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Selenium biofortification on garlic growth and other nutrients accumulation

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ABSTRACT

Selenium (Se) is an essential element for humans and has anti-cancer function. Garlic can accumulate Se, so it is an option to Se supplementation in the human diet. The aim of this research was to study Se uptake and accumulation during garlic growth. Four doses of Na_2SeO_4 and Na_2SeO_3 solution were applied in the substrate (0, 5, 10 and 15 kg ha^{-1} Se) for one time in August 2014, with a random plot design and 3 replicates on garlic clone Rubi INTA. Three harvests were made, in September, October and December 2014. After each harvest, leaves, bulbs and roots were separated and conditioned (peeled and chopped), lyophilized, and finally acid-digested prior to Se, Mg, Zn, Mn, Cu, Fe, P and S determination by inductively coupled plasma mass spectrometry (ICP-MS). The Se accumulation was proportional to Se doses and did not affect garlic growth. Also, Se distribution among different organs was related to the garlic growth cycle. The Se presence decreased accumulation of Mg, Mn, Cu, Fe, P and S but increased Zn accumulation in plants. Garlic can be an important Se source to humans but it is important to consider Se-doses for biofortification.

Keywords: *Allium gender sativum*, selenium uptake, bioaccumulation, enriched crop.

RESUMO

Biofortificação de selênio no crescimento de alho e avaliação de acúmulo de outros elementos nutrientes

O selênio (Se) é elemento essencial para os seres humanos e é considerado anticancerígeno. Alho pode acumular Se, sendo, por isso, uma opção para a suplementação de Se na dieta humana. Assim, o objetivo deste trabalho foi estudar a captação e acúmulo de selênio durante o crescimento do alho e avaliar os efeitos da fortificação de Se. Quatro doses da solução de Se, nas formas de Na_2SeO_4 e Na_2SeO_3 foram aplicadas ao substrato em agosto de 2014 (0, 5, 10 e 15 kg ha^{-1} de Se) de uma única vez, em delineamento de parcela aleatória e 3 repetições no clone de alho Rubi INTA. Três colheitas foram feitas, em setembro, outubro e dezembro de 2014. Após cada colheita, as folhas, bulbos e raízes das amostras de alho resultantes de cada tratamento foram separadas e acondicionadas (descascadas e picadas), liofilizadas, e, finalmente, digeridas com ácido antes da determinação de Mg, Zn, Mn, Cu, Fe, P e S por espectrometria de massa com plasma indutivamente acoplado (ICP-MS). Os resultados confirmaram que o acúmulo de Se foi proporcional à magnitude das doses, e não afetou significativamente o crescimento do alho. Além disso, a distribuição de Se entre os diferentes compartimentos da planta foi relacionada ao ciclo de crescimento da planta de alho. A fortificação do alho com Se causou mudanças significativas, e induziu o acúmulo e distribuição de diferentes nutrientes, como Zn, Mg, Mn, Cu, Fe, P e S, mas aumentou o acúmulo de Zn nas plantas. O alho pode ser considerado fonte importante de Se para aumentar a ingestão deste elemento na dieta humana, mas é importante considerar as doses de Se para a biofortificação.

Palavras-chave: *Allium gender sativum*, captação de selênio, bioacumulação, colheita enriquecida.

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Selenium (Se) is an essential microelement in human and animal nutrition, due to its beneficial effects on health; specially because it protects the immune system and contributes to cardiovascular conditioning, proper thyroid function, fertility in men and women, and above all, anti-cancer function (Bodnar *et al*., 2016). Likewise, Se can be a component of glutathione peroxidase, a biomolecule that protects the organism from the negative actions of free radicals by reducing hydrogen peroxide and organic peroxides (Bodnar *et al*., 2016). The role of Se as potent cancer chemopreventive agent depends on the dose and the form of Se. Moreover, Se is an inducer of apoptosis and inhibitor of cell proliferation which can account for its cancer preventive effect (Sinha & El-Bayoumy, 2004). The most important source of Se in human diet is food. Thus, several foods have been recognized as natural Se sources, including yeast, onion, garlic and Brazil nuts, among others (Ellis & Salt, 2003).

