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# Impact of Nitrogen Deficiency on Biomass Production, Leaf Gas Exchange, and Betacyanin and Total Phenol Concentrations in Red Beet (*Beta vulgaris* L. ssp. *vulgaris*) Plants

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**Summary**

Red beet (*Beta vulgaris* L. ssp. *vulgaris*) is rich in betacyanins and total phenolics, which are desired by consumers due to their considerable free radical scavenger and antioxidant properties. The aim of the present research was to evaluate the effect of N starvation on accumulation of betacyanins and total phenolics in leaves and roots, as well as biomass production, tissue N concentrations, total chlorophyll content and leaf gas exchange. Leaves of hydroponically cultivated red beet plants subjected to nitrogen deprivation (22.4 mg $l^{-1}$ N in the nutrient solution) over 26 d exhibited a dramatic increase of 262 % in betacyanin concentration, in comparison to adequately-fed plants (224 mg $l^{-1}$ N in the nutrient solution). The corresponding increase of betacyanin concentration in the roots of N-starved red beet was also significantly higher, specifically 225 %. Furthermore, total phenolics concentration under N-starvation conditions increased by 39 % in the leaves and 379 % in the roots, in comparison to standard N supply. These results suggest that the biosynthesis of certain secondary plant metabolites, such as total phenolics and betacyanins, can be stimulated by nutritional stress in red beet plants. Dry leaf and root biomass production in N-deficient plants was significantly restricted by 300 and 250 %, respectively, in comparison with the control plants. The total N concentration decreased by 13 % in leaves and 66 % in roots of N-starved plants. Furthermore, the rates of net $CO_2$-assimilation and transpiration, the stomatal conductance, and the concentration of total chlorophyll, were severely restricted by N- deprivation, indicating that the primary metabolism was severely limited by low nitrogen availability. Our results indicate that, in red beet plants grown under prolonged N-deficiency stress, high amounts of total phenolics and betacyanins accumulate especially in the roots, presumably because the allocation of N to secondary metabolic processes aimed at survival takes strong precedence over N utilization in growth processes.

**Key words.** antioxidant – hydroponics – N-starvation – photosynthesis – secondary plant metabolites

## Introduction

Different environmental stresses (including nutrient deficiency, wounding, pathogens, light, temperature, heavy metals, drought etc.) are known to limit plant growth. Under stress conditions, plants respond by stimulating the biosynthesis and accumulation of secondary plant metabolites, especially plant phenolics and pigments, which seem to exhibit considerable free radical scavenger and antioxidant properties (Rice-Evans et al. 1997; Sudha and Ravishankar 2002). Phenolics and pigments, such as betacyanins are widely distributed secondary metabolites in the plant kingdom. Their accumulation in plants is a striking example of metabolic plasticity against biotic and abiotic stress factors, enabling plants to adapt to changing environments (Boudet 2007; Moreno et al. 2008). The interest in phenolic compounds has increased in the last decade, because of their presumed beneficial effects on human health due to their antioxidative and health protective properties (Rice-Evans et al. 1997). Recent studies have proved that the dietary polyphenolic constituents are more effective antioxidants than vitamin E or C (Vinson et al. 1998; Du Toit et al. 2001) and were, therefore, proposed as potential natural food preservatives (Nychas 1995).

The biosynthesis and accumulation of phenolic compounds in plant tissues is strongly stimulated by nitrogen deficiency (Santsez et al. 2000; Scheible et al. 2004). In contrast, under prolonged nitrogen deprivation, chlorophyll content, net assimilation, plant biomass accumulation and crop productivity are dramatically restricted (Santsez et al. 2000; Leser and Treutter 2005).

The red-violet betacyanins are water-soluble, nitrogen-containing pigments contained in species of the botanical order Caryophyllales and some higher fungi (Stark et al. 2003). Due to their strong antioxidant activity, which has been shown in several chemical and biological assays (Escribano 1998), betalains are considered as potential anticancer and anti-aging natural compounds (Ninfali et al. 1997). Red beet (*Beta vulgaris* L. ssp. *vulgaris*), which is a rich source of betacyanins, is ranked among the ten most efficient vegetables with respect to antioxidant capacity and antiproliferate activity against cancer cells (Vinson et al. 1998; Boivin et al. 2009). Several works addressed the extraction and stability of betacyanins in red beet (Kujala et al. 2000; De Azeredo et al. 2009). Betacyanins can be produced also by employing in vitro systems, specifically callus cultures, cell suspensions and hairy roots transformants of red beet root, but their yield is still lower than that obtained from field-grown red beet (Georgiev et al. 2008).

