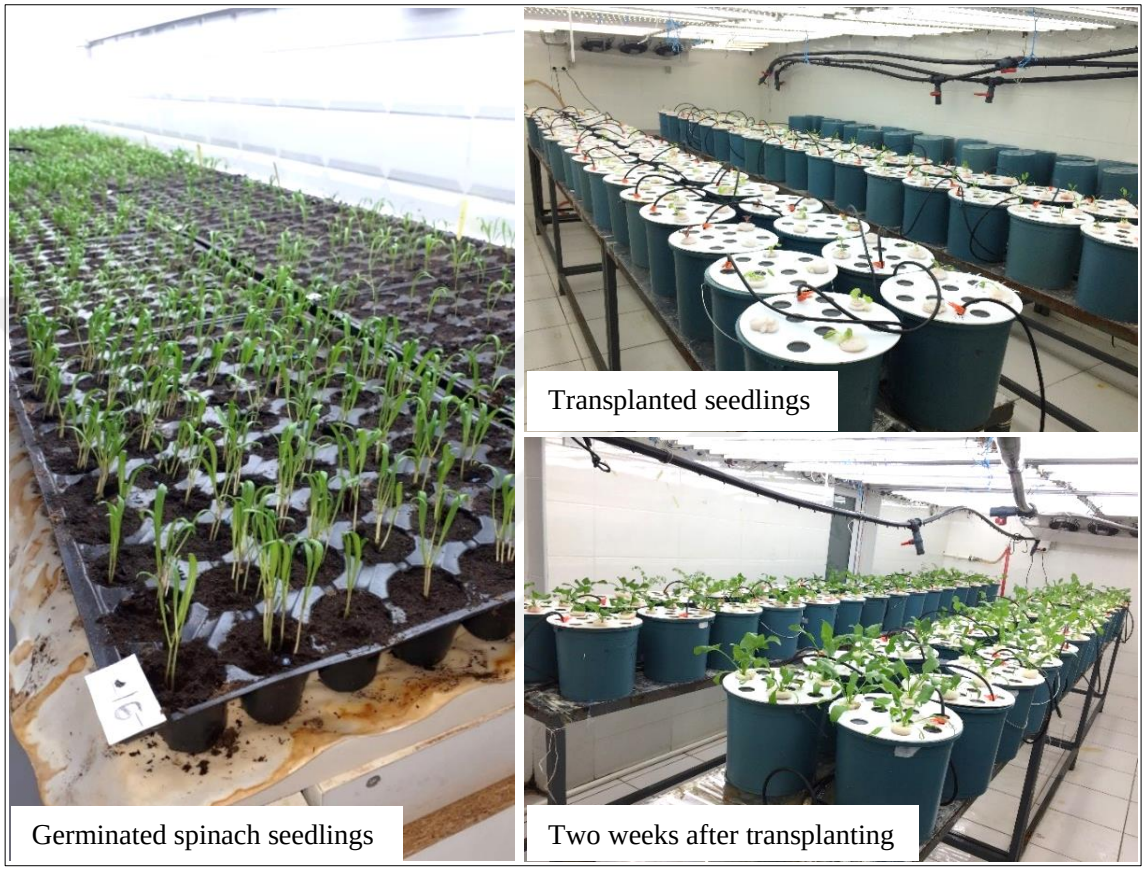


Figure 2.1: Spinach seedling nursery and transplanting onto nutrient solution

2.2 Plant Materials

The plant materials used were some commonly cultivated open-pollinated and hybrid spinach varieties (Table 2.2) in Turkiye, with no pre-information of their physiological and morphological responses to various biotic and abiotic stress conditions. Spinach was chosen as the experimental material for study because of its importance in human diet, short growth period, easy to grow under controlled environments alongside with its potential to accumulate higher amounts of nitrogen especially in NO_3^- nitrogen.

Table 2.2. Plant materials

No.	**Variety Name**	**Variety Characteristics**	**Company**
1	Aras F1	F1	May Tohum
2	Ayaz F1	F1	May Tohum
3	Efsun F1	F1	May Tohum
4	Elevator F1	F1	Westar
5	Eskişehir	Open pollinated	---
6	Hatay-1	Open-pollinated	---
7	Hatay-2	Open-pollinated	---
8	Hatay-3	Open-pollinated	---
9	Kayseri	Open-pollinated	---
10	Matador	Open-pollinated	Green Sohut
11	Nebraska F1	F1	Altın Tohum
12	Reis F1	F1	May Tohum
13	Samsun	Open-pollinated	---
14	Spinoza	Open-pollinated	Syngenta
15	Sprentir F1	F1	May Tohum

2.3. Experimental Setups

The study involves a total of four separate experimental setups as explained below and summarised in Table 2.3.

Experiment 1: Screening Spinach varieties under Optimum Conditions (OLOT)

Spinach varieties were screened under optimum conditions of 20°C and a 10-hour photoperiod at an intensity of 250 μmol m^{-2} s^{-1} and a 55% relative humidity. The aim here is to filter out the non-performing varieties and to select the efficient ones based on biomass production and other morphological parameters. The selected varieties from this screening experiment were used in the second, third and fourth experiments; to study the effect of variant irradiance and temperature on spinach growth, nitrogen metabolism, and accumulation. The results of this experiment would also serve as the control for this study.

2.3.2. Experiment 2: Low Light & Optimum Temperature (LLOT) Experiment

In this experiment, the selected varieties from OLOT experiment were grown under an optimum temperature of 20°C as in the screening experiment but with low light of 130 μmol m^{-2} s^{-1}. All other factors were the same. Our aim here is to investigate the effect of light intensity on spinach growth, nitrogen metabolism, and accumulation. This experiment was based on the assumption that, in greenhouse cultivation of vegetables, they may be problems with lightening which may, in turn, have an effect on the yield and quality of spinach produced.

2.3.3. Experiment 3: Optimum Light & Low Temperature Experiment (OLLT)

What if the greenhouse is poorly constructed that its temperature could not be effectively maintained at optimum levels even though lightening is optimal? With this in mind, we setup a trial, with the selected varieties from Experiment 1, to investigate the effect of low temperature but optimum light intensity on spinach growth and development, nitrogen metabolism, and nitrate accumulation. The experiment was done under a 10°C temperature and 250 μmol m^{-2} s^{-1} light intensity. All other factors were the same.

2.3.4. Experiment 4: Low Light Low Temperature Experiment (LLLT)

Here, the selected efficient varieties were grown under low temperature (10°C) and low light (130 μmol m^{-2} s^{-1}) while keeping all other factors the same as in previous experiments. The objective of this experiment is thus to induce an artificial cold season conditions and to investigate how low temperature and low light influence spinach growth and development, nitrogen metabolism and accumulation, and the genotypic effect under these conditions.

Table 2.3: A Summary of the Experimental Setups.

Experiments	Description	Treatments			
		Temperature	PPFD	Nitrogen	
Experiment 1	Screening under optimum light & temperature	20°C	250 μmol m^{-2} s^{-1}	0.3mM	3mM
Experiment 2	Low light & optimum temperature	20°C	130 μmol m^{-2} s^{-1}	0.3mM	3mM
Experiment 3	Optimum light & low temperature	10°C	250 μmol m^{-2} s^{-1}	0.3mM	3mM
Experiment 4	Low light & low temperature	10°C	130 μmol m^{-2} s^{-1}	0.3mM	3mM

2.4 The Nutrient Solution

The composition of the nutrient solution used for the experiment is shown in Table 2.3. Except for iron (EDDHA-Fe 6% Ortho-Ortho 4.8) from Compo Expert GmBH, all other chemicals used were from Merck KGaA EMSURE grades. Chemicals 1-9 were each prepared separately and later mixed together in a larger container to make a stock solution. $CaSO_4$ was weighed and applied directly before filling the pots; so as to prevent possible damage to roots from the use of deionised water. When necessary, $CaCO_3$ was applied as a buffer to stabilise the pH at around 7.0. Electric conductivity (EC) of the solution was maintained between 0.75 and 1.0. The solution was aerated with air pumps. Periodic monitoring was done to identify and correct any abnormalities. The nutrient solution was replaced once every week.

Table 2.4: Composition of nutrient solution

No.	Name		Molecular Formula	Molecular Weight	µM/L
1	Potassium Sulphate		K_2SO_4	174.26	750
2	Potassium dihydrogen phosphate		KH_2PO_4	136.08	500
3	Magnesium Sulphate		$MgSO_4$	246.49	650
4	Sodium Chloride		NaCl	58.44	50
5	Boric Acid		H_3BO_3	61.83	10
6	Manganese (II) sulphate Monohydrate		$MnSO_4$	169.02	0.5
7	Zinc Sulphate Heptahydrate		$ZnSO_4$	287.54	0.5
8	Copper (II) Sulphate		$CuSO_4$	249.68	0.4
9	Sodium molybdate Dihydrate		$MoNa_2O_4$	241.95	0.4
10	Ethylene diamine- N,N'-bis (2-hydroxy phenyl acetic acid) ferric-sodium complex (EDDHA-Fe O-O 4.8)		$C_{18}H_{16}N_2O_6FeNa$	435.19	80
11	Calcium Sulphate Dihydrate		$CaSO_4$	172.17	1000
High N		Calcium Nitrate Tetrahydrate	$Ca(NO_3)_2$	236.15	2250
		Ammonium Sulphate	$(NH4)_2SO_4$	132.14	750
Low N		Calcium Nitrate Tetrahydrate	$Ca(NO_3)_2$	236.15	225
		Ammonium Sulphate	$(NH4)_2SO_4$	132.14	75

2.4. Data Collection

2.4.2. Biomass (Fresh and Dry)

After seven weeks of growing, the plants were harvested and weighed to obtain the fresh weight (in grams). Samples were then dried in the oven at 70°C for 10 days and weighed to obtain the dry weight (in grams).

2.4.3. Leaf Number and Total Leaf Area

At harvesting, we counted the total number of fully developed spinach leaves per plant. We then measured the Leaf Area (LA) of the plants with LI-COR (LI 3100, model C) leaf area measuring device and recorded in square centimetres (cm^2).

2.4.4. Root Dry Weight

Roots were removed from the nutrient solution and rinsed with tap water and let to drain; after which they were weighed for fresh weight and then dried in the oven at 70°C for 10 days and weighed to obtain the dry weight (in grams).

2.4.5. Root Length

After roots were drained, they were weighed for fresh weight and after which one gram of fresh root sample was taken for measuring root length using WinRhizo (Regular LA2400 of Regent Instruments Inc.) image analysis program in combination with Epson Expression 11000XL scanner. The total root length was then calculated as the ratio of sampled root weight to the total fresh root weight.

2.4.6. Nitrate Reductase Enzyme Activity

Before harvesting, the nitrate reductase enzyme activity (NRA) was measured following Harley's protocol (Harley, 1993) as summarised below.

1. Chemical preparations
    a. Assay solution was prepared (on litre basis) by dissolving: 3.02 g KNO_3^- (30 mM), 2.18 g KH_2PO_4 (100 mM), 14.62 g K_2HPO_4 (100 mM) in 500 mL distilled water together with 5% v/v 2-propanol (50 mL) and topped up with distilled water to the one litre mark.
    b. Sulphanilamide solution (1% w/v) was prepared (on a litre basis) by dissolving 10 g sulphanilamide in 500 mL distilled water together with 125 mL 38% hydrochloric acid and topped up with distilled water to one litre mark.
    c. NEED solution (0.02% w/v) was prepared (on litre basis) by dissolving 200 mg N-(1-naphthyl)-ethylenediamine dihydrochloride in 1000 mL distilled water.

2. Two 50 mL sample tubes were labelled time-0 (T_0) and time-60 (T_{60}).
3. 200 mg of fresh leaf sample was taken from three different leaves and chopped into pieces and placed into each sample tube (T_0 and T_{60}). The tubes were covered with aluminium foil to prevent exposer to light.
4. 10 ml of assay solution was added to each to sample tube (T_0 and T_{60}).
5. The T_0 sample tubes were immediately (within five minutes) place into boiling water bath and boiled for 5 minutes. T_{60} sample tubes were kept in the dark for 60 minutes at room temperature; after which they were also boiled for 5 minutes. All samples were allowed to cool to room temperature.
6. After cooling 5 mL sulphanilamide solution and 5 mL NEED [N-(1-naphthyl)-ethylenediamine dihydrochloride] solution were added and shaken vigorously. Samples were left for 20 minutes for colour to develop.
7. After 20 minutes, the absorbance was measured in the spectrophotometer (UV/VS T80+ from PG Instruments Limited, UK) at 540nm wavelength.
8. The readings were converted to micromoles (μmol) nitrite using the standard curve equation (see step 10). The amount of NR activity was then computed as using the following equation.

$$\text{nmoles } NO_2^- h^{-1} g^{-1} FW = \frac{\text{nmoles } NO_2^- (T_{60}) - \text{nmoles } NO_2^- (T_0) \times 1000}{\text{weight of plant fresh sample (g)}}$$

9. Standard curve was constructed from nitrite standard prepared as follows:
    a. 85 mg potassium nitrite (KNO_2^-) dissolved in 100 mL distilled water (10 mM).
    b. 1mL of 10 mM NO_2^- diluted with distilled water to 400 mL to make 25 μmoles NO_2^- mL^{-1}.
    c. 0,1,2,3…10 mL (0,25,50,75…250 μmoles NO_2^- mL^{-1}) pipetted into labelled falcon tubes and topped up with distilled water to 10 mL mark.

d. Sulphanilamide and NEED solutions were added as in step 7 and measured with UV spectrophotometer as in step 8.
e. Spectrophotometric readings were used to construct standard curve (Figure 2.2).

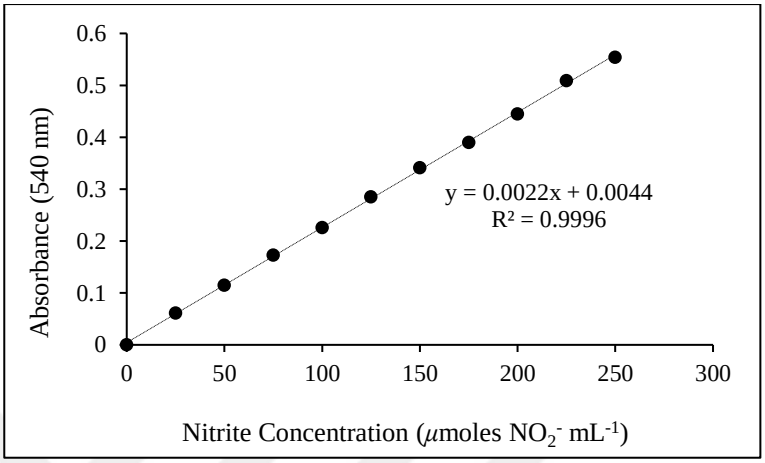


Figure 2.2: Nitrite standard curve

2.4.7. Leaf Chlorophyll

At harvest, 200 mg of fresh leaf samples was taken from three mature leaves of each replication and placed into 15 mL capped tubes. 10 mL of 95% ethylene alcohol (ethanol) was added and kept under darkness at room temperature for 24 hours. Chlorophyll concentration of the extracts were measured using UV/VS T80+ spectrophotometer (from PG Instruments Limited, UK) at absorption spectra of 664.2 nm and 648.6 nm. Chlorophyll (Chl_{a+b}) concentration (mg g^{-1}) was computed using Lichtenthaler (1987) equation for ethanol extract as below.

Chl_{a+b} = (5,24 x $OD_{664.2}$) + (22,24 x $OD_{648.6}$) [mg g^{-1}plant sample]

Note: *$OD_{664.2}$, $OD_{648.6}$ refers to spectrometric readings at wavelength 648.6 nm and 664.2 nm, respectively.*

2.4.8. Photosynthesis

We measured the photosynthetic activity of the plants using LI-COR (LI-6400XTP). One fully matured but not old leaf was randomly selected from a plant in each replication and measured. Repeated measurements were done at weekly intervals and the he average of all the weeks taken for data analysis.

2.4.9. Nitrate Content Measurement

A total nitrate was determined following the protocols of Cataldo et al. (1975) and Zhao & Wang (2017).

1. Chemical preparations
    a. Nitrate standard solution: 500 mg L^{-1} potassium nitrate (KNO_3) standard solution was prepared by dissolving 0.3609 g KNO_3 in 100 mL distilled water. 1, 2, 3, 5, 7, 9, 11 and 12 mL were then transferred into 50 mL falcon tubes and diluted with distilled water to mark to make 10, 20, 30, 50, 70, 90, 110 and 120 mg L^{-1} KNO_3 working standards.
    b. Salicylic-sulphuric acid solution (SA-H_2SO_4): 5% w/v sulfosalicylic acid solution was prepared by dissolving 5 g salicylic acid ($C_7H_6O_3$) in 100 mL 98% concentrated sulphuric acid (H_2SO_4).
    c. Sodium hydroxide solution: 8% (2 normality) sodium hydroxide (NaOH) solution was prepared by dissolving 40 g of NaOH in 500 mL distilled water.
2. 100 mg of dried and ground plant sample was suspended in 10 mL distilled water for one hour at 45 °C, shaken periodically, and filtered with Watman Filter paper.
3. 0.1 mL of filtrate was pipetted into 15 mL falcon tubes.
4. 0.4 mL SA-H_2SO_4 added, vortexed and allowed to rest for 20 minutes.
5. 9.5 mL NaOH solution added and allowed to cool to room temperature.
6. Samples were measured at 410 nm wavelength using spectrophotometer (UV/VS T80+ from PG Instruments Limited, UK).

7. Spectrophotometer readings were converted to milligram nitrate per kilogram of dry weight (*mg NO_3^- kg^{-1} DW*) using the standard curve equation (see step 8).

$$\text{Nitrates } (mg\, kg^{-1}\, DW) = \frac{(OD_{410} + A) \times V}{B \times W}$$

OD_{410} = spectrophotometric reading; V= volume of extract (mL)
A= slope intercept; B= slope; W= weight of sample (g)

8. Nitrate standard curve (Figure 2.3) was prepared same as steps 3 to 6 from the working KNO_3 standards prepared in step 1a.

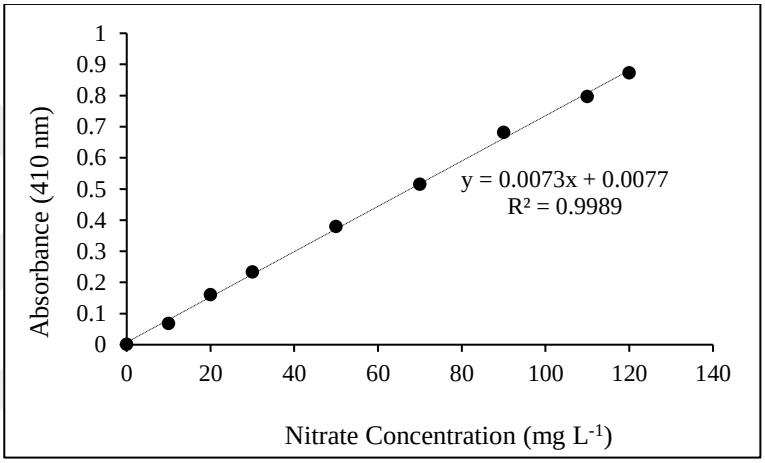


Figure 2.3: Nitrate standard curve

2.4.10. Total Nitrogen Determination

Total nitrogen was determined using the *Kjeldahl Total Nitrogen Determination Method* as described below with reference to Labconco Corporation (1998).

1. Sample preparation
    a. 200 mg of dried and ground plant sample was weighed into digestion tube.
    b. One Kjeldahl tablet and 10 mL of concentrated H_2SO_4 (98%) were added.
    c. Samples were digested for 4 hours at 400 ºC starting from 100 ºC and adjusted by 50 ºC every 30 minutes interval until the 400 ºC mark.
    d. Digested samples were let to cool to room temperature and diluted with 30 ml of distilled water.

e. Samples were distilled against 40 mL (40% w/v) NaOH for two minutes. Distillate was collected into conical flasks containing 10 mL boric acid-indicator solution.

f. Resulting content was titrated with 0.1 normality H_2SO_4.

g. Total N content was calculated as below.

- Nitrogen (%) = $\frac{\text{(Sample titre value – Blank titre value) x (Normality of acid x 1.4007)}}{\text{Weight of plant sample (g)}}$
- Nitrogen Concentration (mg g^{-1}) = N% x 10
- Nitrogen Uptake (mg $plant^{-1}$) = Concentration (mg/g) x plant dry weight (g)

2. Chemical preparations

a. 40% w/v (10 normality) of NaOH was prepared by dissolving 400 g NaOH in one litre distilled water and let to cool.

b. Boric acid - indicator solution was prepared by:

- Dissolving 20g of boric acid (H_3BO_3) in one litre distilled water (2% w/v).
- Dissolving 0.1 g each of Methyl red and Bromocresol green separately in 100 mL of 95% v/v ethanol and rapped with aluminium foil to keep away from light.
- Adding 7 mL and 10 mL of Methyl red and Bromocresol green, respectively, to the boric acid solution. The resultant solution's pH was adjusted to 5.5 by adding drops of 0.1 normality NaOH or H_2SO_4 to increase or decrease pH, respectively.

3. 0.1 normality H_2SO_4 solution (on litre bases) was prepared by adding 2.805 mL of98% concentrated H_2SO_4 into 500 mL distilled water and top up to one litre mark.

2.5. Data Analysis

Data was checked for outliers and tested for homogeneity within replications. Analysis of variance was done using Statistical Analysis Programme (SAS 9.0) at 5% significance level. Results are presented in tables and figures (graphs) where necessary and discussed accordingly.

CHAPTER THREE

RESULTS

3.1. Experiment One (Screening Experiment) Results

A total of 15 spinach genotypes were screened under optimal growth conditions for their responsiveness to variant nitrogen supplies (nitrogen use efficiency). The aim here was to identify and eliminate the non-performing varieties based on their biomass and morphologic parameters in response to nitrogen supply, and to use these best performing varieties for conducting the remaining experiments of the study. This was necessary to reduce the sample size and cost. Results from this screening experiment would also serve as the control experiment of the present study.

3.1.1. Effect of Nitrogen Dose on Shoot Fresh Weight, Leaf Area and Leaf Number

Results of the screening experiment reveals that irrespective of genotype, nitrogen dose had a significant effect on shoot fresh weight, total leaf area and leaf number (Table 3.1). Shoot fresh weight, total leaf area and leaf number increased by 78%, 112% and 63% respectively, under high nitrogen supply (3 mM) compared to low nitrogen (0.3 mM). This observation is true as nitrogen deficiency limits/inhibits cell division and enlargement resulting in stunted growth. It was also observed that, genotypes with higher leaf areas had corresponding higher total fresh weights. Similar trend was observed on leaf number.

Table 3.1: Shoot Fresh Weight, Leaf Area and Leaf Number. Letters denote the Tukey grouping of means in alphabetical order. Means with the same upper or low case letters are not significantly different at 5% LSD. *** = significant ($p < 0.05$).