The fortification of different plants with Se has also been studied in order to obtain Se-enriched foods that have potential functionality to promote health. In fact, it is well established that vegetables derived from *Allium* and *Brassica* plant species, play an important role in Se supplementation because they have the ability of accumulating high amounts of Se even though Se is not a micronutrient for the plants, and hence, they could be used as natural dietary Se supplements (Lavu *et al*., 2012). Among these, garlic (*Allium sativum*) is highly important due to its multiple medicinal effects, for example, it reduces blood cholesterol levels and antiplatelet aggregation, produces anti-inflammatory activity and inhibits cholesterol synthesis (Burba, 2013). Besides, this crop is the most consumed culinary seasoning in the world, 26.5 million tons of garlic are approximately consumed per year (Camargo *et al*., 2010). Garlic is also considered a Se hyper-accumulator and uptakes this element effectively, up to 1000 μg g^{-1} (Ghasemi *et al*., 2015; Kaur *et al*., 2016). This large Se accumulation is mainly because Se and S have some chemical similarity (Kaur *et al*., 2016), which causes that Se can be metabolized through the same assimilation pathways than S. Furthermore, Se is metabolized by garlic into important Se-amino acids like Se-methionine (SeMet), Se-cysteine (SeCys), Se-methylselenocysteine (Se-methylSeCyst) and γ-glutamyl-Se-methylselenocysteine (γ-glutamyl-SemethylSeCyst) (Quinn *et al*., 2011). These Se-amino acids are precursors of methylselenol, which has been demonstrated to be one of the most active species for cancer prevention in humans (Lü *et al*., 2016).

Although Se-enriched garlic is an attractive option to naturally increase Se intake by humans, there are few studies on the uptake and accumulation during the growth of garlic plant (Poldma *et al*., 2011; Ogra *et al*., 2015; Cheng *et al*., 2016). Additionally, the application of Se can improve plant quality and yield, given that, Se has an antioxidative effect on plants which depends on Se doses (Zhao *et al*., 2013). Moreover, an optimal Se dose could play a positive role on plant yield and nutritional quality, whereas a high dose can be harmful to plant (Xue *et al*., 2001). Therefore, it is important to investigate the garlic tolerance to different Se doses and the best time for Se application to obtain an optimum enrichment in the plants without affecting their growth. On the other hand, Se may influence the accumulation of macronutrients and micronutrients involved in oxidative regulation of cells, in a way that biofortification is feasible only if the Se content has no negative influence on uptake of other essential elements (Longchamp *et al*., 2015).

The aim of the present research was to study Se uptake and accumulation in different moments of garlic growth, evaluating its effects on the development of the plant, as well as on uptake and accumulation of other nutrients to a better understand of Se influence on garlic plants.

MATERIAL AND METHODS

Reagents

All reagents used were of analytical grade and all solutions were prepared in ultrapure water with a minimum resistivity of 18.0 MΩ cm obtained from an Osmo ion-U-0.5 ultrapure water equipment (APEME, Buenos Aires, Argentina). The Se solution (169.89 g L^{-1}) used in the different Se biofortifications was prepared with the following proportions: 284.59 g L^{-1} sodium selenate (Na_2SeO_4) 98% and 111.65 g L^{-1} sodium selenite (Na_2SeO_3) 99% from Sigma (St Louis, USA). Sub boiling nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) 30% Merck (New Jersey, USA) were used for sample decomposition. The standard solutions of Se, Mg, Zn, Mn, Cu, Fe, P and S were prepared from stock solutions (1000 mg L^{-1}) by simple dilution. Reagents from Merck were used in the stock solutions. Rhodium (^{103}Rh) mono-elemental standard solution from Perkin Elmer Pure Plus Atomic Spectroscopy Standards was used as internal standard.

Cultivation of Se enriched garlic

Selenium enrichment experiments of garlic were conducted at La Consulta, Experimental Station INTA, Mendoza, Argentina (33°42′30″S, 69°04′22″W, 958 m altitude) during the growing season between April 2014 and December 2014. The garlic clone "Rubi INTA" was used in this work and was fortified with a solution at a concentration of 169 g L^{-1} of Se. Four doses were applied: 0, 5, 10 and 15 kg ha^{-1} of Se, with a random plot design and three replicates. The tested Se doses were similar to those reported in other studies about the *Allium gender* (Sharma *et al*., 2007; Domokos-Szabolcsy *et al*., 2011; Lavu *et al*., 2012). Three samplings were performed: at the beginning (September 2014:103-BBCH), mid-term (October 2014; 203-BBCH scale) and end (December 2014; 409-BBCH scale) of the growth cycle (Lopez-Bellido *et al*., 2016). Garlic was planted in 10 L pots filled with a mixture of 90% sphagnum peat and 10% sandy loam soil (10% clay, 60% fine sand, 25% silt and 5% calcium bicarbonate). A single application of Se to garlic plants was performed in August because garlic plants are in the vegetative growth stage, i.e. an specific time where assimilation and metabolization of nutrients are more efficient (Burba, 2013). The Se dose was applied directly to the substrate. Plants were irrigated daily during that stage with an automatic drip system operated with a timer and fertilized with a 10:1:10 (N:P:K) fertilizer solution reaching the equivalent of 300 kg ha^{-1} of N, 30 kg ha^{-1} of P and 300 kg ha^{-1} of K in the growth cycle. The environmental conditions of the crop were: maximum and minimum temperature between 15 and 30°C and -5 and 10°C respectively; maximum and minimum relative humidity between 80 and 100% and 20 and 50% respectively and rainfall was between 10 and 110 mm. Three useful plants were used for each treatment.

