Effect of nitrogen fertilization on carrot quality

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A study was carried out during the 2005 and 2006 growing seasons to evaluate the effect of increasing nitrogen fertilization rates on nitrate accumulation, vitamin C and β-carotene content in two carrot genotypes. Nitrogen fertilizer (calcium ammonium nitrate, 27% N) was applied at four rates: 0, 60, 120 and 180 kgN/ha¹. After harvesting, root samples were collected and tested for quality. The use of increasing nitrogen rates resulted in increased nitrate accumulation in the roots. The increase in nitrogen fertilizer rate from 60 to 120 kgN/ha¹ led to a reduction in Vitamin C content and an increase in β-carotene. Differences were observed between cultivars and hybrids in all quality parameters tested.

Key words: Carrot, genotypes, nitrogen, nitrates, vitamin C, β-carotene.

INTRODUCTION

Carrots are a very important vegetable crop, widely used in human (especially children) diet due to their high nutritional (Heinonen, 1990) and medicinal value, and their role in disease prevention (Gallichio et al., 2008; Zhang et al., 2009; Arscott and Tanumihardjo, 2010). Being nitrophilous vegetables showing a tendency to accumulate nitrates, carrots require nitrogen fertilization, as one of the most important management practices (John et al., 2003; Ahmadi et al., 2010). Nitrogen is one of the most important yield-limiting nutrients for plants (Ekbic et al., 2010; Xia et al., 2011). Nitrate accumulation is affected not only by the type of nitrate fertilizer used, but also by nitrogen rates, variety, environment, harvesting date and other agronomical factors (Cserni and Prohaszka, 1988; Gutezeit, 2000; Amr and Nadidi, 2001; Kôňa, 2006; Gajewski et al., 2009). Nitrogen fertilizers should be applied in such a way to prevent the excessive supply of this nutrient without limiting the yield potential of different carrot genotypes. Minimum nitrate accumulation in vegetables is affected by choice of low nitrate-accumulating genotypes and proper nitrogen fertilization rates. Wiebe (1987) obtained the best result of carrot yield with 80 to 140 kg/ha¹ of nitrogen, whereas Markovic et al. (2002) reported the highest yield at the application rate of 100 kg/ha¹. As high nitrogen rates cause accumulation of harmful nitrates in the plants (Gutezeit, 1999: John et al., 2003; Chen et al., 2004; Anjana et al., 2007; Mubashir et al., 2010; Ahmadi et al., 2010), it is essential to use genotypes which accumulate a low content of this nutrient. The consumption of foods and beverages high in nitrates is very dangerous to human health since it causes a large number of diseases, most commonly carcinogenic diseases (Mozafar, 1993). The toxic effects of nitrate are due to its endogenous conversion to nitrite, which is related to methaemoglobinemia, gastric cancer and other diseases (Santamaria, 2006). Carrots contain valuable biological substances, most notably β-carotene and vitamin C which has a protective effect and inhibits carcinogenic nitrosamines (Hartmann, 1983; McKnight et al., 1999). High nitrate concentrations reduce the vitamin C level. A review by Mozafar (1993) summarizes the effects of N fertilization on the vitamin content of plants, including carrot. Fertilization with N, especially at high rates, decreases the concentration of vitamin C and increases the concentration of carotenoids. Lisiewska and Kmiecik (1996) determined that an increase in nitrogen rates from 80 to 120 kg/ha results in a 44 and 33% reduction in vitamin C levels in broccoli and cauliflower, respectively. The concentration of β-carotene increases with increasing nitrogen rates. Hocmuth et al. (1999) used nitrogen rates of 0 to 220 kg/ha and obtained the highest content of β-carotene (55 mg/kg) with 160 kg/ha.
In their study on the effect of different nitrogen rates on growth, elemental accumulation and carotenoid production in parsley, Chenard et al. (2005) found that β-carotene content was affected by increasing nitrogen rates. Musa et al. (2010) reported that the applied nitrogen significantly elevated β-carotene content at maturity, while no significant variation was recorded at fruiting. In the same study, the results obtained from the determination of vitamin C content showed that the applied nitrogen fertilizer significantly decreased the vitamin content at both market maturity and fruiting stages of the plant development. The objective of this work was to investigate the effects of different nitrogen fertilizers on nitrate accumulation, and vitamin C and β-carotene content in the root of two carrot genotypes.

MATERIALS AND METHODS

The experiment was carried out at the experimental plot of the Faculty of Agronomy, Central Serbia (43°44′N; 20°7′E), in spring 2005 and 2006. The soil samples were analyzed for chemical properties and their element content using standard methods: nitrate was measured by Kjeldahl method; pH was determined potentiometrically in water and 1 M KCl; available P$_2$O$_5$ and K$_2$O were determined by extraction with Al solution (0.1 M ammonium lactate and 0.4 M acetic acid), and P and K by molybdate colorimetry and flame photometry, respectively. The soil used in the experiment was loam soil, slightly acid in reaction, low in available phosphorus, and having moderate levels of available potassium and nitrate nitrogen (Table 1).