Varieties	Shoot Fresh Weight (g plant^{-1})		Leaf Area (cm^2 plant^{-1})		Leaf Number (per plant)	
	High N	Low N	High N	Low N	High N	Low N
Aras-F1	29.69 ^{G}	18.92 ^{ef}	455.08 ^{FG}	193.56 ^{j}	20 ^{E}	18 ^{cd}
Ayaz-F1	32.28 ^{FG}	9.06 ^{h}	467.35 ^{F}	192.20 ^{j}	25 ^{CDE}	15 ^{def}
Efsun-F1	44.00 ^{BC}	25.08 ^{a}	620.84 ^{D}	324.81 ^{c}	30 ^{C}	15 ^{ef}
Elevator -F1	35.36 ^{EF}	21.56 ^{cde}	473.01 ^{F}	285.00 ^{def}	27 ^{CD}	17 ^{cde}
Eskisehir	49.78 ^{A}	24.36 ^{ab}	771.90 ^{B}	356.30 ^{b}	51 ^{AB}	27 ^{ab}
Hatay-1	36.08 ^{E}	20.78 ^{de}	671.92 ^{C}	251.62 ^{h}	23 ^{DE}	15 ^{def}
Hatay-2	34.69 ^{EF}	23.69 ^{abc}	582.20 ^{E}	368.77 ^{a}	46 ^{B}	28 ^{a}
Hatay-3	44.72 ^{B}	21.19 ^{cde}	677.15 ^{C}	292.89 ^{de}	24 ^{CDE}	15 ^{def}
Kayseri	25.50 ^{H}	20.69 ^{de}	424.04 ^{G}	281.75 ^{efg}	28 ^{CD}	25 ^{b}
Matador	39.28 ^{D}	19.92 ^{def}	816.37 ^{A}	273.67 ^{fg}	27 ^{CD}	15 ^{cdef}
Nebraska-F1	32.53 ^{FG}	21.94 ^{bcd}	384.86 ^{H}	270.63 ^{g}	23 ^{DE}	18 ^{c}
Reis-F1	17.56 ^{I}	13.22 ^{g}	278.17 ^{I}	164.48 ^{k}	24 ^{CDE}	13 ^{f}
Samsun	23.89 ^{H}	10.53 ^{gh}	361.02 ^{H}	115.15 ^{l}	21 ^{E}	13 ^{f}
Spinoza	41.08 ^{CD}	19.17 ^{ef}	820.94 ^{A}	295.12 ^{d}	54 ^{A}	25 ^{b}
Sprentir-F1	25.64 ^{H}	17.69 ^{f}	429.96 ^{G}	225.96 ^{i}	22 ^{DE}	14 ^{f}
Means	**34.14**	**19.19**	**548.99**	**259.46**	**29.67**	**18.20**
Max	49.78	25.08	820.94	368.77	54	28
Min	17.56	9.06	278.17	115.15	20	13
$LSD_{0.05}$	3.09	2.75	31.50	11.4	6.09	2.92
F-test	Variety *** Nitrogen *** Variety x Nitrogen ***		Variety *** Nitrogen *** Variety x Nitrogen ***		Variety *** Nitrogen *** Variety x Nitrogen ***	

3.1.2. Effect of Nitrogen Dose on Shoot and Root Dry Weights and Root-Shoot Ratio

Shoot Dry Weight

As shown in Figure 3.1, shoot dry matter (SDW) accumulation was averagely higher (about 121%) under high nitrogen treatment than low nitrogen treatment irrespective of genotype. Analysis of variance on dry matter accumulation revealed that, significant differences ($p < 0.01$) existed between the N doses and among the varieties. Variety nitrogen interaction was also significant ($p < 0.01$). Under high N treatment Eskişehir and Reis-F1 whereas under low N Efsun-F1 and Ayaz-F1 varieties produced the highest and the lowest dry matter, respectively.

Root Dry Weight

Nitrogen dose had a great effect on root dry weight (RDW). The results (Figure 3.2) showed that, irrespective of genotype, N dose significantly ($p < 0.01$) affected RDW. Averagely there was a 41% higher RDW under high N treatment compared to low N. Among varieties, RDW differed significantly ($p < 0.01$) regardless of N dose. Variety nitrogen interaction was also significant ($p < 0.01$). Under both N treatments, Efsun-F1 produced the highest RDW. Samsun and Ayaz-F1 varieties recorded the lowest RDW under both N doses.

Root-Shoot Ratio

Root-shoot ratios (Figure 3.3) were averagely 63% higher under low nitrogen treatment than under high nitrogen. Analysis of variance revealed that the effect of nitrogen dose was significant ($p < 0.01$). Significant differences also existed among varieties ($p < 0.01$) under both nitrogen treatments. The interaction was also significant ($p < 0.01$).

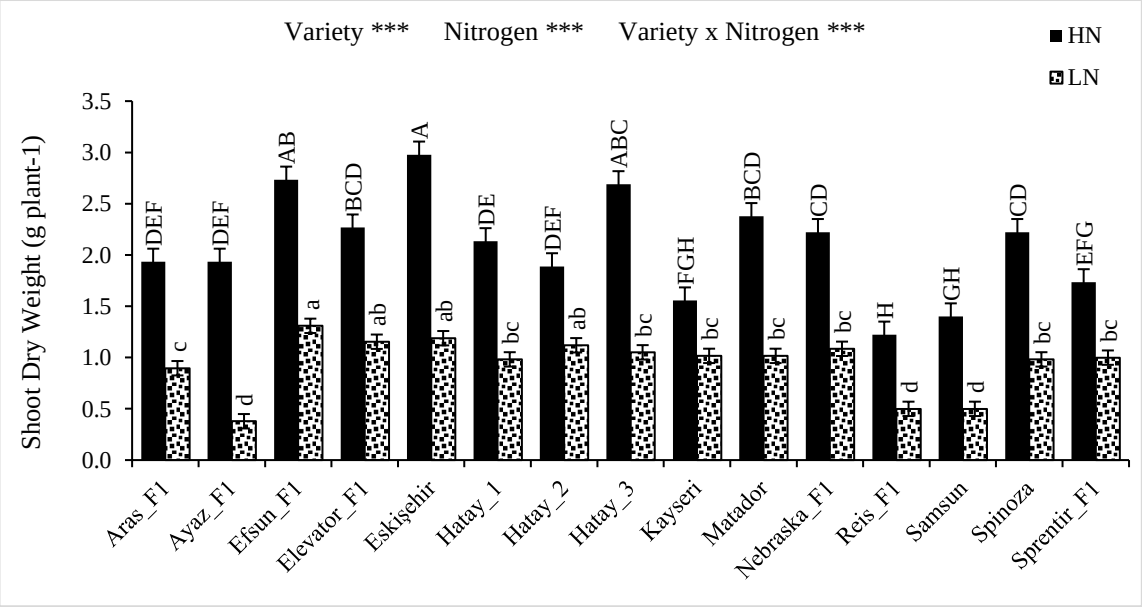


Figure 3.1: Shoot dry weight of spinach plants. Letters denote the Tukey grouping of means in alphabetical order. Means with the same upper or low case letters are not significantly different at 5% LSD. HN = high nitrogen, LN = Low nitrogen, *** = significant ($p < 0.05$).

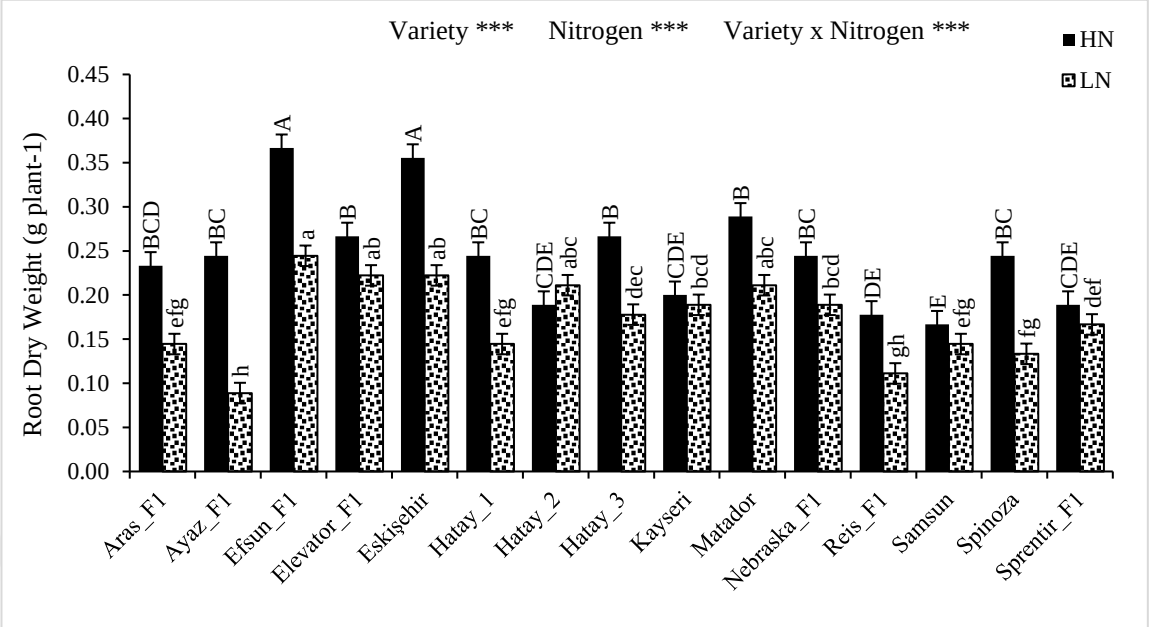


Figure 3.2: Root dry weight of spinach plants. Letters denote the Tukey grouping of means in alphabetical order. Means with the same upper or low case letters are not significantly different at 5% LSD. HN = high nitrogen, LN = Low nitrogen, *** = significant ($p < 0.05$).

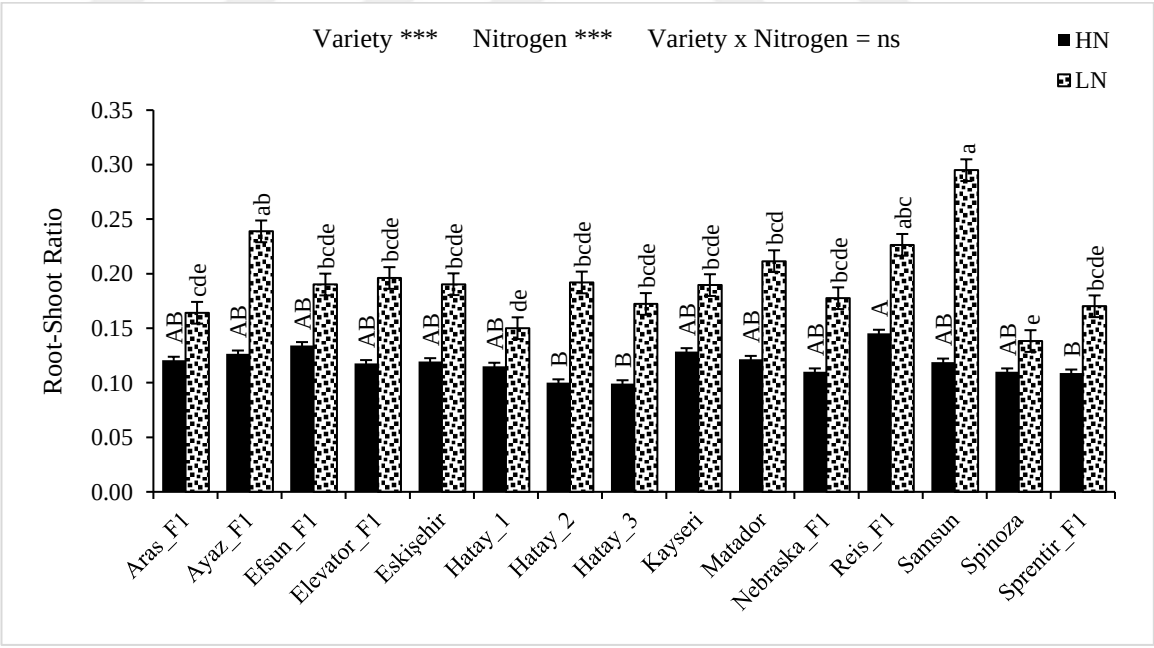


Figure 3.3: Root-shoot ratios of spinach varieties under high and low N treatments. Letters denote the Tukey grouping of means in alphabetical order. Means with the same upper or low case letters are not significantly different at 5% LSD. HN = high nitrogen, LN = Low nitrogen, *** = significant ($p < 0.05$); ns = not significant ($p > 0.05$).

3.1.3. Root Length of spinach plants as affected by different N doses

Though RDW is an important parameter to consider in plant development analysis, it gives a limited information on morphology and as such, it is important to consider other root features such as root length. Similar to RDW, total root length (Figure 3.4) was averagely 13% higher in high N treated plants compared to treated with low N. Significant variations ($p < 0.01$) existed in total root length among the spinach varieties. Under both N treatments, the Eskisehir variety recorded the highest total root length whereas Nebraska-F1 under high N and Ayaz-F1 under low N recorded the lowest.

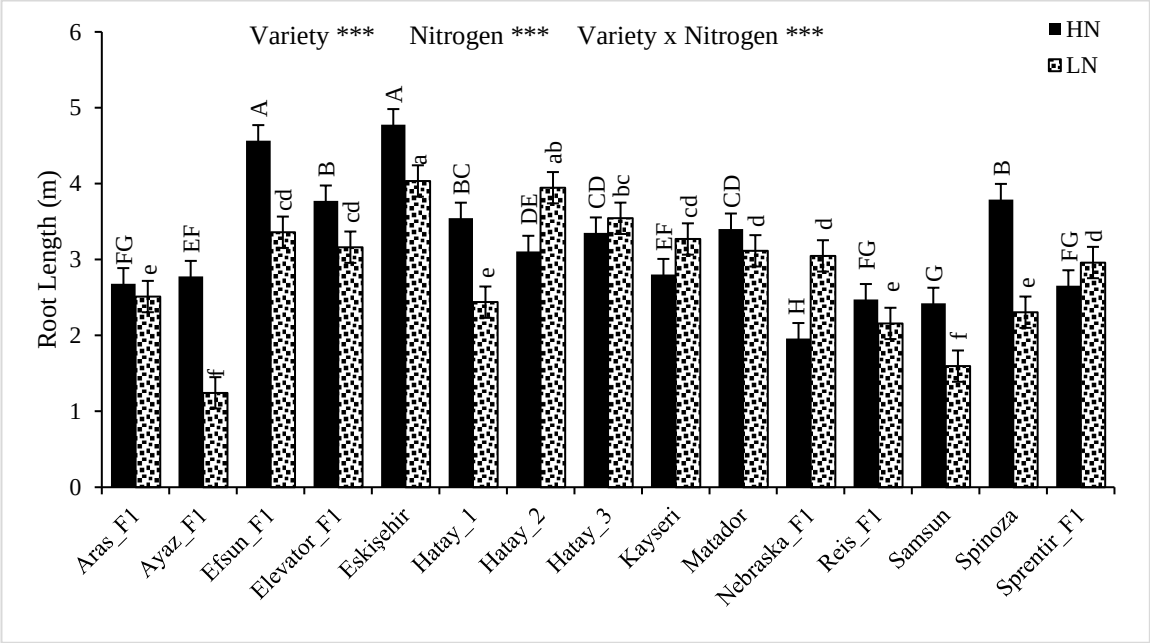


Figure 3.4: Root length of spinach plants under different nitrogen doses. Letters denote the Tukey grouping of means in alphabetical order. Means with the same upper or low case letters are not significantly different at 5% LSD. HN = high nitrogen, LN = Low nitrogen, *** = highly significant ($p < 0.01$).

3.1.4. Nitrogen Concentration, Total N-Uptake and Nitrate Concentration

Nitrogen Concentration

As presented in Table 3.2, nitrogen concertation (mg N g^{-1} DW) varied significantly among the varieties under investigations. Some varieties (Nebraska-F1, Samsun, Spinoza and Sprentir-F1) under low N have higher or similar concentrations compared with their replicates under high N. A significant effect existed between low and high nitrogen treatments with a mean difference of 8% higher N concentration in plants treated with high nitrogen than low nitrogen.

N-Uptake

Results on N-Uptake (NUp) revealed a much clearer picture of nitrogen content in each variety per its dry matter basis. As shown in Table 3.2, NUp under high N is averagely 141% higher than under low N irrespective of variety. Significant differences ($p < 0.01$) among the varieties were observed under both nitrogen treatments. Correlation results showed that N concentration has no significant correlation with total NUp..

Nitrate concentration

Nitrate concentration (mg NO_3^- g^{-1} DW) as presented in Table 3.5 also showed a similar trend as in NUp. Plants treated with high N were found to have higher nitrate concentrations (averagely 51%) than replicates under low N treatment irrespective of genotype. Variations between spinach varieties ($p < 0.01$) existed under both nitrogen conditions. For high N treatment, the highest and lowest total NO_3^- concentration was measured on Hatay-2 and Reis-F1 cultivars, respectively, whereas under low N treatment, NO_3^- concentration was highest in Samsun and lowest in hatay-2 cultivars.

Table 3.2: Nitrogen Concentration, Total N-Uptake and Nitrate Concentration. Letters denote the Tukey grouping of means in alphabetical order. Means with the same upper or low case letters are not significantly different at 5% LSD. *** = significant ($p < 0.05$).

Varieties	N Concentration (mg N g^{-1} DW)		N Uptake (mg N $plant^{-1}$ DW)		Nitrate Concentration (mg NO_3^- kg^{-1} DW)	
	High N	Low N	High N	Low N	High N	Low N
Aras-F1	83.24 ^{B}	79.84 ^{bc}	161.72 $^{D-F}$	71.77 ^{ab}	2438.81 ^{G}	2124.66 $^{d-f}$
Ayaz-F1	79.70 $^{B-D}$	72.63 ^{e}	154.60 $^{D-G}$	27.57 ^{c}	4005.02 $^{C-E}$	2900.91 $^{b-e}$
Efsun-F1	72.17 ^{F}	65.97 ^{f}	197.97 $^{B-D}$	86.20 ^{ab}	4475.34 ^{CD}	2371.23 $^{c-f}$
Elevator -F1	113.0 ^{A}	71.44 ^{e}	256.87 ^{A}	82.48 ^{ab}	4379.45 ^{CD}	2348.40 $^{c-f}$
Eskisehir	80.96 ^{BC}	70.70 ^{ef}	241.23 ^{AB}	84.32 ^{ab}	3776.75 $^{C-F}$	2257.08 $^{c-f}$
Hatay-1	78.75 $^{B-E}$	70.35 ^{ef}	168.37 $^{D-E}$	69.26 ^{b}	4493.61 ^{CD}	2590.41 $^{b-f}$
Hatay-2	78.40 $^{B-E}$	73.22 ^{e}	148.73 $^{E-G}$	82.03 ^{ab}	6854.35 ^{A}	1709.13 ^{f}
Hatay-3	81.38 ^{BC}	73.99 ^{de}	218.94 $^{A-C}$	77.31 ^{ab}	4854.34 ^{BC}	3243.38 ^{bc}
Kayseri	73.12 ^{EF}	70.42 ^{ef}	114.09 $^{F-H}$	71.32 ^{ab}	3425.11 $^{D-G}$	3142.92 $^{b-d}$
Matador	80.16 $^{B-D}$	71.33 ^{e}	190.57 $^{C-E}$	72.41 ^{ab}	5813.24 ^{AB}	2074.43 ^{ef}
Nebraska-F1	76.02 $^{C-F}$	80.02 ^{b}	169.56 $^{D-E}$	87.10 ^{a}	3032.42 $^{E-G}$	2786.76 $^{b-e}$
Reis-F1	77.53 $^{B-F}$	75.08 $^{c-e}$	95.22 ^{H}	37.46 ^{c}	2356.62 ^{G}	3184.02 ^{bc}
Samsun	78.44 $^{B-E}$	83.03 ^{ab}	110.84 ^{GH}	41.76 ^{c}	2836.07 ^{FG}	4371.23 ^{a}
Spinoza	74.69 $^{D-F}$	78.44 $^{b-d}$	166.75 ^{DE}	77.36 ^{ab}	4023.29 $^{C-E}$	3517.35 ^{ab}
Sprentir-F1	82.61 ^{B}	85.55 ^{a}	143.39 $^{E-H}$	85.26 ^{ab}	3100.91 $^{E-G}$	2700 $^{b-f}$
Means	**80.68**	**74.80**	**169.26**	**70.24**	**3991.02**	**2754.79**
Max	113	85.55	256.87	87.10	6854.35	4371.23
Min	72.17	65.97	95.22	27.57	2356.62	1709.13
$LSD_{0.05}$	5.79	4.80	48.54	17.79	1163.09	1034.41
F-test	Variety *** Nitrogen *** Variety x Nitrogen ***		Variety *** Nitrogen *** Variety x Nitrogen ***		Variety *** Nitrogen *** Variety x Nitrogen ***	

3.1.5. Nitrate Reductase Enzyme Activity Rate

Figure 3.5 shows the effect of genotype and nitrogen dose on nitrate reductase enzyme activity (NRA) of spinach varieties under investigation after 35 days growing in nutrient solution. The results revealed that nitrogen dose and variety had strong effect on NRA. Statistical analysis revealed that significant differences ($p < 0.01$) existed between N doses and among varieties. Significant also was variety-nitrogen interaction. Though some varieties (Sprentir-F1, Eskişehir, Nebraska-F1, Spinoza and Samsun) showed higher NRA rates under low N treatment, there was averagely a 45% overall higher NRA rate in plants under high N treatment. Ayaz-F1 at high N and Reis-F1 at low N exhibited the highest NRA rates whereas Samsun and Hatay-1 recorded the lowest NRA under high and low N doses, respectively.

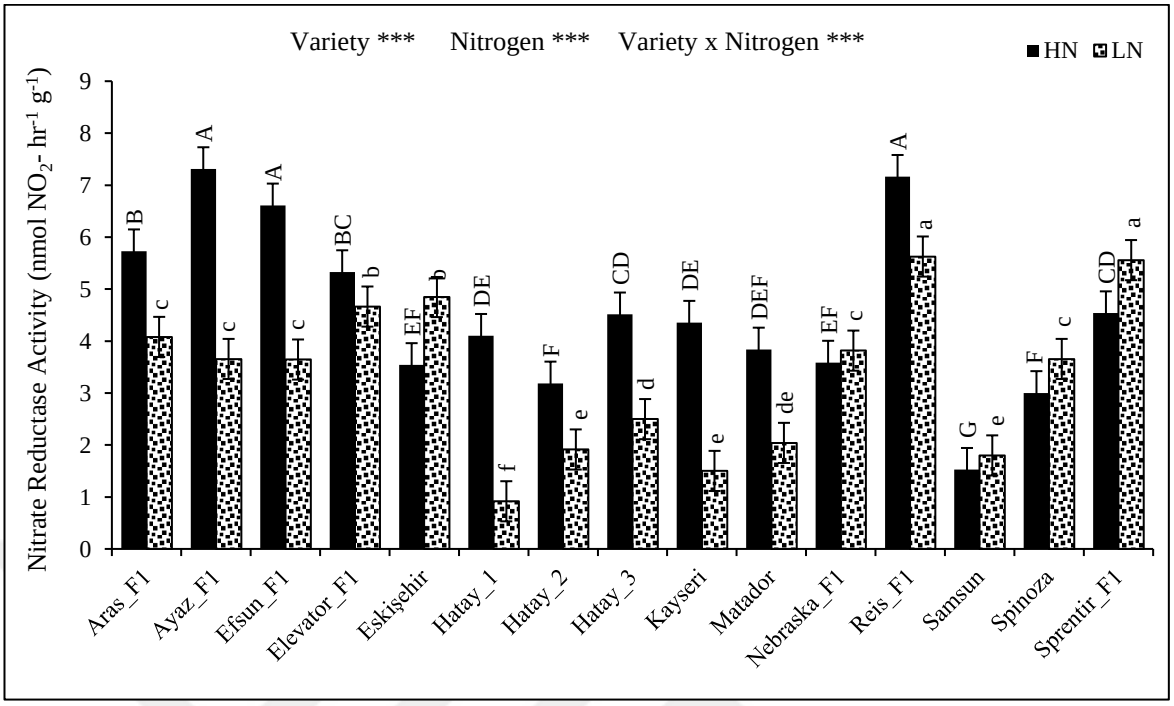


Figure 3.5: Leaf nitrate reductase activity rate as affected by nitrogen dose and genotype. Letters denote the Tukey grouping of means in alphabetical order. Means with the same upper or low case letters are not significantly different at 5% LSD. HN = high nitrogen, LN = Low nitrogen, *** = highly significant ($p < 0.01$).

3.1.6. Total Chlorophyll and Net Photosynthetic Rate

Figures 3.7 and 3.8 shows the effect of nitrogen dose and variety on leaf total chlorophyll content and net photosynthetic rate respectively. Variety had a significant ($p < 0.01$) effect on total chlorophyll (Figure 3.6). Nitrogen on the other hand had no significant effect on total chlorophyll though the average was slightly higher (2%) in plants grown under high N compared to low N treated plants. On net photosynthetic rate, the results showed that irrespective of genotype, photosynthetic rates were higher in plants treated with high N compared to their replicates at low N (Figure 3.7). Analysis of variance revealed that net photosynthetic rate was significantly affected by N dose ($p < 0.01$). Variety and nitrogen-variety interaction were not significant.