The substratum and the water used in these assays were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) before their use and Se was not detected.

Sample processing

After each sampling, leaves, bulb

and roots were separated, cleaned and weighed. Then, samples were lyophilized, homogenized and stored at -18°C until analytical determination. For lyophilization and homogenization, a freezer dryer Virtis freeze mobile (New York, USA) Model 6 Lyophilizer 12l and a grinder Ultracomb (Buenos Aires, Argentina) model MO-8100A were used, respectively.

Digestion of garlic samples

Approximately 0.150 g of dried sample (leaves, bulbs and roots) was digested using 6 mL sub-boiling HNO_3 and 0.5 mL 30% (v/v) H_2O_2. The decomposition of the samples was performed in a microwave oven (DGT, Provecto Analítica, Jundiaí, Brazil) at a nominal power of 1200W and the program comprised four steps: 1) 5 min at 400W, 2) 8 min at 790W, 3) 4 min at 320W, and 4) 3 min at 0W. Finally, sample was filtered and diluted to 50 mL before the analysis.

Determination of Se and nutrients in garlic

The concentration of different elements (Se, Mg, Zn, Mn, Cu, Fe, P and S) in garlic samples was evaluated by inductively coupled plasma mass spectrometry (ICP-MS). The Elan DRC-e ICP-MS from Perkin Elmer (Norwalk, CT, USA) was used. Depending on the analyte, different strategies for interferences removal were adopted, including the application of two different reaction gases: oxygen and methane (Table 1). The instrument was calibrated against external certified standard solution containing each of the elements determined in this work. Also, ^{103}Rh was used as internal standard (Table 1).

Translocation factor (TF)

The translocation factor (TF) was calculated as the ratio of Se concentration in the leaves to the Se concentration in the roots (Renkema *et al*., 2012).

$TF = [Se]_{leaves}/[Se]_{roots}$

Statistical analysis

Four treatments were studied: 0, 5, 10 and 15 kg ha^{-1} of Se, with a random plot design and three replicates. The Se doses were applied directly to the substrate. All instrumental measurements were also performed with three replicates. Data were evaluated using analysis of variance (ANOVA), and means were compared by Tukey's test at 5% probability level using InfoStat/L.

RESULTS AND DISCUSSION

Accumulation of Se in different garlic organs

Garlic has a special ability to accumulate large amounts of Se (Table 2). This is possible because Se has chemical similarity with S. Also, the highest Se concentrations were found in leaves; in fact, Se concentration in leaves was twice that found in the

