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ORIGINAL ARTICLE

The Effects of Nitrate and Phosphate Deficiencies on Certain Biochemical Metabolites in Tomato (*Lycopersicon esculentum* Mill. c.v. Urbana V.F.) Plant

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Nitrogen (N) and phosphorus (P) are two important macronutrients with diverse functions in plants. Therefore, the effects of their deficiencies on different physiological and biochemical characteristics especially in crops have always been investigated. In this study, the effects of nitrate and phosphate deficiencies in two levels of 25% and 35% deficiencies compared to control plants were studied in *Lycopersicon esculentum* Mill. Results were analyzed statistically that showed a significant increase of root soluble and insoluble sugars and peroxidase activity and a significant decrease of root soluble proteins in both levels of nitrate and phosphate deficiencies which have less been studied. Furthermore, reverse relationships between soluble sugars and soluble proteins (r^2=0.996) and between insoluble sugars and soluble proteins (r^2=1) under nitrate deficiencies were developed. Also, by decreasing nitrate, β-caroten and xanthophyll contents decreased. By decreasing phosphate, concentration of β-caroten diminished but xanthophyll contents were not affected significantly. On the whole, biochemical characteristics were affected more in nitrate-deficient treatments in tomato plants than those of control plants.

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Nitrogen (N) and phosphorus (P) are two essential macronutrients to crops which improve their growth, yield and product quality (Togun *et al.* 2004; Chen *et al.* 2007). Nitrate and ammonium are two major sources of N for plants and their uptake occurs at the root level via specific transporters (Togun *et al.* 2004; Chen *et al.* 2008; Yin *et al.* 2006; Masclaux-Daubresse *et al.* 2010). However, plants uptake the majority of their N from the assimilation of nitrate and subsequent reduction to ammonium which then incorporated into amino acids that are necessary for protein synthesis (Sohlenkamp *et al.* 2002; Urbanczyk-Wochniak and Fernie, 2005; Masclaux-Daubresse *et al.* 2010). Also, it has been demonstrated that nitrate has both nutrient and signal metabolite functions which are important in plant metabolism, photosynthesis and growth (Glass *et al.* 2002; Urbanczyk-Wochniak and Fernie, 2005).

Likewise, P as an essential macronutrient for all living organisms, is a limiting factor for crop productivity (Franco-Zorrilla *et al.* 2004; Chen *et al.* 2008). P is taken up by plants from soil preferentially in the orthophosphate forms ($H_2PO_4^-$ and $HPO_4{}^{2-}$) by specific phosphate transporters (Vance *et al.* 2003; Chen *et al.* 2008). P moves symplastically from the root surface to the xylem and then to the cell cytoplasm and from cytoplasm to vacuole of the above-ground organs (Vance *et al.* 2003). This macronutrient has been found in essential molecules such as ATP, nucleic acids and phospholipids. Also, it is important in metabolic processes such as energy transfer, protein activation and carbon (C) metabolism (Wu *et al.* 2003).

Therefore, low availability of these two macronutrients is a major constraint for crop growth and production (Lo´pez-Bucio *et al.* 2003; Chen *et al.* 2008). Falling acid rains in industrial regions and subsequent nitrate leaching results in reduced N concentrations in soil transporters (Khavari-Nejad *et al.* 2009; Masclaux-Daubresse *et al.* 2010). Also, because of insoluble complexes of P with cations in acid-weathered soils, little phosphate is available to plants in most soils (Hammond and White, 2008; Turner, 2008; Vance, 2003). So, plants have evolved developmental and biochemical adaptations to low concentration of N and P in soil (Franco-Zorrilla *et al.* 2004; Masclaux-Daubresse *et al.* 2010). However, most investigations have been done on overground organs and less has been reported in roots. Plant roots sensing and adaptation to changes in the nutrient is important. Also, this organ performs many essential functions such as nutrient uptake and it is important to investigate different biochemical changes of it under nutrient stress of the rhizosphere (Lo´pez-Bucio *et al.* 2003; Shin *et al.* 2005).

In this study, certain biochemical characteristics of tomato (*Lycopersicon esculentum* Mill.) roots and leaves in response to nitrate and phosphate deficiencies have been evaluated. Also, relationships between some of these biochemical parameters were developed.

MATERIALS AND METHODS

Plant materials and treatments. 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 26/17 °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 (l 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. After 42 days of experimental period, for biochemical analysis plants were harvested.

Biochemical assays. Root 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 the method of Hellebust and Craigie (1978). The concentration of leaf chlorophylls were estimated according to Arnon (1949) spectrophotometrically and activity of peroxidase was determined according to Sudhakar *et al*. (2001).

