



***PHD COURSE IN HORTICULTURE***

**XXI CYCLE**

**Growth and quality of leafy vegetables in hydroponics: effect  
of environmental conditions and mineral nutrition**

SSD AGR/04

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# CHAPTER 1

## 1.1 General introduction

Quality of vegetable crops includes appearance (size, shape, color, gloss, and freedom from defects and decay), texture (firmness, crispness, juiciness, mealiness, and toughness), flavor (sweetness, sourness, astringency, aroma, and off-flavors), and nutritive value (vitamins, minerals, dietary fiber, phytonutrients). The relative importance of each quality component depends on the commodity and the individual's interest (Kader, 1992). Most postharvest researchers, producers, and handlers are product-oriented where the quality is described by specific attributes of the product itself, such as sugar content, color, or firmness. In contrast, growers, marketers, and economists are more likely to be consumer-oriented where the quality is described by consumer wants and needs (Shewfelt 1999). Although consumers purchase fresh produce based on appearance and textural quality, their repeat purchases depend upon their satisfaction with flavor (taste and aroma). They are also interested in the health-promoting attributes and nutritional quality of fresh fruits and vegetables. As well as visual characteristics, properties such as texture, the content of minerals and vitamins, flavor and other organoleptic characteristics must be considered. In addition, new knowledge show that vegetables are appreciated for their beneficial health effects in humans and underlines the importance of nutritional quality.

The high variety of compounds in vegetables represents the product of primary and secondary plant metabolism. Primary metabolites such as carbohydrates, amino acids, fatty acids, and organic acids are involved in growth and development, respiration and photosynthesis, and hormone and protein synthesis. Primary metabolites are found across all species within broad phylogenetic groups, and are produced using the same (or nearly the same) biochemical pathways (Taiz and Zeiger, 2006). Secondary metabolites such as flavonoids, carotenoids, sterols, phenolic acids, alkaloids, and glucosinolates determine the color of vegetables, protect plants against herbivores and microorganisms, attract pollinator and seed-dispersing animals, and act as signal molecules under stress conditions (Hounsume et al. 2008).

In human dietary secondary metabolites have beneficial roles against cancers, coronary heart disease, diabetes, high blood pressure, inflammation microbial, viral and parasitic infections, ulcers, etc. (Dillard and German, 2000).

For instance, it is well known that high consumption of tomatoes and tomato products has been linked to reduced carcinogenesis, particularly prostate cancer, and has been

thought to be due to the presence of lycopene, which gives red tomatoes their color (Dumas et al. 2003)

Many research studies have documented methods for achieving a high-quality vegetable product. For example, indoor production for fresh vegetables offers advantages compared to outdoor production with regard to quality assurance principally, because the products are not exposed directly to the rapid changes of climate conditions. On the other hand, vegetable cultivation in greenhouses under artificially creates conditions also affects the internal quality of the product. Moreover, advanced cultivation techniques such as soilless culture can improve product quality due to a better control of water and nutrient availability in the root zone. This method of production has several other advantages: increasing of productivity per area; possibility of using areas not suitable for soil culture, increasing harvesting period due to rapid plant growth; crop rotation not necessary; less consumption of water and fertilizers, greater hygiene and less possibility of contamination with microorganisms, nematodes and insects inherent to the soil (consequently, the use of fungicides, bactericides, and insecticides, as well as herbicides is totally eliminated or reduced), preparation of specific nutrient solution according species, and less manpower needed. However, there are some disadvantages: high cost of installation, dependence on electricity in automated system and need for specialized laborers (Resh 1999; Savvas and Passam 2002).

The main factors influencing the overall quality of vegetable crops are: genotype, environmental conditions, growing system and cultural practices (e.g. soilless system, nutrient and water management).

#### **1.1.1. Genotype**

Genetic factors have a direct influence on nutritional value of vegetables. For instance, there are clear examples showing different phytochemical contents of different species of the same genus and of different cultivars of the same species. In literature the effects of genetic factor are well documented for several crops. For example, among major types of cultivated lettuce (romaine, red leaf, butterhead, crisphead, green or red leaf) there are significant differences on carotenoid concentration. Mou (2005) showed that crisphead lettuce accumulated more lutein than  $\beta$ -carotene, while other lettuce types had more  $\beta$ -carotene than lutein. Several vegetable crops (especially leafy vegetables) accumulate  $\text{NO}_3^-$  as the result of an excess of uptake over reduction. Vegetables represent the major source of dietary intake of  $\text{NO}_3^-$ . Nitrite may be formed from  $\text{NO}_3^-$

after ingestion, causing methaemoglobinemia (Wright and Davinson, 1964). Moreover, presence of  $\text{NO}_3^-$  in blood may result also in formation of nitrosamines which are carcinogenic (Craddock, 1983). Genetic variation has been observed for the accumulation of  $\text{NO}_3^-$  in plants due to the different efficiency of N assimilation. Blom-Zandstra and Eenink (1986) found that the differences in the accumulation of  $\text{NO}_3^-$  in lettuce plants were not only a consequence of differences in availability of reduction capacity between cultivars but to their differences in photosynthetic capacity.

At the cultivar level there are also significant differences in the phenolic composition which affects the quality. For instance, in the case of lettuce cultivar a study reported that some genotypes (icerberg and butter leaf type) were very poor in flavonoids and caffeic acid derivatives while others (lollo rosso and oak leaf) contained large amounts of flavonols, caffeic acid derivatives and anthocyanine (Dupont et al. 2000; Tomàs-Barberà et al. 1997)

It is well known that in tomato fruit the contents of lycopene, b-carotene, vitamin C, vitamins E and various phenolic compounds are depending on genetic factor (Dumas et al 2003). Recently, many cultivars with high lycopene content (“high pigment”) have been released by Seed Companies.

In *Brassicaceae* vegetables (turnip, radish, white cabbage, red cabbage, Brussels sprouts, chinese cabbage, broccoli, cauliflower, etc) the profile and content of glucosinolates show great differences among species. The differences are also evident between cultivars of same species. In broccoli the green spear type has higher contents of anti-oxidative effective carotenoids lutein and b-carotene than the crown type and violet cultivars. According to the coloration, radish cultivars differ in their anthocyanin content. (Schreiner 2005).

A study on eighteen cabbage cultivars, two cauliflower cultivars, two Brussels sprouts, four Chinese cabbage cultivars and six broccoli accessions showed that a substantial and significant variation, both within and between the subspecies for the antioxidant phytochemicals (vitamin C, B-carotene, lutein, a-tocopherol, etc) (Singh et al 2007).

Besides there is a great variation inside the plant depending on the plant development and differences can also occur in the same class of compounds. For instance in a variety of kale Velasco et. al. (2007) detected an increasing concentration of aliphatic glucosinolates in leaves from the early stage until the prebolting stage. Indole glucosinolates were increasing from early stage to five months after sowing and then they started to decrease. In oilseed rape, the same behavior occurred, aliphatic and

indole glucosinolates showed unlike variation throughout the development (Fieldsend and Milford 1994).

### **1.1.2 Soilless culture and cultural practices**

In recent decades, the use of soilless culture in crop production has become common, not only for growing seedlings and propagation of plants but also for vegetable production. The principal objective of soilless culture is to give optimal conditions to roots, with the aims of enhancing the growth and development of the whole plant and obtaining high yields of the economically useful part (fruit, leaf, flower, root, etc.). There is a great variety of organic and inorganic substrates used for soilless production. Peat, perlite, vermiculite, rockwool, agrolite, coir, mixtures of some of these and patented commercial mixtures are widely used.

In the last decades many studies have been carried out to improve the nutritional value of vegetables in soilless culture by changing substrate composition, nutrient solution management, etc. For example, Cross et al. (2007) studied the influence of peat, vermiculite, coir, perlite and two peat and perlite mixtures (3:1 and 1:1 v/v, respectively) in a floating system on the total fatty acids content of purslane (*Portulaca oleracea*). They reported that the highest total fatty acids content was obtained in the peat-grown plants and the lowest with coir and perlite. Moreover, other studies with tomato plants revealed that the concentrations of b-carotene, lycopene and lutein in fruits were almost halved when the plants were grown in rockwool (Petersen et al. 1998) or perlite (Stamatakis et al. 2003), compared with plants grown in soil (De Pascale et al. 2001). However, in broccoli plants the total concentration of glucosinolates was three times lower when plants were grown in a mixture of silica sand, white peat and clay compared to those cultivated in soil (Rangkagilok et al. 2004; Schonhof et al. 2007). Moreover, in other work no differences between substrates and soil have been reported (Abak and Celikel, 1994). In five strawberry cultivar, the concentrations of flavonols and total phenolics obtained in different closed soilless systems were similar and were also similar to the values obtained in soil culture (Hernanz et al. 2007). The above findings pointed out that additional factors besides substrates affected the nutritional value of vegetable crops.

The management of mineral nutrition is a key pre-harvest factor that determines the yield and quality of vegetable crops. The total nutrient concentration, defined by electrical conductivity (EC) is one of the most important aspects for a successful

vegetable production. Too high levels of nutrients induce osmotic stress, ion toxicity and nutrient imbalance, while too low values are mostly accompanied by nutrient deficiencies and decreasing of yield (Savvas and Passam, 2002).

Nitrogen is the most limiting nutrient for crop production. Crop plants usually elicit positive vegetative growth response to nitrogen fertilization. The excessive presence of nitrogen is, however, often damaging to the environment as well as to crop quality, especially in vegetables, thereby making nitrogen the most difficult nutrient to manage where quality is a priority. For example, nitrate is the most critical form of N in leafy vegetables because of the potential toxicity of nitrate to humans. Generally, nitrate-accumulating vegetable belong to the families of *Brassicaceae* (rocket, radish, musterd), *Chenopodiaceae* (beetroot, swiss chard, spinach), *Amarantaceae*, *Asteraceae* (lettuce) and *Apiaceae* (celery, parsely) (Santamaria 2006). Nitrate content in plants is affected by external condition such as nitrate availability in the root zone. Rocket absorbs nitrate very quickly and nitrate concentration in leaves can be much higher than in the the root zone. Santamaria et al (2002) showed that in some rocket salad genotype, with 1 mmol L<sup>-1</sup> nitrate nitrogen in a hydroponic nutrient solution, nitrate concentration in the leaves increased until to 101-fold respect to the concentration nutrient solution.

In some species the level of nitrate is also influenced by level of P and K. In pot experimets it has been found that low available P in soil led to an increase in nitrate levels in cabbage and spinach. Absorption and reduction of nitrate is an energy-consuming process, and the energy is supplied by adenosine triphosphate (ATP). Consequently, the metabolism of nitrate is related to P supply. Hydroponic experiments showed that the addition of K decreased nitrate concentrations in cabbage by 26% compared to the control treatment. In contrast, the addition of K increased nitrate concentrations by 8.2% in spinach. Furthermore, application of K to treatments containing high N levels inhibited spinach growth, but had no significant effect on cabbage (Gao et al. 1989)

Although the major form of N supply in the fertilization is nitrate based fertilizers, addition of some ammonium to the nutrient solution seems to be beneficial to the plant and helps to decrease the pH of the nutrient solution. Moreover the modification of nitrate:ammonium ratio can modulate the relative uptake of anions and cations, changing primary and secondary metabolism, and consequently influence the vegetable quality (Sonneveld, 2002).

The effect of fertilisation on the concentrations of phytochemicals in vegetables and fruits has been studied.

Recent investigations revealed a significant influence of sulphur fertilisation on the amounts of flavonoids and hydroxycinnamic acid derivatives of different broccoli cultivars (Vallejo et al. 2003a). The total glucosinolates present in all broccoli cultivars could be affected by rich or poor sulphur fertilisation, establishing a direct relationship between sulphate in nutrient solution and glucosinolate content (Vallejo et al. 2003b).

Moreover, Schonhof et al. (2007) showed that S supply should always be considered in combination with N application with regard to effects on phytochemicals accumulation. Therefore, vegetables can benefit from having an optimal N supply when sufficient S is available to allow the synthesis of S-containing compounds such as glucosinolates. Rangkagilok et al. (2004) studied the effect of sulphur fertiliser in three broccoli cultivars, showing an increase of total S and glucoraphanin accumulation in these cultivars during plant development, with a large increase in broccoli heads and mature seeds. However, different results were obtained with flavonoids, since they strongly decreased. Moreover, abiotic stress has been shown to induce metabolic disturbances, leading to an increase in phenolic compounds (Ali and Abbas 2003). The effect of fertilisation with micronutrients has been also studied. Zinc availability influenced glucosinolate levels in *Brassicaceae* species (Coolong et al. 2004). The effects of a wide range of Zn levels on detectable glucosinolates in hydroponically grown *Brassica rapa* suggested that Zn influenced glucosinolates content and flavour (bitterness, etc.). Finally, Robbins et al. (2005) showed in broccoli that Se may decrease the contents of the bioactive components studied, independent of other parameters.

Water availability and its salt concentration affect the synthesis and/or accumulation of phytochemical. The major of studies regard the tomato fruit. De Pascale et al. (2001) also found significant increases in fruit quality parameters and lycopene content when tomatoes were irrigated with moderately saline solutions. In studies of the effects of irrigation regimes on the phytochemical content of different tomato cultivar, it was observed that the fruit lycopene content decreased in response to flooding stress (Naphade 1993). Deficit irrigation strategies are relatively a good tool for managing plant growth and improving quality and water use efficiency, while maintaining yields. Peterson et al. (1998) found that tomato B-carotene content increased due to a reduction of plant water content. In Cherry tomatoes both the total carotene and the amount of lycopene increased in response to drought (Matsuzoe et al. 1998). Zushi and Matsuzoe

(1998) showed that water deficits tended to increase the amount of lycopene but had no effect on the amount or distribution of B-carotene and xanthophylls. It has been shown that antioxidant compounds in tomato are sensitive to soil water content and that higher levels were produced when plant experienced drought-like conditions imposed by deficit irrigation practices (Mitchell et.al.1991).

Water shortage seems generally to tend to increase the vitamins C content of the tomato fruit as well as the dry matter and soluble solid content. However, in a work it has been showed that the effects of soil water deficits on the vitamin C may be positive or null depending on the cultivar (Zushi and Matsuzoe 1998).

Proietti et al. (2008) found that fruit quality of mini-watermelon plants was affected by drought with an increase in K, Mg, and spermine concentration.

In broccoli decreasing water availability doubled the glucosinolate content (Paschhold et al. 2000).

Changes of flavonols can occur due to the activities of the enzymes involved in the biosynthesis of phenolic compounds such as L-phenilalanine ammonia-lyase which is more active under higher water stress (Tovar et al. 2002).

Water stress is known to increase glucosinolate content in watercress (Ciska et al.2000).

The soilless culture offers the possibility to better control the water availability in the root zone for specific periods during the growing cycle leading to a better product quality.

However, increasing too much the content of phytochemicals such as phenol compounds in some species (leafy vegetable) can be a disadvantage; it is well known that a high level of bitterness is related to the phenol content and an excessive level of bitterness could cause the rejection of the product by consumers. Thus, to meet product quality and marketing needs an adequate amount of water has to be supplied.

### **1.1.3 Environmental factors**

Climatic conditions, especially temperature and light intensity, have an strong effect on the growth and yield but also on nutritional quality of vegetables (Savvas and Passam, 2002). Radiation intensity has a definite influence on flavonoids metabolism. Vegetables exposed to full sunlight contained more flavonoids than those grown under shading conditions. Generally, the glucosinolates content of broccoli and cauliflower is strongly influenced by ht temperature and to a lesser extent by irradiation during plant

development (Schreiner 2005). In broccoli, the contents of two flavonols (quercetin and kampferol) were highly positively correlated with total solar radiation in the period from planting to the harvest of broccoli inflorescences (Gliszczynska-Swiglo et al. 2007). In five botanical groups of *B. oleracea* high concentration of total glucosinolates generally corresponded to cultivation at higher temperatures and photosynthetic photon flux as well as to longer day length. Elevated atmospheric CO<sub>2</sub> (685-820 ppm) in comparison to ambient CO<sub>2</sub> (430-480) concentration increased the total glucosinolate (Schonhof et al 2007).

In contrast to the flavonoids and glucosinolates, radiation is not essential for inducing carotenogenesis and hence radiation does not influence carotenoid biosynthesis that is strongly temperature-dependent. For example daily mean temperature below 16.5 °C were beneficial for the  $\beta$ -carotene synthesis in broccoli whereas beneficial temperatures for lycopene formation in tomato were found in the range from 16°C to 21 °C. (Schreiner 2005).  $\beta$ -carotene content is reduced if temperatures are higher or lower than this range, principally due to the temperature sensitivity of lycopene, the precursor to  $\beta$ -carotene and lutein (Dumas et al. 2003).

In a study on eight green leaf and eight red leaf lettuce varieties it has been showed that supplemental UV-B increased the carotenoid and chlorophyll concentration of green leaf lettuce while reducing the levels of these compounds in red leaf lettuce (Caldwell and Britz, 2006).

In kale grown under increasing photoperiods, maximum chlorophyll occurred under the 12-hour photoperiod whereas the maximum accumulation of  $\beta$ -carotene and lutein was shifted to the 16-hour photoperiod (Lefsrud et al. 2006a).

There is also a different response to the environmental factors according to the species. For instance, in a study on kale and spinach, it has been reported that the accumulation of lutein and  $\beta$ -carotene were significantly different among radiation levels for kale but were not significantly different for spinach (Lefsrud et al. 2006b).

Moreover, in tomato fruit light exposure is favourable to vitamin C accumulation (Dumas et al. 2003).

The radiation has also a strong influence on nitrate accumulation. Low light levels are associated with nitrate accumulation in some leafy vegetables (lettuce and spinach) whereas high light level have opposite effect (Santamaria, 2006). Moreover, in lettuce growth and nitrate accumulation were observed to increase when the root zone temperature was increased and the air temperature decreased (van der Boon et al. 1990).

## 1.2 Aim of experiments

Starting from the above considerations, taking into account that soilless growing systems are common practice in many countries all over the world and that in these systems a better control of factors is possible, the aim of the thesis was to investigate the influence of nutrient solution and environmental factors on growth and quality of some leafy vegetable such as baby lettuce and Asian Brassicaceae species, grown in floating raft culture and nutrient film technique, respectively. Baby lettuce (*Lactuca sativa* L. var. *acephala*) is considered a primary component of fresh-cut (minimally processed) vegetables which are increasingly marketed for fast food, catering and home consumption because of their convenience as a ready-to-use product.

Instead, Asian Brassica vegetables are very consumed from Chinese population and as all cruciferous vegetables they are a rich source of glucosinolates and flavonoids, compounds that seem to play an essential role in the cancer-preventive effects. In addition, in the literature there is a shortage of information about the influence of pre-harvest factors in these species on some quality parameters and growth.

In particular, the effects of five nutrient solution concentrations (with the same ionic proportion) and two growing seasons on yield and leaf quality of *Lactuca sativa* L. var. *acephala* are discussed in the Chapter 1 while the effects of six nutrient solution at the same concentration but with different macronutrient proportions and two growing seasons on yield and leaf quality of *Lactuca sativa* L. var. *acephala* are discussed in the Chapter 2; the effects of N form and radiation on the growth and mineral content of two *Brassica* species are reported in the Chapter 3; and finally, in the Chapter 4, the influence of N form and radiation on phytochemical concentration in two *Brassica* species are discussed. Final conclusion are reported in the Chapter 6.

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## **CHAPTER 2**

**Nutrient solution concentration and growing season affect  
yield and quality of *Lactuca sativa* L. var. *acephala* in a  
floating raft culture**

## Abstract

**BACKGROUND:** There is a growing interest among consumers in baby leaf vegetables, mostly requested for mixed salads, both as fresh market products and ready-to-use vegetables. Fertilization is one of the most practical and effective ways to control and improve yield and nutritional quality of crops for human consumption. The optimal fertilizer concentration of baby leaf vegetables depends on the environmental conditions. The aim of the present work was to determine the effects of nutrient solution concentration (2, 18, 34, 50 or 66 mequiv L<sup>-1</sup>) during two consecutive growing seasons (spring and summer) on yield and leaf quality of *Lactuca sativa* L. var. *acephala* grown in a floating system.

**RESULTS:** Marketable fresh yield, total dry biomass, leaf area index, macroelement concentrations (N, P, K and Mg), nitrate and total chlorophyll contents increased in response to an increase in the nutrient solution concentration, while an opposite trend was observed for the root-to-shoot ratio, glucose, fructose, starch, total carbohydrates and protein contents. Plants grown in the spring season exhibited a lower yield and growth (total dry biomass and leaf area index) than those grown in the summer season but offered highest leaf quality (higher content of glucose, sucrose and total carbohydrates and lower nitrate content)

**CONCLUSION:** The use of nutrient solution concentration of 37 and 44 mequiv L<sup>-1</sup> for the spring and summer season, respectively could be adopted in our conditions for improving marketable fresh yield and leaf mineral content with a slight reduction of some nutritional parameters.

**Keywords:** growing season; hydroponics; leafy lettuce; nutrient solution concentration; nutritional quality

## 2.1 INTRODUCTION

The minimally processed or fresh-cut leafy vegetables have been gaining importance in the world-wide vegetables market.<sup>1</sup> The consumer demand has been increasing in recent years and many farmers oriented their production plans towards the baby leaf vegetables such as lettuce, spinach, rocket, lamb's lettuce and Swiss chard<sup>2</sup>. The nutritional quality of vegetables can be affected by many pre- and post-harvest factors.<sup>3</sup> The management of mineral nutrition is a key pre-harvest factor that determines the yield and quality of

leafy vegetable crops. In this perspective the soilless culture represents an important tool, because it permits a precise control of plant nutrition.<sup>4</sup>

Leafy vegetables grown in soilless culture require careful management of fertilizers<sup>5</sup> because of limited root substrate; high density of seedlings, also the concentrations of essential plant nutrients in the media are frequently insufficient to sustain plant growth. Therefore, optimizing the nutrient solution concentration is required by farmers in order to maximize yield and quality. The total nutrient concentration of the solutions used in soilless culture is one of the most important aspects for a successful vegetable production. Too high levels of nutrients induce osmotic stress, ion toxicity and nutrient imbalance, while too low values are mostly accompanied by nutrient deficiencies.<sup>6</sup> Several attempts have been made to establish optimal ranges of total ionic concentration in the nutrient solution for the production of floricultural greenhouse crops.<sup>7-12</sup> However, there is a lack of information about the optimal fertilizer concentrations for many vegetable crops especially the baby leaf vegetables.

An optimal fertilizer concentration and water supply of horticultural crops in soilless system also depends on the environmental conditions. For instance, Kang and Van Iersel<sup>13</sup> reported that the optimal fertilizer concentration of potted plants decreased with increasing temperature. Moreover, Roorda van Eysinga and van der Meijs<sup>14</sup> have demonstrated a greater influence of nitrogen supply on the nitrate content of soil-grown lettuce under high light conditions, where crop nitrate content was low and increased considerably with increasing rates of nitrogen application, than under low light, where crop nitrate content was high and increased only slightly with increasing rates of nitrogen application. A similar interaction has also been reported for spinach.<sup>15</sup> Therefore, different temperatures and solar radiation conditions may be good treatment variables to look at possible interactive effects of environmental conditions and nutrient solution concentration on plant growth, yield and quality.

In Italy, leafy lettuce (*Lactuca sativa* L. var. *acephala*) is traditionally cultivated in soil and is considered a primary component of fresh-cut (minimally processed) vegetables which are increasingly marketed for fast food, catering and home consumption because of their convenience as a ready-to-use product. Despite a relatively large market for this crop, alternative cultivation techniques, such as floating system, have not been sufficiently considered for improving the yield and quality of this species. The floating system can provide an alternative system to traditional soil cultivation of *Lactuca sativa* L. var. *acephala*, to obtain both higher sanitary quality without soil contaminants and

higher yield, to standardize cultural practices, to reduce production costs, and to have better control of the cultivation process.<sup>16</sup>

Starting from the above considerations, the objective of this study was to determine the effects of nutrient solution concentration (2, 18, 34, 50 or 66 mequiv L<sup>-1</sup> giving an electrical conductivity values of 0.3, 1.2, 2.0, 2.8 or 3.6 dS m<sup>-1</sup>) during two consecutive growing seasons (spring and summer) on yield and leaf quality (chlorophyll content, colour parameters, carbohydrates and mineral composition) of *Lactuca sativa* L. var. *acephala* grown in a floating system.

## 2.2 MATERIALS AND METHODS

### 2.2.1 Plant material and growth conditions

Two experiments were conducted in two consecutive growing seasons: spring season (experiment 1) and summer season (experiment 2) in a polyethylene 200 m<sup>2</sup> greenhouse situated at the experimental farm of Tuscia University, central Italy (latitude 42°25'N, longitude 12°08'E, altitude 310 m). Inside the greenhouse, ventilation was provided automatically when the air temperature exceeded 26 °C, light was provided only by natural solar radiation. The following climate data inside the greenhouse was determined: dry and wet bulb air temperature by means of wire resistance thermometers in aspirated boxes, solar radiation by means of a pyranometer (CM11 Kipp and Zonen, Netherlands). All measurements were collected on a data logger system (CR10X, Campbell Scientific, Inc., UK), the sensors were scanned every minute and the 30 min average values were recorded.

Seeds of lettuce (*Lactuca sativa* L. var. *acephala* cv. 'Green Salad Bowl', SAIS seed company, Cesena, Italy) were sown on 24 March 2007 (experiment 1) and on 31 May 2007 (experiment 2) into a floating raft growing system, consisting of polystyrene plug trays floating in plastic tanks with a constant volume (65 L) of stagnant nutrient solution, which was continuously aerated with an air compressor in order to maintain the oxygen content above 6.0 mg L<sup>-1</sup>. The planting density was 1857 plants m<sup>-2</sup>, as used commercially for similar leafy vegetables in floating systems.

In both growing seasons (experiments 1 and 2), a randomized complete-block design with three replicates was used to compare five nutrient solution concentrations (2, 18, 34, 50 and 66 mequiv L<sup>-1</sup>). Each experimental unit consisted of 0.147 m<sup>2</sup> (273 plants) container filled with 65 L of aerated nutrient solution.

