The Impact of Ammonium to Nitrare Ratio on the Growth and Nutritional Status of Kale

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Abstract

The effect of four NH₄NO₃ ratios in the nutrient solution (0:100; 25:75; 50:50 and 75:25) on growth and nutrient concentrations of four kale (Brassica oleraceae L. var. acephala) hybrids: 'CN KAL 1029', 'Redbor', 'Winnetou', 'Reflex' and one indigenous cultivar: 'Ntopia Mytilinis' was investigated. In the first four weeks of cultivation none of the NH₄/NO₃ ratios applied induced adverse effects on most growth characteristics determined whereas plants grown with 75% NH₄-N accumulated higher leaf N, P, K, Fe, Mn, Zn and Cu concentrations. After an eight week period, plants under 75% NH₄-N showed significant reductions in many growth parameters suggesting a probable preference of kale plants grown for a prolonged period towards either a complete exclusion of NH₄ from the nutrient solution or a 25:75 or a 50:50 NH₄/NO₃ ratio. Among the genotypes tested, 'Ntopia Mytilinis' produced the greatest yield with the lowest leaf nitrate accumulation.

Keywords: Brassica; N form; nitrates; NO₃:NH₄ ratio; nutrient elements; yield

Introduction

Agriculture is changing in ways that closely link food production to human health and nutritional requirements (Emongor et al., 2004; Assimakopoulou 2006). Moreover, plant foods provide most of the nutrients that feed the developing world. Brassicaceous plants represent one of the major vegetable crops grown worldwide, comprising a dietetic crop that has long been the object of many investigations. Kale (Brassica oleraceae L. var. acephala) is the one of the oldest forms of the cabbage family, originating in the Eastern Mediterranean, having been used as a food crop as early as 2000 BC, whereas Theophrastus described a savoyed form of kale in 350 BC (Balkaya and Yanmaz, 2005). In the last few years, kale has spread quickly in Greece once again, showing increasing economic potential because of its short biological cycle, spicy taste and nutritive value, commonly used as a salad for fresh consumption or as soup in different ways. It comprises a whole food as it can provide significant quantities of daily essential minerals and prebiotic carbohydrates. Relevant studies (Ayaz et al., 2006, 2008; Sikora and Bodzianczyk, 2012) contributed to the knowledge of the nutritional properties and pro-health potential of the plant, as well as of the antioxidant and antibacterial activities of phenolic fractions isolated from the leaves and seeds (Lefsrud et al., 2007; Kopsell et al., 2013). Lotti et al. (2018) studied the diversity of kale in Apulia, Southern Italy, because of its great popularity, especially in the US, as a “superfood” due to its health benefits. Although Thavarajah et al. (2016) reported that kale nutritional quality could be further enhanced to benefit North American consumers; Šamec et al. (2018) supported the aspect that kale can be considered as a superfood, but the same as another cruciferous.

With regard to plant inorganic nutrition and hence plant growth, exceptionally nitrogen plays a pivotal role. However, the application of high concentrations of N not only contaminates the environment, but also causes nitrate accumulation in the leaves of vegetable crops reducing their quality. Given that ammonium and nitrate are the two major N sources taken up by the roots of higher plants, the N form may have significant effect on both the growth and chemical composition of plants, including vegetables (Therios and Sakellariadis, 1988; Abu-Rayyan et al., 2004; Wang and Li, 2004). The effect of these two forms on plant growth is dependent not only on the plant species, but also on their ratios and concentrations (Marschner, 1997). Specifically, the form of N supply, to a great extent, controls the uptake ratio of cations and anions and thus, influences dry matter production and root rhizosphere and apoplastic pH (Mengel et al., 1994; Marschner, 1997). However, few vegetables actually perform well when NH₄-N is provided as the only N source (Santamaria and Elia, 1997); in such a
case, plants may develop some symptoms of ammonia toxicity (Findenegg, 1987; Guo et al., 2002), suggesting that normal growth needs NO₃-N nutrition as well (Smiciklas and Below, 1992a, 1992b; Rideout et al., 1994; Palaniwamy et al., 2002). The appropriate fertilization is one of the most practical and effective ways of controlling and improving the yield and nutritional quality of crops for human consumption. In crops whose commercial yields are the leaves, such as lettuce, spinach, endive, cabbage etc., a great number of studies have been done on the influence of N fertilization (rate and form) on yield, nitrate accumulation and ion composition (Santamaria and Elia, 1997; Wang and Tadashi, 1997; Simonne et al., 2001; Wang and Li, 2004; Gorenjak and Cencič, 2013). Although considerable information is available on the effects of various ratios of NH₄NO₃ on growth for different plants, there is limited or no information on kale. Therefore, the objective of our experiment was to assess the impact of NH₄NO₃ ratio on kale growth, nutrient element and leaf nitrate accumulation.

Materials and Methods

Plant culture and nutrient determinations

On February 1st, 2016, seeds of the four hybrids ‘CN KAL 1029 F1’, ‘Redbor F1’, ‘Winnetou F1’ and ‘Reflex F1’ and the indigenous cultivar ‘Ntopia Mytilinis’ (‘Ntopia’ of kale (Brassica oleracea L. var. acephala) were germinated and grown in sand culture for one month by receiving half strength nutrient solution for macronutrients and full strength for micronutrients (Hoagland and Arnon, 1938). Afterwards, the more uniform seedlings were transplanted to individual 4 L plastic pots (one seedling per pot), filled with medium grade silica sand and perlite (1:1 v/v) and placed in a glasshouse without supplementary heating and lighting at the Technological Educational Institute of Peloponnese (longitude 37.062° E; latitude 22.062° N). The pots were arranged in a completely randomized block factorial design (five genotypes x four N ratios) with 6 replicates. Four treatments (Tr0, Tr25, Tr50 and Tr75) were applied to the plants, with the same total N content (10 mmol L⁻¹), but with the following percent molar nitrate nitrogen (NO₃-N) to ammonium nitrogen (NH₄-N) ratios in the nutrient solutions: Tr0: 0% NH₄-N+100% NO₃-N, Tr25: 25% NH₄-N+75% NO₃-N, Tr50: 50%NH₄-N+50% NO₃-N, Tr75: 75%NH₄-N+25% NO₃-N. In the present study, the effect of an all-NH₄ treatment (100% NH₄ ratio+0% NO₃) was not examined, because of expected poor growth or no growth under the specific treatment and the possibility of missing data while analyzing the growth and nutrient status characteristics. The composition of the macronutrients (mM) of the nutrient solutions applied were in the case of Tr0: Ca(NO₃)₂:4H₂O 2.0, NaNO₃ 2.0, KNO₃ 4.0, MgSO₄.7H₂O 1.0, K₂HPO₄ 1.0; Tr25: KCl 3.0, Ca(NO₃)₂:4H₂O 2.0, KNO₃ 1.0, NH₄NO₃ 2.5, MgSO₄.7H₂O 1.0, K₂HPO₄ 1.0; Tr50: KCl 5.0, (NH₄)₂SO₄ 1.0, Ca(NO₃)₂:4H₂O 2.0, KNO₃ 1.0, (NH₄)PO₄ 3H₂O 1.0, MgCl₂ 1.0; Tr75: KCl 6.0, (NH₄)₂SO₄ 1.0, NH₄NO₃ 2.5, (NH₄)PO₄ 3H₂O 1.0, CaCl₂ 2.0, MgCl₂ 1.0. Micronutrients applied in all treatments were the same according to the aforementioned Hoagland and Arnon (1938) solution.

On March 23rd, the four aforementioned nutrient solutions were applied to the plants. The pH of every nutrient solution was monitored at 1-2 day intervals and maintained within the range 6.0-6.5 by the addition of 0.5 mol L⁻¹ HCl or NaOH when needed. The relevant electrical conductivity (EC) of the solutions ranged from 1.8 to 2.2 dS cm⁻². Each plant was irrigated three times daily with 0.08 L of the appropriate nutrient solution. The mean temperature from 21-31/3/2016 in the glasshouse recorded was 17.4 °C, from 1-30/4/2016 21.1 °C and from 1-20/5/2016 20.7 °C.