The region of Cacak has a temperate continental climate. Climatic characteristics of the study area for the experimental period are shown in Table 2. Four treatments with nitrogen applied as CAN (Calcium Ammonium Nitrate, containing 27% of N$_{tot}$) were employed with basic fertilization (fertilization starting in spring as part of topsoil preparation). A total of 400 kg/ha NPK-fertilizer 0:20:30 (80 kgP$_2$O$_5$ and 120 kgK$_2$Oha$^{-1}$) was applied based on soil fertility and carrot requirements. Nitrogen was used in a single application as follows (kgN/ha$^{-1}$): 0 (T$_1$), 60 (T$_2$) and 180 (T$_4$). The experiment was set up in a randomized block design in three replications (plot size: 10 m$^2$).

Two carrot genotypes were used in the study: *Nantes*, a cultivar having cylindrical straight orange tap roots 12 to 17 cm in length, 140 to 160 g in weight and 2 to 4 cm in diameter. *Nantes* is grown both for fresh consumption and processing. *Almaro F$_1$* is a hybrid cultivar of the *Nantes* type which develops cylindrical firm smooth taproots 19 to 21 cm in length.

Sowing was performed on the 1st April, 2005 and on the 3rd April, 2006 (row spacing 30 cm; within-row spacing 10 cm). Carrot roots were harvested on July 29th, 2005 and July 30th, 2006, upon sampling and preparation for analysis.

Nitrate was determined using the colorimetric Cd-reduction method (Maynard and Kalra, 1993). Vitamin C was measured using the indophenol titration method (Association of Vitamin Chemists. Methods of Vitamin Assay, 3rd ed.; Wiley: London, U.K., 1966.). The carrots were homogenized in an MPA solution (metaphosphoric acid) and extracted. The vitamin C was titrated against a 2.6-dichlorophenol–indophenol solution at pH 6.0 in the presence of formaldehyde, to a pink endpoint. β-carotene was determined by HPLC according to the method described by Bushway (1986). All manipulations were carried out under gold fluorescent lighting, due to the high degradation of carotenoids due to the exposition to light, heat, and air. The chromatograph used consisted of an L-6200A intelligent pump, an L-4500 diode array detector (Hitachi Ltd.), and an Elkonex PC 466/I computer (Elkonex). A 5 μL sample was injected after filtration through a 0.45 μm filter. The isocratic separation was performed using a 5 μm Vydac 218 TP54 column, 250 mm × 4.6 mm i.d. (Phenomenex, Cheshire, U.K.), with a solvent system of acetonitrile/methanol/stabilized tetrahydrofuran (40:56:4, v/v/v) at a flow rate of 1 ml/min and a monitoring wavelength of 454 nm. β-carotene contents were determined on the basis of peak heights through comparison with a calibration curve obtained with the corresponding standard. Results on nitrate and β-carotene were calculated as mgNO$_3$-kg$^{-1}$ on a fresh weight basis, and those on vitamin C content as mg vitamin C100mg$^{-1}$ on a fresh weight basis.

The data obtained were subjected to analysis of variance (ANOVA) and means were compared by LSD test at P≤0.05 using the MSTAT-C statistical computer package (Michigan State University, East Lansing, MI, USA). The correlation curves were

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

<table>
<thead>
<tr>
<th>Soil type</th>
<th>pH</th>
<th>mg kg$^{-1}$ NO$_3$-N</th>
<th>P$_2$O$_5$</th>
<th>K$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loam soil</td>
<td>6.31</td>
<td>5.22</td>
<td>24.50</td>
<td>29.50</td>
</tr>
</tbody>
</table>

### Table 2. Precipitation and mean air temperatures*.

<table>
<thead>
<tr>
<th>Year</th>
<th>April</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>Precipitation (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>12.0</td>
<td>17.2</td>
<td>21.0</td>
<td>23.7</td>
<td>80.5</td>
</tr>
<tr>
<td>2006</td>
<td>14.0</td>
<td>18.2</td>
<td>21.7</td>
<td>24.7</td>
<td>61.0</td>
</tr>
</tbody>
</table>

*Meteorological Station, “Serbia” Institute, Fruit and Grape Research Center, Cacak