Statistical analysis. The research was conducted using completely randomized design with four replications. Data were analyzed by the analysis of variance (ANOVA) using SAS software.

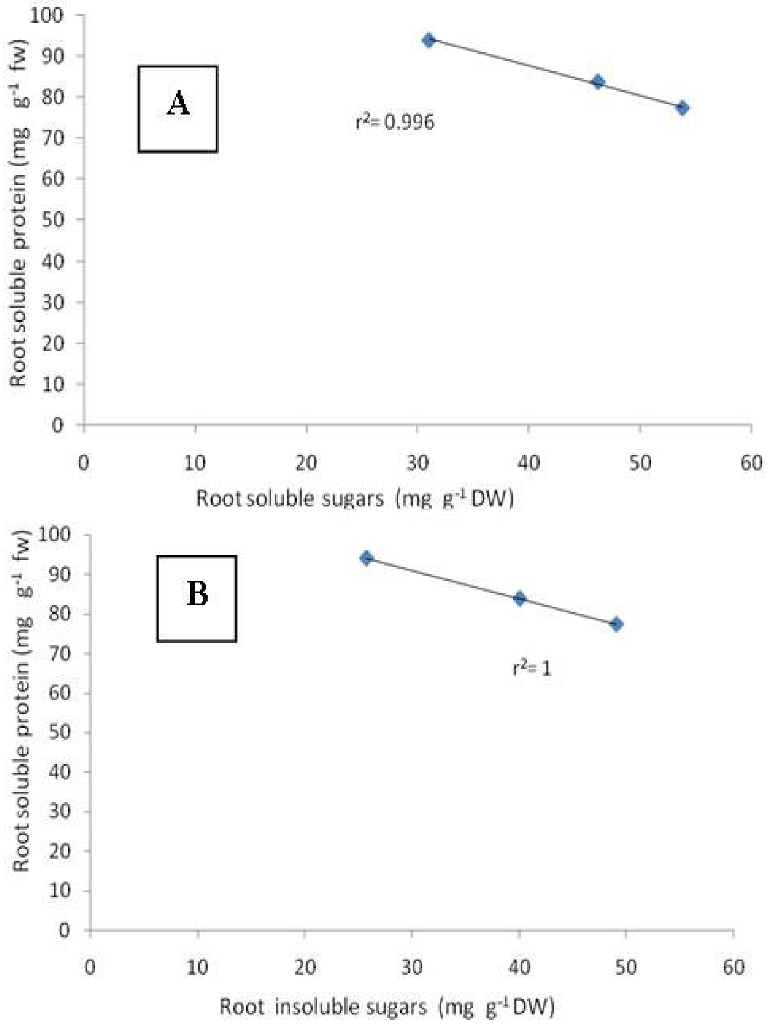
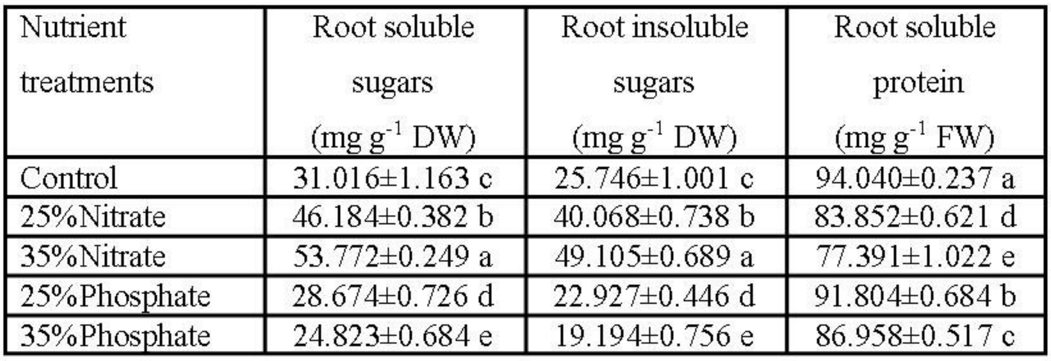