Under field conditions, the yield and quality of betacyanins obtained from red beet roots is significantly influenced by lack of minerals, drought, salinity, and pathogen attacks (Ramachandra Rao and Ravishankar 2002; Ugrinovic 1999). However, specifically the impact of nitrogen starvation on the concentration of betacyanins and phenolics and their partitioning between leaves and roots of red beet plants has been hardly investigated up to date. Akita et al. (2000) showed that the amount and form of N supplied in the in vitro cell suspensions affects betacyanin accumulation. In intact plants, it seems that there is a physiological trade-off between growth and secondary metabolism imposed by developmental constraints in growing cells, and competition between primary and secondary metabolic pathways in mature cells (Loomis 1932; Herms and Mattson 1992). According to this concept, resource availability, such as N supply level in crop plants, may shift the balance between growth and secondary metabolism. In red beet plants, this shift may considerably influence the accumulation of the secondary metabolites betacyanins and total phenolics. To test this hypothesis in red beet, plant growth parameters and concentrations of these secondary metabolites have to be determined under conditions of both standard and limited N supply.

The main objective of the present study was to determine the influence of nitrogen deprivation on the concentration of betacyanins and total phenolics in red beet plants grown hydroponically. In addition, the impact of nitrogen starvation on biomass production, tissue N concentrations, total chlorophyll content and gas exchange parameters were also investigated. Furthermore, the regulatory mechanisms between accumulation of secondary plant metabolites that are characterized by high antioxidant activity and plant growth under nitrogen starvation conditions are also discussed.

## Materials and Methods

### *Growth conditions*

The experiment was carried out from 18 February until 25 April 2008 in a heated glasshouse located at the Technological Institute of Mesolonghi, in Western Greece. The plants were grown under natural light conditions and the air temperature inside the glasshouse was maintained between 16 and 28 °C during the day and 15 to 21 °C during the night.

Red beet (*Beta vulgaris* L. *ssp. vulgaris*) seedlings grown in peat cubes (4×4×4 cm) were transferred to 6 individual gullies (1.10 m length × 0.10 m width × 0.10 m depth, 15 plants per gully) filled with perlite as soon as the third true leaf had expanded. Each gully, which constituted an experimental unit, was connected to a different storage tank which contained 50 l of nutrient solution (Table 1). Two different N treatments (22.4 and 224 mg $l^{-1}$ $NO_3^-$-N in the nutrient solution) were applied and each of them was in triplicate. The culture mediums were continuously aerated and renewed weekly to prevent nutrient depletion. Drip irrigation was given automatically by using small peristaltic pumps.

### *Gas exchange measurements*

Gas exchange measurements were conducted in four plants per experimental unit from 10 to 20 April 2008, using an LCi Portable Photosynthesis System (ADC BioScientific Ltd.). Specifically, net $CO_2$ assimilation rate (μmol $CO_2$ $m^{-2}$ $s^{-1}$), transpiration rate (mmol $m^{-2}$ $s^{-1}$) and stomatal conductance for $CO_2$ diffusion (mol $m^{-2}$ $s^{-1}$) were conducted in leaves of the same physiological age at the same daytime under identical conditions (incident photon flux density on the leaf surface approximately 1000 μmol $m^{-2}$ $s^{-1}$ and leaf surface temperature 28 °C).

### *Total biomass, nitrogen and chlorophyll determinations*

After completion of the gas exchange measurements, the plants were harvested and separated into leaves and roots, wrapped in plastic bags and transferred immediately to the lab. The harvested plants were at the stage of commercial maturity, both in the control and the N-limited treatment. At that stage the roots of all plants were tuber-

Table 1. Composition of the nutrient solutions in the two different N-supply treatments.

| Macronutrient (mM) | Standard N-supply | Deficient N-supply | Micronutrient (μM) | Standard N-supply | Deficient N-supply |
|---|---|---|---|---|---|
| $KNO_3$ | 8.0 | 0.0 | NaFe-EDTA | 40.0 | 40.0 |
| $KH_2PO_4$ | 1.0 | 1.0 | $MnCl_2$ | 9.0 | 9.0 |
| $K_2SO_4$ | 0.0 | 4.0 | $ZnSO_4$ | 3.0 | 3.0 |
| $MgSO_4$ | 2.0 | 2.0 | $CuSO_4$ | 0.5 | 0.5 |
| $Ca(NO_3)_2$ | 4.0 | 0.8 | $H_3BO_3$ | 45.0 | 45.0 |
| $CaCl_2$ | 1.0 | 4.2 | $Na_2MoO_4$ | 0.4 | 0.4 |

ized. Dry mass was determined after oven-drying leaves and roots at 60 °C until constant weight. The total N content was estimated by the Kjeldahl method, which is based on digestion of dry mass in $H_2SO_4$. Total chlorophyll content was estimated according to SHINANO et al. (1996). Initially, the sampled leaves were washed using deionised water and then 6 cyclic leaflets with a diameter of 10.4 mm were obtained from each sample and weighed. Chlorophyll was extracted using an 80 % acetone solution. The determination of chlorophyll a and b was performed by means of a spectrophotometer (Hitachi U-1000, Tokyo, Japan) at 645 and 663 nm, respectively.

