

ORIGINAL ARTICLE

Oxidative Stress Tolerance by Calcium and Histidine in Two Tomato Cultivars Under Nickel Stress

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We investigated calcium (Ca) and L-histidine (His) interaction on nickel (Ni)-induced oxidative stress tolerance in two tomato (*Solanum lycopersicum* Mill.) cultivars including Cal-J N3 and Petoeary CH. CaCl₂ (0 and 300 μM) and L-histidine (0 and 300 μM) effects on the oxidative responses in these cultivars cultured were compared in the hydroponic media under Ni stress (NiSO₄; 0,150 and 300 μM). The activities of antioxidative enzymes including catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), superoxide dismutase (SOD) and total content of proteins, malondialdehyde (MDA), other aldehydes, H₂O₂, Ca²⁺, Ni²⁺, ascorbate (ASC), dehydroascorbate (DHA) and electrolytes leakage (EL) were determined. The obtained results indicated that the application of Ca and His generally reduced oxidative markers such as the contents of EL, H₂O₂, MDA and activity of CAT as well as the Ni²⁺ content of root and shoot organs under nickel toxicity, while application of Ni treatment without Ca+His increased these oxidative parameters and accumulation of Ni²⁺, compared to the control. Applying Ni without Ca and His has resulted in reduction of GPX, APX and SOD activities as well as concentrations of root and shoot Ca²⁺ and ASC in the two mentioned cultivars. Application of Ca and His lead to the elevated contents of Ca²⁺ and ASC, increased activities of GPX, APX and SOD as well as inhibition of Ni²⁺ accumulation differently in both cultivars. Ca and His also alleviated the adverse effects of Ni stress on the selected investigated parameters especially in Petoeary CH cultivar. Thus, interaction of Ca and His appeared to improve adaptive responses to Ni stress leading to decreasing Ni-induced oxidative stress in the tomato plants. Therefore, our results suggest that Ca+His alleviated nickel-induced oxidative stress by uptake and inhibition of translocation of Ni²⁺ plus Ni chelating mechanism improvement in the tomato cultivars.

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Key words: Cal-J N3, Petoeary CH, antioxidative enzymes, malondialdehyde, electrolytes leakage

Soil pollution by heavy metals (HMs) including Cd, Cu, Ni, Pb, and Zn occur by industrial wastes containing toxic heavy metals in environment specially cultivated fields. Most of the wastes

contain heavy metals in an amount enough to cause toxicity to the crop plants (Whitby and Huchinson, 1974). Therefore, abiotic stresses like heavy metal stress, air pollutants stress and others negatively affect processes associated with biomass production and seed yield in almost all major field grown crops. Every metal and plant interacts in a specific way, which depends on several factors such as type of soil, growth conditions, and the presence of other ions (Felix-Henningsen, 2010; Ghani, 2010; Dalvi and Bhalerao, 2013).

Heavy metal toxicity as one of the major abiotic stresses induces hazardous effects in crop plants. A common consequence of HM toxicity is the excessive accumulation of reactive oxygen species (ROS) and methylglyoxal (MG), both of which can cause peroxidation of lipids, oxidation of protein, inactivation of enzymes, DNA damage and/or interact with other vital constituents of plant cells. Higher plants have evolved antioxidant defense systems to scavenge ROS and MG. In addition, HMs may be sequestered by organic acids, glutathione (GSH), or by specific metal-binding ligands in cells. GSH as a central molecule of antioxidant defense systems is involved in either direct or indirect control of ROS and MG and their reaction products in plant cells, thus protecting the plant from HM induced oxidative damage (Hossain *et al.*, 2012).

Nickel is naturally present in soil and water, usually in low concentrations. Most agricultural soils contain an average of 0.005 μg of Ni g^{-1} dry weight (DW), and symptoms of phytotoxicity often become apparent at Ni concentrations as low as 25-30 μg g^{-1} soil (Khalid and Tinsley, 1980). Growth inhibition, chlorosis and reduced water content of tissues are commonly observed in plants exposed to phytotoxics amounts of Ni (Pandolfini *et al.*, 1992). Despite his harmful effect, several plants

nutritionally require Ni for various metabolic activities (Welch, 1981) in very low concentrations. Physiological role of Ni and its toxic effects on higher plants have been observed (Temple and Bisessar, 1981).

At cellular and molecular levels, oxidative stress is widely studied as a key marker of plant stress especially metal toxicity. Studies have reported that toxic levels of Cu, Ni and Zn result in a stimulation of lipid peroxidation leading to a loss of membrane function. It is documented that excessive heavy metal such as Ni exposure may increase reactive oxygen species (ROS) accumulation in plants, and oxidative stress would arise if the balance between ROS generation and destruction were broken (Gajewska and Sklodowska, 2007). Membrane peroxidation has been observed in plants that treated with excess Ni amounts (Gajewska and Sklodowska, 2007). Cells have evolved an elaborate system of enzymatic and non enzymatic antioxidants which help to scavenge these indigenously generated ROS. Various enzymes involved in ROS-scavenging have been manipulated, over expressed or down regulated to add to the present knowledge and understanding the role of the antioxidant systems. (Ahmad *et al.*, 2010). At the time the first mechanisms of chloroplast redox regulation were being discovered, ROS were regarded as by-products of potentially beneficial reactions. Indeed, it remains the case that reduction of O^2 to superoxide by the thylakoid electron transport chain can prevent over reduction (redox poisoning) and contribute to chloroplast ATP pools via pseudo cyclic phosphorylation. Nevertheless, as they became implicated in diverse stress responses, ROS subsequently gained the reputation as damaging molecules (Foyer and Noctor, 2012).

Superoxide Dismutase (SOD) and catalase (CAT) are enzymes that represent the first line of antioxidant defense in plant cells. Whereas SOD is responsible for the removal of superoxide radical ($O_2^{\bullet-}$), CAT metabolizes H_2O_2 into H_2O and O_2 . Both enzymes not only prevent formation of more toxic ROS, but play an essential role in cellular H_2O_2 signaling. Since there are a number of ROS producing sites, both enzymes were found to be present in different isoforms localized in ROS generating organelles. Whereas CAT has been identified in three different isoforms that could be localized in both cytosol and peroxisomes based on their amino-acid targeting sequence, SOD is present in three metallic forms each occupying a specified organelle. Fe_2^+ SODs are located in the chloroplasts, the Mn^{2+} isoform in the mitochondria and Cu^{2+}/Zn^{2+} isoforms are present in the chloroplast, cytosol and possibly in extracellular spaces as well (Skyba *et al.*, 2012).

Plants possess an enzymatic protective mechanism including peroxidases such as GPX and APX, besides CAT and SOD. These anti oxidative enzymes have an important role in radicals and peroxides control that are produced under metals stress such as Ni (Tanyolac *et al.*, 2007). Kumar *et al.* (2007) showed that nickel treatment, $100 \mu M$ $NiSO_4$, caused increase in the H_2O_2 concentration concomitantly with antioxidant enzyme activities in maize. Therefore, SOD catalyzes disproportionation of superoxide radicals ($O_2^{\bullet-}$) into H_2O_2 for decreasing the hydroxyl radical (OH^{\bullet}) content (Foyer, 1994). CAT, GPX and APX are contributed in decreasing the content of plant H_2O_2 . In leaf tissues, H_2O_2 is transformed in chloroplasts through the ascorbate dependent H_2O_2 scavenging pathway which involves oxidations and re-reductions of ascorbate, glutathione and NADPH (Baccouch *et al.*, 1998).

The prevention of toxic metal accumulation in the cytoplasm and organelles is one of the important mechanisms for plant tolerance to heavy metals. In tolerant plants, heavy metals as Ni^{2+} , Cd^{2+} and Cu^{2+} are very often chelated or precipitated inside the cell vacuoles that this indicates a transport of heavy metals through the cytoplasm (Howden *et al.*, 1995). In plants with ability for Ni hyper accumulation, the low molecular chelators including histidine (Kramer *et al.*, 1996), malonate or malate (Brooks *et al.*, 1979) and citrate (Lee *et al.*, 1977) have been contributed for Ni detoxification. It is approved that yeast tolerance (*Saccharomyces cerevisiae*) to Ni^{2+} toxicity was depending on high cellular His concentrations (Joho *et al.*, 1992). Increasing of free histidine and the chelating of the heavy metals ions were found for the Ni transport by the imidazole nitrogen of the amino acid (Kramer *et al.*, 1996). In addition, metal chelators such as phyto siderophores (Von Wiren *et al.*, 1996) or organic acids (Ma *et al.*, 2001) regulate the transportable nutrients availability for metal uptake by plant cells. Phyto siderophores have been identified in the barley (*Hordeum vulgare*) xylem sap by Shah *et al.* (2001).