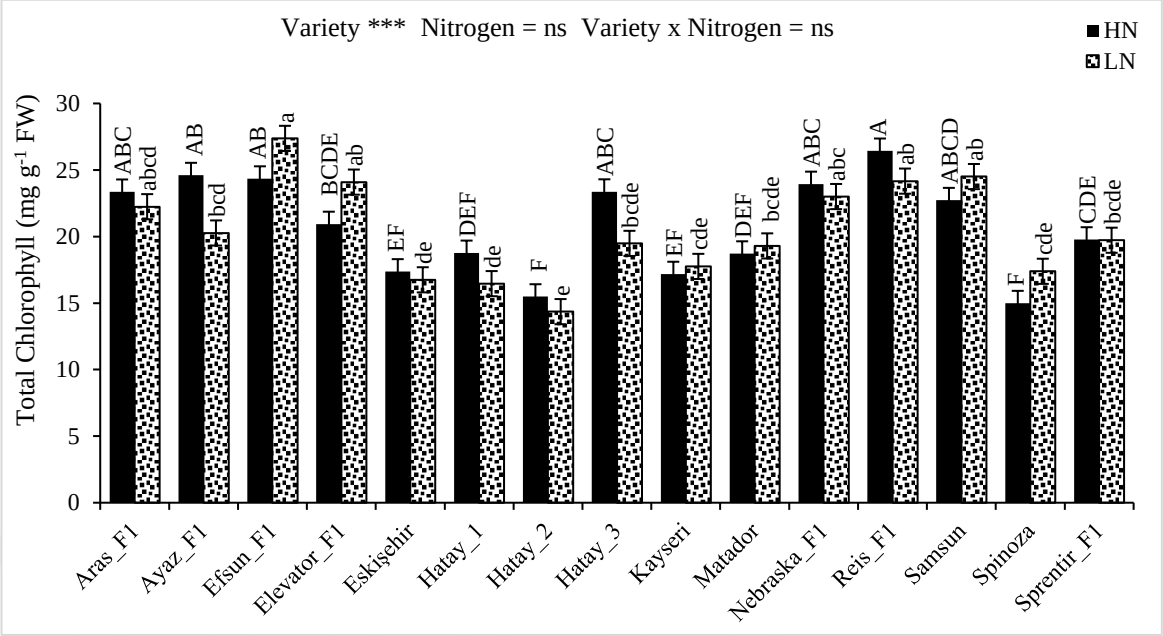


Figure 3.6: Total chlorophyll content of spinach varieties under low and high N doses. Letters denote the Tukey grouping of means in alphabetical order. Means with the same upper or low case letters are not significantly different at 5% LSD. HN = high nitrogen, LN = Low nitrogen, *** = significant ($p < 0.05$); ns = not significant ($p > 0.05$).

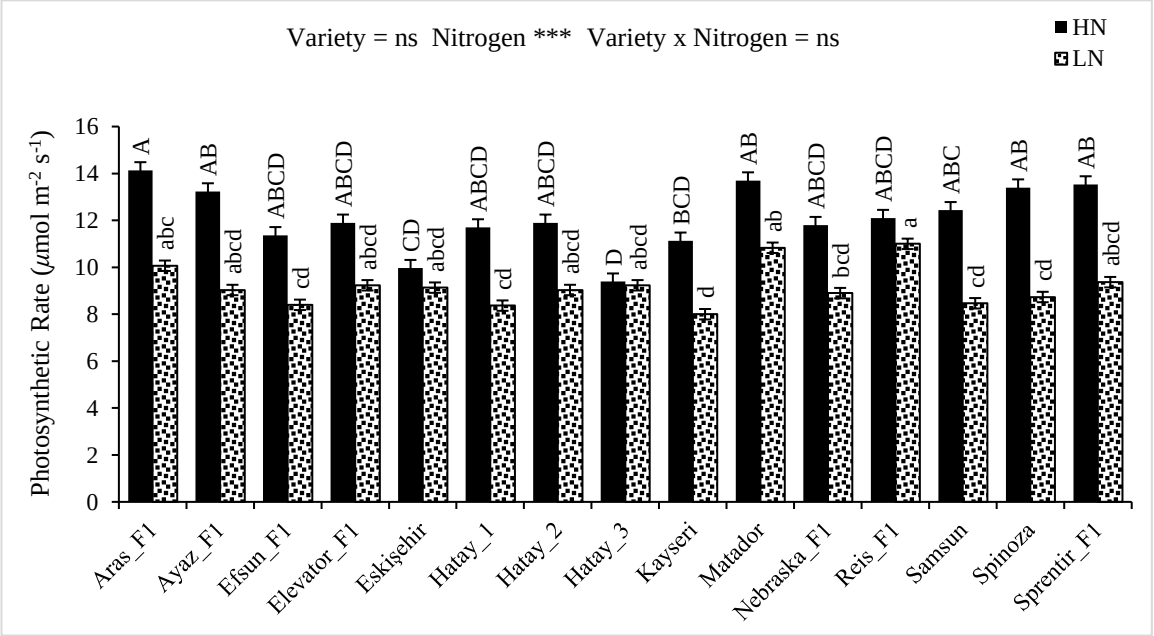


Figure 3.7: Photosynthetic rate of spinach varieties under low and high N doses. Letters denote the Tukey grouping of means in alphabetical order. Means with the same upper or low case letters are not significantly different at 5% LSD. HN = high nitrogen, LN = Low nitrogen, *** = significant ($p < 0.05$); ns = not significant ($p > 0.05$).

3.1.7. Conclusion

From the results of this screening experiment, five spinach varieties (Efsun_F1, Eskisehir, Hatay_3, Matador and Spinoza) with the highest fresh and dry biomass production were selected. They also had corresponding higher total leaf areas, root dry weights, root lengths and total nitrogen uptakes. They varieties were used as the plant materials for the conducting the second (LLOT), third (OLLT) and fourth (LLLT) experiments. The data of these five varieties would also serve as the control experiment for the other experiments.

3.2. Results of LLOT, OLLT and LLLT Experiments.

3.2.1. Shoot Fresh weight

As shown in Figure 3.8 shoot fresh mass was higher under high N treatments compared to their replicates under low N irrespective of the variety or the light-temperature combination. Analysis of variance revealed that N dose had a significant effect ($p < 0.01$) on shoot fresh weights. Similarly, shoot fresh mass significantly varied ($p < 0.01$) among the spinach varieties irrespective of the nitrogen dose or the light-temperature combination. The interaction between variety and nitrogen was significant ($p < 0.01$).

Comparing the shoot fresh mass of spinach grown under the various light-temperature combination experiments showed that fresh biomass production was higher under OLOT followed by LLOT, OLLT and LLLT. In the OLOT experiment, Eskisehir and Efsun-F1 recorded the highest SFW under high N treatment whereas the lowest was recorded from Hatay-3 at high N and Spinoza at low N. In the LLOT experiment, Hatay-3 produced the highest shoot fresh weight at high N and Efsun-F1 the lowest while Spinoza recorded the highest and Matador the lowest at low N. In the OLLT experiment, Spinoza and Efsun-F1 respectively recorded the highest and lowest fresh masses under both N doses. In the LLLT experiment, the Eskisehir and Efsun-F1 varieties recorded the highest and lowest respectively under both high and low N treatments.

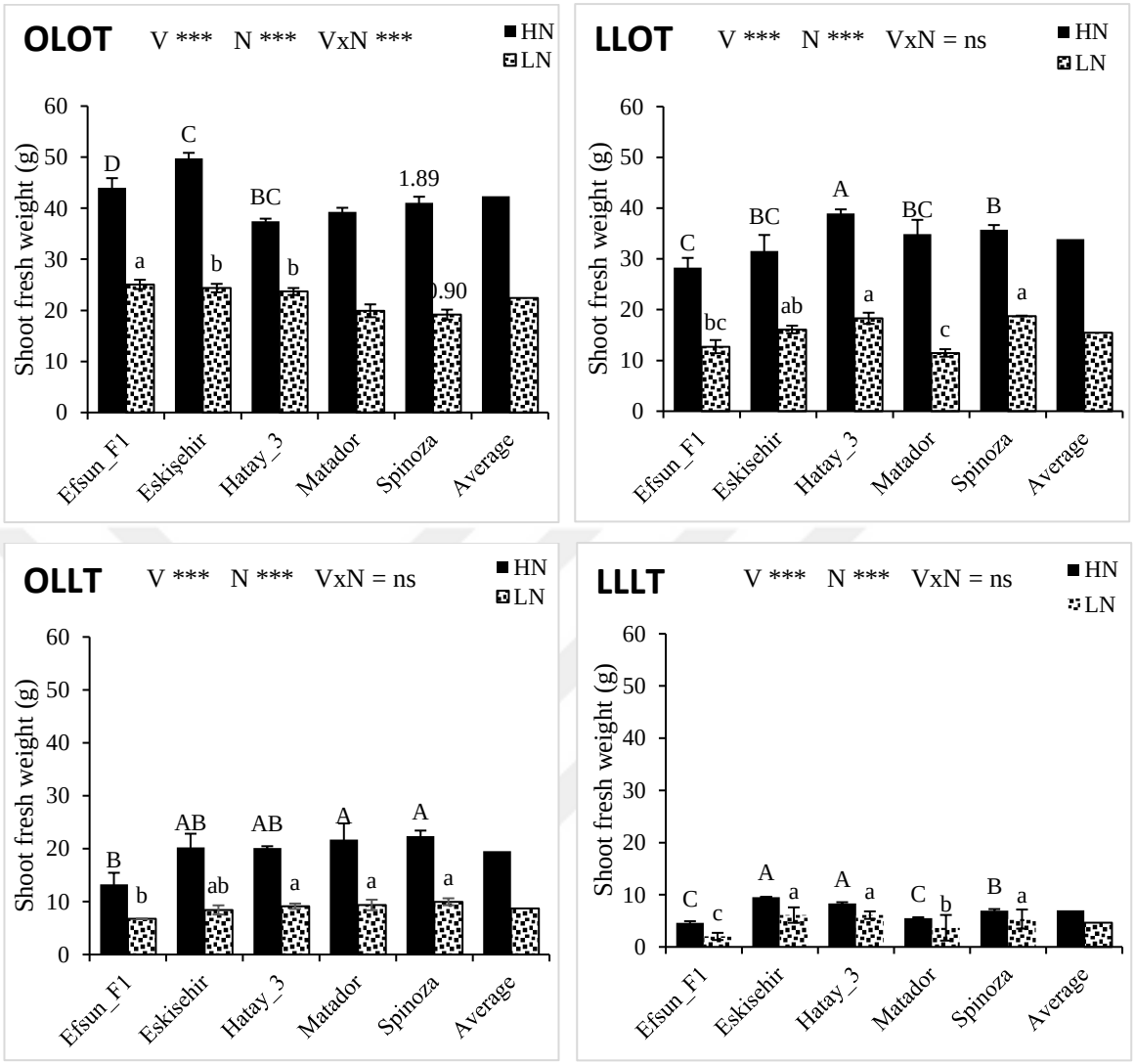


Figure 3.8: Shoot fresh weight of spinach varieties grown under different light-temperature combinations and two N treatments. Letters denote the Tukey grouping of means in alphabetical order. Means with the same upper or low case letters are not significantly different at 5% LSD. V = variety, N = nitrogen, HN = high nitrogen, LN = Low nitrogen, *** = significant ($p < 0.05$) ns = not significant ($p > 0.05$).

3.2.2. Leaf Area

One of the parameters that have a direct effect on plant biomass is leaf area. As seen in figure 3.9, the leaf area of spinach plants was higher in plants grown under high nitrogen supply in comparison with plants grown under deficient nitrogen irrespective of variety or the light-temperature combination. Analysis of variance showed that significant differences ($p < 0.01$) existed between N treatments. Similarly, spinach varieties significantly varied ($p < 0.01$) from one another in response to nitrogen treatments irrespective of the light-temperature combination. Variety-nitrogen interaction was also significant ($p < 0.01$) except in the OLLT experiment. A holistic view of the results of all the four experiments showed that light-temperature combinations significantly affected leaf expansion regardless of variety and/or nitrogen dose. Larger leaf areas were recorded in the OLOT experiment followed by LLOT > OLLT > LLLT in descending order.

Varietal performances under the OLOT experiment saw Spinoza and Hatay-3 at high N recording the highest and lowest total leaf areas, respectively. At low N, Hatay-3 and Eskisehir were the varieties with the highest leaf areas and Matador with the lowest. In the LLOT experiment, higher and lower leaf areas were, respectively, recorded from Spinoza and Efsun-F1 under high N and at low N Hatay-3 and Matador recorded the lowest. In the OLLT conditions, Hatay-3 and Efsun-F1 recorded the highest and lowest leaf areas, respectively, at high N whereas Spinoza and Efsun-F1 recorded the highest and lowest at low N. Finally, in the LLLT experiment, Eskisehir and Efsun-F1 under high N, and Hatay-3 and Efsun-F1 under low N, respectively, recorded the highest and lowest total leaf areas.

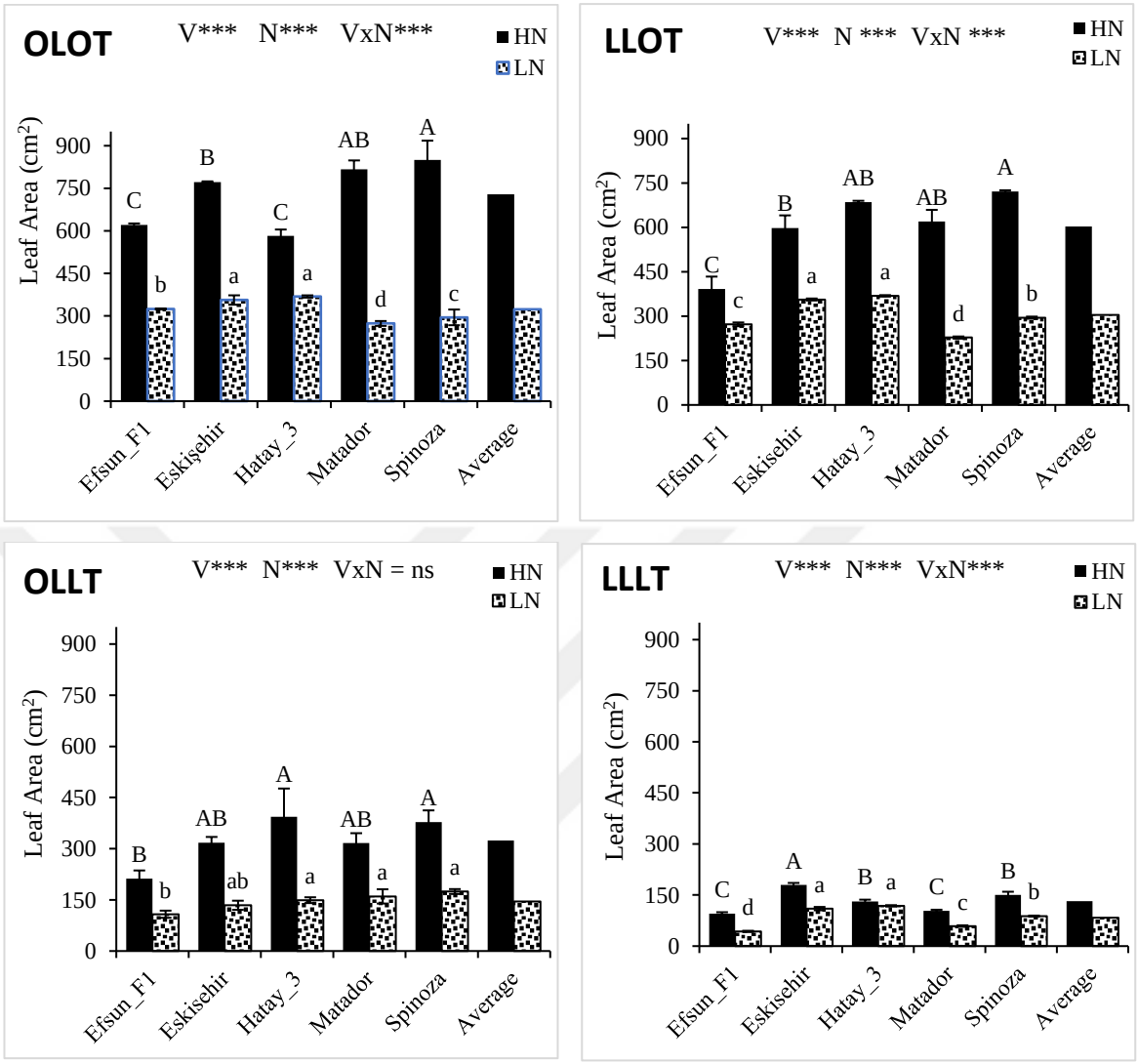


Figure 3.9: Leaf area of spinach plants grown under different light-temperature combinations and two N treatments. Letters denote the Tukey grouping of means in alphabetical order. Means with the same upper or low case letters are not significantly different at 5% LSD. V = variety, N = nitrogen, HN = high nitrogen, LN = Low nitrogen, *** = significant ($p < 0.05$).

3.2.3. Leaf Number

Similar to the total leaf area, the leaf number of spinach plants was generally higher in high N treated plants across all the four experiments except for Matador and Hatay-3 in OLLT and LLLT experiments, respectively (Figure 3.10). Analysis of variance revealded that nitrogen dose significantly affected leaf number ($p < 0.01$) in all experiments except in the LLLT experiment. Significant variations ($p < 0.01$) existed among the spinach varieties regardless of the growing condition. The interaction was also significant.

Light-temperature combinations impacted leaf numbers significantly. Leaf number per plant was generally less in plants grown under low-temperature conditions regardless of light intensity. As such, leaf numbers were higher under OLOT > LLOT > OLLT > LLLT in descending order. Leaf numbers in OLOT experiment saw Spinoza with more leaves and Matador with fewer at high N whereas at low N Hatay-3 had more leaves and Efsun-F1 with the same number as Matador recorded fewer number of leaves. In the LLOT experiment, Hatay-3 and Matador were the varieties with more leaves at high N dose and Eskisehir at low N. Efsun-F1 had fewer number of leaves at both N doses. In the OLLT experiment, Spinoza had more, Matador had fewer leaves at high N, and at low N Hatay-3 had more, and Efsun-F1 had fewer leaves. In the LLLT experiment, Eskisehir, Hatay-3 at high N and Hatay-3 at low N recorded the highest number of leaves whereas Efsun-F1 recorded fewer number of leaves at both N treatments.

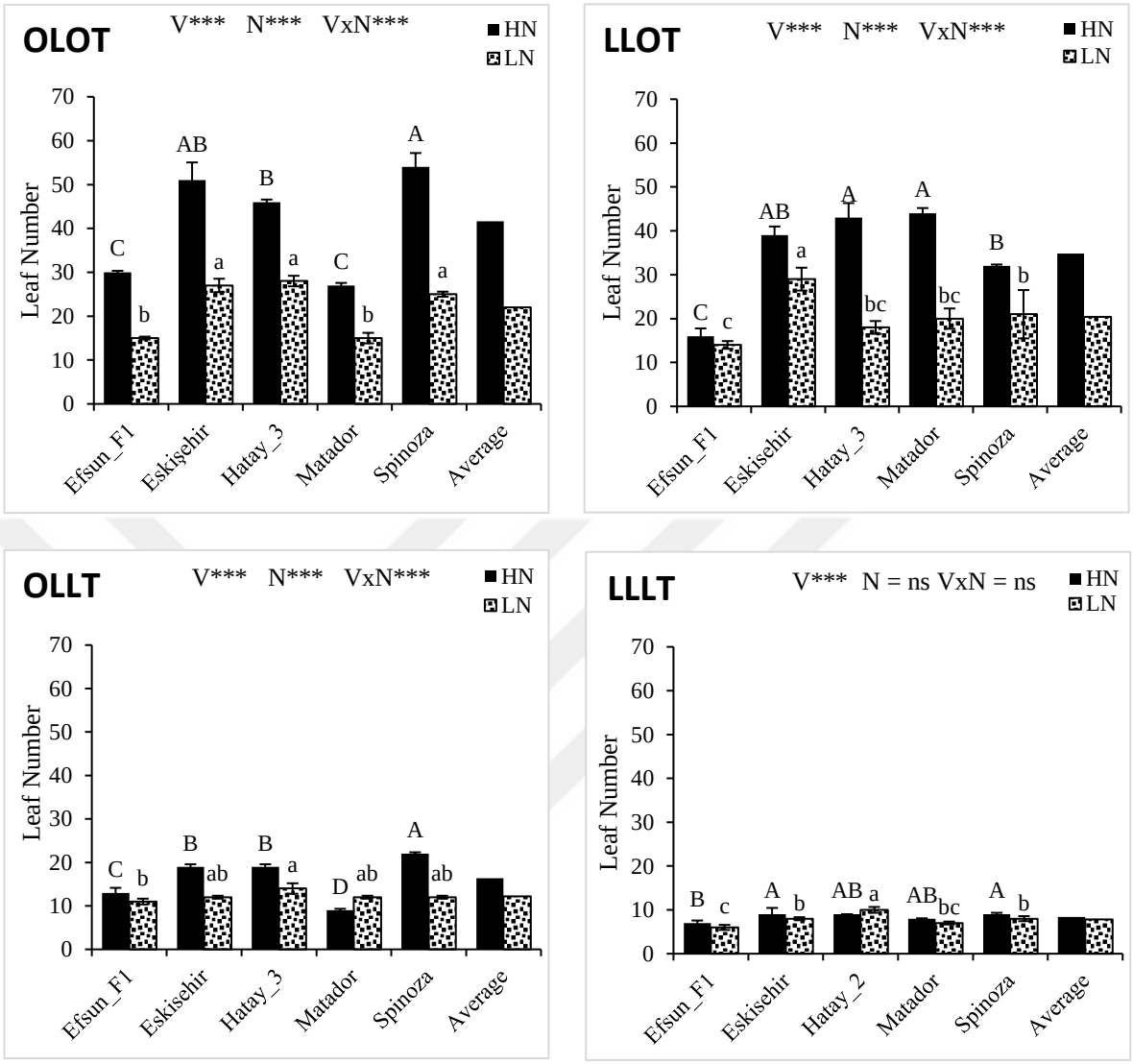


Figure 3.10: Leaf number of spinach plants grown under different light-temperature combinations and two N treatments. Letters denote the Tukey grouping of means in alphabetical order. Means with the same upper or low case letters are not significantly different at 5% LSD. V = variety, N = nitrogen, HN = high nitrogen, LN = Low nitrogen, *** = significant ($p < 0.05$).

3.2.4. Shoot Dry Weight

Results on shoot dry matter (SDW) as shown in Figure 3.11, revealed that light-temperature combination and nitrogen dose significantly affected ($p < 0.01$) SDW regardless of variety. Plants treated with high N produced higher shoot dry biomass than low N treated replicates regardless of the variety and/or the light-temperature combination. Varietal differences in response to nitrogen supply were observed in OLOT, LLOT and LLLT experiments but not in the OLLT experiment. Interaction between variety and nitrogen was also only significant in OLOT and LLLT experiments. It was observed that temperature had a greater effect on dry biomass accumulation in spinach than that light. The trend of SDW accumulation when the data of all four experiments were compared was OLOT > LLOT > OLLT > LLLT in descending order, regardless of N dose.

Tukey test ranking of varietal means showed that in the OLOT experiment Eskisehir and Hatay-3, respectively, had the highest and lowest SDW at high N treatment, whereas at low N, Efsun-F1 recorded the highest and Spinoza the lowest. In the LLOT experiment, Spinoza at high N and Hatay-3 at low N recorded the highest SDW, whereas Efsun-F1 at high N and Matador at low N recorded the lowest SDW. In the OLLT experiment, Spinoza recorded the highest at both N doses whereas Efsun-F1 at high N and Matador at low N recorded the lowest dry weights. In LLLT experiment, Eskisehir and Matador, respectively, recorded the highest and lowest SDW at high N, whereas at low N Hatay-3, Spinoza with the same value recorded the highest and Efsun-F1 with the lowest.