### 2.2.2 Nutrient solution management

In all nutrient solutions, the macroanion proportions were  $0.90\text{NO}_3^-/0.05\text{SO}_4^{2-}/0.05\text{H}_2\text{PO}_4^-$  whereas the macrocation proportions were  $0.25\text{K}^+/0.50\text{Ca}^{2+}/0.25\text{Mg}^{2+}$ , and the ratio between anions ( $\text{NO}_3^- + \text{SO}_4^{2-} + \text{H}_2\text{PO}_4^-$ ) and cations ( $\text{K}^+ + \text{Ca}^{2+} + \text{Mg}^{2+}$ ) was equal to 1. The macronutrients concentrations were 2, 18, 34, 50 or 66 mequiv  $\text{L}^{-1}$  giving electrical conductivity values of 0.3, 1.2, 2.0, 2.8 or 3.6  $\text{dS m}^{-1}$ , respectively. In all treatments, the micronutrients concentrations were Fe, 40.0  $\mu\text{mequiv L}^{-1}$ ; Mn, 18.0  $\mu\text{mequiv L}^{-1}$ ; Cu, 3.0  $\mu\text{mequiv L}^{-1}$ ; Zn, 6.0  $\mu\text{mequiv L}^{-1}$ ; B, 60.0  $\mu\text{mequiv L}^{-1}$  and Mo, 1.8  $\mu\text{mequiv L}^{-1}$ . The pH of the nutrient solution for all treatments was  $6.0 \pm 0.5$ . Demineralized water was used in the preparation of all nutrient solutions.

### 2.2.3 Recording, sampling and analysis

In experiments 1 and 2, lettuce were harvested on 17 April and 21 June, respectively at the same physiological age, expressed as the standard accumulation of growing-degree (base-temperature of 6 °C; ceiling temperature of 30°C) days after sowing, which was in the range of 380-385 degree-days. Fifty plants per plot were separated into shoots to determine marketable fresh yield and roots, and their tissues were dried in a forced-air oven at 80 °C for 72 h for biomass determination. Root to shoot ratio was calculated by dividing root dry weight by the shoot dry weight. Leaf areas (LA) were measured on 8 plants per treatment using an electronic area meter (Delta-T Devices Ltd., Cambridge, UK). Leaf area index (LAI) was computed as the ratio of green leaf area divided by the ground area.

In both experiments, dried leaf tissues were ground separately in a Wiley mill to pass through a 20 mesh screen, then 0.5 g of the dried plant tissues were analyzed for the following macro and micronutrients: N, P, K, Ca, Mg, Fe, Cu, Mn, and Zn. Nitrogen concentration in the plant tissues was determined after mineralization with sulfuric acid by “Regular Kjeldahl method”<sup>17</sup>, P, K, Ca, and Mg concentrations were determined by dry ashing at 400 °C for 24 h, dissolving the ash in 1:25 HCl, and assaying the solution obtained using an inductively coupled plasma emission spectrophotometer (ICP Iris; Thermo Optek, Milano, Italy).<sup>18</sup>

In both experiments, external lettuce leaf color was measured using a Minolta Chromameter (Minolta Chroma Meter CR-200, Minolta Camera Co. Ltd., Osaka, Japan) in CIE  $L^*$ ,  $a^*$ ,  $b^*$  mode with an 8-mm measuring aperture using CIE Standard Illuminant C [in the CIELAB System: the  $L^*$  component represents lightness; the  $a^*$

component represents values from green (–) to red (+); and the b\* component represents values from blue (–) to yellow (+)]. The instrument was calibrated with the Minolta Calibration standard white reflector plate before sampling lettuce leaves. Whole-leaf samples were placed on a white background and single readings were taken on the upper surface of each leaf midway between the apical and basal ends with the handheld unit. Individual leaves were then positioned between two paper towels moistened with distilled water in a plastic bag and held on ice until all readings were completed. L\*, a\*, b\* readings were transformed to those of the L, a, b color space. For the chlorophyll and carotenoids analyses, leaf discs were taken from 24 plants per replicate. Chlorophyll and carotenoids were extracted by grinding the tissue with a mortar and pestle using ammoniacal acetone. The resulting extracts were centrifuged at 3000×g for 3 min. The total chlorophyll and carotenoid contents were determined by UV–vis spectrophotometry (Beckman DU-50 spectrophotometer; Beckman Instruments, Inc., Fullerton, CA). The absorbance of the solution was measured at 470, 647 and 664 nm. Formulae and extinction coefficients used for the determination of chlorophyllous pigments (total chlorophyll and carotenoids) were described by Lichtenhaler and Wellburn.<sup>19</sup>

In both experiments, 16 lettuce plants per experimental unit were harvested, frozen and stored in liquid nitrogen for quality analysis. The frozen samples were reduced to a fine powder with a mortar and pestle under liquid nitrogen. Spare powder was freeze dried and used for measurements of soluble carbohydrates, starch, nitrate and protein contents.

Soluble carbohydrate (glucose, fructose, and sucrose) and starch were extracted on 80 mg dry sample, in 1.5 mL of 80% (v/v) ethanol, 20% (v/v) 100 mmol L<sup>–1</sup> Hepes (pH 7.3), containing 10 mmol L<sup>–1</sup> MgCl<sub>2</sub> at 80 °C for 45min. Glucose, fructose, sucrose and starch determinations were performed by spectrophotometric coupled enzymatic assays as described by Jones et al.<sup>20</sup> including the modification of Antognozzi et al.<sup>21</sup>. To measure nitrate content, 50 mg lettuce samples were extracted by placing in 1 mL water at 70 °C for 40 min. After centrifugation at 12000 × g for 5 min, the supernatant was used for the determination of nitrate content by an enzymatic assay.<sup>22</sup>, the assay was performed with a dualwavelength (340–400 nm) spectrophotometer (Thermo Spectronic-HELIOS, UK). Finally, quantification of proteins was performed according to the principle of protein-dye binding.<sup>23</sup>

### 2.2.4 Statistical analysis

Analysis of variance of the data was calculated using the software package, SPSS 10 for Windows, 2001. Combined analysis of variance was performed using season as a fixed variable.<sup>24</sup> In both experiments, orthogonal polynomial contrasts were used to compare nutrient solution concentration effects<sup>24</sup> on selected parameters. Regression analyses were conducted to identify relationships between marketable yields, shoot dry biomass, leaf area index root-to-shoot ratio and the concentration of the nutrient solution.

## 2.3 RESULTS

### 2.3.1 Climatic conditions inside the greenhouse

Differences between the growing seasons in daily global radiation ( $R_g$ ) and mean air temperature ( $T_a$ ) were observed (Fig. 2.1). During the spring season, the daily  $R_g$  and  $T_a$  ranged from 6.6 and 20.9 MJ m<sup>-2</sup>, 16.2 and 22.7 °C, respectively, while for the summer season, the daily  $R_g$  and  $T_a$  ranged from 5.4 to 23.6 MJ m<sup>-2</sup> and from 19.3 to 29.6 °C, respectively. Moreover, a positive linear correlation between  $R_g$  and  $T_a$  was observed ( $R = 70$ ,  $P < 0.01$ ). This relationship between the climatic parameters inside the greenhouse can be expected especially in non-heated greenhouses because the climatic factors are closely related to each other: the stronger the solar radiation is, the higher the air temperature in the greenhouse will be.<sup>25,26</sup>

### 2.3.2 Plant growth and yield

Marketable yield, total dry biomass, and leaf area index (LAI) of lettuce in both experiments were highly influenced by the growing season ( $P < 0.01$ ) and nutrient solution concentration ( $P < 0.05$ ), whereas no significant difference was observed on the growing season  $\times$  nutrient solution concentration ( $S \times C$ ) interaction. Moreover, the root-to-shoot ratio (R/S) was significantly affected by growing season ( $P < 0.01$ ), nutrient solution concentration ( $P < 0.05$ ), with an  $S \times C$  interaction ( $P < 0.05$ ). Regression analysis indicated a significant quadratic effect of nutrient solution concentration on lettuce marketable yield, total dry biomass, LAI, and the R/S ratio (Fig. 2.2). In both experiments, marketable yield, total dry biomass, and LAI increased quadratically in response to an increase in electrical conductivity, while an opposite trend was observed for the R/S ratio (Fig. 2.2). Moreover, regression analysis indicates maximum yield and total dry biomass at 37 and 38 mequiv L<sup>-1</sup> for the spring season and at 44 and 43 mequiv L<sup>-1</sup> for the summer season, respectively, whereas the lowest values

of the R/S ratio observed at 44 mequiv L<sup>-1</sup> in both growing seasons. Finally, irrespective of the nutrient solution concentration the highest values of yield, total dry biomass and LAI was recorded in the summer season (3.5 kg m<sup>-2</sup>, 196.5 g m<sup>-2</sup>, and 11.6, respectively), in comparison to those recorded during the spring growing season (1.7 kg m<sup>-2</sup>, 123.0 g m<sup>-2</sup>, and 6.8, respectively).

### **2.3.3. Leaf chlorophyll, carotenoids, nitrate contents and color parameters**

Total chlorophyll and nitrate contents were significantly affected by the growing season and nutrient solution concentration, with no significant S × C interaction (Table 2.1). Increasing the nutrient solution concentration from 2 to 66 mequiv L<sup>-1</sup> caused quadratic and linear increases in total chlorophyll and nitrate contents, respectively. Moreover, irrespective of nutrient solution concentration, the total chlorophyll and nitrate contents were significantly higher by 31% and 22%, respectively, in the summer season in comparison with the spring season (Table 2.1).

Color parameters represented by L\*, a\* (greenness) and the b\* (yellowness) components were highly influenced by the nutrient solution concentration (Table 2.1). In both growing seasons, L\*, a\*, and b\* increased quadratically in response to an increase in the electrical conductivity, with the highest values recorded at 34 and 50 mequiv L<sup>-1</sup>.

Irrespective of the nutrient solution concentration, the highest a\* and b\* values were recorded in the spring season, compared to those observed with the summer season. Finally, no significant difference among treatments was observed for total carotenoids content (avg. 139.1 mg kg<sup>-1</sup> fresh weight).

### **2.3.4 Carbohydrates and proteins**

No significant S × C interaction was observed for the reduced sugars (glucose, fructose and sucrose), starch and protein concentrations. Sucrose was the predominant sugar in both seasons, while glucose and fructose were presented in lower quantities (Table 2.2). In both growing seasons, increasing the nutrient solution concentration from 2 to 66 mequiv L<sup>-1</sup> caused linear decreases in glucose, fructose, starch, and proteins whereas the sucrose content was not affected by the increase of the concentration of the nutrient solution. Moreover, irrespective of the nutrient solution, the concentrations of glucose, sucrose and total carbohydrates recorded during the summer cropping season were significantly reduced by 20, 40, and 30%, respectively compared to leafy lettuce picked during the spring season (Table 2.2).

### 2.3.5 Mineral composition

The macroelements concentration of lettuce plants as a function of the growing season and nutrient solution concentration are displayed in Table 2.3. The concentration of N, K, and Mg were highly influenced by the growing season and nutrient solution concentration, with no  $S \times C$  interaction, whereas the P concentration was significantly affected by nutrient solution concentration,  $S \times C$  interaction, but not by growing season. Moreover, no significant difference among treatments was observed for Ca concentration (avg.  $10.0 \text{ g kg}^{-1}$  dry weight). In both experiments, the concentration of N, P, and K increased quadratically and linearly for Mg in response to an increase in electrical conductivity of the nutrient solution (Table 2.3). Finally, irrespective of the nutrient solution, the N, K and Mg concentration in leafy lettuce recorded in the summer season was significantly higher by 9, 13, and 16%, respectively than those harvested during the spring cropping season (Table 2.3).

## 2.4 DISCUSSION

It is well-established that crop growth and yield are negatively affected by too high or too low concentration of the nutrient solution.<sup>6</sup> In the current experiment, marketable fresh yield, dry shoot biomass and leaf area index (LAI) of leafy lettuce, were significantly reduced at low (2 and  $18 \text{ mequiv L}^{-1}$ ) and high ( $66 \text{ mequiv L}^{-1}$ ) fertilizer concentrations, due to nutrient deficiencies and osmotic stress, respectively. The reduction of yield due to osmotic effect has been reported previously for many vegetable crops.<sup>27-29</sup> The main effect of high or low nutrient solution concentration was the reduction of growth rate due to smaller leaves; shorter stature, fewer leaves and imbalance between shoot and root growth also occurred. The imbalance between shoot and root indicated that partitioning in the plants was affected by the fertilizer concentrations. Moreover, the results of this study suggest that *Lactuca sativa* L. var. *acephala* indicated maximum yield at 37 and  $44 \text{ mequiv L}^{-1}$  for the spring and summer growing season, respectively. The higher marketable yield of lettuce in the summer cropping season in comparison to the spring season was due to solar radiation conditions.<sup>30</sup> The higher solar radiation due to the high level of natural light and long photoperiod was presumably responsible for the increased photosynthesis in the summer with respect to the spring season: the mean value of daily global radiation in the greenhouse was  $19.1 \text{ MJ m}^{-2}$  in the summer versus  $14.6 \text{ MJ m}^{-2}$  in the spring season.

In line with the above findings, Marcelis<sup>31</sup> observed that shading cucumber plants grown in a glasshouse during an extended period reduced the yield by 60% in comparison to cucumber plants grown at 100% irradiance level. Moreover, He et al.<sup>32</sup> reported a reduction of tomato yield grown hydroponically by 49% in the fall to winter compared to the spring to summer cropping seasons. Finally, in a study in England on tomatoes,<sup>33</sup> both light shade (6.4%) and heavy shade (23.4%) reduced total fresh weight yield of fruit by 7.5 and 19.9%, respectively compared to unshaded crops.

In both growing seasons, chlorophyll content and the  $a^*$  component (greenness) of leafy lettuce increased with increasing nutrient solution concentration from 2 to 34 mequiv L<sup>-1</sup>, while there was a significant decrease from 50 to 66 mequiv L<sup>-1</sup>. Low fertilizer concentrations generally decrease chlorophyll content<sup>8, 9, 11</sup>, presumably due to lower nitrogen levels in the leaves. In addition, yellowing (the  $b^*$  component) or loss of green colour which is considered the major consequence of chlorophyll degradation was also observed with too high levels of nutrients. An increase in total salt concentration above 50 mequiv L<sup>-1</sup> in the nutrient solution leads to an osmotic stress and consequently to a decrease in N uptake, which may also affect the biosynthesis of plant metabolites, including chlorophyll. A similar response of chlorophyll degradation in relation to biotic and abiotic stress such as water and heat stress, insect feeding and aging, has been reported earlier.<sup>34-36</sup>

Despite the debate about the effect of nitrates on human health, the negative effect of accumulated nitrate on vegetable quality is indisputable.<sup>37</sup> In Europe, widespread evidence of nitrate accumulation in leafy vegetables led to the European Community developing limits on fresh nitrate concentration of lettuce.<sup>38</sup> Both nitrate supply and light intensity are known to be critical factors in determining nitrate levels in leafy vegetables.<sup>38</sup> In the present study, increasing the nutrient solution concentration from 2 to 66 mequiv L<sup>-1</sup> caused a linear increase in nitrate contents, but the nitrate values in leaf tissues were never as high as the limit value of 4500 mg kg<sup>-1</sup> fresh weight imposed by the European Community. The high correlation between nutrient application rates, in particular nitrogen and crop nitrate is in close agreement with the results of several earlier studies with lettuce grown in soil under both field<sup>39, 40</sup> and greenhouse<sup>14, 41</sup>. Moreover, low light levels are associated with nitrate accumulation in some leafy vegetables. For example Chadja et al.<sup>42</sup> and Gaurdeau et al.<sup>43</sup> reported that a reduction in the light level was associated with reduced nitrate reductase activity and increased nitrate accumulation in lettuce and spinach. Nitrate is favoured as an osmoticum at low

light levels, replacing the energy-expensive carbohydrate, to maintain turgor pressure in lettuce.<sup>44, 45</sup> However, Cantliffe<sup>15</sup> observed little effect of light above irradiance levels of about 450-900  $\mu\text{mol m}^{-2} \text{s}^{-1}$  on spinach leaf nitrate concentration. Similarly, Parks et al.<sup>37</sup> showed that the shoot nitrate concentration of Swiss chard was primarily affected by nitrogen supply and not by light level because the light conditions exceeded the critical level ( $\sim 200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) required to increase leaf nitrate. They also observed that higher growth and greater nitrate accumulation of Swiss chard occurred in the spring experiment, compared with the winter experiment, and this was influenced by a higher range of temperatures. Moreover, Laurie and Stewart<sup>46</sup> showed that high temperatures stimulated growth and did not affect root activity of chickpea but reduced shoot nitrate reductase activity, potentially leading to nitrate accumulation. In the current study the higher nitrate concentration in the summer growing season could be associated to a higher range of temperatures. Average minimum to maximum temperatures of 19.3-29.6 °C were obtained for the summer experiment compared with 16.2-22.7 °C for the spring experiment.

In general, increased electrical conductivity (EC) in the nutrient solution reduces the yield of vegetable crops, but in many improves their nutritional quality, as observed in plants grown in both soil and soilless culture.<sup>29,47</sup> In this study, increasing EC of the nutrient solution from 2 to 66 mequiv L<sup>-1</sup> decreased the qualitative characteristics especially when the nutrient solution concentration was above 50 mequiv L<sup>-1</sup>: leafy lettuce with lower content of glucose, fructose, starch, total carbohydrates and protein. It was demonstrated, that high respiration rate in several crops was observed when the ionic strength of the nutrient solution increase.<sup>48</sup> Hence, the reduction of sugars content in leafy lettuce with high nutrient concentration could be related to an increase in tissue vegetable respiration. The reduced sugar content in the high nutrient solution concentration treatment was accompanied by an increased content of nitrate. Similar negative relationship between sugars and nitrate<sup>49, 50</sup> has been previously reported. Moreover, the effect of the growing season significantly affected the quality parameters of leafy lettuce. In comparison to the summer season, our results indicate that during the spring season an improvement of glucose, sucrose and total carbohydrates was observed. The above findings are in line with those reported on tomatoes by<sup>51</sup>, which observed a reduction of sugar concentration during the warm season due to a lower activity level of sucrolytic enzymes as compared with the cool season.

In comparison to the spring season, our results indicate that during the summer season higher concentrations of K and Mg were observed, which is interesting from a nutritional point of view because fruits and vegetables usually contribute to 35 and 24% respectively of the total K and Mg to the dietary intake of humans.<sup>52</sup> The highest macronutrient content (N, K and Mg) of leafy lettuce in summer vs. spring season was mainly related to the total plant biomass. The total plant uptake of N, K, and Mg in greenhouses is usually enhanced by stronger natural radiation or supplemental light.<sup>53</sup>

## 2.5 CONCLUSIONS

It can be concluded that yield and quality of leafy lettuce grown in a floating system was significantly affected by the growing season, where plants grown in the spring season exhibited a lower yield and growth (total dry biomass and leaf area index) than those grown in the summer season but offered highest fruit quality (higher content of glucose, sucrose and total carbohydrates and lower nitrate content). The results also indicate that although, the effect of nutrient solution concentration on yield and quality of leafy lettuce was significant, and more pronounced than the effect of growing season. Increasing fertilizer concentrations increased linearly or quadratically plant growth parameters, marketable fresh yield and leaf mineral content (K and Mg), but negatively affected some quality parameters of leafy lettuce (higher content of nitrates and lower concentration of glucose, fructose, starch, total carbohydrates and protein). The use of nutrient solution concentration of 37 and 44 mequiv L<sup>-1</sup> for the spring and summer season, respectively could be adopted in our conditions for improving marketable fresh yield and leaf mineral concentration (K and Mg) with a slight reduction of some nutritional parameters (carbohydrates and proteins).

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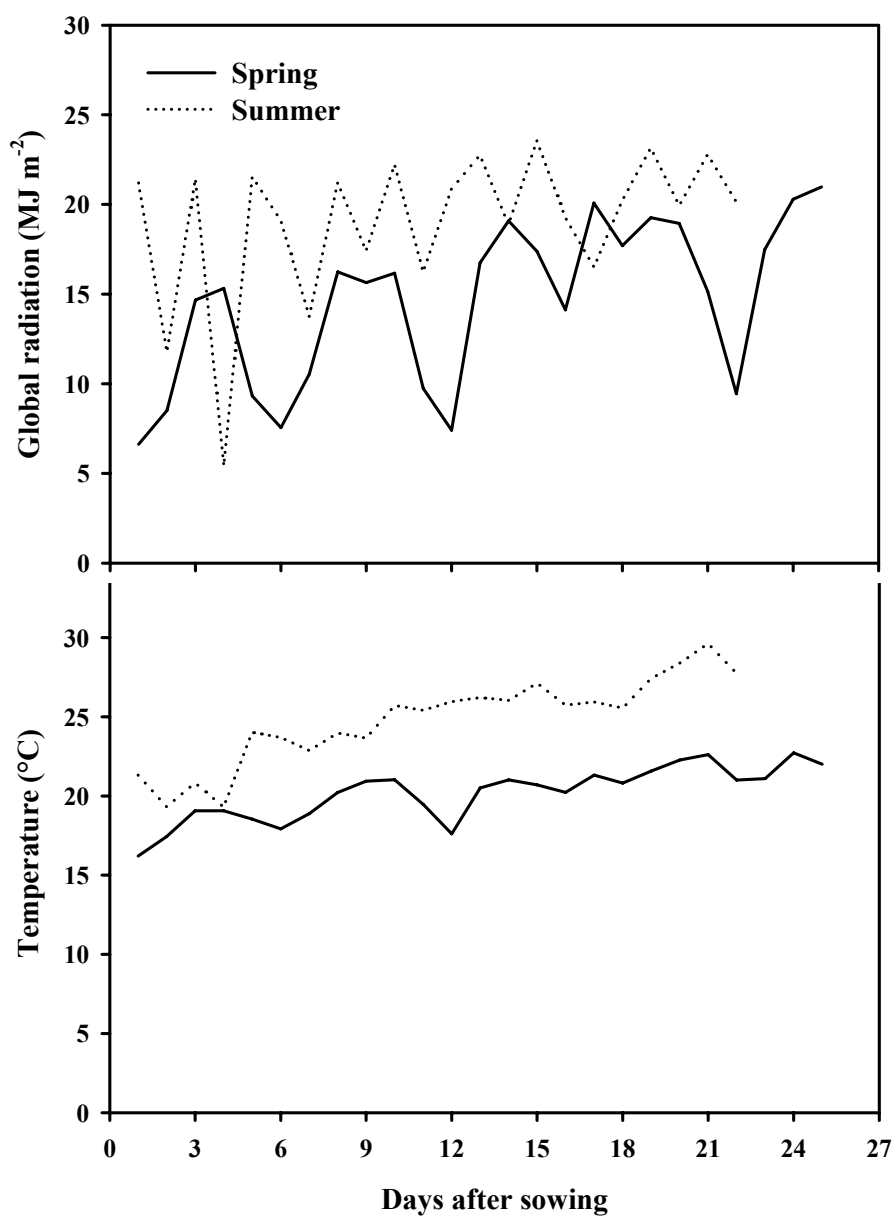


**Figure 2.1.** Daily global radiation and mean air temperatures recorded inside the greenhouse during the spring (24 March-17 April) and summer (31 May-21 June) seasons.

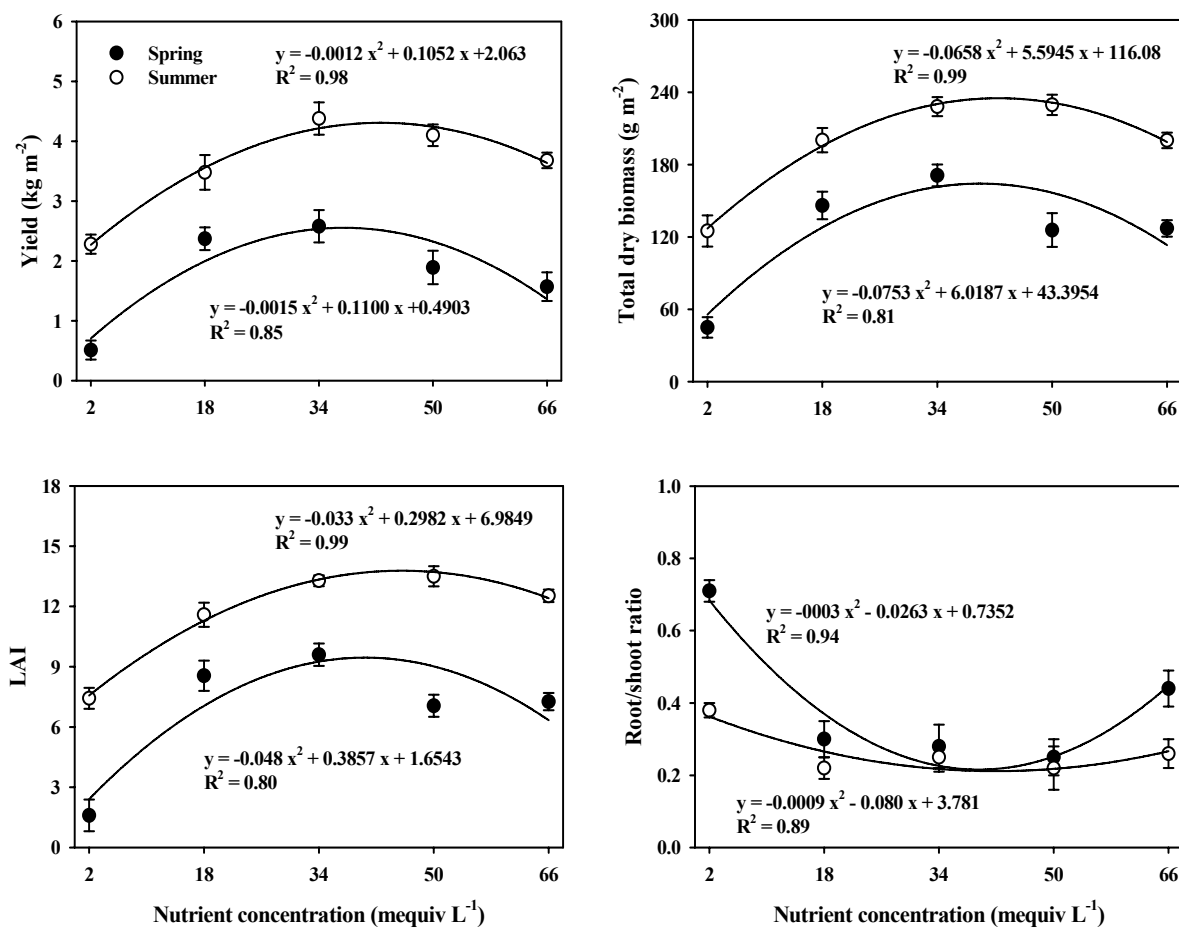


**Figure 2.2.** Relationships between marketable yield, total dry biomass, leaf area index and root-to-shoot ratio and nutrient solution concentration of greenhouse lettuce during the spring and the summer seasons.

