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Full Length Article

Diverse Responses of Tomato to N and P Deficiency

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ABSTRACT

Tomato plants were grown hydroponically under different deficient regimes of nitrogen (N) and phosphorus (P) in the form of nitrate and phosphate. Results showed a severe reduction in growth, soluble protein and chlorophylls contents under N deficiency, which suggested accelerating of leaf senescence. Furthermore, it was accompanied by enhanced activity of peroxidase and sugars contents that were both correlated with increased shoot anthocyanins content. Under P deficiency, similar results were observed except reduced concentration of sugars and chlorophylls and that both peroxidase activity and anthocyanins contents were higher in P-deficient plants. On the whole, tomato showed reduced tolerance to N than P deficiency, although N deficiency was more damaging. Reduced content of soluble proteins was an important criterion of N and P tolerance. Plant tolerance to low P supply was accompanied by increased activity of peroxidases and accumulation of anthocyanins.

Key Words: Macronutrients; Limitation; Growth; Biochemical characteristics; Tomato

INTRODUCTION

Nitrogen (N) and phosphorus (P) are two essential macronutrients in plants. Their deficiency as well as excess quantities may change plant functions (Glass *et al*., 2002; Mahmud *et al*., 2003; Taghavi *et al*., 2004; Montemurro *et al*., 2007). Also because of acid rain falls in industrial regions and extensive agriculture, N is still one of major factor limiting crop yield, which necessitates application N fertilizers. Although sufficient supply of N to crops is fundamental to optimize N application, it can result in contamination of groundwater (Jaynes *et al*., 2001). Likewise, the concentration of P as phosphate is often low (2 to 10 µM; Raghothama, 1999); thus its deficiency is considered as a major abiotic stress that limits growth and productivity of plants throughout the world (Sanchez & Salinas, 1981). Application of inorganic P fertilizers is usually recommended for enhancing soil P availability and crop yields. However, it is problematic for both intensive and extensive agriculture (Vance *et al*., 2003). Furthermore, excess P can pollute local water-courses (Withers *et al*., 2001).

Plants have evolved several strategies for acquisition of N and P from the topsoil (Lynch & Brown, 2001; Jiang *et al*., 2007) or modification of carbon metabolism (Vance *et al*., 2003). In addition the response of plant photosynthesis and growth to N limitation has been demonstrated to be different from the response to P limitation (De Groot *et al*., 2001; De Groot *et al*., 2003). However, little work has been reported for changes in physiological and biochemical characteristics under N and P deficiencies. The present study was undertaken to assess the effects of different deficiency levels of individually applied N and P on the growth and a range of biochemical characteristics of tomato (*Lycopersicon esculentum* Mill.) vegetative plant and also to develop relationships between leaf anthocyanin pigment, an important sign of N and P deficiency and some other metabolic characteristics.

MATERIALS AND METHODS

Experimental details. Tomato seeds (*Lycopersicon esculentum* Mill. cv. Urbana V.F.) were obtained from Falaat Company, Tehran, Iran. The seeds were sterilized in 1% (w/v) sodium hypochlorite (2 min) and washed 5 times with sterile distilled water. Then, they were transferred to petri dishes in darkness at 25°C for germination. Six days old seedlings were transferred to pots containing sterilized sands under a light density of approximately 100 µmol m^{-2} s^{-1}, day/night temperatures of 26±1/17±1°C under a 16 h photoperiod. Plants were grown in half–strength Hogland's nutrient solution for 10 days. At 4th leaf stage, plants were treated with 3.75 and 3.25 mM of KNO_3, defined as 25% and 35% nitrate deficiency, respectively compared to complete solution (5 mM KNO_3) or 0.75 and 0.65 mM of KH_2PO_4, defined as 25% and 35% phosphate deficiency respectively compared to complete solution (1 mM KH_2PO_4), for 23 days before being harvested. Nutrient solutions were changed twice a week and the pH was adjusted to 6.5–6.8 regularly performed at 48 day interval.

Growth assay. Harvested plants were divided into leaves, stems and roots and their fresh weight were determined and leaf area (LA) and specific leaf area (SLA) were measured. Other growth characteristics including root to shoot dry weight, relative growth rate (RGR), shoot (SHTI), root (RTI) and total (TTI) tolerance index of plants were measured.

Biochemical assays. Soluble protein content was measured according to the method of Bradford (1979) and activity of peroxidase was determined according to Sudhakar *et al*. (2001). Soluble and insoluble sugars contents were determined according to method of Hellebust and Craigie (1978). The concentration of chlorophylls were estimated according to Arnon (1949) and leaf and stem anthocyanin contents were estimated spectrophotometrically according to method of Diaz *et al*. (2006) using cyanidin-3-glucoside (Kuromanin chloride) as a standard.