The first 60 plants were harvested four weeks after the beginning of the treatments (harvest 1) when kale leaves had reached the marketable size whereas the other 60 plants were harvested 4 weeks later than the first harvest and eight weeks from the beginning of the treatments (harvest 2). At each harvest, the chlorophyll content of younger fully mature leaves was recorded by using a chlorophyll-meter (SPAD-502, Tokyo, Minolta, Japan). Every plant was separated into the upper plant part (stem and leaves) and the root. The root was washed carefully, three times with deionised water and the fresh weight (FW) of the stem, leaves and root, the stem length as well as the number of the leaves per plant were recorded. Then, the aforementioned plant material was dried to constant weight in a forced draught air oven at 80 °C and the relevant dry weights (DW) were recorded as well. Then, the dry plant material was either wet-ashed (Kjeldahl method) or dry-ashed in a furnace at 500 °C. The concentrations of N were determined by using the indophenol-blue method in the wet digest, P by the molybdenum-blue method and K, Ca, Mg, Fe, Mn, Zn, Cu by using a Varian A220 atomic absorption spectrometer, in the dry digest (Allen, 1989). The nitrate content of the leaves was determined by the colorimetric determination of nitrate in plant tissues by nitration of salicylic acid (Cataldo et al., 1975).

Statistics

Data were subjected to analysis of variance (ANOVA). Where a significant F-test was observed, significant differences in mean values between the treatments were evaluated by the ANOVA, Least Significant Difference (LSD) test at P<0.05.

Results

Ammonium toxicity symptoms

Although plants, especially under the treatments Tr50 and Tr75, were grown with a high quantity of NH₄-N in the nutrient solution, no visual symptoms of ammonium toxicity were observed in the aboveground plant part till the end of the experiment. However, a number of lateral roots of plants grown under Tr75 were brown and dead at the end of the experiment. Contrary to our results, Simonne et al. (1993) reported symptoms of NH₄ toxicity (reduced growth and curly leaves with dark-green areas surrounding yellow spots) in the leaves of turnip plants (Brassica rapa L.) grown in sand culture when NH₄ was the dominant nitrogen form.
Plant growth

Data concerning the main effects of cultivar/hybrid and N form and their interaction on several plant growth parameters are shown in the Tables 1 and 2 (1st and 2nd harvest respectively) whereas some leaf and stem characteristics are shown in Table 3 (harvests 1, 2).

At the 1st harvest, the main effects of the nitrogen form and ratio in the nutrient solution on several plant growth parameters like plant leaves, upper plant part and total plant FWs, were their non-significant differentiation despite the high presence of NH$_4$-N in the nutrient solution with 75% NH$_4$-N (Table 1). At the 2nd harvest, two months from the beginning of the treatments and one month after the 1st harvest, plants under Tr25 and Tr50 compared to plants under Tr0 did not present any significant differentiation of the aforementioned growth parameters whereas plants grown with 75% NH$_4$-N did. The latter ones had significantly (sign.) decreased leaves, stem, upper plant part and total plant FWs compared to plants grown under the other three ammonium nitrate ratios (Table 2). However, at both harvests, by increasing the NH$_4$-N concentration in the nutrient solution, the root FW presented the trend to increase; i.e. at harvest 1, plants under Tr75 presented significantly greater root FW compared to plants under Tr0 (Tables 1, 2). The RT/SHT ratios increased by increasing the NH$_4$-N concentration in the nutrient solution, as well; at harvest 1, plants under Tr50 showed significantly higher RT/SHT ratio compared to plants under Tr0 whereas at harvest 2, plants under Tr75 showed significantly higher RT/SHT ratio compared to the other three treatments. With regard to the upper plant part, root and total plant DWs, there were no significantly differences among plants grown under Tr0, Tr25 and Tr50, at both harvests; only plants grown with 75% NH$_4$-N showed the aforementioned parameters significantly decreased. The same trend was observed in the case of leaves DW (Table 2). Leaf water content, at harvest 1, was found to be significantly higher in plants under Tr75 compared to that of Tr0, Tr25 and Tr50 but not significantly differentiated among the four treatments at harvest 2. The plant leaf number and the leaf chlorophyll content (as expressed in SPAD units) did not differ among the four treatments, at both harvests (Table 3).

Contrary to the results of the main effects of nitrogen form on plant growth parameters determined, the main effect of the cultivar/hybrid showed significantly differentiations in most cases. The results of the plant leaf biomass, which comprises the edible plant part, showed that the indigenous cultivar ‘Ntopia’ presented the significantly highest one, the hybrid ‘CN KAL 1029’ intermediate values (31.1% lower than ‘Ntopia’) whereas the hybrid ‘Redbor’ the lowest ones (41.9% lower than ‘Ntopia’). ‘Ntopia’ presented the highest upper plant part and whole plant FW whereas the hybrid ‘Redbor’ the lowest ones (Table 1). Similar trends were observed at harvest 2 (Table 2). Although the root FW of ‘Ntopia’ followed by ‘Winnetou’ was significantly greater compared to the root of the other hybrids at harvest 1, the RT/SHT ratio of the cultivar presented lower values, significantly lower compared to the relevant ratio of ‘Winnetou’ (Table 1). With regard to leaf water content (LWC) at harvest 1, ‘Ntopia’ showed the significantly highest LWC, ‘CN KAL 1029’ and ‘Redbor’ intermediate, whereas ‘Winnetou’ and ‘Reflex’ showed the lowest ones (Table 3). At harvest 2, the root FW, RT/SHT ratio and LWC were not significantly differentiated among the five genotypes tested (Table 2, 3). The main effect of the cultivar/hybrid on plant leaf number was that ‘Ntopia’ showed the lowest one, ‘Reflex’, ‘CN KAL 1029’ and ‘Redbor’ intermediate and ‘Winnetou’ the greatest one (Table 3); at this point, it must be reported that the leaves of ‘Ntopia’ are much wider compared to the four hybrids. The leaf chlorophyll content of ‘Ntopia’ was found to be the greatest one at both harvests while that of ‘Reflex’ the lowest one at the end of the experiment (Table 3). The relevant growth parameter results expressed on DW basis were similar to FW trends, at both harvests. Specifically, ‘Ntopia’ followed by ‘Winnetou’ and ‘Reflex’ showed significantly highest total plant, upper plant part and root DWs compared to ‘CN KAL 1029’ and ‘Redbor’ (Tables 1,2). The stem length of ‘CN KAL 1029’ followed by ‘Ntopia’ was significantly lower compared to the other three hybrids; similar results were taken in the case of the stem FW (Tables 1, 2, 3). The interaction between hybrid/cultivar and N form was not found to be significant in any growth parameter determined, at any harvest (Tables 1, 2).

Elemental concentration

Data concerning the main effects of cultivar/hybrid and N form and their interaction on leaf and root nutrient element concentrations are shown in Tables 4,5,6 and 7 whereas the leaf nitrate content is in Table 8 and Fig 1.

Leaf Nitrogen-Phosphorus-Potassium-Calcium

Magnesium concentrations

Regarding the leaf N concentration at both harvests, the main effect of N form in the nutrient solution was that plants grown either with 0, 25 or 50% NH$_4$-N in the nutrient solution presented similar N level; however, plants grown with 75% NH$_4$-N showed the sign. highest N concentration. Leaf P concentration increased gradually by increasing NH$_4$-N in the nutrient solution at both harvests, whereas leaf K did not vary among plants under Tr0, Tr25 and Tr50, but increased under Tr75. On the contrary, leaf Mg decreased gradually by increasing NH$_4$-N in the nutrient solution, at both harvests. Leaf Ca increased by increasing NH$_4$-N in the nutrient solution till 50% but decreased by increasing NH$_4$-N in the nutrient solution at 75%, at both harvests (Tables 4, 5).