**Table 2.1.** Effects of growing season and nutrient solution concentration on leaf chlorophyll, nitrate content and colour parameters of lettuce leaves

| Growing season             | Nutrient concentration<br>(mequiv L <sup>-1</sup> ) | Total chlorophyll<br>(mg kg <sup>-1</sup> FW) | Nitrate<br>(mg kg <sup>-1</sup> FW) | L*          | a*           | b*          |
|----------------------------|-----------------------------------------------------|-----------------------------------------------|-------------------------------------|-------------|--------------|-------------|
| Spring                     | 2                                                   | 482.5 ± 18.9                                  | 1336 ± 115                          | 55.0 ± 0.4  | -11.4 ± 0.3  | 39.5 ± 0.2  |
|                            | 18                                                  | 585.8 ± 17.3                                  | 1823 ± 173                          | 57.0 ± 0.7  | -13.6 ± 0.2  | 40.7 ± 0.5  |
|                            | 34                                                  | 677.4 ± 19.9                                  | 2092 ± 189                          | 57.6 ± 0.6  | -13.7 ± 0.1  | 42.0 ± 0.3  |
|                            | 50                                                  | 577.0 ± 21.8                                  | 2322 ± 152                          | 58.8 ± 0.3  | -13.4 ± 0.1  | 42.8 ± 0.5  |
|                            | 66                                                  | 558.8 ± 10.3                                  | 2490 ± 112                          | 56.9 ± 0.8  | -13.3 ± 0.2  | 40.9 ± 0.4  |
|                            | <i>mean</i>                                         | <i>566.3</i>                                  | <i>2013</i>                         | <i>57.1</i> | <i>-13.1</i> | <i>41.2</i> |
| Summer                     | 2                                                   | 655.3 ± 5.9                                   | 1200 ± 215                          | 54.7 ± 0.4  | -14.6 ± 0.1  | 35.5 ± 0.5  |
|                            | 18                                                  | 714.8 ± 17.5                                  | 1837 ± 129                          | 57.3 ± 0.5  | -16.1 ± 0.3  | 39.8 ± 0.2  |
|                            | 34                                                  | 821.6 ± 18.9                                  | 2510 ± 162                          | 58.3 ± 0.2  | -16.5 ± 0.2  | 39.9 ± 0.3  |
|                            | 50                                                  | 775.7 ± 13.4                                  | 2910 ± 190                          | 57.3 ± 0.1  | -15.8 ± 0.1  | 38.5 ± 0.6  |
|                            | 66                                                  | 753.5 ± 11.3                                  | 3684 ± 267                          | 55.7 ± 0.4  | -15.2 ± 0.8  | 36.9 ± 0.3  |
|                            | <i>mean</i>                                         | <i>744.2</i>                                  | <i>2448</i>                         | <i>56.6</i> | <i>-15.7</i> | <i>38.1</i> |
| Significance               |                                                     |                                               |                                     |             |              |             |
| Growing season (S)         |                                                     | NS                                            | **                                  | **          | ***          | *           |
| Nutrient concentration (C) |                                                     | Q*                                            | Q*                                  | Q**         | Q*           | L**         |
| S x C                      |                                                     | NS                                            | NS                                  | NS          | NS           | NS          |

L = linear, Q = quadratic; NS, \*, \*\*, \*\*\* Non significant or significant at  $P \leq 0.05$ , 0.01 and 0.001, respectively. Means of three replicates ± SE.

**Table 2.2.** Effects of growing season and nutrient solution concentration on soluble sugars, starch, total carbohydrates and proteins of lettuce leaves

| Growing season             | Nutrient concentration<br>(mequiv L <sup>-1</sup> ) | Glucose                             | Fructose   | Sucrose    | Starch     | Total<br>carbohydrates | Proteins    |
|----------------------------|-----------------------------------------------------|-------------------------------------|------------|------------|------------|------------------------|-------------|
|                            |                                                     | ----- (mg g <sup>-1</sup> FW) ----- |            |            |            |                        |             |
| Spring                     | 2                                                   | 2.9 ± 0.1                           | 3.9 ± 0.3  | 11.7 ± 1.0 | 1.9 ± 0.4  | 20.5 ± 1.4             | 16.5 ± 1.5  |
|                            | 18                                                  | 2.8 ± 0.2                           | 3.8 ± 0.2  | 8.6 ± 0.7  | 1.4 ± 0.1  | 16.7 ± 1.0             | 14.8 ± 1.1  |
|                            | 34                                                  | 2.8 ± 0.2                           | 3.2 ± 0.3  | 8.0 ± 0.6  | 0.8 ± 0.2  | 14.9 ± 0.4             | 13.7 ± 0.5  |
|                            | 50                                                  | 2.2 ± 0.3                           | 2.6 ± 0.1  | 9.9 ± 0.9  | 0.8 ± 0.3  | 15.6 ± 1.4             | 13.6 ± 0.7  |
|                            | 66                                                  | 2.1 ± 0.3                           | 2.6 ± 0.5  | 8.2 ± 0.6  | 0.5 ± 0.3  | 13.6 ± 1.2             | 13.2 ± 1.4  |
|                            | <i>mean</i>                                         | <i>2.5</i>                          | <i>3.2</i> | <i>9.3</i> | <i>1.1</i> | <i>16.3</i>            | <i>14.4</i> |
| Summer                     | 2                                                   | 2.3 ± 0.1                           | 5.0 ± 0.6  | 6.3 ± 0.3  | 1.1 ± 0.1  | 14.9 ± 0.8             | 15.2 ± .09  |
|                            | 18                                                  | 2.2 ± 0.2                           | 3.1 ± 0.4  | 5.4 ± 0.7  | 0.9 ± 0.1  | 11.8 ± 1.1             | 15.5 ± 1.4  |
|                            | 34                                                  | 2.1 ± 0.2                           | 2.5 ± 0.4  | 6.9 ± 0.9  | 0.7 ± 0.2  | 12.3 ± 0.9             | 14.1 ± 0.8  |
|                            | 50                                                  | 1.7 ± 0.1                           | 1.8 ± 0.1  | 5.0 ± 0.4  | 0.6 ± 0.3  | 9.3 ± 0.8              | 11.6 ± 0.5  |
|                            | 66                                                  | 1.7 ± 0.2                           | 1.9 ± 0.5  | 4.3 ± 0.9  | 0.7 ± 0.1  | 8.7 ± 1.1              | 10.2 ± 0.8  |
|                            | <i>mean</i>                                         | <i>2.0</i>                          | <i>2.9</i> | <i>5.6</i> | <i>0.8</i> | <i>11.4</i>            | <i>13.3</i> |
| Significance               |                                                     |                                     |            |            |            |                        |             |
| Growing season (S)         |                                                     | *                                   | NS         | **         | NS         | **                     | NS          |
| Nutrient concentration (C) |                                                     | L*                                  | L**        | NS         | L*         | L**                    | L*          |
| S x C                      |                                                     | NS                                  | NS         | NS         | NS         | NS                     | NS          |

L = linear; NS, \*, \*\* Non significant or significant at  $P \leq 0.05$  and  $0.01$ , respectively. Means of three replicates  $\pm$  SE

**Table 2.3.** Effects of growing season and nutrient solution concentration on macronutrient composition of lettuce leaves

| Growing season             | Nutrient concentration (mequiv L <sup>-1</sup> ) | Macronutrients (g kg <sup>-1</sup> DW) |            |             |             |            |
|----------------------------|--------------------------------------------------|----------------------------------------|------------|-------------|-------------|------------|
|                            |                                                  | N                                      | P          | K           | Ca          | Mg         |
| Spring                     | 2                                                | 43.9 ± 0.5                             | 3.4 ± 0.3  | 38.0 ± 1.7  | 9.0 ± 0.7   | 4.2 ± 0.3  |
|                            | 18                                               | 45.5 ± 0.6                             | 5.8 ± 0.2  | 52.3 ± 1.9  | 10.6 ± 1.3  | 4.8 ± 0.4  |
|                            | 34                                               | 47.8 ± 0.7                             | 6.1 ± 0.4  | 70.6 ± 2.0  | 10.1 ± 1.1  | 5.2 ± 0.4  |
|                            | 50                                               | 49.5 ± 0.2                             | 5.8 ± 0.2  | 66.9 ± 1.5  | 10.2 ± 1.2  | 5.4 ± 0.2  |
|                            | 66                                               | 48.9 ± 0.3                             | 5.5 ± 0.1  | 67.3 ± 2.0  | 9.7 ± 0.4   | 5.7 ± 0.6  |
|                            | <i>mean</i>                                      | <i>47.1</i>                            | <i>5.3</i> | <i>59.0</i> | <i>9.9</i>  | <i>5.0</i> |
| Summer                     | 2                                                | 48.3 ± 0.7                             | 4.5 ± 0.91 | 52.7 ± 2.3  | 10.8 ± 0.9  | 4.2 ± 0.4  |
|                            | 18                                               | 50.4 ± 0.1                             | 5.1 ± 0.27 | 66.2 ± 2.7  | 9.5 ± 1.8   | 5.6 ± 0.1  |
|                            | 34                                               | 51.9 ± 0.8                             | 5.5 ± 0.39 | 72.0 ± 1.5  | 9.9 ± 1.3   | 6.1 ± 0.2  |
|                            | 50                                               | 53.0 ± 0.7                             | 6.4 ± 0.15 | 80.8 ± 0.9  | 10.3 ± 0.6  | 6.3 ± 0.2  |
|                            | 66                                               | 52.4 ± 0.5                             | 5.3 ± 0.21 | 61.9 ± 1.1  | 10.4 ± 0.5  | 6.6 ± 0.2  |
|                            | <i>mean</i>                                      | <i>51.2</i>                            | <i>5.4</i> | <i>66.7</i> | <i>10.2</i> | <i>5.8</i> |
| Significance               |                                                  |                                        |            |             |             |            |
| Growing season (S)         |                                                  | *                                      | NS         | **          | NS          | *          |
| Nutrient concentration (C) |                                                  | Q*                                     | Q*         | Q**         | NS          | L**        |
| S x C                      |                                                  | NS                                     | Q**        | NS          | NS          | NS         |

L = linear, Q = quadratic; NS, \*, \*\* Non significant or significant at  $P \leq 0.05$  and 0.01, respectively. Means of three replicates ± SE.

## **CHAPTER 3**

### **Yield and quality of leafy lettuce in response to nutrient solution composition and growing season**

## Abstract

The aim of this study was to investigate the effect of macro-anion ( $\text{NO}_3^-$ ,  $\text{H}_2\text{PO}_4^-$ , and  $\text{SO}_4^{2-}$ ) and macro-cation ( $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Mg}^{2+}$ ) proportions in the nutrient solution during two consecutive growing seasons (spring and summer) on yield and leaf quality (chlorophyll content, color parameters, carbohydrates and mineral composition) of *Lactuca sativa* L. var. *acephala* grown in a floating system. Marketable yield, shoot biomass and leaf area index were unaffected by nutrient solution composition. A high proportion of calcium in the nutrient solution increased the quality attributes in particular calcium, chlorophyll, glucose and fructose concentrations. Plants grown in the spring season exhibited a lower yield, growth (total dry biomass and leaf area index), leaf mineral content (N, K, and Mg), total carotenoids and water use efficiency than those grown in the summer season but influenced positively some quality parameters (higher content of glucose, and fructose and lower nitrate content). The results demonstrated, that the effect of growing season on leafy lettuce performance (yield and quality) was more pronounced than the effect of nutrient solution composition.

**Key words:** growing season, floating system, leafy lettuce, nutrient solution composition, nutritional quality.

## 3.1 Introduction

Baby leaf vegetables (i.e. rocket, lamb's lettuce, headless lettuce, endive, escarole, water cress) are the subject of increased interest in consumers and are mostly requested for mixed salads, both as fresh market products and ready-to-use vegetables. The nutritional quality of vegetables can be affected by many pre- and post-harvest factors<sup>1</sup>. Fertilization is one of the most practical and effective pre-harvest ways to control and improve yield and nutritional quality of crops for human consumption. In this perspective the soilless culture represents an important tool, because it permits a precise control of plant nutrition<sup>2-7</sup>. Several authors<sup>8-10</sup> have shown a significant influence of cationic and anionic ratio on yield and quality attributes of several vegetable crops. For instance, Fanasca *et al.*<sup>10</sup> have reported that a high proportion of K in the nutrient solution increased the quality attributes (fruit dry matter, total soluble solids content) and the lycopene content of tomato fruit, whereas a high proportion of Ca improved tomato fruit yield and reduced the incidence of blossom-end rot (BER). Similarly, Trudel and Ozbun<sup>11</sup> showed a 40% increase in lycopene concentration when potassium concentration in the nutrient solution has been

increased from 0 to 8 mM, whereas a 26% depression of  $\beta$ -carotene concentration has been observed. Nutrient solution composition also affects crop growth and yield. Soundy *et al.*<sup>9</sup> demonstrated that at least 15 mg L<sup>-1</sup> P, supplied via floatation irrigation to a peat + vermiculite mix, was required to produce lettuce plantlets with the highest fresh and dry shoot biomass. Soundy and Cantliffe<sup>12</sup> have reported that shoot growth of lettuce plantlets increased as N concentration increased from 0 to 60 mg L<sup>-1</sup> in a floating irrigation system. Moreover, fresh and dry shoot weight of lettuce plantlets grown in a floating system were unaffected by applied K from 15 to 60 mg L<sup>-1</sup><sup>13</sup>. However, the former studies focused on the influence of nutrients on biometric characteristics of head lettuce transplants while there is a lack of information on the influence of nutrient solution composition on yield and quality of leafy lettuce (*Lactuca sativa* L. var. *acephala*) that is considered a primary component of fresh-cut (minimally processed) vegetables and it is one of the most important horticultural crops grown in a floating (hydroponic) system in Italy.

An optimal nutrient solution composition for vegetable crops in closed fertigation systems also depends on the environmental conditions. Unfortunately, most recommendations for the fertilization of vegetables do not take into account environmental conditions. Therefore, different temperatures and solar radiation conditions may be good treatments variable to look at possible interactive effects of environmental conditions and ionic proportions in the nutrient solution on plant growth, yield and quality.

The objective of this work was to assess the effect of macro-anion ( $\text{NO}_3^-$ ,  $\text{H}_2\text{PO}_4^-$ , and  $\text{SO}_4^{2-}$ ) and macro-cation ( $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Mg}^{2+}$ ) proportions in the nutrient solution during two consecutive growing seasons (spring and summer) on yield and leaf quality (chlorophyll content, colour parameters, carbohydrates and mineral composition) of *Lactuca sativa* L. var. *acephala* grown in a floating system.

### 3.2 Materials and Methods

**Location, experimental design and crop management:** Two experiments were conducted in two consecutive growing seasons: spring season (experiment 1) and summer season (experiment 2) in a polyethylene 200 m<sup>2</sup> greenhouse situated at the experimental farm of Tuscia University, central Italy (latitude 42°25'N, longitude 12°08'E, altitude 310 m). Inside the greenhouse, ventilation was provided automatically when the air temperature exceeded 26 °C, light was provided only by natural solar radiation. The following climate data inside the greenhouse was determined: dry and wet bulb air temperature by means of wire resistance thermometers in aspirated boxes, solar radiation by means of a

pyranometer (CM11 Kipp and Zonen, Netherlands). All measurements were collected on a data logger system (CR10X, Campbell Scientific, Inc., UK), the sensors were scanned every minute and the 30 min average values were recorded.

Seeds of lettuce (*Lactuca sativa* L. var. *acephala* cv. 'Green Salad Bowl', SAIS seed company, Cesena, Italy) were sown on 24 March 2007 (experiment 1) and on 31 May 2007 (experiment 2) into a floating raft growing system, consisting of polystyrene plug trays floating in plastic tanks with a constant volume (65 L) of stagnant nutrient solution, which was continuously aerated with an air compressor in order to maintain the oxygen content above 6.0 mg L<sup>-1</sup>. The planting density was 1857 plants m<sup>-2</sup>, as used commercially for similar leafy vegetables in floating systems.

In both growing seasons (experiments 1 and 2), a randomized complete-block design with three replicates was used to compare six nutrient solution having the same total concentration of nutrients (40 mequiv L<sup>-1</sup>) but different proportions among macro-anions (NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, and H<sub>2</sub>PO<sub>4</sub><sup>-</sup>) and macro-cations (K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup>). For both anionic and cationic groups, the nutrient formulations were defined by a high proportion of one nutrient and a equally low proportion of the others giving the same anion:cation ratio (1) (Fig. 1). In all anionic treatments the concentrations of K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> were (mequiv L<sup>-1</sup>) 10.20, 5.04, and 4.76, respectively. In all cationic treatments, the concentrations of NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup> and H<sub>2</sub>PO<sub>4</sub><sup>-</sup> were (mequiv L<sup>-1</sup>) 14.96, 4.02, and 1.02, respectively. Each experimental unit consisted of 0.147 m<sup>2</sup> (273 plants) container filled with 65 L of aerated nutrient solution. In all treatments, the micronutrients concentrations were Fe, 40.0 µmequiv L<sup>-1</sup>; Mn, 18.0 µmequiv L<sup>-1</sup>; Cu, 3.0 µmequiv L<sup>-1</sup>; Zn, 6.0 µmequiv L<sup>-1</sup>; B, 60.0 µmequiv L<sup>-1</sup> and Mo, 1.8 µmequiv L<sup>-1</sup>. The pH of the nutrient solution for all treatments was 6.0 ± 0.5. Demineralized water was used in the preparation of all nutrient solutions.

**Measurements and analysis:** During both growing cycles, the water used by the crop was monitored in all treatments. The amount of water used was recorded by a flowmeter. Water use efficiency for harvested yield was calculated as the marketable fresh weight divided by the evapotranspiration losses. In experiments 1 and 2, lettuce were harvested on 17 April and 21 June, respectively at the same physiological age, expressed as the standard accumulation of growing-degree (base-temperature of 6 °C; ceiling temperature of 30°C) days after sowing, which was in the range of 380-385 degree-days. Fifty plants per plot were separated into shoots to determine marketable fresh yield and roots, and their tissues were dried in a forced-air oven at 80 °C for 72 h for biomass determination. Root to shoot

ratio was calculated by dividing root dry weight by the shoot dry weight. Leaf areas (LA) were measured on 8 plants per treatment using an electronic area meter (Delta-T Devices Ltd., Cambridge, UK). Leaf area index (LAI) was computed as the ratio of green leaf area divided by the ground area.

In both experiments, dried leaf tissues were ground separately in a Wiley mill to pass through a 20 mesh screen, then 0.5 g of the dried plant tissues were analyzed for the following macro and micronutrients: N, P, K, Ca, Mg, Fe, Cu, Mn, and Zn. Nitrogen concentration in the plant tissues was determined after mineralization with sulfuric acid by “Regular Kjeldahl method”<sup>14</sup>, P, K, Ca, and Mg concentrations were determined by dry ashing at 400 °C for 24 h, dissolving the ash in 1:25 HCl, and assaying the solution obtained using an inductively coupled plasma emission spectrophotometer (ICP Iris; Thermo Optek, Milano, Italy)<sup>15</sup>.

The color parameters L\*, a\* and b\* [in the CIELAB-System: the L\* component represents lightness; the a\* component represents values from green (–) to red (+); and the b\* component represents values from blue (–) to yellow (+)] were evaluated on leaves from marketable plants using a portable colorimeter (Minolta Chroma Meter CR-200, Minolta Camera Co. Ltd., Osaka, Japan). For the chlorophyll and carotenoids analyses, leaf discs were taken from 24 plants per replicate. The total chlorophyll and carotenoid contents were determined by UV–vis spectrophotometry (Beckman DU-50 spectrophotometer; Beckman Instruments, Inc., Fullerton, CA). The absorbance of the solution was measured at 470, 647 and 664 nm. Formulae and extinction coefficients used for the determination of chlorophyllous pigments (total chlorophyll and carotenoids) were described by Lichtenhaler and Wellburn<sup>16</sup>.

In both experiments, 16 lettuce plants per experimental unit were harvested, frozen and stored in liquid nitrogen for quality analysis. The frozen samples were reduced to a fine powder with a mortar and pestle under liquid nitrogen. Spare powder was freeze dried and used for measurements of soluble carbohydrates, starch, nitrate and protein contents.

Soluble carbohydrate (glucose, fructose, and sucrose) and starch were performed by spectrophotometric coupled enzymatic assays as described by Jones *et al.*<sup>17</sup> including the modification of Antognozzi *et al.*<sup>18</sup>. The nitrate concentration was measured with enzymatic assay<sup>19</sup>, the assay was performed with a dual wavelength (340–400 nm) spectrophotometer (Thermo Spectronic-HELIOS, UK). Finally, quantification of proteins was performed according to the principle of protein-dye binding<sup>20</sup>.

**Statistical analysis:** All data were statistically analyzed by ANOVA using the SPSS software package (SPSS 10 for Windows, 2001). Combined analysis of variance was performed using season as a fixed variable<sup>21</sup>. Duncan's multiple range test was performed at  $P = 0.05$  on each of the significant variables measured.

### 3.3 Results

Differences between the cropping seasons in daily solar radiation ( $R_g$ ), mean air temperature ( $T_a$ ) and relative humidity (RH) inside the greenhouse were observed. During the spring season, the daily  $R_g$ ,  $T_a$  and RH ranged from 6.6 to 20.9 MJ m<sup>-2</sup>, 16.2 to 22.7 °C, and 48 to 75%, respectively. Moreover, during the summer season, the daily  $R_g$ ,  $T_a$  and RH ranged from 5.4 to 23.6 MJ m<sup>-2</sup>, 19.2 to 29.6 °C, and 48 to 82%, respectively.

Since no significant interaction was recorded between the growing season and the nutrient solution composition for growth, yield and quality parameters, the main effects of growing season and nutrient solution composition were separately reported.

Marketable yield, total dry biomass, leaf area index (LAI), and the root-to-shoot ratio (R/S) of lettuce were highly influenced by the growing season but not by the nutrient solution composition (Table 3.1). When averaged over nutrient solution composition, the marketable yield, total dry biomass, and LAI were significantly higher by 77%, 33%, and 44%, respectively in the summer season in comparison to the spring season, while an opposite trend was observed for the R/S ratio with the highest values recorded in the spring season in comparison to the summer cropping season (Table 3.1).

Total chlorophyll, carotenoids, nitrate concentration, and color parameters represented by  $a^*$  (greenness) and the  $b^*$  (yellowness) components were highly influenced by the growing season, whereas the effect of nutrient solution composition was less pronounced since only the total chlorophyll and the nitrate concentrations were affected by the ionic proportions in the nutrient solution (Table 3.2). Irrespective of nutrient solution composition, the total chlorophyll, total carotenoids, and nitrate contents were significantly higher by 22%, 60%, and 19%, respectively, in the summer season in comparison with the spring season (Table 3.2), whereas an opposite trend was observed for the colour parameters, where the highest  $a^*$  and  $b^*$  values were recorded in the spring season, compared to those observed with the summer season (Table 3.2). Moreover, the highest total chlorophyll and nitrate concentrations were recorded on plants grown in nutrient solution with a high proportion of Ca ( $T_{Ca}$ ) and N ( $T_N$ ), respectively, whereas the lowest values were observed with  $T_P$  and  $T_{Mg}$  for total chlorophyll and with  $T_{Ca}$  for nitrate concentrations (Table 3.2).

No significant difference among treatments was observed for starch (avg. 0.8 mg g<sup>-1</sup> of fresh weight) and total protein concentrations (avg. 14.4 mg g<sup>-1</sup> of fresh weight). Sucrose was the predominant sugar in both seasons, while glucose and fructose were presented in lower quantities (Table 3.3). Concentrations of glucose, fructose, sucrose and total carbohydrates were highly influenced by the growing season. In the leafy lettuce harvested during the summer cropping season, the concentrations of glucose, fructose, sucrose and total carbohydrates were reduced by 16%, 21%, 32%, and 25%, respectively compared to leafy lettuce picked during the spring season (Table 3.3). Moreover, irrespective of the growing season, the highest concentrations of glucose and fructose were recorded on leafy lettuce grown in nutrient solution with proportion of Ca (T<sub>Ca</sub>).

The macroelements concentration of lettuce plants as a function of the growing season and nutrient solution composition are displayed in Table 3.4. The concentration of N, K, and Mg were highly influenced by the growing season and nutrient solution concentration, whereas the Ca concentration was only affected by nutrient solution composition. Moreover, no significant difference among treatments was observed for P concentration (avg. 5.7 g kg<sup>-1</sup> dry weight). The highest concentration of N, K, Ca and Mg were recorded on plants with a high proportion of N, K, Ca, and Mg, respectively (Table 3.4). Irrespective of the nutrient solution composition, the N, K and Mg concentration in leafy lettuce recorded in the summer season was significantly higher by 4%, 21%, and 15%, respectively than those harvested during the spring cropping season (Table 3.4).

Finally, at the end of the cultural cycle, the water uptake and the water use efficiency (WUE) was significantly ( $P < 0.01$ ) affected by the growing season, but not by nutrient solution composition and nutrient solution  $\times$  growing season interaction (data not shown). The water requirement of leafy lettuce in the summer season (93.0 L m<sup>-2</sup>) was significantly higher by 58% in comparison with those grown in the spring season (59.0 L m<sup>-2</sup>). To produce 1 kg of lettuce 23.5 L of nutrient solution were necessary in the summer season and 26.1 L was in the spring season.

### 3.4 Discussion

In the current experiment, the nutrient solution composition did not significantly affect the marketable yield and the growth parameters of leafy lettuce. These results are consistent with the findings of Soundy *et al.*<sup>13</sup> who observed that fresh and dry shoot weight, leaf area and relative growth rate of lettuce transplants using a floatation system were unaffected by applied K (15, 30, 45, or 60 g L<sup>-1</sup>). Moreover, other researchers<sup>10, 22</sup> have

reported a significant effect of nutrient proportion on yield and quality of chicory and tomato plants grown in soilless system. Explanations for this disagreement could be the variations between species in their sensitivity to ionic proportions; leafy lettuce is characterized by a short growing cycle (25 and 22 days for spring and summer season, respectively) and consequently a low nutrient requirement leading to a scarce influence of nutrient proportions in the solution on marketable yield and growth.

The higher marketable yield of lettuce in the summer cropping season in comparison to the spring season was due to solar radiation conditions<sup>23</sup>. The higher solar radiation due to the high level of natural light and long photoperiod was presumably responsible for the increased photosynthesis in the summer with respect to the spring season: the mean value of daily global radiation in the greenhouse was 19.1 MJ m<sup>-2</sup> in the summer versus 14.6 MJ m<sup>-2</sup> in the spring season. In line with the above findings, Rouphael and Colla<sup>5</sup> reported a reduction zucchini squash marketable yield grown hydroponically by 33% in the summer to fall compared to the spring to summer cropping seasons. Moreover, Marcelis<sup>24</sup> observed that shading cucumber plants grown in a glasshouse during an extended period reduced the yield by 60% in comparison to cucumber plants grown at 100% irradiance level.