### *Betacyanins and phenolics determination*

The concentrations of total phenolics and betacyanins in fresh leaves and roots were measured in six randomly selected samples per N supply treatment (two samples per experimental unit). The extraction of total phenolics and betacyanins from the plant tissue samples and their quantitative determination were carried out according to MAZZA et al. (1999). Each sample, which consisted of 10 g of fresh plant material, was homogenized at a controlled temperature level of 4 °C using a blender at low speed for 1 min, after addition of 100 ml of extraction solution comprising methanol, formic acid and milli q water (M:F:M) at a ratio of 50:1.5:48.5. After 10 min of incubation, the mixture was centrifuged at 10.000 rpm (type: Centra-MP4R of IEC, USA) for 10 min at 4 °C. The supernatant was then used for the determination of total phenolics and betacyanins via a UV spectrophotometer (UV-1601, Shimadzu Corp., Japan). Standard solutions for the determination of total phenols and betacyanins were prepared by dissolving 10 mg gallic acid (100 g, Sigma, USA), and 500 mg betanin (Red Beet extract diluted with Dextrin, 25 g, TCI Europe nv, Belgium) in 100 ml M:F:W solution to obtain concentrations of 0.59 mM and 9 mM, respectively.

### *Statistics*

The experiment was set as a completely randomized design with each channel comprising one replicate. Statistical analysis was conducted using the statistical package Statgraphics 5.1.Plus (Statistical Graphics Corporation). The significance of differences between treatment means was evaluated by applying one-way analysis of variance ($P \leq 0.05$).

## Results

### *Total phenolics accumulation*

The concentration of total phenolics, expressed as gallic acid equivalents, increased significantly in both the leaves and the roots of the N-stressed red beet plants in comparison with the control plants which were adequately supplied with N (Fig. 1). In particular, after 26 d of N-deprivation, the concentration of total phenolics in the leaves rose by 39 % as compared with that measured in plants grown under unlimited N supply. In the roots of N-deficient plants, the total phenolics concentration increased dramatically to a level that was 379 % higher than that measured in the N replete plants. Furthermore, N-starvation enhanced the exudation of phenolic compounds by the roots of red beet in comparison with standard N-supply, as indicated by measurements in extracts from perlite particles (140 $\mu g\ g^{-1}$ versus 41 $\mu g\ g^{-1}$, respectively).

### *Betacyanin accumulation*

The prolonged deprivation of N resulted in a strong accumulation of betacyanins in red beet plants. In particular, under low nitrogen supply, the betacyanin concentration in plant leaves and roots increased by 262 % and 225 % respectively, in comparison to those measured in plants grown under standard N-supply conditions (Fig. 2).

### *Total biomass, nitrogen and total chlorophyll*

The plants grown under N-limited conditions were very small and their leaves tended to be red violet in color, while those of the plants grown under standard N supply conditions were green with red violet veins.The N-depri-

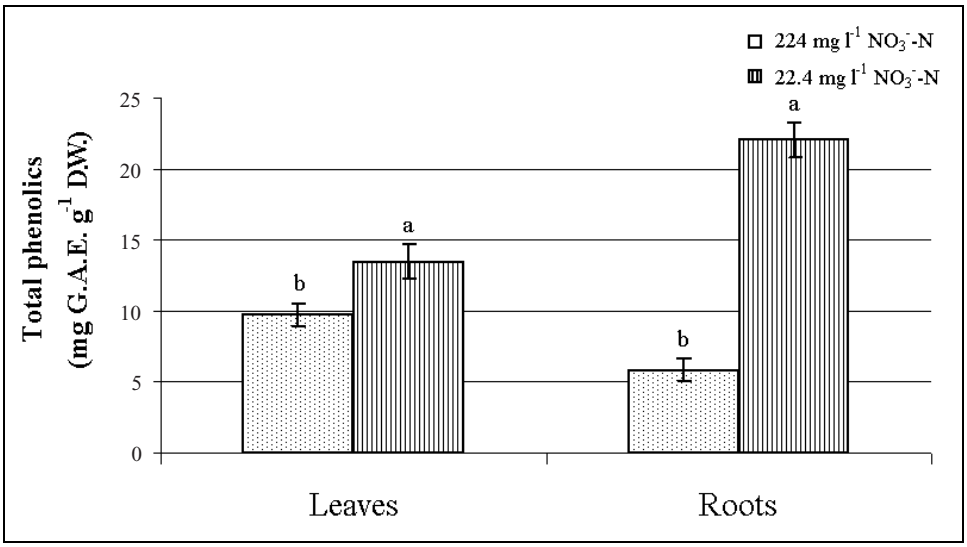


Fig. 1. Impact of N starvation on the accumulation of total phenolics in leaves and roots of hydroponically-grown red beet plants, as influenced by low or adequate N supply (22.4 or 224 mg $l^{-1}$ as $NO_3$-N) via the nutrient solution. Total phenolics are expressed as gallic acid equivalents (GAE). Significant differences between means are indicated by different letters ($P \leq 0.05$).

