The modification in quality of parsley (*Petroselinum crispum*) by selenium and amino acids

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Abstract

This research was conducted with the purpose of appraising the effect of Selenium (Se) and/or amino acid fertilizers (AA) on improving growth, physiology, and biofortification of parsley grown under high EC condition. Plants were foliarly supplemented with four levels of Se (0, 25, 50, 100 mgl^{-1}) and/or two concentrations of AA (0 and 0.2%) three times with a week interval. The significantly increases in the leaf fresh and dry masses as well as improvements in leaf area and stem heights were recorded in Se and/or AA-supplemented plants, as opposed to Se 100. However, the growth inhibiting effects of Se 100 were relieved by AA. In contrast to Se 100, Se 25 AA and Se 50 AA were the most effective treatments to improve the chlorophyll contents. The significant inductions in the activities of antioxidant enzymes, peroxidase, and polyphenol oxidase were observed in Se and/or AA treated plants. The usage of Se and/or AA, especially the combined ones, led to the significant stimulations in the activities of phenylalanine ammonia lyase (PAL). The significantly higher contents of phenolics, a suitable quality indicator with great significance for cardiovascular diseases in humans, were observed in Se and/or AA supplemented plants. The foliar supplementation of amino acids accelerated and/or declined the physiological alterations triggered by Se dependent on the applied concentrations. It could be concluded that the simultaneous supplementation of Se and AA at appropriate levels represents an environmentally friendly way of promoting growth, alleviating stress, and fortifying in parsley.

Key words: Antioxidant; biofortification; heavy metal; nutrition; stress

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Introduction

As opposed to some other living organisms, Selenium (Se) has not been classified as an essential nutrient for plants yet (Lindblom et al., 2012) in spite of the fact that Se at the appropriate levels may represent the considerable beneficial impacts on the plant growth and metabolism (Hartikainen, 2005; Lyons et al., 2009; Pilon-Smits et al., 2009). It has been postulated that Se-related metabolism has been lost during evolution of higher plants (Pilon-Smits and Quinn, 2010). In higher plants, the absorption and reduction process of Se, is mainly mediated by sulfur related proteins, thereby producing selenocystein (SeCys) and selenomethionine (SeMet) (Terry et al., 2000; Tamaoki et al., 2008; Pilon-Smits and Quinn, 2010; Van Hoewyk, 2013).

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The probability of occurrence of some critical human diseases like cancers correlates with selenium shortage (Diwadkar-Navsariwala et al., 2006; White and Broadley, 2009). However, the boundary between the Se requirement and the toxicity amounts is narrow (Germ et al., 2007; Pilon-Smits and Quinn, 2010) and Se nutrition is globally regarded as a common issue. The research for better understanding of Se effects and the involved mechanisms is of great significance for the development of Se-fortified agricultural products (Han et al., 2013) which contain organic Se, selenocysteine (SeCys), and selenomethionine (SeMet). The consumptions of the fortified foods or plants with Se have been considered as alternative ways of improving the total dietary intake of Se (Kápolna et al., 2012). For Se enrichment of plants, herbal crop species are considered as suitable substitutes of Brassica and Allium species, Se accumulators, due to regularly consumptions in the human diet and reductions in selenosis possibility (Kopsell et al., 2009).

Amino acids (AA), as important primary metabolites are implicated in the synthesis of plant growth regulators (Glawischnig et al., 2000) as well as other critical organic compounds, such as proteins, amines, alkaloids, vitamins, enzymes, isopernoids (Hounsome et al., 2008; Ibrahim et al., 2010). Moreover, various amino acids are involved in different aspects of plant metabolism like acting as buffers and protecting cells against ammonia toxicity, with amid formation (Abdel Aziz et al., 2010). As selenium is particularly implicated in amino acid related metabolism, therefore it is hypothesized that the foliar supplementation of amino acids might accelerate and/or decline the physiological alterations triggered by Se dependent on the applied concentrations.

The foliar application is considered as a more advantageous method compared to soil fertilization, mainly due to the fact that direct foliar uptake route assures a high degree of assimilation by the plant (Kápolna et al., 2009) and reducing soil adsorption or microbial mediated conversions (Kápolna et al., 2012). Nowadays, with respect to the environmental issues the focus of interest in agriculture is the efficacy of applying natural and safety compounds as well as the methods of supplementations of these organic and inorganic fertilizers to improve plant growth and mitigate signs of different biotic and abiotic stresses.