Calcium is an essential macronutrient with diverse functions in plants (Wu and Hendershot, 2010). Calcium plays an important regulatory role in cell division, cell extension, cell wall and membrane synthesis and function (Hanson, 1984; McLaughlin and Wimmer, 1999; Girija *et al.*, 2002). Calcium also functions as a second messenger at low concentrations in the signal transduction between environmental factors and plant responses (Marschner, 1995). The concentration of Ca in the cytosol is very low as $1 \mu M$ approximately, since Ca is cytotoxic in higher concentrations at millimolar range (Marschner, 1995). The various roles of Ca in

plant systems depend on its unique chemical properties that allowed it to exist in a wide variety of binding states (Hepler and Wayne, 1985). In the cell wall, Ca is mainly bound to exchangeable sites in the middle lamella. By binding to carboxylic groups of the poly galacturonic acids (pectins) and cross linking the pectic chains of the middle lamella, Ca strengthens the cell wall and controls its rigidity (Demarty *et al.*, 1984). The cell wall selectivity property correlates with optimum calcium content in plant tissues and rhizosphere (Girija *et al.*, 2002). The cell wall is important chelator of toxic metals such as nickel, as far as the metal is transported apoplastically especially in root tissue. Pectic sites, histidyl groups and extracellular carbohydrates present in plants cell wall causes immobilization of heavy metals and prevent their uptake in to cytosol. The heavy metals are bound to carboxylic groups of poly galacturonic acids restricting the plant uptake. Recent studies indicated that the chemical properties of the cell wall might modulate metal uptake and consequently metal tolerance (Dalvi and Bhalerao, 2013). The cell walls inevitably come into direct contact with the heavy-metal-containing medium in soil and act as a cation exchanger in plant roots (Küpper *et al.*, 2001). It has been described that calcium may decrease the uptake, translocation and accumulation of heavy metals in plants (Osteras and Greger, 2006).

There is little information about the sensitivity and physiological responses of different tomato cultivars to Ni toxicity, although tomato rank is the second important vegetables in terms of planting areas and production. Moreover, results from effect of Ni toxicity on the antioxidant enzymes are contradictory and both increase and decrease in their activities have been observed under different treatments. This warrants the further study of Ni-

induced antioxidant systems in other plants such as tomato cultivars. The objectives of the present study are to evaluate the effects of Ca, His and Ni treatments on the activities of four anti oxidative enzymes (CAT, GPX, APX, SOD), and accumulation of protein, MDA, other aldehydes, H₂O₂, Ca²⁺, Ni²⁺, ASA, DHA and membrane ion leakage in the two probable Ni sensitive tomato cultivars in hydroponic culture. Thus, the aim of this research is to determine the Ca and His role in the alleviation of oxidative stress and improvements of the antioxidant enzyme activities in the two mentioned tomato cultivars from Iran. Moreover, we evaluated and compared Ni stress sensitivity and tomato cultivars response to Ca, His and Ni treatments between these two important tomato cultivars.

MATERIALS AND METHODS

Plant Growth: Two tomato (*Solanum lycopersicum* Mill.) cultivars including Cal-J N3 and Petoearly CH, being widely planted in southeastern of Iran, were used in this study. Seeds of recent tomato cultivars were placed on two sheets of filter paper in petri dishes (90 mm) containing Hoagland solution for optimum seedling growth (Hoagland and Arnon, 1950). After 7 d of culture, uniform seedlings of tomato were selected and transferred into dark polyethylene vessels (two plants per vessel), each supplied with 50 mL of a modified one-tenth-strength Hoagland solution containing 0.5 mM KNO₃, 0.4 mM Ca(NO₃)₂, 10 μM Fe-EDTA, 0.2 mM MgSO₄, 0.1 mM KH₂PO₄, 10 μM H₃BO₃, 2 μM MnCl₂, 2 μM ZnSO₄, 0.1 μM Na₂MoO₄ and 0.2 μM CuSO₄ buffered pH 5.8±0.1. The growth medium was continuously aerated and nutrient solutions were exchanged once a day. Seedlings and plants were grown in a greenhouse with supplementary light provided by sodium vapor lamps at a 200 μmol m⁻² s⁻¹ during the day, a

photoperiod of 16/8 h and temperature of 25°C and 22°C (day/night), respectively, and 60% constant relative humidity.

Experimental design: We evaluated several treatments (containing excess Ni, Ca and His) effects on seed germination and plantlet growth parameters at independent experiment in tomato cultivars for 20 d. Thus, we observed primitive response of tomato plants to the Ni, Ca and His concentrations and the final Ni, Ca and His levels were selected for main project (data not shown in results and discussion parts). After this stage, the optimum time (3 weeks) and concentrations of Ca, His and Ni resulted for treatments application on the tomato plants.

Therefore, tomato plants were grown in a modified one-tenth-strength Hoagland solution for 3 weeks after transplanting. After this period, the effect of Ni exposure and other compound (L-histidine and CaCl₂) on growth parameters, membrane properties and oxidative parameters were investigated by replacing the nutrient with a fresh solution containing the respective added compounds for 10 d. The indicated total treatments concentrations were supplied as NiSO₄ (0, 150 or 300 μM) and Ca as CaCl₂ (0 or 300 μM) and L-histidine (0 or 300 μM) (extra pure, Merck Co., Germany) that adjusted to pH 5.8±0.1 with 0.1 M KOH or 0.1 M HCl as required. The tomato plants under Hoagland media without adding NiSO₄, CaCl₂ and L-histidine were presented as "Control" in our results description. At the end of the treatment period, 3rd leaf, whole root and shoot organs were fresh weighted (FW) and length per plant in both shoot and root organs were determined. After that, these organs were washed in deionized water, blotted dry with tissue paper, weighted, frozen in liquid nitrogen and stored at -70°C until analysis.

Electrolyte leakage: The electrolyte leakage was determined as described by Ben Hamed *et al.* (2007) in leaves tissue of the tomato plants. The leave samples (0.2 g) were placed in test tubes containing 10 ml of double distilled water. The leaves were cut into discs of uniform size (5 mm length). The tubes were incubated in a water bath at 32°C for 2 h and the initial electrical conductivity of the medium (EC₁) measured. The samples were autoclaved at 121°C for 20 min to release all the electrolytes, cooled to 25°C and the final electrical conductivity (EC₂) measured. The electrolyte leakage (EL) was calculated by using the formula: EL = 100 (EC₁/ EC₂).

H₂O₂ content: For determination of H₂O₂ concentration, leaf fresh tissue (0.1 g) was extracted with 3 ml TCA (0.1 %, w/v) in an ice bath and centrifuged at 12,000 x g for 15 min (Velikova *et al.*, 2000). The content of H₂O₂ was expressed as μM g⁻¹ FW using the extinction coefficient (ε=0.028 mM⁻¹ mm⁻¹) at 390 nm.

Analysis of ASC and DHA: ASC and DHA of leaves were measured as described by de Pinto (1999). Briefly, total ascorbate was determined after reduction of DHA to ASC with DTT, and the concentration of DHA was estimated from the difference between total ascorbate pool (ASC plus DHA) and ASC. A standard curve was developed based on ASC in the range of 0-20 mg L⁻¹.

MDA and other aldehydes assay: The level of lipid peroxidation in tomato leaves was determined as the amount of 2-thiobarbituric acid-reactive substances (TBARS) by means of malondialdehyde (MDA) and other aldehydes content formed as described by Heath and Packer (1969) and Meirs *et al.* (1992) respectively. The concentration of MDA and other aldehydes was calculated using an extinction coefficient of 15.5 mM⁻¹ mm⁻¹ and

0.00457 mM⁻¹ mm⁻¹ expressed as mmol g⁻¹ FW.

Enzyme activity determinations: The total protein content in leaves and roots was assayed according to the method of Bradford (1976) using bovine serum albumin as a standard. All spectrophotometric analyses of enzyme were conducted in a total volume of 3 mL in a Carry UV/visible light spectrophotometer at 25°C. CAT (E.C 1.11.1.6) activity of leaves was measured according to the modified method of Dhindsa *et al.* (1981). The activity was calculated using an extinction coefficient of 4 mM⁻¹mm⁻¹. The unit (U) of CAT activity was defined as the amount of enzyme that decomposed 1 mM H₂O₂ per minute per mg protein in 100 µL enzyme extract are given in U mg⁻¹ protein. The activities of peroxidases were assayed using two different electron donors guaiacol and ascorbate. The GPX (EC 1.11.1.7) activity of leaves was determined by using method derived from Plewa *et al.* (1991). The increase of absorbance, due to tetra guaiacol formation, was recorded at 470 nm and enzyme activity was determined using an extinction coefficient of 2.55 mM⁻¹mm⁻¹. The unit (U) of GPX activity was defined as the amount of enzyme that produced 1 mM tetra guaiacol per minute per mg protein in 20 µL enzyme extract that given in U mg⁻¹ protein. The APX (EC 1.11.1.11) activity of leaves was assayed by monitoring the decrease in absorbance at 290 nm due to ascorbate oxidation (Nakano and Asada, 1987). The activity of APX was calculated using the extinction coefficient of 0.28 mM⁻¹mm⁻¹. The unit (U) of APX activity was defined as the amount of enzyme that decomposed 1 mM ascorbate per minute per mg protein in 150 µL enzyme extract that given in U mg⁻¹ protein.

