Volume 57(2):131-144, 2013
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

Calcium and L-histidine interaction on growth improvement of three tomato cultivars under nickel stress

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ABSTRACT Nickel is considered to be an essential micronutrient for many plants; however, it is very toxic at excess concentration. In this investigation the interaction between L-histidine (His) and calcium on improvements of growth and K^+ nutrition was studied under Ni^{2+} stress in hydroponic media in 3 tomato cultivars (*Cal-J N3*, *Early Urbana Y* and *Petoearly CH*) from Iran. The treatments contained Ca^{2+} (400 and 700 µM), L-histidine (0 and 300µM) and $NiSO_4$ (0, 150 and 300 µM). The following parameters were determined: root and shoot length, fresh weight, pigment concentration, leaf area index, K^+ accumulation, reducing sugars, proline, free amino acids (FAA) and leaf relative water content (RWC). The results showed that Ni^{2+} treatments significantly decreased the shoot and root length, the pigment content of leafs and the K^+ content of root and shoot in all cultivars, whilst application of Ca^{2+} and His elevated these growth and nutritional parameters irrespectively of the presence of Ni. The effect of Ca^{2+} on increasing of leaf area and other parameters in *Early Urbana Y* and *Cal-J N3* cultivars was more pronounced than in *Petoearly CH* cultivar. Therefore, application both Ca^{2+} and His can affect on nutrition improvement and increasing of the tolerance and growth of agronomic plants under Ni^{2+} stress.
Acta Biol Szeged 57(2):131-144 (2013)

KEY WORDS

calcium
L-histidine
hydroponic media
nickel stress
tomato

Heavy metals such as nickel, copper, cadmium and mercury are toxic to most organisms and a variety of mechanisms have been evolved for coping with these toxic elements (Scheller et al. 1987). Ni is an essential micronutrient for plants, since it is in the active centre of the enzyme urease, which is required for nitrogen metabolism in higher plants. However, it is also showed that at elevated levels, Ni is a toxic metal in various plant species. The most decisive symptoms of Ni-induced toxicity in plants are the inhibition of growth, photosynthesis, mineral nutrition, sugar transport and water relation (Seregin and Kozhevnikova 2006). During Ni-induced toxicity, plants develop different resistance mechanism to avoid or tolerate Ni stress, including the changes of the lipid composition, the profiles of isozymes and enzyme activity, sugar or amino acid contents, and the level of soluble proteins (Schützendübel and Polle 2002).

Metal chelation by specific low-M_r ligands is one of the major processes that determine metal tolerance of a plant. All plants are able to produce phytochelatins, which can bind and detoxify heavy metals (Ha et al. 1999). In nickel-tolerant plants histidine has been implicated in metal detoxification (Sagner et al. 1998).

Accepted January 17, 2014
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Generally, plants develop a complex network of highly effective homeostatic mechanisms that serve to control the uptake, accumulation, trafficking and detoxification of metals. Chelators are involved in metal detoxification via buffering the cytosolic metal concentration (Clemens 2001). Reactive interaction between metal ions and organic acids or amino acids for metal chelating was reported (Wagner 1993; Delhaize and Ryan 1995; Sagner et al. 1998).

The binding of Ni with His has been confirmed with the analyses of Ni-hyper accumulation and non-accumulation species. Under Ni toxication, increasing of the His content was detected in xylem sap in the Ni-hyper accumulation *Alyssum lesbiacum* (Kramer et al. 1996). When His treatments had been used for non-accumulation plant *A. montanum*, these treatments increased the Ni tolerance (Kramer et al. 1996). Tolerance of yeasts to Ni and other heavy metals has been reported to correlate with high cellular His levels (Joho et al. 1992). Metal chelators like phytosiderophores or organic acids also play an important role in regulating metal uptake by plant cells (Curie et al. 2001). Phytosiderophores have been detected in the xylem sap of barley plants (Alam et al. 2001). Chelators mediate and control the partitioning and translocation of the heavy metal such as Ni between the roots and shoot organs.

Calcium is an essential macronutrient with diverse functions in plants (McLaughlin and Wimmer 1999). Ca plays an important regulatory role in cell division, cell extension, cell wall- and membrane-synthesis and function (Hanson 1984; McLaughlin and Wimmer 1999). Ca also functions as a second messenger at low concentrations in signal transduction between environmental factors and plant responses (Marschner 1995). The concentration of Ca in the cytosol is very low (1 µM 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 allow 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 polygalacturonic 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 selective property of cell wall correlates with optimum Ca content in plant tissues and rhizosphere (Girija et al. 2002). The cell wall is important chelator of toxic metals such as Ni, as far as the metal is transported apoplastically especially in root tissue. The cell walls inevitably come into direct contact with the heavy metal-containing medium in the soil and act as a cation exchanger in the plant root (Küpper et al. 2001). It has been described that Ca may decrease the uptake, translocation and accumulation of heavy metals in plants (Österas and Greger 2006).

As hypothesized, metals were found to interact and negatively affecting the accumulation of each other in the stem and roots of plants. Furthermore, in nutrient solutions Ca was found to decrease the accumulation of Cd, Cu, Mn and Zn in stem and roots. Even at low elevated Ca or Cu addition interactions were found (Saleh et al. 1999). Similarly, heavy metals, like Cu and Cd, have been found to reduce the Ca, Mn and Zn contents in roots, shoots and leaves of trees (Arduini et al. 1998).

Heavy metal ions are supposed to enter into plant cells through systems devoted to the uptake of essential cations. The uptakes of many toxic metals are happened via Ca channels by plant cells (Wu and Hendershot 2010). Moreover, it was shown that heavy metals compete with Ca at both Ca channels (Nelson 1986) and intracellular Ca binding proteins (Rivetta et al. 1997). Metals with similar physiochemical properties, such as ion-size and charge, compete with each other for binding sites in plants (Marschner 1995), thereby affecting the uptake, translocation and accumulation of each other.

Supplementing the medium with Ca alleviates growth inhibition under metal stress conditions (Yan et al. 1992; Kinraide 1998). The concentration of different cations in the uptake solution is decisive for the resulting transport across the membrane (Lu et al. 2010). Raising the Ca^{2+} concentration was shown to block heavy metal transport into rice roots as a result of the competition of toxic metals with Ca^{2+} for Ca transporters (Clemens 2006).

Permeability of plasma membrane to toxic ions correlates with cell wall activity and Ca content. The main part of Ca in plant tissues is located in the apoplast, bound to the cell wall, the outer surface of the plasma membrane and other structures (Marshner 1995). Ca blocks the symplastic uptake of Ni in root tissue. Generally, Ca ion plays an important role in regulating ion transfer into plant cells growing in stress conditions, like salinity and toxic metals (Greenway and Munns 1980). In addition, Ca is very effective in detoxifying high concentration of other toxic elements under stress condition (Ashraf and Akhtar 2004). Thus, Ca sustains potassium transport and K^+/Na^+ selectivity in plant membrane. Ca also plays a key role in control of production of proline and glycine-betaine (Charest and Phan 1990; Wu et al. 2009).

This study aimed at analysing the interaction effect between exogenous Ca (as a very important nutrient with diverse functions specially in cell wall and membrane) and His (as a specific Ni ligand) on the probable higher alleviation effect of Ni stress in three cultivars of tomato from Iran and improving growth and nutrition of these plants under Ni^{2+} stress conditions. On the other hand, we attempt to clarify that non-toxic concentration of exogenous Ca with His probably can alleviate Ni toxication in tomato than His application alone. For this reason, we evaluated growth and biochemical parameters, in three tomato cultivars under Ca, His and $NiSO_4$ treatments in hydroponic media under standard conditions and optimized treatments.