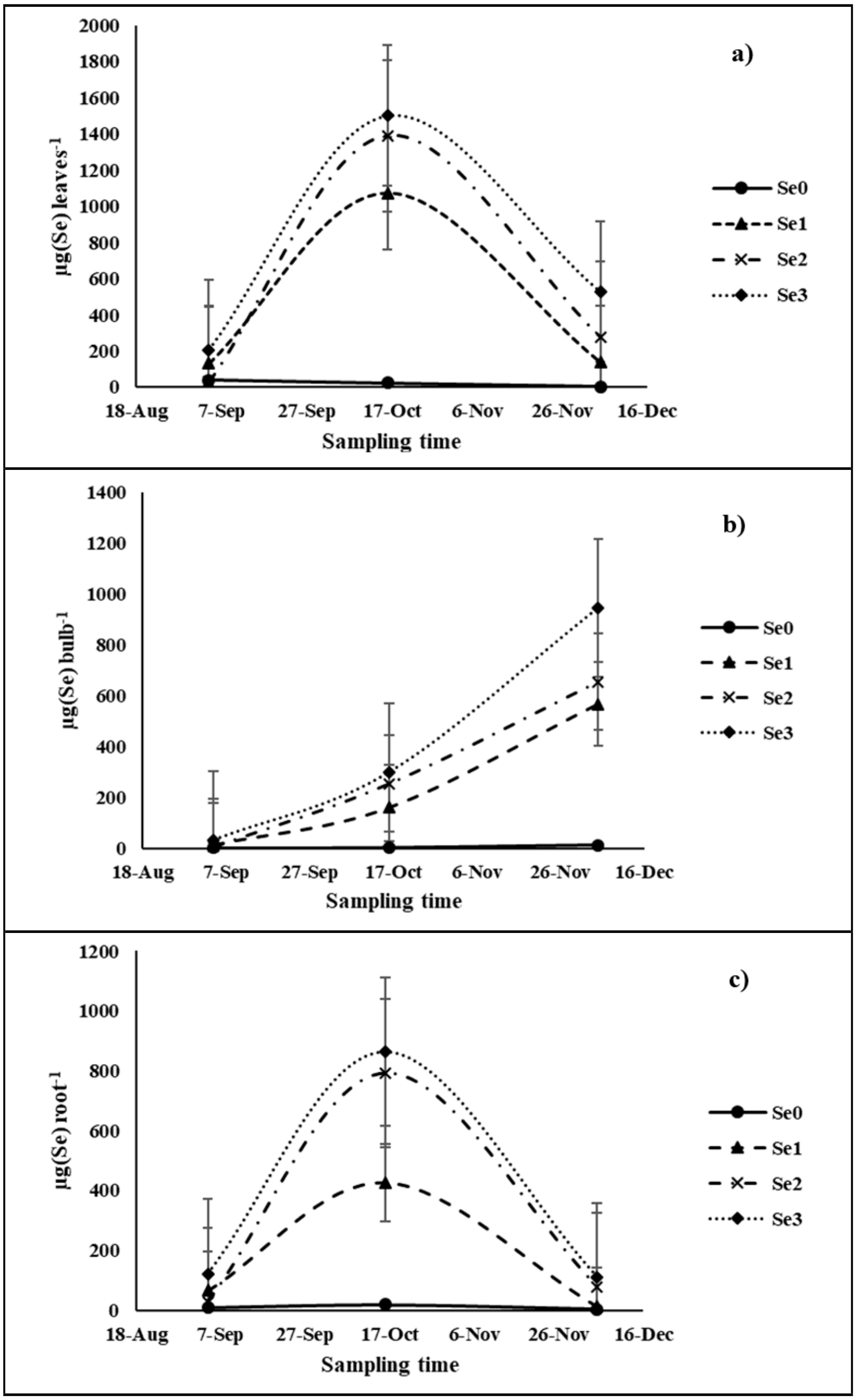

Figure 1. Total Se accumulated (µg) during growing season in different garlic organs. a) leaves; b) bulb; c) roots; Se0= 0 kg ha^{-1}; Se1= 5 kg ha^{-1}; Se2= 10 kg ha^{-1}; Se3= 15 kg ha^{-1}. Mendoza, INTA, 2014.

other organs. This result was expected as leaves are the termination of the vascular tissue, i.e. where it is expected that Se would be accumulated with the highest magnitude. Furthermore, we observed that Se content in garlic plants was increased thirty times by Se fertilization (Table 2). Similar results have been reported for other *Allium* plants like ramps, leek and onion where Se accumulation was also around 1000 µg g^{-1} (Montes-Bayón *et al*., 2002; Lavu *et al*., 2012; Schiavon & Pilon-Smits, 2017). For this reason, the *Allium* plants are considered as Se hyperaccumulaters and represent natural Se sources to humans. In our experiments we also observed that Se concentration dropped between the second and the third sampling period, most probably due to the formation of volatile Se compounds after the initial accumulation of Se. In fact, it has to be mentioned that *Allium* plants are able to form highly volatile Se compounds, such as dimethylselenides (DMSe) and dimethyldiselenides (DMDSe). The results reported in the present work have demonstrated that Se accumulation in garlic plants not only depends on the Se doses but also on each specific organ and the time of garlic growth. On the other hand, Se is not considered essential for plants but they can obtain benefits from this element due to its antioxidant power against reactive oxygen species. These free radicals are very reactive producing oxidative stress, which results in H_2O_2 accumulation that can induce a sequence of reactions and/or trigger unspecific oxidation of proteins, membrane lipids or DNA injury (Mora *et al*., 2015). All this is avoided with Se because it is a better antioxidant than S. Therefore, Se supplementation is not only good for human health but also for plants.