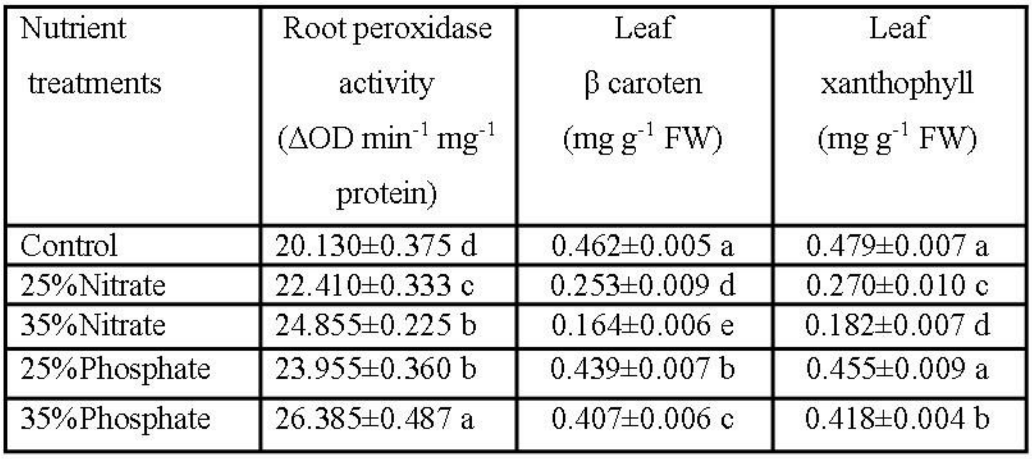
Figure 1: Relationships between soluble protein content and root insoluble sugars (A) and soluble sugars concentrations (B) of tomato roots grown in nitrate-deficient solution (n=4).

Table 1. Effects of nitrogen and phosphorus deficiencies on root soluble protein, soluble and insoluble sugars. Means (±SE) of four replicates, numbers followed by the same are not significantly different (P<0.05).



Nutrient treatments	Root soluble sugars (mg g^{-1} DW)	Root insoluble sugars (mg g^{-1} DW)	Root soluble protein (mg g^{-1} FW)
Control	31.016±1.163 c	25.746±1.001 c	94.040±0.237 a
25%Nitrate	46.184±0.382 b	40.068±0.738 b	83.852±0.621 d
35%Nitrate	53.772±0.249 a	49.105±0.689 a	77.391±1.022 e
25%Phosphate	28.674±0.726 d	22.927±0.446 d	91.804±0.684 b
35%Phosphate	24.823±0.684 e	19.194±0.756 e	86.958±0.517 c

Table 2. Effects of nitrogen and phosphorus deficiencies on leaf carotenoids content and root peroxidase activity. Means (±SE) of four replicates, numbers followed by the same are not significantly different (P<0.05).



Nutrient treatments	Root peroxidase activity (ΔOD min^{-1} mg^{-1} protein)	Leaf β caroten (mg g^{-1} FW)	Leaf xanthophyll (mg g^{-1} FW)
Control	20.130±0.375 d	0.462±0.005 a	0.479±0.007 a
25%Nitrate	22.410±0.333 c	0.253±0.009 d	0.270±0.010 c
35%Nitrate	24.855±0.225 b	0.164±0.006 e	0.182±0.007 d
25%Phosphate	23.955±0.360 b	0.439±0.007 b	0.455±0.009 a
35%Phosphate	26.385±0.487 a	0.407±0.006 c	0.418±0.004 b

RESULTS AND DISCUSSION

Sugars. Soluble and insoluble sugars contents in the roots increased significantly (Table I) in N-deficient plants. Similar observations in *Glycine max* showed that nitrate deficiency results in accumulation of sugars in roots (Rufty *et al*. 1988). Another study on *Glycine max* showed increased concentration of sucrose and starch in nitrate- and ammonium-deficient plants (Robinson, 1996). Also, these results were consistent with studies on *Solanum lycopersicum* (Urbanczyk-Wochniak & Fernie, 2005) and *Olea europaea* L. (Boussadia *et al.* 2010). However, less results have been reported for changes in biochemical parameters under N deficiencies. Because of closely relation of carbon (C) and N assimilation with the rates of plant growth, it seems that N deficiencies would induce enzymatic activity of carbohydrates biosynthesis pathway. Also, decreased growth in N-deficient plants induces sink limitation within the whole plant which reduces photosynthesis. Therefore, higher levels of C would allocate to the roots (Paul and Foyer, 2001; Remans *et al.* 2006 ; Boussadia *et al.* 2010).

Results showed significant decreased concentrations of sugars in roots of P-deficient tomato plants (Table I). However, responses to P limitation seem to vary in different plants and species. A decrease in starch and soluble sugars concentrations has been reported in P-deficient tomato plants cultivar Capita (De Groot *et al*. 2003; Khavari-Nejad *et al.*2009) and rice plants (Nanamori *et al.* 2004). Our results were in conformity with these studies so that it seems decreasing in sugars of P-deficient tomato plants cultivar Urbana resulted from decreased activity of Calvin cycle enzymes, which then reduced CO_2 fixation and carboxylation capacity (Pieters *et al*. 2001). However, less studies have been shown an increase in sugar concentration of roots under P-deficient condition (Ciereszko *et al.* 1996; Sarker and Karmoker, 2011).