Materials and Methods

Plant growth

Seeds of the tomato cultivars (*Solanum lycopersicon* Mill. CVs; *Petoearly CH, Cal-J N3, Early Urbana Y*), that were a gift from Falaat Ghaareh Company (Tehran, Iran), were placed on two sheets filter paper in Petri dishes (9 cm), that contain Hoagland solution for optimum seedling growth. After 7 days and emergence, uniform seedlings of tomato were selected and transferred into dark polyethylene vessels (two plants per vessel), each supplied with 50 ml of a modified Hoagland solution (Hoagland and Arnon 1950) containing 0.5 mM KNO_3, 400 µM $Ca(NO_3)_2$, 10 µM Fe-EDTA, 0.2 mM $MgSO_4$, 0.1 mM KH_2PO_4, 10 µM H_3BO_3, 2 µM $MnCl_2$, 2 µM $ZnSO_4$, 0.1 µM Na_2MoO_4 and 0.2 µM $CuSO_4$ buffered to pH 5.8±0.1. The growth medium was continuously aerated and the nutrient solutions were exchanged once a day. Seedlings and plants were grown in greenhouse with supplementary light provided by sodium vapor lamps at a photon flux density of 10 klx during the day, a photoperiod of 16/8 hours, day and night temperature of 25°C and 22°C respectively, and 60% constant relative humidity.

Experimental treatments

Tomato plants were grown under control conditions in a modified Hoagland solution for 3 weeks that was continuously aerated and exchanged once a day. After this period, the effect of Ni exposure and other compounds (L-histidine and $CaCl_2$) on growth have been studied. Nutritional and physiological parameters were investigated by replacing the nutrient with a fresh solution containing the respective compounds for 10 days that was continuously aerated and was exchanged once a day. The indicated total treatment concentrations were supplied as $NiSO_4$ (0, 150 and 300 µM), as $CaCl_2$ (400 and 700 µM; total Ca^{2+} concentration in nutritional media) and L-histidine (0 and 300 µM; extra pure, Merck Co, Germany), that were solved in the Hoagland solution and adjusted to pH 5.8±0.1 with KOH. In this experiment the root and shoot samples were collected after treatments exposure for growth and physiological analyses. At the end of the treatment period, root and shoot organs were washed in deionised water, blotted dry with tissue paper, measured, frozen in liquid nitrogen and stored at -80°C until analysis.

Morphological analysis

The morphological parameters determined in this research included fresh weight (FW), length of both shoot and root organs and leaf area.

Biochemical analysis

The concentration of the main photosynthetic pigments (chlorophyll a+b and total carotenoids) were measured quantitatively in acetone extract from untreated and Ni-treated (with or without Ca and His) tomato cultivars using absorption coefficient at specific wavelengths (470, 646 and 663 nm) given by Lichtenthaler (1987) and recalculated per gram of fresh weight. The relative water content (RWC) of leaves was determined by the method of Turkan et al. (2005). The method for proline determination was essentially as described by Bates et al. (1973). Free amino acids (FAA) was measured in shoot and root tissues using the method of Hwang and Ederer (1975). For the determination of the reduced carbohydrates the method of Somogyi (1952) was used.

Element analysis

Samples of root and shoot were oven dried at 70 °C for 72 h, and after the determination of dry biomass 0.5 g samples were dissolved in 10 ml 65% (w/v) nitric acid (supra pure, Merck Co, Germany). Total concentration of K^+ was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Varian) by the method of Sagner et al. (1998).

Experimental design and statistical data analysis

The experimental design was a completely randomized design with 12 treatments, 3 cultivars and 4 replications per treatments. Samples were collected from two plants per culture vessels. Data were analysed using analysis of variance (four-way ANOVA), followed by Duncan test. Differences between means were considered significant at confidence level of P 0.05. All statistical analyses were done using the software SPSS package Version 18.0 (SPSS 2009).

Results

The effects of various treatments on root and shoot length in the three tomato cultivars are shown in Figure 1. When the concentrations of the external His and Ca^{2+} were low, an increase from 0 to 150 or 300 µM Ni^{2+} significantly decreased the shoot length in all cultivars compared to the control. A further increase in the concentrations of Ca^{2+} and His from 0 to 300 µM, separately or together, had increasing positive effect on shoot length. In general, both Ca^{2+} and His significantly increased the root growth in two cultivars (*Petoearly CH* and *Early Urbana Y*) under Ni^{2+} toxication, however, *Cal-J N3* cultivar had no improving growth after treatment with 300 µM Ni^{2+} beside Ca^{2+} and His. Ni^{2+} treatment (150 µM) without Ca^{2+} and His decreased the shoot length, while treatments containing Ca^{2+} and His resulted increasing growth compared to the control. Generally, Ca^{2+} and His significantly increased the root growth in all studied cultivars under Ni^{2+} toxication; only it had not observed any improving effect on the growth of *Cal-J N3* cultivar, when 300 µM Ni^{2+} was applied beside Ca^{2+} and His (Figs. 1 and 2).

In the treatment containing 150 µM Ni^{2+} beside Ca^{2+} and His, shoot fresh weight (FW) was not significantly different in *Cal-J N3* cultivar compared to the control. But shoot and root FW was increased under 300 µM Ni^{2+} in the other two cultivars compared to the control. However, in treatments containing only His, root FW was higher than under Ca^{2+} treatments (Fig. 2 D-F). Similar results were found also at the dry weight determination (data not shown).

Figure 3 A-C indicated the leaf RWC at different Ni^{2+} concentrations affected by Ca^{2+} and His treatments. In both cultivars, *Early Urbana Y* and *Cal-J N3*, the Ca^{2+} and His improved the leaf RWC under 150 and 300 µM Ni^{2+} treatments. However, Ca^{2+} and His had negative effects on leaf RWC in tomato *Petoearly CH* cultivar compared to the control (Fig. 3C).

In this research, leaf pigments, especially chlorophylls were also determined (Fig. 4 A-C). The application of Ca^{2+} and His increased the level of chlorophyll pigments under Ni^{2+} stress. Our data indicated that His increased the level of chlorophyll (a+b) under Ni^{2+} stress. In *Cal-J N3* cultivar under 150 µM Ni^{2+} treatment, application 300 µM Ca^{2+} and His increased the level of chlorophyll (a+b) compared to other treatments. Chlorophyll (a+b) content was significantly increased under Ca^{2+} treatment in unstressed conditions (Fig. 4 A-C). On the other hand, this pigment increase shows that