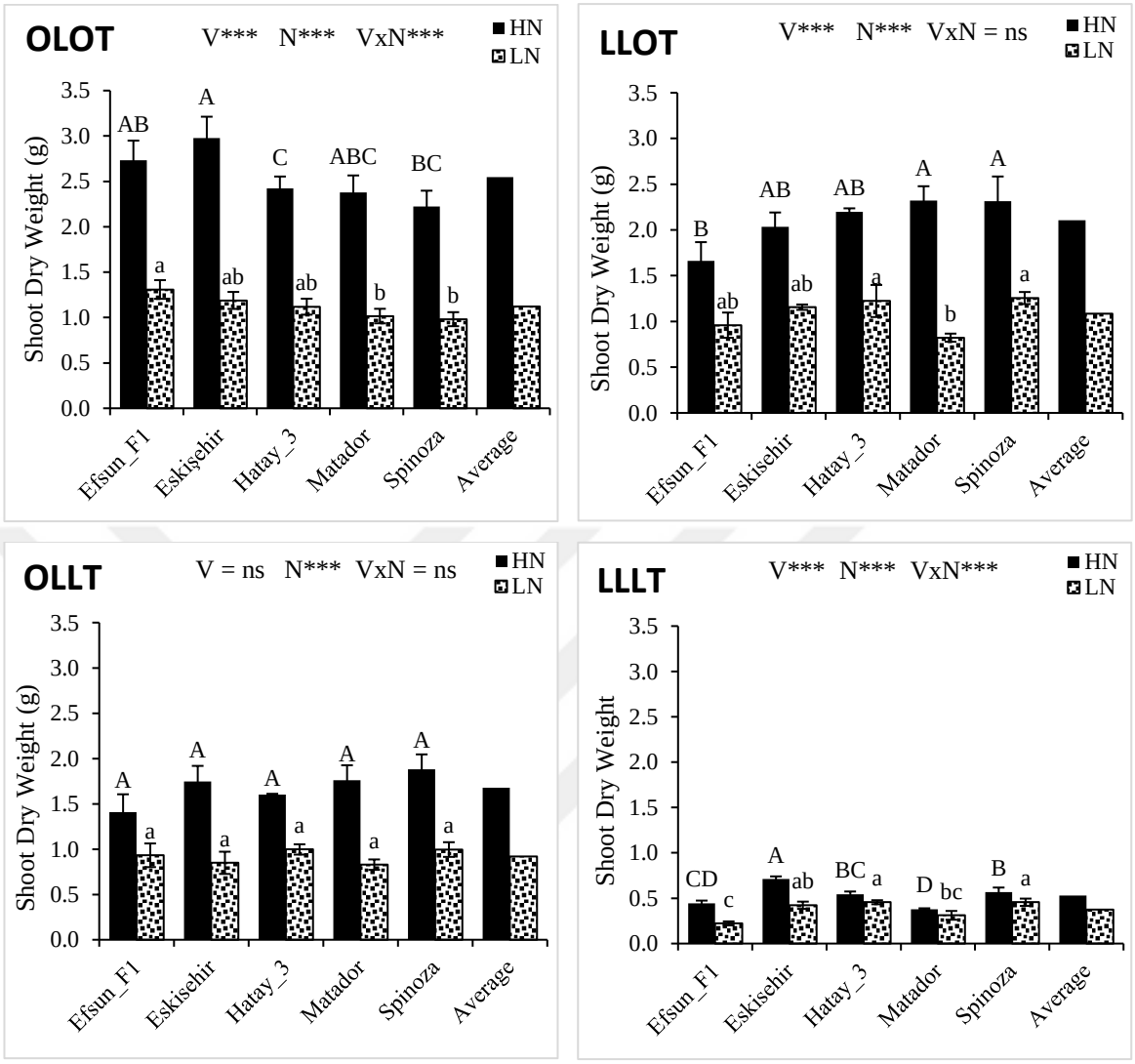


Figure 3.17: Shoot dry weight of spinach plants grown under different light-temperature combinations and two N treatments. Letters denote the Tukey grouping of means in alphabetical order. Means with the same upper or low case letters are not significantly different at 5% LSD. V = variety, N = nitrogen, HN = high nitrogen, LN = Low nitrogen, *** = significant ($p < 0.05$).

3.2.5. Root Dry Weight

Analysis of variance on root dry weight (RDW) of spinach varieties under investigation showed that RDW (Figure 3.12) varied significantly ($p < 0.01$) between nitrogen doses except in the LLLT experiment. Significant variations among the varieties were also observed in all four experiments. The interaction between variety and nitrogen was also significant except in the LLOT experiment. Comparing data of all the four experiments showed that irrespective of N treatment, the average RDW was high in OLLT followed by LLT, OLOT and LLLT, in descending order. At optimum temperature (OT) conditions irrespective of light intensity, RDW was averagely higher in high N treated plants than low N treated replicates. The opposite was observed for low temperature (LT) conditions irrespective of light intensity.

In the OLOT experiment, Efsun-F1 recorded the highest RDW under both N doses whereas the lowest was recorded from Hatay-3 at high N and Spinoza at low N. In LLOT Experiment, Hatay-3 and Efun-F1, respectively, recorded the highest and lowest RDW at high N, whereas at low N Eskisehir and Efsun-F1 recorded the highest and lowest RDW, respectively. In the OLLT experiment, Spinoza and Hatay-3, respectively, had higher and lower RDW at high N treatment while at low N Spinoza with the same figure as Hatay-3 recorded the highest and Efsun-F1 with the lowest. And in the LLLT experiment, the highest RDW was recorded from Spinoza at both N doses while Matador at high N and Efsun-F1 at low N recorded the lowest.

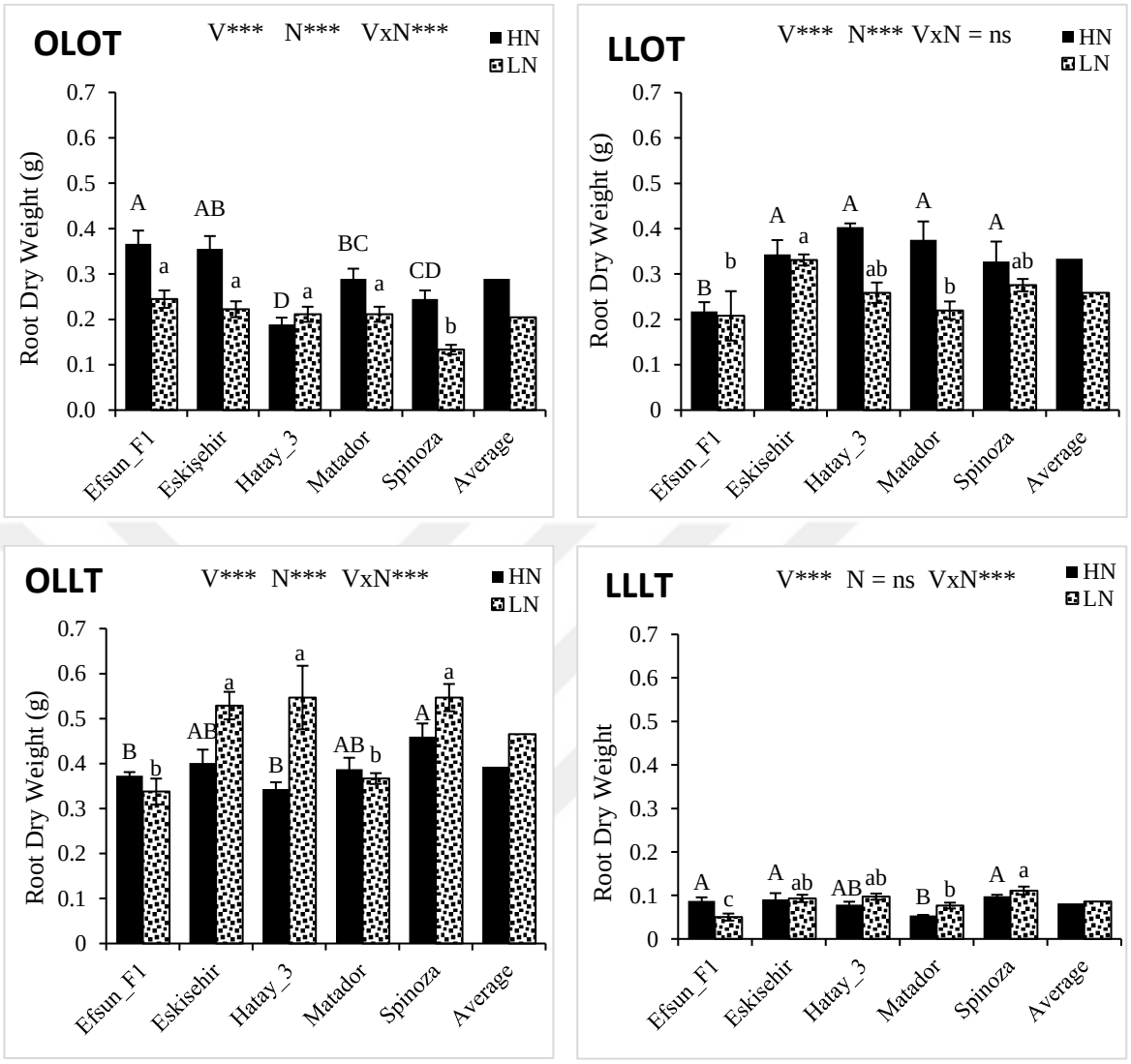


Figure 3.12: Root dry weight of spinach plants grown under different light-temperature combinations and two N treatments. Letters denote the Tukey grouping of means in alphabetical order. Means with the same upper or low case letters are not significantly different at 5% LSD. V = variety, N = nitrogen, HN = high nitrogen, LN = Low nitrogen, *** = significant ($p < 0.05$).

3.2.6. Root: Shoot Ratio

Analysis of variance showed that the light-temperature combination and nitrogen dose had a significant effect on root-shoot ratios (Figure 3.13). Varietal (genotypic) effect was not significant in all experiments. Irrespective of the growing environment (light-temperature combination) and/or variety, root-shoot ratios were higher in low N treated

plants than in high N treated replicates. At high N, root-shoot ratios were averagely higher in the OLLT experiment, followed by LLLT, LLOT and OLOT while at low the trend was LLOT > LLOT > LLLT > OLOT in descending order. Interaction between variety, and nitrogen dose was not significant.

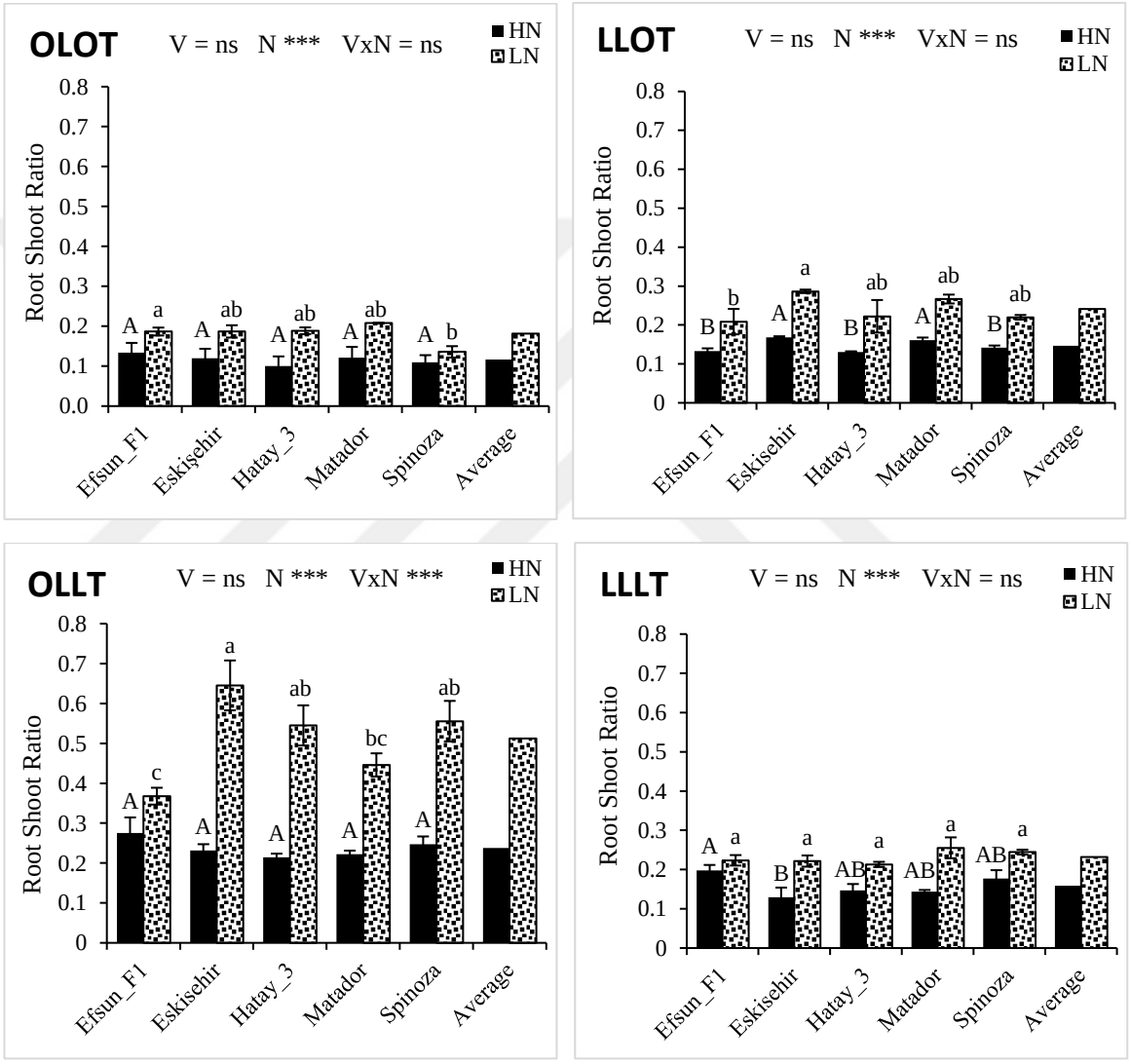


Figure 3.13: Root-shoot ratios of spinach plants grown under different light-temperature combinations and two N treatments. Letters denote the Tukey grouping of means in alphabetical order. Means with the same upper or low case letters are not significantly different at 5% LSD. V = variety, N = nitrogen, HN = high nitrogen, LN = Low nitrogen, *** = significant ($p < 0.05$), ns = not significant ($p > 0.05$).

3.2.7. Root Length

Figure 3.14 shows the root lengths of spinach varieties as affected by nitrogen dose in four experiments of varying light-temperature combinations. Analysis of variance revealed that significant differences ($p < 0.01$) in root length existed among spinach varieties in the OLOT and OLLT experiments but not in OLLT and LLLT experiments. Statistically, the effect of nitrogen dose was significant only in optimum temperature (OLOT and LLOT) experiments. Variety-nitrogen interaction was only significant in the OLOT experiment. The trend of root elongation under the various growing conditions, was LLOT > OLLT > OLOT > LLLT at high N whereas at low N the trend was OLLT > LLOT > OLOT > LLLT in descending order. Similar to that of RDW, root length at optimum temperature (OT) conditions was averagely higher in plants treated with high N than replicates at low N irrespective of light intensity. The opposite was observed in low temperature (LT) conditions irrespective of light intensity.

Ranking the root lengths of individual varieties as affected by light-temperature combination and N dose shows that; in the OLOT experiment, the Eskisehir variety had the highest root lengths under both N doses whereas Hatay-3 at high N and Spinoza at low N recorded the lowest. In the LLOT experiment, Hatay-3 and Efsun-F1, respectively, recorded the highest and lowest root lengths under high N treatment whereas at low N Eskisehir recorded the highest and Matador with the lowest. In the OLLT experiment, Spinoza at high N and Matador at low N recorded the highest root lengths. Efsun-F1 variety recorded the lowest at both N doses. Lastly, in the LLLT experiment, the highest and lowest root lengths were recorded from Hatay-3 and Matador at high N and Spinoza and Efsun-F1 at low N.

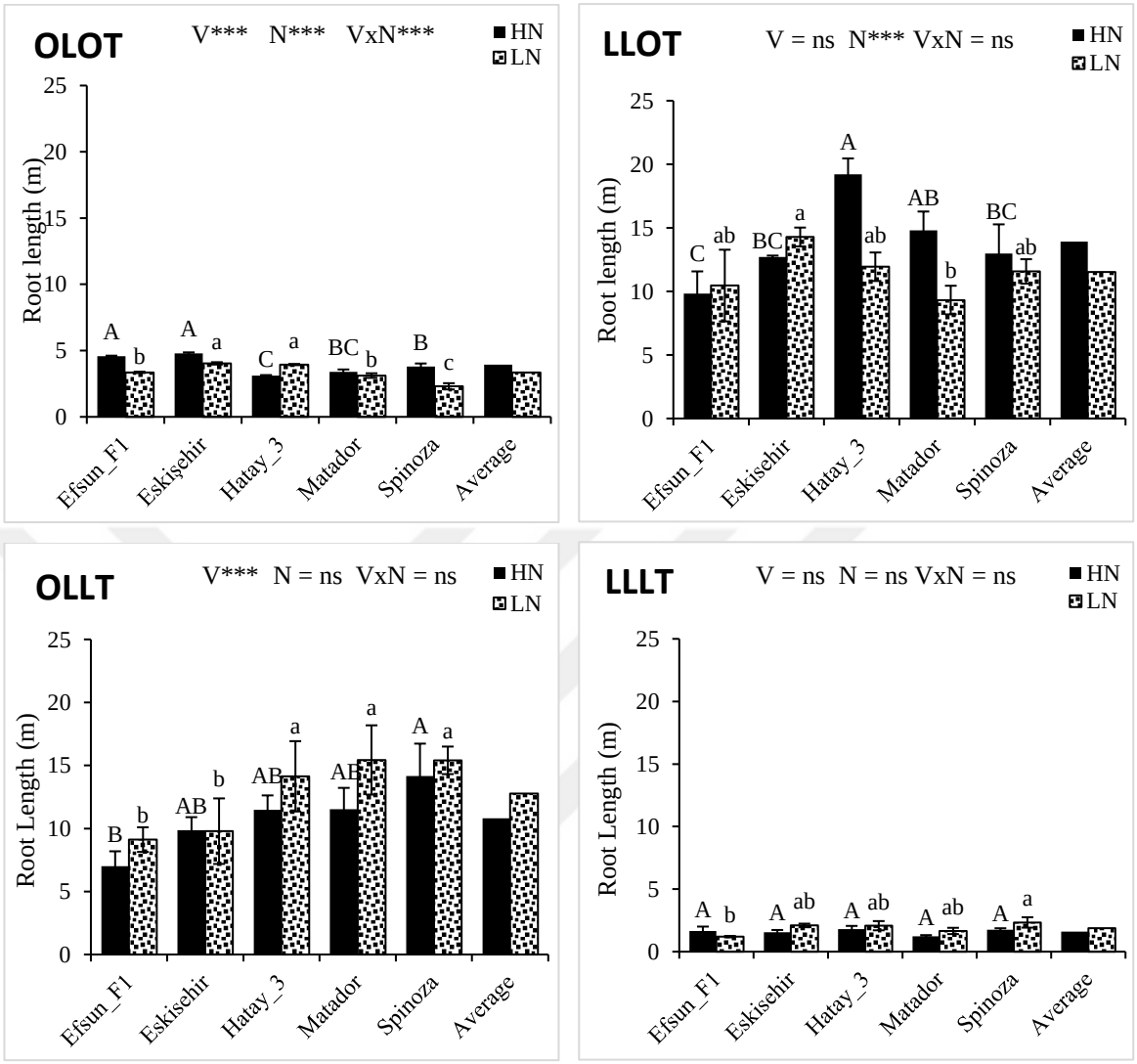


Figure 3.14: Root length of spinach plants grown under different light-temperature combinations and two N treatments. Letters denote the Tukey grouping of means in alphabetical order. Means with the same upper or low case letters are not significantly different at 5% LSD. V = variety, N = nitrogen, HN = high nitrogen, LN = Low nitrogen, *** = significant ($p < 0.05$), ns = not significant ($p > 0.05$).

3.2.8. Nitrogen Concentration

As presented in Figure 3.15, results showed that nitrogen concentrations of the spinach plants were averagely higher in high N-treated plants in all the four experiments. Statistically, nitrogen dose had a significant effect ($p < 0.01$) on spinach nitrogen concentration in all experiments but the LLLT experiment. Varietal (genotypic) effect on

nitrogen concentration was only significant in the OLOT experiment. Comparatively, nitrogen concentrations were averagely higher in plants in the OLOT experiment, followed by LLLT, LLOT and OLLT in decreasing order irrespective of the variety and/or N dose.

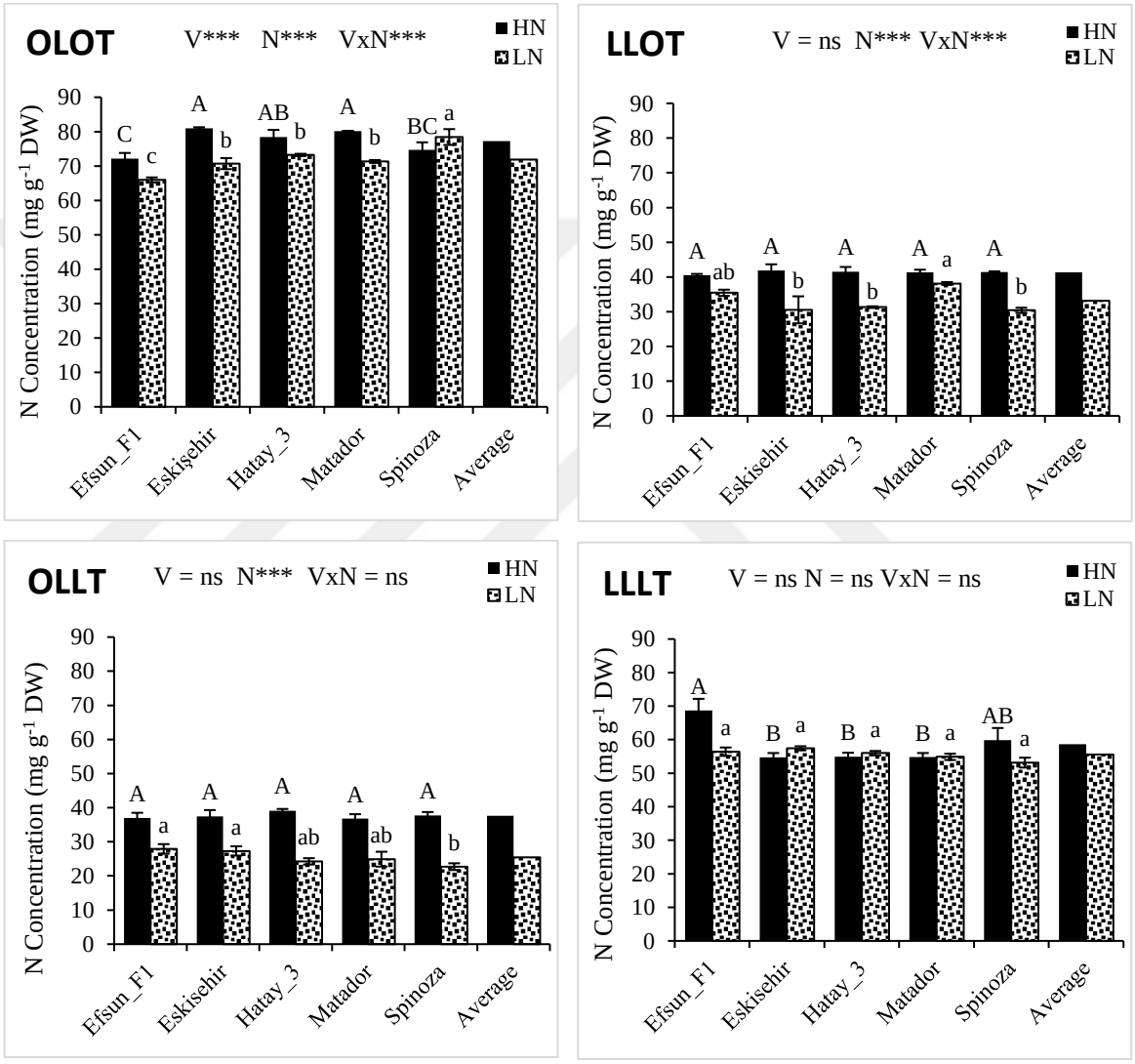


Figure 3.15: Nitrogen concentration in spinach grown under different light-temperature combinations and two N treatments. Letters denote the Tukey grouping of means in alphabetical order. Means with the same upper or low case letters are not significantly different at 5% LSD. V = variety, N = nitrogen, HN = high nitrogen, LN = Low nitrogen, *** = significant ($p < 0.05$), ns = not significant ($p > 0.05$).

3.2.9. Nitrogen Uptake

To get a clearer picture of how much nitrogen is accumulated by the spinach plants, we calculated the total nitrogen uptake (NUp) of each plant. The results (Figure 3.16) showed that irrespective of the light-temperature combination, nitrogen dose and/or variety, NUp was higher in plants treated with high N compared to their replicates treated with low N. Statistically, NUp varied significantly ($p < 0.01$) between N treatments. Among the varieties, significant variations were only observed in OLOT and LLLT experiments. Again, the variety-nitrogen interaction was significant only in OLOT and LLLT experiments. Overall, in decreasing order, NUp was higher in plants in the OLOT experiment than LLOT, OLLT and LLLT.

In the OLOT experiment, Eskisehir and Spinoza, respectively, recorded the highest and lowest NUp under high N treatment whereas at low N treatment Efsun-F1 and Matador, respectively, recorded the highest and lowest. In the LLOT Experiment, Spinoza and Efun-F1, respectively, recorded the highest and lowest NUp at high N; at low N Hatay-3 and Matador recorded the highest and lowest NUp, respectively. In the OLLT experiment, Spinoza and Efsun-F1, respectively, had higher and lower NUp at high N treatment while at low N Efsun-F1 recorded the highest and Matador with the lowest. And in the LLLT experiment, Eskisehir recorded the highest NUp and Matador the lowest at high N treatment while Hatay-3 and Efsun-F1 recorded the highest and the lowest, respectively, at low N treatment.