Total SOD (EC 1.15.1.1) activity of leaves was determined by the ability of SOD to inhibit reduction of Nitro Blue Tetra zolium (NBT) to

furmazone at pH=7.0 (Giannopolitis and Ries, 1977). The unit (U) of SOD activity was defined as the amount of enzyme that inhibits the NBT photo reduction by 50 %. SOD activity values that given in U mg⁻¹ protein.

Ca and Ni accumulation Analysis: Samples of whole root and shoot were oven dried at 70°C for 72 h and after determination of dry biomass. Dry root and shoot biomass (0.5 g was digested in an acid mixture of nitric acid:sulphuric acid:perchloric acid (2:1:1, v/v). Acid mixture was allowed to evaporate and the metal residues were dissolved in 10 ml of 0.1 N HCl. Ni and Ca concentrations in the plant digest was measured using coupled plasma atomic emission spectroscopy (ICP, OES, Varian CO) on dry weight basis (Ganje and Page, 1974).

Experimental Design and statistical data analysis: The experimental design was a completely randomized design with 12 treatments, 2 cultivars and 4 replications per treatments. Samples were collected from two plants per culture vessel. Data were analyzed by using one-way analysis of variance (ANOVA). Differences between means were considers significant at confidence level of P≤0.05. All statistical analyses were done using the software SPSS package, Version 18.0. The Duncan test analysis was done to determine the significant difference between treatments.

RESULTS

In general, the interactive effects between Ca and His were significant to alleviation of most growth and oxidative stress markers under Ni 300 µM especially in Petoearly CH cultivar compared to Cal-J N3. Although, application of excess Ca or His in hydroponic media also improved some growth parameters and oxidative markers especially in Cal-J N3 under Ni stress. Whenever external histidine and calcium are low at each Ni²⁺ levels, an increase

from 0 to 150 and 300 μM nickel concentrations significantly decreases length and FW of shoot and root in the two studied cultivars compared to the control plants. A further increase in Ca^{2+} and His concentration from 0 to 300 μM , separately or together, had positive effects on shoot and root length especially in Petoeearly CH cultivar. However, histidine, root FW was more in the treatments containing only than calcium treatments in this Petoeearly CH cultivar (Table 1).

Effect of exogenous Ca, His and Ni on total protein, ASC and DHA content

As it is shown in Table 2, nickel treatments without application of histidine and calcium, resulted in significant increase and decrease of total leaf protein in Cal-J N3 and Petoeearly CH cultivars respectively, compared to the control, but the application of histidine adversely declined leaf protein content. Furthermore, addition of Ca plus Ni 150 μM decreased root total protein significantly and comparable to stress conditions.

Moreover, the interaction of Ca and His had significant effect on decreasing the total protein content markedly under high Ni 300 μM conditions at 0.05 levels in Cal-J N3 cultivar compared to Petoeearly CH. Exogenous His without addition of Ca^{2+} was effective in tomato cultivar Petoeearly CH (Table 2). Generally, exogenous application of Ca and His increased leave ASC content under non-Ni conditions compared to the control samples especially in Petoeearly CH cultivar (Table 2). Utilization of Ca and His increased levels of ASC significantly under 300 μM Ni stress in Petoeearly CH cultivar and we determined highest concentration of ASC in this condition. The interaction of Ca and His had no significant effect on ASC content in the absent of Ni. Under high Ni stress conditions (300 μM), histidine elevated ASC concentration in Cal-J

N3 cultivar.

Ni treatment (150 μM) led to asignificant decrease in DHA content by separate applications of Ca and His compared to this stress condition without Ca and His. 300 μM of nickel treatment along with 300 μM of Ca and His, DHA content was substantially reduced more than stress condition while this interactive effect of Ca and His was not observed in Petoeearly CH cultivar under the mentioned toxic nickel level (Table 2).

H_2O_2 , EL and TBA reactive substances

The applied toxic nickel levels increased EL percentage compared to the control in both cultivars of tomato. Exogenous application of His alone in Cal-J N3 cultivar and also Ca with or without His in Petoeearly CH cultivar under 150 μM of Ni improved the membrane integrity. In 300 μM of nickel treatment containing both components, EL was significantly decreased compared to stress conditions in Petoeearly CH cultivar (Table 3).

It is generally regarded that H_2O_2 and other aldehydes content in both cultivars decreased at the concentrations of 150 and 300 μM of Ni containing Ca and His compared to the control. The Ni levels of 150 and 300 μM containing separate treatments of Ca and His decreased the MDA content only in Petoeearly CH cultivar. The interactive effects between Ca and His was significant in decreasing of H_2O_2 and other aldehydes contents in the Petoeearly CH cultivar under 300 μM of Ni at the 95% confidence level ($P \leq 0.05$) (Table 3).

Response of antioxidant enzymes

The results illustrate that both Ca and His could up-regulate the antioxidant mechanisms in the tomato cultivars under unstressed and Ni toxicity conditions. Nickel 150 μM treatment containing Ca

and/or His increased CAT activity in both cultivars of tomato (Table 4). CAT activity decreased under 300 μM of Ni treatment with applications of both Ca and His but APX activity increased under similar conditions compared to the toxic level (300 μM) of Ni in both cultivars of tomato. Under high Ni stress condition (300 μM), SOD activity decreased comparing to the control in Cal-J N3 cultivar (Table 4). Excessive histidine increased the activity of GPX and SOD under Ni treatments (150 and 300 μM) compared to the control in Cal-J N3 cultivar. In this cultivar, the interaction effects of Ca and His stimulated APX activity in Ni applications that was significant statistically. In Petoeary CH cultivar, Ca increased GPX and SOD activities under Ni 150 μM (Table 4).

Ca and Ni accumulation in root and shoot of the cultivars

The results from ICP, OES showed that high concentrations of Ca in shoots and roots of the tomato cultivars under CaCl_2 treatments and non stress conditions (Figures 1A-D). The treatments containing NiSO_4 without Ca and His, increased Ni accumulation in root and shoot of the two cultivars similarly (Figures 2A-D). Figures 2A and 2B show that after treatment with Ni 150 μM , Ni content in the shoots was about 1.28-fold higher in cv. Cal-J N3 compared to cv. Petoeary CH (cv. Cal-J N3: approximately 0.068 %DW, cv. Petoeary CH: approximately 0.053 %DW). Furthermore, there were considerable differences between the cultivars in terms of Ni accumulation of shoots after treatment with 300 μM of nickel. Accumulation of Ni in the roots also differed between the cultivars. Assimilation of Ni induces a greater decrease in the Ca uptake of root and shoot in both cultivars of tomato (Figures 1A-D). The application of Ca and His in hydroponic media increased calcium content

of shoot and root in Petoeary CH and Cal-J N3 cultivars compared with the control plants (Figures 1A-D). In root tissues, Ca uptake declined slightly with the increase in Ca and His concentration and Ca concentration increased in shoot under similar conditions. The relationship between Ca content of root and shoot was inversed with respect to Ca accumulation in the tomato plants especially in Petoeary CH cultivar under treatments with 300 μM of Ni (Figures 1A-D). However, Ca content of roots improved under both nickel toxic levels 150 μM and 300 μM containing CaCl_2 without His in Cal-J N3 and Petoeary CH, respectively compared to same stress conditions (Figures 1C and D).

Our data showed that Ni accumulation decreased significantly in shoot of the tomato cultivars under 150 and 300 μM of nickel treatments containing Ca+His (Figures 2 A and B). Furthermore, the interactive effect of Ca and His on Ni content was observed in roots of the cultivars treated by 300 μM of Ni. We also observed this interaction effect only about decrease of Ni content of root only in Petoeary CH cultivar under 150 μM of Ni treatment (Figure 2D). The application of Ca with His led to high decreasing in shoot nickel accumulation in Cal-J N3 cultivar treated by 150 μM toxic level. On other hand, our data from root and shoot Ni analysis showed that nickel translocation from roots to shoots was declined. This was observed specially under Ni stress (150 μM) in both cultivars and also in Cal-J N3 cultivar treated by Ni 300 μM (Figures 2A-D). According to Table 1, it seems that Petoeary CH cultivar was more Ni-sensitive compared to other tomato cultivar. Thus, we observed significant effects for interaction of Ca and His in Petoeary CH cultivar compared to Cal-J N3 under Ni stress at 0.05 level. In other hand, the data concerning growth parameters presented in

Tab. 1 clearly show that Ni-induced growth inhibition especially in the case of roots was more pronounced in cv. Petoeary CH. In the latter cultivar treatment with Ni 300 μ M, root length and fresh weight were reduced by 94% and 66%, respectively, comparing to the control (length control: 141 mm,

Ni 300: 9 mm; FW control: 1.42 g, Ni 300: 0.49 g), while the same Ni dose decreased root length and weight by 20% and 31%, respectively (length control: 145.6 mm, Ni 300: 116.6 mm; FW control: 1.26 g, Ni 300: 0.87 g) in cv. Cal-J N3.