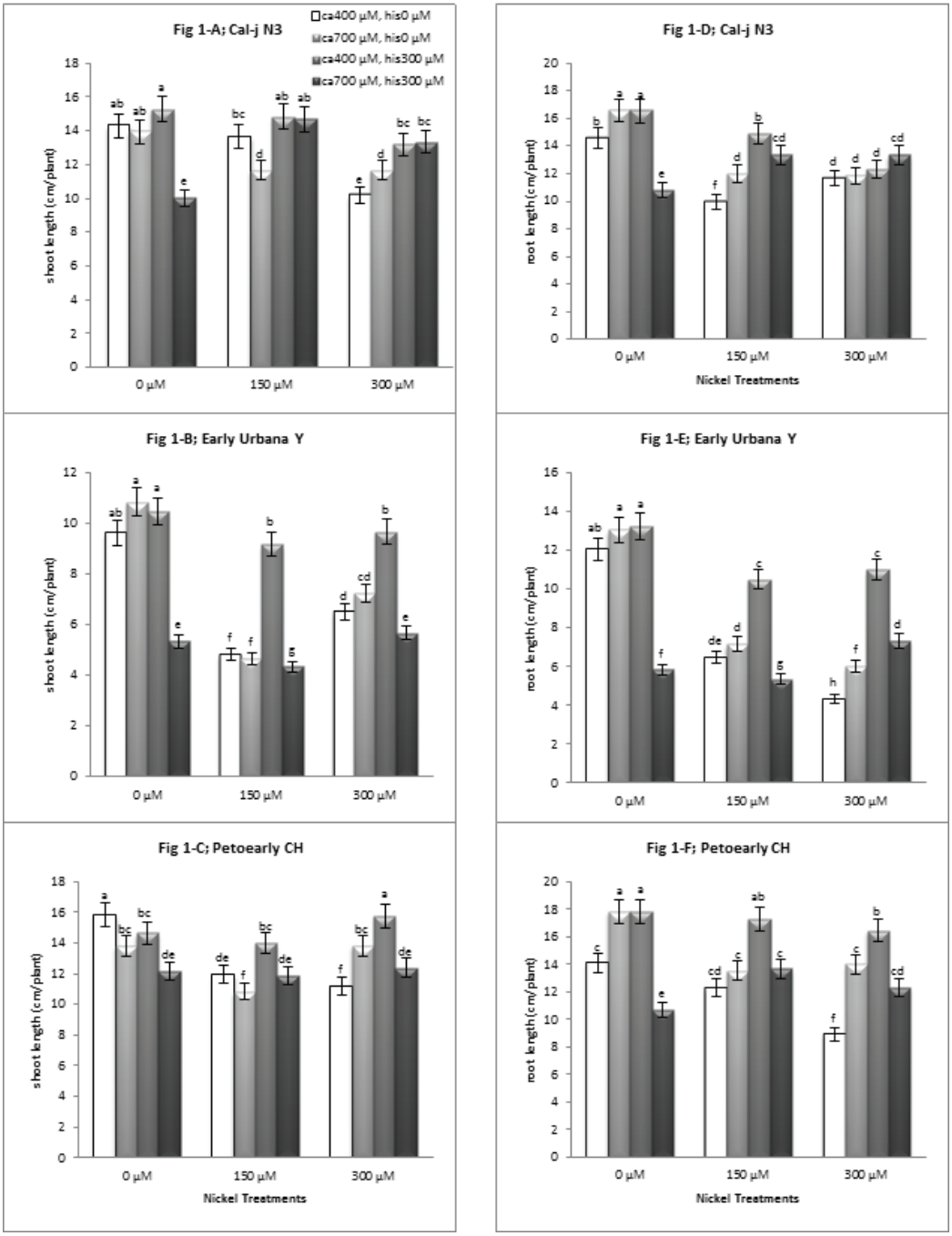

Figure 1. The mean of shoot and root length (A-F) determined and three-way ANOVA with multiple but equal number of observations per test tube for the effects of individual treatments and their interactive effects on the growth prarmeter changes in the tomato cultivars treated with a nutrient solution containing different concentrations of nickel, calcium and histidine ($P<0.05$). Vertical bars indicate the mean of four replications ± SE (n=4). Different letters indicate significantly different values among the experimental treatments.

leaf growth and expanding can depend upon calcium ion. The total carotenoid determination showed that Ca^{2+} and His increased the concentration of this leaf pigment under 150 µM Ni^{2+} treatment. The incubation with Ni^{2+} (300 µM) beside Ca^{2+} and His resulted in a decrease of carotenoid content in leaf tissue in all the tomato cultivars. In two cultivars (*Early Urbana Y* and *Cal-J N3*) leaf area was increased under Ni^{2+}, Ca and His treatments compared to Ni^{2+} treatment without Ca^{2+} and His. In summary, Ca^{2+} with or without His, improved growth and leaf area index in the studied cultivars of tomato. In ad-

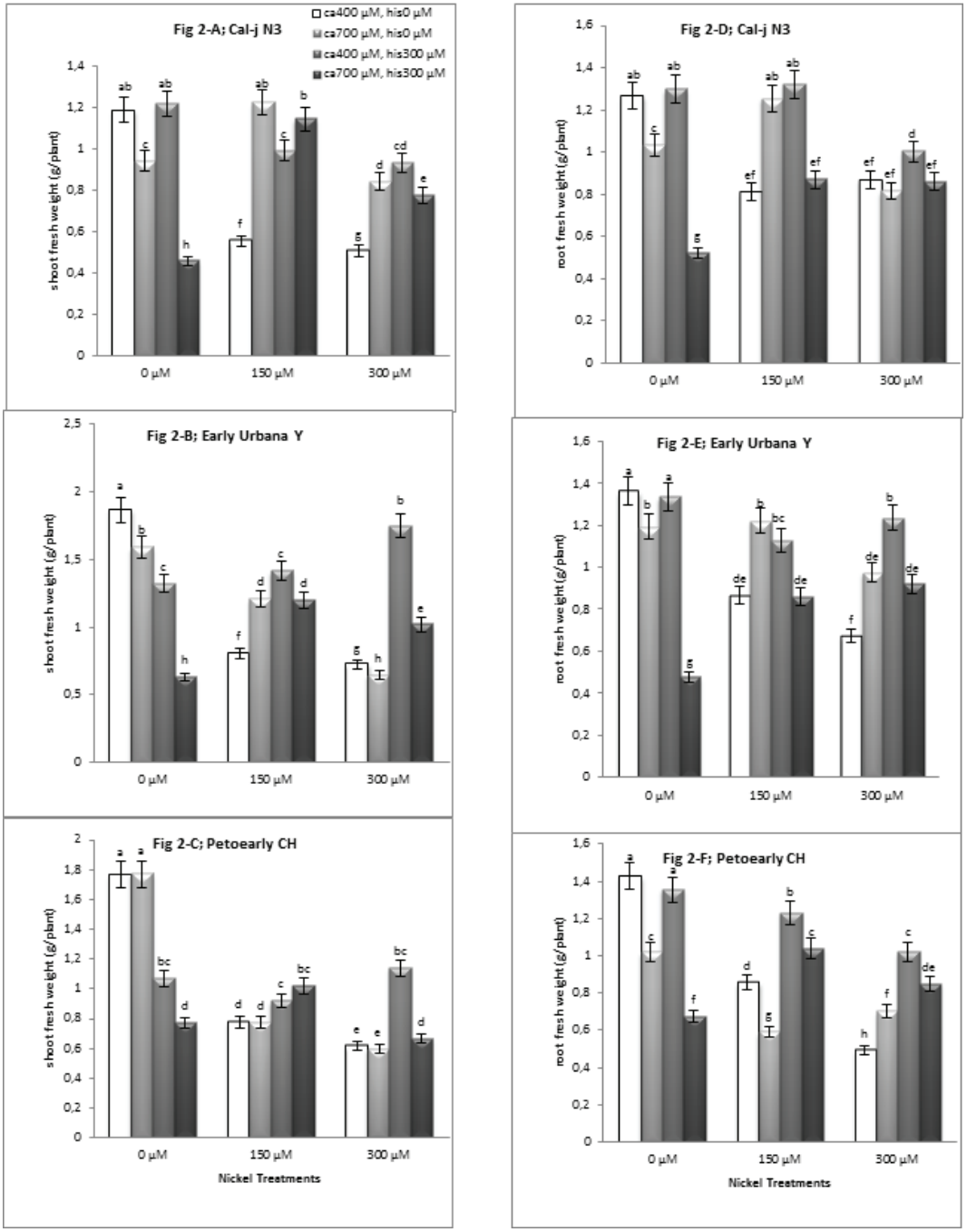

Figure 2. The mean of shoot and root fresh weight (A-F) determined and three-way ANOVA with multiple but equal number of observations per test tube for the effects of individual treatments and their interactive effects on fresh weight changes in the tomato cultivars treated with a nutrient solution containing different concentrations of nickel, calcium and histidine ($P<0.05$). Vertical bars indicate the mean of four replications ± SE (n=4). Different letters indicate significantly different values among the experimental treatments.

dition, it has been observed that His treatments have positive effect on leaf area increase under Ni^{2+} treatments in the *Early Urbana Y* and *Cal-J N3* cultivars of tomato (Fig. 4 D-F).