The main effects of the cultivar/hybrid on leaf nutrient concentrations were significant in most cases. At harvest 1, leaf N was higher in ‘Reflex’ and lower in ‘Ntopia’ whereas at harvest 2, it remained higher in ‘Reflex’ but lowered in ‘CN KAL 1029’ and ‘Winnetou’ whereas ‘Ntopia’ and ‘Redbor’ presented intermediate N values. Leaf P at harvest 1, was sign. higher in ‘CN KAL 1029’ and ‘Redbor’ compared to ‘Reflex’ and ‘Ntopia’ whereas ‘Winnetou’ presented intermediate values; at harvest 2, ‘Reflex’ presented the highest P. At both harvests, ‘CN KAL 1029’ presented the highest leaf K, Ca and Mg whereas ‘Reflex’ and ‘Ntopia’ presented the lowest ones (Tables 4, 5). The interaction between hybrid/cultivar and N form was found to be significant only in the case of K and Ca concentrations at harvest 1 and in the case of N, P, K, Mg, at harvest 2 (Tables 4, 5).
Table 1. Growth parameters of four hybrids 'CN KAL 1029', 'Redbor', 'Winnetou' and 'Reflex' and one indigenous cultivar 'Ntopia Mytilinis' of kale plants grown with different N forms and rates in the nutrient solution, at harvest 1.

| Harvest 1 | Cultivar | Leaf FW | Stem FW | Shoot FW | Root FW | Rate FW (g) | Root DW | Plant DW |
|-----------|----------|---------|---------|----------|---------|------------|---------|---------|
| CN KAL 1029 | 343.8 | 194.4 | 872.1 | 180.4 | 443.2 | 0.23 | 55.4 | 2.4 |
| REDBOR | 209.6 | 44.1 | 355.7 | 78.3 | 412.1 | 0.24 | 35.1 | 7.5 |
| WINNETOU | 335.4 | 45.4 | 308.7 | 101.8 | 482.0 | 0.27 | 43.7 | 23.9 |
| REFLEX | 324.5 | 40.8 | 365.2 | 85.8 | 451.0 | 0.24 | 43.3 | 9.3 |
| NTOPIA | 498.4 | 38.0 | 536.4 | 105.9 | 640.3 | 0.20 | 47.7 | 10.7 |

N form (% NH4-N)

|       | 0% NH4-N | 50% NH4-N | 75% NH4-N | 0% NH4-N | 50% NH4-N | 75% NH4-N |
|-------|----------|------------|------------|----------|------------|------------|
| CN KAL 1029 | 343.8 | 194.4 | 872.1 | 180.4 | 443.2 | 0.23 | 55.4 | 2.4 |
| REDBOR | 209.6 | 44.1 | 355.7 | 78.3 | 412.1 | 0.24 | 35.1 | 7.5 |
| WINNETOU | 335.4 | 45.4 | 308.7 | 101.8 | 482.0 | 0.27 | 43.7 | 23.9 |
| REFLEX | 324.5 | 40.8 | 365.2 | 85.8 | 451.0 | 0.24 | 43.3 | 9.3 |
| NTOPIA | 498.4 | 38.0 | 536.4 | 105.9 | 640.3 | 0.20 | 47.7 | 10.7 |

Means within the same column and for the same cultivar followed by the same letter do not differ significantly based on LSD at P<0.05.

Table 2. Growth parameters of four hybrids 'CN KAL 1029', 'Redbor', 'Winnetou' and 'Reflex' and one indigenous cultivar 'Ntopia Mytilinis' of kale plants grown with different N forms and rates in the nutrient solution, at harvest 2.

| Harvest 2 | Cultivar | Leaf FW | Stem FW | Shoot FW | Root FW | Rate FW (g) | Root DW | Plant DW |
|-----------|----------|---------|---------|----------|---------|------------|---------|---------|
| CN KAL 1029 | 301.8 | 176.0 | 634.1 | 145.5 | 417.5 | 0.17 | 42.7 | 9.3 |
| REDBOR | 192.1 | 86.6 | 285.0 | 67.3 | 251.7 | 0.13 | 39.6 | 8.1 |
| WINNETOU | 321.3 | 183.9 | 690.1 | 151.3 | 455.1 | 0.19 | 44.9 | 9.5 |
| REFLEX | 290.5 | 170.0 | 620.0 | 140.0 | 420.0 | 0.16 | 40.0 | 8.7 |
| NTOPIA | 437.2 | 243.2 | 777.4 | 180.4 | 537.4 | 0.26 | 48.7 | 10.4 |

N form (% NH4-N)

|       | 0% NH4-N | 50% NH4-N | 75% NH4-N | 0% NH4-N | 50% NH4-N | 75% NH4-N |
|-------|----------|------------|------------|----------|------------|------------|
| CN KAL 1029 | 301.8 | 176.0 | 634.1 | 145.5 | 417.5 | 0.17 | 42.7 | 9.3 |
| REDBOR | 192.1 | 86.6 | 285.0 | 67.3 | 251.7 | 0.13 | 39.6 | 8.1 |
| WINNETOU | 321.3 | 183.9 | 690.1 | 151.3 | 455.1 | 0.19 | 44.9 | 9.5 |
| REFLEX | 290.5 | 170.0 | 620.0 | 140.0 | 420.0 | 0.16 | 40.0 | 8.7 |
| NTOPIA | 437.2 | 243.2 | 777.4 | 180.4 | 537.4 | 0.26 | 48.7 | 10.4 |

Means within the same column and for the same cultivar followed by the same letter do not differ significantly based on LSD at P<0.05.
Table 3. Leaf characteristics of four hybrids ‘CN KAL 1029’, ‘Redbor’, ‘Winnetou’ and ‘Reflex’ and one indigenous cultivar ‘Ntopia Mytilinis’ of kale plants grown with different N forms and rates in the nutrient solution, at harvests 1 and 2.

| Harvest | Cultivar | Leaf Water Content (%) | Leaf Water Content (%) | Plant Leaf Content (%) | Plant Leaf Content (%) | Harvest 1 Chlorophyll Content (g FAD/m²) | Harvest 1 Chlorophyll Content (g FAD/m²) | Harvest 2 Leaf Length (cm) | Harvest 2 Leaf Length (cm) |
|---------|----------|------------------------|------------------------|------------------------|------------------------|------------------------------------------|------------------------------------------|--------------------------|--------------------------|
| 0% NH₄ | CN KAL 1029 | 85.4 | b | 89.2 | a | 77 | h | 752 | e | 45 | a | 52 | ab | 58.3 | b | 166 | a |
| 5% NH₄ | REDBOR | 97.0 | a | 95.2 | b | 74 | b | 519 | c | 45 | a | 52 | ab | 58.3 | b | 166 | a |
| 10% NH₄ | WINNETOU | 85.4 | a | 85.3 | a | 89 | c | 584 | d | 45 | a | 52 | ab | 58.3 | b | 166 | a |
| 15% NH₄ | REFLEX | 85.2 | a | 95.3 | a | 81 | b | 549 | c | 46 | a | 52 | ab | 58.3 | b | 166 | a |
| 20% NH₄ | NYTOP | 97.1 | c | 96.6 | a | 89 | a | 252 | a | 51 | b | 15 | a | 20 | a | 20 | a |

Means within the same column and for the same cultivar followed by the same letter do not differ significantly based on LSD at P<0.05.