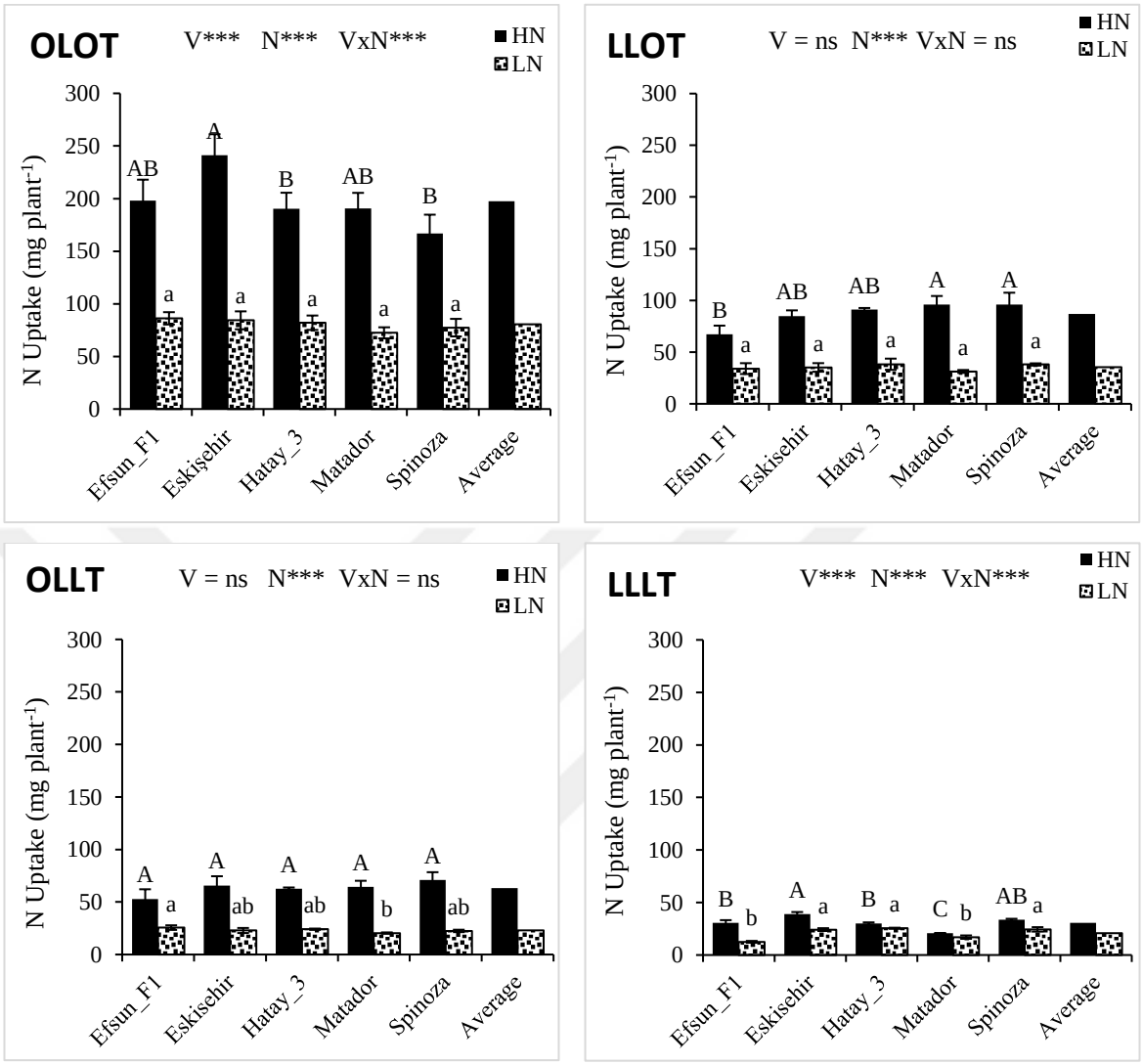


Figure 3.16: Nitrogen uptake of spinach plants grown under different light-temperature combinations and two N treatments. Letters denote the Tukey grouping of means in alphabetical order. Means with the same upper or low case letters are not significantly different at 5% LSD. V = variety, N = nitrogen, HN = high nitrogen, LN = Low nitrogen, *** = significant ($p < 0.05$).

3.2.10. Nitrate concentration

Many leafy vegetables are known for their nitrate (NO_3^-) accumulation capabilities, especially under certain environmental conditions. In the current study, we measured the NO_3^- concentrations of spinach plants as influenced by different growing environments (light-temperature combinations), variety and nitrogen dose. The results showed that NO_3^- concentration (Figure 3.17) was higher in plants treated with high N than those treated with low N regardless of the variety and/or the growing environment. Statistical analysis revealed that highly significant differences ($p < 0.01$) existed between N treatments, and among the varieties. The interaction between variety and nitrogen dose was also significant. Irrespective of nitrogen dose, the average nitrate content was higher in plants in the LLOT followed by LLLT, OLOT and OLLT, in descending. The nitrate levels in spinach plants grown under low light (LL) conditions were averagely higher than that in plants grown under optimum light (OL) conditions irrespective of temperature.

Tukey test ranking of means showed that in the OLOT experiment Hatay-3 and Eskisehir varieties, respectively, had the highest and lowest NO_3^- concentrations at high N treatment, whereas, at low N, Spinoza recorded the highest and Hatay-3 the lowest. In the LLOT experiment, Efsun-F1 at high N and Matador at low N recorded the highest NO_3^- concentrations, whereas Matador at high N and Spinoza at low N recorded the lowest NO_3^- concentrations. In the OLLT experiment, Hatay-3 recorded the highest at both N treatments, whereas Efsun-F1 at high N and Eskisehir at low N recorded the lowest NO_3^- concentrations. In the LLLT experiment, Hatay-3 at high N and Matador at low N recorded the highest NO_3^- concentrations, whereas, Efsun-F1 had the lowest NO_3^- concentration at both N treatments.

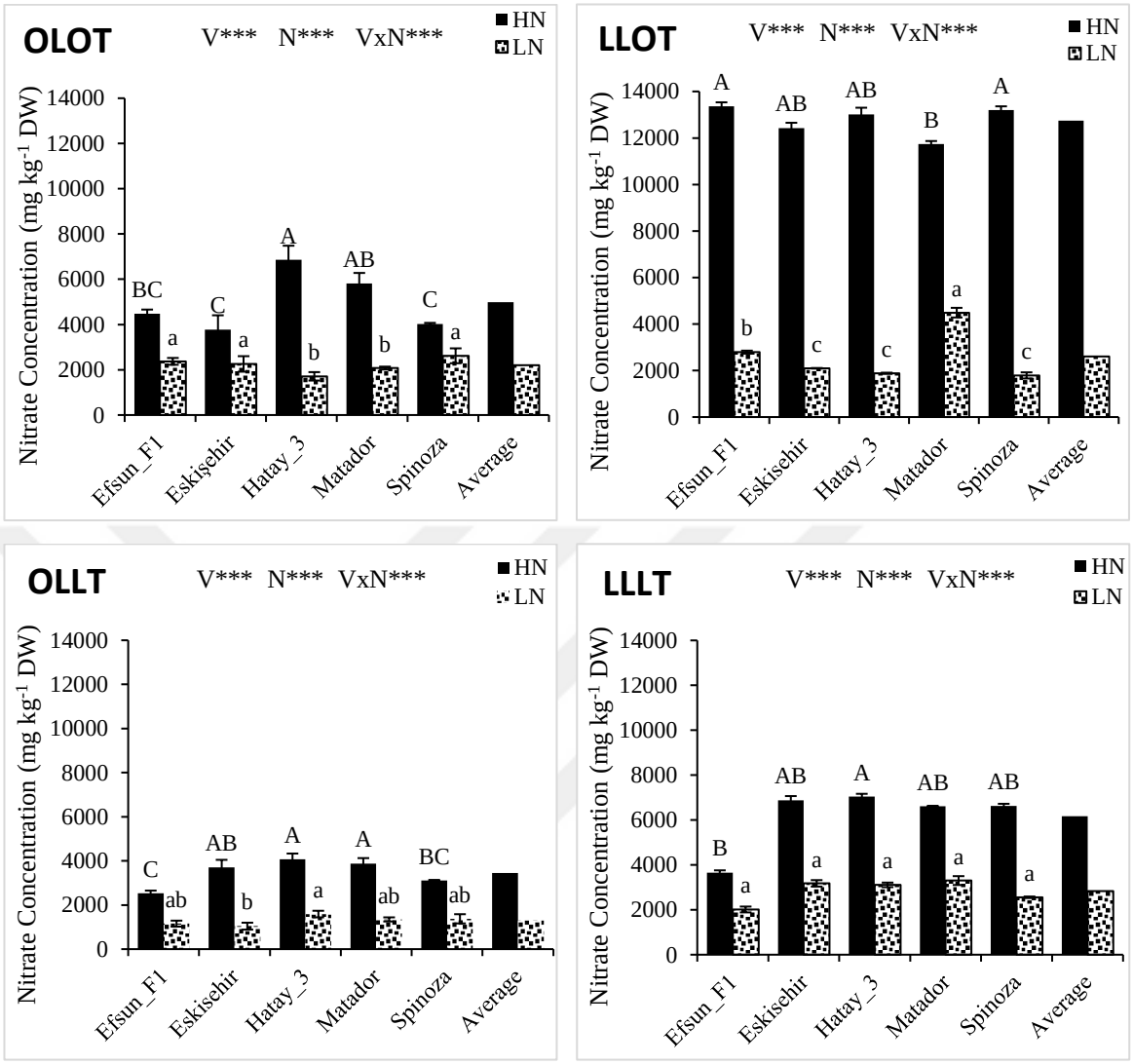


Figure 3.17: Nitrate concentration in spinach grown under different light-temperature combinations and two N treatments. Letters denote the Tukey grouping of means in alphabetical order. Means with the same upper or low case letters are not significantly different at 5% LSD. V = variety, N = nitrogen, HN = high nitrogen, LN = Low nitrogen, *** = significant ($p < 0.05$).

3.2.11. Nitrate Reductase Enzyme Activity

One of the important processes in nitrogen metabolism is the reduction of nitrates to ammonium by the nitrate reductase enzyme especially if nitrogen is taken up in the form of nitrates. Measuring nitrate reductase activity (NRA) of plants is thus vital to understand how efficient a genotype is in transforming nitrates to usable forms under certain growing conditions. Analysis of variance on leaf NRA of spinach varieties (Figure 3.18) grown under different environments (light-temperature combinations) showed that NRA was significantly ($p < 0.01$) affected by the growing environment. The effect of nitrogen dose was significant in OLOT, LLOT and LLLT experiments. Significant difference also existed between spinach varieties in all the experiments except in the LLLT experiment. Again, except in the LLLT experiment, variety-nitrogen interaction was significant in all other experiments. Averagely, NRA rate under the various growing conditions was OLLT, LLOT, OLOT and LLLT in descending order for high N and OLLT, LLLT, LLOT and OLOT for low N.

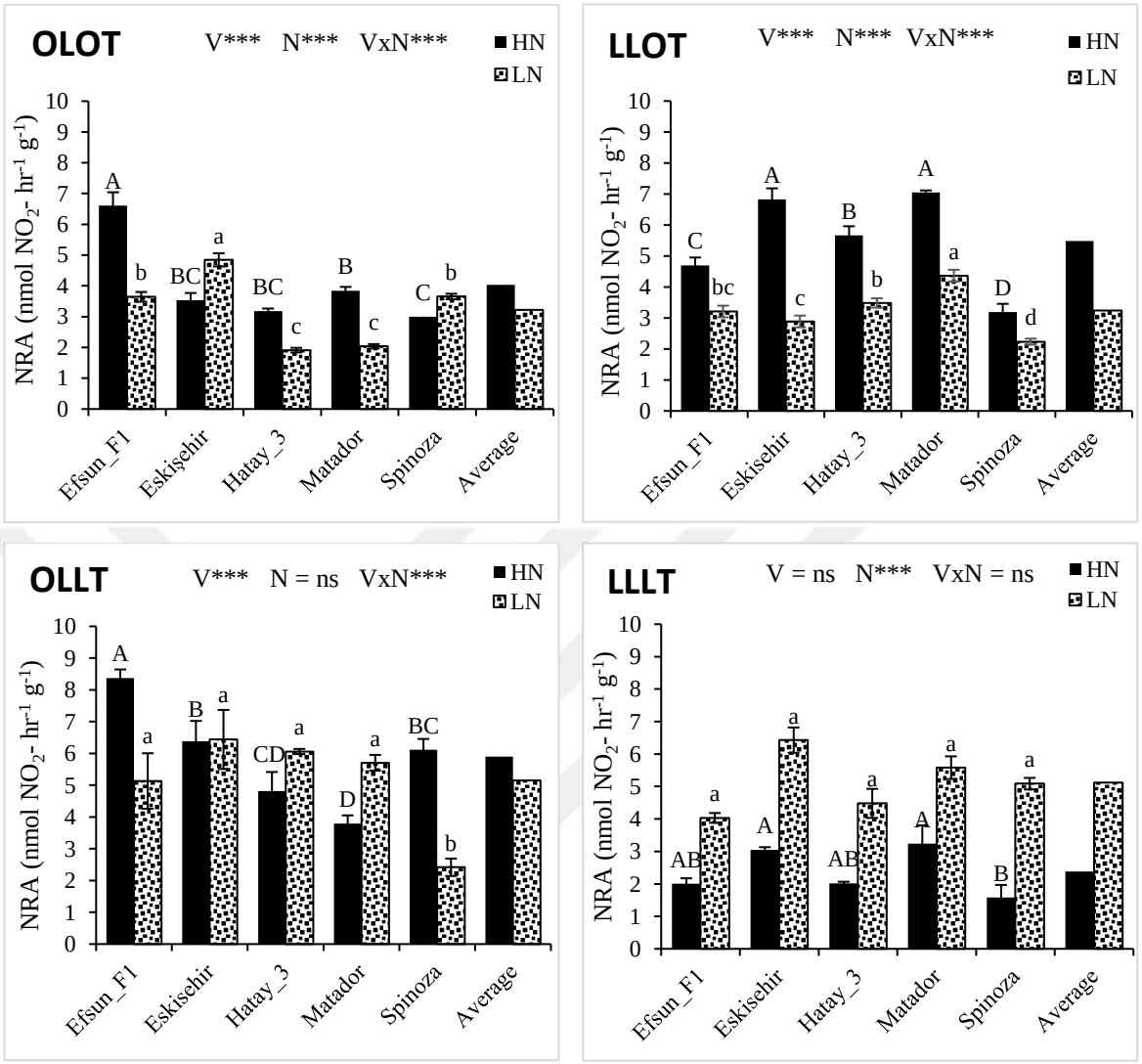


Figure 3.18: Leaf nitrate reductase activity rate in spinach grown under different light-temperature combinations and two N treatments. Letters denote the Tukey grouping of means in alphabetical order. Means with the same upper or low case letters are not significantly different at 5% LSD. V = variety, N = nitrogen, HN = high nitrogen, LN = Low nitrogen, *** = significant ($p < 0.05$), ns = not significant ($p > 0.05$).

3.2.12. Chlorophyll Concentration

Results (Figure 3.19) showed that significant variations ($p < 0.01$) existed among the spinach varieties under investigation. However, N dose and the interaction between variety and nitrogen were not significant. Averagely, leaf chlorophyll contents were slightly higher in plants grown under low temperature (LT) conditions than optimum temperature (OT) conditions irrespective of light intensity and N dose. This is because at low-temperature conditions leaf expansion is reduced resulting in the concentration of more pigments per unit area of the leaf. Overall, variant light-temperature combinations affected leaf chlorophyll content. Leaf chlorophyll contents were higher under the OLLT experiment, followed by LLLT, LLOT and OLOT in decreasing order irrespective of N treatment.

3.2.13. Net Photosynthetic Rate

Results showed that in all the four experiments, spinach plants treated with high nitrogen had higher net photosynthetic rates than low nitrogen treated replicates (Figure 3.20). Analysis of variance revealed that significant differences ($p < 0.01$) existed between nitrogen treatments in all but LLLT experiment. Varietal effect was only significant in plants grown under optimum temperatures (OLOT and LLOT experiments). Variety-nitrogen interaction was not significant. Comparing the average net photosynthetic rate of all the four experiments shows that at high N treatment, rates were higher in OLLT, OLOT, LLLT and LLOT in descending order; while in low N treated plants the trend was OLLT, LLLT, LLOT and OLOT.

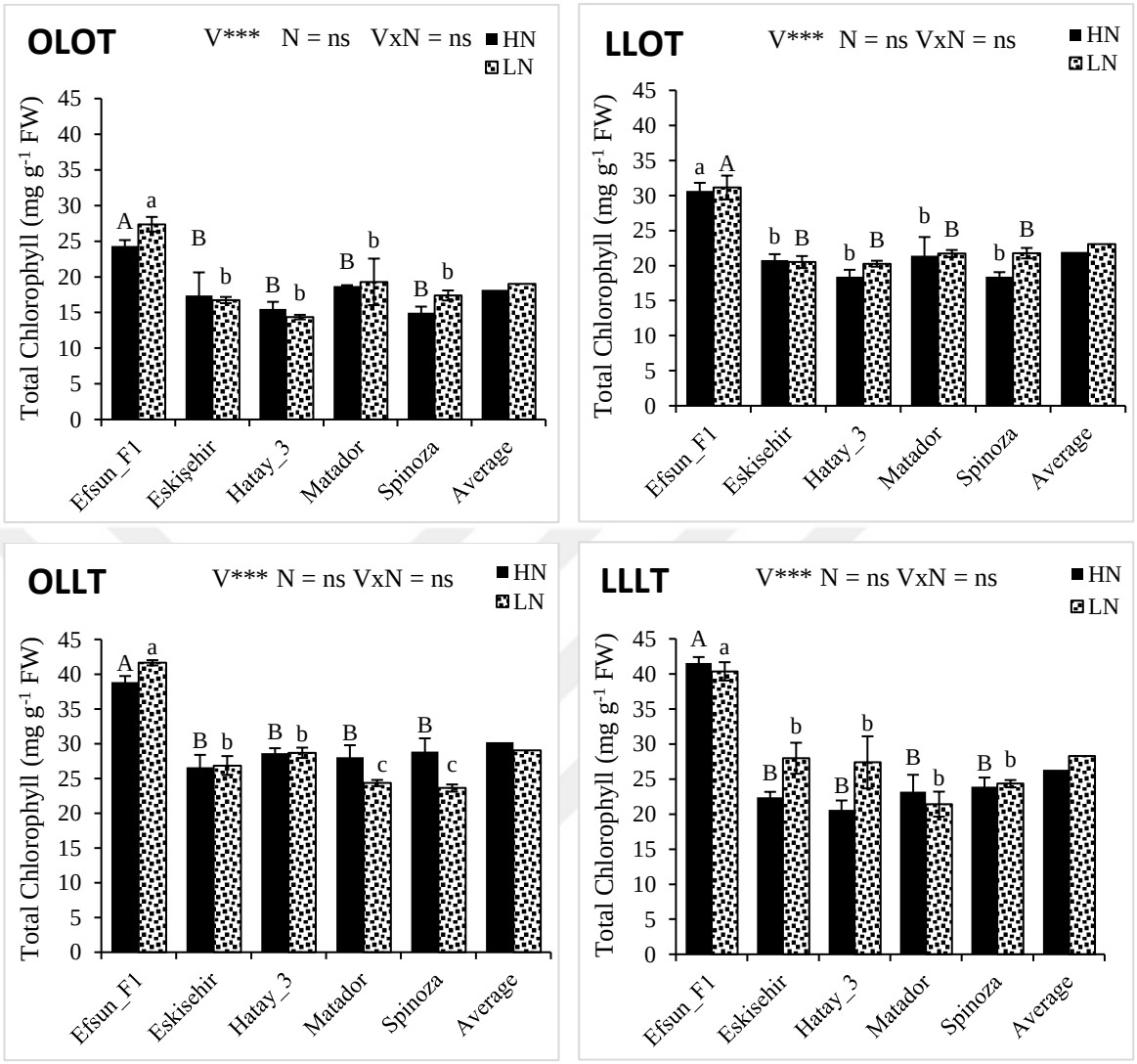


Figure 3.19: Total chlorophyll content in leaves of spinach grown under different light-temperature combinations and two N treatments. Letters denote the Tukey grouping of means in alphabetical order. Means with the same upper or low case letters are not significantly different at 5% LSD. V = variety, N = nitrogen, HN = high nitrogen, LN = Low nitrogen, *** = significant ($p < 0.05$), ns = not significant ($p > 0.05$).

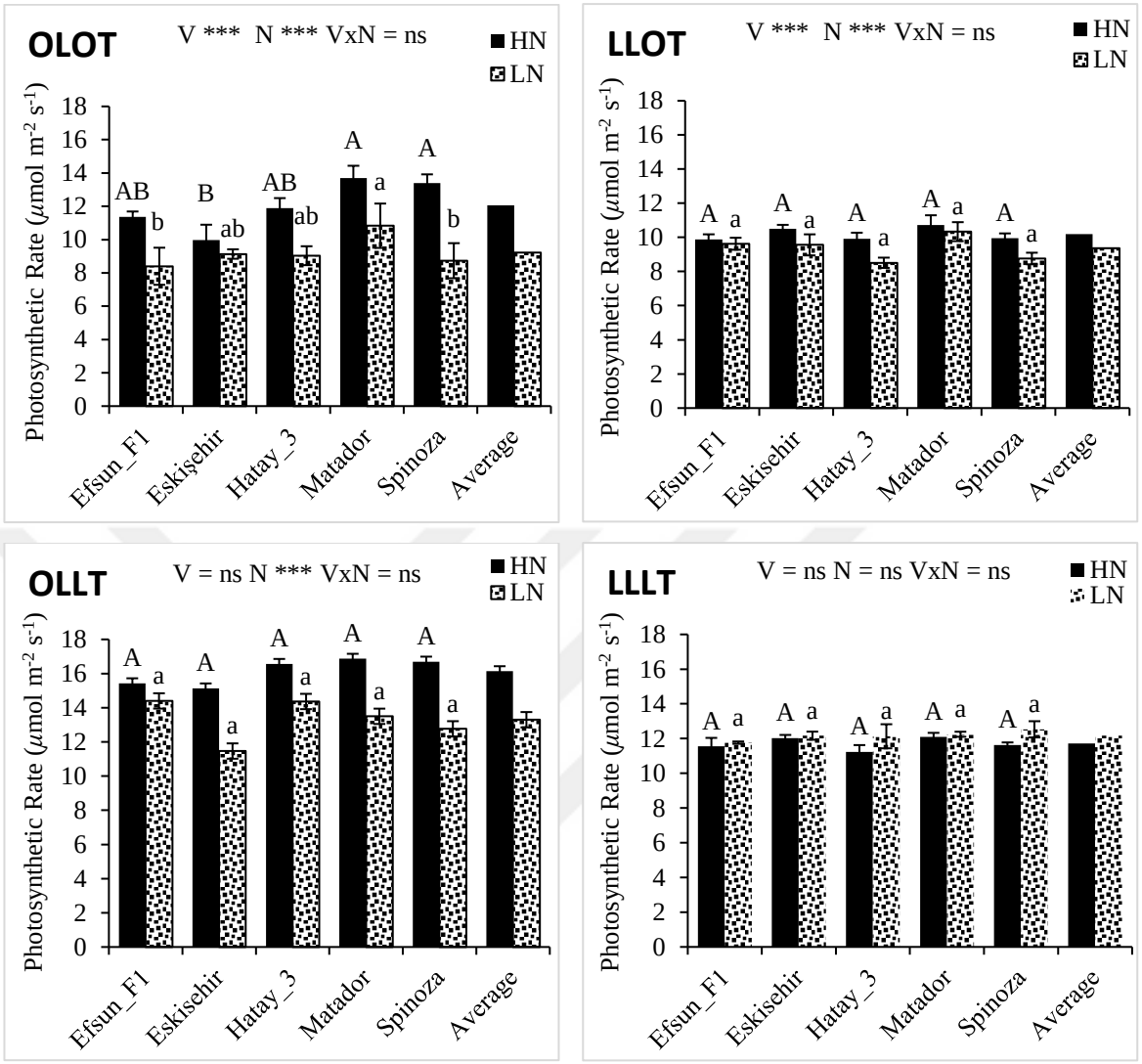


Figure 3.20: Photosynthetic rate in leaves of spinach plants grown under different light-temperature combinations and two N treatments. Letters denote the Tukey grouping of means in alphabetical order. Means with the same upper or low case letters are not significantly different at 5% LSD. V = variety, N = nitrogen, HN = high nitrogen, LN = Low nitrogen, *** = significant ($p < 0.05$), ns = not significant ($p > 0.05$).