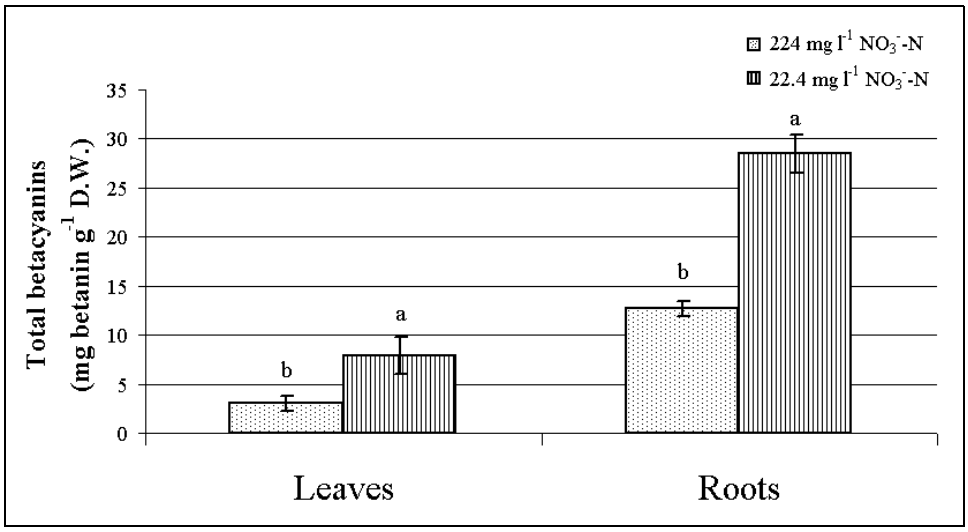


Fig. 2. Impact of N starvation on the accumulation of betacyanins in leaves and roots of hydroponically-grown red beet plants, as influenced by low or adequate N supply (22.4 or 224 mg $l^{-1}$ as $NO_3$-N) via the nutrient solution. For each plant part, significant differences between means are indicated by different letters ($P \leq 0.05$).

vation restricted appreciably the dry biomass accumulation in both leaves and roots by 300 and 250 %, respectively, in comparison with the plants grown under unlimited N supply (Fig. 3). Moreover, the severe limitation in N supply via the nutrient solution restricted significantly the total N concentrations by 13 % in the leaves and 66 % in the roots, in comparison with those measured in the N replete plants (Fig. 4).

The total chlorophyll concentration and the rates of net assimilation and transpiration, as well as the stomatal conductance in the leaves of N-stressed red beet plants were strongly restricted by the limited supply of nitrogen (Table 2). In particular, the limited N supply decreased the total leaf chlorophyll content by 28 %, in comparison with standard N supply. Furthermore, the N-deprivation suppressed the rates of net $CO_2$ assimilation and transpiration, as well as the stomatal conductance were reduced by 66, 70, and 83 %, respectively, in comparison with those determined in N replete plants.

## Discussion

### *Effects of N starvation on total phenolics accumulation*

Our results are in agreement with previous findings which showed that the accumulation of phenolic compounds in plant tissues is often enhanced under conditions of restricted nitrogen nutrition (Mercure et al. 2004; Kováčik and Bačkor 2007). Lower levels of phenolic compounds in leaves and needles of plants grown under high N supply has been reported for many other crop plants, including basil (Nguyen and Niemeyer 2008), apple trees (Leser and Treutter 2005) and potato (Mittelstrass et al. 2006). It is well known that environmental stresses, including nitrogen starvation (Kováčik and Bačkor 2007), may increase the production of reactive oxygen species (ROS). Plants have evolved a complex array of detoxification mechanisms to combat oxidative damage caused by ROS. One of these mechanisms de-