Table 4. Leaf nutrient element concentrations of four hybrids ‘CN KAL 1029’, ‘Redbor’, ‘Winnetou’ and ‘Reflex’ and one indigenous cultivar ‘Ntopia Mytilinis’ of kale plants grown with different N forms and rates in the nutrient solution, at harvest 1.

| Harvest | Cultivar | N | P | K | Ca | Mg | B | Cu | Mn |
|---------|----------|---|---|---|---|---|---|---|---|
| 0% NH₄ | CN KAL 1029 | 54.6 | ab | 6.3 | a | 0.9 | a | 15.8 | a | 0.9 | a |
| 5% NH₄ | REDBOR | 56.3 | c | 6.4 | b | 1.1 | b | 12.1 | c | 1.5 | c |
| 10% NH₄ | WINNETOU | 55.9 | b | 5.8 | b | 0.9 | c | 10.8 | c | 0.2 | a |
| 15% NH₄ | REFLEX | 64.5 | c | 5.2 | a | 3.0 | a | 10.2 | b | 3.0 | a |
| 20% NH₄ | NYTOP | 56.9 | a | 4.7 | a | 3.0 | a | 12.6 | c | 4.3 | b |
| 0% NH₄ | CN KAL 1029 | 55.2 | a | 5.4 | a | 6.8 | a | 11.6 | c | 0.9 | a |
| 5% NH₄ | REDBOR | 55.6 | a | 5.4 | a | 6.8 | a | 11.6 | c | 0.9 | a |
| 10% NH₄ | WINNETOU | 58.5 | b | 3.3 | b | 73.0 | b | 15.7 | b | 3.7 | b |
| 15% NH₄ | REFLEX | 56.5 | a | 4.2 | a | 6.4 | a | 7.8 | a | 0.9 | a |
| 20% NH₄ | NYTOP | 54.2 | a | 5.8 | a | 6.6 | a | 15.5 | c | 4.6 | b |
| 0% NH₄ | CN KAL 1029 | 60.3 | b | 8.9 | b | 55.9 | b | 11.6 | b | 3.1 | a |
| 5% NH₄ | REDBOR | 57.9 | a | 6.3 | a | 8.1 | a | 11.6 | c | 0.9 | a |
| 10% NH₄ | WINNETOU | 55.2 | b | 5.9 | a | 6.8 | a | 11.6 | c | 0.9 | a |
| 15% NH₄ | REFLEX | 61.7 | a | 5.7 | a | 6.8 | a | 11.6 | c | 0.9 | a |
| 20% NH₄ | NYTOP | 54.9 | a | 6.6 | a | 6.6 | a | 15.2 | b | 4.1 | a |

Means within the same column and for the same cultivar followed by the same letter do not differ significantly based on LSD at P<0.05.
### Table 5. Nutrient element concentrations of four hybrids ‘CN KAL 1029’, ‘Redbor’, ‘Winnetou’ and ‘Reeflex’ and one indigenous cultivar ‘Ntopia Mytillus’ of kale plants grown with different N forms and rates in the nutrient solution, at harvest 2

| Cultivar | N form ( % NH4) | 0% | 25% | 50% | 75% | Harvest 1 | Harvest 2 |
|----------|-----------------|-----|-----|-----|-----|-----------|-----------|
| WINNETOU | 0%              | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |
|          | 25%             | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |
|          | 50%             | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |
|          | 75%             | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |
| REFLEX   | 0%              | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |
|          | 25%             | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |
|          | 50%             | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |
|          | 75%             | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |
| NTOPIA   | 0%              | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |
|          | 25%             | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |
|          | 50%             | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |
|          | 75%             | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |

Means within the same column and for the same cultivar followed by the same letter do not differ significantly based on LSD at P<0.05.

### Table 6. Root nutrient element concentrations of four hybrids ‘CN KAL 1029’, ‘Redbor’, ‘Winnetou’ and ‘Reeflex’ and one indigenous cultivar ‘Ntopia Mytillus’ of kale plants grown with different N forms and rates in the nutrient solution, at harvest 1

| Cultivar | N form ( % NH4) | 0% | 25% | 50% | 75% | Harvest 1 | Harvest 2 |
|----------|-----------------|-----|-----|-----|-----|-----------|-----------|
| WINNETOU | 0%              | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |
|          | 25%             | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |
|          | 50%             | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |
|          | 75%             | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |
| REFLEX   | 0%              | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |
|          | 25%             | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |
|          | 50%             | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |
|          | 75%             | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |
| NTOPIA   | 0%              | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |
|          | 25%             | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |
|          | 50%             | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |
|          | 75%             | 3.8 | a   | 3.6 | a   | 28.4      | 30.3      |

Means within the same column and for the same cultivar followed by the same letter do not differ significantly based on LSD at P<0.05.
Table 7. Root nutrient element concentrations of four hybrids 'CN KAL 1029', 'Redbor', 'Winnetou' and 'Reflex' and one indigenous cultivar 'Ntopia Mytilinis' of kale plants grown with different N forms and rates in the nutrient solution, at harvest 1 and harvest 2

| Cultivar | P | K | Ca | Mg | Fe | Mn | Zn | Cu | B |
|----------|---|---|----|----|----|----|----|----|----|
| CN KAL 1029 | 46.4 | 35.4 | 108.4 | 1.5 | 20.6 | 31 | 17 | 4.7 | 34.5 |
| REDBOR | 81 | b | 6.5 | c | 13.7 | c | 1.7 | b | 24.1 | a |
| 50% NH4-N | 6.2 | a | 5.5 | b | 5.1 | c | 1.4 | a | 19.1 | a |
| 75% NH4-N | 7.1 | ab | 4.5 | ab | 10.3 | bc | 1.9 | b | 21.4 | ab |
| Ntopia | 81.6 | c | 4.1 | a | 13.6 | c | 1.6 | b | 24.1 | a |
| 0% NH4-N | 59.9 | a | 5.7 | a | 14.1 | d | 2.1 | d | 25.9 | b |
| 25% NH4-N | 70 | b | 5.9 | a | 11.4 | c | 1.7 | c | 26.9 | c |
| 50% NH4-N | 7.5 | k | 5.5 | b | 6.7 | h | 1.4 | h | 19.4 | a |
| 75% NH4-N | 81 | c | 6.1 | b | 4.1 | a | 1.3 | a | 22.6 | ab |
| Ntopia | 70.1 | c | 5.9 | a | 11.7 | c | 1.7 | c | 26.9 | c |
| CN KAL 1029 | 46.4 | 35.4 | 108.4 | 1.5 | 20.6 | 31 | 17 | 4.7 | 34.5 |
| REDBOR | 81 | b | 6.5 | c | 13.7 | c | 1.7 | b | 24.1 | a |
| 50% NH4-N | 6.2 | a | 5.5 | b | 5.1 | c | 1.4 | a | 19.1 | a |
| 75% NH4-N | 7.1 | ab | 4.5 | ab | 10.3 | bc | 1.9 | b | 21.4 | ab |
| Ntopia | 81.6 | c | 4.1 | a | 13.6 | c | 1.6 | b | 24.1 | a |
| 0% NH4-N | 59.9 | a | 5.7 | a | 14.1 | d | 2.1 | d | 25.9 | b |
| 25% NH4-N | 70 | b | 5.9 | a | 11.4 | c | 1.7 | c | 26.9 | c |
| 50% NH4-N | 7.5 | k | 5.5 | b | 6.7 | h | 1.4 | h | 19.4 | a |
| 75% NH4-N | 81 | c | 6.1 | b | 4.1 | a | 1.3 | a | 22.6 | ab |
| Ntopia | 70.1 | c | 5.9 | a | 11.7 | c | 1.7 | c | 26.9 | c |

Means within the same column and for the same cultivar followed by the same letter do not differ significantly based on LSD at P<0.05.