Long-term mean (LTM): Cacak Weather Bureau

<table>
<thead>
<tr>
<th>Year</th>
<th>April</th>
<th>May</th>
<th>June</th>
<th>July</th>
</tr>
</thead>
<tbody>
<tr>
<td>1965-2003</td>
<td>11.3</td>
<td>16.8</td>
<td>20.3</td>
<td>21.7</td>
</tr>
<tr>
<td></td>
<td>56.2</td>
<td>73.7</td>
<td>87.4</td>
<td>79.2</td>
</tr>
</tbody>
</table>
Table 3. Contents of nitrate, vitamin C and β-carotene in carrot roots treated with increasing levels of calcium ammonium nitrate.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Nitrate content (mg NO₃ kg⁻¹ on a fresh weight basis)</th>
<th>Vitamin C (mg vitamin C 100 g⁻¹ on a fresh weight basis)</th>
<th>β-carotene (mg β-carotene kg⁻¹ on a fresh weight basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nantes</td>
<td>338.4  a</td>
<td>515.9  a</td>
<td>27.35  a</td>
</tr>
<tr>
<td>Almaro F₁</td>
<td>241.6  b</td>
<td>271.1  b</td>
<td>26.25  b</td>
</tr>
<tr>
<td>N-fertilization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (T₁)</td>
<td>122.0  d</td>
<td>241.2  d</td>
<td>28.75  a</td>
</tr>
<tr>
<td>60 (T₂)</td>
<td>187.0  c</td>
<td>335.0  c</td>
<td>27.60  b</td>
</tr>
<tr>
<td>120 (T₃)</td>
<td>358.4  b</td>
<td>420.3  b</td>
<td>26.40  c</td>
</tr>
<tr>
<td>180 (T₄)</td>
<td>492.4  a</td>
<td>577.7  a</td>
<td>25.50  d</td>
</tr>
<tr>
<td>Root part</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/3</td>
<td>332.5  a</td>
<td>524.1  a</td>
<td></td>
</tr>
<tr>
<td>2/3</td>
<td>247.5  b</td>
<td>262.8  b</td>
<td></td>
</tr>
</tbody>
</table>

Values in the same column followed by different letters are significantly different (P<0.05) according to the LSD-test. The analysis in all treatments included 30 carrot roots. The values for the content of nitrates, vitamin C and β-carotene as affected by increasing nitrogen application rates are presented as average values for both genotypes.

RESULTS AND DISCUSSION

The concentration of root nitrate showed differences with the increasing levels of nitrogen for the both years evaluated (Table 3). The lowest nitrate content was found in non-fertilized soil (122.0 mg NO₃ kg⁻¹ FW in 2005, that is, 241.2 mg NO₃ kg⁻¹ FW in 2006), whereas the highest level was obtained at 180 kgNha⁻¹ supply, a higher nitrate accumulation in carrot is possibly due to a greater uptake of nitrate than its utilization in plant physiological processes (Cantliffe, 1973). The statistically different nitrate content in carrot roots between the two years was probably induced by different quantities and dates of precipitation across months (Table 2). Our results in 2006 confirmed Viets and Hageman (1971) findings of increased soil nitrate concentrations and nitrate accumulation in plants under drought and inadequate watering conditions. Similar results were obtained by Augustin et al. (1977), Who reported a two-fold increase in nitrate content due to insufficient irrigation. Allaire-Leung et al. (2001) found that nitrate leaching was positively correlated to soil NO₃-N content but was not correlated to irrigation depth, irrigation uniformity, or deep percolation. Van Der Boon et al. (1988) determined that increased soil and air temperatures reduce nitrate reductase activity consequently leading to an increase in nitrate content, as confirmed by the results of Calatayud et al. (2008). The carrot genotypes tested had a significant effect (P<0.05) on nitrate content in the root. Lower nitrate content was found in the hybrid Almaro in both years, which was expected considering the genetic predisposition of the hybrid to reduce nitrate accumulation (Cserni et al., 1983; Anikeenko and Vintsunas, 1986; Lee et al., 1992; Gutezeit and Fink, 1999).
Amr and Nadidi (2001) reported a statistically significant effect of cultivar (P<0.05) on the nitrate content in vegetables grown under both greenhouse and open field conditions. The distribution of accumulated nitrates in carrot roots in both years was uneven, gradually decreasing from the top to the bottom of the root. Higher nitrate levels were measured in the upper part of the root (332.5 mg NO3kg-1 FW in 2005, that is, 524.1 mg NO3 kg-1 FW in 2006), which was statistically significant as compared to the lower part (247.5 mg NO3kg-1 FW in 2005, that is, 262.8 mg NO3 kg-1 FW in 2006). Similar nitrate distribution within carrot root was previously reported by Steer (1982), who found 90% of totally accumulated nitrate in the upper third of the root, that is, just below the top.