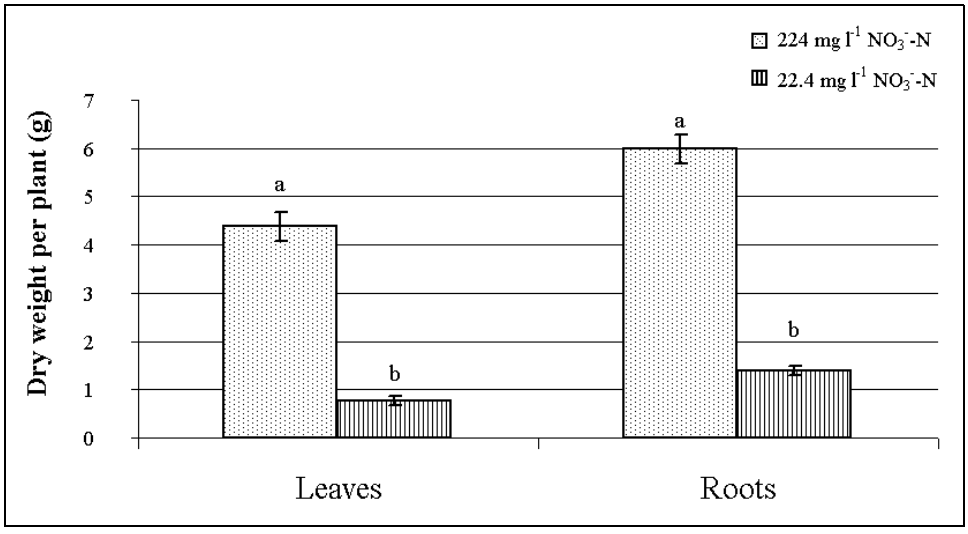


Fig. 3. Dry matter accumulation in the leaves and roots of hydroponically grown red beet plants in response to the level of N supply (solely $NO_3$-N) via the nutrient solution. For each plant part, significant differences between means are indicated by different letters ($P \leq 0.05$).

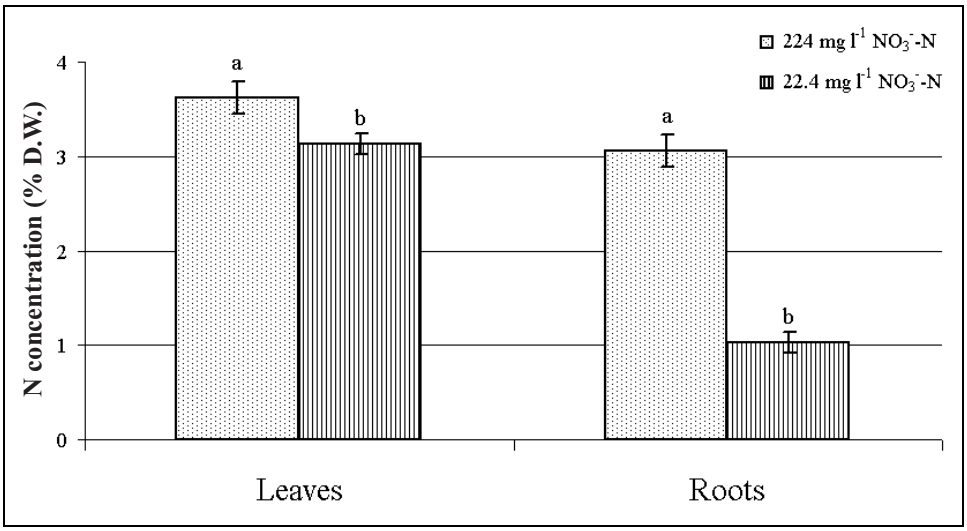


Fig. 4. Impact of N starvation on the total-N concentration in leaves and roots of hydroponically grown red beet plants, as influenced by low or adequate N supply (22.4 or 224 mg $l^{-1}$ as $NO_3$-N) via the nutrient solution. For each plant part, significant differences between means are indicated by different letters ($P \leq 0.05$).

Table 2. Total chlorophyll content and gas exchange parameters in leaves of hydroponically grown red beet, as influenced by N deprivation. N was supplied to the plants via the nutrient solution at two concentration levels, specifically 22.4 and 224 in form of $NO_3$-N.

| $NO_3$-N concentration in the nutrient solution (mg $l^{-1}$) | Total chlorophyll content (µg $cm^{-2}$) | Net $CO_2$ assimilation rate (µmol $m^{-2}$ $s^{-1}$) | Transpiration rate (mmol $m^{-2}$ $s^{-1}$) | Stomatal conductance (mol $m^{-2}$ $s^{-1}$) |
|---|---|---|---|---|
| 224.0 | 38.5 a | 20.97 a | 6.51 a | 0.47 a |
| 22.4 | 27.6 b | 7.12 b | 1.98 b | 0.08 b |

Means followed by different letters within each column differ significantly at $P \leq 0.05$

ploys the high antioxidative capacity of phenolic compounds which act as scavengers of free radicals and other oxidative species (Rice-Evans et al. 1997). In particular, phenols seem to be involved in the detoxification of $H_2O_2$ and other oxygen radicals through their phenolic hydrogen (Rice-Evans et al. 1997). In this cycle, phenolic compounds are oxidized to phenoxyl radicals, which can be subsequently reduced again by ascorbate (Yamasaki and Grace 1998). Hence, it seems that the appreciable increase of total phenolics content in red beet shoot and roots caused by N-starvation stress is aimed at protecting the plant cells from excessive production of ROS. Furthermore, the enhanced production of phenolic compounds under N-deficiency conditions may be facilitated by concomitant release of N-free carbon skeletons that are shunted into the phenylpropanoid metabolism (Xiong et al. 2010).