In Europe, widespread evidence of nitrate accumulation in leafy vegetables led to the European Community developing limits on fresh nitrate concentration of lettuce<sup>25</sup>. Both nitrate supply and light intensity are known to be critical factors in determining nitrate levels in leafy vegetables<sup>25</sup>. In the present study, a high proportion of N in the nutrient solution caused a linear increase in nitrate contents, but the nitrate values in leaf tissues were never as high as the limit value of 4500 mg kg<sup>-1</sup> fresh weight imposed by the European Community. The high correlation between nitrogen application rates and crop nitrate is in close agreement with the results of several earlier studies with lettuce grown in soil under both field<sup>26</sup> and greenhouse<sup>27</sup>. Moreover, low light levels are associated with nitrate accumulation in some leafy vegetables. For example Chadjaa *et al.*<sup>28</sup> and Gaurdeau *et al.*<sup>29</sup> reported that a reduction in the light level was associated with reduced nitrate reductase activity and increased nitrate accumulation in lettuce and spinach. Nitrate is favoured as an osmoticum at low light levels, replacing the energy-expensive carbohydrate, to maintain turgor pressure in lettuce<sup>30, 31</sup>. However, Cantliffe<sup>32</sup> observed little effect of light above irradiance levels of about 450-900  $\mu\text{mol m}^{-2} \text{s}^{-1}$  on spinach leaf nitrate concentration. Similarly, Parks *et al.*<sup>33</sup> showed that the shoot nitrate concentration of Swiss chard was primarily affected by nitrogen supply and not by light level because the light conditions

exceeded the critical level ( $\sim 200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) required to increase leaf nitrate. They also observed that higher growth and greater nitrate accumulation of Swiss chard occurred in the spring experiment, compared with the winter experiment, and this was influenced by a higher range of temperatures. Finally, Laurie and Stewart <sup>34</sup> showed that high temperatures stimulated growth and did not affect root activity of chickpea but reduced shoot nitrate reductase activity, potentially leading to nitrate accumulation. In the current study the higher nitrate concentration in the summer growing season could be associated to a higher range of temperatures. Average minimum to maximum temperatures of 19.3-29.6 °C were obtained for the summer experiment compared with 16.2-22.7 °C for the spring experiment.

From a nutritional point of view, a high proportion of calcium in the nutrient solution increased the quality attributes in particular calcium, glucose and fructose concentrations. Calcium plays an essential role in plant development and overall plant health because it is a structural component of cell wall and it is necessary for cell growth and division. In lettuce increasing of calcium in the leaf tissues can increase photosynthetic capacity and also chlorophyll synthesis <sup>35, 36</sup>. This might lead to an increasing of primary product of photosynthesis such as glucose and fructose. In leafy vegetables, these soluble sugars have an influence on taste. Lettuce can be an excellent dietary source of calcium and is a good alternative for individuals with a diet low in dairy products. Hence, increasing calcium, glucose and fructose contents in lettuce could further improve their nutritional benefits, which is most likely to be of value to consumers. Moreover, the effect of the growing season significantly affected the quality parameters of leafy lettuce. In comparison to the summer season, our results indicate that during the spring season an improvement of glucose, fructose, sucrose and total carbohydrates was observed. The above findings are in line with those reported on tomatoes by Islam and Khan <sup>37</sup>, which observed a reduction of sugar concentration during the warm season due to a lower activity level of sucrolytic enzymes as compared with the cool season.

The N, K, Ca, and Mg concentrations in the leafy lettuce were positively affected by using nutrient solutions with high proportion of N, K, Ca, and Mg, respectively. Our results are in line with a previous study on butterhead lettuce, where the uptake of Ca and K was linearly related to the high proportion of Ca and K in the nutrient solution <sup>38</sup>. In comparison to the spring season, our results indicate that during the summer season higher concentrations of K and Mg was observed, which is interesting from a nutritional point of view because fruits and vegetables usually contribute to 35 and 24% respectively of the

total K and Mg to the dietary intake of humans<sup>39</sup>. The highest macronutrient content (N, K and Mg) of leafy lettuce in summer vs. spring season was mainly related to the total plant biomass. The total plant uptake of N, K, and Mg in greenhouses is usually enhanced by stronger natural radiation or supplemental light<sup>4</sup>.

Finally, the daily water use of leafy lettuce in the spring season was lower in comparison to the summer season due to the reduced evaporative demand of the environment (lower global radiation and air temperature). Our results are in line with those recorded by Roupael and Colla<sup>4,5</sup>, who observed that the transpiration rates of hydroponically grown zucchini squash were positively correlated with solar radiation and temperature. Moreover, the lower water use efficiency (WUE) observed in the summer in comparison to the spring season may also be due to the high radiation and temperature that may have reduced the rate of photosynthesis and increased respiratory losses<sup>40</sup>. A previous study<sup>41</sup> on greenhouse rose crop showed that WUE was inversely proportional to solar radiation and to vapor pressure deficit.

### **3.5 Conclusions**

To summarize, we can conclude that marketable yield, shoot biomass and leaf area index were unaffected by nutrient solution composition. A high proportion of calcium in the nutrient solution increased the quality attributes in particular calcium, chlorophyll, glucose and fructose concentrations. The results also indicate that, the effect of growing season on yield and quality of leafy lettuce was significant, and more pronounced than the effect of nutrient solution composition. Plants grown in the spring season exhibited a lower yield, growth (total dry biomass and leaf area index), and mineral composition (N, K, and Mg) than those grown in the summer season but offered the highest fruit quality (higher content of glucose and fructose and lower nitrate content).

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**Table 3.1.** Main effects of growing season and nutrient solution composition on marketable yield, total dry biomass, leaf area index (LAI) and root-to-shoot ratio of lettuce plants.

| Treatments                    | Marketable yield<br>(kg m <sup>-2</sup> ) | Total dry biomass<br>(g m <sup>-2</sup> ) | LAI    | Root:shoot |
|-------------------------------|-------------------------------------------|-------------------------------------------|--------|------------|
| Growing season                |                                           |                                           |        |            |
| Spring                        | 2.2 b                                     | 160.8 b                                   | 7.8 b  | 0.23 a     |
| Summer                        | 3.9 a                                     | 214.5 a                                   | 11.2 a | 0.16 b     |
| Nutrient solution composition |                                           |                                           |        |            |
| T <sub>N</sub>                | 3.1                                       | 183.8                                     | 10.0   | 0.17       |
| T <sub>P</sub>                | 3.0                                       | 192.7                                     | 8.7    | 0.21       |
| T <sub>S</sub>                | 3.1                                       | 176.1                                     | 8.5    | 0.21       |
| T <sub>K</sub>                | 3.2                                       | 179.0                                     | 10.4   | 0.17       |
| T <sub>Ca</sub>               | 2.9                                       | 187.9                                     | 8.9    | 0.21       |
| T <sub>Mg</sub>               | 3.1                                       | 206.7                                     | 10.5   | 0.15       |
| Significance                  |                                           |                                           |        |            |
| Growing season (S)            | ***                                       | ***                                       | ***    | **         |
| Nutrient solution (N)         | NS                                        | NS                                        | NS     | NS         |
| S x N                         | NS                                        | NS                                        | NS     | NS         |

T<sub>N</sub>, T<sub>P</sub>, T<sub>S</sub>, T<sub>K</sub>, T<sub>Ca</sub>, T<sub>Mg</sub> are nutrient formulations with increased proportion of NO<sub>3</sub><sup>-</sup>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, respectively.

NS, \*\* and \*\*\* non significant or significant at  $P \leq 0.01$  and 0.001, respectively.

Means separated using Duncan's test at  $P=0.05$

**Table 3.2.** Main effects of growing season and nutrient solution composition on chlorophyll, carotenoids, nitrate contents and colour parameters of lettuce leaves

| Treatments                    | Total chlorophyll<br>(mg kg <sup>-1</sup> FW) | Total carotenoids<br>(mg kg <sup>-1</sup> FW) | Nitrate<br>(mg kg <sup>-1</sup> FW) | L*   | a*      | b*     |
|-------------------------------|-----------------------------------------------|-----------------------------------------------|-------------------------------------|------|---------|--------|
| Growing season                |                                               |                                               |                                     |      |         |        |
| Spring                        | 558.9 b                                       | 99.5 b                                        | 1698 b                              | 57.0 | -13.6 b | 41.0 a |
| Summer                        | 679.3 a                                       | 159.6 a                                       | 2015 a                              | 56.9 | -16.4 a | 39.5 b |
| Nutrient solution composition |                                               |                                               |                                     |      |         |        |
| T <sub>N</sub>                | 647.2 ab                                      | 128.2                                         | 2207 a                              | 57.6 | -14.9   | 40.9   |
| T <sub>P</sub>                | 553.1 b                                       | 115.1                                         | 1702 ab                             | 57.4 | -14.9   | 40.3   |
| T <sub>S</sub>                | 601.3 ab                                      | 153.2                                         | 1885 ab                             | 55.9 | -15.2   | 39.5   |
| T <sub>K</sub>                | 598.0 ab                                      | 126.2                                         | 2054 ab                             | 57.2 | -15.1   | 41.0   |
| T <sub>Ca</sub>               | 793.6 a                                       | 153.0                                         | 1592 b                              | 56.8 | -15.1   | 40.0   |
| T <sub>Mg</sub>               | 521.4 b                                       | 101.7                                         | 1699 ab                             | 56.8 | -14.9   | 40.0   |
| Significance                  |                                               |                                               |                                     |      |         |        |
| Growing season (S)            | *                                             | ***                                           | *                                   | NS   | ***     | **     |
| Nutrient solution (N)         | *                                             | NS                                            | *                                   | NS   | NS      | NS     |
| S x N                         | NS                                            | NS                                            | NS                                  | NS   | NS      | NS     |

T<sub>N</sub>, T<sub>P</sub>, T<sub>S</sub>, T<sub>K</sub>, T<sub>Ca</sub>, T<sub>Mg</sub> are nutrient formulations with increased proportion of NO<sub>3</sub><sup>-</sup>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, respectively. NS, \*, \*\* and \*\*\* non significant or significant at  $P \leq 0.05$ , 0.01 and 0.001, respectively. Means separated using Duncan's test at  $P=0.05$ .

**Table 3.3.** Main effects of growing season and nutrient solution composition on glucose, fructose, sucrose, starch, total carbohydrates and proteins of lettuce.

| Treatments                    | Glucose                            | Fructose | Sucrose | Starch | Total carbohydrates | Proteins |
|-------------------------------|------------------------------------|----------|---------|--------|---------------------|----------|
|                               | ------(mg g <sup>-1</sup> FW)----- |          |         |        |                     |          |
| Growing season                |                                    |          |         |        |                     |          |
| Spring                        | 3.1 a                              | 3.4 a    | 9.3 a   | 0.7    | 16.7 a              | 14.2     |
| Summer                        | 2.6 b                              | 2.7 b    | 6.3 b   | 0.9    | 12.6 b              | 14.6     |
| Nutrient solution composition |                                    |          |         |        |                     |          |
| T <sub>N</sub>                | 2.5 bc                             | 2.9 b    | 7.5     | 0.9    | 14.0                | 14.7     |
| T <sub>P</sub>                | 3.2 ab                             | 3.1 ab   | 7.7     | 0.8    | 14.9                | 14.6     |
| T <sub>S</sub>                | 2.9 abc                            | 3.1 ab   | 8.3     | 0.7    | 15.2                | 14.1     |
| T <sub>K</sub>                | 2.0 c                              | 2.2 b    | 7.8     | 0.8    | 12.9                | 15.8     |
| T <sub>Ca</sub>               | 3.8 a                              | 3.8 a    | 8.0     | 0.9    | 16.7                | 12.2     |
| T <sub>Mg</sub>               | 2.7 bc                             | 2.9 ab   | 7.5     | 0.8    | 14.1                | 15.2     |
| Significance                  |                                    |          |         |        |                     |          |
| Growing season (S)            | *                                  | **       | ***     | NS     | ***                 | NS       |
| Nutrient solution (N)         | *                                  | *        | NS      | NS     | NS                  | NS       |
| S x N                         | NS                                 | NS       | NS      | NS     | NS                  | NS       |

T<sub>N</sub>, T<sub>P</sub>, T<sub>S</sub>, T<sub>K</sub>, T<sub>Ca</sub>, T<sub>Mg</sub> are nutrient formulations with increased proportion of NO<sub>3</sub><sup>-</sup>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, respectively.

NS, \*, \*\* and \*\*\* non significant or significant at  $P \leq 0.05$ , 0.01 and 0.001, respectively. Means separated using Duncan's test at  $P=0.05$ .

**Table 3.4.** Main effects of growing season and nutrient solution composition on macronutrient composition of lettuce leaves

| Treatments                    | Macronutrients (g kg <sup>-1</sup> DW) |     |          |        |       |
|-------------------------------|----------------------------------------|-----|----------|--------|-------|
|                               | N                                      | P   | K        | Ca     | Mg    |
| Growing season                |                                        |     |          |        |       |
| Spring                        | 46.2 b                                 | 5.5 | 58.6 b   | 8.9    | 4.8 b |
| Summer                        | 48.0 a                                 | 5.9 | 71.1 a   | 9.4    | 5.5 a |
| Nutrient solution composition |                                        |     |          |        |       |
| T <sub>N</sub>                | 48.0 a                                 | 5.7 | 69.7 ab  | 9.0 b  | 4.7 b |
| T <sub>P</sub>                | 46.7 b                                 | 6.1 | 65.1 abc | 8.9 b  | 4.8 b |
| T <sub>S</sub>                | 45.8 b                                 | 5.7 | 66.9 abc | 9.2 b  | 4.5 b |
| T <sub>K</sub>                | 47.3 ab                                | 6.0 | 74.7 a   | 8.4 b  | 4.5 b |
| T <sub>Ca</sub>               | 47.6 ab                                | 5.4 | 53.7 c   | 11.3 a | 5.3 b |
| T <sub>Mg</sub>               | 47.3 ab                                | 5.4 | 59.0 bc  | 8.0 b  | 7.0 a |
| Significance                  |                                        |     |          |        |       |
| Growing season (S)            | *                                      | NS  | **       | NS     | *     |
| Nutrient solution (N)         | *                                      | NS  | *        | *      | **    |
| S x N                         | NS                                     | NS  | NS       | NS     | NS    |

T<sub>N</sub>, T<sub>P</sub>, T<sub>S</sub>, T<sub>K</sub>, T<sub>Ca</sub>, T<sub>Mg</sub> are nutrient formulations with increased proportion of NO<sub>3</sub><sup>-</sup>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, respectively.

NS, \* and \*\* non significant or significant at  $P \leq 0.05$  and 0.01, respectively.

Means separated using Duncan's test at  $P=0.05$ .

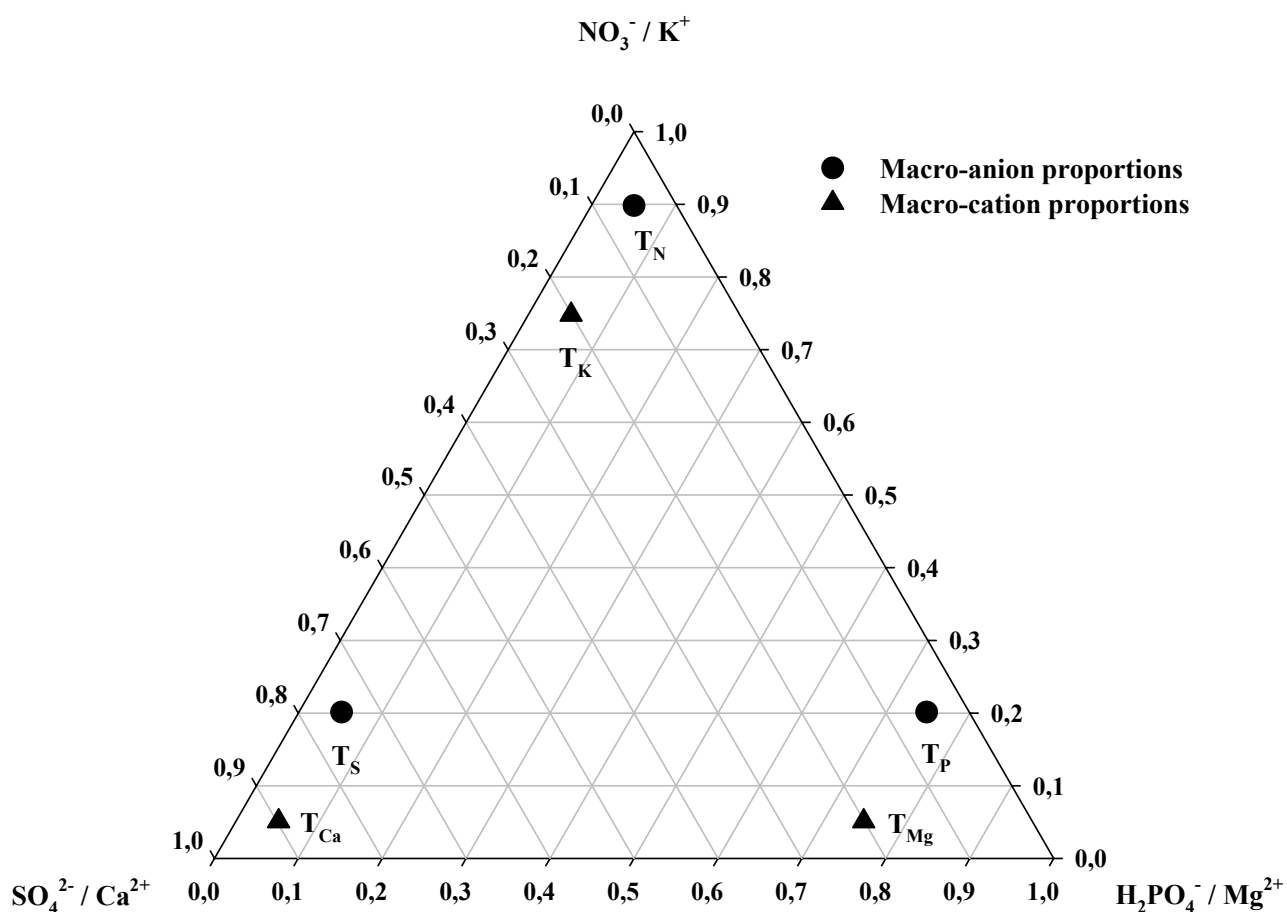


**Figure 3.1.** Nutrient proportions in the nutrient formulation treatments. T<sub>N</sub>, T<sub>P</sub>, T<sub>S</sub>, T<sub>K</sub>, T<sub>Ca</sub>, T<sub>Mg</sub> are nutrient formulations with increased proportion of NO<sub>3</sub><sup>-</sup>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, respectively.

## **CHAPTER 4**

**Effect of nitrogen forms and radiation on growth and  
mineral content of two *Brassica* species**

## Abstract

Three greenhouse experiments were carried out to determine the growth, yield, nitrate-, N- and S- concentrations in shoots, and water uptake of *Brassica rapa* L. subsp. *nipposinica* var. *chinoleifera* and *Brassica juncea* L. plants grown in hydroponics under three daily photosynthetically active radiation (PAR) levels, 5.0 (low), 6.8 (medium) and 9.0 (high) mol m<sup>-2</sup>. In each experiment, plants were supplied with nutrient solutions having equal N concentrations of 11 mM in different forms (100 % NH<sub>4</sub>, 50 % NH<sub>4</sub> + 50 % NO<sub>3</sub>, 100 % NO<sub>3</sub>). Nitrogen supplied as 100% NH<sub>4</sub> reduced fresh and dry shoot biomass, leaf area, and leaf number in both *Brassica* species, especially at low and medium PAR levels. In both *Brassica* species, S concentrations were highest, while nitrate concentrations were lowest in leaves of plants grown at N supplied as 100% NH<sub>4</sub>. No differences in leaf nitrate concentrations were observed between 50% NH<sub>4</sub> + 50% NO<sub>3</sub> and 100% NO<sub>3</sub> treatments. Low and high PAR levels increased the nitrate concentrations and decreased the N/S ratio in leaves of both crops compared to medium PAR level. Fresh shoot biomass was maximised in *Brassica rapa* when nitrate was supplied in the nutrient solution, either solely as NO<sub>3</sub> or as a mixture of 50% NO<sub>3</sub> and 50% NH<sub>4</sub>, and when at exposure to either a medium or a high PAR level while in *Brassica juncea* the highest fresh shoot biomass was observed at high PAR level without significant differences among nutrient solutions containing the different N forms.

**KEYWORDS:** *Brassica rapa* L., *Brassica juncea* L., hydroponics, nitrate, ammonium, Photosynthetically Active Radiation.

## 4.1 Introduction

In recent years, interest has grown in cultivating *Brassicaceae* due to their high nutritional value. These vegetables have high concentrations of vitamins, minerals, and phytochemicals such as glucosinolates, flavonoids, chlorophylls, and carotenoids that have antioxidant and anticarcinogenic properties (Knekt et al., 2002; Erlund, 2004; Artemyeva and Solovyeva, 2006). Several studies have been carried out on common *Brassica* crops (cabbage, broccoli, cauliflower, turnip, Brussels sprouts, etc) to investigate the influence of preharvest factors on growth and yield (Guo et al., 2007), however information on Asian *Brassica* species such as *Brassica rapa* L. subsp. *nipposinica* var. *chinoleifera* and *Brassica juncea* L., is rare. These two *Brassica* species are used for human consumption

either as fresh or processed food. They are very important due to the wide spectrum of health-promoting substances and their greater concentration of ascorbic acid compared to other leafy vegetable (Krumbein et al., 2005).

It is well known that nitrogen is one of the most important nutrients affecting plant growth and yield. Plants can utilize both nitrate and ammonium as a nitrogen source. However, compared to nitrate, ammonium as the sole nitrogen source can be deleterious to many plants species. The addition of small amounts of ammonium to nutrient solutions increased growth of several plant species and decreased the pH of the nutrient solution (Sonneveld, 2002). Ammonium nutrition was reported to be superior to nitrate nutrition in regards to plant growth of blueberry, rice, maize and sweet pepper (Guo et al., 2007). In some crops maximum growth and yield was obtained by supplying a mixture of ammonium and nitrate (Guo et al., 2007). On chicory cultivars however, the main growth characteristics were not affected by the N form (Santamaria et al., 1998).

The benefits of mixed N nutrition compared to sole nitrate supply are lower energy costs of ammonium uptake and the altered distribution of assimilated N between leaves and roots (Marschner, 1995; Guo et al., 2007). Furthermore, as compared to nitrate supply, sole ammonium application decreased the uptake of cations such as K, Ca, and Mg and increased the uptake of anions such as P and S (Barker and Mills, 1980; Salsac, 1987).

Although yield of most vegetables belonging to the families of *Brassicaceae*, *Chenopodiaceae*, *Asteraceae* and *Apiaceae*, can be increased by the application of high levels of nitrate in the nutrient solution, this leads to an accumulation of nitrate in leaves and the nitrate application itself can cause environmental pollution (Santamaria, 2006). High nitrate concentrations in leaves are considered to be harmful for human health. There is a risk of stomach cancer though recently there is evidence contradicting this view (Santamaria, 2006). These studies highlight a negative correlation between nitrate intake and gastric cancers because vegetables are an excellent source of vitamins, minerals and biologically active compounds (Santamaria, 2006). There are also studies reporting of beneficial effects of nitrate in food mainly related to the control of the gut flora; other studies show that nitrate consumed with acid ascorbic and glutathione, contained in vegetable, increase the activity of stomach acid against food-borne pathogens (Addiscott and Benjamin, 2004; Fite et al., 2004; Lunderberg et al., 2004).

In vegetables, the use of ammonium as nitrogen source can decrease the level of nitrate in leaf tissues. Zhang et al. (1990) showed in a water culture experiment that nitrate concentrations in spinach was decreased by 79-98% when fertilized with ammonium or

urea compared to treatments fertilized with nitrate. On the other hand, Wang and Li (2003) reported that ammonium chloride, ammonium nitrate, sodium nitrate, and urea had no significant effects on nitrate N levels in cabbage and spinach regardless of harvest time, although nitrate N fertilizers tended to increase nitrate accumulation compared to the ammonium N fertilizers.

*Brassica* species require more sulphur than most other crops due to its role in the synthesis of glucosinolates as well as sulphur aminoacids and proteins (Rosa et al., 1997). Moreover, several studies show that glucosinolate concentration can be influenced by nitrogen and sulphur supplied amount; a proper balance between nitrogen and sulphur played an important role in the regulation of the glucosinolate synthesis in turnip, mustards, kale, broccoli (Schonhof et al., 2007; Shumin et al., 2007).

The effects of the nitrogen form on growth, yield, and nutrient accumulation in plant depend on environmental conditions, such as light intensity, pH and temperature in the root environment, nitrogen concentration (Guo et al., 2007). A low light level is generally associated with reduced nitrate reductase activity and nitrate accumulation in some leafy vegetables (Gaudreau et al., 1995; Chadja et al., 1999). Moreover, to maintain turgor pressure in the cells nitrate is favoured as an osmoticum at low light levels, replacing energy-expensive carbohydrates (Buwalda and Warmenhoven, 1999). However, increasing the light level above radiation levels of 450-900  $\mu\text{mol m}^{-2} \text{s}^{-1}$  did not always reduce nitrate concentration in leaves (Cantliffe, 1972). In a greenhouse study, higher nitrate accumulation in Swiss chard occurred in a spring experiment compared to a winter experiment and it was attributed to a negative effect of high temperatures on nitrate reductase activity (Parks et al., 2008).

Light intensity also changes the crop response to N form. In strawberry, the use of a low  $\text{NO}_3:\text{NH}_4$  ratio caused a reduction of photosynthetic rate which was more pronounced in shaded plants than in unshaded plants (Tabatabaei et al., 2008). Therefore, different radiation intensities may be good treatment variables to look at possible interactive effects of environmental conditions and nitrogen form in nutrient solution on plant growth, yield, and quality.

Starting from the above considerations, we hypothesized that partial or total replacement of nitrate by ammonium in the nutrient solution especially under higher radiation intensity should have no adverse effects on growth and yield of two *Brassica* species with different quality characteristics cultivated in soilless culture. Moreover, by replacing partially or totally nitrate by ammonium in the solution we expected that nitrate concentrations in

leaves was lowered at low radiation too. The aim of this research was to investigate the influence of different forms of nitrogen and radiation intensities on growth, nitrate, nitrogen, and sulphur concentrations of two *Brassica* species.