The proline content in both root and shoot was increased under Ni^{2+} stress and it was the highest in the presence of Ni^{2+} without Ca^{2+} and His in tomato plants. In the root and shoot of *Cal-J N3* cultivar treatment with 300 µM Ni^{2+} increased the proline concentration (Fig. 6 A-F). In the other two cultivars (*Petoearly CH* and *Early Urbana Y*) the effect of 150 µM Ni^{2+} on proline content was significantly higher (Fig. 6

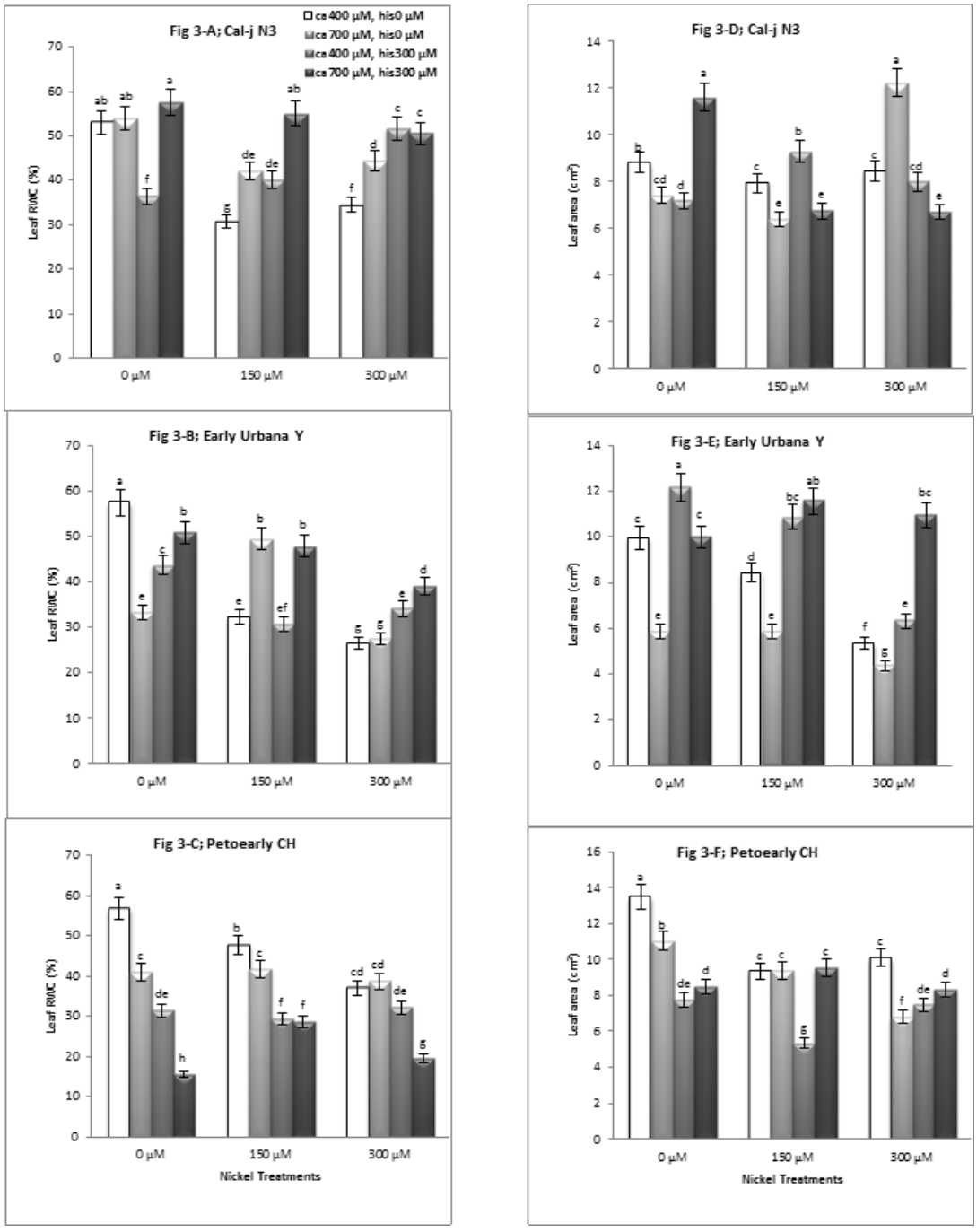

Figure 3. The mean of leaf RWC (A-C) and leaf area (D-F) determined and three-way ANOVA with multiple but equal number of observations per test tube for the effects of individual treatments and their interactive effects on the leaf parameters changes in the tomato cultivars treated with a nutrient solution containing different concentrations of nickel, calcium and histidine ($P<0.05$). Vertical bars indicate the mean of four replications ± SE (n=4). Different letters indicate significantly different values among the experimental treatments.

A-F). The proline content was also the highest in the presence of Ni^{2+} without Ca^{2+} and His in all tomato cultivars. Based on the lower effect of 150 Ni^{2+} µM on proline concentration in *Cal-J N3* cultivar, proline content was not diminished by the addition of Ca^{2+} and His as ligands. Proline content was decreased by the addition of 300 µM His under both concentrations (150 and 300 µM) of Ni^{2+} treatments, while the effect of His combined with Ni^{2+} was similar to the control in *Cal-J N3* cultivar. Interaction effect of His and Ca^{2+} on proline decline was observed in all cultivars under Ni-stress. In the