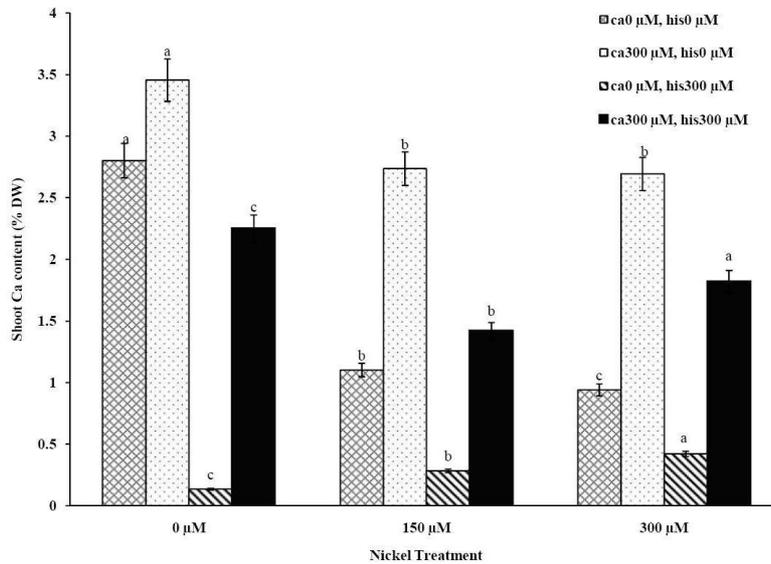


Figure 1A. The Mean of **shoot calcium accumulation** determined and three-way ANOVA with multiple but equal number of observations per test tube for the effects of individual treatments and their interactive effects on Ca content changes in **Cal-J N3** cultivar of tomato treated with a nutrient solution containing different concentrations of nickel, calcium and histidine. Vertical bars indicate the mean of four replications \pm SD (n=4). Different letters indicate significantly different values at a particular treatment component containing calcium and histidine ($P \leq 0.05$).

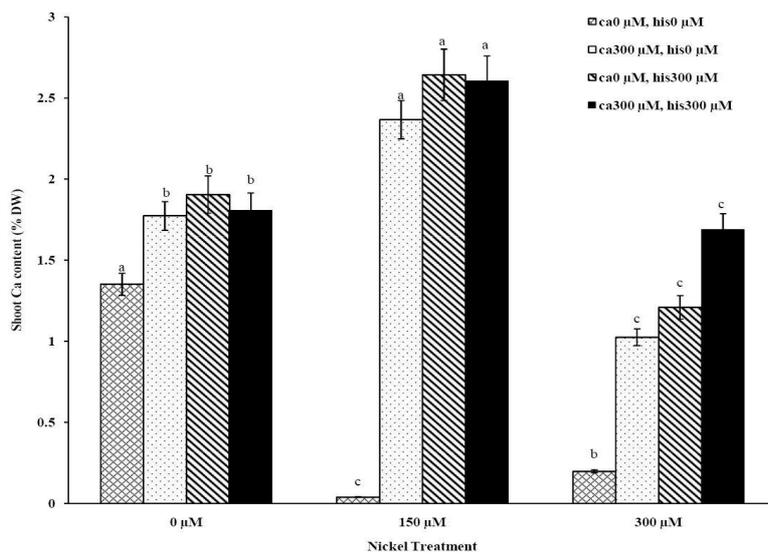


Figure 1B. The Mean of **shoot calcium accumulation** determined and three-way ANOVA with multiple but equal number of observations per test tube for the effects of individual treatments and their interactive effects on Ca content changes in **Petoeary CH** cultivar of tomato treated with a nutrient solution containing different concentrations of nickel, calcium and histidine. Vertical bars indicate the mean of four replications \pm SD (n=4). Different letters indicate significantly different values at a particular treatment component containing calcium and histidine ($P \leq 0.05$).

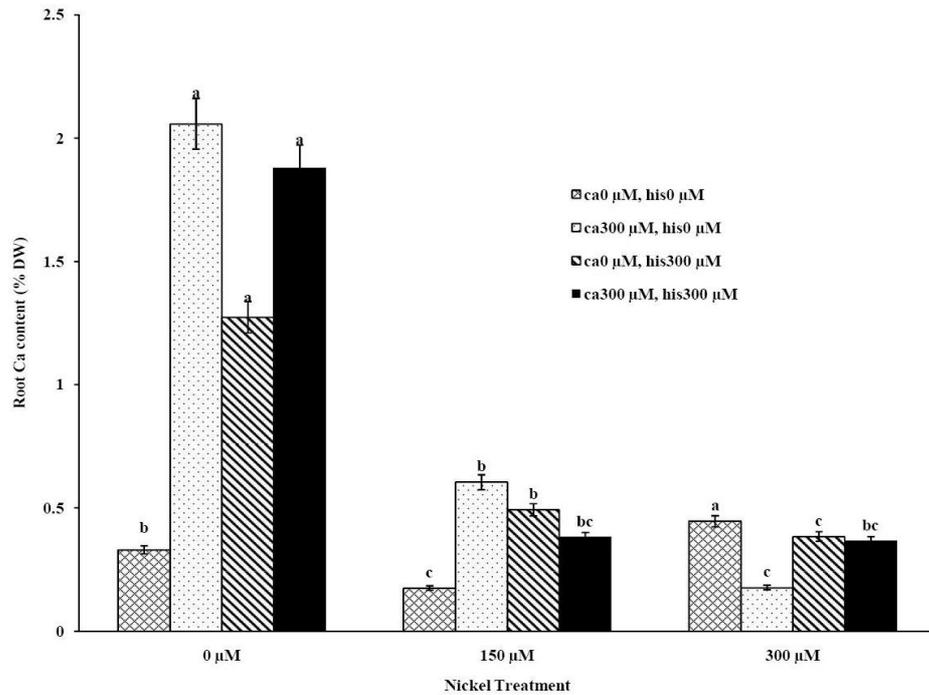


Figure 1C. The Mean of **root calcium content** determined and three-way ANOVA with multiple but equal number of observations per test tube for the effects of individual treatments and their interactive effects on **Root Ca content** changes in **Cal-J N3** cultivar of tomato treated with a nutrient solution containing different concentrations of nickel, calcium and histidine. Vertical bars indicate the mean of four replications \pm SD (n=4). Different letters indicate significantly different values at a particular treatment component containing calcium and histidine ($P \leq 0.05$).

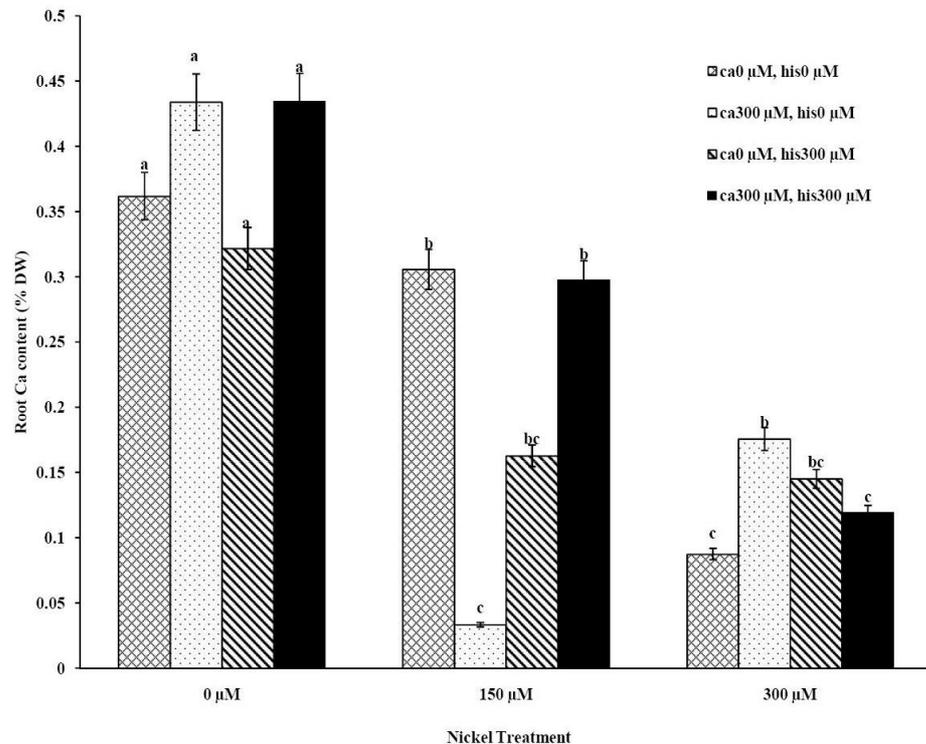


Figure 1D. The Mean of **root calcium content** determined and three-way ANOVA with multiple but equal number of observations per test tube for the effects of individual treatments and their interactive effects on **Root Ca content** changes in **Petoearly CH** cultivar of tomato treated with a nutrient solution containing different concentrations of nickel, calcium and histidine. Vertical bars indicate the mean of four replications \pm SD (n=4). Different letters indicate significantly different values at a particular treatment component containing calcium and histidine ($P \leq 0.05$).

