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Effect of nitrogen concentration and growth regulators on growth and nitrate content of lettuce

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Abstract

Lettuce plants (*Lactuca sativa* L. cv. 'Paris Island') were grown in an unheated plastic greenhouse to determine the effects of solution nitrogen concentration and growth regulators (gibberellin and kinetin) on growth (fresh and dry head weight) and tissue nitrate content (TNC). The plants were grown in plastic containers with perlite and supplied with a basic nutrient solution supplemented with nitrogen (N) corresponding to 50, 100, 150, and 200 ppm NO_3-N. Growth regulators; gibberellin (GA_3) and kinetin were applied at different doses independently and in combination. Fresh, dry weight and TNC were responsive to N application level. However, fresh and dry weights were similar at 150 and 200 ppm and TNC at 100 and 150 ppm nitrogen supply. Gibberellin (GA_3) and gibberellin and kinetin in combination (GA_3+kinetin) enhanced fresh and dry weight and TNC compared to the control. There were few differences in response to application rates. Therefore, where lettuce plants are grown in similar conditions and low NO_3 accumulation is desirable, together with high yield and good size, the best N application level is 150 ppm NO_3-N and growth regulators application may enhance yield. However their use in reducing the nitrate content is not recommended.

Key words: *Lactuca sativa*, nitrogen, gibberellin, kinetin, growth, TNC

Introduction

The presence of nitrates in vegetables (Peck *et al.*, 1971), as in water and generally in other food products is a serious threat to man health (WHO, 1985). Not so much due to its toxicity, which is low, but for giving rise to the dangerous compounds in the organism (*e.g.* methaemoglobin, nitrosamines) (Van Der Boon *et al.*, 1986, 1990; Boink and Speijers, 2001).Vegetables constitute the major source of nitrate providing 72-74% of the average daily human dietary intakes (Santamaria, 1997). Generally leafy vegetables tend to accumulate nitrates and the European Commission has issued the Commission Regulation (EC) No. 563/2002 setting maximum acceptable nitrate concentration in lettuce and spinach. For winter grown lettuce the value is 4500 mg kg^{-1} fresh weight and for summer-grown lettuce 2500 mg kg^{-1} fresh weight.

To find ways to reduce nitrate accumulation in lettuce, many experiments were conducted by controlling some factors like the nitrogen form and concentration, light intensity, CO_2 concentration, genetic variation and cultivation techniques. (Blom-Zandstra and Lampe, 1985; Reinink and Groenwold, 1987; Reinink *et al.*, 1987; Kaiser and Brendle-Behnisch, 1991; Economakis, 1992; Jaervau, 1994, Pasda *et al.*, 2001; Premuzic *et al.*, 2002, 2003).

There are reports focusing on nitrate content in lettuce and other leafy vegetables known as nitrate accumulators by growth regulators (Rozek *et al.*, 1989; Rozek and Wojciechowska, 1990; Myczkowski *et al.*, 1990, 1991; Sady *et al.*, 1995). Encouraged by these observations, EU recommendations to lower nitrate content of vegetables and the growing interest in soilless culture, our research was focused on the influence of solution nitrogen concentration and two plant growth regulators (gibberellin and kinetin) on growth and tissue nitrate content (TNC) in lettuce grown in soilless culture under eastern Mediterranean climatic conditions (Cyprus).

Materials and methods

Plant material and culture: The experiment was conducted at the Agricultural Research Institute of Cyprus (32°24′ E, 35°09′ N) in a plastic greenhouse under natural daylight conditions and temperature. Lettuce seedlings at four true leaves stage (cv. 'Paris Island', a romaine type) were transplanted into plastic containers filled with 2 kg of horticultural perlite. The N treatments corresponded to nutrient solutions nitrogen concentration of 50, 100, 150, and 200 ppm NO_3-N and electrical conductivity values 1.5, 2.0, 2.6 and 2.8 mS cm^{-1}. It does appear that although lettuce plants can be grown over a wide range of solution conductivities, EC levels within 2.0-3.0 mS cm^{-1} give more satisfactory results (Economakis, 1991). The pH of the nutrient solutions was adjusted around 6.5 (Bres, 1992). Treatments were arranged in a randomized complete block design. Each nitrogen treatment corresponded to a block with 32 plants and replicated three times. Each block consisted of four rows with four plastic containers in each row and two plants per container. Within every block the growth regulators (GA_3, kinetin, GA_3+kinetin) were applied.