Vegetables are the considerable part of the human diet and contain crucial essential components like vitamins, dietary fiber, antioxidants, and cholesterol-lowering substances (Hounsome et al., 2008); therefore, improvement of the nutritional quality of vegetables is of importance.

The high EC (electrical conductivity) condition which has unfavorable impacts on plant is a worldwide restricting factor in plant cultivation, especially in arid and semiarid climates. Nowadays, there are more interests in introducing ecofriendly alternative ways to improve plant growth and nutritional qualities as well as relieve the symptoms of physicochemical stresses in plants.

There are limited studies about physiological alterations induced by the interactions of Se and AA, especially in plant exposed to stress conditions. Considering the highlighted importance of Se and AA above, the present research was conducted to appraise the efficacies of Selenium (Se) and/or amino acid fertilizer (AA) to improve growth, metabolism and alleviate the symptoms of high EC stress in *Petroselinum crispum* as well as to evaluate the potential of Se to fortify parsley.

Material and Methods

Experimental design

The experimental design was completely randomized with three replications. The details of formulations of the applied amino acid fertilizer, Aminol-Forte, supplied by INAGROPARS (Agro-Biological Industries Co.) are presented in Table 1. The seeds were surface sterilized and planted in pots. The applied soil was examined (EC: 5.81 dsm^{-1}, pH: 7.8 and N: 0.47%). Two month-old seedlings of parsley (*Petroselinum crispum*) in 8 different groups were sprayed with 4 levels of Se (0, 25, 50,

Table 1
The details of the formulations of Aminol-Forte product

Supplementary	Compounds
Uric nitrogen	1.1%
Total nitrogen	0.8%
Organic nitrogen	0.3%
Organic matter	2%
P2O5 (soluble in water)	6%
Free amino acids	3750 mgL^{-1}

and 100 mgl^{-1}) and/or 2 levels of amino acid fertilizer (0 and 0.2% (v/v)) 3 times with a week interval. Plants were harvested two weeks after the last treatment for the biochemical analysis.

Measurements of Photosynthetic pigments and growth-related characteristics

Photosynthetic pigments were extracted using 80% (v/v) acetone as a solvent. The determination of chlorophyll was performed according to the method of Arnon (1949). The fresh and dry weights of leaves, leaf area, and stem height were measured.

Preparation of enzyme extracts

Enzymes were extracted at four centigrade degree in a mortar and pestle from 1 g leaf tissue using phosphate buffer, 0.1 M pH 7.5 containing Na2-EDTA 0.5 mM and ascorbic acid 0.5 mM, as an extraction buffer. The homogenate was centrifuged for fifteen minutes at 4 °C and the supernatants were applied as enzyme extracts.

Determination of peroxidase activity

Peroxidase activity was determined as described by Abeles and Biles (1991). The enzyme reaction mixture consisted of acetate buffer (0.2 M, pH 4.8) containing 3% H_2O_2 and 0.04 M benzidine in 50% (v/v) methanol. The reaction was recorded by adding the enzyme extract. The peroxidase activity was expressed as an increase in the absorbance at 530 nm per min per gram fresh weight ($\Delta Amin^{-1}g^{-1}fw$).

Assessment of polyphenol oxidase (PPO) activity

PPO activity was assayed according to the method of Raymond et al. (1993). The reaction mixture consisted of 2.6 ml of 0.2 M phosphate buffer PH 7.6 and 200 µl of pyrogallol as a substrate. The enzyme extract was added and the rate of increase in absorbance at 430 nm was measured. The activity was expressed as an increase in the absorbance per min per gram fresh weight ($\Delta Amin^{-1}g^{-1}fw$).

Measuring phenylalanine ammonia lyase (PAL) Aactivity

The reaction mixture for PAL activity consisted of 6 mM phenylalanine, Tris-HCl buffer (0.5 M pH 8) and 200 µl of enzyme extract. After 60 min at 37 °C, the reaction was terminated by the addition of 50 µl of 5 N HCl. PAL activity was determined based on the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm. PAL activity was expressed in microgram of Cinnamate per min per gram fresh weight (µg Cin. $min^{-1}g^{-1}fw$), according to the procedure described by Beaudoin-Eagan and Thrope (1985).

Determining total soluble phenols

Total soluble phenols in the leaf tissues were extracted using 70% (v/v) ethanol as a solvent and assayed using the Folin-Ciocalteu reagent method. Finally, the total soluble phenols were calculated using standard curve of tannic acid.

Statistical procedure

The obtained data were analyzed as a factorial experiment by analysis of variance using SPSS software. Mean differences were determined by Duncan's multiple range test at $P < 0.05$.