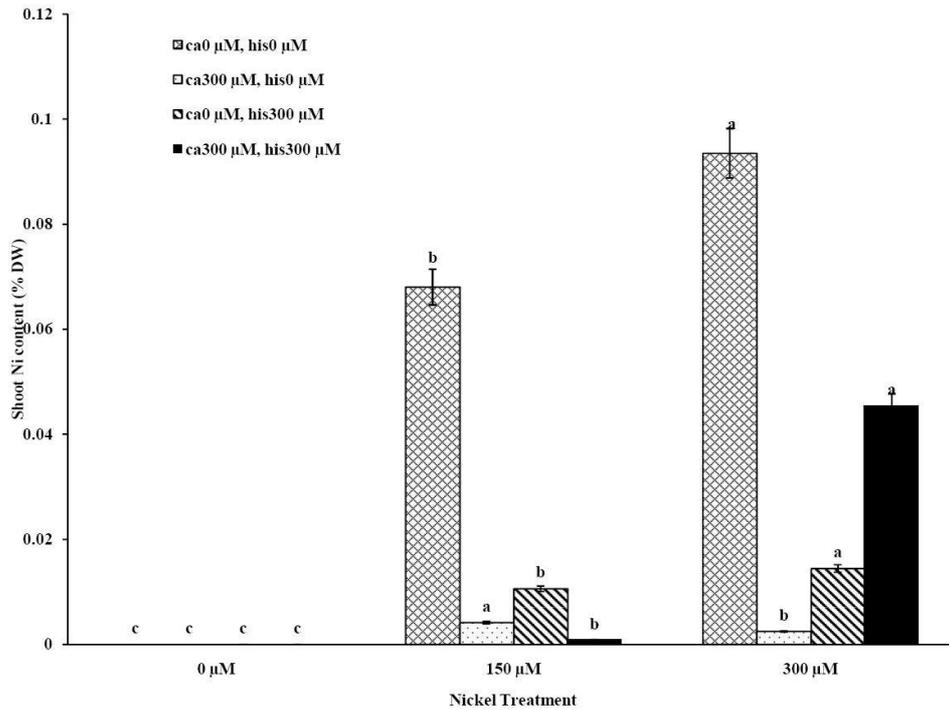


Figure 2A. The Mean of **shoot nickel accumulation** determined and three-way ANOVA with multiple but equal number of observations per test tube for the effects of individual treatments and their interactive effects on Ni content changes in **Cal-J N3** cultivar of tomato treated with a nutrient solution containing different concentrations of nickel, calcium and histidine. Vertical bars indicate the mean of four replications ± SD (n=4). Different letters indicate significantly different values at a particular treatment component containing calcium and histidine ($P \leq 0.05$).

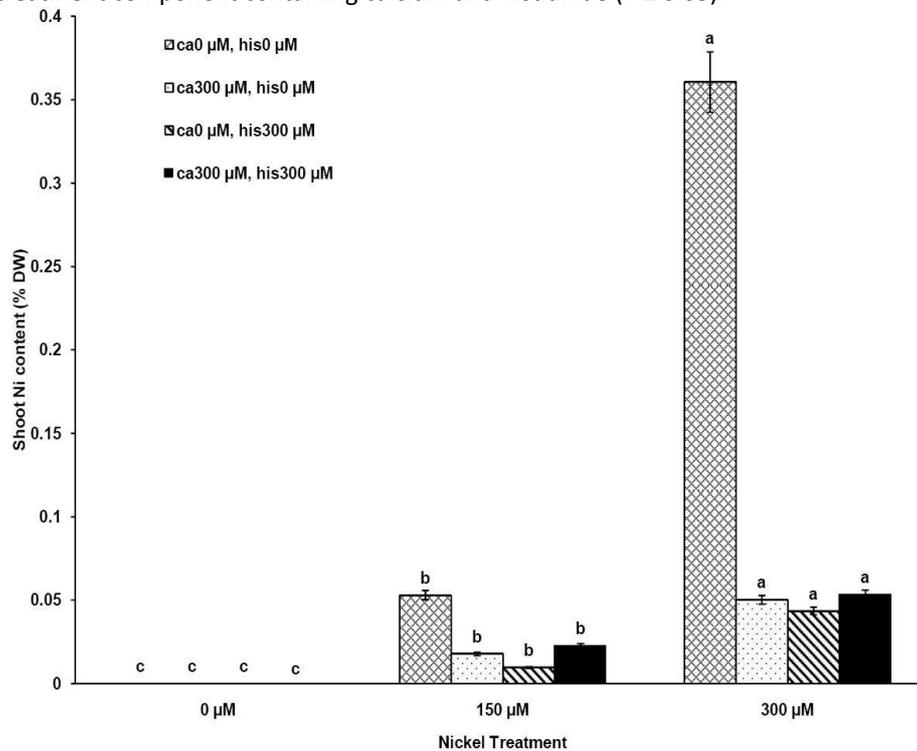


Figure 2B. The Mean of **shoot nickel accumulation** determined and three-way ANOVA with multiple but equal number of observations per test tube for the effects of individual treatments and their interactive effects on Ni content changes in **Petoearly CH** cultivar of tomato treated with a nutrient solution containing different concentrations of nickel, calcium and histidine. Vertical bars indicate the mean of four replications ± SD (n=4). Different letters indicate significantly different values at a particular treatment component containing calcium and histidine ($P \leq 0.05$).

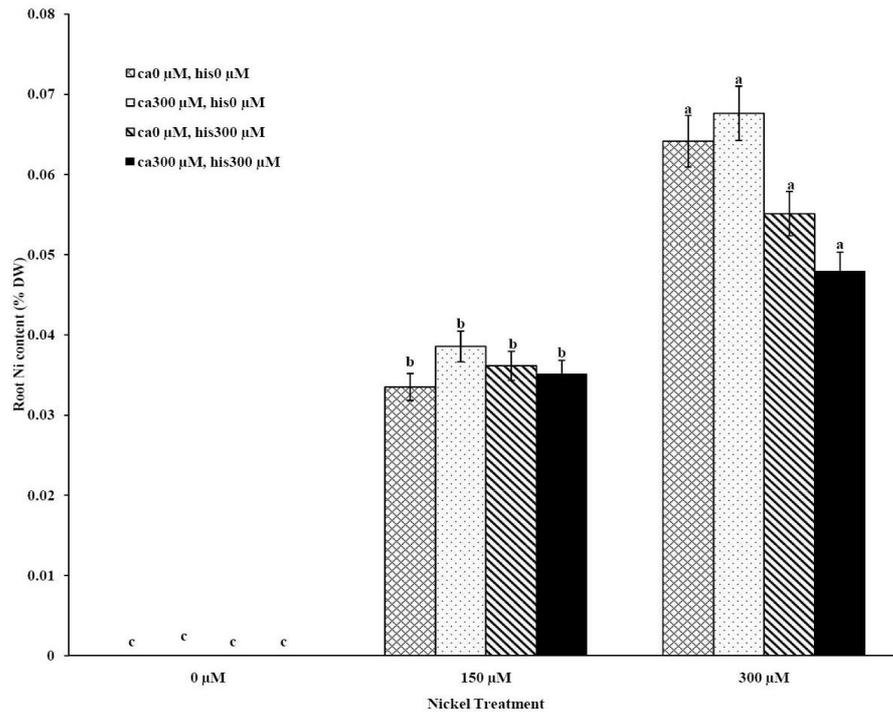


Figure 2C. The Mean of **root nickel content** determined and three-way ANOVA with multiple but equal number of observations per test tube for the effects of individual treatments and their interactive effects on **Root Ni content** changes in **Cal-J N3** cultivar of tomato treated with a nutrient solution containing different concentrations of nickel, calcium and histidine. Vertical bars indicate the mean of four replications \pm SD ($n=4$). Different letters indicate significantly different values at a particular treatment component containing calcium and histidine ($P \leq 0.05$).