In-line drip irrigation, providing 2 L h^{-1}, was placed at the top of the containers. Small holes were cut on the bottom of the plastic containers to allow excess nutrient solution to flow out and the plastic containers were laid on a catchment plate to gather the excess nutrient solution. The nutrient solution applied and the number of trickling turns per day was adjusted depending on the amount of over drained solution. The plants were fertigated with a basic nutrient solution (Table 1) supplemented with the nitrogen concentration corresponding to the treatment.

Table 1. Nutrient solution basic composition

Nutrient	Concentration (mg L^{-1})
P	30
K	150
Mg	25
Ca	50
Hoagland solution*	50 mL m^{-3}

*Micro-nutrients were supplied from the micro-nutrient Hoagland stock solution ($MnSO_4.H_2O$=34 g L^{-1}, $CuSO_4.5H_2O$=1 g L^{-1}, $ZnSO_4.7H_2O$=2.2 g L^{-1}, $(NH_4)_6Mo_7O_{24}.4H_2O$=1 g L^{-1}).

The air temperature and relative humidity (R.H.) inside the greenhouse are given in Table 2. The greenhouse was ventilated when the temperature exceeded 25°C. Global solar radiation fluctuated from 19.3 to 20.4 MJ m^{-2} and sunshine duration from 7.9 to 9.8 h day^{-1} over the whole experiment. There were no disease problems during the growth cycle. The plants were sprayed once, at an early stage of growth, with the systematic insecticide Mospilan (Acetamiprid) to prevent any pest problems. On the 29th day the plants were sprayed with 40 mL per plant solution of gibberellic acid (GA_3) or kinetin (Kinetin®, Sigma) independently (20, 100 and 200 ppm) or in combination (100 + 100, 20 + 200 and 200 + 20 ppm). Plants were harvested after a growing period of 40 days with a good market size and a relatively well-formed head (romaine type). At harvest, the lettuce heads were weighed, damaged leaves were removed, and the marketable weight was determined. The dry weight and tissue nitrate content were recorded.

Table 2. Mean minimum and maximum air temperature (°C) and relative humidity (%) inside the greenhouse during the growing period

Growing period (days)	Air temperature (°C)		RH(%)	
	Maximum	Minimum	Maximum	Minimum
1-8	37.8	5.0	95.1	32.1
9-16	40.6	5.7	85.0	20.0
17-24	36.1	4.9	95.0	22.1
25-32	38.9	6.1	89.5	18.9
33-40	40.7	7.7	94.2	27.1

Sample preparation: Lettuce heads were cut in four pieces put in paper bags and placed in an oven at 65°C temperature and air circulation to dry until reaching a constant weight. The dried material was recorded, finely mixed, ground and closed hermitically in glass vessels.

Determination of nitrates in plant tissue: For the determination of TNC a method reported by Paul and Carlson (1968), modified by Baker and Smith (1969) and Hadjidemetriou (1982) was used. An amount of 0.25 g of dried ground head was added to 25 mL extracting solution (0.02 N copper sulphates containing the amount of silver sulphate needed for the precipitation of chlorides), homogenized by shaking for 15 min and filtered through Watman (No.2) filter paper. The samples potential (me) was measured with a nitrate combination ion selective electrode (924-300, Jenway, UK) connected with the pH and conductivity meter apparatus measuring in millivolt (mv) with the help of a standard solution of known concentrations of nitrate. To avoid interference by chloride ion, silver sulphate was added in the extractant for precipitation of chlorides (Hadjidemitriou, 1982) because chlorides are possibly the most serious interfering ion (Baker and Smith, 1969).

Statistical analysis: The yield data and nitrate content in plant tissue were subjected to ANOVA in SAS (SAS Inc., Cary, NC) for analysis of variance. Means were compared using Duncan's multiple range test at the 5% level of significance.