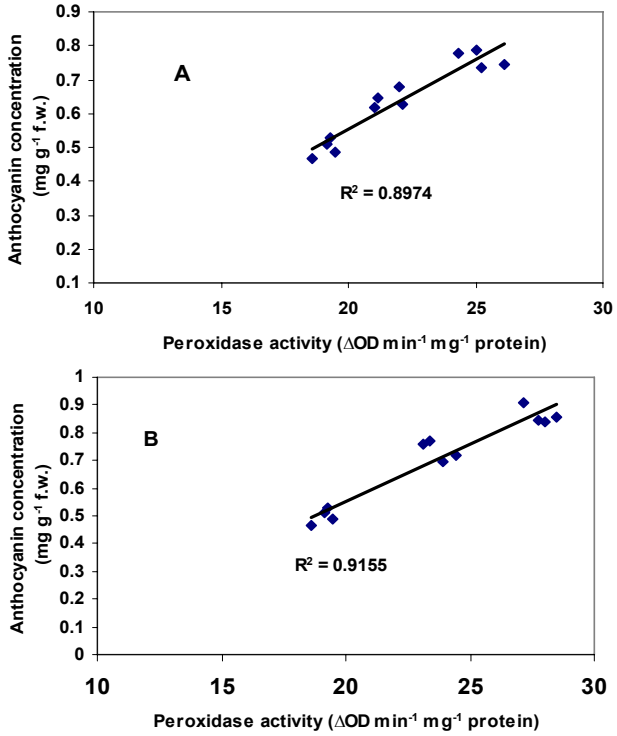
Statistical analysis. The research was conducted using completely randomized design with four replications. Data were analyzed using SAS software.

RESULTS AND DISCUSSION

Growth characteristics. The response of plant growth to N deficiency differed from the response to P deficiency, which was most likely due to their different physiological roles. Compare to control plants, decreasing the N and P supply at both levels (25 & 35%) reduced most growth characteristics (Tables I & II). Root to shoot dry weight ratio increased in both N and P deficiency treatments (Table II). LA, SLA, shoot and root dry mass and also RGR (Table I) decreased under phosphate deficiency, because less structural biomass was produced. However, N deficiency suppressed total plant biomass production, because N deficiency was associated with reduction in both LA and leaf photosynthetic capacity (Zhao *et al*., 2005). Hence, greater reduction in plant growth could be observed under N deficiency. By contrast, root dry mass was not affected much by N- and P-deficiency treatments. P-deficient roots indicated increased root to shoot dry weight ratio (Wissuwa *et al*., 2005). Plant tolerance indexes for analyzed for plant production showed reduced tolerance of tomato plants to P and especially N deficiencies. This might be due to greater effect of N on growth, productivity and protein synthesis in plants.

Biochemical characteristics. Soluble and insoluble sugars contents in the leaves significantly increased (Table IV) in nitrate-deficient plants, which was consistent with studies on *Glycine max* (Rufty *et al*., 1988) and *Solanum lycopersicum* (Urbanczyk-Wochniak & Fernie, 2005). The activities of carbon (C) and N assimilation are closely related to the rates of plant and development. Results showed that by decreased P application decreased the concentrations of sugars in leaves significantly (Table IV). In tomato plants cultivar Capita, a decrease in starch and soluble sugars concentration, with decreasing P application has been observed (De Groot *et al*., 2003). In these tomato plants, P

Fig. 1. Relationship between anthocyanin accumulation and peroxidase activity of tomato leaves grown in nitrate-deficient (A) or phosphate-deficient (B) solution (n=4)



deficiency plausibly affected photosynthesis through charges in the activity of calvin cycle enzymes, which in turn reduce CO_2 fixation and carboxylation capacity (Pieters *et al*., 2001). Results of this study with tomato cv. Urbana are also in conformity with these findings.

Peroxidase activity on a soluble protein basis in leaves significantly (Table III) increased in all nutrient-deficient treatments, which was higher in P-deficient plants. Also the soluble protein content of leaves significantly decreased both under N and P deficiencies (Table III). Concentration of chlorophylls (a, b & total) were significantly decreased under N-deficiency treatments (Table IV), whereas in P-deficient plants content of chlorophylls did not significantly change. However, total chlorophylls were significantly decreased in plants grown in treatments defined as 35% phosphate solution (Table IV). It appears that high peroxidase activity may be induced to protect cells from generated free radicals. Studies have shown that N deficiency stress leads to the increase in excitation pressure in PSII centers, subsequent overproduction of reactive oxygen species (ROS) so activity of a scavenger system such as perodixase as an antioxidant enzyme per mg^{-1} protein increases (De Groot & Rauen, 1998). Nitrogen deficiency accelerates leaf senescence and production of ROS, which leads to degradation of macromolecules like proteins and chlorophylls in plant (Crafts–Brandner, 1992). Also due to the pivotal role of N in protein biosynthesis, it is