Variations of Se content in garlic during growth season

Despite the nutritional importance of garlic and its high content of Se, the current information regarding Se accumulation and metabolism in garlic is scarce. In the present work, garlic plants were cultivated under the earlier mentioned conditions and Se content was determined in different growth cycle moments. Selenium was found to be accumulated differently in leaves, bulbs and roots depending on the growing period of garlic (Figure 1). The content of Se increased in bulbs as time passed while in leaves and roots the Se accumulation increased to a maximum value and after that it decreased (Figure 1). The Se accumulation in bulbs reached a maximum value of 1000 µg/ $bulb^{-1}$ when the Se dose was the highest while the Se accumulation in leaves went from 1600 to 600 µg/$leaves^{-1}$, and roots went from 800 to 200 µg/ $root^{-1}$. This behavior can be explained considering that during growth cycle garlic plants have four different stages

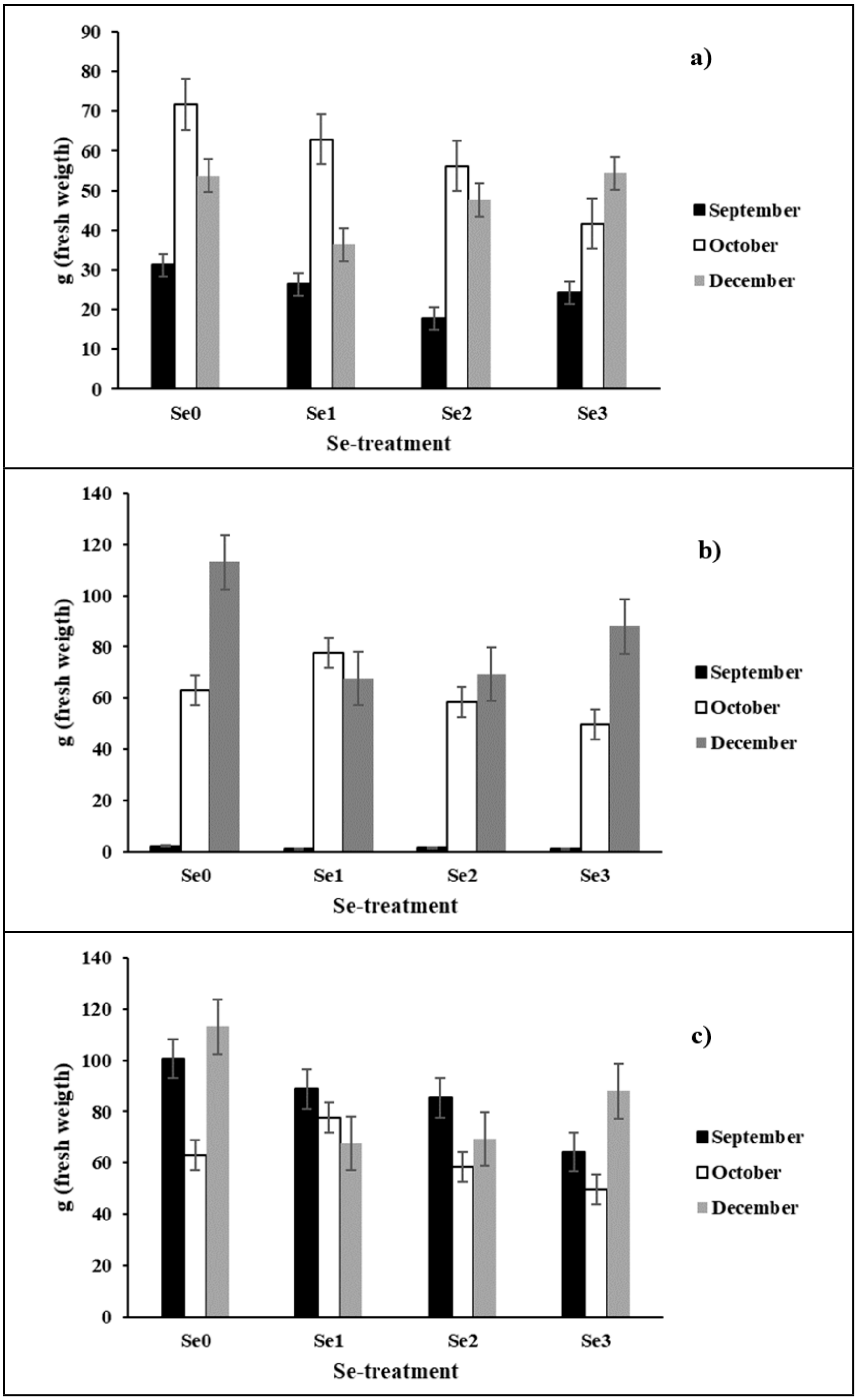

Figure 2. Fresh weight (g) during growing season of garlic fortified with Se. a) leaves, b) bulb and c) roots; Se0= 0 kg ha^{-1}; Se1= 5 kg ha^{-1}; Se2= 10 kg ha^{-1}; Se3= 15 kg ha^{-1}. Mendoza, INTA, 2014.