## 4.2 Material and Methods

### 4.2.1 Plant material and growing conditions

Three hydroponic experiments were carried out in a greenhouse at the Institute of Vegetable and Ornamental Crops, located at Grossbeeren (Germany): experiment I, from December 6, 2007 to January 22, 2008; experiment II, from January 23, 2008 to February 25, 2008; experiment III, from March 6, 2008 to April 9, 2008.

All experiments were conducted at the same mean temperature (15°C), relative humidity (74%) and CO<sub>2</sub> concentration (400 µmol mol<sup>-1</sup>). The mean daily photosynthetic active radiation (PAR) was 5.0 (low, Exp. I), 6.8 (medium, Exp. II) and 9.0 (high, Exp. III) mol m<sup>-2</sup>. For each trial seeds of *Brassica juncea* L. cultivar Red Giant and *Brassica rapa* L. subsp. *nipposinica* var. *chiloneifera*. cultivar Mibuna Early were sown in rockwool cubes under greenhouse conditions. The plants at the two-true leaf stage, 25 days after sowing, were transferred into eight meter long gullies supplied continuously with nutrient solution. Distance between the 46 plants in each gully amounted to 0.17 m. Total nitrogen concentration was the same in all treatments but treatments differed in nitrogen forms (100 % NH<sub>4</sub>, 50% NH<sub>4</sub> + 50 % NO<sub>3</sub>, 100 % NO<sub>3</sub>). In 100% NH<sub>4</sub> treatment, NH<sub>4</sub> was supplied using NH<sub>4</sub>Cl while NH<sub>4</sub>NO<sub>3</sub> was used in the 50% NH<sub>4</sub> + 50 % NO<sub>3</sub> treatments. Treatments were arranged in a randomized complete block design with three replications. Macronutrients were supplied in all treatments at the following concentrations (mM): K 3.6, Ca 4.2, P 0.6, Mg 1.0, S 1.3; micronutrients had the following concentrations (µM): Fe 40.0, Mn 5.0, Zn 4.0, B 30, Cu 0.5, Mo 0.5. During all experiments, the electrical conductivity was kept at 2 dS m<sup>-1</sup> by adding stock solution or deionised water according to the variations (± 10% of the target value). In all nutrient solutions a MES buffer was applied at 1.5 mM to keep the pH in the range of 5.6-5.8. Moreover, phosphoric acid or potassium hydroxide was added when the solution pH drifted above or below the threshold.

### 4.2.2. Sampling and data analysis

At commercial maturity, (45, 32 and 33 days after transplanting for experiment I, II and III) a sample of 20 plants was harvested and the shoot was weighed. Subsequently, shoot

was dried in an oven at 70 °C for 72 h and weighed to determine dry mass and calculate dry matter content.

To follow growth during each experiment at four date maximum leaf length ( $L_{length}$ ) and width ( $L_{width}$ ) were measured and number of leaves was counted. First measurement was carried out at 14, 7, and 9 days after transplanting for experiment I, II and III, respectively; the last measurement was made the day before the final harvest. The area of a single leaf ( $A_{leaf}$ ) was calculated using an equation adapted to Schwarz and Klaering (2001):

$$A_{leaf} = a + L_{length}^b \times L_{width}^c$$

where  $a$  is the constant of integration and  $b$ ,  $c$  are allometry coefficients. Total plant leaf area was calculated as the sum of all single leaf area.

A sample of fresh shoot (300 g of f. wt) was harvested and immediately frozen (-28° C), freeze-dried, and finally reduced to a fine powder by a centrifugal mill for N, S, and nitrate analyses. Total N was determined after dry oxidation by the Dumas method (Elementar Vario EL, Hanau, Germany). Nitrate was measured potentiometrically with a NO<sub>3</sub> ion plus Sure-Flow® electrode (Orion-Research, Beverly; USA). Total S was analyzed by an elementary analyzer (high-temperature oxidation) and detected with a non dispersive infrared (NDIR sensor). All data were statistically analysed by ANOVA using SPSS software package (SPSS version 15.0 for Windows). When the interaction between N form and PAR was significant, ANOVA was performed separately for each experiment. The means were separated by Tukey's HSD test (5% significance level).

### 4.3 Results

In *B. rapa*, shoot fresh and dry mass, dry matter concentration, leaf area, and number of leaves were affected by N form and PAR level but no interactions between N form and PAR were observed (**Table 4.1**). When N was exclusively provided as NH<sub>4</sub>, shoot fresh and dry mass were reduced by 70 % and 50 %, respectively. On the other hand, the NH<sub>4</sub> treatment resulted in the highest shoot dry matter percentage. Related to PAR the highest shoot fresh mass was recorded at medium and high PAR, while the highest shoot dry mass and dry matter concentration were observed at medium PAR. In the early growth stage, leaf area was similar in all N form treatments (**Figure 4.1**). Differences were noted later on, 36, 24, and 23 days after transplanting at low, medium, and high PAR level. The lowest leaf area was observed in plants exposed to a low PAR and where N was exclusively provided as ammonium (**Table 4.1; Fig. 4.1**). Number of leaves showed a similar trend as the leaf area (**Table 4.1**).

In *B. juncea*, shoot fresh and dry mass, leaf area, and leaf number were affected by N form, PAR level, and the interaction of N form and PAR; except for shoot dry mass, where N form and PAR had no interactive effects (Table 2). Nitrogen form affected shoot fresh mass and leaf area differently depending on the PAR. At low and medium PAR, the 100 % NO<sub>3</sub> and 50 % NH<sub>4</sub> + 50 % NO<sub>3</sub> treatments had the highest shoot fresh mass and leaf area whereas at high PAR no significant differences were recorded among the nutrient solutions containing the different N forms. On the other hand, the 100 % NH<sub>4</sub> treatment resulted in the highest dry matter concentration at low and medium PAR while dry matter concentration was not affected by N form at high PAR. At low and medium PAR, leaf area was affected by the N form in the later growing stages. Plants grown with nutrient solution containing exclusively 100 % NH<sub>4</sub> had the lowest leaf areas (**Table 4.2; Figure 4.1**). No differences among N form treatments were recorded at high PAR level. The leaf number showed a similar trend of leaf area (**Table 4.2**).

Nitrogen and N/S ratio of *B. rapa* leaves were affected by N form, PAR, and their interactions (**Table 4.3**). Sulphur and nitrate concentrations (both, on fresh and dry mass basis) were affected by N form and PAR level but not by the interaction of N form and PAR. Leaf nitrogen concentrations differed only among N forms when plants grew at medium PAR. The highest leaf nitrogen concentration was observed in the 100 % NH<sub>4</sub> treatment, the lowest in the 100 % NO<sub>3</sub> treatment. The highest S concentration in leaves was recorded in the NH<sub>4</sub> treatment followed by NH<sub>4</sub>+NO<sub>3</sub>, whereas the NO<sub>3</sub> treatment had the lowest value. Moreover, increasing PAR above the medium value reduced the S leaf concentration. The lowest nitrate concentration on both fresh and dry mass basis was recorded in leaves of plants grown under medium PAR conditions using a nutrient solution containing NH<sub>4</sub> as sole N form. In *B. juncea*, a similar trend was observed for all characteristics considered compared with *B. rapa* (**Table 4.4**).

Both, N form and PAR affected the total water uptake ( $P \leq 0.05$ ) but no interactive effects between the two treatments were observed (data not shown). Total water uptake of the two *Brassica* species was significantly lower in the 100% NH<sub>4</sub> treatment (3.0 L plant<sup>-1</sup> for *B. rapa*, 1.2 L plant<sup>-1</sup> for *B. juncea*) compared with 50 % NH<sub>4</sub> + 50% NO<sub>3</sub> and 100% NO<sub>3</sub> treatments (mean 3.6 L plant<sup>-1</sup> for *B. rapa*, 1.8 L plant<sup>-1</sup> for *B. juncea*). In both species, total water uptake was significantly higher in plants grown at high PAR (3.6 L plant<sup>-1</sup> for *B. rapa*, 1.8 L plant<sup>-1</sup> for *B. juncea*) than in those grown at low and medium PAR (mean 3.0 L plant<sup>-1</sup> for *B. rapa* and 1.3 L plant<sup>-1</sup> for *B. juncea*).

#### 4.4 Discussion

It is often reported that the use of ammonium as sole or dominating N source results in impaired growth and yield restrictions of many plant species (Claussen, 2002; Kotsiras et al., 2005; Guo et al., 2007). Our experiments confirmed that shoot fresh and dry mass production of both nitrate-fed *Brassica* species were higher than those of ammonium-fed plants. However, in *B. juncea*, at high PAR the N form supplied did not affect fresh shoot biomass significantly. Hence, it can be concluded that for this species growth response to N form depends on radiation, while it is less important for *B. rapa*.

In several studies growth inhibition by ammonium uptake has been related to the pH decrease in the root environment leading to an impairment of the root plasma membrane (Marschner, 1995). In the present experiments the pH was kept constant between 5.6-5.8 and therefore, could not have caused growth inhibition. As frequently reported, plants supplied solely with ammonium have smaller leaf areas and/or leaf numbers compared to those supplied with nitrate (Raab and Terry, 1994; Walch-Liu et al., 2000). Our results are in agreement with the above findings showing a reduction in leaf area and leaf number in both species when N was supplied exclusively as ammonium. The growth reduction could be explained by a higher demand of carbohydrates for ammonium assimilation and subsequently detoxification in the roots (Marschner, 1995). However, at high PAR level no negative effect of ammonium on fresh and dry shoot biomass was observed in *B. juncea*. Increased ammonium supply to strawberries resulted an inhibitory effect on growth under shading compared with unshaded conditions because of the shortage of carbohydrate (Tabatabei et al., 2008). Moreover, studies on sugar beet and tobacco, reported that leaf rubisco concentration was higher with ammonium supply than with nitrate supply (Raab and Terry, 1994; Terce-Laforguea et al., 2004). In addition, total amount and activity of rubisco increased with higher light intensity (Taiz and Zeiger, 1998). Hence, the lack of negative effects of ammonium supply on growth of *B. juncea* may be explained by a better ammonium detoxification capacity of roots at high PAR level together with a higher photosynthetic activity.

In our experiments N form did not influence the leaf area and number of leaves during the early growth stages of *B. rapa*. However, phytotoxic effects or reduction of growth by ammonium nutrition should appear soon during the early stage because the roots are not able to detoxify ammonium (Barker and Mills, 1980). Instead, we observed differences of leaf area within N form treatments only in the late growing stages. These findings are in agreement with a study on avocado plants in hydroponic where the plants were less

affected by N form during early stage of growth (Lobit et al., 2007). Higher growth rate and photosynthetic activity in the early growth stage may have improved the ammonium tolerance in both species increasing the carbohydrate availability.

Nitrate and ammonium uptake influences also the uptake of other ions; the uptake of all ions is involved in maintaining electro-neutrality within the plant (Kotsiras et al., 2002). Increased ammonium supply enhanced the levels of phosphorus in leaves of lettuce (Savvas et al., 2006) and sulphur in tobacco (Kruse et al., 2007). On the other hand, increasing ammonium supply decreased potassium, calcium, and magnesium concentrations in leaf tissues (Guo et al., 2007). In our experiments, sulphur concentration in the leaves of both species increased with ammonium supply in the nutrient solution and this lead to the lowest N/S ratio (Table 3, 4). Generally, the uptake of sulphate that is in unspecific competition with nitrate, has been reported to increase with increasing ammonium supply (Van den Berg et al., 2005). In *Brassica* species, N/S ratio plays an important role on glucosinolates concentrations of leaves because they contain S- and N- in their structure. In fact, sulphur deficiency affects the amino acid profile in plants with a decrease in the share of amino acid rich in sulphur such as cysteine and methionine which are the precursor of aliphatic glucosinolates; on the contrary, indole glucosinolates concentration can increase at the highest nitrogen level because they are derived from tryptophan a N-containing amino acid (Mortensen, 1994; Hesse et al., 2004; Grubb and Abel, 2006). In *Brassica oleracea* it has been reported that with S concentrations above 6 mg g<sup>-1</sup> of dry matter and an N/S ratio lower than 10:1, the glucosinolate concentrations were on average around 0.33 g kg<sup>-1</sup> of fresh matter and differed significantly from those plants characterized by a S concentration below 6 mg g<sup>-1</sup> of dry matter and an N/S ratio above 10:1. Moreover, N/S ratios between 7:1 and 10:1 promoted plant yield and enhanced overall appearance (Schonhof et al., 2007). In the present experiments, N/S ratio higher than about 5 and 6 improved fresh and dry shoot biomass of *B. rapa* and *B. juncea*.

In both *Brassica* species, leaf N concentration increased with ammonium supply especially at low PAR level. This is consistent with the findings on pepper, strawberry, and roses (Lorenzo et al., 2000; Bar-Tal et al., 2001; Tabatabei et al., 2008) where an addition of NH<sub>4</sub> to nutrient solution produced an increasing of total N concentration in leaves. Moreover, it has been reported that where little growth occurred, nutrients are often concentrated in leaves and deficiencies may not apparent (Righetti et al., 1990). This was the case since plants grown at low PAR level exhibited poor growth and high leaf N

concentration compared to those grown at higher PAR levels especially using a nutrient solution with  $\text{NH}_4$  as sole nitrogen source.

Ammonium in the nutrient solution as the only nitrogen source inhibited water uptake producing imbalance in plant water relations (Ragab, 1980). In our experiments, the lower water uptake by plants supplied with ammonium compared with those supplied with nitrate may also be due to the smaller leaf area and number of leaves. In line with the findings of Schröder and Lieth (2002) and Rouphael and Colla (2004), water uptake of both *B. rapa* and *B. juncea* increased under higher PAR levels since transpiration rate is mainly affected by radiation intensity.

In both *Brassica* species high levels of nitrate were recorded in nitrate-fed plants and nitrate-ammonium fed plants, about 2.700-6.300  $\text{mg kg}^{-1}$  of fresh weight, whereas ammonium-fed plants showed low values, < 1.100  $\text{mg kg}^{-1}$  of fresh weight. In other leafy vegetables, such as lettuce and spinach, nitrate thresholds have been imposed by European Community: 3.500 and 4500  $\text{mg kg}^{-1}$  of fresh weight for greenhouse lettuce grown from 1 October to 31 March and from 1 April to 30 September; 2.500 and 3.000  $\text{mg kg}^{-1}$  of fresh weight for spinach grown from 1 November to 31 March and from 1 April to 31 October. Actually, there are no thresholds imposed by European Community regulation for both *Brassica* species although they exhibited a high capacity to accumulate nitrate above the highest EU threshold of 4,500  $\text{mg kg}^{-1}$ .

In both species, the highest nitrate concentration in leaves occurred at low and high PAR level while medium PAR level gave the lowest values. Several factors are responsible for a higher nitrate uptake and accumulation in leaves. Generally, the concentration of nitrate in leafy vegetable shows a variation according to light intensity and growth rate with high levels in winter time and considerably lower level in spring and summer time (Santamaria, 2006). Chadjaa et al. (1999) and Gaurdeau et al. (1995) reported that a reduction in the light level was associated with reduced nitrate reductase activity and increased nitrate accumulation in lettuce and spinach. Nitrate is favoured as an osmoticum at low light levels, replacing the energy-expensive carbohydrate to maintain turgor pressure in lettuce. The above findings explain the higher nitrate levels recorded in leaves of both *Brassica* species grown at low PAR level in comparison with those observed in leaves of *Brassicaceae* grown at medium PAR level. However, the high nitrate concentrations observed in both *Brassica* species grown at high PAR level indicated that additional factors besides light intensity have influenced nitrate accumulation. Cantliffe (1972) observed little effect of light above irradiance levels of about 450-900  $\mu\text{mol m}^{-2} \text{s}^{-1}$  on

spinach leaf nitrate concentration. Similarly, Parks et al. (2008) showed that the shoot nitrate concentration of Swiss chard was primarily affected by nitrogen supply and not by light level because the light conditions exceeded the critical level ( $\sim 200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) required to increase leaf nitrate. The authors attributed the shoot nitrate variation to the different temperatures recorded during the season, 18-39° C for the spring and 14-28°C for the winter (Parks et al., 2008). In the present study, the air temperature was about the same in all experiments ( $15 \pm 1.0^\circ \text{C}$ ), but the plants grown at high PAR level exhibited higher water uptake than those grown at low and medium PAR. In a greenhouse zucchini crop, Rouphael and Colla (2004) showed that leaf temperature was lower (up to 5°C) than air temperature during daytime when high transpiration rates were observed. Similarly, the high transpiration rate of *Brassica* plants grown at high PAR level may have reduce the leaf temperature leading to a reduction of nitrate reductase activity and an accumulation of nitrate in shoots.

Finally, the results showed that a medium PAR level improved the plant growth and reduced the leaf nitrate concentration of *Brassica rapa* while the use of a nutrient solution containing 100% of  $\text{NO}_3$  or 50%  $\text{NO}_3$  and 50%  $\text{NH}_4$  lead to a better plant growth but increased the leaf nitrate concentration. In *Brassica juncea*, a high PAR level was required to maximise plant growth; at high PAR level, growers can use a nutrient solution containing 100%  $\text{NH}_4$  to reduce the leaf nitrate concentration, the water requirement and to increase the N e S concentration of leaves without adverse effects on plant growth.

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**Table 4.1.** Effect of nitrogen form and photosynthetic active radiation (PAR) on fresh and dry shoot mass, dry matter content (DMC), leaf area ( $A_{\text{leaf}}$ ), and leaf number ( $N_{\text{leaf}}$ ) of *Brassica rapa* plants.

| PAR          | N form                           | Fresh shoot<br>g plant <sup>-1</sup> | Dry shoot<br>g plant <sup>-1</sup> | DMC<br>% | $A_{\text{leaf}}$<br>m <sup>2</sup> plant <sup>-1</sup> | $N_{\text{leaf}}$<br>no plant <sup>-1</sup> |
|--------------|----------------------------------|--------------------------------------|------------------------------------|----------|---------------------------------------------------------|---------------------------------------------|
| Low          | NH <sub>4</sub>                  | 48 b                                 | 3.4 b                              | 6.7 a    | 0.083 b                                                 | 23.8 b                                      |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 114 a                                | 7.8 a                              | 5.3 b    | 0.189 a                                                 | 45.2 a                                      |
|              | NO <sub>3</sub>                  | 117 a                                | 8.0 a                              | 5.5 b    | 0.191 a                                                 | 45.9 a                                      |
| Medium       | NH <sub>4</sub>                  | 108 b                                | 6.8 b                              | 7.1 a    | 0.159 b                                                 | 29.5 b                                      |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 194 a                                | 11.9 a                             | 5.7 b    | 0.261 a                                                 | 53.0 a                                      |
|              | NO <sub>3</sub>                  | 184 a                                | 11.8 a                             | 6.0 ab   | 0.252 a                                                 | 51.5 a                                      |
| High         | NH <sub>4</sub>                  | 121 b                                | 6.7 b                              | 6.2 a    | 0.163 b                                                 | 37.2 b                                      |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 150 a                                | 7.8 ab                             | 5.7 b    | 0.224 ab                                                | 46.8 a                                      |
|              | NO <sub>3</sub>                  | 186 a                                | 8.7 a                              | 5.7 b    | 0.268 a                                                 | 48.0 a                                      |
| Main effect  |                                  |                                      |                                    |          |                                                         |                                             |
| N form       | NH <sub>4</sub>                  | 93 b                                 | 6.0 b                              | 6.7 a    | 0.135 b                                                 | 31.2 b                                      |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 153 a                                | 8.8 a                              | 5.6 b    | 0.225 a                                                 | 48.4 a                                      |
|              | NO <sub>3</sub>                  | 162 a                                | 9.5 a                              | 5.7 b    | 0.237 a                                                 | 48.4 a                                      |
| PAR          | Low                              | 93 b                                 | 6.4 b                              | 5.8 b    | 0.154 b                                                 | 39.4 b                                      |
|              | Medium                           | 162 a                                | 10.2 a                             | 6.3 a    | 0.224 a                                                 | 44.8 a                                      |
|              | High                             | 152 a                                | 7.8 b                              | 5.9 ab   | 0.218 a                                                 | 43.9 a                                      |
| N form       |                                  | ***                                  | **                                 | ***      | ***                                                     | ***                                         |
| PAR          |                                  | ***                                  | **                                 | *        | ***                                                     | **                                          |
| N form x PAR |                                  | ns                                   | ns                                 | ns       | ns                                                      | ns                                          |

ns, \*, \*\* and \*\*\* not significant or significant at  $P \leq 0.05$ , 0.01, or 0.001. Means separated by HSD test at  $P = 0.05$ .

**Table 4.2.** Effect of nitrogen form and photosynthetic active radiation (PAR) on fresh and dry shoot mass, dry matter content (DMC), leaf area ( $A_{\text{leaf}}$ ) and leaf number ( $N_{\text{leaf}}$ ) of *Brassica juncea* plants.

| PAR          | N form                           | Fresh shoot<br>g plant <sup>-1</sup> | Dry shoot<br>g plant <sup>-1</sup> | Dry matter<br>% | $A_{\text{leaf}}$<br>m <sup>2</sup> plant <sup>-1</sup> | $N_{\text{leaf}}$<br>no plant <sup>-1</sup> |
|--------------|----------------------------------|--------------------------------------|------------------------------------|-----------------|---------------------------------------------------------|---------------------------------------------|
| Low          | NH <sub>4</sub>                  | 14.3 b                               | 1.5 b                              | 8.4 a           | 0.033 b                                                 | 4.0 b                                       |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 71.5 a                               | 3.7 a                              | 5.5 b           | 0.090 a                                                 | 6.7 a                                       |
|              | NO <sub>3</sub>                  | 57.5 a                               | 3.9 a                              | 5.8 b           | 0.095 a                                                 | 6.7 a                                       |
| Medium       | NH <sub>4</sub>                  | 65.0 b                               | 4.4 b                              | 7.3 a           | 0.095 b                                                 | 6.8 b                                       |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 97.5 a                               | 7.4 a                              | 6.6 ab          | 0.130 a                                                 | 7.3 a                                       |
|              | NO <sub>3</sub>                  | 84.3 ab                              | 6.1 ab                             | 6.1 b           | 0.127 a                                                 | 7.5 a                                       |
| High         | NH <sub>4</sub>                  | 90.6                                 | 5.6                                | 5.9             | 0.118                                                   | 6.5                                         |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 106.7                                | 6.1                                | 6.1             | 0.119                                                   | 6.8                                         |
|              | NO <sub>3</sub>                  | 92.1                                 | 4.9                                | 6.1             | 0.129                                                   | 6.5                                         |
| Main effect  |                                  |                                      |                                    |                 |                                                         |                                             |
| N form       | NH <sub>4</sub>                  | 56.6 c                               | 3.9 b                              | 7.2 a           | 0.082 b                                                 | 5.7 b                                       |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 91.9 a                               | 5.7 a                              | 6.1 b           | 0.116 a                                                 | 7.0 a                                       |
|              | NO <sub>3</sub>                  | 77.9 b                               | 5.0 a                              | 6.0 b           | 0.113 a                                                 | 6.9 a                                       |
| PAR          | Low                              | 47.7 c                               | 3.0 b                              | 6.6 a           | 0.072 b                                                 | 5.7 c                                       |
|              | Medium                           | 82.3 b                               | 6.0 a                              | 6.7 a           | 0.117 a                                                 | 6.6 b                                       |
|              | High                             | 96.5 a                               | 5.5 a                              | 6.1 b           | 0.122 a                                                 | 7.2 a                                       |
| N form       |                                  | ***                                  | **                                 | ***             | ***                                                     | ***                                         |
| PAR          |                                  | ***                                  | ***                                | ***             | ***                                                     | ***                                         |
| N form x PAR |                                  | *                                    | ns                                 | ***             | ***                                                     | ***                                         |

ns, \*, \*\* and \*\*\* not significant or significant at  $P \leq 0.05$ , 0.01 and 0.001.  
Means separated by HSD test at  $P = 0.05$ .

**Table 4.3.** Effect of nitrogen form and photosynthetic active radiation (PAR) on N, S, N/S ratio, and nitrate content in leaves of *Brassica rapa* plants.

| PAR          | N form                           | N<br>mg g <sup>-1</sup> dry<br>wt | S<br>mg g <sup>-1</sup> dry<br>wt | N/S<br>ratio | NO <sub>3</sub> <sup>-</sup><br>mg kg <sup>-1</sup><br>fresh wt | NO <sub>3</sub> <sup>-</sup><br>g kg <sup>-1</sup> dry<br>wt |
|--------------|----------------------------------|-----------------------------------|-----------------------------------|--------------|-----------------------------------------------------------------|--------------------------------------------------------------|
| Low          | NH <sub>4</sub>                  | 72.70                             | 13.48 a                           | 5.41         | 184 b                                                           | 2.29 b                                                       |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 74.87                             | 12.51 ab                          | 6.01         | 5380 a                                                          | 67.14 a                                                      |
|              | NO <sub>3</sub>                  | 63.90                             | 10.69 b                           | 6.11         | 5728 a                                                          | 82.05 a                                                      |
| Medium       | NH <sub>4</sub>                  | 69.33 a                           | 13.33 a                           | 5.21 a       | 527 b                                                           | 5.76 b                                                       |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 60.37 b                           | 13.84 a                           | 4.37 b       | 3067 a                                                          | 37.63 a                                                      |
|              | NO <sub>3</sub>                  | 54.53 c                           | 11.19 b                           | 4.88 ab      | 2930 a                                                          | 33.76 a                                                      |
| High         | NH <sub>4</sub>                  | 55.80                             | 13.10 a                           | 4.26 b       | 967 b                                                           | 11.66 b                                                      |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 61.53                             | 10.63 b                           | 5.80 a       | 5176 a                                                          | 70.54 a                                                      |
|              | NO <sub>3</sub>                  | 60.13                             | 10.00 c                           | 6.02 a       | 5222 a                                                          | 70.09 a                                                      |
| Main effect  |                                  |                                   |                                   |              |                                                                 |                                                              |
| N form       | NH <sub>4</sub>                  | 65.94 a                           | 13.30 a                           | 4.96 b       | 560 b                                                           | 6.57 b                                                       |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 65.59 a                           | 12.33 b                           | 5.39 ab      | 4541 a                                                          | 58.44 a                                                      |
|              | NO <sub>3</sub>                  | 59.52 b                           | 10.62 c                           | 5.67 a       | 4627 a                                                          | 61.97 a                                                      |
| PAR          | Low                              | 70.49 a                           | 12.23 a                           | 5.84 a       | 3764 a                                                          | 50.49 a                                                      |
|              | Medium                           | 61.41 b                           | 12.79 a                           | 4.82 b       | 2175 b                                                          | 25.71 b                                                      |
|              | High                             | 59.16 b                           | 11.24 b                           | 5.36 ab      | 3789 a                                                          | 50.76 a                                                      |
| N form       |                                  | **                                | ***                               | *            | ***                                                             | ***                                                          |
| PAR          |                                  | ***                               | **                                | **           | **                                                              | **                                                           |
| N form x PAR |                                  | **                                | ns                                | *            | ns                                                              | ns                                                           |

ns, \*, \*\* and \*\*\* not significant or significant at  $P \leq 0.05$ , 0.01 and 0.001.  
Means separated by HSD test at  $P = 0.05$ .