Results and discussion

Effect of solution nitrogen concentrations: The effect of nitrogen concentrations on fresh and dry weight per head and TNC of lettuce plants are shown in Table 3. Average head weight (fresh weight) was very responsive to N application level (Pearson correlation coefficient $r = 0.917$*). Kunsch *et al.* (1993) reported that lettuce heads can be determined as commercial when the fresh weight of the lettuce head is above 250 g. According to this, the lettuce heads of the treatment 50 ppm NO_3-N were not as per commercial standards whereas the lettuce heads of the treatments 150 and 200 ppm NO_3-N had more marketable heads. The dry matter weight per head increased significantly related to the amount of N applied (Pearson correlation coefficient $r = 0.882$*) but differences were not significant between application levels of 150 and 200 ppm NO_3-N (Table 3). This could be expected based on fresh weight results. Increasing the N application level increased the TNC (Table 3) with a significant correlation coefficient ($r = 0.798$*). The maximum value was observed at 200 ppm NO_3-N whereas; at 100 and 150 ppm NO_3-N TNC remained at significant lower values (Table 3). The lowest significant value was at 50 ppm NO_3-N but growth was consistently depressed in contrary with results of Koleilat (1993) suggesting that lettuce can be grown at low nitrogen level (50 ppm) without reduction in yield. Finally, it could be stressed that TNC in lettuce heads was below the recommended limit by the European Commission at all nitrogen concentrations levels.

Effect of GA_3, kinetin and GA_3+kinetin: The effect of growth regulators, GA_3, kinetin and GA_3+kinetin on fresh and dry weight per head and TNC of lettuce plants are shown in Table 3. GA_3 and GA_3+kinetin treatments had the highest averages of fresh, dry weight and TNC. Control gave significant lower values and kinetin had non significant differences (Table 3). The interaction between N application level and growth regulators was not significant (Table 3).

There were few differences in response to application rates. GA_3 at different application rates had a non significant influence on fresh and dry weight. However, increased supply of GA_3

Table 3. Effect of nutrient solution nitrogen concentration (NC) and growth regulators (GR): gibberellin (GA_3), kinetin and GA_3+kinetin, on fresh head weight (F.H.W.), dry head weight (D.H.W.) and tissue nitrate content (TNC) of lettuce

Nitrogen concentration	F.H.W. (g)	D.H.W. (g)	TNC [mgNO_3 (kg F.W.)$^{-1}$]
N-50 ppm NO_3-N	212c	10.9c	381c
N-100 ppm NO_3-N	325b	12.2b	1173b
N-150 ppm NO_3-N	553a	19.0a	1204b
N-200 ppm NO_3-N	590a	19.7a	1371a
GR			
Control	380b	14.3b	893c
Kinetin	416ab	15.2ab	1002bc
GA_3 + Kinetin	430a	16.1a	1073ab
GA_3	454a	16.3a	1161a
NS × GR	NS	NS	NS

NS: non-significant at $P \leq 0.05$.

Mean values followed by the same letter in the same column are not significantly different at $P \leq 0.05$.

Table 4. Effect of growth regulators (GR): gibberellin (GA_3), kinetin and GA_3 + kinetin combination at different application rates on fresh head weight (F.H.W.), dry head weight (D.H.W.) and tissue nitrate content (TNC) of lettuce

GR (ppm)	F.H.W. (g)	D.H.W. (g)	TNC [$mgNO_3$ (kg F.W.)$^{-1}$]
GA_3			
20	417	15.3	1095b
100	478	17.3	1141ab
200	466	16.3	1248a
Significance	NS	NS	*
Kinetin			
20	418	15.3	978
100	428	15.3	983
200	403	15.0	1044
Significance	NS	NS	NS
GA_3 + kinetin			
100 + 100	409	15.5	1317a
20 + 200	415	15.5	906b
200 + 20	466	16.2	996b
Significance	NS	NS	*

NS, non-significant, * significant at $P \leq 0.05$.

Means within columns for a given treatment followed by the same letter are not significantly different at $P \leq 0.05$.

enhanced the accumulation of nitrates (Table 4). The TNC was highest at 200 ppm and lowest at 20 ppm GA_3 application. The GA_3 at 100 ppm gave non significant differences. The effects of kinetin application rates on fresh and dry weight and TNC of lettuce plants were not significant as shown in Table 4. Similarly, the effects of GA_3+kinetin at different application rates on fresh and dry weight were not significant. However, the exposure of plants at application rate of 100+100 ppm resulted in significantly higher TNC (Table 4).

