NITROGEN FERTILIZATION INFLUENCES ON NITRATE CONTENTS IN SPINACH

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Introduction

Genotype and management practice, especially fertilization, have important influences on vegetables yield and quality. Application of high nitrogen (N) doses could be resulted by increased nitrates uptake in plants (Mohanty and Kanwar, 1994; Gaudreau, 1995; Pavlovic et al., 1997; Belanger, 2002; Németh and Izsáki, 2005). Also, nitrate contents in plants are under influences of plant organ, age of plants and genotype (Corré and Breimer, 1989; Belanger, 2002; Wadas et al., 2005; Pepó et al, 2005, Venter, 1979). Spinach is one of the most sensitive vegetable crops concerning inclination to nitrate accumulation in plant (Pavlovic et al., 1997; Ramachandran et al., 2005). Cumulative nitrate consumption by food and drinking water is dangerous for human health. For example, chemical reaction of nitrates with amines in human and animal digestive tract resulting with forming cancerogenic substances nitrosamines (Hildebrandt, 1976). Aim of this study was testing response of spinach genotypes to nitrogen fertilization (yield, nitrates in leaf blade and stem).

Material and methods

Field experiment, sampling, chemical and statistical analysis

Four spinach genotypes (three hybrids: *Tiptiek*, *Triathlon* i *Vivat;* variety*: Matador*) were grown under field conditions on Trbusani smonica soil (nunicipality Cacak, central Serbia) in spring growing cycle for two growing seasons. Four nitrogen treatments in form of calcium ammonium nitrate (27% N) were applied on constant fertilization (30 t manure ha^{-1} ploughed in autumn; spring starting fertilization = 70 kg P_2O_5 and 100 kg K_2O ha^{-1}) as follows (kg N ha^{-1}): 0, 80, 120 and 250. The experiment was conducted by randomized block design in three replicates (basic plot 10 m^2). Spinach was sown in terms March 15, 2003 and March 12, 2004 (interrow spacing 25 cm; distance in row 10 cm). One phasing harvest of spinach was made in stage of maximal development of rosette in terms May 10 2003 and May 14, 2004. Leaves samples (separately blades and stems) were prepared for nitrate determination by colorimetric method using Cd-reduction (Maynard and Kalra, 1993). Results were calculated in mg NO_3 kg^{-1} on fresh mass basis. The statistical analysis were made as three-factorial experiment (A=spinach genotype; B=nitrogen fertilization; C= year of testing) by analysis of variance (ANOVA, Microsoft STATISTICA Ver. 5.0., StatSoft Inc, 1995).

Soil characteristics and weather conditions

The experiment was conducted on smonitza soil characterizing neutral reaction, adequate humus contents, very high levels of plant available phosphorus and potassium as well (Table 1). Mean soil sample was taken by auger in autumn before organic manure distribution. Soil was moderately supplied with NO_3-N in amount without of serious influences on nitrate uptake by plants.

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Table 1. Chemical characteristics of the soil (0-30 cm depth)

Year of testing	pH (1nKCl)	% Humus	mg kg^{-1} NO_3-N	mg100g^{-1} of soil (AL-method)	
				P_2O_5	K_2O
2003	6.8	4.2	18.9	59.6	63.0
2004	6.5	3.9	16.5	56.7	65.0

Table 2. Precipitation and mean air-temperatures *

Years	Air temperature (^{0}C)			Precipitation (mm)		
	March	April	May	March	April	May
Meteorological Station, "Serbia" Institute, Fruit and Grape Research Centre, Cacak						
2003.	3.3	9.3	18.3	31.7	68.7	66.0
2004.	4.9	11.4	14.3	2.5	20.0	42.7
Long-term mean (LTM): Cacak Weather Bureau						
LTM 1961-1990	6.2	11.5	16.2	53.8	57.8	88.6

Results and discussions

In our study, considerable influences of genotype, nitrogen fertilization and growing season characteristics (weather conditions) on nitrate contents (NO_3-c) in spinach leaves were found (Table 3). Mean NO_3-c in blade (132 mg NO_3 kg^{-1}) was for 55% lower than corresponding value for stem (290 mg NO_3 kg^{-1}). Vivat genotype had very significantly lowest NO_3-c both in blade and stem, in comparison with remaining tree tested genotypes, while Triathlon characterized the highest NO_3-c. For example, NO_3-c in Vivat was lower for 40% (blade) and 35% (stem) than in Triathlon.

Nitrogen fertilization was the most influencing factors of NO_3-c in spinach because by the lowest applied N rate NO_3-c were increased for 4.9-fold and 5.7-fold compared to unfertilized treatment, for blade and stem, respectively. By additional increasing of N rates, NO_3-c were increased up to 12.3-fold (blade) and 8.2-fold, (stem) compared to control. However, in our study, nitrate concentrations in spinach remained in permissible ranges according European Community regulative (Anonymous, 1997).

Nitrate accumulation in plants is also depending on weather conditions. For example under conditions of 2004 it was higher for 17% (blade) and 19% (stem) than in previous year. This phenomenon is possible to explain by lower precipitation for the 2004 growing season (Table 2). Our findings concerning precipitation influences on nitrate contents in plants are in accordance with data of other authors. For example, Amelina et al. (2002) reported that the higher nitrate accumulation is in connection with more coldly and dry periods. Also, Cantliffe (1972) found that temperature influences on nitrate accumulation in plants are less compared to precipitation because it increase to temperature up to maximal 25 ^{o}C and than has decreasing trend.

Table 3. Influences of genotype (A), nitrogen fertilization (B) and year (C) on nitrate contents in spinach leaves and yield

Factor A	Factor B	Factor C: year of testing								
		Nitrate contents: mg NO_3 kg^{-1} on fresh mass basis						t $ha^{-1}$of fresh mass		
Spinach genotype	N kg ha^{-1}	Leaf blade			Leaf stem			Yield		
		2003	2004	Mean	2003	2004	Mean	2003	2004	Mean
		AC		A	AC		A	AC		A
Matador		108.0	128.4	118.2	245.8	344.2	295.0	16.5	14.0	15.2
Triptiek		126.7	153.0	139.9	250.1	344.6	297.4	33.1	31.2	32.1
Triathlon		140.8	192.6	166.8	316.5	370.6	343.6	45.4	41.7	43.5