3.2.14. Correlations

A 2-tailed Pearson's correlation analysis was performed on the measured parameters as presented in Tables 3.3, 3.4, 3.5 and 3.6 for OLOT, LLOT, OLLT and LLLT experiments, respectively. Correlations in white (below the diagonal) and grey (above the diagonal) represents high and low nitrogen, respectively.

In OLOT experiment, significant positive correlations existed between SFW, SDW, RDW, RL and NUp in high N treated plants whereas in low N treated plants, significant correlations existed between SFW, LA, SDW, RL and NUp. Nitrate content was negatively correlated with almost all the measured parameters in both high and low N treatments. (Table 3.3).

In LLOT experiment, significant positive correlations existed between SFW, SDW, RDW, LA, RL and NUp, regardless of nitrogen treatment. Again, nitrate content was negatively correlated with almost all other parameters irrespective of N dose (Table 3.4).

In OLLT experiment, significant positive correlations existed between SFW, SDW, RDW and NUp in high N treated plants. In low N treated plants, significant correlations existed between SFW, LA, RDW, RL and NC. Correlation between nitrate content and other parameters was mostly weak and negative, especially in high N treated plants (Table 3.5).

In the LLLT experiment, SFW and SDW were significantly correlated with LA and NUp irrespective of N treatment. Correlations in low N treated plants were however stronger. In low N condition, RDW and LN also correlated strongly with the forementioned parameters (Table 3.6).

Table 3.3. OLOT Experiment Results Correlation Table. Correlations between shoot fresh weight (SFW), leaf area (LA), leaf number (LN), shoot dry weight (SDW), root dry weight (RDW), root shoot ratio (R:S), root length (RL), nitrogen concentration (NC), nitrogen uptake (NUp), average, nitrate content, nitrate reductase activity (NRA), total chlorophyll (Chl_{a+b}) and net photosynthesis (NP) of high N and low N treated plants. Correlations in white and grey below and above the diagonal refer to low and high nitrogen levels respectively. * = significant correlation ($p < 0.05$).

	SFW	LA	LN	SDW	RDW	R:S	RL	NC	NUp	Nitrate Content	NRA	Chl_{a+b}	NP
SFW		0.709 *	0.100	0.296	0.404	0.183	0.770 *	-0.649 *	0.041	-0.209	0.285	0.247	-0.169
LA	0.253		0.625 *	0.307	0.283	0.066	0.761 *	-0.202	0.261	-0.331	0.207	-0.326	-0.304
LN	0.111	0.269		-0.237	-0.385	-0.287	0.305	0.506	-0.010	-0.071	0.167	-0.692 *	-0.154
SDW	0.733 *	0.160	-0.033		0.849 *	0.313	0.209	-0.404	0.905 *	-0.039	0.258	0.422	-0.302
RDW	0.736 *	0.086	-0.251	0.959 *		0.552 *	0.490	-0.657 *	0.638 *	-0.283	-0.012	0.378	-0.017
R:S	0.409	-0.097	-0.281	0.519 *	0.603 *		0.257	-0.373	0.174	-0.542 *	-0.217	0.101	0.114
RL	0.878 *	0.072	0.047	0.722 *	0.768 *	0.430		-0.488	0.043	-0.442	0.054	-0.134	0.010
NC	0.030	0.169	0.145	0.263	0.072	-0.196	-0.254		0.020	0.203	-0.074	-0.557 *	0.027
NUp	0.683 *	0.200	0.026	0.972 *	0.884 *	0.429	0.606 *	0.478		0.047	0.247	0.183	-0.301
Nitrate Content	-0.729 *	-0.449	-0.287	-0.627 *	-0.597 *	-0.241	-0.722 *	0.094	-0.552 *		0.434	0.216	0.041
NRA	0.246	-0.405	-0.639 *	0.37	0.586 *	0.337	0.479	-0.491	0.198	-0.197		0.187	-0.349
TC	0.223	-0.291	-0.568 *	0.511	0.658 *	0.524 *	0.464	-0.299	0.381	-0.065	0.747 *		-0.280
NP	-0.263	0.309	-0.227	-0.279	-0.255	0.007	-0.419	-0.085	-0.276	0.230	-0.187	-0.059	

Table 3.4. LLOT Experiment Results Correlation Table. Correlations between shoot fresh weight (SFW), leaf area (LA), leaf number (LN), shoot dry weight (SDW), root dry weight (RDW), root shoot ratio (R:S), root length (RL), nitrogen concentration (NC), nitrogen uptake (NUp), average, nitrate content, nitrate reductase activity (NRA), total chlorophyll (Chl_{a+b}) and net photosynthesis (NP) of high N and low N treated plants. Correlations in white and grey below and above the diagonal refer to low and high nitrogen levels respectively. * = significant correlation ($p < 0.05$).

	SFW	LA	LN	SDW	RDW	R:S	RL	NC	NUp	Nitrate Content	NRA	Chl_{a+b}	NP
SFW		0.685 *	0.378	0.894 *	0.574 *	-0.155	0.442	-0.713 *	0.610 *	-0.793 *	-0.597 *	-0.412	-0.707 *
LA	0.789 *		0.353	0.590 *	0.534 *	0.058	0.561 *	-0.653 *	0.301	-0.797 *	-0.394	-0.396	-0.551 *
LN	0.602 *	0.639 *		0.372	0.652 *	0.425	0.458	-0.416	0.155	-0.161	-0.165	-0.538 *	0.083
SDW	0.750 *	0.732 *	0.672 *		0.636 *	-0.203	0.462	-0.591 *	0.814 *	-0.697 *	-0.519 *	-0.247	-0.602 *
RDW	0.834 *	0.748 *	0.848 *	0.854 *		.609*	0.885 *	-0.541 *	0.416	-0.409	-0.376	-0.322	-0.402
R:S	-0.009	0.141	0.400	0.211	0.334		0.624 *	-0.027	-0.253	0.255	0.172	-0.217	0.074
RL	0.741 *	0.579 *	0.670 *	0.716 *	0.858 *	-0.063		-0.548 *	0.210	-0.383	-0.334	-0.132	-0.455
NC	0.183	0.106	0.225	0.089	0.148	0.032	0.159		-0.018	0.727 *	0.677 *	0.392	0.519 *
NUp	0.763 *	0.719 *	0.688 *	0.978 *	0.848 *	0.207	0.715 *	0.295		-0.378	-0.178	0.012	-0.394
Nitrate Content	-0.002	-0.132	-.602 *	-0.25	-0.364	-0.678 *	-0.131	-0.135	-0.263		0.774 *	0.157	0.624 *
NRA	-0.047	-0.082	0.472	0.017	0.288	0.478	0.206	0.244	0.06	-0.725 *		0.038	0.528 *
TC	-0.600 *	-0.822 *	-0.774 *	-0.564 *	-0.615 *	-0.096	-0.498	-0.176	-0.571 *	0.238	-0.044		0.223
NP	0.012	0.128	0.228	0.02	0.209	0.606 *	0.113	0.337	0.083	-0.520 *	0.451	-0.043	

Table 3.5. OLLT Experiment Results Correlation Table. Correlations between shoot fresh weight (SFW), leaf area (LA), leaf number (LN), shoot dry weight (SDW), root dry weight (RDW), root shoot ratio (R:S), root length (RL), nitrogen concentration (NC), nitrogen uptake (NUp), average, nitrate content, nitrate reductase activity (NRA), total chlorophyll (Chl_{a+b}) and net photosynthesis (NP) of high N and low N treated plants. Correlations in white and grey below and above the diagonal refer to low and high nitrogen levels respectively. * = significant correlation ($p < 0.05$).

	SFW	LA	LN	SDW	RDW	R:S	RL	NC	NUp	Nitrate Content	NRA	Chl_{a+b}	NP
SFW		0.918 *	0.279	0.487	0.532 *	0.086	0.815 *	-0.886 *	-0.111	0.467	-0.39	-0.729 *	-0.218
LA	0.630 *		0.210	0.392	0.448	0.107	0.749 *	-0.879 *	-0.232	0.368	-0.394	-0.725 *	-0.253
LN	0.307	0.421		0.382	0.536 *	0.226	0.437	-0.296	0.286	0.534 *	0.115	-0.266	0.073
SDW	0.913 *	0.483	0.310		0.448	-0.319	0.537 *	-0.701 *	0.767 *	0.550 *	-0.463	0.027	0.120
RDW	0.542 *	0.399	0.329	0.635 *		0.678 *	0.383	-0.471	0.177	0.129	-0.256	-0.493	-0.13
R:S	-0.658 *	-0.317	-0.152	-0.658 *	0.135		-0.109	0.088	-0.404	-0.340	0.121	-0.496	-0.332
RL	0.456	0.444	0.335	0.319	0.156	-0.334		-0.804 *	0.058	0.549 *	-0.324	-0.458	0.102
NC	0.284	0.279	0.361	0.320	-0.029	-0.466	0.228		-0.089	-0.556 *	0.548 *	0.520 *	0.162
NUp	0.863 *	0.482	0.377	0.958 *	0.554 *	-0.685 *	0.334	0.573 *		0.285	-0.150	0.504	0.331
Nitrate Content	0.256	0.242	0.028	-0.001	-0.327	-0.352	0.241	-0.011	-0.027		-0.123	-0.215	0.137
NRA	-0.494	-0.475	0.173	-0.219	0.120	0.446	-0.397	0.183	-0.108	-0.684 *		0.218	0.023
TC	-0.481	-0.470	-0.331	-0.239	-0.093	0.333	-0.479	-0.029	-0.194	-0.709 *	0.683 *		0.376
NP	0.515 *	0.398	-0.087	0.280	0.402	0.065	0.211	-0.138	0.197	0.13	-0.380	-0.068	

Table 3.6. LLLT Experiment Results Correlation Table. Correlations between shoot fresh weight (SFW), leaf area (LA), leaf number (LN), shoot dry weight (SDW), root dry weight (RDW), root shoot ratio (R:S), root length (RL), nitrogen concentration (NC), nitrogen uptake (NUp), average, nitrate content, nitrate reductase activity (NRA), total chlorophyll (Chl_{a+b}) and net photosynthesis (NP) of high N and low N treated plants. Correlations in white and grey below and above the diagonal refer to low and high nitrogen levels respectively. * = significant correlation ($p < 0.05$).

	SFW	LA	LN	SDW	RDW	R:S	RL	NC	NUp	Nitrate Content	NRA	Chl_{a+b}	NP
SFW		0.921 *	0.757 *	0.898 *	0.830 *	-0.193	0.674 *	0.060	0.911 *	0.593 *	0.308	-0.527 *	0.089
LA	0.867 *		0.724 *	0.810 *	0.703 *	-0.256	0.491	0.084	0.823 *	0.501	0.207	-0.330	0.171
LN	0.602 *	0.533 *		0.816 *	0.735 *	-0.153	0.509	0.021	0.816 *	0.446	0.011	-0.459	-0.076
SDW	0.844 *	0.908 *	0.467		0.929 *	-0.232	0.639 *	-0.091	0.986 *	0.403	0.313	-0.432	0.096
RDW	0.258	0.450	0.477	0.429		0.130	0.704 *	-0.237	0.890 *	0.357	0.321	-0.553 *	0.101
R:S	-0.545 *	-0.404	-0.105	-0.480	0.560 *		0.165	-0.301	-0.28	-0.003	-0.008	-0.358	0.008
RL	0.213	0.266	0.356	0.320	0.720 *	0.365		0.208	0.689 *	0.259	0.001	-0.440	-0.079
NC	-0.476	-0.343	-0.467	-0.284	0.283	0.667 *	0.202		0.072	0.110	-0.224	0.241	-0.237
NUp	0.599 *	0.733 *	0.206	0.861 *	0.550 *	-0.165	0.418	0.239		0.423	0.276	-0.39	0.057
Nitrate Content	0.717 *	0.615 *	0.536 *	0.403	-0.127	-0.578 *	-0.020	-0.660 *	0.075		0.362	-0.702 *	0.273
NRA	0.168	0.012	-0.077	0.081	-0.568 *	-0.581 *	-0.417	-0.225	-0.025	0.169		-0.33	-0.017
TC	-0.671 *	-0.573 *	-0.495	-0.345	0.149	0.543 *	0.034	0.674 *	-0.004	-0.969 *	-0.213		-0.091
NP	-0.374	-0.285	-0.243	-0.173	0.027	0.238	-0.028	0.539 *	0.109	-0.611 *	0.301	0.622 *	

CHAPTER FOUR

DISCUSSION

4.1. Temperature Effect on Spinach Biomass Production

One of the environmental factors that affects plant development is temperature. Plant growth, metabolism, reproduction and distribution across regions is greatly influenced by temperature (Sinha, 2004). Plants neither like extreme higher nor lower temperatures as both conditions affect development. Spinach is well known for its adaptiveness to cooler temperatures, however, its growth, development is retarded and quality is compromised when grown under temperatures below optimum. As seen in our results, shoot fresh and dry biomasses of all spinach varieties were higher under optimum temperatures compared to low temperatures irrespective of light intensity and/or nitrogen dose. This observation is in agreement with Zhou et al. (2019) who reported a decrease in biomass production of lettuce grown under low temperature conditions compared higher temperatures irrespective light intensity. Similar results were reported by Galieni et al. (2016) on lettuce grown at different seasons (spring and summer) using same light intensity. They observed an increase in leaf area and dry biomass in summer than in spring regardless of light intensity. These observations are true because, plant physiological activities decline at lower temperatures resulting in the shortage of metabolites needed for growth. This is evident in our results presented: leaf area and leaf number were lower at low temperature conditions than at optimum temperature, and thus confirms the reasons for the observed

higher shoot fresh and dry biomasses in plants grown under optimum temperature conditions. Even though spinach is known for its tolerance to cool climates, its rate of development is affected by temperatures below 10°C and. Comparing the developmental ages of the second pair of spinach leaves, Boese & Huner (1990) reported that, it took 92 days for plants grown under 5°C to reach full leaf expansion compared to 32 days under 16°C. At lower temperatures growth and yield of crops is usually reduced due to cell division inhibition, deactivation of enzymes, reduced water and mineral absorption, inhibition of photosynthesis etc. (Sinha, 2004; Gruda, 2005; Zhou et al., 2019).

The effect of nitrogen dose on plant growth and development cannot be overlooked. As seen in our results, nitrogen dose has positively affected spinach biomass production, leaf area and leaf number irrespective of growing condition. Under limiting nitrogen conditions stunted growth occur in plants due to inhibition of cell division, cell enlargement and reduce respiration (Sinha, 2004) leading to fewer leaves, smaller leaf area (D. Zhao et al., 2003), accelerates leaf senescence, reduces light use efficiency (Uhart & Andrade, 1995) and consequently causing low biomass production and yield.

Temperature also has a significant effect on root growth as well as nutrient uptake. A plant survival under various environmental stress conditions is closely connected to its root architecture (Bohn et al., 2006). A strong root system improves nutrient uptake and provides anchorage (Sen et al., 2012). During the fruit development stage in tomato, bell pepper and cucumber, Schnitzler & Gruda (2003) observed an improved water and nutrient uptake under optimum temperatures, due to a constant new growth of roots. At suboptimal temperatures on the other hand, root growth is reduced which limits the plants' ability to absorb nutrients, which in turn affects growth and development of the plant. This is evident in our results; root growth was heavily reduced at low temperature

conditions thereby causing a decrease in the growth of spinach plants under investigation. However, root growth does not always decrease at low temperatures if other factors such as light is abundant as was observed in spinach plants grown in optimum light and low temperature (OLLT) experiment. We believe this increment in root growth at low temperature condition is due to increased photosynthetic rate at the presence of high light intensity, making available large quantities of photo assimilates to the roots in quest for more ion/nutrient uptake. In order to withstand a certain environmental stress, plants do prioritise the allocation of photo assimilates (Markesteijn & Poorter, 2009) to parts responsible for acquiring the limiting resource (Deng et al., 2020). For instance, it can be seen from our results that, spinach plants treated with low nitrogen has higher root-shoot ratios than their replicates treated with high nitrogen regardless of the light-temperature combination. Our results further showed that the allocations of photoassimilates to the roots is further increased by low temperature stress, resulting in increased root-shoot ratios in plants growing under low temperature. Thornton & Robinson (2005) reported a similar observation on pepper.

4.2. Light Effect on Spinach Biomass Production

Aside temperature, light (intensity, duration and form/spectrum) is one of the major environmental factors that has a great influence on plant growth, development, distribution, yield and quality. Plant growth, development and physiology is significantly affected by the quality of light (Ohashi-Kaneko et al., 2007; Xu et al., 2021) and adjusting it appropriately helps improve nutrient absorption (Xu et al., 2021), yield and quality of vegetables (Hanyu & Shoji, 2000; Xu et al., 2021).

As our results revealed, the average performance of spinach plants growing under optimum light intensity were better in terms of biomass productions irrespective of temperature and nitrogen dose. This increased in growth and dry matter accumulation is as a result of increase photosynthetic rates at optimal light intensities. Under poor lightening conditions, full stomatal opening does not occur and thus gas diffusion and for that matter, photosynthesis is less, resulting in a reduction in dry matter accumulation. In our experiments, we observed a decrease in biomass production in plants grown under low light intensities irrespective of temperature and nitrogen dose. This observation of ours in agreement with Proietti et al. (2004) who in a similar experiment on spinach grown under two light intensities (high = 800 μmol m^{-2} s^{-1} and low = 200 μmol m^{-2} s^{-1}) observed a decreased in growth and leaf area in plants grown under low intensity compared to those grown under high intensity. This decrease in biomass production according to Proietti et al. (2004) may be due to a reduction in photosynthetic rate. Lower daily light intensities is said to be contributing to suboptimal yields of greenhouse vegetables (Gruda, 2005). Other researchers have reported on the effect of different forms of light on plant development. Supplementing natural light with artificial light is reported an increased growth and dry matter accumulation in various vegetables (Grimstad, 1991; Hanyu & Shoji, 2002; Hikosaka et al., 2013, 2017). By increasing the light intensity with supplemental light, Gaudreau et al. (1994) observed a more than 270% increase in biomass production and a 30% shortened production cycle in lettuce grown in a controlled environment compared to natural light only. Galieni et al. (2016) also reported a reduction in leaf number and biomass in lettuce plants grown under artificial shading induced low light conditions irrespective of growing season (spring or summer).

4.3. Temperature and Light Effect on N Uptake and N Concentration

Aside moisture, temperature and light are the other major environmental factors influencing nutrient absorption and metabolism in plants. Plants have specific temperature and light requirements, outside which their physiological and morphological developments will be hampered. The interaction between temperature, light and water controls CO_2 assimilation, nutrient uptake and partitioning. In this present study, we observed that irrespective of nitrogen dose, nitrogen uptake was generally higher in spinach plants grown under optimum light or optimum temperature conditions compared to the suboptimal low conditions of both factors. The main contribution factor to this increase in nitrogen uptake is enhanced growth (biomass accumulation) of spinach plants grown under either optimum temperature or optimum light intensity.

Nitrogen uptake is regulated by plant metabolic activities (photosynthesis, respiration etc.) and as such is affected by temperature, which has a great influence on them. Nitrogen absorption increases with increasing temperature due active new root growth (Schnitzler & Gruda, 2003) and increase metabolic rates and growth. In an experiment on conifers, Boczulak et al. (2014) reported an increase in nitrogen uptake with increasing temperature. Similarly, in a solution culture experiment, Thornton & Robinson (2005) found that the nitrogen content of grafted pepper plants grown under high temperature conditions were more than double of that grown under low temperature conditions, which they attributed to high dry mass production.

On the other hand, the quality of light, its intensity and duration also has a significant influence in the uptake and utilisation of nutrients (Lin et al., 2020; Sakuraba & Yanagisawa, 2018; Cui et al., 2019; Xu et al., 2021). Nitrogen uptake, translocation and assimilation are regulated by light signals (Lillo, 2008). At an optimum temperature,

increasing light intensity usually results in an increase photosynthetic activity in plants, which also translates to active and rapid growth and hence the need for more nutrient uptake. Joniyas et al. (2016) reported an increased in nitrogen (nitrate and ammonium) and phosphorus absorption in filiform sea moss (*Gracilaria manilaensis*) at increased light intensity.

4.4. Temperature and Light Effect on Nitrate Content of Spinach

Spinach is known for its many health benefits in human diet however it is also known to be a vegetable that can accumulate higher amounts of nitrates if cultivated under suboptimal environmental conditions of temperature, light, water and nutrition. In addition, plant internal factors (e.g. metabolic activity) (Márquez-Quiroz et al., 2014) and plant species/cultivars (Gil et al., 2012) influences nitrate accumulation in leafy vegetables. The results of this study indicates that nitrate content of spinach plants was affected by temperature, light, nitrogen dose and cultivar.

Excessive nitrogen fertiliser use, especially nitrate fertilizers and the timing of application may result in higher nitrate accumulation. Leafy vegetables does accumulate large amounts of nitrogen in the form of nitrate (NO_3^-) especially with excess application of NO_3^- , to increase green colour and succulence (Ryder, 1979). Spinach may accumulate high concentrations of nitrates when supplied with non-limiting nitrate concentrations in the growth medium (Steingröver et al., 1982). Data from our experiments confirms this, as spinach plants treated with high nitrogen (3 mM) had more nitrates content than those grown under low nitrogen conditions (0.3 mM) regardless of growing condition and spinach cultivar.

On the effect of temperature, it was observed that spinach plants grown under higher temperature conditions had higher nitrate contents than their replicates at low temperature irrespective of light intensity. Our observations is in agreement with Van Der Boon et al. (1990) who reported higher nitrate contents in lettuce grown at higher temperatures compared to low temperatures. Similar results were reported in radish by Nieuwhof (1994). The reason for this high nitrate accumulation according to Laurie & Stewart (1993) is that, high temperatures do not alter root activity but does cause a reduction in leaf nitrate reductase activity, and thus resulting in excess nitrate accumulation.

Light intensity is another factor that influences nitrate content in plants. In lettuce grown at different light intensities in a nutrient solution with constant concentration, (Fang, 2018 as cited in Xu et al., 2021) observed that nitrate content of plants treated with low light intensity had higher nitrate content compared to those treated with high light intensity. Similar results were recorded from our experiments on spinach: nitrate content were high in plants grown under low light intensity than plants under high light intensity regardless of temperature. Proietti et al. (2004) in a research on the effect of two light intensities (800 and 200 μmol quanta $m^{-2}s^{-1}$) on spinach reported that, the nitrate content in spinach leaves was lower at high light and higher at low light intensities. The reasons for higher nitrate contents of plants grown under low light intensities can be related to: (1) reduce nitrate reductase activity rate and (2) low photosynthesis. Nitrate reduction rate is decreased under low light conditions (Chadjaa et al., 2019; Dapoigny et al., 2000; Gaudreau et al., 1994; Lillo, 1994, 2008) and thus results in higher nitrate contents in leaves. Photosynthetic efficiency on the other hand is reduced at low light intensities (Xu et al., 2021) resulting in less photoassimilates to maintain osmotic pressure. This causes increased nitrate uptake to fill up the vacuum created (Anjana et al., 2007; Rouphael et al., 2012) thereby leading to higher nitrate contents in the plant.

4.5. Genotypic Difference in Biomass Production and Nitrate Accumulation

It is an established fact that genotypes of a plant species could be influenced differently by the same growing environment as each genotype's mode of interaction with the environment may differ. Varietal differences in biomass production and NUE (Chan-Navarrete et al., 2014; Giordano et al., 2021; Stagnari et al., 2007) as well as difference in nitrate accumulation (Barker et al., 1971; Maynard & Barker, 1979; Tang et al., 2013; Wang et al., 2018) among spinach genotypes grown under same environmental conditions and nitrogen treatments have been reported. Our findings are in line with these reports. The results from this study showed that genotypic differences existed in almost all the parameters measured, more especially in biomass production and nitrate accumulation. The observed factors that contributed to genotypic differences in biomass accumulation was leaf area and root morphology, as evident in the correlation tables (Section 3.2.14). It can thus be said that the observed differences among the varieties across all the experiments is mainly due to their genotypic capabilities to absorb nutrients (a strong root system) and light interception efficiency by leaves. Root morphology has been reported to have a linear correlation with biomass production and yield in rice (Yang et al., 2012). Similarly, light interception and use efficiency has been associated with increased dry matter accumulation and yield in various crops (Haverkort, 2007; Li et al., 2013) The Varieties with higher biomasses also have corresponding higher nitrogen uptakes. It is thus important to consider the uptake system and light interception efficiency when selecting spinach varieties for production in suboptimal conditions.