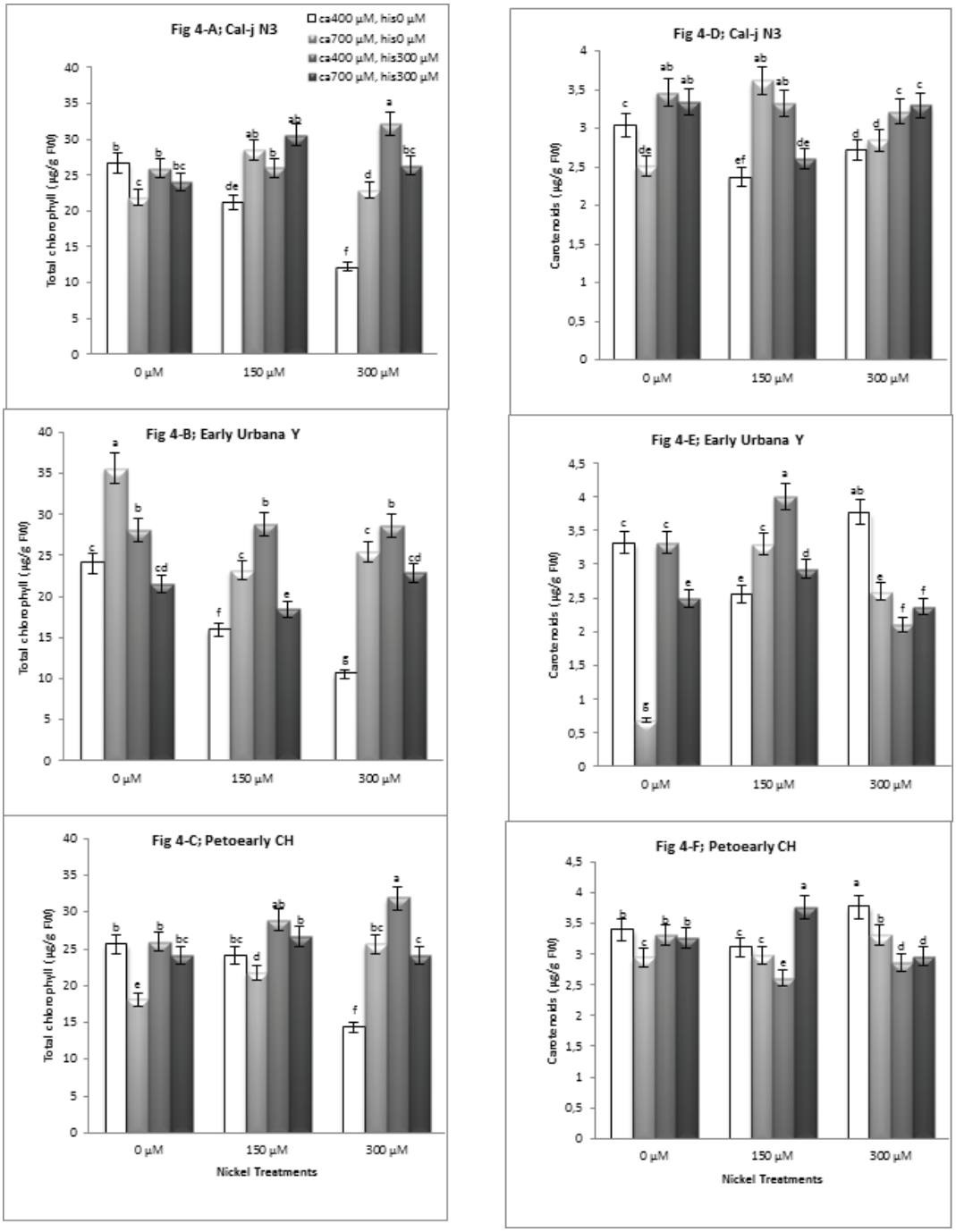

Figure 4. The mean of leaf total chlorophyll (A-C) and carotenoids (D-F) determined and three-way ANOVA with multiple but equal number of observations per test tube for the effects of individual treatments and their interactive effects on the leaf parameters changes in the tomato cultivars treated with a nutrient solution containing different concentrations of nickel, calcium and histidine ($P<0.05$). Vertical bars indicate the mean of four replications ± SE (n=4). Different letters indicate significantly different values among the experimental treatments.

shoot of the *Petoearly CH* cultivar proline accumulation was lower under Ni^{2+}, Ca^{2+} and His treatments than under Ni^{2+} treatment alone.

The interaction effect of Ca^{2+} and His on FAA content was significant compared to the control. The influence of Ca^{2+} and His on the decreasing of FAA content was observed in *Cal-J N3* cultivar under 150 µM Ni^{2+} compared to unstressed conditions. The treatment with 300 µM Ni^{2+} beside Ca^{2+} and His resulted in the decreasing of the FAA content in the root and shoot tissues in all tomato cultivars. It seems that Ca^{2+}

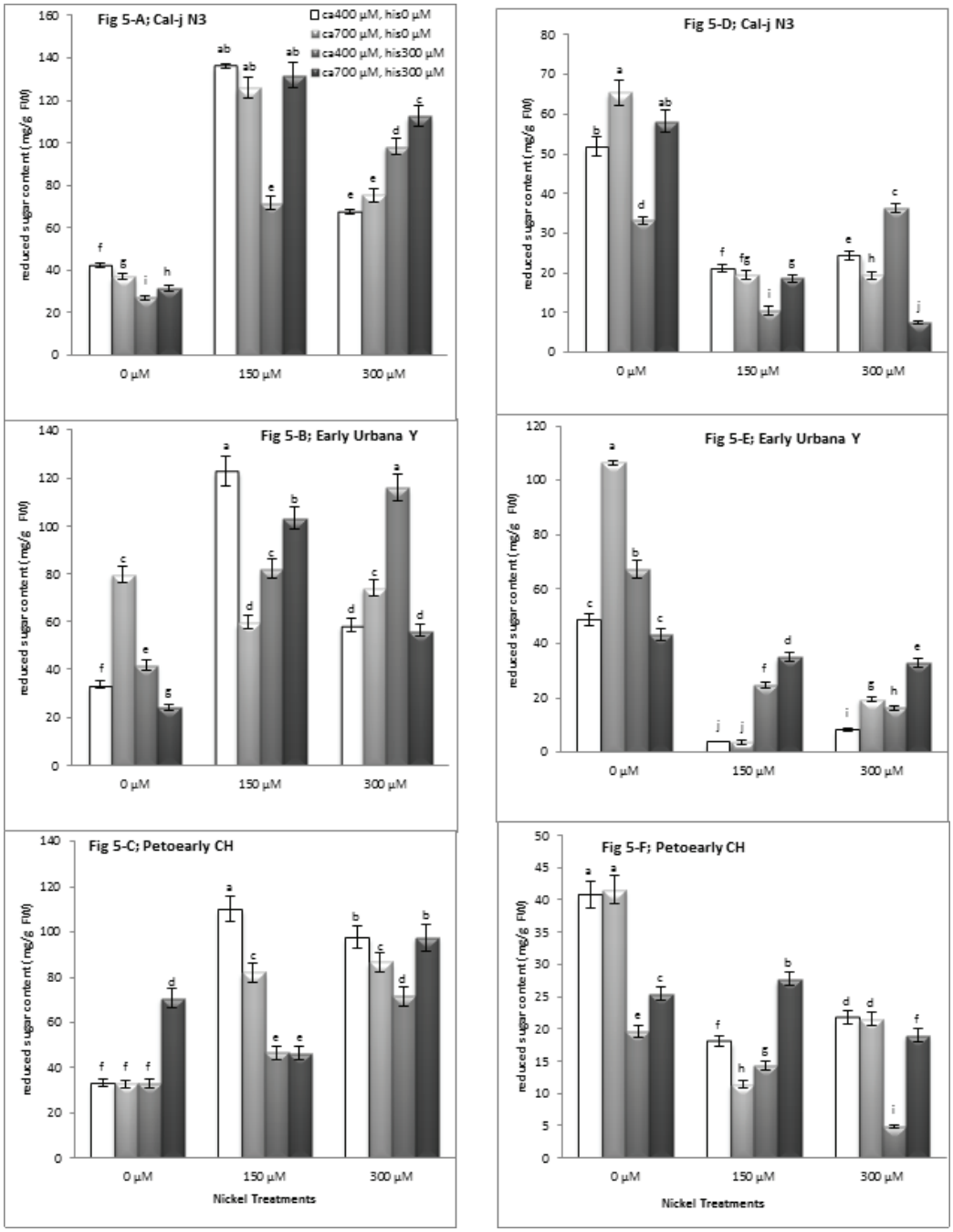

Figure 5. The mean of shoot and root reduced sugars (A-F) determined and three-way ANOVA with multiple but equal number of observations per test tube for the effects of individual treatments and their interactive effects on reduced sugars content changes in the tomato cultivars of treated with a nutrient solution containing different concentrations of nickel, calcium and histidine ($P<0.05$). Vertical bars indicate the mean of four replications ± SE (n=4). Different letters indicate significantly different values among the experimental treatments.

effect on FAA decrease is significant under 150 µM Ni^{2+} and interaction between Ca^{2+} and His on the FAA concentration was found at 300 µM Ni^{2+} treatment (Fig. 7 A-C).