**Table 4.4.** Effect of nitrogen form and photosynthetic active radiation (PAR) on N, S, N/S ratio, and nitrate content in leaves of *Brassica juncea* plants.

| PAR          | N form                           | N<br>mg g <sup>-1</sup> dry<br>wt | S<br>mg g <sup>-1</sup> dry<br>wt | N/S<br>ratio | NO <sub>3</sub> <sup>-</sup><br>mg kg <sup>-1</sup><br>fresh wt | NO <sub>3</sub> <sup>-</sup><br>g kg <sup>-1</sup> dry<br>wt |
|--------------|----------------------------------|-----------------------------------|-----------------------------------|--------------|-----------------------------------------------------------------|--------------------------------------------------------------|
| Low          | NH <sub>4</sub>                  | 77.60 a                           | 10.40                             | 7.47         | 229 b                                                           | 2.07 b                                                       |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 74.73 ab                          | 8.80                              | 8.51         | 6266 a                                                          | 76.73 a                                                      |
|              | NO <sub>3</sub>                  | 66.90 b                           | 8.91                              | 7.60         | 5698 a                                                          | 69.36 a                                                      |
| Medium       | NH <sub>4</sub>                  | 73.43 a                           | 12.44 a                           | 5.90         | 592 b                                                           | 5.98 b                                                       |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 67.27 b                           | 10.25 b                           | 6.59         | 3200 a                                                          | 37.63 a                                                      |
|              | NO <sub>3</sub>                  | 59.10 c                           | 9.93 b                            | 5.97         | 2759 a                                                          | 28.96 a                                                      |
| High         | NH <sub>4</sub>                  | 63.33                             | 10.87 a                           | 5.77 b       | 1060 b                                                          | 12.10 b                                                      |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 67.60                             | 8.09 b                            | 8.37 a       | 5322 a                                                          | 63.31 a                                                      |
|              | NO <sub>3</sub>                  | 64.53                             | 7.81 b                            | 8.28 a       | 5831 a                                                          | 68.32 a                                                      |
| Main effect  |                                  |                                   |                                   |              |                                                                 |                                                              |
| N form       | NH <sub>4</sub>                  | 71.12 a                           | 11.24 a                           | 6.38 b       | 627 b                                                           | 6.71 b                                                       |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 69.87 a                           | 9.05 b                            | 7.82 a       | 4929 a                                                          | 59.22 a                                                      |
|              | NO <sub>3</sub>                  | 63.51 b                           | 8.88 b                            | 7.29 a       | 4763 a                                                          | 55.50 a                                                      |
| PAR          | Low                              | 73.08 a                           | 9.37 b                            | 7.86 a       | 4064 a                                                          | 49.39 a                                                      |
|              | Medium                           | 66.60 b                           | 10.87 a                           | 6.15 b       | 2184 b                                                          | 24.11 b                                                      |
|              | High                             | 64.82 b                           | 8.92 b                            | 7.47 a       | 4071 a                                                          | 47.91 a                                                      |
| N form       |                                  | ***                               | ***                               | **           | ***                                                             | ***                                                          |
| PAR          |                                  | ***                               | ***                               | ***          | ***                                                             | ***                                                          |
| N form x PAR |                                  | **                                | ns                                | *            | **                                                              | *                                                            |

ns, \*, \*\* and \*\*\* not significant or significant at  $P \leq 0.05$ , 0.01, and 0.001.  
Means separated by HSD test at  $P = 0.05$ .

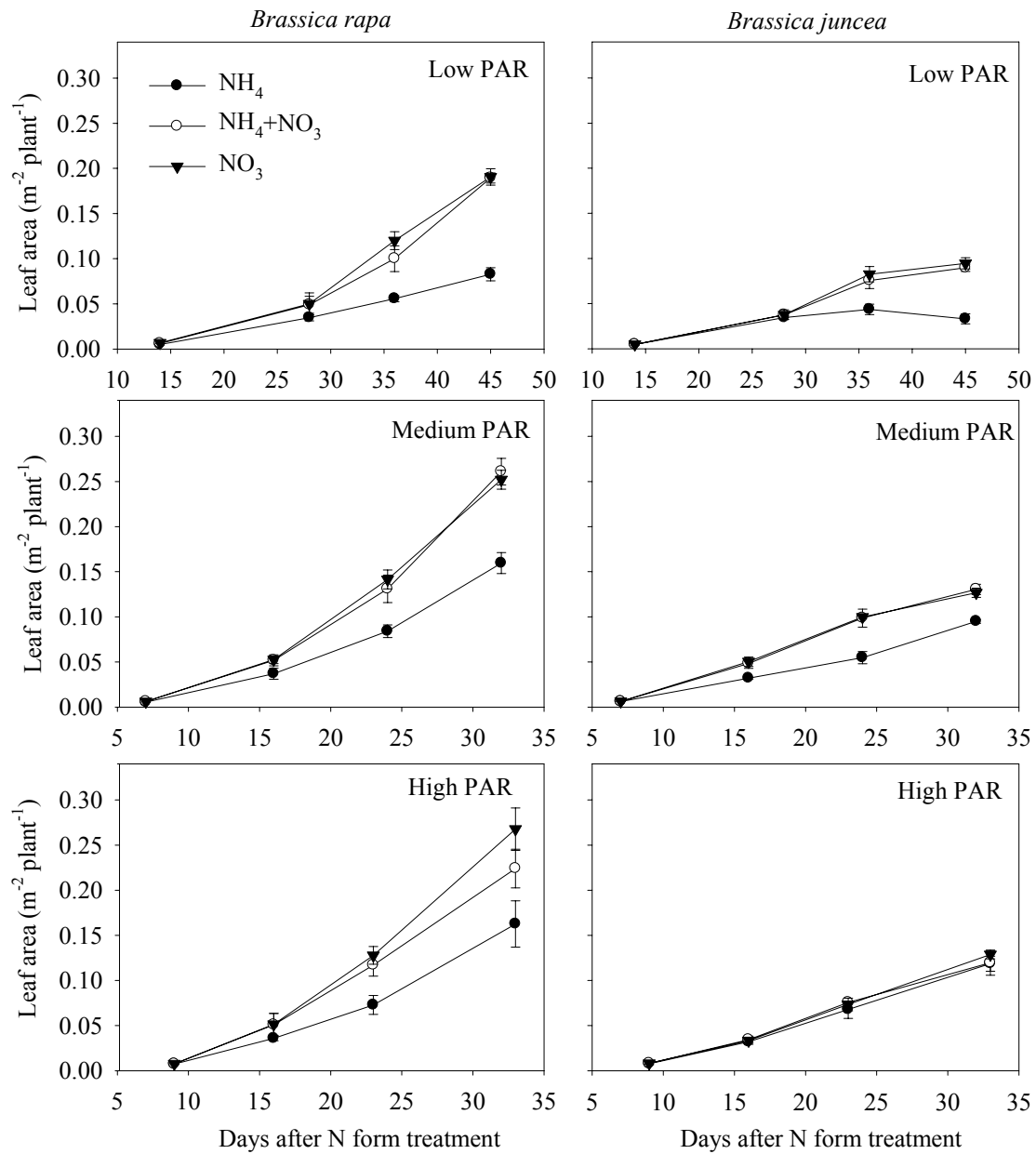


**Figure 4.1.** Trend of leaf area in response to nitrate form in plants of *Brassica rapa* and *Brassica juncea* grown at low, modest, and medium photosynthetic active radiation (PAR). Data are means of three replicates. Vertical bars indicate  $\pm$  S.E. of the mean.

## **CHAPTER 5**

### **Phytochemicals of two *Brassica* species affected by N form and radiation**

## Abstract

The aim of the present work was to determine the effects of three N form (100 %  $\text{NH}_4$ , 50%  $\text{NH}_4$  + 50 %  $\text{NO}_3$ , 100 %  $\text{NO}_3$ ) in the nutrient solution during consecutive experiments under three daily Photosynthetic Active Radiation (PAR) levels, 5.0 (low), 6.8 (medium) and 9.0 (high)  $\text{mol m}^{-2}$  on flavonoids, glucosinolates, carotenoids and chlorophylls in *Brassica rapa* L. subsp. *nipposinica* var. *chinoleifera* and *Brassica juncea* L. plants grown in hydroponics. The results showed that in both species the use of a nutrient solution containing 100% of N as  $\text{NH}_4$  influenced negatively the flavonoid concentration of quercetin and kaempferol at low and medium PAR. Highest concentration of total flavonoids (kaempferol, quercetin, isorhamnetin) was found at high PAR. In contrast, in *B. rapa*,  $\text{NH}_4$  supply influenced positively the concentrations of total, alkyl, alkenyl and indole glucosinolates compared to supply of equal amounts of  $\text{NH}_4$  and  $\text{NO}_3$ , as well as  $\text{NO}_3$  supply caused by a low N/S ratio in the leaves. There was a different glucosinolate response on PAR level between the two investigated *Brassica* species. The highest concentration of total glucosinolates occurred at medium PAR in *B. juncea* and at medium and high PAR in *B. rapa*. Furthermore, low concentrations of carotenoids and chlorophylls were found in *Brassica* species grown at  $\text{NO}_3$  supply in combination with medium and high PAR as well as in *Brassica* species grown at  $\text{NH}_4$  supply in combination with low PAR. Thus, the data provided here have implications for using crop managing strategies aiming to optimize desired health promoting compound concentrations.

Key words: *Brassica rapa* L., *Brassica juncea* L., phytochemicals, nitrate supply, ammonium supply, Photosynthetic Active Radiation

## 5.1 INTRODUCTION

In the last years phytochemicals aroused particular interest due to their beneficial properties for human health. Phytochemicals include a wide variety of compounds produced by plants and found in all kind of fruits and vegetables. Among the common known phytochemicals are glucosinolates, flavonoids, carotenoids, and vitamin C. The prevention of diseases is of great interest particularly in developed nations. The amount and character of phytochemicals with biological activities can be determined by careful selection of food.

Asia *Brassica* vegetables are a good source of health-promoting compounds since they contain glucosinolates, flavonoids, carotenoids, vitamin C. The species *Brassica rapa* L.

subsp. *nipposinica* var. *chinoleifera* and *Brassica juncea* L. gain more and more attention because they are characterized by a good content of glucosinolates, carotenoids, chlorophyll and vitamin C (Krumbein et al., 2005). Moreover, as other leafy vegetables, they are characterized by high water content, low caloric value, high quality protein and fibre (O'Hare et al., 2005; Artemyeva and Solovyeva, 2006).

Flavonoids are antioxidants and play an important role against several chronic diseases: ischemic heart disease, cerebrovascular disease, lung and prostate cancer, diabetes, and asthma. The potential beneficial effects of flavonoids are mainly ascribed to quercetin, the most potent antioxidant but also to other compounds such as kaempferol, myricetin, hesperitin, naringenin, and isorhamnetin (Erlund, 2004; Knekt et al. 2002; Harborne et al., 2000).

Glucosinolates are a group of plant secondary metabolites found in 15 botanical families of the order of Capparales. In Brassicaceae they are abundant and responsible for the specific and pungent flavours. At least 120 different glucosinolates have been identified in Brassica vegetables. They are amino acid-derived and can be grouped into aliphatic, aromatic and indolyl glucosinolates based on whether they are derived from amino acids, methionine, phenylalanine or tyrosine, or tryptophan, respectively. They are located in all organs of the plant and have been mainly found within the vacuole of the cell. Generally glucosinolates are biologically inactive but after disruption of plant cell they are hydrolysed by a  $\beta$ -thioglucosidase enzyme, called myrosinase, resulting in the release of numerous compounds with different biological activities: isothiocyanates, nitriles, epithionitriles and oxazolidines. In the plants, they are responsible of many physiological effects such as protection against general herbivores and pathogens attack. In human they have been shown health beneficial reducing the risk of several cancers (Cartea and Velasco 2008; Moreno et al. 2006; Yan and Chen 2007).

Carotenoids are a group of lipid-soluble pigments synthesized in plants, fungi and bacteria but they can not be produced by mammals. In plants they are used as antenna pigments to funnel light energy and they are in close proximity to the chlorophyll molecules where they perform a protective function against oxidative damage. The antioxidant activity of carotenoids has been suggested to play a role in the underlying mechanisms preventing several kinds of cancer and age-related macular degeneration (Stahl et al. 2005).

Chlorophylls and its various derivatives have been widely investigated for a range of beneficial biological effects including antioxidant and antimutagenic activity, apoptosis in cancer cells (Sass-Kiss et al. 2005; Ferruzzi and Blakeslee, 2007).

In *Brassica* vegetables there is a wide variability of phytochemicals in quantity and quality caused by genetic and environmental factors, plant age, mineral nutrients, and water availability (Verkerk et al. 2009). For instance, in broccoli, cabbage, cauliflower, and kale glucosinolate content can be influenced by maturity stage, temperature, season, rainfall rate (Krumbein et al. 2007; Ciska et al. 2000; Charron et al. 2005). In kale photoperiod and irradiance can influence pigment accumulation. The content of carotenoids and chlorophyll responded positively on increased photoperiod and irradiance (Lefsrud et al. 2006a). In inflorescences of three broccoli cultivars the content of quercetin and kaempferol was highly correlated with solar radiation in the period between planting and harvest (Gliszczyńska-Swigłowa et al. 2007).

Fertilization management plays an important role on biosynthesis and accumulation of phytochemicals in Brassicaceae. Since glucosinolates are sulphur containing compounds, their accumulation in the plant is strongly affected by S and N supply. In general species of the Brassicaceae are considered to have a high sulphur requirement to accumulate glucosinolates, while glucosinolate content declines with high levels of nitrogen (Falk et al. 2007; Shumin et al. 2007; Schonhof et al. 2007).

Studies on several crops show that the flavonoid concentration in leaf tissue is strongly related to the N status. For instance in apple and tomato leaves the flavonoids decline at high N level (Strissel, 2005; Bounge-Bartelsman and Phillips 1995).

Nitrate and ammonium are the predominant forms of inorganic nitrogen taken up by plant roots. Although plants can use both forms, nitrate is the preferred source for crop growth (Guo et al. 2007). Most plants can tolerate high nitrate concentration without any sign of toxicity because the leave vacuoles have high capability to accumulate nitrate. On the other hand the high nitrate concentration in the leaves reduces the produce quality due to the negative effect on human health though recently there is evidence contradicting this view. Studies highlight a negative correlation between nitrate intake and gastric cancers because vegetables are an excellent source of vitamins, minerals and biologically active compounds (Santamaria, 2006).

Contrary, the ammonium accumulation in most plants can not be tolerated and its translocation to the leaves is dangerous giving the phytotoxicity phenomena of ammonia such as chlorosis and necrosis of leaves, epinasty, and stem lesions (Barker and Mills, 1980). The uptake of nitrate and ammonium influences uptake of other ions (Guo et al. 2007). Uptake of ammonium by plants, compared to nitrate, has been reported to decrease

the uptake of cations, such as K, Mg, Ca and to increase the uptake of anions, such as P and S, (Kirkby and Knight, 1977; Salsac 1987; Barker and Mills, 1980).

The effects of nitrogen form on plant growth, morphology and physiological processes have been investigated in numerous experiments. In most crops ammonium results in small root and small leaf areas which contribute to a low carbon gain and to an inhibition on growth. However, the effects of nitrogen form on plant growth, primary and secondary metabolism are mainly dependent by genotype but also environmental conditions such as light intensity, temperature, nitrogen concentration, medium pH (Guo et al. 2007).

Studies of the N form effects on phytochemicals content are limited to a few Brassica species. In rocket salad the highest content of glucosinolates occurred with a well-balanced ratio of ammonium and nitrate in the nutrient solution (Kim et al. 2006). In two kale cultivars the carotenoid and chlorophyll pigments increased when nitrate increased in the nutrient solution and decreased with increasing portion of ammonium (Kopsell et al. 2007). In another study on kohlrabi (*Brassica oleracea* var. *gongylodes*) the ammonium fertilization increased the chlorophyll content in the leaves by 20% compared plants supplied with nitrate (Blanke et al. 1996). However, no information is available if and how nitrogen form affects phytochemicals in Brassica vegetables from Asia.

Since this is not known and since N supply can interact with environmental conditions, it was the aim of our investigation to determine the effect of ammonium and nitrate supply under different radiation on the phytochemical content of *Brassica rapa* subsp. *nipposinica* var. *chinoleifera* and *Brassica juncea* species which were selected due to their different phytochemical profile. The comprehensive investigations included the most important phytochemicals, such as flavonoids, glucosinolates, carotenoids and chlorophylls.

## **5.2 MATERIAL AND METHODS**

### **5.2.1 Plant material and experimental design**

Three hydroponic experiments were carried out in a greenhouse at the Institute of Vegetable and Ornamental Crops, located at Grossbeeren (Germany): experiment I, from December 6, 2007 to January 22, 2008; experiment II, from January 23, 2008 to February 25, 2008; experiment III, from March 6, 2008 to April 9, 2008.

All experiments were conducted at the same mean temperature (15°C), relative humidity (74%) and CO<sub>2</sub> concentration (400 µmol mol<sup>-1</sup>). The mean daily photosynthetic active radiation (PAR) was 5.0 (low, Exp. I), 6.8 (medium, Exp. II) and 9.0 (high, Exp. III) mol m<sup>-2</sup>. For each trial seeds of *Brassica juncea* L. cultivar Red Giant and *Brassica rapa*

*L. subsp. nipposinica* var. *chiloneifera*. cultivar Mibuna Early were sown in rockwool cubes under greenhouse conditions. The plants at the two-true leaf stage, 25 days after sowing, were transferred into eight meter long gullies supplied continuously with nutrient solution. Distance between the 46 plants in each gully amounted to 0.17 m. Total nitrogen concentration was the same in all treatments but treatments differed in nitrogen forms (100 %  $\text{NH}_4$ , 50%  $\text{NH}_4$  + 50 %  $\text{NO}_3$ , 100 %  $\text{NO}_3$ ). Treatments were arranged in a randomized complete block design with three replications. Nutrients were supplied in all treatments at the following concentrations. Macronutrients in mM: K 3.6, Ca 4.2, P 0.6, Mg 1.0,  $\text{SO}_4$  1.3; micronutrients in  $\mu\text{M}$ : Fe 40.0, Mn 5.0, Zn 4.0, B 30, Cu 0.5, Mo 0.5. During all experiments, the electrical conductivity was kept at  $2 \text{ dS m}^{-1}$  by adding stock solution or deionised water according to the variations ( $\pm 10\%$  of the target value). In all nutrient solutions a MES buffer was applied at 1.5 mM to keep the pH in the range of 5.6-5.8. Moreover, phosphoric acid or potassium hydroxide was added when the solution pH drifted above or below the threshold.

### **5.2.2 Sample preparation for analysis**

At commercial maturity, (45, 32 and 33 days after transplanting for experiment I, II and III) a sample of 20 plants was harvested and immediately transported to the laboratory. For the carotenoid and chlorophyll determinations a fresh sample was cut into small pieces and used soon for the analyses.

A sample of fresh shoot (300 g of f. wt) was harvested and immediately frozen ( $-28^\circ \text{C}$ ), freeze-dried, and finally reduced to a fine powder by a centrifugal mill for glucosinolates, flavonoid, N, and S analysis.

### **5.2.3 Carotenoid and chlorophyll analyses**

Carotenoids ( $\beta$ -carotene and lutein) and chlorophylls (chlorophyll a and chlorophyll b) were determined by HPLC (Krumbein et al., 2005). To 15 g of cut broccoli, 1 g calcium carbonate, 30 g sodium sulfate, and 30 ml acetone were added, and the samples were homogenized for 2 min. The extract was then filtered under suction, and the solid materials were extracted repeatedly with acetone until the solid materials were colorless. The extract was then filtered through a  $0.45 \mu\text{m}$  filter for HPLC analyses. Carotenoid and chlorophyll compositions and concentrations were determined by HPLC using a C-18 reversed-phase column Lichosphere 100 ( $5 \mu\text{m}$ ,  $250 \times 4 \text{ mm}$ ; Merck, Darmstadt, Germany) with an isocratic eluent of 75% acetonitrile, 15% methanol, and 10% methylene chloride. The analysis was carried out at a

flow rate of 1 ml min<sup>-1</sup>. Wavelengths of 448, 455, 432, and 464 nm were used to determine lutein,  $\beta$ -carotene, chlorophyll a, and chlorophyll b, respectively. Concentrations were quantitatively determined by calibration curves of the related pure standards. The results were converted on dry mass basis (mg g<sup>-1</sup> DM). Chemical analyses were performed in duplicate.

#### 5.2.4 Glucosinolate analysis.

Glucosinolates were analyzed by HPLC as their desulfoglucosinolates (Krumbein et al. 2005). Freeze-dried sample material (0.5 g) was heated to and incubated at 75°C for 1 min, extracted with 4 ml of a methanol/water mixture (v/v=7:3, T=70°C) and then, after adding 1 ml 0.4 M barium acetate, centrifuged at 4000 rpm for 10 min. The residue was extracted twice more with 3 ml of the methanol/water mixture (v/v=7:3, T=70°C). The supernatants were pooled and made up to 10 ml with the methanol/water mixture. From this supernatant, 5 ml were applied to a 250  $\mu$ l DEAE-Sephadex A-25 ion-exchanger (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany, acetic acid-activated) and rinsed with 10 ml deionized water. Next, 250  $\mu$ l of a purified solution of aryl sulfatase (Boehringer-Mannheim GmbH, Mannheim, Germany) was applied and left for 12 h. The desulfo-compounds were then flushed with 5 ml deionized water. The analysis was conducted using a Merck-Hitachi HPLC system (Merck-Hitachi, Darmstadt, Germany) with a Spherisorb ODS2 column (Bischoff, Leonberg, Germany, 5  $\mu$ m, 250 x 4 mm). A gradient of 0 to 20% acetonitrile in water was selected from minutes 2 to 34, followed by 20% acetonitrile in water until minute 40, and then 100% acetonitrile for 10 min until minute 50. Determination was conducted at a flow rate of 1.3 ml min<sup>-1</sup> and a wavelength of 229 nm. Glucosinolate concentration was calculated using sinigrin as internal standard, and using the response factor of each compound relative to sinigrin (Official Journal of the European Communities, 1990, *L 170*, 28-34). The results were calculated in on dry mass basis (mg g<sup>-1</sup> DM). Determination of glucosinolate concentration was performed in duplicate. Desulfoglucosinolates were identified according to previous work (Zimmermann et al. 2007) from the protonated molecular ions [M + H]<sup>+</sup> and the fragment ions corresponded to [M + H - glucose]<sup>+</sup> by HPLC-APCI-MS<sup>2</sup> using Agilent 1100 series (Agilent Technologies, Waldbronn, Germany) in the positive ionization mode.

#### 5.2.5 Flavonoid analysis

Flavonols were determined as their aglycones after acid hydrolyses (Krumbein et al. 2007). 40 ml of 62.5% aqueous methanol was added to 0.5 g of the freeze-dried sample. 10

ml of 8 M HCl was added to this extract. Thus, the extraction solution consisted of 1.6 M HCl in 50% aqueous methanol (v/v). After refluxing at 90°C for 2h, the extract was allowed to cool, was adjusted to 100 ml with 50% methanol, and sonicated for 5 min. The extract was then filtered through a 0.45 µm filter for HPLC analyses.

The flavonoid composition and concentration were determined using a series 1100 HPLC (Agilent, Waldbronn, Germany) equipped with a diode array detection system. A Prodigy column ODS 3 (250 x 4.6 mm, 5 µ, 100Å) (Phenomenex, Aschaffenburg, Germany) was used with a security guard C18 (4 x 3.0 mm) at a temperature of 25°C. Solvent A was water + 0.1% TFA (trifluoroacetic acid) + 2% THF (tetrahydrofuran); solvent B was acetonitrile. The following gradient was used: 30 - 35% B (5min), 35 - 39% B (12 min), 39 - 90% B (5min), 90% B isocratic (2 min), 90 - 30% B (5min), 30% B isocratic (5 min). The chromatogramme were monitored at 370 nm with a flow rate of 1 ml min<sup>-1</sup>. Contents were quantitatively determined by calibration curves of the related pure standards. The results were converted on dry mass basis (mg g<sup>-1</sup> DM). Determination of flavonoid concentration was performed in duplicate. Quercetin, kaempferol and isorhamnetin were identified by HPLC-ESI-MS<sup>2</sup>, using Agilent 1100 series (ion trap) in the negative ionisation mode. Nitrogen was used as dry gas (12 l min<sup>-1</sup>, 350°C) and nebuliser gas (40 psi). To compare the results with the HPLC-DAD, the same column material with a shorter length (150 x 4.6 mm, 5 µ, 100 Å) was used. TFA in solvent A was replaced by 0.5% (v/v) acetic acid, THF in solvent A was omitted and the same water/acetonitril gradient and a flow rate of 0.6 ml min<sup>-1</sup> were used. Quercetin, kaempferol and isorhamnetin were identified from the deprotonated molecular ions [M - H]<sup>-</sup> with m/z 301, 285 and 315, respectively.

#### **5.2.6 N and S analysis**

Total N was determined after dry oxidation by the Dumas method (Elementar Vario EL, Hanau, Germany).. Total S was analyzed by an elementary analyzer (high-temperature oxidation) and detected with a non dispersive infrared (NDIR sensor).

#### **5.2.7 Statistical analysis**

All data were statistically analysed by ANOVA using SPSS software package (SPSS version 15.0 for Windows). When the interaction between N form and PAR was significant, ANOVA was performed separately for each experiment. The means were

separated by Tukey's HSD test (5% significance level). Regression analysis were carried out at significant level  $P \leq 0.05$ .