Table 2
The effects of Se and/or AA supplementations on some characteristics related to growth and photosynthetic pigments in parsley exposed to the high EC condition

Treatments	Leaf fresh mass (g)	Leaf dry mass (g)	Leaf area (mm^2)	Plant height (Cm)	Chla ($\mu gg^{-1}fw$)	Chlb ($\mu gg^{-1}fw$)	Total Chl ($\mu gg^{-1}fw$)
C	.048000^{c}	.012333^{d}	16.3333^{b}	17.3333^{d}	563.7882^{c}	244.6570^{bc}	808.2387^{cd}
AA	.080667^{b}	.018667^{c}	19.0000^{a}	19.3333^{c}	644.6715^{b}	277.7292^{b}	922.1655^{b}
Se25	.084667^{b}	.019333^{c}	19.3333^{a}	19.3333^{c}	665.3491^{b}	253.7509^{bc}	918.8723^{b}
Se25AA	.089000^{b}	.024000^{b}	19.3333^{a}	20.3333^{bc}	668.1899^{b}	270.5962^{bc}	938.5502^{b}
Se50	.106000^{a}	.028333^{ab}	19.6667^{a}	20.6667^{b}	634.8587^{b}	243.0050^{bc}	877.646^{bcd}
Se50AA	.114000^{a}	.029667^{a}	20.0000^{a}	22.3333^{a}	765.8581^{a}	356.2715^{a}	1121.838^{a}
Se100	.035667^{d}	.006333^{e}	13.3333^{c}	15.3333^{e}	569.4607^{c}	227.7417^{c}	797.0027^{d}
Se100AA	.039000^{d}	.007667^{e}	14.3333^{c}	15.6667^{e}	646.9909^{b}	241.0903^{bc}	887.8623^{bc}

*: the mean values followed by different letters are significantly different, according to the Duncan's test.

Table 3
The effects of Se and/or AA supplementations on some characteristics related to enzymatic antioxidant system and phenylpropanoid metabolism in parsley exposed to the high EC condition

Treatments	Peroxidase activities ($\Delta Amin^{-1}g^{-1}fw$)	PPO activities ($\Delta Amin^{-1}g^{-1}fw$)	PAL activities ($\mu gCin.min^{-1}g^{-1}fw$)	The soluble phenols ($mgg^{-1}fw$)
C	1.2500^{f}	.5550^{c}	.9615^{d}	8.5960^{e}
AA	1.6900^{e}	.7350^{b}	1.1572^{c}	9.6887^{a}
Se25	2.3500^{d}	.5150^{c}	1.1207^{c}	9.2815^{bc}
Se25AA	3.0700^{b}	.8300^{b}	1.1610^{c}	9.1242^{cd}
Se50	2.4000^{d}	1.0050^{a}	1.3336^{b}	9.2653^{bc}
Se50AA	3.6000^{a}	.7900^{b}	1.5638^{a}	9.6242^{a}
Se100	2.9600^{bc}	.9650^{a}	1.0478^{cd}	8.9589^{d}
Se100AA	2.7200^{c}	1.0750^{a}	1.3183^{b}	9.5113^{ab}

*: the mean values followed by different letters are significantly different, according to the Duncan's test.

Results

Application of Se and/or AA by spraying the highest level of Se (Se 100) led to the significant promotions in biomass, as it was indicated by the recorded higher amounts of the fresh and dry weights of leaves in treated plants, compared to control (Table 2). In contrast with Se 100, considerable improvements in the leaf area and increases in the stem heights were observed in Se and/or AA-treated groups, compared to control (Table 2). The reductions in various growth related characteristics in combination with necrotic lesions were recorded as signs of stress induced by the highest levels of Se (Se 100) applied.

The evaluations of the changes induced by the applied concentrations of Se and/or AA in photosynthetic pigments revealed that in contrast to Se 100, Se 25 AA and Se 50 AA were the most effective treatments to improve chlorophyll contents, in comparison with control (Table 2). In addition, based on the obtained results Chl. a was more affected by Se than Chl. b (Table 2).

In comparison to control, the significant induced activities of peroxidase were found in Se and/or AA supplied plants, among which Se 25 AA and Se 50 AA had the highest results (Table 3). Except for individual treatment of Se 25, Se and/or AA-treated plants possessed significantly higher activities of polyphenol oxidase, compared to control (Table 3).