Our data showed that Ni^{2+} stress resulted in the accumulation of the reducing sugars in the shoot of the cultivars, whereas in root tissue they are decreased in comparison to the control (Fig. 5; A-F). The level of the reducing sugar content was considerably greater in the shoot of *Cal-J N3*, than other cultivars treated with Ni^{2+} at toxic levels. However, sugar concentration was lower in root of *Cal-J N3*, than in other

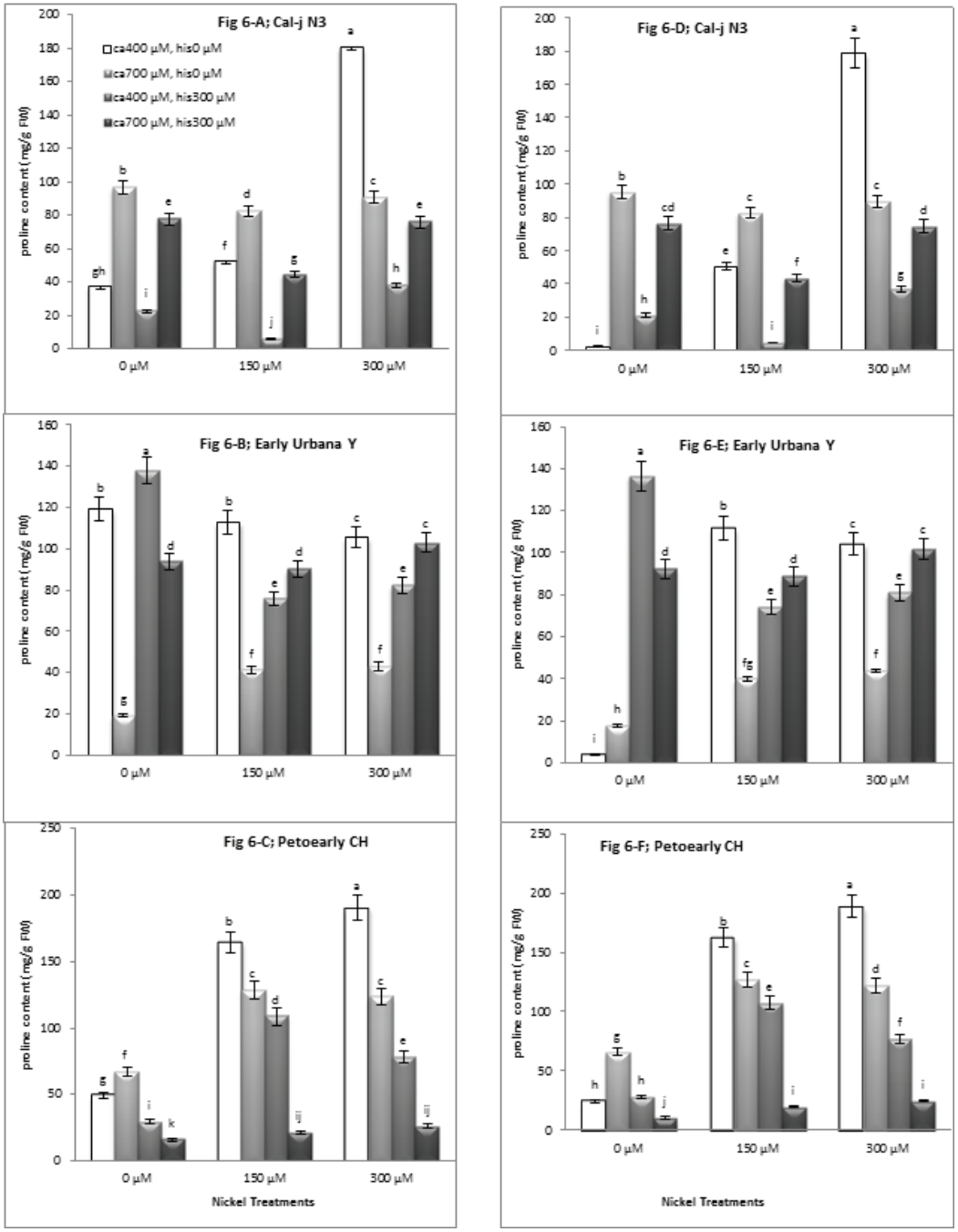

Figure 6. The mean of shoot and root proline (A-F) determined and three-way ANOVA with multiple but equal number of observations per test tube for the effects of individual treatments and their interactive effects on proline content changes in the tomato cultivars treated with a nutrient solution containing different concentrations of nickel, calcium and histidine ($P<0.05$). Vertical bars indicate the mean of four replications±SE (n=4). Different letters indicate significantly different values among the experimental treatments.

cultivars. Independent application of Ca^{2+} and His decreased the sugar content in the shoot of *Cal-J N3* cultivar, which was similar to the control. His alone also increased the sugar concentration in root of two cultivars (*Petoearly CH* and *Cal-J N3*) under 150 µM Ni^{2+} treatment.

Figure 8 shows the effect of the treatments on potassium concentration of the shoot and root tissues in the tomato cultivars resulted from ICP determination. When the concentration of the external Ca^{2+} and His was low beside the 300 µM Ni^{2+} level (Ni^{2+} treatments without Ca^{2+} and His), an increase in

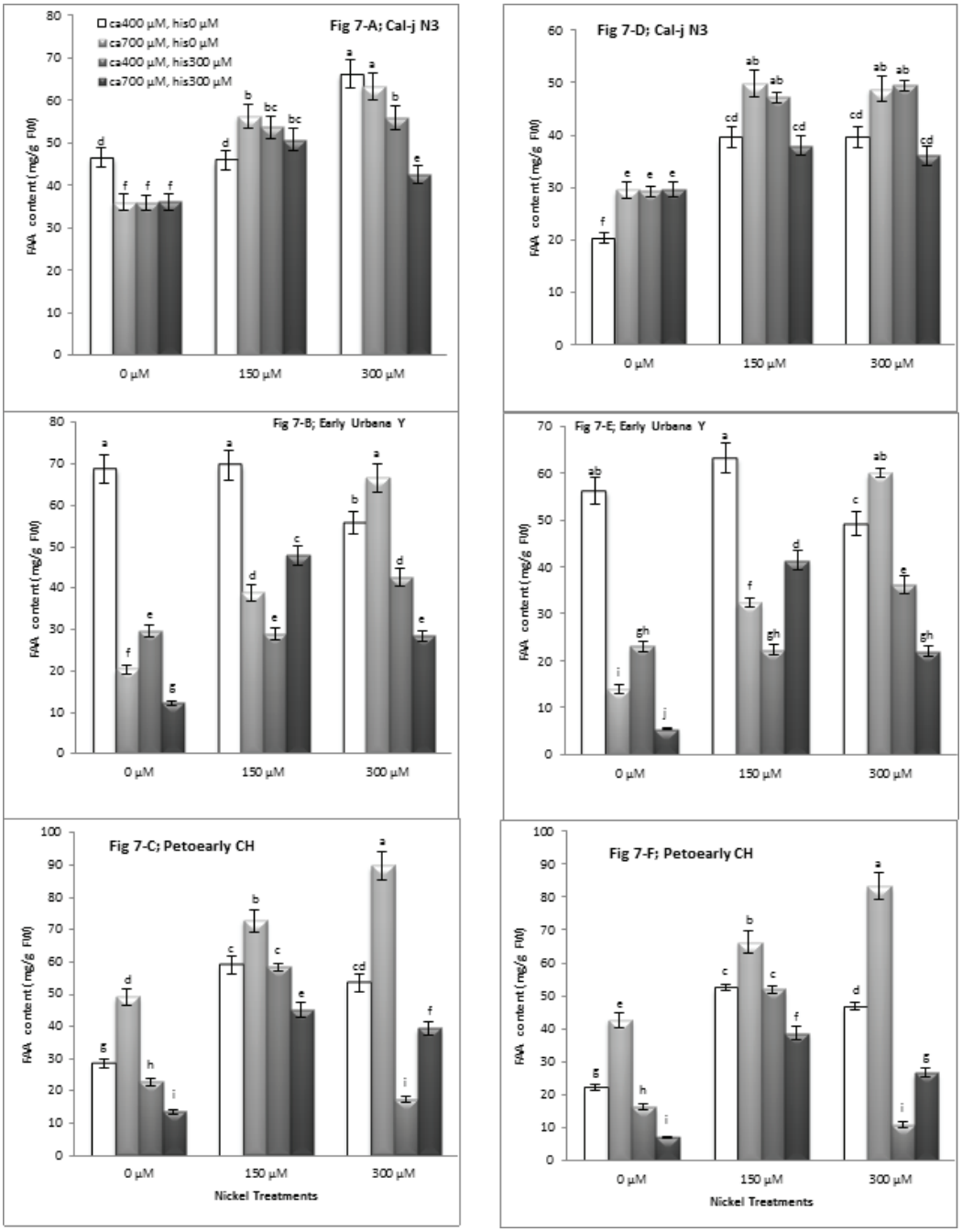

Figure 7. The mean of shoot and root FAA (A-F) determined and three-way ANOVA with multiple but equal number of observations per test tube for the effects of individual treatments and their interactive effects on FAA content changes in the tomato cultivar treated with a nutrient solution containing different concentrations of nickel, calcium and histidine ($P<0.05$). Vertical bars indicate the mean of four replications ± SE ($n=4$). Different letters indicate significantly different values among the experimental treatments.