Table 8. Leaf nitrate concentration of four hybrids 'CN KAL 1029', 'Redbor', 'Winnetou' and 'Reflex' and one indigenous cultivar 'Ntopia Mytilinis' of kale plants grown with different N forms and rates in the nutrient solution, at harvest 1 and harvest 2

| Cultivar | Harvest 1 | Harvest 2 |
|----------|-----------|-----------|
|          | Nitrate ppm dw leaf | Nitrate ppm dw leaf |
| CN KAL 1029 | 260.9 | c | 354.9 | b |
| REDBOR | 245.9 | c | 324.4 | b |
| WINNETOU | 173.3 | a | 435.0 | c |
| REFLEX | 218.2 | bc | 353.8 | b |
| Ntopia | 190.9 | ab | 149.8 | a |
| N form (% NH4) |          |          |          |          |
| 0% NH4-N | 338.2 | c | 462.2 | d |
| 25% NH4-N | 272.3 | b | 377.6 | c |
| 50% NH4-N | 144.8 | a | 312.4 | b |
| 75% NH4-N | 118.9 | a | 157.4 | a |

Means within the same column and for the same cultivar followed by the same letter do not differ significantly based on LSD at P<0.05.
Among the four N treatments whereas at harvest 2, it increased by increasing NH$_4$-N in the nutrient solution (Tables 4,5). Regardless of the N form, the main effect of cultivar/hybrid on leaf Fe was that the concentration of the element at harvest 1 did not significantly vary among the five genotypes (Table 4); at harvest 2, 'Ntopia' and 'Redbor' followed by 'CN KAL 1029' presented the highest Fe level whereas 'Winnetou' presented the lowest one (Table 6). At harvest 1, 'CN KAL 1029', 'Redbor' and 'Winnetou' presented higher leaf Mn, Zn and Cu compared to 'Reflex' and 'Ntopia', and 'Winnetou' showed the lowest leaf boron concentration (Table 4). At harvest 2, 'CN KAL 1029' presented lower Mn level, 'Winnetou' higher Zn whereas 'CN KAL 1029' and 'Redbor' higher leaf Cu and B concentrations (Table 5). The interaction between hybrid/cultivar and N form was found to be significant in the cases of leaf Mn, Cu and B concentrations at harvest 1 and in the cases of Mn and Zn, at harvest 2 (Tables 4, 5).

**Root Nitrogen-Phosphorus-Potassium-Calcium-Magnesium concentrations**

At both harvests, the main effect of N form on root P and K concentrations was that they increased gradually by increasing NH$_4$-N in the nutrient solution with the majority of the differences being significant; the only exception was the root P of plants under Tr75 which was found to be lower compared to the relevant one of plants grown under Tr50, at harvest 1. On the contrary, root Ca and Mg decreased by increasing NH$_4$-N in the nutrient solution at both harvests, with the majority of the differences being significant, as well (Tables 6, 7). Regardless of the N form, the main effect of cultivar/hybrid on root P and Ca at harvest 1 was their non-significant differentiation; 'Redbor' presented the highest K and 'CN KAL 1029' high Mg level (Table 6). At harvest 2, root P, K and Ca concentrations were higher in 'Redbor' whereas 'Ntopia', and root Mg highest in 'CN KAL 1029' and 'Redbor' (Table 7). The interaction between hybrid/cultivar and N form was found to be significant only in the case of root Ca and Mg concentrations, at harvest 2.

**Leaf Iron, Manganese, Zinc, Copper and Boron concentrations**

According to ANOVA results, the main effect of N form was that leaf Fe, Mn, Zn and Cu concentrations of kale plants increased by increasing NH$_4$-N in the nutrient solution, at both harvests. In the case of Fe at harvest 1, the leaf Fe concentrations of plants grown under Tr25, Tr50 and Tr75 were found to be significantly greater compared to plants grown under Tr0, without being significantly differentiated among them (Table 4); however, at harvest 2, the concentration of the element did not significantly differ among the four N treatments (Table 5). Leaf Mn and Zn concentrations of plants grown under Tr75 were also found to be elevated compared to the three other treatments Tr0, Tr25 and Tr50, at harvest 1; however, at harvest 2, elevated leaf Mn and Zn concentrations were noticed not only in plants grown under Tr75 but also under Tr50 compared to plants grown under Tr0 and Tr25 (Table 4). Boron concentration at harvest 1, was not significantly differentiated among the four N treatments whereas at harvest 2, it increased by increasing NH$_4$-N in the nutrient solution (Tables 4,5). Regardless of the N form, the main effect of cultivar/hybrid on leaf Fe was that the concentration of the element at harvest 1 did not significantly vary among the five genotypes (Table 4); at harvest 2, 'Ntopia' and 'Redbor' followed by 'CN KAL 1029' presented the highest Fe level whereas 'Winnetou' presented the lowest one (Table 6). At harvest 1, 'CN KAL 1029', 'Redbor' and 'Winnetou' presented higher leaf Mn, Zn and Cu compared to 'Reflex' and 'Ntopia', and 'Winnetou' showed the lowest leaf boron concentration (Table 4). At harvest 2, 'CN KAL 1029' presented lower Mn level, 'Winnetou' higher Zn whereas 'CN KAL 1029' and 'Redbor' higher leaf Cu and B concentrations (Table 5). The interaction between hybrid/cultivar and N form was found to be significant in the cases of leaf Mn, Cu and B concentrations at harvest 1 and in the cases of Mn and Zn, at harvest 2 (Tables 4, 5).
whereas root Cu concentration was not significantly differentiated among the five genotypes. ‘Ntopia’, at harvest 1, presented low root Fe, Mn, Zn and Cu but high B (Tables 6, 7). The interaction between hybrid/cultivar × N form was found to be significant only in the case of root B concentration, at harvest 2 (Table 7).

**Leaf nitrate concentration**

Regardless of the genotype, the main effect of N form on leaf nitrate concentration was that by increasing NH₄-N in the nutrient solution, the leaf nitrate concentration decreased gradually, at both harvests; all the relevant differences were significant, except the lower but non-sign. differentiated nitrate content of plants under Tr75 compared to plants under Tr 50, at harvest 1 (Table 8). Regardless of the N form, the main effect of genotype on leaf nitrate concentration was that ‘CN KAL 1029’ and ‘Redbor’ at harvest 1, as well as ‘Winnetou’ at harvest 2, showed the highest leaf nitrate contents whereas ‘Ntopia’ at both harvests presented the relevant lowest concentrations. It should be mentioned that four weeks after the beginning of the treatments (harvest 1) ‘Winnetou’ presented the lowest leaf nitrate concentration but eight weeks later the same hybrid presented the highest nitrate content (Table 8). Given that the Fpr of the interaction cultivar/hybrid and N form for leaf nitrate concentration at harvest 2 was found to be statistically significant (P<0.05), it should be noticed that when ‘Ntopia’ was grown with 25% NH₄-N+75% NO₃-N in the nutrient solution, the leaf nitrate concentration decreased by 45% compared to plants grown with 100% NO₃-N; when the same cultivar was grown with 50% NH₄-N, the relevant nitrate concentration decreased by 54% whereas when it was grown with 75% NH₄-N, the nitrate concentration of the cultivar decreased by 73%. The relevant decreases in leaf nitrate concentration of ‘Redbor’ were found to be 3%, -3% and 42%, respectively, whereas those of the other three hybrids presented intermediate decrease percentages (Fig. 1).

**Discussion**

The nutrient concentrations of kale leaves determined in the present work were found to be higher compared to those reported by the National Nutrient Database for Standard Reference Legacy Release for kale (Agricultural Research Service, United States Department of Agriculture 2018). Among the macronutrients determined, the concentration of N followed by that of K were the highest ones whereas the most abundant micronutrients were Fe and B followed by Mn and Zn (Tables 4, 5). Ayaş et al. (2006) analyzing kale leaves harvested from six different fields in Trabzon (Turkey) determined nutrient levels similar to ours, whereas Grace et al. (2000) reported deficient in Mn and Zn kale crops in New Zealand.