The significantly induced activities of PAL were found in Se and/or AA applied plants among which Se 50 AA, Se 50 and Se 100 AA had respectively the highest records (Table 3). Similarly, significant promotions were observed in the contents of total soluble phenols caused by supplementation of Se and/or AA, compared to control (Table 3). There was a positively significant correlation between the total soluble phenol contents and PAL activities (the correlation coefficient of 0.630 significant at $P<0.01$).

Discussion

Growth and toxicity

The plant growth and development were affected by the applied treatments, as it was reflected by the significantly enhanced leaf biomasses, the increased leaf area, as well as the elevated stem height observed in Se and/or AA-supplemented plants, except for the plants sprayed by the highest applied level. These results in combination with the observed necrotic symptoms recorded in plants treated by Se 100 clearly indicated that in contrast to the other applied levels of Se, this concentration had relatively toxic and inhibiting impacts on the plant growth and development which were slightly relieved by AA supplementation. These findings about the growth inhibiting effects of selenium at inappropriate concentrations are consistent with earlier studies in different plant, including mustard (Fargašová, 2003) and lettuce (Ramos et al., 2010). Also, the promoting effects of Se on the growth and/or photosynthesis have been recorded in various species of higher plants like lettuce (Xue *et al.,* 2001), soybean (Djanaguiraman *et al*., 2005), and barley (Habibi, 2013) as well as different algal species, including Marine coccolithophorids, *Emiliania huxleyi*, *Gephyrocapsa oceanica*, and *Helladosphaera* spp. (Danbara and Shiraiwa, 1999). Given that, the alterations in hormones, especially ethylene, salicylic acid and jasmonate are triggered by Se (Tamaoki et al., 2008), the changed hormonal balances may be responsible for promoting or preventing plant growth in Se-treated samples. The foliar application of nitrogen led to the modifications in vegetative growth in *Thuja orientalis* (Abdel Aziz *et al*., 2010).

Photosynthetic pigments

Evaluation of the changes induced by the applied concentrations of Se and/or AA in photosynthetic pigments revealed that in contrast to Se 100, other used levels of Se in combination with AA were effective to improve chlorophyll contents. In addition, based on the obtained results it seems that Chl a is more affected by Se than Chl b. The enhancing effects of Se on chlorophyll contents found in the present research is in agreement with findings of Xue *et al*. (2001) in lettuce, Germ *et al*. (2007) in potato, and Djanaguiraman *et al*. (2005) in soybean. The enhanced photosynthetic pigments and probably higher photosynthesis rates as well as the induced antioxidant system due to the suitable treatments of Se and/or AA might be responsible for improving qualities and quantities of parsley leaves and mitigating the signs of the high EC. It has been postulated that ethylene, salicylic acid, and jasmonate implicate in Se-triggered responses (Tamaoki *et al.*, 2008). Thus, the modified hormonal balance, depending on Se concentration, may be responsible for promoting or inhibiting effects of Se on the growth related characteristics. Since Se is chemically similar to sulfur (S), nonspecific incorporation of S by Se into proteins and other sulfur components may result in non-functional proteins and Se toxicity at elevated levels (Pilon-Smits and Quinn, 2010; Habibi, 2013). The excess Se may be detrimental to photosynthesis apparatus and inhibit photosynthesis (Vitova *et al*., 2011; Łabanowska *et al*., 2012; Wang *et al*., 2012). Reductions in the absorbed light energy, the stream of excitation energy, the transport of electrons through PSII, the quantum yield of electron transport in PSII, and the density of active centers of photochemical reactions in PSII were mentioned as mechanisms involving in stress caused by the excess Se in three genotypes of wheat (Łabanowska *et al.,* 2012). A high rate of photosynthesis caused by the supplemented nitrogen provoked higher biomass production (Neuberg *et al.*, 2010).