Table I. Comparison of some growth characteristics as influenced by different nutrient treatments

Nutrient treatments	RGR (g kg^{-1} d^{-1})	LA (cm^2)	SLA (cm^2 g^{-1})	Root dry weight (g $plant^{-1}$)	Shoot dry weight (g $plant^{-1}$)
Control	274.75±1.93 a	198.31±1.78 a	346.87±3.78 a	0.09±0.00 a	0.89±0.01 a
25%Nitrate	128.50±3.80 c	97.00±1.70 d	272.03±2.48 d	0.08±0.00 c	0.57±0.00 c
35%Nitrate	108.00±3.76 d	65.695±1.24 e	224.95±2.41 e	0.07±0.00 d	0.45±0.00 d
25%Phosphate	174.50±1.62 b	138.97±1.15 b	324.23±2.66 c	0.08±0.00 b	0.68±0.00 b
35%Phosphate	116.70±4.41 d	124.43±1.17 c	333.70±3.03 b	0.09±0.00 b	0.58±0.00 c

Means sharing same letter differ non-significantly

Table II. Comparison of different growth characteristics as influenced by different nutrient treatments

Nutrient treatments	SHTI	RTI	TTI	Root to Shoot d.w. Ratio
Control	1.00±0.00 a	1.00±0.00 a	1.00±0.00 a	0.11±0.00 e
25%Nitrate	0.64±0.01 d	0.82±0.01 c	0.65±0.00 d	0.14±0.00 c
35%Nitrate	0.51±0.01 e	0.73±0.00 d	0.53±0.00 e	0.16±0.00 a
25%Phosphate	0.76±0.00 b	0.90±0.00 b	0.77±0.00 b	0.13±0.00 d
35%Phosphate	0.65±0.00 c	0.92±0.01 b	0.67±0.00 c	0.15±0.00 b

Means sharing same letter differ non-significantly

Table III. Comparison of some metabolic characteristics as influenced by different nutrient treatments

Nutrient treatments	Leaf Anthocyanins (mg g^{-1} fresh weight)	Stem Anthocyanins (mg g^{-1} fresh weight)	Leaf soluble protein (mg g^{-1} fresh weight)	Leaf peroxidase activity (ΔOD min^{-1} mg^{-1} protein)
Control	0.49±0.01d	0.29±0.02d	94.16±1.05a	19.12±0.19e
25%Nitrate	0.64±0.01c	0.41±0.01c	61.48±0.42d	21.58±0.28d
35%Nitrate	0.76±0.01b	0.54±0.01b	47.94±0.53e	25.17±0.38b
25%Phosphate	0.73±0.01b	0.51±0.01b	85.34±0.55b	23.70±0.28c
35%Phosphate	0.86±0.01a	0.65±0.01a	72.96±0.73c	27.85±0.28a

Means sharing same letter differ non-significantly

Table IV. Comparison of different metabolic characteristics as influenced by different nutrient treatments

Nutrient treatments	Leaf chl. a (mg g^{-1} fresh weight)	Leaf chl. b (mg g^{-1} fresh weight)	Leaf total chl. (mg g^{-1} fresh weight)	Leaf soluble sugars (mg g^{-1} dry weight)	Leaf insoluble sugars (mg g^{-1} dry weight)
Control	0.62±0.00a	0.41±0.00a	0.88±0.00a	36.39±0.26c	30.99±0.45c
25%Nitrate	0.41±0.00b	0.36±0.00b	0.65±0.00c	42.32±0.28b	36.43±0.42b
35%Nitrate	0.28±0.00c	0.31±0.01c	0.49±0.00d	50.81±0.52a	43.49±0.40a
25%Phosphate	0.62±0.00a	0.41±0.00a	0.87±0.00ab	28.06±0.12d	22.97±0.44d
35%Phosphate	0.61±0.00a	0.40±0.00a	0.86±0.00b	21.99±0.51e	17.12±0.16e