Ca^{2+} and His (from 0 to 300 µM) significantly increased the K^+ uptake in shoot and root plants. In the *Cal-J N3* cultivar 150 µM Ni^{2+} combined with Ca^{2+} and His decreased the K^+ content in shoot compared to Ni^{2+}-free Ca^{2+} and His treatment (Fig. 8A). However, in the *Cal-J N3* cultivar, Ca^{2+} and His increased the K^+ concentration in shoot and root differently in stress and control conditions (Fig. 8A, 8D). In other cultivars we also observed similar results in K^+ accumulation in the *Cal-J N3* cultivar plants. Our data showed that, Ca^{2+} and His decreased K^+ accumulation in both root and shoot of

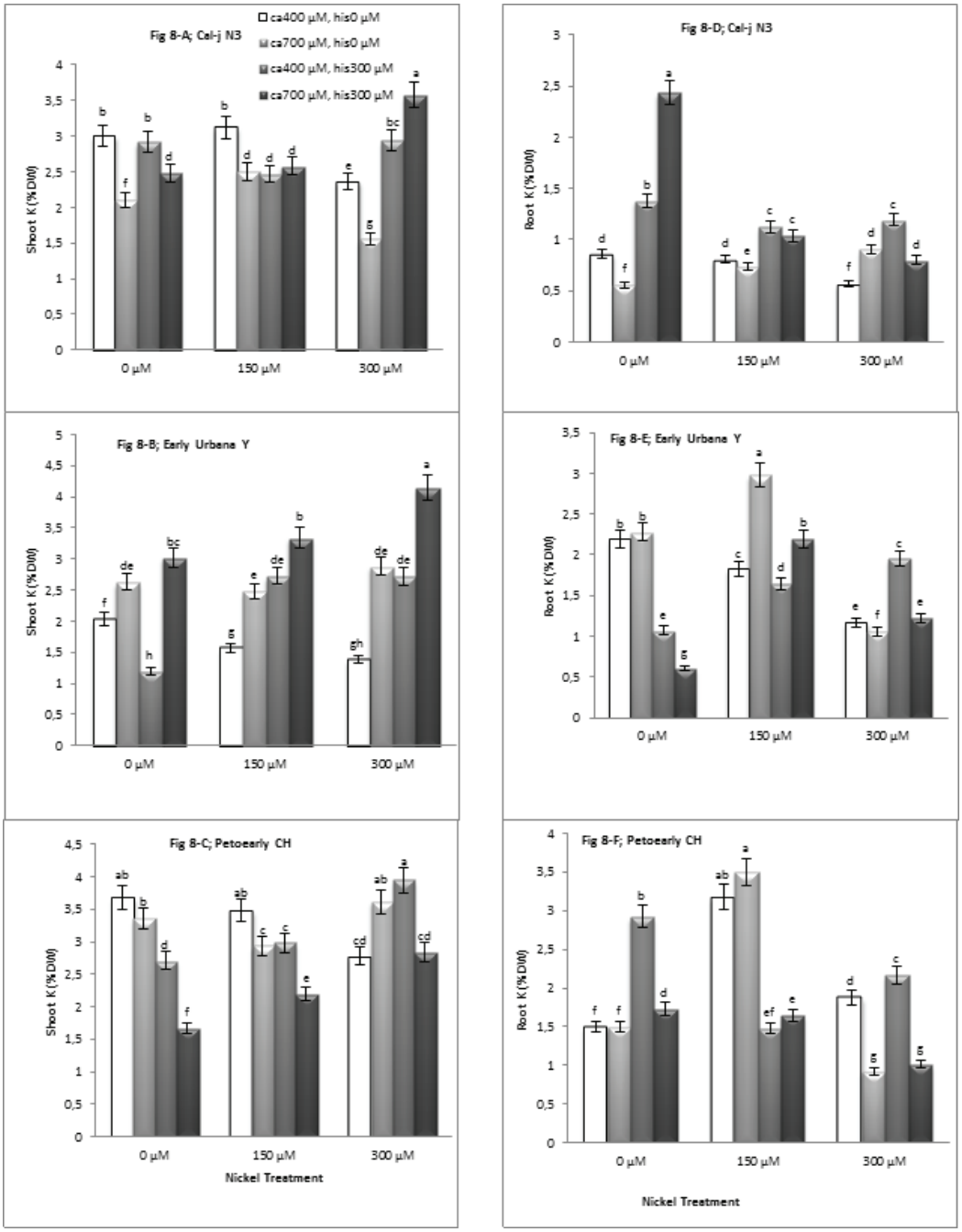

Figure 8. The mean of shoot and root K accumulation (A-F) determined and three-way ANOVA with multiple but equal number of observations per test tube for the effects of individual treatments and their interactive effects on K content changes in the tomato cultivars treated with a nutrient solution containing different concentrations of Nickel, calcium and histidine ($P<0.05$). Vertical bars indicate the mean of four replications ± SE (n=4). Different letters indicate significantly different values among the experimental treatments.

Petoearly CH cultivar under 150 µM Ni^{2+} (Fig. 8C, 8F). But K^+ accumulation and nutrition under 300 µM Ni^{2+} treatment (also containing Ca^{2+} and His) increased compared to the same Ni^{2+} treatment without Ca^{2+} and His.

Discussion

Ni is among those metals that the most cause for immediate concern in environment (Zafar et al. 2007). It is also phytotoxic causing growth inhibition, disturbances in nutrient

uptake and other metabolic and physiological processes of plants (Sanita di Toppi and Gabbrielli 1999, Molas 2002). In this study, we have shown that Ca^{2+} and His are highly effective in protecting tomato plants from growth inhibition caused by the high concentrations of Ni^{2+} under hydroponic culture experiments. Heavy metal tolerance in plants often include binding of metals by chelators such as His or Ca in cell wall, phytosiderophores and phytochelatins, volatilization and enhanced export from the cell (Cataldo et al. 1988). The ligands (such as cell wall and His), bind considerable amounts of Ni^{2+} in rhizosphere, root tissue and cell wall in pericycle layer cells under both resting and growing conditions (Cataldo et al. 1988). The described interactions could play a significant role in metal availability by absorbing and immobilizing toxic ions from soil solution (Wu et al. 2006).