Our results indicated that when plants had reached their marketable size (four weeks from the beginning of the treatments-1st harvest), the presence of 25%, 50% and 75% ammonium to nitrate N in the nutrient solution did not affect leaf biomass produced (which comprises the edible plant part), upper plant part and total plant FWs of kale. The only traits that were affected were the root biomass which increased and the stem FW that decreased by growing plants under 75% NH₄-N in the nutrient solution (Table 1). Although the fresh matter of plant leaves, upper plant part and total plant were not affected up to 75% ammonium nitrogen at harvest 1, the relevant dry matter decreased; it may be related to the significant increase of leaf water content under 75% ammonium N (Table 3). At the 1st harvest, the Root/Shoot ratio increased by increasing ammonium up to 50% but decreased by the further ammonium increase. The imbalance between shoot and root growth indicated that partitioning in the plants is affected by the ammonium concentration even when the other growth parameters remained stable. The aforementioned results showed that kale leaves for at least a four week culture can tolerate even 75% NH₄-N in the nutrient solution without showing any adverse leaf growth effects. Given that, the complete exclusion of NH₄ from the nutrient solution (plants under 100% NO₃-N) did not differentiate the growth of kale plants, it could be assumed that during this period there is no preference of the plant toward the N form and rate up to 75% NH₄-N+25% NO₃-N ratio in the hydroponic solution.

With regard to nutrient concentrations, leaf N and K did not vary up to 50% NH₄-N but increased in plants under 75% NH₄-N. Leaf P increased gradually from 0% to 75% NH₄-N while leaf Mg decreased gradually. Leaf Ca increased by increasing NH₄-N up to 50% NH₄-N but decreased under 75% NH₄-N. Consequently, kale leaves harvested up to the first four weeks accumulate higher quantities of N, P and K when grown with 75% NH₄-N compared to plants grown with 0, 25 and 50% NH₄-N; the same trend was observed in the case of leaf Fe, Mn, Zn and Cu contents whereas B was not differentiated because of the ammonium to nitrate ratio (Table 4). The results of the 2nd harvest indicated that after eight weeks from the beginning of the treatments and four from the 1st harvest, kale plants grown with 75% NH₄-N compared to plants with 0, 25 and 50% NH₄-N showed sign. reductions in most of the growth parameters determined (plant leaves, shoot, total plant FWs and DWs, stem length) (Tables 2, 3), as well as symptoms of ammonium toxicity in the root system. In particular, there was an approximately 25% reduction in the leaf fresh and dry matter of plants grown with 75% NH₄-N compared to plants grown with either 0%, 25% or 50% NH₄-N, suggesting a probable preference of kale plants grown for a prolonged period towards either a complete exclusion of NH₄-N from the nutrient solution or an ammonium to nitrate N ratio up to 50%. The opposing effect on the growth of kale plants fed with 75% NH₄-N might be due to the unavailability of NO₃ as a N source and the higher demand for carbohydrates channelled for NH₄ assimilation and detoxification (Bevers and Hageman, 1969; Tabatabaei et al., 2006). The present work showed that the ammonium threshold that kale plants can tolerate for normal growth for a prolonged period of cultivation is the presence of 50% NH₄-N. Similar findings were found by Zhang et al. (2007) for cabbage grown with five NH₄-N/NO₃-N ratios in the nutrient solution (1:0; 0.75:0.25; 0.5:0.5; 0.25:0.75; and 0:1); their results showed that cabbage growth was reduced much more (by 87%) when the proportion of NH₄-N in the nutrient solution was more than 75% compared with the ratio of 0.5:0.5, 35 days after the transplanting, suggesting a possible toxicity due to the
accumulation of a large amount of free ammonia in the leaves.

An imbalance in Root/Shoot ratio occurred at harvest 2 as well, as it increased by increasing the ammonium concentration in the nutrient solution at 75% of the total nitrogen whereas the relevant ratios of plants grown under 0%, 25% and 50% NH$_4$-N remained non-differentiated (Table 2). It has repeatedly been reported that an increased Root/Shoot ratio has physiological advantages because it may not only increase the quantity of nutrient element absorbing sites with a resultant higher source-sink ratio in the plant, but also renders lower the total requirement by decreasing the proportion of shoot, in which most of the nutrient element of the plant is utilized. Similar results were taken by Wei et al. (1994) and Hajboland et al. (2003) in recent experiments with susceptible and resistant to Fe-deficiency subclovers and rice cultivars, respectively, as the Root/Shoot ratio of those plants increased with increasing chlorosis resistance. However, despite the prolonged imposition of plants under 75% NH$_4$-N there was no significant differentiation in the plant leaf number, the leaf water content and chlorophyll content (Table 3).

Leaf nutrient concentrations at harvest 2 presented similar trends compared to the relevant ones at harvest 1; leaf N and K did not vary up to 50% NH$_4$-N but increased in plants under 75% NH$_4$-N; the N and K increase of plants grown under 75% NH$_4$-N could be due to the greater accumulation of the elements in the smaller leaf biomass of those plants (Table 2). Leaf P increased gradually from Tr0 to Tr75; the leaf P gradual increase by increasing NH$_4$-N in the nutrient solution could be due to the acidification of the rhizosphere because of the excretion of H$^+$ from plant roots fed with NH$_4$-N. Leaf Ca increased gradually by increasing NH$_4$-N up to Tr 50 but decreased under Tr75. The ratio 50% NH$_4$-N/50% NO$_3$-N not only did not cause adverse effects in kale plants growth but also increased many leaf nutrient concentrations; Zhang et al. (2007) results were similar to ours as they reported higher leaf P, K, Ca and Mg concentrations in cabbage plants grown under 50% NH$_4$-N/50% NO$_3$-N. An appropriate NH$_4$/NO$_3$ ratio improves the absorption of many nutrients and maintains a suitable proportion of N assimilation and storage that should benefit the plant growth and quality of kale as a vegetable. Adversely to Zhang et al. (2007) results, our work showed that leaf Mg decreased gradually by increasing NH$_4$-N in the nutrient solution. Reduced Ca and Mg concentrations in kale plants grown with high ammonium N in the nutrient solution may have been due to the reduced uptake of the elements as an antagonism effect to the high presence of the cation NH$_4^+$ in the nutrient solution; our results are in accordance with the findings of Pill and Lambeth (1977), Allan (1989), Mills and Jones (1996), Fageria (2001), Kotsiras et al. (2002) and Tabatabaei et al. (2006).

Leaf Mn, Zn, Cu and B contents increased by increasing NH$_4$-N in the nutrient solution whereas Fe did not vary. The high pH in the rhizosphere owing to NO$_3$-N supply reduced the shoot P concentration and markedly suppressed the Mn, Zn and Cu contents (Tables 4, 5); similar results were obtained by Savvas et al. (2003). The well-known effect of high rhizosphere pH on the uptake of P, Fe, Mn, Zn, Cu has also been referred to by Marschner (1997). P, K, Mn, Zn and Cu results similar to ours were obtained by Simonne et al. (1993) when working with turnip plants (Brassica rapa L.) grown in sand culture under five NH$_4$-N/NO$_3$-N ratios (1:0, 3:1, 1:1, 1:3, 0:1).

Taking into consideration that brassicas are consumed in part for their nutritional values of K, Ca, Mg, Fe and Zn, Kopsell et al. (2004) have reported an average of a twofold difference in elemental accumulation among cultivars and selections measured by them. The present work showed a significant variability among the five genotypes tested for their leaf macro- and micronutrients contents, however these differences were lower than double; i.e. the leaf K, Ca and Mg concentrations of the hybrid ‘CN KAL 1029’ were higher by 33%, 35% and 27%, respectively, compared to ‘Reflex’ hybrid whereas the relevant micronutrient concentration differences were even lower (Table 4). Moreover, Kopsell et al. (2004) had reported that among the 22 kale and collard cultivars and selections tested, the hybrid with the highest leaf elemental accumulation was ‘Redbor’. In our experiment ‘Redbor’ presented intermediate nutrient contents compared to the other genotypes included. The information on genotypic variability for elemental accumulation may be important for producers and consumers looking to select kale cultivars with higher nutritional levels of beneficial dietary elements.