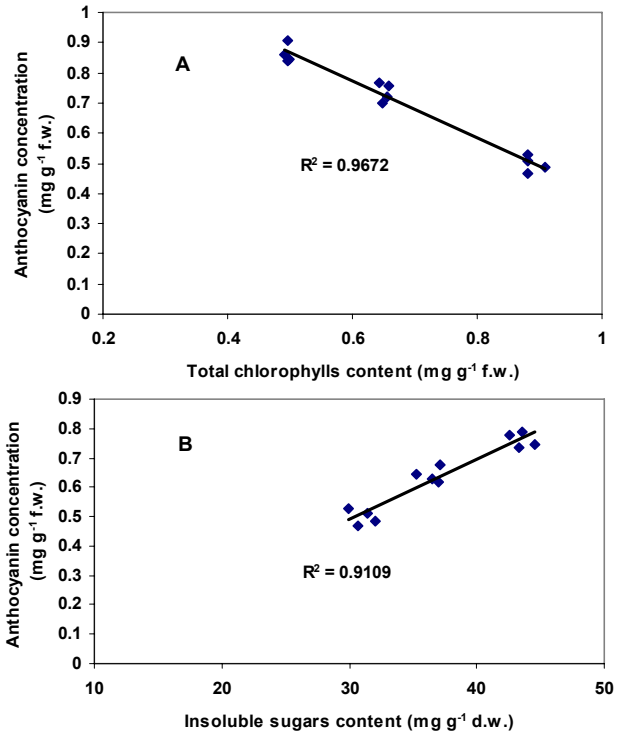
Means sharing same letter differ non-significantly

not surprising that N deficiency could reduce amino acids and proteins, as has been observed in *Solanum lycopersicum* (Urbanczyk-Wochniak & Fernie, 2005) and *Oryza sativa* (Huang *et al*., 2004).

Soluble proteins decreased under low P supply, because its deficiency decreases phosphorylation of different metabolic reactions in protein biosynthesis. Leaf chlorophylls contents decreased in N-deficient plants due to their reduced synthesis. Similar effects has been observed in *Oryza sativa* (Huang *et al*., 2004) and *Sorghum bicolor* (Zhao *et al*., 2005) under N-deficient condition, whereas P deficiency did not affect chlorophylls in *Arabidopsis* (Ticconi *et al*., 2001) and *Phaseolus vulgaris* (Lima *et al*., 2000). It seems that the rate of leaf area development is slower than chlorophyll biosynthesis, so that their concentrations per area leaf increase. As compared to control leaf and stem tissues, anthocyanins increased significantly in all N-deficient treatments (Table III). The results showed that anthocyanin accumulation was inversely related with N and P availability in shoot tissues of tomato resulting in the highest anthocyanin content of 35% P-deficient plants. Bongue–Bartelsman and Phillips (1995) displayed accumulation of anthocyanins as petunidin in N–deficient tomato leaves using a HPLC–based approach. Increased levels of anthocyanins as peonine were demonstrated in bean leaves under phosphate deficiency (Juszczuk *et al*., 2004). The present results were consistent with above studies and hypothesis that flavonoids accumulate under nitrogen deficiency due to induction of genes in the flavonoid biosynthetic pathway (Margna *et al*., 1989).

Another purpose was to determine probable relationships between anthocyanin accumulation and other metabolic processes. Anthocyanin accumulation was accompanied by enhanced peroxidase activity in both N– and P–deficient plants (Fig. 1A & 1B). Zhou *et al.* (2002) displayed higher peroxidase activity in the calli of *Prunus incise* but little has been reported regarding these relations especially on whole plant level. On the other hand, N deficiency led to overproduction of reactive oxygen species (ROS), while the enhanced peroxidase activity and the anthocyanin accumulation helped the cells to overcome this stress. In this study, highest content of anthocyanin, in 35%

Fig. 2. Relationship between anthocyanin accumulation and total chlorophylls concentration (A) and anthocyanin accumulation and insoluble sugars contents (B) of tomato leaves grown in nitrate-deficient solution (n=4)



phosphate-deficient plant leaves, was accompanied by highest activity of peroxidase (Table III). Furthermore, in N-deficient plants, increased accumulation of anthocyanins was accompanied by decreased content of total chlorophylls, although these changes were more explicit in the former. The anthocyanins increased to protect the senescing leaves against external damages especially caused by UV radiation (Fig. 2A). In addition, in N–limiting condition, leaf anthocyanin concentration was correlated with insoluble sugar content (Fig. 2B). It has been shown that under nitrogen limitation, sugars increased by decreased utilization of assimilate (De Groot *et al*., 2003). More work is needed to explain the correlation between soluble sugars and anthocyanin accumulation under N and P deficiency focusing on biochemical basis.

CONCLUSION

There were diverse effects of N and P deficiency on the growth and metabolic characteristics of vegetative tissues of tomato. On the whole, tomato showed reduced tolerance to N than P deficiency. Further studies are needed to determine whether other metabolic processes and antioxidants could regulate anthocyanin accumulation under different nutrient stresses in order to enhance their content especially in vegetative crops with health benefits.

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