Soluble proteins. Soluble proteins decreased in both N and P treatments (Table II). Our results were in conformity with findings in *Oryza sativa* (Huang *et al*. 2004), *Sorghum bicolor* (Zhao *et al*. 2005) and *Solanum lycopersicum* (Urbanczyk-Wochniak and Fernie, 2005) in N-deficient condition. N deficiency induces the degradation of proteins by production of ROS (Crafts–Brandner, 1992 ; Xu *et al.* 2011). On the other hand, we can refer the decreased concentrations of soluble proteins to the reduction in production of amino acids in protein biosynthesis process because N is a structural element of chlorophyll and protein molecules (Ray Tucker, 2004). Also, soluble proteins decreased in P-deficient tomato roots which were related to decreased phosphorylation of metabolic reactions in protein biosynthesis pathway. These findings were in consistent with observations in *Zea mays* L. (Usuda and Shimogawara, 1992; Yun and Kaeppler, 2001), *Lens culinaris* **(**Sarker and Karmoker, 2011**)** and *Phaseolus vulgaris* (Lima *et al.,*2000; Zafar *et al*. 2011). Also, our results revealed an inverse relationship between root soluble (r^2=0.996) and insoluble (r^2=1) contents and root soluble protein concentrations in N-deficient treatments which confirms the competition between N and P in metabolism processes (Figure 1).

Peroxidase activity. Root peroxidase activity on a soluble protein basis significantly increased in both N- and P-deficient treatments (Table 2). N deficiency can increase in excitation pressure in PSII centers, and overproduction of reactive oxygen species (ROS) which enhance the activity of such as perodixase as an antioxidant enzyme (De Groot & Rauen, 1998). ROS also play a role in regulating gene expression in response to the deficiency of several macronutrients including N and P (Shin *et al.* 2005; Kováčik and Bačkor, 2007). It has been shown that certain genes can be induced more specifically upon the deprivation of some nutrient. For example, a peroxidase gene, TPX1, has been identified in tomato roots which can be induced in P-deficient condition (Quiroga *et al.* 2000). Peroxidase activity analyzed was also remarkably higher in low N plants which detoxify the ROS produced (Asada, 1999; Logan *et al.* 2006). Accumulation of the antioxidant systems including peroxidase has been observed in several plants such as *Prunus* incise (Zhou *et al*. 2002) *Coffea arabica* L. under nutrient deficiency (Pompelli *et al.* 2010),

Carotenoids. Concentration of leaf β carotenes were significantly decreased in both N- and P-deficiency treatments (Table II). Although carotenoids decreased remarkably with a diminishing N supply and decreased concentrations of xanthophylls was observed in N-deficient plants but in P-deficient ones, the content of xanthophyll did not significantly change. Similar effects has been observed in *Caspicum annuum* L. (Doncheva *et al.* 2001), *Oryza sativa* (Huang *et al*., 2004) and *Coffea arabica* L. (Pompelli *et al.* 2010), whereas P deficiency did not affect *Phaseolus vulgaris* (Lima *et al*. 2000). It has been demonstrated that carotenoid content depended on the presence and ratio of macronutrients especially N as one of the most essential element (Bojovic and Stojanovic, 2005) and its deficiency decreases the accumulation of protective carotenoids. Therefore, the biosynthesis of these compounds is tightly regulated by environmental conditions such as nutrient availability (López-Ráez and Bouwmeester, 2008). Enhanced employment of xanthophyll cycle-dependent energy dissipation under N- deficient conditions has been observed (Verhoeven *et al.* 1997). Also, N deficiency can induce leaf senescence and production of ROS, which leads to degradation of some leaf macromoleculs which can oxidize some pigments (Crafts–Brandner, 1992). Also, it has been shown that P starvation can induce changes in gene expression of some carotenoids including β carotenes and compounds derived from them in tomato roots (López-Ráez and Bouwmeester, 2008). However, It is supposed that xanthophylls did not affect significantly for their photoprotective roles in leaves which need more investigation.

CONCLUSION

Finally, these results suggested that N and P deficiency can alter some root and leaf metabolic characteristics. Root system may be important in detecting or sensing changes in soil N and P conditions which can result in metabolic and developmental responses. Also, roots tended to accumulate more soluble and insoluble sugars in N-deficient roots which showed reverse relationships with soluble protein contents. Leaf carotenoid content especially β caroten, also, depends on the presence and ratio of macronutrients and it would decrease in N- and P- deficient condition. On the whole, biochemical parameters were affected more in nitrate-deficient treatments in tomato plants. More detailed research will be required to determine other biochemical parameters including antioxidative enzymes and the signaling pathways that mediate molecular and developmental responses of plants to N and P deficiency.

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