Gibberellin and GA_3+kinetin enhanced fresh and dry weight per head and similar results were reported by Rozek *et al.* (1989) and Neocleous *et al.* (2007), who found that growth regulators GA_3, kinetin and GA_3+kinetin combination stimulated fresh weight growth in lettuce heads, being the most intensive in plants sprayed with the combination of the two regulators. In addition, GA_3 and GA_3+kinetin enhanced TNC. These results are in agreement with those found by Neocleous *et al.* (2007) but in conflict with those found by Rozek and Wojciechowska (1990), Myczkowski *et al.* (1990) and Sady *et al.* (1995) who reported that lettuce plants sprayed with GA_3 and kinetin had the greatest reduction in nitrate content. Attributed to different weather conditions (light intensity, sunshine duration and temperature) and reports from the literature (Roorda-Van-Eysinger, 1984; Blom-Zandstra and Lampe, 1985; Kaiser and Forster, 1989; Van der Boon *et al.*, 1990; Talon *et al.*, 1991; Lacertosa *et al.*, 1997; Neocleous *et al.*, 2007) showing that there is an interaction between the amount of light or time of the year in which crop is grown and the nitrate content, it can be argued that under east Mediterranean (Cyprus) climatic conditions, where light intensity and temperature are high even in winters, the role of growth regulators in lowering the tissue nitrate content might be less important. Similarly, Behr and Wiebe (1992) reported a close negative correlation between photosynthesis and nitrate content in lettuce cultivars and this explaination may be suitable in present study also. Tissue nitrate content was between 381 and 1371 mg NO_3 (kg fresh weight)$^{-1}$ all over the experiment which is lower than European standards and comparable with biologically produced lettuce (Leuzzi *et al.*, 1997).

In conclusion, in lettuce plants low NO_3 accumulation is desirable together with high fresh and dry head weight for which the best N-application level is 150 ppm NO_3-N. Also the use of growth regulators, GA_3 and GA_3+kinetin, may enhance yield. However, their use for reducing the nitrate content is not recommended.

References

Baker, A.S. and R. Smith, 1969. Extracting solution for potentiometer determination of nitrate in plant tissue. *J. Agric. Food Chem*., 17: 1284.

Behr, U. and H.J. Wiebe, 1992. Relationship between photosynthesis and nitrate content of lettuce cultivars. *Sci. Hort*., 49: 175-179.

Blom-Zandstra, M. and J.E.M. Lampe, 1985. The role of nitrate in the osmoregulation of lettuce (*Lactuca sativa* L.) grown at different light intensities. *J. Exp. Bot*., 36: 1043-1052.

Boink, A. and G. Speijers, 2001. Health effects of nitrates and nitrites, a review. *Acta Hort*., 563: 29-36.

Bres, W. 1992. Nutrient accumulation and tip burn in NFT-grown lettuce at several potassium and pH levels. *HortScience*, 27: 790-792.

Economakis, C.D. 1991. Effect of solution conductivity on growth and yield of lettuce in nutrient film culture. *Acta Hort*., 287: 309-316.

Economakis, C.D. 1992. Effect of climatic conditions and time of harvest on growth and tissue nitrate content of lettuce in Nutrient Film Technique. *Acta Hort*., 323: 75-80.

Hadjidemetriou, D.G. 1982. Comparative study of determination of nitrates in calcareous soils by the ion selective electrode, chromotropic acid and phenoldisulphonic acid methods. *The Analyst*, 107: 25-29.

Jaervau, M. 1994. The dependence of nitrate content of vegetables on biological peculiarities and growth conditions. *J. Agric. Sci*., 5: 149-169.

Kaiser, W.M. and J. Forster, 1989. Low CO_2 presents nitrate reduction in leaves. *Plant Physiol*., 91: 970-974.

Kaiser, W.M. and E. Brendle-Behnisch, 1991. Rapid modulation of spinach leaf nitrate reductase activity by photosynthesis. I. Modulation *in vivo* by CO_2 availability. *Plant Physiol*., 96: 363-367.

Koleilat, R. 1993. The effects of different solution nitrogen concentrations on growth and tissue nitrate content of lettuce plants (*Lactuca sativa* L.) grown in solution culture (NFT). M.Sc. thesis. Mediterranean Agronomic Institute of Chania, Greece.

Kunsch, U., H. Scharer, J. Hurter and P. Konrad, 1993. Moglichkeiten zur Reduktion des Nitratgehalts in Kopfasalat durch erdelosen Anbau. *Kongreßband,* 37: 453-456.

Lacertosa, G., G. Capotorti, D. Palazzo and F. Montemurro, 1997. Effects of enviromental parameters and nitrogen fertilization on nitrate content of lettuce (*Lactuca sativa* L.) (Basilicata). *Rivista -di-Agronomia*, 31: 72-77.