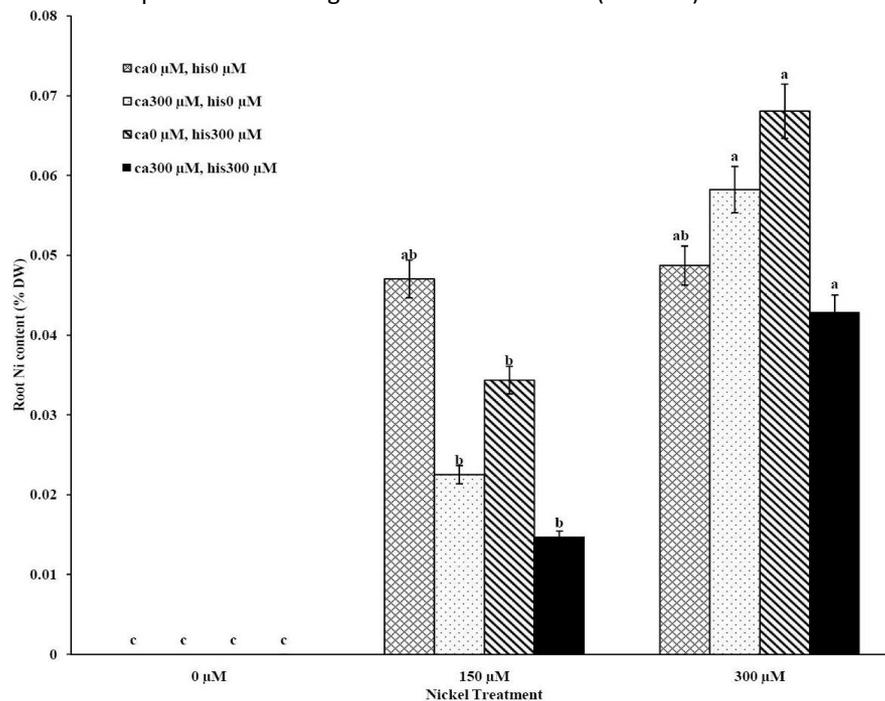


Figure 2D. The Mean of **root nickel content** determined and three-way ANOVA with multiple but equal number of observations per test tube for the effects of individual treatments and their interactive effects on **Root Ni content** changes in **Petoeary CH** cultivar of tomato treated with a nutrient solution containing different concentrations of nickel, calcium and histidine. Vertical bars indicate the mean of four replications \pm SD ($n=4$). Different letters indicate significantly different values at a particular treatment component containing calcium and histidine ($P \leq 0.05$).

Table 1. Mean±SE of the Length and FW from shoot and root organs determined in our research and three-way ANOVA with multiple but equal number of observations per test tube for the effects of individual treatments and their interactive effects on parameters changes in the two cultivars of tomato including **Cal-J N3** and **Petoearly CH**. Means followed by different letter within columns are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

Petoearly CH cultivar			Cal-J N3 cultivar				Treatments components (μM)
Shoot FW (g / plant)	Root Length (mm / plant)	Shoot Length (mm / plant)	Root FW (g/ plant)	Shoot FW (g per plant)	Root Length (mm / plant)	Shoot Length (mm/ plant)	Ni +His+ Ca
1.76±0.02 a	141.0±0.76 bc	158.3±0.32 a	1.26±0.04 ab	1.18±0.06 ab	145.6±0.60 ab	143±0.70 ab	0+0+0
0.77±0.01 cd	123.0±6.5 d	119.6±0.35 cd	0.81±0.01 c	0.55±0.02 e	99.3±0.44 d	136.6±0.66 ab	150+0+0
0.61±0.05 d	90.0±0.04 e	112.0±0.65 d	0.87±0.03 c	0.50±0.03 e	116.6±0.55 cd	101.6±0.41 d	300+0+0
1.77±0.05 a	178.3±0.48 a	138.3±0.28 ab	1.03±0.02 bc	0.94±0.05 cd	165.9±0.74 ab	139.6±0.80 ab	0+0+300
0.77±0.06 cd	135.3±0.54 c	108.3±0.45 d	1.25±0.02 ab	1.22±0.05 ab	120.0±0.59 cd	116.6±0.51 cd	150+0+300
0.59±0.03 e	140.0±0.62 bc	138.3±0.65 ab	0.81±0.03 c	0.84±0.03 d	118.3±0.52 d	116.6±0.62 cd	300+0+300
1.06±0.01 b	178.0±0.22 a	146.6±0.48 ab	1.29±0.04 a	1.21±0.06 ab	165.4±0.78 ab	152.6±0.81 ab	0+300+0
0.91±0.02 bc	173.3±0.52 ab	140.0±0.68 ab	1.32±0.04 a	0.98±0.04 bc	149.0±0.74 ab	148.3±0.33 ab	150+300+0
1.13±0.05 b	164.6±0.46 ab	157.6±0.45 a	1.00±0.05 c	0.93±0.03 cd	123.0±0.61 cd	132.1±0.32 bc	300+300+0
0.77±0.02 cd	106.6±0.44 d	121.6±0.77 bc	0.52±0.01 d	0.45±0.01 e	108.0±0.52 cd	100.1±0.59 d	0+300+300
1.01±0.02 bc	36.6±0.16 f	118.3±0.19 c	0.87±0.03 c	1.14±0.05 ab	133±0.04 d	146.6±0.68 ab	150+300+300
0.66±0.04 d	123.3±0.61 d	123.6±0.64 b	0.86±0.03 c	0.77±0.03 d	133.3±0.67 bc	133.3±0.64 bc	300+300+300
0.0	0.0005	0.0231	0.01015	0.006	0.018	0.016	ANOVA Sig. ($\alpha \leq 0.05$)

Table 2. Mean±SE of the total protein, ASC and DHA parameters determined in our research and three-way ANOVA with multiple but equal number of observations per test tube for the effects of individual treatments and their interactive effects on parameters changes in the two cultivars of tomato including **Cal-J N3** and **Petoearly CH**. Means followed by different letter within columns are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

Petoearly CH cultivar				Cal-J N3 cultivar			Treatments components (μM)
Leaf ASC (mg/ g FW)	Root protein (mg/ g FW)	Leaf protein (mg/ g FW)	Leaf DHA (mg/ g FW)	Leaf ASC (mg/ g FW)	Root protein (mg/ g FW)	Leaf protein (mg/ g FW)	Ni +His+ Ca
2.08±0.12 f	1.73±0.04 de	7.51±0.31 a	4.57±0.25 b	3.93±0.19 d	2.51±0.05 cd	5.61±0.24 d	0+0+0
2.25±0.11 f	3.64±0.13 bc	7.14±0.32 a	1.70±0.04 c	3.55±0.16 d	4.04±0.16 b	5.16±0.23 d	150+0+0
8.15±0.41 bc	5.09±0.24 a	4.51±0.22 c	4.63±0.21 b	5.04±0.20 cd	7.16±0.34 a	8.62±0.74 ab	300+0+0
2.71±0.11 f	0.55±0.01 f	4.86±0.23 c	4.18±0.26 b	8.76±0.26 a	1.22±0.04 d	5.57±0.15 d	0+0+300
2.48±0.15 f	0.52±0.02 f	6.13±0.28 ab	0.93±0.01 de	2.89±0.10 d	5.47±0.27 b	9.01±0.44 ab	150+0+300
2.33±0.14 f	3.11±0.12 c	7.70±0.29 a	1.48±0.02 d	4.18±0.18 d	7.64±0.31 a	6.39±0.31 cd	300+0+300
9.27±0.34 bc	2.37±0.13 cd	5.50±0.11 bc	2.83±0.13 c	7.05±0.28 b	0.75±0.01 e	1.70±0.26 f	0+300+0
2.62±0.16 f	5.80±0.24 a	7.26±0.23 a	0.56±0.01 e	3.51±0.21 d	3.19±0.12 bc	5.09±0.11 d	150+300+0
5.76±0.24 d	1.19±0.03 ef	4.97±0.24 c	1.00±0.04 c	6.36±0.29 c	2.01±0.13 cd	2.58±0.10 f	300+300+0
1.61±0.01 g	0.76±0.01 f	5.36±0.26 bc	4.40±0.17 b	4.26±0.17 d	4.08±0.24 b	4.73±0.18 de	0+300+300
16.20±0.74 a	2.89±0.12 cd	6.48±0.31 ab	7.66±0.28 a	2.09±0.11 de	7.70±0.25 a	6.16±0.27 c	150+300+300
4.76±0.26 de	4.83±0.21 ab	3.65±0.11 d	0.69±0.01 e	1.63±0.04 e	2.80±0.15 cd	7.43±0.32 c	300+300+300
0.026	0.015	0.00	0.011	0.004	0.013	0.00014	ANOVA Sig. ($\alpha \leq 0.05$)