Antioxidant enzymes

The assessment of the alterations in two vital enzymatic antioxidants caused by the applied treatments indicated that Se and AA, especially the former, induced the activities of peroxidase and PPO. These results clearly indicated that the enzymatic antioxidant system is influenced by Se and/or AA. Ionic and osmotic (Munns and Tester, 2008) as well as oxidative stresses (Palma *et al.,* 2013) are known as the main reasons why salinity condition suppresses growth and causes damage. Antioxidant system which includes non-enzymatic and enzymatic antioxidants may provide a strategy to improve tolerance in the plants exposed to the various abiotic and biotic stress conditions (Gill and Tuteja, 2010). The inductions in the activities of antioxidant enzymes caused by Se have been recorded in varieties of plants (Hartikainen *et al.,* 2000; Xue *et al.,* 2001; Seppanen *et al*., 2003; Kong *et al.,* 2005; Filek *et al*., 2008; Ramos *et al.,* 2010) and proposed as a main mechanism triggered by Se to counteract lesion signs in plants exposed to different physicochemical stresses (Djanaguiraman *et al.,* 2010; Hasanuzzaman *et al.,* 2011; Feng *et al.,* 2013). Se mitigated the signs of lead toxicity in Coleus blumei by the improved glutathione levels and the activities of catalase and glutathione peroxidase (Yuan *et al*., 2013). Selenium at low levels promoted the growth of tobacco by improving the antioxidant system and declining lipid peroxidation rate, in contrast to high levels (Han *et al.,* 2013). The excessive inorganic Se might enhance lipid peroxidation, thereby causing damage to the plant growth (Han *et al.,* 2013). The recorded inducing effects of exogenously applied amino acid fertilizer on the activities of peroxidase and polyphenol oxidase are consistent with the findings of Oraghi Ardebili et al. (2012) in *Aloe vera*.

Phenylpropanoid metabolism

Phenylpropanoid metabolism was influenced by the supplementations of Se and/or AA as it was reflected in the modified activities of PAL (a main key enzyme in phenylpropanoid pathway) and the soluble phenol contents. The foliar usage of Se and/or AA, especially the mixed treatment, led to significant stimulations in PAL activities and there was a positively significant correlation between soluble phenol contents and PAL activities. The enhanced phenolic contents may be regarded as a suitable quality indicator because of their antioxidant properties.

It has been suggested that Genins, aglycone flavonoids purified from parsley leaves, possess a potent antiplatelet activity and may reduce thrombosis and cardiovascular issues (Gadi *et al.,* 2012). Flavonoids potentially represent beneficial effects, including antiviral, anti-inflammatory, antihistamine, and antioxidant activities, preventing lipid peroxidation, scavenging free radicals as well as chelating iron and copper ions (Hounsome *et al.,* 2008). The stimulation in phenylpropanoid metabolism could be attributed to the Se modified hormonal alterations, particularly salicylic acid, jasmonic acid and ethylene, thereby stimulating defense correlated reactions. The Se triggered expressions of the ethylene and/or jasmonate related genes have been recorded (Tamaoki *et al*., 2008). Plant secondary metabolites have the critical role in the special plant interaction with the environment as well as adaptation (Treutter, 2006; Ramakrishna and Ravishankar, 2011). Phenolic compounds which are primarily derived from cinnamate formed by the action of PAL have been considered as the most important secondary metabolites (Michalak, 2006). Phenolic compounds, especially flavonoids, have been considered as dietary components for their significant contribution to the nutritional and antioxidant characteristics of plants (Çirak *et al.*, 2014) because of their possible role in restricting cardiovascular diseases and several kinds of cancer (Chu *et al.,* 2000). The shikimate pathway occurred in microorganisms and plants, but not in animals, gives rise to aromatic compounds, particularly the aromatic amino acids, including L-phenylalanine, L-tyrosine and L-tryptophan, which are essential amino acids for humans and should be obtained through the diet (Fraser and Chapple, 2011). The phenylpropanoid derivatives are known to be implicated in all aspects of plant responses to biotic and physicochemical stimuli (Vogt, 2010). It has been stated that the accumulations of phenylpropanoids are affected by a vast range of biotic and abiotic stresses (Ramakrishna and Ravishankar, 2011). The promotions in antioxidant activities recorded in *Aloe vera* supplemented by the amino acids have been attributed to increases in antioxidants, ascorbate, reduced glutathione, and phenolic compounds (Oraghi Ardebili *et al*., 2012). The foliar supplementation of amino acids improved the contents of secondary metabolites in Aloe vera (Oraghi Ardebili *et al.,* 2012) and Ocimum basilicum (Azad Khankandi *et al*., 2013).

Conclusion

These findings obviously reflected that the foliar simultaneous applications of Se 25 or Se 50 and AA were the most effective treatments to improve growth and qualities of parsley plants grown under unfavorable condition while Se 100 adversely affected some parameters related to growth. According to the obtained results from the present research it could be postulated that the foliar supplementation of Se and/or AA potentially represents an environmentally friendly way of promoting growth, alleviating the symptoms of stresses as well as improving qualities of plants via the possible mechanisms, including inductions in antioxidant system, enhancements in photosynthesis pigments and stimulations in the production of secondary metabolites, especially phenolics which are suitable quality indicator with great significance for cardiovascular diseases in humans.

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