Leuzzi, U., G. Cimino and G. Dugo, 1997. Application of ionic chromatography to the determination of the anionic composition in vegetables (lettuce, spinach) produced with biological method. *Rivista-di-Scienza-dell'Alimentazione*, 25: 349-355.

Myczkowsci, J., S. Rozek and W. Sady, 1991. The effects of fertilization with different forms of nitrogen on yield and nitrate metabolism in leaves of greenhouse lettuce. II. The effect of growth regulators. *Folia Hort*., 3: 13-25.

Myczkowsci, J., S. Rozek, W. Sady and T. Wojtaszek, 1990. The effect of some factors on the content of nitrates and nitrite in lettuce leaves grown with the nutrient film technique. II. Effect of light and growth regulators in conditions of NPK fertilization discontinued before harvesting. *Folia Hort*., 2: 7-16.

Neocleous, D., I. Papadopoulos and C. Olympios, 2007. The effects of growth regulators on growth and tissue nitrate content of lettuce plants (*Lactuca sativa*) grown in Cyprus. Technical Bulletin 230, Agricultural Research Institute, Nicosia.

Pasda, G., R. Hahndel and W. Zerulla, 2001. Effect of fertilizers with the new nitrification inhibitor DMPP (3,4-dimethylpyrazolephosphate) on yield and quality of agricultural and horticultural crops. *Biol. Fertil. Soils*, 34: 85-97.

Paul, J.L. and R.M. Carlson, 1968. Nitrate determination in plant extracts by the nitrate electrode. *J. Agric. Food Chem*., 16: 766.

Peck, N.H., A.V. Barker, G.E. MacDonald and R.S. Shallenberger, 1971. Nitrate accumulation in vegetables. *Agron. J*., 63: 130-132

Premuzic, Z., A. Garate and I. Bonilla, 2002. Production of lettuce under different fertilisation tratments, yield and quality. *Acta Hort*., 571: 65-72.

Premuzic, Z., F. Vilella, A. Garate and I. Bonilla, 2003. Production and quality of winter field and greenhouse grown lettuce (*Lactuca sativa*). *Acta Hort*., 607: 149-155.

Reinink, K., R. Groenwold and A. Bootsma, 1987. Genotypic differences in nitrate content in *Lactuca sativa* L. and related species and correlation with dry matter content. *Euphytica*, 36: 11-18.

Reinink, K. and R. Groenwold, 1987. The inheritance of nitrate content in lettuce (*Lactuca sativa* L.). *Euphytica,* 36: 733-744.

Roorda-Van-Eysinger, J.P.N.L.1984. Nitrate and glasshouse vegetables. *Fert. Res*., 5: 149-156.

Rozek, S., J. Myczkowsci, W. Sady and T. Wojtaszek, 1989. The effect of some factors on the content of nitrate and nitrite in lettuce leaves grown with the nutrient film technique. I. The effect of light and growth regulators. *Folia Hort*., 1: 31-34.

Rozek, S. and R. Wojciechowska, 1990. Effect of light and growth regulators on the circadian rhythmicity of nitrate reductase and nitrite reductase activities in greenhouse lettuce leaves. *Folia Hort*., 2: 53-64.

Sady, W., S. Rozek and J. Myczkowsci, 1995. Effects of different forms of nitrogen on the quality of lettuce yield. *Acta Hort*., 401: 409-418.

Santamaria, P. 1997. Occurrence of nitrates in vegetables and total dietary intakes (nitrate-nitrite-nitrosamine). *Industrie-Alimentari*, 36: 1324-1334.

Talon, M., J.A.D. Zeerant and D.A. Gage, 1991. Identification of gibberellins in spinach and effects of light and darkness on their levels. *Plant Physiol*., 97: 1521-1526.

Van der Boon, J., J.W. Steenhuizen, H. Lampers, J.J. Neeteson and I. Stulen, 1986. Reduction of the nitrate concentration of lettuce grown in recirculating nutrient solution. Fundamental-ecological and agricultural aspects of nitrogen metabolism in higher plants. *J. Hort. Sci*., 61: 485-489.

Van der Boon, J., J.W. Steenhuizen and *E.g.* Steingrover, 1990. Growth and nitrate concentration of lettuce as affected by total nitrogen and chloride concentration, NH_4/NO_3 ratio and temperature of the recirculating nutrient solution. *J. Hort. Sci*., 65: 309-321.

WHO, 1985. Health hazards from nitrates in drinking water. *Report on a WHO Meeting*, Copenhagen 5-9 March, 1984.