Table 3. Mean±SE of the some of leafs biochemical parameters determined in our research and three-way ANOVA with multiple but equal number of observations per test tube for the effects of individual treatments and their interactive effects on parameters changes in the two cultivars of tomato including **Cal-J N3** and **Petoearly CH**. Means followed by different letter within columns are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

Petoearly CH cultivar			Cal-J N3 cultivar				Treatments components (μM)
Leaf MDA ($\mu\text{M}/\text{g FW}$)	Leaf EL (%)	Leaf H_2O_2 content ($\mu\text{M}/\text{g FW}$)	Leaf aldehydes ($\mu\text{M}/\text{g FW}$)	Leaf MDA ($\mu\text{M}/\text{g FW}$)	Leaf EL (%)	Leaf H_2O_2 content ($\mu\text{M}/\text{g FW}$)	Ni +His+ Ca
0.16±0.02 cd	6.86±0.24 e	13.92±0.64 g	2.22±0.12 c	0.07±0.01 f	8.11±0.42 d	61.10±2.88 e	0+0+0
0.43±0.02 b	27.59±1.24 bc	191.36±5.14 a	2.80±0.11 a	0.19±0.01 e	27.69±1.28 ab	127.0±5.54 a	150+0+0
0.49±0.03 a	41.38±2.36 a	152.13±6.24 b	3.08±0.13 b	0.28±0.01 c	27.14±1.33 ab	101.1±4.11 b	300+0+0
0.13±0.01 f	31.19±1.08 b	128.54±5.88 d	1.69±0.02 d	0.27±0.01 c	10.97±0.54 c	87.26±3.21 c	0+0+300
0.27±0.01 c	23.04±1.14 c	165.72±8.47 b	1.60±0.01 d	0.22±0.01 d	33.69±1.66 ab	78.03±2.19 d	150+0+300
0.20±0.01 d	40.15±2.99 a	140.08±7.24 c	4.31±0.18 a	0.40±0.02 b	25.41±0.97 ab	86.74±2.45 c	300+0+300
0.16±0.01 e	14.51±0.48 d	40.85±2.08 f	2.18±0.19 bc	0.28±0.01 c	18.95±0.95 ab	70.85±3.02 d	0+300+0
0.16±0.01 e	45.43±2.54 a	42.38±2.56 f	2.19±0.21 bc	0.28±0.01 c	19.52±1.05 ab	82.38±1.02 c	150+300+0
0.21±0.02 d	42.20±1.26 a	43.67±2.33 f	2.62±0.10 a	0.38±0.02 b	37.74±1.98 a	53.92±2.05 f	300+300+0
0.19±0.01 d	41.55±2.17 a	48.54±2.47 f	2.39±0.12 a	0.27±0.01 c	15.31±0.74 bc	79.05±3.54 d	0+300+300
0.28±0.03 c	21.85±1.45 c	193.67±6.75 a	3.34±0.14 ab	0.26±0.01 c	33.74±1.44 ab	124.1±4.56 a	150+300+300
0.30±0.02 c	32.08±1.67 b	82.90±4.12 e	2.81±0.16 a	0.54±0.03 a	26.41±1.11 ab	61.10±3.21 e	300+300+300
0.004	0.0	0.001	0.005	0.026	0.028	0.004.	ANOVA Sig. (a≤0.05)

Table 4. Mean±SE of the antioxidative enzymes activity of leaf determined in our research and three-way ANOVA with multiple but equal number of observations per test tube for the effects of individual treatments and their interactive effects on parameters changes in the two cultivars of tomato including **Cal-J N3** and **Petoearly CH**. Means followed by different letter within columns are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

Petoearly CH cultivar Leaf			Cal-J N3 cultivar Leaf				Treatments components (μM)
APX (U/mg protein)	CAT (U/mg protein)	GPX (U/mg protein)	SOD (U/ mg protein)	APX (U/ mg protein)	CAT (U/mg protein)	GPX (U/mg protein)	Ni +His+ Ca
3.50±0.14 de	10.97±0.42 e	18.39±0.87 d	8.67±0.34 c	1.30±0.03 de	2.46±0.13 d	6.55±0.28 g	0+0+0
2.11±0.16 e	7.19±0.34 f	17.85±0.56 e	8.46±0.41 c	0.98±0.02 e	7.44±0.28 c	9.76±0.51 f	150+0+0
9.05±0.33 c	16.26±0.75 c	68.95±2.77 a	5.81±0.31 e	1.93±0.06 d	13.05±0.1 b	17.72±0.32 d	300+0+0
0.97±0.02 f	26.21±1.04 b	21.26±0.99 d	8.52±0.34 c	4.42±0.26 a	2.92±0.17 d	14.70±0.65 de	0+0+300
12.91±0.24 b	1.51±0.03 h	70.40±2.11 a	5.37±0.24 e	1.76±0.03 d	9.17±0.37 bc	9.84±0.39 f	150+0+300
1.76±0.04 f	5.50±0.18 f	17.55±0.64 e	7.77±0.34 d	3.84±0.25 a	9.85±0.15 bc	8.38±0.33 f	300+0+300
4.06±0.15 de	5.53±0.24 f	34.61±1.33 b	28.08±1.5 a	2.27±0.06 c	2.03±0.13 d	52.36±1.13 b	0+300+0
1.49±0.03 f	14.47±0.41 c	27.20±1.24 c	13.35±0.54 b	2.19±0.05 c	24.34±1.3 a	26.35±1.02 c	150+300+0
1.66±0.03 f	10.13±0.62 e	19.44±0.57 d	26.70±1.24 a	4.03±0.14 a	6.51±0.22 c	58.50±2.34 a	300+300+0
0.41±0.01 g	41.83±1.24 a	18.11±0.84 d	11.61±0.64 bc	1.98±0.05 d	2.97±0.17 d	7.79±0.28 g	0+300+300
6.44±0.28 cd	4.85±0.19 g	26.09±1.29 c	8.70±0.4 6c	2.85±0.05 ab	9.09±0.45 bc	24.86±1.18 c	150+300+300
16.88±0.71 a	12.14±0.55 d	27.85±1.18 c	6.60±0.34 d	2.81±0.06 ab	6.88±0.52 c	19.42±0.88 d	300+300+300
0.0311	0.0	0.0003	0.001	0.0	0.003	0.02	ANOVA Sig. (a≤0.05)

DISCUSSION

Although the Ca and Ni accumulation in roots are usually inversely correlated, root Ca accumulation is more often accounted for a higher share of variability in root elongation compare with Ni accumulation in root tissue at high concentration of Ca compared with the control conditions (Wu and Hendershot, 2010). Therefore, the evaluation of root elongation requires including Ni and Ca as predictor whenever environmental conditions (low Ca concentrations) significantly affect the amount of Ca accumulated. Root elongation is highly positively correlated with total Ca content in the roots (Wu and Hendershot, 2010). In this study our data showed that Ca plus Ni increased length and FW of root in the tomato cultivars significantly in comparison to Ni treatments without excess CaCl₂. Thus, the treatments containing excess Ca (300 µM) has positive effects on shoot and root length elongation (Table 1, Figures 1A-D). However, Ca and His interaction effect on alleviation of growth and Ca accumulation of tomato plants were significant especially in Petoearyly CH cultivar under Ni stress. Additionally, Ca plus His alleviated calcium content of shoots and roots, more than control plants in both cultivars (Figures 1A-D). In root tissue of the cultivars, Ca uptake declined slightly with the increase in Ca and His concentration and Ca concentration increased in shoot under similar conditions. Therefore, the relationship between root and shoot Ca was inversed because of higher Ca accumulation in shoot especially in Petoearyly CH cultivar under Ni 300 µM treatments (Figures 1A-D). However, Ca content of roots increased under nickel levels 150 µM and 300 µM containing CaCl₂ without His in Cal-J N3 and Petoearyly CH, respectively which is compared to same stress conditions (Figures 1C and D).