## 5.3 RESULT AND DISCUSSION

### 5.3.1 Flavonoid concentration.

The most important flavonoid was quantitatively represented by kaempferol (range from 1.05 to 3.31 mg g<sup>-1</sup> DM in *B. rapa* and from 1.35 to 4.04 mg g<sup>-1</sup> DM in *B. juncea*), following by isorhamnetin (range from 0.35 to 1.83 mg g<sup>-1</sup> DM in *B. rapa* and from 0.27 to 0.96 mg g<sup>-1</sup> DM in *B. juncea*) and quercetin (range from 0.10 to 0.39 mg g<sup>-1</sup> DM in *B. rapa* and from 0.09 to 0.47 mg g<sup>-1</sup> DM in *B. juncea*) (**Table 5.1**).

In *B. juncea*, total flavonoids, quercetin and kaempferol concentrations were affected by N form and PAR, whereas the isorhamnetin concentration was only affected by PAR. For all flavonoids no interaction was observed (**Table 5.1**). Regarding to the N form, NH<sub>4</sub> influenced negatively the total flavonoids, quercetin and kaempferol concentrations. Compared to NO<sub>3</sub>, the total flavonoid concentration was reduced from 3.19 to 1.70 mg g<sup>-1</sup> DM at low PAR, from 4.16 to 2.84 mg g<sup>-1</sup> DM at medium PAR, and from 5.19 to 4.51 mg g<sup>-1</sup> DM at high PAR. NH<sub>4</sub>+NO<sub>3</sub> had similar concentrations to NO<sub>3</sub>. A similar trend of reduction was observed for individual flavonoids, quercetin and kaempferol. The PAR influenced strongly the concentrations of flavonoids. The total flavonoids concentrations were 4.99, 3.68 and 2.68 mg g<sup>-1</sup> DM at high, medium and low PAR, respectively. Individual flavonoids, quercetin, kaempferol and isorhamnetin showed clearly the trend above mentioned with high concentrations at high PAR

In *B. rapa*, the total flavonoid concentration was influenced by PAR and interaction N form x PAR. The quercetin and kaempferol concentrations were affected by N form, PAR and their interaction. The isorhamnetin concentration was solely affected by PAR (**Table 5.1**). NH<sub>4</sub> influenced negatively the total flavonoids, quercetin and kaempferol concentration at low and medium PAR. Regarding to NO<sub>3</sub>, the total flavonoid concentration was reduced from 2.69 to 1.72 mg g<sup>-1</sup> DM and from 3.95 to 2.69 mg g<sup>-1</sup> DM at low PAR and medium PAR, respectively. For these PAR levels, the reduction of concentrations due to NH<sub>4</sub> was also observed for individual flavonoids, kaempferol and quercetin. Intermediate concentrations were noticed for NH<sub>4</sub>+NO<sub>3</sub>. At high PAR the concentration of flavonoids was not affected by nitrogen form. This could be the reason that explains because the total flavonoid concentration was not influenced by N form when the data were pooled. Regarding to the PAR, the total flavonoids, quercetin, kaempferol and

isorhamnetin concentrations were strongly influenced. The highest total flavonoid concentration resulted at high PAR (5.05 mg g<sup>-1</sup> DM). Intermediate concentration occurred at medium PAR (3.44 mg g<sup>-1</sup> DM) whereas the lowest values of concentration resulted at low PAR (2.24 mg g<sup>-1</sup> DM). A similar trend was observed for the predominant flavonoid kaempferol with high concentration at high PAR.

Interestingly in both species, there was an influence by N form on total N concentration in plants (**Table 5.2**). In *B. juncea*, NH<sub>4</sub> determined the highest concentrations of N in plants at low and medium PAR, whereas no significant differences were found at high PAR. A high content of N was also found in leaves of *B. rapa* grown at NH<sub>4</sub> and at medium PAR. Our results showed that there was a clear relationship between the concentrations of N in leaves and the concentrations of the flavonoids in both species. Low concentration of N in leaves determined an increase of flavonoids, whereas high concentration of N had a contrary effect with a decrease of flavonoids. (**Figure 5.1**).

Most of studies to the regard on different crops draw attention on N supply and the content of flavonoids without investigating the N form used and content of N in the plant. In tronchuda cabbage high phenolic content was obtained with minimum nitrogen fertilization (Sousa C. et al. 2008). In a study on mature tomato leaf tissue the concentrations of total flavonoids, quercetin, kaempferol and isorhamnetin increased in response to reduced N availability (Stewart et al., 2001). In other study carried out on tomato leaves the levels of quercetin were doubled in response to nitrogen stress (Bongue-Bartelsman and Phillips, 1995). A similar behaviour was recorded in studies on apple leaves in which the accumulation of flavonoids decreased with high N nutrition (Strissel et al., 2005). Furthermore, the levels of eleven flavonols increased in *Matricaria camomilla* leaves subjected to N deficiency (Kováčik et al., 2007). Moreover, studies in *Arabidopsis thaliana* showed that nitrogen deficiency resulted in the accumulation of not only anthocyanins but also flavonoids such as quercetin and kaempferol. In the same study, nitrogen deficiency resulted in enhanced transcript levels of some genes important for accumulation of anthocyaninn and flavonoids (Lea et al.2007). Also in aerial parts of stinging nettle (*Urtica dioica*) the concentrations of flavonoids were significantly reduced with high N levels. (Grevsen et al. 2008).

Different enzymes of flavonoid biosynthesis seem to be influenced by nitrogen level. Most of the studies proved decreasing activity of PAL (phenylalanine ammonia-lyase) with increasing availability of N. PAL is the key enzyme of the phenylpropanoid pathway which provides one of the precursor for flavonoids formation. In apple leaves PAL activity

seems to be down regulated, hence resulting in a decreased flavonoids accumulation with high level of N. (Strissel et al., 2005). Moreover, N deprivation greatly increased the mRNA levels of chalcone synthase (CHS) and dihydroflavonol reductase (DFR) in tomato leaves which are two important enzymes involve in the biosynthesis of flavonoids. (Bongue-Bartelsman and Phillips, 1995).

The results of the present study concerning the influence of nitrogen form show that the content of flavonoids decrease with high N content in the leaves which resulted by use of  $\text{NH}_4$ . The trend was observed in two different species belonging to the same genus. In our study, we used the same concentration of N in the nutrient solution ( $12 \text{ mmol L}^{-1}$ ).

Recently, a study on medicinal plant (*E. angustifolia*) carried out to investigate the content of phenylalanine-derived substances, showed that PAL activity in the leaves was not influenced by two  $\text{NO}_3:\text{NH}_4$  ratio (1:0 and 1:1) in the nutrient solution. Interestingly, in the same work the authors show that PAL activity increased solely in root tissues of plants grown with  $\text{NO}_3$  (Montanari et al.2008).The study does not highlight whether there is a relation with the content of N in tissue.

In the phenylpropanoid pathway, the PAL carries out the elimination of ammonia from phenylalanine to produce cinnamic acids which serves as the precursor for all phenylpropanoids of secondary metabolism. The level of phenylalanine in the different vegetable tissue can change when the plants are subjected to several external stimulus (Forkmann and Heller, 1999). Changes at level of substrate could be determining factor to accumulate flavonoids in the plants. Hence, in our study the high level of flavonoids recorded with lower level of N in the vegetable tissue could be due to level of phenylalanine decidedly better. A lower level of phenylalanine induced by a high level of N in the plant could be the reason of decreasing of flavonoids. Therefore the level of the amino acid phenylalanine in the leaves can affect the content of flavonoids within vegetable tissue. A study on seven salad green (escarole, kale, lettuce, mibuna, minutina, mustard and spinach) show how the composition of free amino acids in leaves can change due to genotype, fertilization and season (Gent, 2005).

Regarding to the effects of PAR, our results are in agreement with several researches in *Brassica* species regarding the radiation effect on flavonoid content. In broccoli the contents of quercetin and Kaempferol were positively correlated with radiation (Gliszczyńska-Swigłoa et al., 2007). In another research, exposure of leaves of *Brassica napus* to supplementary UV radiation determined an overall increase in the amount of flavonoids of 150 % with a specific increase in the amount of quercetin of 36-fold (Olsson

et al. 1998). The radiation seems to be the determining factor for flavonoid biosynthesis in Brassica species. Numerous studies have largely demonstrated that the accumulation of flavonoids by plants is the main defence mechanism against several environmental stress including the light. Flavonoids together to other antioxidant compounds present in green leaves absorb in the UV bands representing a selective filter which protect plant tissue against harmful rays.(Treutter, 2006; Harborne et al. 2000; Larson, 1988).

The results show clearly that the choice of nitrogen form is an important strategy to increase the content of flavonoids in *B. rapa* and *B. juncea* improving health-promoting compounds. Because the accumulation of flavonoid and other phenylalanine-derived polyphenols is a process which mainly controlled at the enzymic level, these results are a positive basement to further studies. Molecular and genetic approaches are necessary to understand really the flavonoid pathway in *B. rapa* and *B. juncea*. In fact, how some abiotic stress and external stimulus promote high concentration of flavonoids in several species is not yet clear.

### 5.3.2 Glucosinolate concentration

Nine individual glucosinolates were quantitatively determined in the investigated *Brassica* species: aliphatic (alkyl: glucoraphanin and glucoalyssin; alkenyl: gluconapin and glucobrassicinapin), indole (glucobrassicin, 4-hydroxyglucobrassicin, 4-methoxyglucobrassicin and neoglucobrassicin) and aromatic (aryl: gluconasturtiin). The two cultivars can be differentiated by their glucosinolate profile. *B. juncea* was characterized by high concentrations of sinigrin up to 90% of the total glucosinolate concentration while in *B. rapa* the predominant glucosinolate was gluconapin followed by glucobrassicin (Tables 5.3 and 5.4).

***B. rapa*.** The total, alkyl, alkenyl, indole and aryl glucosinolate concentrations were affected by N form and PAR. Interactions were observed for alkyl, indole and aryl glucosinolates but not for the predominant alkenyl glucosinolates, gluconapin and glucobrassicinapin (Table 5.3).

NH<sub>4</sub> supply influenced positively the concentrations of total, alkyl, alkenyl and indole glucosinolates compared to supply of equal amounts of ammonium and nitrate as well as nitrate supply. On the contrary, the concentration of the aryl glucosinolate showed the highest value at NO<sub>3</sub> supply. The corresponding individual glucosinolates clearly showed similar trend. The results can be discussed in relation with N and S concentrations as well as N/S ratio in leaves of *B. rapa* (Table 5.2). NH<sub>4</sub> supply resulted in high N and S

concentrations and a low N/S ratio in leaves (**Table 5.2**). Moreover there was a strict relationship between the total glucosinolate concentration and N/S ratio (**Figure 5.2**). The total glucosinolate concentration decreased with increasing N/S ratio.

In Brassica vegetables, it is well known that N and S affect the glucosinolate concentration because they are S- and N-containing compounds. There are several studies on the importance of N, S and N/S ratio with wide range of variability mainly depending by genotype and other factors. In broccoli plants total glucosinolate concentrations are high at insufficient N, independent of the S level and low at insufficient S in combination with an optimal N supply. In addition N/S ratio lower than 10:1 determine a positive effect on alkyl and indole glucosinolate concentrations (Schonhof et al., 2007). In our experiment no relationship was found between total glucosinolate concentration and N and S concentration (data not shown). There was solely a relationship with N/S ratio. Accordingly the proper balance between N and S play an important role in the biosynthesis of the glucosinolates as shown in several studies on turnip, broccoli, cabbage and other *Brassica* species (Shumin et al., 2007; Schonhof et al. 2007; Rosen, 2005; Falk et al. 2007). Interactive effects between N and S assimilatory pathways are well documented (Clarkson et al., 1999; Koprivova et al., 2000; Nikiforova et al., 2003). O-acetylserine play a key role because it is derived from S and N assimilation pathways. Moreover it is a precursor of the N and S containing amino acids cysteine and methionine which are important precursors of glucosinolate synthesis. With insufficient S, O-acetylserine incline to accumulate whereas with insufficient N the increase of O-acetylserine is reduced. High N and low S concentrations (high N/S ratio) could lead to an accumulation of O-acetylserine and reduced cysteine and methionine synthesis resulting in a lack of precursors for glucosinolate synthesis. Furthermore metabolomic and transcriptomic studies demonstrate that sulfur deficiency leads to the reduced expression of all the major glucosinolate biosynthetic genes reducing the content of glucosinolates in the plants (Falk et al. 2007). Therefore, in the present work the highest concentration of total glucosinolates with low N/S ratio could be attributed to the accumulation of essential precursor such as methionine. Specifically, methionine is one of the most important precursor for aliphatic glucosinolates (alkyl and alkenyl glucosinolates). In *B. rapa*, total glucosinolate concentration was mainly determined by alkenyl and alkyl glucosinolates.

Regarding the influence of PAR, the highest concentration of total glucosinolates was observed at medium and high PAR which was mainly caused by the major aliphatic

glucosinolates gluconapin and glucobrassicinapin. Conversely, the concentration of the indole glucosinolate glucobrassicin was higher at low PAR.

Considering the chemical classes, previous studies have highlighted the behaviour of various enzymes involved in the biosynthesis of glucosinolates regarding the environmental factors temperature and radiation. Wallsgrove and Bennet (1995) found that low radiation intensity reduces the glucosinolate content of rape leaves due to decreasing flavin-containing monooxygenases which catalyse the formation of aliphatic aldoxime, a key regulator step in aliphatic glucosinolate biosynthesis. Our current study confirms the results found by Wallsgrove and Bennet (1995) since at steady temperature the predominant alkenyl glucosinolates were lowest at low PAR and higher at medium and high PAR. In contrast, the main indole glucosinolate glucobrassicin was higher at low PAR which was also found in a study on broccoli grown under controlled environment in a greenhouse (Schonhof et al., 2007). The peroxidases that catalyse the conversion from tryptophan to indole glucosinolate are rather temperature dependent (Starzynska et al., 2003); therefore, in our experiment the radiation could have a scarce influence on indole glucosinolate glucobrassicin. Surprisingly, this could not be confirmed for the indole glucosinolates 4-hydroxyglucobrassicin, 4-methoxyglucobrassicin and neoglucobrassicin which were found in relatively low concentrations. Furthermore, no clear radiation effect was found on the aryl glucosinolate gluconasturtiin. In another work on various cultivar of *Brassica oleracea*, the glucoraphanin concentrations were significantly affected by photosynthetic photon flux and daylength but not by temperature (Charron et al., 2005). Our results are also in agreement with previous study on *B. campestris* in which the concentrations of total glucosinolates were highest in the plants cultivated in spring-summer compared with those grown in autumn-winter (He et al., 2000)

***B. juncea***. The total and alkenyl glucosinolate concentrations were not affected by N form and interaction but only by PAR level (**Table 5.4**). Indole glucosinolate concentration were only affected by N form, whereas the aryl glucosinolate concentration was affected by N form and interaction. Concerning N form, the predominant alkenyl glucosinolate sinigrin, showed similar trend to total glucosinolate concentration. The lower contents of gluconapin and glucobrassicin were affected by NH<sub>4</sub> supply that showed the highest values.

Similarly to *B. rapa*, a linear relationship was found between total glucosinolate concentration and N/S ratio in leaves (**Figure 5.2**). Increasing of N/S ratio determined a reduction of total glucosinolate concentration. However there was a difference between

two species because the optimal N/S ratio that maximized total glucosinolate concentration was higher for *B. juncea* compared to *B. rapa* (6:1 vs 4:1). This result draws attention to an important point regarding *Brassica* species-specific responses to N/S ratio. Therefore, the concentrations of N and S in the plant have a great influence in determining a proper N/S ratio; this matter is important in the management of crop systems to obtain the highest glucosinolate content.

The highest concentration of total glucosinolates occurred at medium PAR. At low and high PAR no significant difference was observed. The same trend was noted for the predominant alkenyl glucosinolates, sinigrin and gluconapin. These results are in contrast to *B. rapa* which showed the highest concentration of total glucosinolates at medium and high PAR which was mainly caused by the alkenyl glucosinolates gluconapin and glucobrassicinapin. That means there was a different alkenyl glucosinolate response to high PAR level between the two investigated *Brassica* species. A different behaviour was also noted in various types of broccoli and cauliflower. Increasing irradiation led to rising contents of aliphatic glucosinolates in green broccoli and cauliflower and in white cauliflower. On the contrary the glucosinolate content of violet broccoli and cauliflower was nearly unaffected by irradiation (Schreiner, 2005). Furthermore, a strong interaction between cultivar and season was found in levels of aliphatic and aromatic glucosinolates in eleven cultivars of broccoli (Rosa and Gomes, 2001) as well as in five varieties of *Brassica oleracea* such as broccoli, Brussels sprouts, cabbage, cauliflower, and kale (Charron et al., 2005). Moreover, the last study also indicates that variations of climatic factors near the time of harvest influence the glucosinolate concentration.

Hence, on the basis of above mentioned reports, it is possible to conclude that in some *Brassica* species the genetic factors are more important than environmental factors such as the radiation and temperature.

Furthermore, other many factors can influence the glucosinolate content. The accumulation of glucosinolates occurs also in the root. In any *Brassica* cultivar the total glucosinolate content is higher in the root than in the shoot (Castro et al., 2004). Fluctuation of aliphatic and indole glucosinolates happens along growth with opposite trend (Bellostas et al., 2007).

Previous studies in *Arabidopsis thaliana* showed that glucosinolates can be transported by phloem due to suitable physicochemical properties and glucosinolate exchange between the plant organs is possible. Glucosinolates synthesized in mature leaves are transported together with photoassimilates in the osmotically driven translocation stream from source

to sink. Other plant secondary metabolites (e.g. cytokinin) produced in roots are transported to the leaves (Chen and Andreasson 2001). Hence, it is also possible that in any *Brassica* species the glucosinolates help the plant to defend itself against environmental stress acting as protective and exchangeable resource between various organs.

Present investigation highlights the behaviour of two interesting *Brassica* species with different glucosinolate profiles in relation to N form and PAR level. In both species, the highest concentrations of total glucosinolates occur at low N/S ratio in plants (4:1 for *B. rapa* and 6:1 for *B. juncea*). Furthermore, in our investigations medium PAR level increased the glucosinolate content in both investigated species while high concentrations of glucosinolates were also found in *B. rapa* grown at high PAR level.

From the investigated glucosinolates potential candidates with health promoting effect seem to be glucoraphanin, sinigrin, gluconapin and gluconasturtiin (Verkerk et al. 2009). The possible health promoting effect of few indole glucosinolates is further in discussion.

### **5.3.3 Carotenoids and chlorophylls concentration**

In both species the carotenoids lutein and  $\beta$ -carotene as well as the chlorophylls a and b were quantified. In both species, lutein predominated slightly on  $\beta$ -carotene whereas the concentration of chlorophyll a was higher than chlorophyll b concentration (**Table 5.5**).

In *B. rapa* total carotenoid concentration was affected by N form, PAR and their interaction, whereas total chlorophyll concentration was affected by PAR and N form x PAR interaction (**Table 5.5**).

In *B. juncea* total carotenoid and chlorophyll concentrations were affected by N form, PAR and their interaction (**Table 5.5**). No relationships were found with N and S concentration and N/S ratio for both species (data not shown).

In *B. rapa*,  $\text{NH}_4$  supply determined the highest concentrations of total carotenoids, lutein,  $\beta$ -carotene and chlorophyll a. But these effects were mainly evident at medium and high PAR. At low PAR, excepting chlorophyll b, N form had no effects on concentrations of carotenoids and chlorophylls. Instead in *B. juncea*  $\text{NH}_4$  supply and supply of equal amounts of  $\text{NH}_4$  and  $\text{NO}_3$  caused the highest concentration of total carotenoids, lutein and  $\beta$ -carotene at medium PAR. At low PAR there was an opposite trend; the  $\text{NH}_4$  determined the lowest concentrations of total carotenoids, lutein and  $\beta$ -carotene. The same trend was observed for total chlorophylls, chlorophyll a and chlorophyll b. In general low concentrations of carotenoids and chlorophylls were found in *Brassica* species grown at

NO<sub>3</sub> supply in combination with medium and high PAR as well as in Brassica species grown at NH<sub>4</sub> supply in combination with low PAR.

The lack of relationships between S content and carotenoid and chlorophyll contents was recorded in other studies. In some cultivar of kale and in watercress the contents of lutein, β-carotene, chlorophyll a and chlorophyll b were unaffected by increasing of S supply. Instead, increasing of carotenoids and chlorophylls were observed under increasing N supply (Kopsell et al., 2003; Kopsell et al., 2007a; Kopsell et al., 2007b). However, a review highlights that high rate of N usually have a negative effect on vitamins and phytonutrients (Mozafar, 1993).

In a nitrogen form study on kale, the content of lutein on dry mass basis was increased due to increasing of NO<sub>3</sub> in nutrient solution; however a quadratic trend was observed with first increasing and then decreasing. The highest concentration was not observed under 100% NO<sub>3</sub> in nutrient solution but at 25%NH<sub>4</sub>:75% NO<sub>3</sub> (Kopsell et al., 2007). However knowledge of the influence of N form and radiation on carotenoid and chlorophyll contents are limited in *Brassica* species.

In strawberry, a recent study about the effects of shading and N form on yield and quality shows that the ratio of NH<sub>4</sub>:NO<sub>3</sub> should be adjusted with respect to light intensity for successfully growing (Tabatabaei et al., 2008).

In our experiment interactions between N form and PAR were found for total carotenoid and chlorophyll concentration in both species. These results indicate that content of carotenoids and chlorophylls respond differently to N form depending on the PAR level. Generally, the accumulation of carotenoids and chlorophylls is favoured by NH<sub>4</sub> and NH<sub>4</sub>+NO<sub>3</sub> at medium and high PAR. NO<sub>3</sub> and NH<sub>4</sub>+NO<sub>3</sub> affect the contents of carotenoids and chlorophylls at low PAR. The trend is more pronounced for *B. juncea*.

Several other factors can influence the levels of carotenoids and chlorophylls such as the concentrations of other nutrient. Previous researches in many crops have shown that high concentrations of NH<sub>4</sub> in nutrient solution induce a decreased concentration of cations such as K, Ca and Mg whereas NO<sub>3</sub> shows opposite effects. Moreover, NH<sub>4</sub> is used scarcely or not at all during early growth of plant under poor light condition (Marschner, 1995; Adams, 2002). For example Mg has an important role in photosynthesis involving the control of thylakoid stacking and being a part of chlorophyll. Thus a reduction of Mg levels in the plants can affect the concentrations of chlorophylls.

A review examined the effect of different N form on growth, photosynthesis, photorespiration and water relation in higher plants. Several studies show that NH<sub>4</sub>,

compared to  $\text{NO}_3$ , determines an increased chloroplast volume (Guo et al., 2007). Hence, greater concentration of chlorophylls could occur in the chloroplasts.

Contradictory results were found in literature regarding the influence of PAR on carotenoid and chlorophyll contents. In an experiment on broccoli under controlled environment, the contents of lutein,  $\beta$ -carotene, chlorophyll a and chlorophyll b were not affected by radiation, but the contents of lutein and  $\beta$ -carotene were temperature-dependent (Schreiner, 2005; Schonhof et al., 2007). In contrast, kale and spinach grown in a controlled environment had the highest contents of carotenoids and chlorophylls at medium PAR levels (Lefsrud et al., 2006; Lefsrud and Kopsell, 2006). At high PAR levels the content of carotenoids and chlorophylls decreased. Moreover, the authors showed that in kale grown under different radiation cycles with same irradiance-to-dark ratios, maximum accumulation of lutein,  $\beta$ -carotene and chlorophyll a occurred with 12-hour radiation cycle. At high radiation cycles the concentration fell down.

In an other study on common cultivar kale, all four principal carotenoids ( $\beta$ -carotene, lutein, violaxanthin, and neoxanthin) had higher concentrations in the summer than in the winter (De Azevedo and Rodriguez-Amaya, 2005).

In our experiment the highest concentrations of carotenoids and chlorophylls were observed at medium PAR level for both species and at low PAR level for *B. rapa*..

Another important matter concerns the variation of carotenoids and chlorophylls along the growth of plant. A study on kale show that carotenoids and chlorophylls concentration increase in leaf tissue with maturity and subsequently decrease mainly during senescence (Lefsrud et al. 2007). Moreover, the changes are strongly affected by genetic factors, morphological features and chemical composition. Hence it is difficult to understand exactly when the maximum accumulation of carotenoids and chlorophylls occur. In photosynthetic tissue there are two important processes with opposing effects on carotenoid levels: biosynthesis and photodegradation. Biosynthesis and photodegradation processes can alternate during leaf ontogeny due to several factors including trend of environmental condition before harvesting stage, nutrient concentration in vegetable tissues, genetic influence and also their interactions (Schonhof et al., 2007; Kopsell et al., 2003; Kopsell et al., 2007 Schreiner, 2005). Another important source of variation in these two species of *Brassica* could be the proper harvesting stage relate to different radiation levels.

To summarize, the results clearly show the interaction between N form and other factors like PAR level. Moreover, they confirm partly the data of other studies about the lack

effects of radiation and another part underline that further researches are necessary above all to give responses to influence by N form.

## 5.4 Conclusion

To summarize, the results showed that in both species the use of  $\text{NH}_4$  influenced negatively the flavonoid concentration of quercetin and kaempferol at low and medium PAR. Highest concentration of total flavonoids was found at high PAR. In contrast, in *B. rapa*,  $\text{NH}_4$  supply influenced positively the concentrations of total, alkyl, alkenyl and indole glucosinolates compared to supply of equal amounts of  $\text{NH}_4$  and  $\text{NO}_3$ , as well as  $\text{NO}_3$  supply caused by a low N/S ratio in the leaves. There was a different glucosinolate response on PAR level between the two investigated *Brassica* species. The highest concentration of total glucosinolates occurred at medium PAR in *B. juncea* and at medium and high PAR in *B. rapa*. Concerning carotenoids and chlorophylls, interactive effects between N form and radiation were found in both species.

Thus, the data provided here have implications for using crop managing strategies aiming to optimize desired health promoting compound concentrations.