The physiological role of vitamin C is to produce protective effects in vegetables by decreasing nitrosamine levels (Beyers and Peery, 1992; Mc Knight et al., 1999). The vitamin C content in the present study was reduced by increasing rates of nitrogen in both years (Table 3). In 2005, statistically significant differences (P<0.05) were observed between all fertilization treatments, whereas in 2006 significant differences were found only between T1 and T2 as compared to T3 and T4. The highest level of vitamin C in both years (28.75 and 23.15 mg vitamin C 100 g-1 FW, respectively) was found upon treatment with the lowest nitrate rate in T1 (122.0, that is, 241.2 mg NO3 kg-1 FW), which complied with the findings previously reported by Mozafar (1996). Stefanelli et al. (2010) reported that increased N applications resulted in increased vegetative growth and larger fruits, suggesting that the decline in ascorbic acid could, in part, be due to a dilution effect. Cieślik (1994) found that the vitamin C content was highest at the lowest nitrate content, whereas Lisiewska and Kmiecik (1996) reported a decrease in vitamin C content as induced by the nitrogen rate increase from 80 to 120 kg/ha. Lee and Kader (2000) associated the low vitamin C level in treatments with increased nitrate rates with rapid plant growth which provoked biological dilution of vitamin C. Cultivar Nantes had a statistically higher vitamin C level than Almaro, as expected by the genetic variability between these two genotypes. The present conclusion is similar to those of Kurlich et al. (1999) and Kalt (2005). All treatments in 2006 gave lower vitamin C content than in 2005, as correlated with the higher nitrate content in carrot root during the year. However, higher temperatures were recorded in 2006, which also led to a decrease in vitamin C content (Dumas et al., 2003). Zushi and Matsuzoe (1998) reported a variable effect of water deficiency on vitamin C content. Vitamin C content is reduced by low water tension (Rudich et al., 1977) as well as by PRD (Partial Root Zone Drying) (Du et al., 2008). In view of the physiological importance of vitamin C, the use of high rates of nitrogen fertilizers should be avoided in carrot cultivation.

The level of β-carotene in carrot roots was found to increase upon treatment with higher rates of nitrogen, which was in agreement with a previous study conducted by Csernii and Prohaszka (1988) and Chenard et al. (2005). The lowest content of β-carotene (95.74 mg β-carotene kg⁻¹ FW in 2005 and 92.15 mg β-carotene kg⁻¹ FW in 2006) was obtained in the untreated control. No statistically significant differences were observed between T1 and T2 whereas the rate of 120, that is, 180 kgNha⁻¹ caused a significant increase in β-carotene. The highest level (111.82 mg β-carotene kg⁻¹ FW in 2005, and 115.74 mg β-carotene kg⁻¹ FW in 2006) resulted from the use of 180 kgNha⁻¹, which was similar to that obtained by treatment with 160 kgNha⁻¹ in a study by Hochmuth et al. (1999). These results are in agreement with the findings of Mengel (1979) and Venter (1979) who reported that fertilization increased carotene concentration of carrots. A lower carotene content was obtained by Evers (1989) using the nitrogen rate of 150 kg/ha, which had no effect on carotene content as compared to the 80 kg/ha rate, similarly to the results of the present study. In 2006, β-carotene content was slightly lower than in 2005 in all treatments excepting the treatment with 180 kgN/ha. Previous studies reported few data on the effect of water deficiency on β-carotene content, except in tomatoes. Matsuzoe et al. (1998) found that water deficiency resulted in an increase in β-carotene content in three cherry tomato cultivars and produced no effect in one cultivar. Zushi and Matsuzoe (1998) suggested that water stress had no effect on β-carotene content in tomatoes, which is in agreement with the results of the present study on carrot. The carrot genotypes tested showed a statistically significant difference in β-carotene level in both years. Genetic variability regarding this quality trait was determined by Hart and Scott (1995), Alasalvar et al. (1998, 2001) and Kalt (2005). The interdependence of nitrate content in carrot roots and the contents of vitamin C and β-carotene is presented in Figures 1 and 2.

The vitamin C content of carrot roots was statistically significantly decreased with increasing nitrogen rates (Figure 1), showing negative correlation (r = -0.584; r² = 0.341). The effect of nitrate concentration on β-carotene content (Figure 2) exhibited direct dependence (r = 0.792; r² = 0.626), with statistically significant differences in β-carotene content being observed at higher nitrogen application rates as compared to both the 60 kgN/ha rate and the control.

**Conclusion**

The results of the present study on the effect of increasing nitrate fertilization rates on the vitamin C and β-carotene content of the root in two carrot genotypes suggest that: Nitrate accumulation in carrot roots was directly affected by nitrogen rate, with nitrate level being statistically different (P<0.05) at all rates applied. The highest level of vitamin C was found in non-fertilized soil with significant differences between the increasing rates of nitrogen. β-carotene content increased with increasing...
rates of nitrogen and was found to be statistically significant even at 120 and 180 kgN ha$^{-1}$, as compared to both the control and 60 kgN ha$^{-1}$ rate.

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