In this investigation, the MDA and other aldehydes as lipid peroxidation markers were higher than the control under Ni toxic levels (Table 3). Membrane degradation was reported as a consequence of Ni stress in roots of *Triticum aestivum* (Pandolfini *et al.*, 1992). Highly reactive oxygen species could be responsible for Ni-induced membrane damage that increasing ROS content has been reported for roots and leaves of wheat, hairy roots of *Alyssum bertolonii* and *Nicotiana tabacum* (Gajewska and Sklodowska, 2007). Thompson *et al.* (1987) documented that lipoxygenase activation precedes lipid peroxidation. This mechanism might underlie Ni induced peroxidation as seen in primary leaves of *Phaseolus vulgaris* under Cu and Zn stress (Weckx *et al.*, 1997). In addition, Pandolfini *et al.* (1992) showed that Ni causes membrane degradation leading to a marked K⁺ and other cation leakage probably. In our research, electrolytes leakage percentage (as an important membrane damage index) increased in both cultivars under nickel stress compared to the control plants (Table 3). It has been suggested that the extent of lipid peroxidation and membrane permeability were closely related to higher levels of ROS during either senescence or under stress conditions (Weckx and Clijsters, 1997).

Our findings shown that Ca and His interaction inhibited MDA and other aldehydes generation compared to stress condition in the tomato cultivars especially in Petoearyly CH compare with other cultivar (Table 3).

Application of His alone in Cal-J N3 cultivar and also Ca and/or His in Petoearyly CH cultivar during 150 µM Ni, declined EL percentage. EL was significantly decreased, compared to stress conditions in Petoearyly CH cultivar that treated with

300 μM of nickel treatment containing both Ca and His (Table 3).

We evaluated the activities of SOD, CAT, GPX and APX that involved in free radicals and H_2O_2 detoxification under the experiment treatments. Higher activity of the SOD led to production of H_2O_2 . Subsequently, increasing in activity of CAT, GPX and APX can remove high H_2O_2 significantly in the tomato plants (Table 4).

In the present study, a significant increase in leaf SOD activity was observed in both tomato cultivars treated with Ca and His treatments during different levels of nickel (Table 4), suggesting that SOD may function as a ROS scavenger (Alscher *et al.*, 2002). Recent studies have demonstrated that over expression of chloroplastic Cu/Zn-SOD in transgenic *Nicotiana tabacum* (Badawi *et al.*, 2004) can provide enhanced ion toxicity tolerance. Even though a high SOD activity (compared in equal total protein content of tissue) protects plants against the superoxide radical, it cannot be considered solely responsible for membrane protection against peroxidation. This ROS should be then scavenged by other enzymes such as catalase and peroxidases.

The fact that leave CAT activity of the Cal-J N3 cultivar in the control and Ni stress treatments was lower than Petoeearly CH cultivar could be the result of genetic differences and higher Ni sensitivity of Cal-J N3 cultivar to Ni toxicity. However, when the tomato plants were subjected to NiSO_4 , the increases in CAT activity were higher under Ni stress 300 μM than the control but Ca and His stimulated its activity during Ni stress (Table 4).

It seems that up regulation of CAT is a requirement for the removal of H_2O_2 in roots and leaves of stressed plants or decreasing in CAT activity may result from enzyme deactivation (Dat

et al., 2000). The contradiction between CAT activation and suppression in the presence of metals may depend upon the element, its concentration and the plant species and cultivars (Gratao *et al.*, 2005).

Higher activity of APX was recorded after application of Ni, Ca and His treatments in the tomato cultivars. The higher level in APX activity was observed in the tomato cultivar Petoeearly CH than other cultivar (Table 4). Further more GPX activity stimulation was considerably higher than APX in our research (Table 4). Consequently, APX may remain in a saturated state for its substrate. In addition, the increase in activities of APX and GPX has been found by Jocsak *et al.*, (2008) in barley seedlings. However, the activities of APX and GPX in both tomato cultivars were also affected by Ca and His treatments compared to Ni 150 μM lacking of Ca and His. The similar results were found for other plant species by Mittova *et al.* 2002 and also elevated APX activity in plants exposed to toxic levels of Ni has been reported earlier in wheat and rice (Gajewska and Skłodowska, 2008). It has been speculated that under stress conditions, H_2O_2 acts as a systemic intracellular signal for the induction of APX (Hernandez *et al.*, 2004). Therefore, H_2O_2 is considered to playing a central role in physiological functions such as plant signaling, regulating plant development and adaptation to biotic and abiotic stresses. Increased availability of H_2O_2 is a commonly observed feature of plant stress response signature. Stresses such as high light, heat, salinity, cold and pathogen attack, besides others, are all considered to trigger H_2O_2 accumulation that contributes to the regulation the expression of many genes. H_2O_2 -mediated regulation of cell division, differentiation and growth must be viewed not only within the context of the basic redox

chemistry of the plant cell that is governed by photosynthesis and respiration but also within the bigger picture of the physiological environment. The physiological context involves a continuous supply of environmental stimuli that can trigger intracellular H_2O_2 accumulation or modulate the response to such accumulation (Foyer and Noctor, 2012; Chakraborty and Pradhan, 2012).

These antioxidative changes could be related to complexity of the ascorbate-glutathione cycle that have enzymes coded by multigenic families whose products are localized in different cell compartments and are regulated differently by stress conditions. For instance, in the cytosolic fraction of sorghum leaves subjected to water stress there was a decrease in monodehydro ascorbate reductase (MDHAR) activity, and increased dehydro ascorbate reductase (DHAR) activity (Mittova *et al.*, 2002). Our data also showed that under high Ni stress conditions (300 μ M), application of histidine resulted in more increased concentration of ASC than the control in Cal-J N3 cultivar while Ca and His interaction on increasing ASC content was significant at 0.05 Level in Petoeary CH cultivar plants (Table 2).

Generally, the obtained data (Figures 2A-D) indicated that Ca and His inhibited Ni accumulation and translocation in root and shoot in the tomato cultivars under Ni stress via ion chelating mechanisms. However, heavy metal detoxification in plants is linked to the synthesis of cysteine-rich poly- peptides, so called phytochelatins (PCs) (Grill *et al.*, 1985). Similar to these compounds, L-histidine are able to chelate Ni^{2+} in different parts of plants (Howden *et al.*, 1995). The function of calcium in preventing toxic effects of HMs during the transport across the cell wall is controversially discussed, very often the optimum Ca content in

plant tissue correlate with HM tolerance (de Knecht *et al.*, 1994).

The tomato plants accumulated appreciable amounts of Ni in both roots and shoots under Ni stress without Ca and His. Our results shown that inhibition of shoot and root Ca uptake was detected after lipid peroxidation began (Figures 1A-D and Table 3). This latter phenomenon appears to be a primary effect of Ni stress that may result in inhibition of tomato plants growth and nutrition especially in Cal-J N3 cultivar that this cultivar was less Ni sensitive compared to the other cultivar in our work (Table 1). Similar to the results obtained by the earlier studies (Madhava and Sresty, 2000), we found that the Ni uptake in the two cultivars (especially Petoeary CH cultivar) is dependent upon nickel concentration in treatments (Figures 2A-D). Accumulated calcium in root tissue increased tolerance of plants to Ni toxicity and also decrease Ni uptake and its accumulation especially in tomato cultivar Petoeary CH (Figures 1A-D and 2A-D).

The Ni and Ca internalization process within the root is physiologically controlled and root growth is a complicated process (Table 1). Our study showed that the measured accumulations of Ni and Ca correlate best with the treatments whenever data collected under varied concentration Ca and His treatments. This suggests that uptake of Ni and Ca is affected by influence of Ca and His on Ni uptake and translocation under the stress conditions (Wu and Hendershot, 2010).

Generally, based on the obtained results it can be suggested that Ni toxicity induced oxidative stress in the Ni sensitive cultivars of tomato resulting in increased generation of H_2O_2 and activation of antioxidant enzymes. The different increases in CAT, APX, SOD and GPX activities under the treatments suggest their roles in scavenging

ROS in tomato plants especially in Petoeary CH cultivar. The data shown that Ca and His elevated antioxidative responses in studies on tomato cultivars under Ni toxicity especially Petoeary CH through inhibition the Ni toxic effects by decreasing nickel uptake and its translocation. For the first time our results showed that the interaction effects between Ca and His could alleviate nickel stress and growth promoting in sensitive tomato cultivar plants such as Petoeary CH. Finally, exogenous Ca+His improved tolerance of this Ni-sensitive tomato cultivar under oxidative stress and higher accumulated Ni of shoots compared to Cal-J N3 cultivar. Furthermore, new researches to understand the basic mechanism under Ca interaction effects are essential in cell wall with heavy metal ligands such as His for alleviation of oxidative stress. Also, determination of the differences in activity among the isoforms of SOD, CAT, GPX and other antioxidative enzymes is our next aim in future researches through the PAGE under Ca, His and Ni-induced oxidative stress in the tomato plants. Thus, field experiments are necessary at contaminated soils with nickel pollution for oxidative stress decreasing and plants tolerance in these conditions. In conclusion, the application of Ca+His as chelators was beneficial to higher oxidative stress tolerance in the tomato cultivars (especially Petoeary CH comparing to Cal-J N3) and increasing growth of the tomato plants under Ni stress conditions.

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