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**Table 5.1.** Flavonoids concentration on dry mass basis (mg g<sup>-1</sup> DM) in *B. juncea* and *B. rapa*.

| PAR          | N form                           | <i>B. juncea</i> |           |            |              | <i>B. rapa</i> |           |            |              |
|--------------|----------------------------------|------------------|-----------|------------|--------------|----------------|-----------|------------|--------------|
|              |                                  | Total            | Quercetin | Kaempferol | Isorhamnetin | Total          | Quercetin | Kaempferol | Isorhamnetin |
| Low          | NH <sub>4</sub>                  | 1.70 b           | 0.09 b    | 1.35 b     | 0.27         | 1.72 c         | 0.10 b    | 1.05 c     | 0.56         |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 3.15 a           | 0.22 a    | 2.64 a     | 0.29         | 2.31 b         | 0.17 a    | 1.79 b     | 0.35         |
|              | NO <sub>3</sub>                  | 3.19 a           | 0.27 a    | 2.60 a     | 0.32         | 2.69 a         | 0.20 a    | 2.07 a     | 0.42         |
| Medium       | NH <sub>4</sub>                  | 2.84 b           | 0.24 b    | 2.17 b     | 0.43         | 2.69 b         | 0.12 b    | 1.86 b     | 0.72         |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 4.05 a           | 0.34 a    | 3.23 a     | 0.48         | 3.67 a         | 0.23 a    | 2.64 a     | 0.80         |
|              | NO <sub>3</sub>                  | 4.16 a           | 0.35 a    | 3.29 a     | 0.52         | 3.95 a         | 0.24 a    | 2.90 a     | 0.81         |
| High         | NH <sub>4</sub>                  | 4.51 b           | 0.42      | 3.30 b     | 0.80         | 5.52           | 0.39      | 3.31       | 1.83         |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 5.27 a           | 0.38      | 4.04 a     | 0.85         | 4.74           | 0.33      | 2.96       | 1.45         |
|              | NO <sub>3</sub>                  | 5.19 a           | 0.47      | 3.76 ab    | 0.96         | 4.88           | 0.34      | 3.06       | 1.48         |
| N form       | NH <sub>4</sub>                  | 3.02 B           | 0.25 B    | 2.28 B     | 0.50         | 3.31           | 0.20 B    | 2.07 B     | 1.04         |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 4.16 A           | 0.31 A    | 3.30 A     | 0.54         | 3.57           | 0.24 AB   | 2.46 A     | 0.87         |
|              | NO <sub>3</sub>                  | 4.18 A           | 0.37 A    | 3.21 A     | 0.60         | 3.84           | 0.26 A    | 2.68 A     | 0.90         |
| PAR          | Low                              | 2.68 C           | 0.19 C    | 2.20 C     | 0.29 C       | 2.24 C         | 0.16 B    | 1.64 C     | 0.45 B       |
|              | Medium                           | 3.68 B           | 0.31 B    | 2.90 B     | 0.48 B       | 3.44 B         | 0.20 B    | 2.47 B     | 0.78 B       |
|              | High                             | 4.99 A           | 0.42 A    | 3.70 A     | 0.87 A       | 5.05 A         | 0.35 A    | 3.11 A     | 1.58 A       |
| N form       |                                  | *                | *         | *          | ns           | ns             | *         | *          | ns           |
| PAR          |                                  | *                | *         | *          | *            | *              | *         | *          | *            |
| N form x PAR |                                  | ns               | ns        | ns         | ns           | *              | *         | *          | ns           |

ns, \*, not significant or significant at  $P \leq 0.05$ . Means separated by HSD test at  $P = 0.05$ .

**Table 5.2.** N, S and N/S ratio concentrations on dry mass basis (mg g<sup>-1</sup> DM) in *B. juncea* and *B. rapa*

| PAR          | N form                           | <i>B. juncea</i> |         |        | <i>B. rapa</i> |          |         |
|--------------|----------------------------------|------------------|---------|--------|----------------|----------|---------|
|              |                                  | N                | S       | N/S    | N              | S        | N/S     |
| Low          | NH <sub>4</sub>                  | 77.60 a          | 10.40   | 7.47   | 72.70          | 13.48 a  | 5.41    |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 74.73 ab         | 8.80    | 8.51   | 74.87          | 12.51 ab | 6.01    |
|              | NO <sub>3</sub>                  | 66.90 b          | 8.91    | 7.60   | 63.90          | 10.69 b  | 6.11    |
| Medium       | NH <sub>4</sub>                  | 73.43 a          | 12.44 a | 5.90   | 69.33 a        | 13.33 a  | 5.21 a  |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 67.27 b          | 10.25 b | 6.59   | 60.37 b        | 13.84 a  | 4.37 b  |
|              | NO <sub>3</sub>                  | 59.10 c          | 9.93 b  | 5.97   | 54.53 c        | 11.19 b  | 4.88 ab |
| High         | NH <sub>4</sub>                  | 63.33            | 10.87 a | 5.77 b | 55.80          | 13.10 a  | 4.26 b  |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 67.60            | 8.09 b  | 8.37 a | 61.53          | 10.63 b  | 5.80 a  |
|              | NO <sub>3</sub>                  | 64.53            | 7.81 b  | 8.28 a | 60.13          | 10.00 c  | 6.02 a  |
| N form       | NH <sub>4</sub>                  | 71.12 A          | 11.24 A | 6.38 B | 65.94 A        | 13.30 A  | 4.96 B  |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 69.87 A          | 9.05 B  | 7.82 A | 65.59 A        | 12.33 B  | 5.39 AB |
|              | NO <sub>3</sub>                  | 63.51 B          | 8.88 B  | 7.29 A | 59.52 B        | 10.62 C  | 5.67 A  |
| PAR          | Low                              | 73.08 A          | 9.37 B  | 7.86 A | 70.49 A        | 12.23 A  | 5.84 A  |
|              | Medium                           | 66.60 B          | 10.87 A | 6.15 B | 61.41 B        | 12.79 A  | 4.82 B  |
|              | High                             | 64.82 B          | 8.92 B  | 7.47 A | 59.16 B        | 11.24 B  | 5.36 AB |
| N form       |                                  | *                | *       | *      | *              | *        | *       |
| PAR          |                                  | *                | *       | *      | *              | *        | *       |
| N form x PAR |                                  | *                | ns      | *      | *              | ns       | *       |

ns, \*, not significant or significant at  $P \leq 0.05$ . Means separated by HSD test at  $P = 0.05$ .

**Table 5.3.** Glucosinolates concentration on dry mass basis (mg g<sup>-1</sup> DM) in *B. rapa*

| PAR          | N form                           | Total   | Alkyl  | Alkeny | Indole | Aryl    | Glucora-<br>phanin | Glucos-<br>alyssin | Gluconapin | Glucobrassi-<br>canapin | Glucobrassicin | 4-Hydroxy-<br>glucobrassicin | 4-Methoxy<br>glucobrassicin | Neoglucobrassicin | Glucobrassicin<br>nasturtiin |
|--------------|----------------------------------|---------|--------|--------|--------|---------|--------------------|--------------------|------------|-------------------------|----------------|------------------------------|-----------------------------|-------------------|------------------------------|
| Low          | NH <sub>4</sub>                  | 3.58 a  | 0.68 a | 2.11   | 0.50 a | 0.30    | 0.35 a             | 0.33 a             | 1.63       | 0.58 a                  | 0.27 a         | 0.08                         | 0.08                        | 0.06              | 0.30                         |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 2.65 b  | 0.58 b | 1.43   | 0.35 b | 0.28    | 0.30 b             | 0.28 b             | 1.05       | 0.38 b                  | 0.12 b         | 0.09                         | 0.08                        | 0.06              | 0.28                         |
|              | NO <sub>3</sub>                  | 2.85 ab | 0.57 b | 1.63   | 0.35 b | 0.29    | 0.30 b             | 0.28 b             | 1.28       | 0.35 b                  | 0.12 b         | 0.09                         | 0.08                        | 0.06              | 0.29                         |
| Medium       | NH <sub>4</sub>                  | 4.55 a  | 0.65 a | 3.17 a | 0.42 a | 0.30 b  | 0.32 a             | 0.34 a             | 2.55 a     | 0.63 a                  | 0.18 a         | 0.09                         | 0.09                        | 0.06              | 0.30 b                       |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 3.36 b  | 0.59 b | 2.13 b | 0.37 b | 0.28 b  | 0.30 b             | 0.28 b             | 1.65 b     | 0.48 b                  | 0.13 b         | 0.09                         | 0.09                        | 0.06              | 0.28 b                       |
|              | NO <sub>3</sub>                  | 3.71 b  | 0.58 b | 2.43 b | 0.36 b | 0.34 a  | 0.30 b             | 0.28 b             | 1.97 b     | 0.46 b                  | 0.12 b         | 0.09                         | 0.09                        | 0.06              | 0.34 a                       |
| High         | NH <sub>4</sub>                  | 4.87 a  | 0.60   | 3.56 a | 0.42 a | 0.29 b  | 0.31               | 0.29               | 2.94 a     | 0.62 a                  | 0.16 a         | 0.10 a                       | 0.09                        | 0.08 a            | 0.29 b                       |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 3.11 b  | 0.58   | 1.87 b | 0.38 b | 0.28 b  | 0.30               | 0.28               | 1.47 b     | 0.41 b                  | 0.13 b         | 0.09 b                       | 0.09                        | 0.07 b            | 0.28 b                       |
|              | NO <sub>3</sub>                  | 3.11 b  | 0.58   | 1.82 b | 0.37 b | 0.34 a  | 0.30               | 0.28               | 1.41 b     | 0.42 b                  | 0.13 b         | 0.09 b                       | 0.09                        | 0.07 b            | 0.34 a                       |
| N form       | NH <sub>4</sub>                  | 4.33 A  | 0.64 A | 2.95 A | 0.45 A | 0.30 B  | 0.32 A             | 0.32 A             | 2.37 A     | 0.58 A                  | 0.20 A         | 0.09 A                       | 0.09                        | 0.07 A            | 0.30 B                       |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 3.04 B  | 0.58 B | 1.81 B | 0.37 B | 0.28 C  | 0.30 B             | 0.28 B             | 1.39 B     | 0.42 B                  | 0.13 B         | 0.08 B                       | 0.09                        | 0.07 A            | 0.28 C                       |
|              | NO <sub>3</sub>                  | 3.22 B  | 0.58 B | 1.96 B | 0.36 B | 0.32 A  | 0.30 B             | 0.28 B             | 1.55 B     | 0.41 B                  | 0.12 B         | 0.08 B                       | 0.09                        | 0.06 B            | 0.32 A                       |
| PAR          | Low                              | 3.03 B  | 0.61 A | 1.72 B | 0.40 A | 0.29 B  | 0.31               | 0.30 A             | 1.32 B     | 0.40 B                  | 0.17 A         | 0.08 B                       | 0.08 B                      | 0.06 B            | 0.29 B                       |
|              | Medium                           | 3.87 A  | 0.61 A | 2.58 A | 0.38 B | 0.31 A  | 0.31               | 0.30 A             | 2.06 A     | 0.52 A                  | 0.14 B         | 0.09 A                       | 0.09 A                      | 0.06 B            | 0.31 A                       |
|              | High                             | 3.70 A  | 0.59 B | 2.42 A | 0.39B  | 0.30 AB | 0.30               | 0.28 B             | 1.94 A     | 0.48 A                  | 0.14 B         | 0.09 A                       | 0.09 A                      | 0.07 A            | 0.30 AB                      |
| N form       |                                  | *       | *      | *      | *      | *       | *                  | *                  | *          | *                       | *              | *                            | ns                          | *                 | *                            |
| PAR          |                                  | *       | *      | *      | *      | *       | ns                 | *                  | *          | *                       | *              | *                            | *                           | *                 | *                            |
| N form x PAR |                                  | ns      | *      | ns     | *      | *       | *                  | *                  | ns         | ns                      | *              | ns                           | ns                          | ns                | ns                           |

ns, \*, not significant or significant at  $P \leq 0.05$ . Means separated by HSD test at  $P = 0.05$ .

**Table 5.4.** Glucosinolates concentration on dry mass basis (mg g<sup>-1</sup> DM) in *B. juncea*.

| PAR          | N form                           | Total  | Alkenyl | Indole  | Aryl   | Sinigrin | Gluconapin | Gluco-brassicin | 4-Hydro-xygluco-brassicin | 4-Metho-Xygluco-brassicin | Gluco-nasturtiin |
|--------------|----------------------------------|--------|---------|---------|--------|----------|------------|-----------------|---------------------------|---------------------------|------------------|
| Low          | NH <sub>4</sub>                  | 4.90   | 4.09    | 0.38    | 0.42   | 3.61     | 0.48       | 0.21            | 0.08                      | 0.10                      | 0.42             |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 5.21   | 4.56    | 0.32    | 0.32   | 4.12     | 0.44       | 0.14            | 0.09                      | 0.09                      | 0.32             |
|              | NO <sub>3</sub>                  | 5.26   | 4.62    | 0.32    | 0.32   | 4.17     | 0.44       | 0.14            | 0.09                      | 0.09                      | 0.32             |
| Medium       | NH <sub>4</sub>                  | 8.40   | 7.71    | 0.38 a  | 0.32 b | 7.12     | 0.59       | 0.19 a          | 0.10                      | 0.09                      | 0.32 b           |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 6.63   | 5.99    | 0.34 b  | 0.30 b | 5.49     | 0.50       | 0.15 b          | 0.10                      | 0.10                      | 0.30 b           |
|              | NO <sub>3</sub>                  | 8.59   | 7.84    | 0.36 ab | 0.39 a | 7.27     | 0.57       | 0.16 b          | 0.10                      | 0.10                      | 0.39 a           |
| High         | NH <sub>4</sub>                  | 6.49   | 5.76    | 0.43 a  | 0.31 b | 5.22     | 0.54       | 0.23 a          | 0.10                      | 0.09                      | 0.31 b           |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 5.15   | 4.49    | 0.37 b  | 0.29 b | 4.01     | 0.48       | 0.18 b          | 0.11                      | 0.09                      | 0.29 b           |
|              | NO <sub>3</sub>                  | 5.45   | 4.70    | 0.34 b  | 0.40 a | 4.21     | 0.49       | 0.18 b          | 0.11                      | 0.09                      | 0.40 a           |
| N form       | NH <sub>4</sub>                  | 6.60   | 5.85    | 0.40 A  | 0.35 A | 5.32     | 0.54 A     | 0.21 A          | 0.09                      | 0.09                      | 0.35 A           |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 5.66   | 5.01    | 0.35 B  | 0.30 B | 4.54     | 0.47 B     | 0.16 B          | 0.09                      | 0.09                      | 0.30 B           |
|              | NO <sub>3</sub>                  | 6.43   | 5.72    | 0.34 B  | 0.37 A | 5.22     | 0.50 B     | 0.15 B          | 0.09                      | 0.09                      | 0.37 A           |
| PAR          | Low                              | 5.12 B | 4.42 B  | 0.34    | 0.35   | 3.97 B   | 0.46 B     | 0.16            | 0.08 B                    | 0.09 B                    | 0.35             |
|              | Medium                           | 7.88 A | 7.18 A  | 0.36    | 0.33   | 6.63 A   | 0.55 A     | 0.17            | 0.10 A                    | 0.10 A                    | 0.33             |
|              | High                             | 5.70 B | 4.98 B  | 0.38    | 0.33   | 4.48 B   | 0.50 AB    | 0.19            | 0.11 A                    | 0.09 B                    | 0.33             |
| N form       |                                  | ns     | ns      | *       | *      | ns       | *          | *               | ns                        | ns                        | *                |
| PAR          |                                  | *      | *       | ns      | ns     | *        | *          | ns              | *                         | *                         | ns               |
| N form x PAR |                                  | ns     | ns      | ns      | *      | ns       | ns         | ns              | *                         | ns                        | *                |

ns, \*, not significant or significant at  $P \leq 0.05$ . Means separated by HSD test at  $P = 0.05$ .

**Table 5.5.** Carotenoids and chlorophylls concentration on dry mass basis (mg g<sup>-1</sup> DM) in *B. rapa* and *B. juncea*

| PAR          | N form                           | <i>B. rapa</i>    |         |            |                    |               |               | <i>B. juncea</i>  |         |            |                    |               |               |
|--------------|----------------------------------|-------------------|---------|------------|--------------------|---------------|---------------|-------------------|---------|------------|--------------------|---------------|---------------|
|              |                                  | Total carotenoids | Lutein  | β-catotene | Total chlorophylls | Chlorophyll a | Chlorophyll b | Total carotenoids | Lutein  | β-catotene | Total chlorophylls | Chlorophyll a | Chlorophyll b |
| Low          | NH <sub>4</sub>                  | 1.72              | 1.04    | 0.68       | 14.16              | 11.30         | 2.86 b        | 1.26 b            | 0.75 b  | 0.51 c     | 10.84 b            | 8.50 b        | 2.34 b        |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 1.81              | 1.04    | 0.77       | 17.71              | 13.67         | 4.04 a        | 1.75 a            | 0.93 a  | 0.82 a     | 17.38 a            | 13.41 a       | 3.97 a        |
|              | NO <sub>3</sub>                  | 1.77              | 1.01    | 0.76       | 17.31              | 13.28         | 4.02 a        | 1.55 a            | 0.84 ab | 0.71 b     | 15.68 a            | 12.00 a       | 3.68 a        |
| Medium       | NH <sub>4</sub>                  | 2.02 a            | 1.11 a  | 0.91 a     | 17.15              | 13.61 a       | 3.54          | 2.11 a            | 1.06 a  | 1.05 a     | 18.99 a            | 15.16 a       | 3.83 a        |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 1.81 ab           | 1.01 b  | 0.80 ab    | 16.55              | 12.70 ab      | 3.85          | 1.92 a            | 1.01 a  | 0.91 b     | 18.49 a            | 14.31 a       | 4.18 a        |
|              | NO <sub>3</sub>                  | 1.55 b            | 0.89 ab | 0.66 b     | 14.08              | 10.78 b       | 3.30          | 1.26 b            | 0.68 b  | 0.58 c     | 11.97 b            | 9.25 b        | 2.72 b        |
| High         | NH <sub>4</sub>                  | 1.89              | 1.03 a  | 0.85 a     | 16.19 a            | 12.71 a       | 3.48 a        | 1.52 a            | 0.78 a  | 0.74 a     | 13.87 a            | 10.78 a       | 3.09          |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 1.56              | 0.87 b  | 0.69 b     | 14.20 b            | 10.86 b       | 3.34 ab       | 1.31 ab           | 0.69 ab | 0.62 ab    | 12.06 ab           | 9.26 ab       | 2.80          |
|              | NO <sub>3</sub>                  | 1.44              | 0.81 b  | 0.63 b     | 12.85 c            | 9.79 c        | 3.06 b        | 1.16 b            | 0.64 b  | 0.52 b     | 10.43 b            | 7.98 b        | 2.45          |
| N form       | NH <sub>4</sub>                  | 1.87 A            | 1.06 A  | 0.81 A     | 15.84              | 12.54 A       | 3.30 B        | 1.63 A            | 0.86 A  | 0.76 A     | 14.56 B            | 11.48 B       | 3.09 B        |
|              | NH <sub>4</sub> +NO <sub>3</sub> | 1.72 AB           | 0.97 B  | 0.75 AB    | 16.15              | 12.41 A       | 3.74 A        | 1.66 A            | 0.88 A  | 0.79 A     | 15.97 A            | 12.32 A       | 3.65 A        |
|              | NO <sub>3</sub>                  | 1.58 B            | 0.90 B  | 0.68 B     | 14.74              | 11.28 B       | 3.46 AB       | 1.32 B            | 0.72 B  | 0.60 B     | 12.70 C            | 9.74 C        | 2.95 B        |
| PAR          | Low                              | 1.76 AB           | 1.03 A  | 0.74       | 16.39 A            | 12.75 A       | 3.64 A        | 1.52 B            | 0.84 B  | 0.68 B     | 14.63 B            | 11.30 B       | 3.33 B        |
|              | Medium                           | 1.79 A            | 1.01 A  | 0.79       | 15.93 A            | 12.36 A       | 3.57 AB       | 1.76 A            | 0.91 A  | 0.85 A     | 16.48 A            | 12.91 A       | 3.58 A        |
|              | High                             | 1.62 B            | 0.90 B  | 0.72       | 14.41 B            | 11.12 B       | 3.29 B        | 1.33 C            | 0.70 C  | 0.63 C     | 12.12 C            | 9.34 C        | 2.78 C        |
| N form       |                                  | *                 | *       | *          | ns                 | *             | *             | *                 | *       | *          | *                  | *             | *             |
| PAR          |                                  | *                 | *       | ns         | *                  | *             | *             | *                 | *       | *          | *                  | *             | *             |
| N form x PAR |                                  | *                 | ns      | *          | *                  | *             | *             | *                 | *       | *          | *                  | *             | *             |

ns, \*, not significant or significant at  $P \leq 0.05$ . Means separated by HSD test at  $P = 0.05$ .

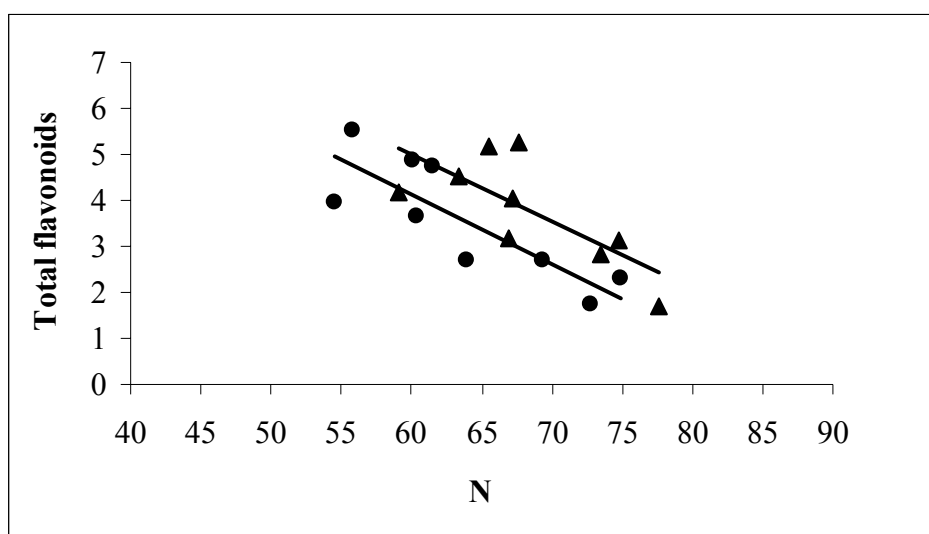


**Figure 5.1.** Relationship between total flavonoids concentration (mg g<sup>-1</sup> DM) and N concentration (mg g<sup>-1</sup> DM) in *B. juncea* (▲) and *B. rapa* (●). Total flavonoid in *B. juncea* =  $13.70 - 0.14N$  ( $R^2 = 0.53^*$ ;  $n=9$ ). Total flavonoid in *B. rapa* =  $13.20 - 0.15N$  ( $R^2 = 0.70^*$ ;  $n=9$ ). \* indicates significance at  $P \leq 0.05$ .

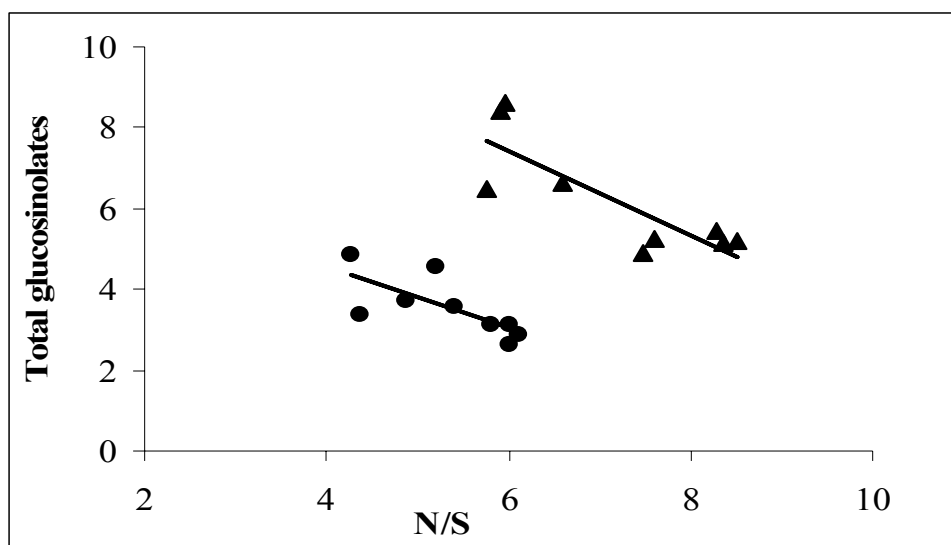


**Figure 5.2.** Relationship between total glucosinolates concentration ( $\text{mg g}^{-1}$  DM) and N/S in *B. juncea* (▲) and *B. rapa* (●)  
 Total glucosinolates in *B. juncea* =  $13.72 - 1.05\text{N/S}$  ( $R^2 = 0.69^*$ ;  $n=9$ ).  
 Total glucosinolates in *B. rapa* =  $7.60 - 0.76\text{N/S}$  ( $R^2 = 0.52^*$ ;  $n=9$ ).  
 \* indicates significance at  $P \leq 0.05$ .

## CHAPTER 6

### Conclusion

To summarize, we can conclude that during the summer season the yield and growth of baby lettuce were increased due to the better climate conditions (mainly radiation and temperature). However, the overall quality of baby lettuce in the summer season was decreased (lower content of carbohydrates and higher nitrate content). Optimal nutrient solution concentrations were determined for the spring and summer season to improve leaf mineral content but with a slight reduction of some nutritional parameters. The effects of macronutrient proportions on marketable fresh yield were not evident; the macronutrient proportions influenced only the mineral content of leafy lettuce.

In *Brassica* experiments, the results showed that a mixture of ammonium and nitrate in the nutrient solution can be used without reducing the marketable yield. However, the content of nitrate in leafy tissues with mixture of ammonium and nitrate did not decrease. *Brassica juncea* would seem more tolerant to ammonium nutrition than *Brassica rapa* at high PAR level.

Ammonium nutrition had a positive effect on some quality characteristics by reducing the nitrate content of leaves and increasing glucosinolate concentration; however, a negative influence on flavonoid concentration was observed with high ammonium concentration in the nutrient solution. Flavonoid concentration increased with higher radiation levels. Interactive effects of N form and PAR level on carotenoid and chlorophyll concentration were found between found in both *Brassica* species.

For a practical point of view, the results obtained in the present thesis can help the growers in the crop management of these leafy vegetables; they can help the consumer in the knowledge of the overall quality of leafy vegetables in different growing seasons; and they are a good basement for other researches. This can be helpful to understand some unknown mechanisms that lead to the biosynthesis of phytochemical compounds in these leafy vegetables.

Actually, the fact that GMOs are not accepted by part of society makes the agronomic techniques a good method for improving the yields and/or the quality aspects of leafy vegetables. In this sense, management of the nutrient solution and environmental factors, together with a perfect knowledge of the crop responses to these factors, could be a good approach to improve the product quality.

## CHAPTER 7

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