Though high biomass yield is important in spinach production, one thing of a major concern is nitrate accumulation because of the numerous health risks it poses. While environmental factors and cultural practices are well known for their role in nitrate

accumulation in spinach, less is spoken about the varietal (genotypic) effect. As shown in our results, variety had a significant effect in nitrate accumulation across all the four experiments. also Genotypic differences in nitrate accumulation was reported by Barker et al. (1971), Huang et al. (2010), Tang et al. (2013), and Wang et al. (2018). The differences could be attributed to variations in nitrate uptake and assimilation (reduction) capabilities of the genotypes. This was the view of many other authors on this topic (Burns et al., 2011; Maynard & Barker, 1979; Reinink & Eenink, 1988; Tang et al., 2013; Wang et al., 2018). Plants may turn to use nitrates to maintain osmoticum other than photo assimilates especially when nitrate is available in excess. Therefore, if a genotype is inefficient in light interception (radiation use efficiency) there would be less photo assimilates to maintain osmoticum leading to absorption of more nitrates. If nitrate absorption is greater than assimilation nitrate concentrations would be higher (Olday et al., 1976).

4.6. Conclusions And Recommendations

Moisture, nutrition, temperature and light are the major abiotic factors that have a great influence on plant growth, development, distribution, yield and quality. Vegetables such as spinach is known for its many health benefits in human diet however it is also known to be a vegetable that has the potential to accumulate higher amounts of nitrates if cultivated under suboptimal environmental conditions of temperature, light and nutrition. In this present study we sort to investigate how different temperature and light combinations affect nitrogen especially nitrate accumulation and metabolism. Based on the results, the following deductions were made.

1. Biomass production and total nitrogen accumulation were enhanced by optimum temperature and light irrespective of nitrogen dose and variety.
2. Low temperatures cause retarded growth but has less effect on nitrate accumulation provided light intensity is optimum.
3. Growing spinach under low light intensities results in the accumulation of higher amounts of nitrates even if temperatures are kept high.
4. Light intensity is the major factor influencing nitrate accumulation in spinach.
5. Though biomass production is high in LLOT experiment, in terms of quality, it is not an ideal light-temperature combination for spinach production as nitrate accumulation was highest under such a condition. Low light low temperature (LLLT) condition is neither ideal for either spinach growth nor quality.
6. Aside environmental factors, variety (genotype) also has an effect on spinach growth and nitrate concentration when grown under suboptimal conditions.
7. Eskisehir and Spinoza varieties are suitable for both optimum and low temperature cultivation as they could give optimum yields but accumulates less

nitrate per dry matter. Nitrate accumulation in the Efsun-F1 variety was low, especially under low temperature conditions than all other varieties, however its biomass production was also low. It is thus not suitable for commercial production but could be used for experimental and breeding purposes.

8. More studies should be done to establish the best lightening (spectrum and source) and temperature combinations that are ideal for giving optimum yields but less nitrate accumulation in spinach, taking into consideration the effect of variety and nitrogen supply.
9. In this present study, only two nitrogen levels were used and hence we could not make a recommendation on a rate for optimum yield but also ideal for less nitrate accumulation. In future experiments nitrogen levels should be increased in order to be able to make a recommendation for producers and researchers.
10. We believe that our findings could be used as a reference for further researches and indoor production of spinach.

REFERENCES

1. Anjana, Umar, S., Iqbal, M. (2007). Nitrate accumulation in plants, factors affecting the process, and human health implications. A review. **Agronomy for Sustainable Development, 27**(1): 45–57.
2. Barker, A. V., Peck, N. H., MacDonald, G. E. (1971). Nitrate accumulation in vegetables. I. Spinach grown in upland soils 1. **Agronomy Journal, 63**(1): 126–129.
3. Beevers, L., Hageman, R. H. (1969). Nitrate reduction in higher plants. **Annual Review of Plant Physiology, 20**(1): 495–522.
4. Blom-zandstra, M., Lampe, J. E. M. (1985). The role of nitrate in the osmoregulation of lettuce (Lactuca sativa L.) grown at different light intensities. **Journal of Experimental Botany, 36**(7): 1043–1052.
5. Boczulak, S. A., Hawkins, B. J., Roy, R. (2014). Temperature effects on nitrogen form uptake by seedling roots of three contrasting conifers. **Tree Physiology, 34**(5): 513–523.
6. Boese, S. R., Huner, N. P. A. (1990). Effect of growth temperature and temperature shifts on spinach leaf morphology and photosynthesis. **Plant Physiology, 94**(4): 1830–1836.
7. Bohn, M., Novais, J., Fonseca, R., Tuberosa, R., Grift, T. E. (2006). Genetic evaluation of root complexity in maize. **Acta Agronomica Hungarica, 54**(3): 291–303.
8. Bot, G. P. A. (2004). The solar greenhouse; technology for low energy consumption. **Acta Horticulturae, 633**, 29–33.

9. Burns, I. G., Zhang, K., Turner, M. K., Meacham, M., Al-Redhiman, K., Lynn, J., Broadley, M. R., Hand, P., Pink, D. (2011). Screening for genotype and environment effects on nitrate accumulation in 24 species of young lettuce. **Journal of the Science of Food and Agriculture, 91**(3): 553–562.
10. Cantliffe, D. J. (1973). Nitrate accumulation in table beets and spinach as affected by nitrogen, phosphorus, and potassium nutrition and light intensity 1. **Agronomy Journal, 65**(4): 563–565.
11. Cataldo, D. A., Haroon, M. H., Schrader, L. E., Youngs, V. L. (1975). Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. **Communications in Soil Science and Plant Analysis, 6**(1): 71–80.
12. Chadjaa, H., Vézina, L.-P., Dubé, S., Gosselin, A. (2019). Effects of supplementary lighting on growth and primary nitrogen metabolism of greenhouse lamb's lettuce and spinach. **HortScience, 30**(4): 883G – 884.
13. Chan-Navarrete, R., Kawai, A., Dolstra, O., Lammerts van Bueren, E. T., van der Linden, C. G. (2014). Genetic diversity for nitrogen use efficiency in spinach (Spinacia oleracea L.) cultivars using the Ingestad model on hydroponics. **Euphytica, 199**(1–2): 155–166.
14. Corre, W. J. (1983). Growth and morphogenesis of sun and shade plants. III. The combined effects of light intensity and nutrient supply. **Acta Botanica Neerlandica, 32**(4): 277–294.
15. Corré, W. J., Breimer, T. (1979). Nitrate and nitrite in vegetables. Centre for Agricultural Publishing and Documentation, Wageningen, 89pp.
16. Cui, J., Lei, B., Liu, H. (2019). Effect of daily light integral on plant growth and development. **Acta Horticulturae Sinica, 46**(9): 1670–1680.

17. Dapoigny, L., De Tourdonnet, S., Roger-Estrade, J., Jeuffroy, M. H., Fleury, A. (2000). Effect of nitrogen nutrition on growth and nitrate accumulation in lettuce (Lactuca sativa L.), under various conditions of radiation and temperature. **Agronomie, 20**(8): 843–855.

18. Deng, X., Xiao, W., Shi, Z., Zeng, L., Lei, L. (2020). Combined effects of drought and shading on growth and non-structural carbohydrates in Pinus massoniana lamb. Seedlings. **Forests, 11**(1): 18.

19. Fallovo, C., Rouphael, Y., Cardarelli, M., Rea, E., Battistelli, A., Colla, G. (2009). Yield and quality of leafy lettuce in response to nutrient solution composition and growing season. **Journal of Food, Agriculture and Environment, 7**(2): 456–462.

20. FAOSTAT. (2020). Food and Agriculture Organization of the United Nations. **https://www.fao.org/faostat/en/#data/QCL**

21. Galieni, A., Stagnari, F., Speca, S., Pisante, M. (2016). Leaf traits as indicators of limiting growing conditions for lettuce (Lactuca sativa). **Annals of Applied Biology, 169**(3): 342–356.

22. Gaudreau, L., Charbonneau, J., Vezina, L. P., Gosselin, A. (1994). Photoperiod and photosynthetic photon flux influence growth and quality of greenhouse-grown lettuce. **HortScience, 29**(11): 1285–1289.

23. Geissler, C., & Powers, H. J. (2009). Fundamentals of human nutrition : for students and practitioners in the health sciences. Churchill Livingstone, Elsevier Health Sciences, 324pp.

24. Gil, P. M., Bonomelli, C., Schaffer, B., Ferreyra, R., Gentina, C. (2012). Effect of soil water-to-air ratio on biomass and mineral nutrition of avocado trees. **Journal of Soil Science and Plant Nutrition, 12**(3): 609–630.

25. Giordano, M., El-Nakhel, C., Colonna, E., Pannico, A., Maiello, R., de Pascale, S., Rouphael, Y. (2021). Effects of genotypes, plant density and nitrogen rates on yield and quality of spinach. **Acta Horticulturae, 1326**, 223–230.
26. Grimstad, S. O. (1987). Supplementary lighting of early tomatoes after planting out in glass and acrylic greenhouses. **Scientia Horticulturae, 33**(3–4): 189–196.
27. Grimstad, S. O. (1991). The efficiency of fluorescent lamps in young lettuce plant production. **Norwegian Journal of Agricultural Sciences, 5**(3): 261–267.
28. Gruda, N. (2005). Impact of environmental factors on product quality of greenhouse vegetables for fresh consumption. In **Critical Reviews in Plant Sciences** 24(3): pp. 227–247).
29. Gull, A., Ahmad Lone, A., Ul Islam Wani, N. (2019). Biotic and abiotic stresses in plants. In **Abiotic and Biotic Stress in Plants** (pp. 1–19).
30. Hanyu, H., Shoji, K. (2000). Effects of blue light and red light on kidney bean plants grown under combined radiation from narrow-band light sources. **Environment Control in Biology, 38**(1): 13–24.
31. Hanyu, H., Shoji, K. (2002). Acceleration of growth in spinach by short-term exposure to red and blue light at the beginning and at the end of the daily dark period. **Acta Horticulturae, 580**, 145–150.
32. Harley, S. M. (1993). Use of a simple, colorimetric assay to demonstrate conditions for induction of nitrate reductase in plants. **American Biology Teacher, 55**(3): 161–164.
33. Haverkort, A. J. (2007). Potato crop response to radiation and daylength. In **Potato Biology and Biotechnology: Advances and Perspectives** (pp. 353–365). Elsevier Science B.V.

34. Hikosaka, S., Iwamoto, N., Goto, E., Ching-Hui, C. (2017). Effects of supplemental lighting on growth & medicinal compounds of Japanese honeysuckle (Lonicera japonica Thunb.). **Environmental Control in Biology, 55**(2): 71–76.

35. Hikosaka, S., Iyoki, S., Hayakumo, M., Goto, E. (2013). Effects of light intensity and amount of supplemental LED lighting on photosynthesis and fruit growth of tomato plants under artificial conditions. **Journal of Agricultural Meteorology, 69**(2): 93–100.

36. Hopkins, W. G., Huner, N. P. A. (2008). Introduction to Plant Physiology (4th ed.). John Wiley & Sons, 523pp.

37. Huang, C., Wang, Z., Li, S., Malhi, S. S. (2010). Nitrate in leaf petiole and blade of spinach cultivars and its relation to biomass and water in plants. **Journal of Plant Nutrition, 33**(8): 1112–1123.

38. Joniyas, A., Surif, M., Dehgahi, R. (2016). Effect of nutrient and light intensity on nutrient uptakes of gracilaria manilaensis. **International Journal of Scientific Research in Environmental Sciences, 4**(6): 173–185.

39. Labconco Corporation. (1998). A guide to Kjeldahl nitrogen determination methods and apparatus. Labconco Corporation: Houston, TX, USA, 2.

40. Lambers, H., Steingröver, E. (1978). Growth respiration of a flood-tolerant and a flood-intolerant senecio species: correlation between calculated and experimental values. **Physiologia Plantarum, 43**(3): 219–224.

41. Laurie, S., Stewart, G. R. (1993). Effects of nitrogen supply and high temperature on the growth and physiology of the chickpea. **Plant, Cell & Environment, 16**(6): 609–621.

42. Li, L., Zhang, L., Zhang, F. (2013). Crop mixtures and the mechanisms of overyielding. *In* Encyclopedia of Biodiversity: Second Edition (Eds: S.A. Levin) (pp. 382–395). Academic Press.

43. Lichtenthaler, H. K. (1987). Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes. **Methods in Enzymology, 148**(C): 350–382.
44. Lillo, C. (1994). Light regulation of nitrate reductase in green leaves of higher plants. **Physiologia Plantarum, 90**(3): 616–620.
45. Lillo, C. (2008). Signalling cascades integrating light-enhanced nitrate metabolism. In **Biochemical Journal 415(**1): pp. 11–19).
46. Lin, L., Li, Z., Yu, L., Wang, H., Niu, Z. (2020). Photosynthetic responses to interaction of light intensity and CO_2 concentration and photoinhibition characteristics of two apple canopy shapes. **Acta Horticulturae Sinica, 47**(11): 2073–2085.
47. Markesteijn, L., Poorter, L. (2009). Seedling root morphology and biomass allocation of 62 tropical tree species in relation to drought- and shade-tolerance. **Journal of Ecology, 97**(2): 311–325.
48. Márquez-Quiroz, C., López-Espinosa, S. T., Sánchez-Chávez, E., García-Bañuelos, M. L., De la Cruz-Lázaro, E., Reyes-Carrillo, J. L. (2014). Effect of vermicompost tea on yield and nitrate reductase enzyme activity in saladette tomato. **Journal of Soil Science and Plant Nutrition, 14**(1): 223–231.
49. Marschner, H. (1995). Mineral nutrition of higher plants (2nd ed.). Academic Press.
50. Maynard, D. N., Barker, A. V. (1979). REGULATION OF NITRATE ACCUMULATION IN VEGETABLES. **Acta Horticulturae, 93**, 153–162.
51. Miano, T. F. (2016). Nutritional value of spinacia oleraecea spinach-an overview. **Review Article International Journal of Life Sciences and Review, 2**(12): 172–174.
52. Nieuwhof, M. (1994). Effects of temperature and light on nitrate content of radish (Raphanus sativus L.). **Gartenbauwissenschaft, 59**(5): 220–224.

53. Ohashi-Kaneko, K., Tarase, M., Noya, K. O. N., Fujiwara, K., & Kurata, K. (2007). Effect of light quality on growth and vegetable quality in leaf lettuce, spinach and komatsuna. **Environmental Control in Biology, 45**(3): 189–198.

54. Olday, F. C., Barker, A. V, Maynard, D. N. (1976). A physiological basis for different patterns of nitrate accumulation in two spinach cultivars. **Journal of the American Society for Horticultural Science, 101**(3): 217–219.

55. Oulai, P., Zoue, L., Megnanou, R.-M., Doue, R., Niamke, S. (2014). Proximate Composition and Nutritive Value of Leafy Vegetables Consumed in Northern Côte D ' Ivoire. **Europian Scientific Journal, 10**(6): 212–227.

56. Pramanik, K., Pradhan, J., Mohapatra, P., Acharya, C. (2020). Impact of Temperature: An Important Climate Changing Factor on Vegetable Crops. In Researchgate.Net (2020 December 18). Immortal publications 105-134.

57. Prohens, J., & Nuez, F. (Eds.). (2008). Vegetables I: Asteraceae, Brassicaceae, Chenopodicaceae, and Cucurbitaceae. Springer New York, 428pp.

58. Proietti, S., Moscatello, S., Colla, G., Battistelli, Y., Leccese, A., Battistelli, A. (2004). The effect of growing spinach (Spinacia oleracea L.) at two light intensities on the amounts of oxalate, ascorbate and nitrate in their leaves. **The Journal of Horticultural Science and Biotechnology, 79**(4): 606–609.

59. Reinink, K., Eenink, A. H. (1988). Genotypical differences in nitrate accumulation in shoots and roots of lettuce. **Scientia Horticulturae, 37**(1–2): 13–24.

60. Rouphael, Y., Cardarelli, M., Bassal, A., Leonardi, C., Giuffrida, F., Colla, G. (2012). Vegetable quality as affected by genetic, agronomic and environmental factors. In **Journal of Food, Agriculture and Environment 10**(3–4): pp. 680–688).

61. Ryder, E. J. (1979). Leafy Salad Vegetables. In Leafy Salad Vegetables. Springer, Dordrecht, 268pp.

62. Sakuraba, Y., Yanagisawa, S. (2018). Light signalling-induced regulation of nutrient acquisition and utilisation in plants. In **Seminars in Cell and Developmental Biology 83**, pp. 123–132). Academic Press.

63. Santamaria, P., Gonnella, M., Elia, A., Parente, A., Serio, F. (2001). Ways of reducing rocket salad nitrate content. **Acta Horticulturae, 548**, 529–536.

64. Schnitzler, W. H., & Gruda, N. (2003). Quality issues of greenhouse production. **Acta Horticulturae, 614**, 663–674.

65. Sen, S., Setter, T., Smith, M. (2012). Maize root morphology and nitrogen use efficiency - A review. **Agricultural Review, 33**(1): 16–26.

66. Sinha, R. K. (2004). Modern plant physiology. Alpha Science International Ltd, Pangbourne, 635pp.

67. Stagnari, F., Di Bitetto, V., Pisante, M. (2007). Effects of N fertilizers and rates on yield, safety and nutrients in processing spinach genotypes. **Scientia Horticulturae, 114**(4): 225–233.

68. Steingröver, E., Oosterhuis, R., Wieringa, F. (1982). Effect of light treatment and nutrition on nitrate accumulation in spinach (*Spinacia oleraceae* L.). **Zeitschrift Für Pflanzenphysiologie, 107**(2): 97–102.

69. Taiz, L., Zeiger, E. (2002). Plant Physiology (3rd ed.). Sinauer Associates, Inc. Publishers, Sunderland, MA, USA 690pp.

70. Tang, Y., Sun, X., Hu, C., Tan, Q., Zhao, X. (2013). Genotypic differences in nitrate uptake, translocation and assimilation of two Chinese cabbage cultivars [*Brassica campestris* L. ssp. Chinensis(L.)]. **Plant Physiology and Biochemistry, 70**, 14–20.

71. Thornton, B., Robinson, D. (2005). Uptake and assimilation of nitrogen from solutions containing multiple N sources. **Plant, Cell and Environment, 28**(6): 813–821.

72. Uhart, S. A., Andrade, F. H. (1995). Nitrogen deficiency in maize: I. Effects on crop growth, development, dry matter partitioning, and kernel set. **Crop Science, 35**(5): 1376–1383.

73. Van Der Boon, J., Steenhuizen, J. W., Steingrover, E. G. (1990). Growth and nitrate concentration of lettuce as affected by total nitrogen and chloride concentration, NH 4 /N0 3 ratio and temperature of the recirculating nutrient solution. **Journal of Horticultural Science, 65**(3): 309–321.

74. Verma, S., Nizam, S., Verma, P. K. (2013). Biotic and abiotic stress signaling in plants. In **Stress Signaling in Plants: Genomics and Proteomics Perspective, 1** (pp. 25–50). Springer New York.

75. Wang, F., Yan, J., Chen, X., Jiang, C., Meng, S., Liu, Y., Xu, T., Qi, M., Li, T. (2019). Light regulation of chlorophyll biosynthesis in plants. **Acta Horticulturae Sinica, 46**(5): 975–994.

76. Wang, X., Cai, X., Xu, C., Wang, S., Dai, S., & Wang, Q. (2018). Nitrate accumulation and expression patterns of genes involved in nitrate transport and assimilation in spinach. **Molecules, 23**(9): p2231.

77. Wheeler, T. R., Craufurd, P. Q., Ellis, R. H., Porter, J. R., Vara Prasad, P. V. (2000). Temperature variability and the yield of annual crops. **Agriculture, Ecosystems and Environment, 82**(1–3): 159–167.

78. Xu, J., Guo, Z., Jiang, X., Ahammed, G. J., Zhou, Y. (2021). Light regulation of horticultural crop nutrient uptake and utilization. **Horticultural Plant Journal, 7**(5): 367–379.

79. Yang, J. chang, Zhang, H., Zhang, J. hua. (2012). Root Morphology and Physiology in Relation to the Yield Formation of Rice. In **Journal of Integrative Agriculture 11**(6): pp. 920–926. Elsevier.

80. Yoneda, K., Suzuki, N. (1998). Effect of Temperature and Light Intensity on the Growth and Flowering of Odontoglossum Intergeneric Hybrids. **Journal of the Japanese Society for Horticultural Science, 67**(4): 619–625.

81. Zhao, D., Reddy, K. R., Kakani, V. G., Read, J. J., Carter, G. A. (2003). Corn (Zea mays L.) growth, leaf pigment concentration, photosynthesis and leaf hyperspectral reflectance properties as affected by nitrogen supply. **Plant and Soil, 257**(1): 205–218.

82. Zhao, L., Wang, Y. (2017). Nitrate assay for plant tissues. **Bio-Protocol, 7**(2): p2029.

83. Zhou, J., Li, P. P., Wang, J. Z., Fu, W. (2019). Growth, photosynthesis, and nutrient uptake at different light intensities and temperatures in lettuce. **HortScience, 54**(11): 1925–1933.