In our experiment, N starvation raised the accumulation of total phenolics to a much higher level in the roots than in the leaves of red beet plants. Under unlimited N supply, the total phenolics content is lower in the roots than in the leaves, where they function as protective UV-filters (Gould and Lister 2005). Kováčik and Bačkor (2007) also found a stronger increase in the total phenolics content in roots than in leaves of N-deficient *Matricaria chamomilla* as compared with N-replete plants. The more marked response of roots than leaves to N-deficiency with respect to phenol accumulation may be ascribed to the role of phenols as solubilizing agents for different nutrients being unavailable in the soil, thereby facilitating their uptake (Dakora and Phillips 2002). Exudation of phenolic compounds in the rhizosphere has been recorded under both phosphate- and nitrogen-deficiency conditions (Juszczuk et al. 2004). Increased root exudation of phenolic compounds in N-starved red beet plants has been observed also in our experiment.

### *Effects of N starvation on betacyanin accumulation*

The concentration of betacyanin was strongly increased in both leaves and roots of N-starved plants. In cell suspension culture of table beet, the increase of the total N supply above a threshold level reduced the betacyanin content (Akita et al. 2000). The present research revealed that N-deficient red beet plants grown hydroponically accumulate appreciably more betacyanins in their tissues in comparison to N-replete plants. This was surprising, given that betacyanins are nitrogenous pigments. Betalains are rather scarce in nature in comparison to other natural pigments and, therefore, they have not been much explored as bioactive compounds (Azeredo 2009). However, some studies have indicated that betalains are capable of acting as potent antioxidants both in vitro and in vivo (Wettasinghe et al. 2002; Moreno et al. 2008). The biosynthetic pathway of the nitrogen-containing water-soluble betalains, which are derived from the amino

acid tyrosine, is only partially understood (Stark et al. 2003; Tanaka et al. 2008). The antioxidative ability of betacyanins is ascribed to their cyclic amine, which is similar to that of the antioxidant ethoxyquine (Lin and Olcott 1995). The profound increase in the concentrations of betacyanins in both shoot and root of red beet under conditions of limited N supply, in addition to its possible relevance to other physiological processes, constitutes also a stress adaptation aimed at increasing the antioxidant potential inside the cells. This response indicates that red beet plants exposed to severe nutrient stress set survival in much higher order of precedence than growth when allocating the limited nutrient sources to the different metabolic functions. As stated by Scheible et al. (2004), N-starvation imposes a wide reprogramming between primary and secondary plant metabolism, by induction of many genes controlling functions of the secondary metabolism, while repressing genes assigned to functions related to plant growth and development.

In previous studies, it has generally been asserted that N-deficiency induces a wide re-allocation between primary and secondary plant metabolism (Scheible et al. 2004) by stimulating phenolic biosynthesis and accumulation and depressing plant growth and development in several crops (Leser and Treutter 2005; Kováčik and Bačkor 2007; Nguyen and Niemeyer 2008). In the present study, the prolonged nitrogen deprivation exhibited negative effects on biomass accumulation, both in roots and leaves, in agreement with previous findings (Santsez et al. 2000; Leser and Treutter 2005). Under low nitrogen supply, in accordance with the findings of this study, the limited growth is strongly associated with the reduction in chlorophyll content and photosynthetic characteristics (Bettmann et al. 2006). Comparable decreases were found in stomatal conductance and transpiration, suggesting strong stomatal regulation of gas exchange under N stress (Maslova et al. 2010).

Many hypotheses have been put forward to explain patterns and variations in the concentration of carbon-based secondary compounds in plant tissues, according to the availability of resources. Most studied are the carbon-nutrient balance hypothesis (CNBH) (Mckey 1979), and the growth-differentiation balance hypothesis (GDBH) (Herms and Mattson 1992). Both of them suggest that plants continuously make an effective use of costly versus beneficial investments towards defence versus growth processes, the trade-off being mainly conditioned by resource availability, such as N and P (Le Bot et al. 2009). Our results are in accordance with these considerations.

The results of the present research demonstrate that hydroponically-cultivated red beet plants accumulated high amounts of total phenolics and betacyanins under prolonged N-stress, especially in the roots. Since hydroponics enables rapid and efficient manipulation of the nutrient supply, this cropping system might be utilized for large-scale production of plants that are rich in health promoting secondary metabolites such as betalains. Nevertheless, further research is needed to develop a scheme of N supply that can maximize their production by efficiently balancing the two contrasting consequences of reduced N supply, namely growth restriction and enhanced phenol and betacyanin concentrations. In addition to the level of N supply, the form of N provided to in vitro cell suspensions of red beet also affected betacyanin accumulation (Akita et al. 2000). Hence, future research work concerning the impact of N supply on the accumulation of total phenols and betacyanins in red beet crops needs to address their responses not only to the N fertilization dosage, but also to the N source (ammonium-N *vs.* nitrate-N).