Regarding leaf nitrate accumulation, leafy vegetables including kale, accumulate fairly large nitrate quantities. The data given in literature concerning nitrate content in kale range from 300 to 1283 mg kg$^{-1}$ fresh matter (Chweya, 1988; Kim and Yoon, 2003) whereas our results showed that the nitrate content of kale leaves ranged from 700 to 6000 mg kg$^{-1}$ fresh matter. Moreover, our study showed that the leaf nitrate content of kale plants decreased by increasing ammonium N in the nutrient solution. Zhang et al. (2007) reported lower leaf nitrate in cabbage plants grown with 50% NH$_4$-N/50% NO$_3$-N, as well. Our investigation showed also that when ‘Ntopia’ was grown with 25%, 50% and 75% NH$_4$-N in the nutrient solution, the leaf nitrate concentration decreased by 45%, 54% and 73% respectively, as compared to plants fed with 100% NO$_3$-N; the relevant decreases of ’Redbor’ were 3%, -3% and 42% and those of the other three hybrids presented intermediate values (Fig. 1). Despite the aforementioned large decreases of leaf nitrate of the indigenous cultivar ‘Ntopia’ when growing with increasing ammonium nitrogen in the nutrient solution, the growth of the same cultivar was not sign. affected even when the concentration of NH$_4$-N was up to 50%.

Comparing the level of total nitrogen and nitrites not only in relation to the cultivar/hybrid but also to the time of harvest, we observed that leaf nitrate concentration between harvest 1 and harvest 2 (28 days interval) increased. In particular, ‘Winnetou’ increased nitrate concentration by 51%, ‘Reflex’ by 62%, ‘CN KAL 1029’ by 36% and ‘Redbor’ by 32% whereas the indigenous cultivar ‘Ntopia’ decreased its leaf nitrate content by 22% (Table 8). Adversely, the results of the comparison of leaf total nitrogen concentration between harvest 1 and harvest 2 showed that leaf N decreased. The correlation coefficient between leaf nitrites and leaf total nitrogen was found to be significantly negative (r=-0.36). Korus and Lisienska (2009) study with kale plants grown in the field, showed an opposite trend; they reported that kale plants obtained at the 2nd harvest (14 weeks after the planting of the seedlings in the field) contained 9% more total nitrogen and 67%
fewer nitrates compared to the 1st harvest (10 weeks after the planting of the seedlings in the field). A similar to leaf total N decrease from harvest 1 to harvest 2 was also shown in the cases of leaf K, Ca and Mg. Among the genotypes tested, 'Winnetou' and 'CN KAL' presented the greatest N decreases from harvest 1 to harvest 2 whereas leaf P of 'Reflex' and 'Ntopia' increased. Leaf Fe, Cu and B concentrations at harvest 2 compared to those at harvest 1 decreased as well, whereas leaf Mn and Zn increased. Root Fe level was not differentiated between the two harvests whereas root Mn, Zn and Cu increased and root B decreased. The higher nutrient and the lower nitrate contents of kale leaves at the first harvest that took place four weeks after the treatments comparing to the eight week harvest, is interesting from a nutritional point of view, because it is well known that fruit and vegetables usually contribute about 35 and 24% respectively to the total K and Mg dietary intake of humans (Levander, 1990).

Conclusions

The effect of four different ammonium to nitrate nitrogen ratios (0:100, 25:75, 50:50 and 75:25 NH₄/NO₃) in the nutrient solution on growth parameters, nutrient element and leaf nitrates accumulation of four kale hybrids ‘CN KAL 1029 F1’, ‘Redbor F1’, ‘Winnetou F1’, ‘Reflex F1’ and one indigenous Greek cultivar ‘Ntopia Mytilinis’ was that during the first four weeks of cultivation none of the four NH₄/NO₃ ratios applied adversely affected plant growth. Moreover, kale plants grown with 75% NH₄- N+25%NO₃- accumulated higher N, P, K, Fe, Mn, Zn and Cu quantities and lower nitrates in their foliage. However, kale plants grown for longer than one month period could not tolerate 75% NH₄- N in the nutrient solution without adverse effects on plant development, suggesting their probable preference towards a complete exclusion of ammonium from the nutrient solution or to 25:75 and 50:50 NH₄/NO₃ ratios. Among the genotypes tested, 'Ntopia Mytilinis' produced the greatest yield with the lowest leaf nitrate accumulation as compared to the four hybrids tested whereas the hybrid 'CN KAL 1029 F1' presented the highest leaf K, Ca, Mg concentrations. These results indicate that producers wishing to maximize yield and elemental uptake as well as minimize leaf nitrate content of kale plants need to consider the ratio of NH₄/NO₃ in their fertilizer programs in combination to the cultivar/hybrid used.

References

Allen SE (1989). Chemical Analysis of Ecological Materials. Oxford (UK), Blackwell Scientific Publications.

Assimakopoulou A (2006). Effect of iron supply and nitrogen form on growth, nutritional status and ferric reducing activity of spinach in nutrient solution culture. Scientia Horticulturae 110(1):21-29.

Ayaz FA, Glew RH, Million M, Huang HS, Chuang LT, Sarz G, Hayrughlo-Ayaz S (2006). Nutrient contents of kale (Brassica oleracea L. var. acephala DC). Food Chemistry 96(4):572-579.

Ayaz FA, Hayrughlo-Ayaz S, Alpay-Kanaoglu S, Gruj J, Valokova K, Ulrichova J, Srdm M (2008). Phenolic acid contents of kale (Brassica oleracea L. var. acephala DC) extracts and their antioxidant and antibacterial activities. Food Chemistry 107(1):19-25.

Balayka A, Yannou Z (2005). Promising kale (Brassica oleracea var. acephala) populations from Black Sea region, Turkey. New Zealand Journal of Crop and Horticultural Science 33(1):1-7.

Beevers L, Hageman RH (1969). Nitrate reduction in higher plants. Plant Physiology 20(1):495-522.

Cataldo DA, Maroon M, Schrader LE, Youngs VL (1975). Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Communications in Soil Science and Plant Analysis 6(1):71-80.

Chweya JA (1988). Contents of nitrate-N and thiocyanate ions in kale (Brassica oleracea var. acephala DC) leaves from kale growing areas in Kenya. Acta Horticulturae 218:181-190.

Espenbergs V, Pule-Muekenberg F, Phole O (2004). Effect of promalin on growth and development of kale (Brassica oleracea L. var. acephala). Journal of Agronomy 3(3):208-214.

Fageria ND, Alago PB (2001). Nutrient interactions in crop plants. Journal of Plant Nutrition 24(8):1269-1290.

Finkenegg GR (1987). A comparative study of ammonium toxicity at different constant pH of the nutrient solution. Plant and Soil 103(2):239-243.

Grace ND, Craighead M, Warr B (2000). The macro- and micro-element content of weeds and kales in Southland, New Zealand, and the effect of nitrogen fertilizer application on their Co, Se, and Cu concentrations. New Zealand Journal of Agricultural Research 43(4):533-540.

Gorenjak AH, Cencić A (2013). Nitrate in vegetables and their impact on human health. A review. Acta Alimentaria 42(2):158-172.

Guo S, Berck H, Sattelmacher B (2002). Effects of supplied nitrogen form on growth and water uptake of French bean (Phaseolus vulgaris L.) plants. Plant and Soil 239(2):267-275.

Hajiboland R, Xang XE, Römheld V (2003). Effects of bicarbonate and high pH on growth of Zn-efficient and Zn-inefficient genotypes of rice, wheat and rye. Plant and Soil 250(2):349-357.