LIST OF APPENDIXES

Appendix 1: ANOVA Tables

OLOT Experiment Results ANOVA Tables

SFW

Source	DF	SS	MS	F Value	Pr > F
Variety	4	295.404	73.851	21.69	<.0001
Nitrogen	1	2800.627	2800.627	822.37	<.0001
Variety*Nitrogen	4	169.887	42.472	12.47	0.0007
Rep	2	8.633	4.316	1.27	0.3232
Rep*Variety	8	28.341	3.543	1.04	0.4672
Error	10	34.056	3.406		
Corrected Total	29	3336.947			

Leaf Area

Source					
Variety	4	63531.721	15882.930	12.63	0.0006
Nitrogen	1	1256542.816	1256542.816	999.16	<.0001
Variety*Nitrogen	4	146641.621	36660.405	29.15	<.0001
Rep	2	2194.285	1097.142	0.87	0.4475
Rep*Variety	8	6179.511	772.439	0.61	0.7492
Error	10	12575.997	1257.600		
Corrected Total	29	1487665.949			

Leaf Number

Source	DF	SS	MS	F Value	Pr > F
Variety	4	2090.333333	522.583333	41.15	<.0001
Nitrogen	1	2861.633333	2861.633333	225.33	<.0001
Variety*Nitrogen	4	302.866667	75.716667	5.96	0.0102
Rep	2	15.266667	7.633333	0.60	0.5669
Rep*Variety	8	55.066667	6.883333	0.54	0.8014
Error	10	127.000000	12.700000		
Corrected Total	29	5452.166667			

SDW

Source	DF	SS	MS	F Value	Pr > F
Variety	4	1.58504667	0.39626167	22.62	<.0001
Nitrogen	1	12.98892000	12.98892000	741.52	<.0001
Variety*Nitrogen	4	0.80831333	0.20207833	11.54	0.0009
Rep	2	1.18098000	0.59049000	33.71	<.0001
Rep*Variety	8	0.01965333	0.00245667	0.14	0.9948
Error	10	0.17516667	0.01751667		
Corrected Total	29	16.75808000			

RDW

Source	DF	SS	MS	F Value	Pr > F
Variety	4	0.06651333	0.01662833	166.28	<.0001
Nitrogen	1	0.05547000	0.05547000	554.70	<.0001
Variety*Nitrogen	4	0.02518000	0.00629500	62.95	<.0001
Rep	2	0.02244667	0.01122333	112.23	<.0001
Rep*Variety	8	0.00088667	0.00011083	1.11	0.4307
Error	10	0.00100000	0.00010000		
Corrected Total	29	0.17149667			

Source	DF	SS	MS	F Value	Pr > F

Root: Shoot Ratio	Variety	4	0.00651333	0.00162833	1.65	0.2370
	Nitrogen	1	0.03201333	0.03201333	32.45	0.0002
	Variety*Nitrogen	4	0.00462000	0.00115500	1.17	0.3803
	Rep	2	0.00288667	0.00144333	1.46	0.2772
	Rep*Variety	8	0.00924667	0.00115583	1.17	0.3993
	Error	10	0.00986667	0.00098667		
	Corrected Total	29	0.06514667			

	Source	DF	SS	MS	F Value	Pr > F
Root Length	Variety	4	7.20961333	1.80240333	34.96	<.0001
	Nitrogen	1	2.48832000	2.48832000	48.27	<.0001
	Variety*Nitrogen	4	5.00008000	1.25002000	24.25	<.0001
	Rep	2	0.24052667	0.12026333	2.33	0.1474
	Rep*Variety	8	0.41090667	0.05136333	1.00	0.4920
	Error	10	0.51550000	0.05155000		
	Corrected Total	29	15.86494667			

	Source	DF	SS	MS	F Value	Pr > F
Nitrogen Concentration	Variety	4	232.4679333	58.1169833	18.39	0.0001
	Nitrogen	1	214.1340833	214.1340833	67.75	<.0001
	Variety*Nitrogen	4	179.5090000	44.8772500	14.20	0.0004
	Rep	2	52.1322200	26.0661100	8.25	0.0077
	Rep*Variety	8	41.3096467	5.1637058	1.63	0.2297
	Error	10	31.6080667	3.1608067		
	Corrected Total	29	751.1609500			

	Source	DF	SS	MS	F Value	Pr > F
Nitrogen Uptake	Variety	4	8318.06250	2079.51563	11.66	0.0009
	Nitrogen	1	88437.32485	88437.3249	495.94	<.0001
	Variety*Nitrogen	4	6840.51205	1710.12801	9.59	0.0019
	Rep	2	9114.14953	4557.07476	25.56	0.0001
	Rep*Variety	8	131.33764	16.41720	0.09	0.9988
	Error	10	1783.2203	178.3220		
	Corrected Total	29	114624.6069			

	Source	DF	SS	MS	F Value	Pr > F
Nitrate Concentration	Variety	4	6194441.77	1548610.44	6.50	0.0076
	Nitrogen	1	58077297.61	58077297.61	243.79	<.0001
	Variety*Nitrogen	4	15670320.34	3917580.08	16.44	0.0002
	Rep	2	939851.73	469925.87	1.97	0.1896
	Rep*Variety	8	4669267.82	583658.48	2.45	0.0926
	Error	10	2382247.18	238224.72		
	Corrected Total	29	87933426.45			

	Source	DF	SS	MS	F Value	Pr > F
Nitrate Reductase Activity	Variety	4	25.73368667	6.43342167	50.90	<.0001
	Nitrogen	1	5.00208333	5.00208333	39.58	<.0001
	Variety*Nitrogen	4	18.71743333	4.67935833	37.02	<.0001
	Rep	2	0.15000000	0.07500000	0.59	0.5708
	Rep*Variety	8	0.83053333	0.10381667	0.82	0.6021
	Error	10	1.26393333	0.12639333		
	Corrected Total	29	51.69767000			
	Source	DF	SS	MS	F Value	Pr > F

Chlorophyll Concentration	Variety	4	447.5815533	111.89539	13.27	0.0005
	Nitrogen	1	5.4613333	5.4613333	0.65	0.4396
	Variety*Nitrogen	4	19.9531667	4.9882917	0.59	0.6765
	Rep	2	14.5398067	7.2699033	0.86	0.4513
	Rep*Variety	8	53.9526267	6.7440783	0.80	0.6166
	Error	10	84.3017000	8.4301700		
	Corrected Total	29	625.7901867			

	Source	DF	SS	MS	F Value	Pr > F
Photosynthesis	Variety	4	27.71133333	6.92783333	3.45	0.0509
	Nitrogen	1	60.49200000	60.49200000	30.16	0.0003
	Variety*Nitrogen	4	11.07133333	2.76783333	1.38	0.3086
	Rep	2	3.78466667	1.89233333	0.94	0.4213
	Rep*Variety	8	16.07866667	2.00983333	1.00	0.4887
	Error	10	20.0566667	2.0056667		
	Corrected Total	29	139.1946667			

LLOT Experiment Results ANOVA Tables

	Source	DF	SS	MS	F Value	Pr > F
Shoot Fresh Weight	Variety	4	254.694918	63.673729	8.88	0.0025
	Nitrogen	1	2541.610972	2541.610972	354.28	<.0001
	Variety*Nitrogen	4	73.244973	18.311243	2.55	0.1046
	Rep	2	4.217484	2.108742	0.29	0.7515
	Rep*Variety	8	86.862050	10.857756	1.51	0.2648
	Error	10	71.740201	7.174020		
	Corrected Total	29	3032.370597			

	Source	DF	SS	MS	F Value	Pr > F
Leaf Area	Variety	4	147841.5298	36960.3825	20.94	<.0001
	Nitrogen	1	670966.8426	670966.8426	380.05	<.0001
	Variety*Nitrogen	4	91113.6507	22778.4127	12.90	0.0006
	Rep	2	84.0998	42.0499	0.02	0.9765
	Rep*Variety	8	13964.2681	1745.5335	0.99	0.4965
	Error	10	17654.6397	1765.4640		
	Corrected Total	29	941625.0309			

	Source	DF	SS	MS	F Value	Pr > F
Leaf Number	Variety	4	1360.866667	340.216667	21.22	<.0001
	Nitrogen	1	1442.133333	1442.133333	89.95	<.0001
	Variety*Nitrogen	4	575.533333	143.883333	8.97	0.0024
	Rep	2	1.400000	0.700000	0.04	0.9575
	Rep*Variety	8	228.933333	28.616667	1.78	0.1926
	Error	10	160.333333	16.033333		
	Corrected Total	29	3769.200000			

Shoot Dry Weight

Source	DF	SS	MS	F Value	Pr > F
Variety	4	0.78522953	0.19630738	3.64	0.0444
Nitrogen	1	7.83771853	7.83771853	145.22	<.0001
Variety*Nitrogen	4	0.53239513	0.13309878	2.47	0.1126
Rep	2	0.22817947	0.11408973	2.11	0.1715
Rep*Variety	8	0.57154287	0.07144286	1.32	0.3324
Error	10	0.53970633	0.05397063		
Corrected Total	29	10.49477187			

Root Dry Weight

Source	DF	SS	MS	F Value	Pr > F
Variety	4	0.05946753	0.01486688	5.19	0.0159
Nitrogen	1	0.04203763	0.04203763	14.66	0.0033
Variety*Nitrogen	4	0.02999287	0.00749822	2.62	0.0992
Rep	2	0.00698460	0.00349230	1.22	0.3362
Rep*Variety	8	0.01979607	0.00247451	0.86	0.5744
Error	10	0.02867200	0.00286720		
Corrected Total	29	0.18695070			

Root: Shoot Ratio

Source	DF	SS	MS	F Value	Pr > F
Variety	4	0.01524287	0.00381072	3.25	0.0593
Nitrogen	1	0.06617603	0.06617603	56.50	<.0001
Variety*Nitrogen	4	0.00192847	0.00048212	0.41	0.7966
Rep	2	0.00196127	0.00098063	0.84	0.4611
Rep*Variety	8	0.00548573	0.00068572	0.59	0.7701
Error	10	0.01171300	0.00117130		
Corrected Total	29	0.10250737			

Root Length

Source	DF	SS	MS	F Value	Pr > F
Variety	4	96.26250587	24.06562647	2.81	0.0845
Nitrogen	1	42.90530430	42.90530430	5.01	0.0492
Variety*Nitrogen	4	88.96468587	22.24117147	2.59	0.1009
Rep	2	14.98334927	7.49167463	0.87	0.4469
Rep*Variety	8	43.52739773	5.44092472	0.63	0.7343
Error	10	85.7239723	8.5723972		
Corrected Total	29	372.3672154			

Nitrogen Concentration

Source	DF	SS	MS	F Value	Pr > F
Variety	4	61.0735678	15.2683919	2.56	0.1042
Nitrogen	1	498.4214320	498.4214320	83.47	<.0001
Variety*Nitrogen	4	85.4423645	21.3605911	3.58	0.0465
Rep	2	27.5658342	13.7829171	2.31	0.1499
Rep*Variety	8	48.9361348	6.1170169	1.02	0.4760
Error	10	59.7151710	5.9715171		
Corrected Total	29	781.1545043			

Nitrogen Uptake

Source	DF	SS	MS	F Value	Pr > F
Variety	4	984.91505	246.22876	2.14	0.1505
Nitrogen	1	20004.20012	20004.20012	173.61	<.0001
Variety*Nitrogen	4	836.25467	209.06367	1.81	0.2027
Rep	2	415.69870	207.84935	1.80	0.2143
Rep*Variety	8	716.47410	89.55926	0.78	0.6323
Error	10	1152.21813	115.22181		
Corrected Total	29	24109.76078			

Nitrate Concentration

Source	DF	SS	MS	F Value	Pr > F
Variety	4	3653993.9	913498.5	9.64	0.0018
Nitrogen	1	771383155.2	771383155.2	8140.54	<.0001
Variety*Nitrogen	4	16787389.7	4196847.4	44.29	<.0001
Rep	2	116732.3	58366.2	0.62	0.5594
Rep*Variety	8	532232.4	66529.1	0.70	0.6855
Error	10	947582.4	94758.2		
Corrected Total	29	793421086.0			

Nitrate Reductase Activity

Source	DF	SS	MS	F Value	Pr > F
Variety	4	29.96362480	7.49090620	77.73	<.0001
Nitrogen	1	37.90577613	37.90577613	393.34	<.0001
Variety*Nitrogen	4	7.97916653	1.99479163	20.70	<.0001
Rep	2	0.61931127	0.30965563	3.21	0.0836
Rep*Variety	8	1.38581940	0.17322743	1.80	0.1898
Error	10	0.96369933	0.09636993		
Corrected Total	29	78.81739747			

Chlorophyll Concentration

Source	DF	SS	MS	F Value	Pr > F
Variety	4	543.3753101	135.8438275	29.77	<.0001
Nitrogen	1	9.6889467	9.6889467	2.12	0.1757
Variety*Nitrogen	4	12.6533435	3.1633359	0.69	0.6134
Rep	2	16.5791886	8.2895943	1.82	0.2123
Rep*Variety	8	28.2045891	3.5255736	0.77	0.6355
Error	10	45.6291343	4.5629134		
Corrected Total	29	656.1305123			

Photosynthesis

Source	DF	SS	MS	F Value	Pr > F
Variety	4	6.98076667	1.74519167	8.24	0.0033
Nitrogen	1	4.32060750	4.32060750	20.40	0.0011
Variety*Nitrogen	4	2.38268000	0.59567000	2.81	0.0842
Rep	2	0.03220167	0.01610083	0.08	0.9273
Rep*Variety	8	7.89547333	0.98693417	4.66	0.0133
Error	10	2.11827500	0.21182750		
Corrected Total	29	23.73000417			

OLLT Experiment Results ANOVA Tables

Shoot Fresh Weight

Source	DF	SS	MS	F Value	Pr > F
Variety	4	141.6217260	35.4054315	6.99	0.0059
Nitrogen	1	878.3892741	878.3892741	173.37	<.0001
Variety*Nitrogen	4	36.7316372	9.1829093	1.81	0.2031
Rep	2	59.0822913	29.5411456	5.83	0.0210
Rep*Variety	8	36.7542614	4.5942827	0.91	0.5463
Error	10	50.666901	5.066690		
Corrected Total	29	1203.246091			

Leaf Area

Source	DF	SS	MS	F Value	Pr > F
Variety	4	52182.1262	13045.5316	4.98	0.0180
Nitrogen	1	238907.2597	238907.2597	91.24	<.0001
Variety*Nitrogen	4	16392.8076	4098.2019	1.57	0.2573
Rep	2	26118.3101	13059.1551	4.99	0.0314
Rep*Variety	8	11285.4901	1410.6863	0.54	0.8037
Error	10	26184.3728	2618.4373		
Corrected Total	29	371070.3665			

Leaf Number

Source	DF	SS	MS	F Value	Pr > F
Variety	4	187.1333333	46.7833333	56.14	<.0001
Nitrogen	1	154.1333333	154.1333333	184.96	<.0001
Variety*Nitrogen	4	135.5333333	33.8833333	40.66	<.0001
Rep	2	6.0666667	3.0333333	3.64	0.0649
Rep*Variety	8	12.2666667	1.5333333	1.84	0.1808
Error	10	8.3333333	0.8333333		
Corrected Total	29	503.4666667			

Shoot Dry Weight

Source	DF	SS	MS	F Value	Pr > F
Variety	4	0.21511420	0.05377855	1.13	0.3956
Nitrogen	1	4.31908963	4.31908963	90.86	<.0001
Variety*Nitrogen	4	0.25110153	0.06277538	1.32	0.3273
Rep	2	0.27544640	0.13772320	2.90	0.1017
Rep*Variety	8	0.26277660	0.03284707	0.69	0.6935
Error	10	0.47538033	0.04753803		
Corrected Total	29	5.79890870			

Root Dry Weight

Source	**DF**	**SS**	**MS**	**F Value**	**Pr > F**
Variety	4	0.09084900	0.02271225	12.54	0.0007
Nitrogen	1	0.03924083	0.03924083	21.67	0.0009
Variety*Nitrogen	4	0.06127500	0.01531875	8.46	0.0030
Rep	2	0.02364087	0.01182043	6.53	0.0153
Rep*Variety	8	0.02087880	0.00260985	1.44	0.2886
Error	10	0.01810567	0.00181057		
Corrected Total	29	0.25399017			

Root: Shoot Ratio

Source	DF	SS	MS	F Value	Pr > F
Variety	4	0.05558353	0.01389588	2.35	0.1247
Nitrogen	1	0.56197453	0.56197453	94.97	<.0001
Variety*Nitrogen	4	0.08944847	0.02236212	3.78	0.0401
Rep	2	0.00821207	0.00410603	0.69	0.5222
Rep*Variety	8	0.02572427	0.00321553	0.54	0.8004
Error	10	0.05917300	0.00591730		
Corrected Total	29	0.80011587			

Root Length

Source	DF	SS	MS	F Value	Pr > F
Variety	4	183.2944385	45.8236096	3.44	0.0515
Nitrogen	1	29.1225121	29.1225121	2.18	0.1702
Variety*Nitrogen	4	13.5498975	3.3874744	0.25	0.9006
Rep	2	73.8894091	36.9447045	2.77	0.1102
Rep*Variety	8	17.9412749	2.2426594	0.17	0.9907
Error	10	133.2917253	13.3291725		
Corrected Total	29	451.0892575			

Nitrogen Concentration

Source	**DF**	**SS**	**MS**	**F Value**	**Pr > F**
Variety	4	22.563749	5.640937	1.03	0.4377
Nitrogen	1	1118.216222	1118.216222	204.36	<.0001
Variety*Nitrogen	4	43.807003	10.951751	2.00	0.1703
Rep	2	15.892356	7.946178	1.45	0.2795
Rep*Variety	8	43.436493	5.429562	0.99	0.4944
Error	10	54.718465	5.471846		
Corrected Total	29	1298.634287			

Nitrogen Uptake

Source	DF	SS	MS	F Value	Pr > F
Variety	4	184.90306	46.22576	0.57	0.6882
Nitrogen	1	12164.27842	12164.27842	150.94	<.0001
Variety*Nitrogen	4	403.63653	100.90913	1.25	0.3504
Rep	2	272.17821	136.08910	1.69	0.2334
Rep*Variety	8	509.40234	63.67529	0.79	0.6234
Error	10	805.89351	80.58935		
Corrected Total	29	14340.29205			

Nitrate Concentration

Source	DF	SS	MS	F Value	Pr > F
Variety	4	3374683.2	843670.8	13.45	0.0005
Nitrogen	1	34836652.8	34836653	555.50	<.0001
Variety*Nitrogen	4	1920539.87	480135	7.66	0.0043
Rep	2	245257.8	122628.9	1.96	0.1920
Rep*Variety	8	1439352.2	179919	2.87	0.0607
Error	10	627119.33	62711.93		
Corrected Total	29	42443605.2			

Nitrate Reductase Activity

Source	DF	SS	MS	F Value	Pr > F
Variety	4	26.91175353	6.72793838	5.83	0.0110
Nitrogen	1	4.16045280	4.16045280	3.61	0.0868
Variety*Nitrogen	4	39.99537020	9.99884255	8.66	0.0027
Rep	2	2.49306327	1.24653163	1.08	0.3761
Rep*Variety	8	2.76673207	0.34584151	0.30	0.9496
Error	10	11.54046400	1.15404640		
Corrected Total	29	87.86783587			

Chlorophyll Concentration

Source	DF	SS	MS	F Value	Pr > F
Variety	4	870.8535453	217.7133863	28.99	<.0001
Nitrogen	1	10.3699681	10.3699681	1.38	0.2672
Variety*Nitrogen	4	63.6462619	15.9115655	2.12	0.1530
Rep	2	16.1209617	8.0604808	1.07	0.3782
Rep*Variety	8	11.9563727	1.4945466	0.20	0.9843
Error	10	75.099867	7.509987		
Corrected Total	29	1048.046977			

Photosynthesis

Source	DF	SS	MS	F Value	Pr > F
Variety	4	16.96466667	4.24116667	1.47	0.2818
Nitrogen	1	60.49200000	60.49200000	20.99	0.0010
Variety*Nitrogen	4	8.74466667	2.18616667	0.76	0.5751
Rep	2	2.19800000	1.09900000	0.38	0.6924
Rep*Variety	8	9.83533333	1.22941667	0.43	0.8799
Error	10	28.8133333	2.8813333		
Corrected Total	29	127.0480000			

LLLT Experiment Results ANOVA Tables

Shoot Fresh Weight

Source	DF	SS	MS	F Value	Pr > F
Variety	4	83.57445287	20.89361322	79.70	<.0001
Nitrogen	1	41.22158520	41.22158520	157.25	<.0001
Variety*Nitrogen	4	3.15570780	0.78892695	3.01	0.0718
Rep	2	0.66047047	0.33023523	1.26	0.3251
Rep*Variety	8	1.87614153	0.23451769	0.89	0.5541
Error	10	2.6214860	0.2621486		
Corrected Total	29	133.1098439			

Leaf Area

Source	DF	SS	MS	F Value	Pr > F
Variety	4	24010.30718	6002.57679	77.31	<.0001
Nitrogen	1	17504.59710	17504.59710	225.44	<.0001
Variety*Nitrogen	4	2866.61679	716.65420	9.23	0.0022
Rep	2	393.00824	196.50412	2.53	0.1290
Rep*Variety	8	120.84847	15.10606	0.19	0.9853
Error	10	776.47033	77.64703		
Corrected Total	29	45671.84811			

Leaf Number

Source	DF	SS	MS	F Value	Pr > F
Variety	4	28.46666667	7.11666667	5.77	0.0113
Nitrogen	1	4.80000000	4.80000000	3.89	0.0768
Variety*Nitrogen	4	4.86666667	1.21666667	0.99	0.4578
Rep	2	3.46666667	1.73333333	1.41	0.2898
Rep*Variety	8	7.53333333	0.94166667	0.76	0.6419
Error	10	12.33333333	1.23333333		
Corrected Total	29	61.46666667			

Shoot Dry Weight

Source	DF	SS	MS	F Value	Pr > F
Variety	4	0.26753820	0.06688455	23.85	<.0001
Nitrogen	1	0.18174083	0.18174083	64.81	<.0001
Variety*Nitrogen	4	0.05467433	0.01366858	4.87	0.0193
Rep	2	0.02343260	0.01171630	4.18	0.0480
Rep*Variety	8	0.01957140	0.00244642	0.87	0.5683
Error	10	0.02804133	0.00280413		
Corrected Total	29	0.57499870			

Root Dry Weight

Source	DF	SS	MS	F Value	Pr > F
Variety	4	0.00644913	0.00161228	6.85	0.0064
Nitrogen	1	0.00011213	0.00011213	0.48	0.5056
Variety*Nitrogen	4	0.00351753	0.00087938	3.74	0.0413
Rep	2	0.00014220	0.00007110	0.30	0.7457
Rep*Variety	8	0.00131547	0.00016443	0.70	0.6877
Error	10	0.00235233	0.00023523		
Corrected Total	29	0.01388880			

Root: Shoot Ratio

Source	DF	SS	MS	F Value	Pr > F
Variety	4	0.00671367	0.00167842	2.30	0.1298
Nitrogen	1	0.03967603	0.03967603	54.43	<.0001
Variety*Nitrogen	4	0.00629247	0.00157312	2.16	0.1477
Rep	2	0.00560407	0.00280203	3.84	0.0578
Rep*Variety	8	0.00738093	0.00092262	1.27	0.3564
Error	10	0.00728900	0.00072890		
Corrected Total	29	0.07295617			

Root Length

Source	DF	SS	MS	F Value	Pr > F
Variety	4	1.93745333	0.48436333	2.32	0.1282
Nitrogen	1	0.55651320	0.55651320	2.66	0.1338
Variety*Nitrogen	4	1.09634947	0.27408737	1.31	0.3303
Rep	2	0.13257140	0.06628570	0.32	0.7353
Rep*Variety	8	2.82147427	0.35268428	1.69	0.2157
Error	10	2.09002833	0.20900283		
Corrected Total	29	8.63439000			

Nitrogen Concentration

Source	DF	SS	MS	F Value	Pr > F
Variety	4	103.4757057	25.8689264	2.12	0.1526
Nitrogen	1	30.5061168	30.5061168	2.50	0.1447
Variety*Nitrogen	4	105.4727389	26.3681847	2.16	0.1470
Rep	2	29.2841858	14.6420929	1.20	0.3408
Rep*Variety	8	60.1232685	7.5154086	0.62	0.7475
Error	10	121.9007123	12.1900712		
Corrected Total	29	450.7627280			

Nitrogen Uptake

Source	DF	SS	MS	F Value	Pr > F
Variety	4	300.9347798	75.2336950	14.04	0.0004
Nitrogen	1	333.9336033	333.9336033	62.31	<.0001
Variety*Nitrogen	4	107.3803330	26.8450833	5.01	0.0177
Rep	2	33.6453989	16.8226994	3.14	0.0875
Rep*Variety	8	49.6068258	6.2008532	1.16	0.4063
Error	10	53.5937047	5.3593705		
Corrected Total	29	879.0946455			

Nitrate Concentration

Source	DF	SS	MS	F Value	Pr > F
Variety	4	21564865.87	5391216	99.81	<.0001
Nitrogen	1	83146771.2	83146771	1539.29	<.0001
Variety*Nitrogen	4	5825792.13	1456448	26.96	<.0001
Rep	2	27755.47	13877.73	0.26	0.7784
Rep*Variety	8	375202.53	46900.32	0.87	0.5710
Error	10	540162.7	54016.3		
Corrected Total	29	111480549.9			

Nitrate Reductase Activity

Source	DF	SS	MS	F Value	Pr > F
Variety	4	14.22961953	3.55740488	2.26	0.1352
Nitrogen	1	56.43311053	56.43311053	35.81	0.0001
Variety*Nitrogen	4	2.61222780	0.65305695	0.41	0.7947
Rep	2	0.35503980	0.17751990	0.11	0.8946
Rep*Variety	8	11.17440587	1.39680073	0.89	0.5594
Error	10	15.7599117	1.5759912		
Corrected Total	29	100.5643152			

Chlorophyll Concentration

Source	DF	SS	MS	F Value	Pr > F
Variety	4	1421.107461	355.276865	48.77	<.0001
Nitrogen	1	29.075239	29.075239	3.99	0.0736
Variety*Nitrogen	4	93.630756	23.407689	3.21	0.0611
Rep	2	95.351431	47.675715	6.55	0.0152
Rep*Variety	8	56.265237	7.033155	0.97	0.5102
Error	10	72.841367	7.284137		
Corrected Total	29	1768.271491			

Photosynthesis

Source	DF	SS	MS	F Value	Pr > F
Variety	4	1.07250000	0.26812500	0.53	0.7184
Nitrogen	1	0.43200000	0.43200000	0.85	0.3782
Variety*Nitrogen	4	3.22883333	0.80720833	1.59	0.2516
Rep	2	1.13550000	0.56775000	1.12	0.3648
Rep*Variety	8	1.45950000	0.18243750	0.36	0.9200
Error	10	5.08166667	0.50816667		
Corrected Total	29	12.4100000			

CURRICULUM VITAE

Personal Information

Name: Abdul-Quddus Bayuko ABUBAKAR
Nationality: Ghanaian

Education

Degree	Institution	Graduation Year
Master's Degree	Erciyes University	2017
Bachelor Degree	University for Development Studies	2014
High School	Tumu Sec Tech	2009

Work Experience

Year	Institution	Roles
2018 - date	Dogatech Ar-Ge	Sales

Languages

English, Turkish

Academic Works

1. Abubakar A. B. (2017). Genotypic Differences in Nitrogen Efficiency of Some Selected Local Maize Varieties of Ghana (MSc thesis). Erciyes University, Kayseri/Turkey
2. Abubakar A. B. Ulaş A., Dogancı E. (2017). Root-growth Characteristics Contributing to Genotypic Variation in Nitrogen Efficiency of Maize. Ecology 2017 International Symposium, Erciyes University, KAYSERİ, TÜRKIYE, 11-13 May 2017, pp.762-762
3. Abubakar A. B. (2014). Effects of Different Soil Fertility Management Options on the Grain Yield of Cowpea, Soya Bean and Maize (BSc thesis). University for Development Studies, Tamale, Ghana.