## References


Akita, T., Y. Hina and T. Nishi 2000: Production of betacyanins by a cell suspension culture of table beet (*Beta vulgaris* L.). Biosci. Biotechol. Biochem. **64**, 1807–1812.

Azeredo, H.M.C. 2009: Betalains: properties, sources, applications, and stability – a review. International J. Food Sci. Technol. **44**, 2365–2376.

Bettmann, G.T., H.H Ratnayaka, W.T. Molin and T.M. Sterling 2006: Physiological and antioxidant responses of cotton and spurred anoda (*Anoda cristata*) under nitrogen deficiency. Weed Sci. **54**, 641–650.

Boivin, D., S. Lamy, S. Lord-Dufour, J. Jackson, E. Beaulieu, M. Côté, A. Moghrabi, S. Barrette, D. Gingras and R. Béliveau 2009: Antiproliferative and antioxidant activities of common vegetables: A comparative study. Food Chem. **112**, 374–380.

Boudet, A.M. 2007: Evolution and current status of research in phenolic compounds. Phytochemistry **68**, 2722–2735.

Dakora, F.D. and D.A. Phillips 2002: Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant Soil **245**, 35–47.

De Azeredo, H.M.C., A.C. Pereira, A.C.R. De Souza, S.T. Gouveia and K.C.B. Mendes 2009: Study on efficiency of betacyanin extraction from red beetroots. Intern. J. Food Sci. Technol. **44**, 2464–2469.

Du Toit, R., Y. Volsteedt and Z. Apostolides 2001: Comparison of the antioxidant content of fruits, vegetables and tea measured as vitamin C equivalents. Toxicology **166**, 63–69.

Escribano, J. 1998: Characterization of the antiradical activity of betalains from *Beta vulgaris* L. roots. Phytochem. Anal. **9**, 124–127.

Georgiev, V., M. Ilieva, T. Bley and A. Pavlov 2008: Betalain production in plant in vitro systems. Acta Physiol. Plantarum **30**, 581–593.

Gould, K. and C. Lister 2005: Flavonoid functions in plants. In: Andersen O.M. and K.R. Markham (eds.): Flavonoids Chemistry, Biochemistry and Application. Taylor and Francis, Boca Raton, FL, USA. pp. 397–442.

Herms, D.A. and W.J. Mattson 1992: The dilemma of plants: to growth or to defend. The Quaterly Rev. Biol. **67**, 283–335.

Juszczuk, I.M., A. Wiktorowska, E. Malusá and A.M. Rychter 2004: Changes in the concentration of phenolic compounds and exudation induced by phosphate deficiency in bean plants. (*Phaseolus vulgaris* L.). Plant Soil **267**, 41–49.

Kováčik, J. and M. Bačkor 2007: Changes of phenolic metabolism and oxidative status in nitrogen-deficient *Matricaria chamomilla* plants. Plant Soil **297**, 255–265.

Kujala, T.S., J.M. Loponen, K.D. Klika and K. Pihlaja 2000: Phenolics and betacyanins in red beetroot (*Beta vulgaris*) root: Distribution and effect of cold storage on the content of total phenolics and three individual compounds. J. Agric. Food Chem. **48**, 5338–5342.

Le Bot, J.L., C. Bénard, C. Robin, F. Bourgaud and S. Adamowicz 2009: The 'trade-off' between synthesis of primary and secondary compounds in young tomato leaves is altered by nitrate nutrition: Experimental evidence and model consistency. J. Exp. Bot. **60**, 4301–4314.

Leser, C. and D. Treutter 2005: Effects of nitrogen supply on growth, contents of phenolic compounds and pathogen (scab) resistance of apple trees. Physiol. Plant. **123**, 49–56.

Lin, J.C. and H.S. Olcott 1995: Ethoxyquin nitroxide. J. Agric. Food Chem. **23**, 798–800.

Loomis, W.E. 1932: Growth-differentiation balance vs. carbohydrate-nitrogen ratio. Proc. Am. Soc. Hortic. Sci. **29**, 240–245.

Maslova, S.P., G.N. Tabalenkova and T.K. Golovko 2010: Respiration and nitrogen and carbohydrate contents in perennial rhizome-forming plants as related to realization of different adaptive strategies. Russian J. Plant Physiol. **57**, 631–640.

Mazza, G., L. Fukumoto, P. Delaquis, B. Girard and B. Ewert 1999: Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Columbia. J. Agric. Food Chem. **47**, 4009–4017.

Mckey, D. 1979: The distribution of secondary compounds within plants. In: Rosenthal, G.A. and D.H. Janzen (eds.): Herbivores. Their Interactions with Secondary Plant Constituents. Academic Press, New York, USA, pp. 55–133.