Hoagland DR, Arnon DI (1938). The water culture method for growing plants without soil. Circular. California Agricultural Experiment Station, 347(2nd edn).

Hu J, Ye J, Liao W, Zhang G, Xie J, LV J, ... Bu R (2015). Moderate ammonium-nitrate alleviates low light intensity stress in mini Chinese cabbage seedling by regulating root architecture and photosynthesis. Scientia Horticulturae 186:143-153.

Kim BY, Yoon S (2003). Analysis of nitrate contents of Korean common foods. Journal of the Korean Society for Food Science and Nutrition 32(6):77984.
Kim SJ, Kawaharada C, Ishii G (2006). Effect of ammonium: nitrate nutrient ratio on nitrate and glucosinolate contents of hydroponically-grown rocket salad (Eruca sativa Mill). Soil Science and Plant Nutrition 52(3):387-393.

Kopsell DE, Kopsell DA, LeRued MG, Curran-Celentano J (2004). Variability in elemental accumulations among Leafy Brassica oleracea cultivars and selections. Journal of Plant Nutrition 27(10):1813-1826.

Kopsell DE, Dean A, Kopsell Carl F, Sams, Barickman T, Casey (2013). Ratio of calcium to magnesium influences biomass, elemental accumulations, and pigment concentrations in kale. Journal of Plant Nutrition 36(14):2154-2165.

Korus A, Lisievska Z (2009). Effect of cultivar and harvest date of kale (Brassica oleracea L. var. acephala) on content of nitrogen compounds. Polish Journal of Environmental Studies 18(2):235-241.

Kotsiras A, Olympios CM, Drosopoulou J, Passam HC (2002). Effects of nitrogen form and concentration on the distribution of ions within cucumber fruits. Scientia Horticulurea 95:175-183.

LeRued M, Kopsell D, Wenzel A, Sheehan J (2007). Changes in kale (Brassica oleracea L. var. acephala) carotenoid and chlorophyll pigment concentrations during leaf ontogeny. Scientia Horticulurea 112:136-141.

LeVander OA (1990). Fruit and vegetable contributions to dietary mineral intake in human health and disease. HortScience 25(12):1486-1488.

Lotti C, Iovieno P, Centomani I, Marcotrigiano AR, Fanelli V, Mimiola G, ... Ricciardi L (2018). Genetic, bio-agronomic, and nutritional characterization of kale (Brassica Oleracea L. var. acephala) diversity in Apulia, Southern Italy. Diversity 10(2):25.

Lu Ying Lin, Yang Chun Xu, Qi Rong Shen, Cai Xia Dong (2009). Effects of different nitrogen forms on the growth and cytokinin content in xylem sap of tomato (Lycopersicon esculentum Mill) seedlings. Plant and Soil 315(1-2):67-77.

Manschner H (1997). Mineral nutrition of higher plants. London (UK): Academic Press.

Mengel K, Planker R, Hoffman B (1994). Relationship between leaf apoplast pH and Fe chlorosis of sunflowers (Helianthus annuus L.). Journal of Plant Nutrition 17(6):1053-1064.

Mills HA, Jones Jr JB (1996). Plant analysis handbook II. Athens (GA): MicroMacro Publishing. Chapter, Essential micronutrients pp 8-35.

Palaniswamy UR, Bernard BB, McAvoy RJ (2002). Trends in new crops and new uses. Alexandria: ASHS Press. Chapter. Effect of nitrate: ammonium nitrogen ratio in hydroponics on the oxalate levels of Purslane pp 453-455.

Pill WG, Lambeth VN (1977). Effects of NH$_4$ and NO$_3$ nutrition with and without pH adjustment on tomato growth, ion composition, and water relationships. Journal of the American Society for Horticultural Science 100:78-81.

Rideout JW, Chailleau S, Rape CD, Morot-Gaudry JF (1994). Ammonium and nitrate uptake by soybean during recovery from nitrogen deprivation. Journal of Experimental Botany 45(1):23-33.

Šomec D, Uličić B, Šalopk-Sordić B (2018). Kale (Brassica oleracea var. acephala) as a superfood: Review of the scientific evidence behind the statement. Critical Reviews in Food Science and Nutrition https://doi.org/10.1080/10408398.2018.1454400.

Santamaria P, Elia A (1997). Producing nitrate free endive heads: Effect of nitrogen form on growth, yield and ion composition of endive. Journal of the American Society for Horticultural Science 122(1):140-145.

Santamaria P, Elia A, Papa G, Serio F (1998). Nitrate and ammonium nutrition in chicory and rocket salad plants. Journal of Plant Nutrition 21(9):1779-1789.

Savvas D, Karagianni V, Kotsiras A, Demopoulos V, Karkamis I, Palou P (2003). Interactions between ammonium and pH of the nutrient solution supplied to gerbera (Gerbera jamesonii) grown in punice. Plant and Soil 254(2):393-402.

Silkova E, Bodziarczyk I (2012). Composition and antioxidant activity of kale (Brassica oleracea L. var. acephala) raw and cooked. Acta Scientarium Polonorum Technologia Alimentaria 11(3):239-248.

Simonne EH, Smirile DA, Mills HA (1993). Turnip growth, leaf yield, and leaf nutrient composition responses to nitrogen forms. Journal of Plant Nutrition 16(12):2341-2351.

Simonne F, Simonne A, Wells L (2001). Nitrogen source affects crunchiness, but not lettuce yield. Journal of Plant Nutrition 24(4-5):743-751.

Smidklaas KD, Below FE (1992a). Role of nitrogen form in determining yield of field-grown maize. Crop Science 32(5):1220-1225.

Smidklaas KD, Below FE (1992b). Role of cytokinin in enhanced productivity of maize supplied with NH$_4^+$ and NO$_3^-$. Plant and Soil 142(2):307-313.

Tabatabai SJ, Fatemi LS, Fallahi E (2006). Effect of ammonium:nitrate ratio on yield, calcium concentration, and photosynthesis rate in strawberry. Journal of Plant Nutrition 29(7):1273-1285.

Tabatabai SJ, Yusefi M, Hajiloo J (2008). Effects of shading and NO$_3$-NH$_4$ ratio on the yield, quality and N metabolism in strawberry. Scientia Horticulurea 116:264-272.

Thavarajah D, Thavarajah P, Abuare A, Basnagala S, Lacher C, Smith P, Combs Jr GF (2016). Mineral micronutrient and prebiotic carbohydrate profiles of USA-grown kale (Brassica oleracea L. var. acephala), Journal of Food Composition and Analysis 52:9-15.

Therios IN, Sakellariadis SD (1988). Effects of nitrogen form on growth and mineral composition of olive plants (Olea europaea L.). Scientia Horticulurea 35:167-177.

Thavarajah D, Thavarajah P, Abuare A, Basnagala S, Lacher C, Smith P, Combs GF (2016). Mineral micronutrient and prebiotic carbohydrate profiles of USA-grown kale (Brassica oleracea L. var. acephala), Journal of Food Compositions and Analysis 52:9-15.

Wang XF, Tadashi I (1997). Effect of NO$_3$-N in the additional nutrient solution on the growth, yield, and NO$_3$ content in spinach plant grown in hydroponics. Journal of the Japanese Society for Horticultural Sciences 66(2):313-319.

Wang Z, Li S (2004). Effects of nitrogen and phosphorus fertilization on plant growth and nitrate accumulation in vegetables. Journal of Plant Nutrition 27(3):539-556.

Wei LC, Ocumpaugh WR, Loepert RH (1994). Plant growth and nutrient uptake characteristics of Fe deficiency chlorosis susceptible and resistant subclones. Plant and Soil 165(2):235-240.

Zhang FC, Kang SZ, Li FS, Zhang JH (2007). Growth and major nutrient concentrations in Brassica campestris supplied with different NH$_4^+$/NO$_3^-$ ratios. Journal of Integrative Plant Biology 49(4):455-462.