Although the Ca^{2+} and Ni^{2+} accumulation in the root are usually inversely proportional, root ion accumulation more often accounted for a higher share of variability in root elongation than Ni^{2+} accumulation in root tissue at high concentration of Ca^{2+} than control conditions (Wu and Hendershot 2010). Therefore, in the evaluation of root elongation, certain predictors, such as Ni^{2+} and Ca^{2+} must have accounted, when environmental conditions (low Ca^{2+} concentrations) significantly affect the amount of the accumulated Ca^{2+}. The root Ca^{2+} and Ni^{2+} concentration can be determined from total Ca^{2+} and total Ni^{2+} solution. Root elongation revealed a strong positive correlation with total Ca^{2+} content in the roots (Wu and Hendershot 2010). Our data also showed that Ca^{2+} and Ni^{2+} together increased the root length in all tomato cultivars more significantly than the Ni^{2+} treatments without Ca^{2+} (Fig. 1 A-F). The treatments containing Ca^{2+} (300 µM), similar to root elongation, had positive effect on shoot length.

Excess concentration of Ni^{2+} in the growth medium of plants competes with other essential metals, such as potassium and iron, causing their deficiency and oxidative stress. These resulted in the decrease of the chlorophyll biosynthesis and the damage of the photosynthetic system (Buchanan et al. 2002). The visible effects of these changes, observed in our study, were chlorosis of leaves and a reduction in the biomass (FW) of shoot and root tissues. Ca^{2+} addition improved the effect of Ni^{2+} on the cultivars of tomato, especially on *Cal-J N3* cultivar. When tomato cultivars have grown in hydroponic media containing Ni^{2+}, the plants under stress showed the effects of Ni^{2+} toxication that increased in severity. At high Ni^{2+} concentration (300 µM), the leaves appear yellow and have necrotic edges. The amount of chlorophyll a and a+b is an indication of plant stress and they increase in line with the level of stress. During oxidative stress resulted after Ni^{2+} toxication, the decrease of chlorophyll b occurs first, due to its higher redox potential compared to chlorophyll a (Stearns et al. 2007). Our data showed that chlorophyll (a+b) pigment content significantly increased under Ca^{2+} treatment in unstressed conditions (Fig. 4 A-C). On the other hand, this pigment increasing shows that leaf growth and expanding can depend on Ca ion. In this condition, the accumulation of malonaldehyde (MDA) from the cell membrane decreased the oxidative stress. In our research, Ca^{2+} and His could not decrease MDA content in leaves of *Cal-J N3* cultivar under Ni^{2+} stress, but the effects of Ca^{2+} and His were observed on MDA, which caused decreasing in the other two cultivars, especially on the leaves of *Petoearly CH* (data not shown).

Metabolic stress caused by Ni^{2+} may result in decreasing plant growth (Epron et al. 1999; Dodd and Donavan 1999). Cellular events, such as ion compartmentation and osmotic adjustment in tolerant plants may allow continuous growth in the presence of toxic ions (Volkmar et al. 1998). Proline accumulation may be a general response to toxic ion stress (Fig. 6 A-F). Many investigators found the accumulation of amino acids, especially proline in plants exposed to stress, such as salinity, heavy metals, etc. Proline accumulation may contribute to osmotic adjustment at the cellular level (Perez Alfocea et al. 1993). Proline may act as an enzyme protectant, stabilizing the structure of macromolecules and organelles. Proline also acts as a major reservoir of energy and nitrogen upon exposure to Na ions. Energy for growth and survival may help in tolerance of salt stress in barley (Chandrasekhar and Sandhyarani 1996).

In our research, Ni stress increased the proline content in the tomato plants in all cultivars. Proline content was also high in the presence of Ni^{2+} without Ca^{2+} and His in all tomato cultivars compared to the control. Addition of $CaCl_2$ together Ni^{2+} caused increased proline oxidase activity in the plants under stress (Chandrasekhar and Sandhyarani 1996). Under some combined Ni^{2+} and Ca^{2+} treatments, the proline concentration was lower in the tomato cultivars than that of the control (Fig. 6 A-F).

Free amino acids (FAA), such as glycine-betaine act as an osmotic substrate. In different plants, an increase in glycine-betaine under stress can be observed (Sudhakar et al. 1993). Subcellular compartmentation of glycine-betaine biosynthesis in rice is important for increased toxic ion Na tolerance (Sakamoto et al. 1998). To draw conclusions from proline and FAA determination, we can say that both proline and FAA production were promoted by Ni stress in the tomato cultivars. Adding Ca^{2+} (300 µM) to hydroponic system leads to a decrease in the concentration of these two osmoprotectants. The proline oxidase activity is promoted, while the activity of γ–glutamyl kinase is decreased by Ca and proline synthesis (Girija et al. 2002). Because Ni is chelated by histidine, it does not take effect on proline biosynthesis pathway.

Treatments with low concentration of heavy metals, such as Cu, Ni and Cd exhibit an increase in the amounts of total carbohydrates in the root of the plants, and its reverse is true at treatments with high concentrations (Deef 2007). Heavy metal stress affects the enzyme activity by reducing the antioxidant glutathione pool and affecting the iron me-

diated defence processes (Pinto et al. 2003). Heavy metal, such as Ni toxication greatly impaired not only the decrease of soluble sugars, but also their translocation from the root to the shoot (Kuriakose and Prasad 2008). Hopkins (1995) reported that the moderate levels of heavy metals generally play an important role in plant growth and productivity. They act as activators or co-factors in all vital processes, but relatively elevated level of heavy metals induced harmful effects on all physiological processes of plants (Bonnet et al. 2000). Our experimental data showed that 150 μM Ni^{2+} treatment increased sugar accumulation in shoot of *Cal-J N3* and *Early Urbana Y* cultivars in comparison to the control. At the same time, the carbohydrate content decreased in root of *Early Urbana Y* and *Petoearly CH* cultivars under 150 and 300 μM Ni^{2+} stress. It seems that sugar translocation occurred from the root to the shoot as the sugar content in the roots of the tomato plants was declined (Bonnet et al. 2000).

The accumulation of Cd and Cu in bark decreased with increasing addition of Ca^{2+}. Ca^{2+} has earlier been found to reduce the absorption, uptake, translocation and/or accumulation of different plants (Kawasaki and Moritsugu 1987). Thus, it has been demonstrated that putative tonoplast Ca^{2+}/H^{+} antiporters encoded by *calcium exchanger 1* (CAX1) and *calcium exchanger 2* (CAX2) from *Arabidopsis* are involved in the transport of heavy metals from the cytoplasm to the vacuole (Manohar et al. 2011). Another non-selective transmembrane transporter of Ca^{2+}, the low affinity cation transporter (LCT1) is expressed in wheat, also appears to mediate Cd^{2+} transport into the cell (Clemens 2006). Moreover, interactions between Ca^{2+} and other elements, such as Mn, Cd, Zn, Ni and Fe, have been reported in lettuce (Zorrig et al. 2010).

Thus, if the Ca^{2+} pool in the soil and nutrient media is decreased, the availability of heavy metals is increased. This may result in a deficiency of Ca^{2+} in plants caused by the competition for uptake, translocation and binding with heavy metals by interaction effect with ligands, which may negatively affect plant growth and nutrition (Österas and Greger 2006). Instead, the bioavailability of Ca^{2+} is increased in the soil and nutrient media it may decrease the uptake and accumulation of toxic metals in plants, thereby, ameliorating the toxicity of heavy metals in growth parameters similar to our research.

Our results suggest that Ca^{2+} and His interaction improved the growth of the tomato cultivars and K^{+} nutrition conclusively, and decrease the Ni^{2+} toxication in tomato cultivars, especially in *Cal-J N3* cultivar through reducing the detrimental effects of heavy metal via His and Ca^{2+} effects in the tomato plants. Our results also put forward for the first time that, plant growth promoting interaction effect between Ca^{2+} and His could alleviate the heavy metal, such as Ni stress induced in plants. Further investigations aimed at understanding the basic mechanism underlying Ca^{2+} (as a plant multifunctional nutrient) effects on plant growth and nutrition under heavy metal stress, and field trials are warranted at contaminated soils with Ni ions for study the growth changes and plant tolerance in these sites.

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