Mercure, S.-A., B. Daoust and G. Samson 2004: Causal relationship between growth inhibition, accumulation of phenolic metabolites, and changes of UV-induced fluorescences in nitrogen-deficient barley plants. Can. J. Bot. **82**, 815–821.

Mittelstrass, K., D. Treutter, M. Plessl, W. Heller, E.F. Elstner and I. Heiser 2006: Modification of primary and secondary metabolism of potato plants by nitrogen application differentially affects resistance to *Phytophthora infestans* and *Alternaria solani*. Plant Biol. **8**, 653–661.

Moreno, D.A., C. Garcia-Viguera, J.I. Gil and A. Gil-Izquierdo 2008: Betalains in the era of global agri-food science, technology and nutritional health. Phytochem. Rev. **7**, 261–280.

Nguyen, P.M. and E.D. Niemeyer 2008: Effects of nitrogen fertilization on the phenolic composition and antioxidant properties of basil (*Ocimum basilicum* L.). J. Agric. Food Chem. **56**, 8685–8691.

Ninfali, P., M. Bacchiocca, A. Antonelli, E. Biagiotti, A.M. Di Gioacchino, G. Piccoli, V. Stocchi and G. Brandi 1997: Characterization and biological activity of the main flavonoids from Swiss Chard (*Beta vulgaris* subsp. *cicla*). Phytomedicine **14**, 216–221.

Nychas, G.J.E. 1995: Natural antimicrobials from plants. In: Gould, G.W. (ed.): New Methods of Food Preservation. Blackie Academic Professional, London, pp. 58–89.

Ramachandra Rao, S. and G.A. Ravishankar 2002: Plant cell cultures: Chemical factories of secondary metabolites. Biot. Advan. **20**, 101–153.

Rice-Evans, C.A., N.J. Miller and G. Paganga 1997: Antioxidant properties of phenolic compounds. Trends in Plant Science **2**, 152–159.

Santsez, E., J.M. Soto, P.C. Garcia, L.R. Lopez-Lefebre, R.M. Rivero, J.M. Ruiz and L. Romero 2000: Phenolic and oxidative metabolism as bioindicators of nitrogen deficiency in French bean plants (*Phaseolus vulgaris* L. cv. strike). Plant Biol. **2**, 272–277.

Scheible, W.-R., R. Morcuende, T. Czechowski, C. Fritz, D. Osuna, N. Palacios-Rojas, D. Schindelasch and M. Stitt 2004: Genome-wide reprogramming of primary and secondary metabolism, protein synthesis, cellular growth processes, and the regulatory infrastructure of arabidopsis in response to nitrogen. *Plant Physiol.* **136**, 2483–2499.

Shinano, T., T.T. Lei, T. Kawamukai, M.T. Inoue, T. Koike and T. Tadano 1996: Dimethylsulfoxide method for the extraction of chlorophylls a and b from the leaves of wheat, field bean, dwarf bamboo, and oak. Photosynthetica **32**, 409–415.

Stark, D., T. Vogt and W. Schliemann 2003: Recent advances in betalain research. Phytochemistry **62**, 247–269.

Sudha, G. and G.A. Ravishankar 2002: Involvement and interaction of various signaling compounds on the plant metabolic events during defence response, resistance to stress factors, formation of secondary metabolites and their molecular aspects. Plant Cell, Tissue Organ Cult. **71**, 181–212.

Tanaka, Y., N. Sasaki and A. Ohmiya 2008: Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. The Plant Journal **54**, 733–749.

Ugrinovic, K. 1999: Effect of nitrogen fertilization on quality and yield of red beet (*Beta vulgaris* var. *conditiva* Alef). Acta Hort. **506**, 99–104.

Vinson, J.A., Y. Hao, X. Su and L. Zubik 1998: Phenol antioxidant quantity and quality in foods: Vegetables. J. Agric. Food Chem. **46**, 3630–3634.

Wettasinghe, M., B. Bolling, L. Plhak, H. Xiao and K. Parkin 2002: Phase II enzyme-inducing and antioxidant activities of beetroot (*Beta vulgaris* L.) extracts from phenotypes of different pigmentation. J. Agric. Food Chem. **50**, 6704–6707.

Xiong, J., H.B. Wang, L. Qiu, H.W. Wu, R.S. Chen, H.E. He, R.Y. Lin and W.X. Lin 2010: QRT-PCR analysis of key enzymatic genes related to phenolic acid metabolism in rice accessions (*Oryza sativa* L.) exposed to low nitrogen treatment. Allelopathy J. **25**, 345–356.

Yamasaki, H. and S.C. Grace 1998: EPR detection of phytophenoxyl radicals stabilized by zinc ions: evidence for the redox coupling of plants phenolics with ascorbate in the $H_2O_2$- peroxidase system. FEBS Letters **422**, 377–380.

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