(Burba, 2013). The first one is dormancy when there is no biological activity (Maroto-Borrego, 2002; Portela & Cavagnaro, 2004). The next one is sprouting when leaves and roots start to grow using the nutrients stored in the bulb. After that, it starts the vegetative growth when the plant elaborates important metabolites, like Se and S compounds with biological properties for humans. Likewise, leaves and roots grow fast during that stage (Maroto-Borrego, 2002; Portela & Cavagnaro, 2004). Finally, bulbing is initiated when the elaboration of metabolites is stopped and these substances are transported from leaves to the bulb to start its development (Maroto-Borrego, 2002). These stages are strongly affected by environmental conditions (e.g. temperature and photoperiod) of the site where garlic plants grow (Portela & Cavagnaro, 2004). The obtained results showed that the accumulation of Se in garlic plants was similar to that observed for others nutrients such as S, where its accumulation in bulbs increased fast when the bulbing stage started, while in leaves and roots decreased. Therefore, aiming to maximizing the accumulation of Se in the edible parts of the garlic plant, i.e. bulbs, Se must be applied before the bulbing stage because at this specific time the Se accumulation and its metabolic process are stopped.

Furthermore, the ability of garlic plants to transport and accumulate Se through time was calculated and expressed as TF values (Table 3). Garlic plants showed a very high TF which indicates that Se is transported and accumulated in plants quite easily (Puccinelli *et al.*, 2017). The TF was increased throughout growth (LSD= 9.4) and decreased while Se dose was higher than 10 kg ha^{-1} (LSD= 4.5). This decrease may be due to a decrease in Se tolerance due to its toxicity at high concentrations (Brown & Shrift, 1982). Similar results were found in sunflower crop, while in basil plants the results were opposite with TF being decreased in time during root senescence (Garousi *et al.*, 2016; Puccinelli *et al.*, 2017).

Effect of Se on fresh weight of garlic organs

Fresh weight is an important parameter to be evaluated as profits generated from garlic cultivation are based on the amount of garlic produced. Fresh weight of garlic organs (leaves, bulbs and roots) was evaluated at the beginning (September 2014), mid-term (October 2014) and end (December 2014) of the growth cycle, to study the tolerance to Se by these organs. There were no significant differences in fresh weight of garlic organs treated with different Se doses (Figure 2). Similar results have been reported for onions where the Se effect on growth was very small and in wheat where Se did not affect fresh weight within a given range of concentrations (Arscott & Goldman, 2012; Guerrero *et al.*, 2014). In both cases, Na_2SeO_4 was tested as Se source. In onion, two doses of Se were applied, 127 µmol L^{-1} and 1270 µmol L^{-1} while in wheat, Se doses between 1 and 100 µmol L^{-1} were applied (Arscott & Goldman, 2012; Guerrero *et al.*, 2014). According to the result reported in this work, Se did not affect garlic growth, which could indicate its greater aptitude to be used in biofortification than wheat or onion.

However, there are reports where high content of Se was linked to decreased plant weight because it becomes toxic to plants in high concentrations (Garousi *et al.*, 2016; Haghighi *et al.*, 2016). At bulbing stage there is a decrease in the weight of leaves. In this stage, all metabolic process is stopped and the leaves senescence starts with the bulb development (Brewster, 2008).

On the other hand, Se supplementation

Table 1. ICP-MS instrumental parameters. Mendoza, INTA, 2014.

ICP-MS operational parameters	
Spray chamber	Cyclonic
Nebulizer	Meinhard®
Forward power	1200 W
Plasma gas flow rate	15 L min^{-1}
Nebulizer gas flow rate	0.74 L min^{-1}
Auxiliary gas flow rate	1.0 L min^{-1}
Dwell time	60 ns
Monitored isotopes	^{78}Se, ^{80}Se, ^{64}Zn, ^{66}Zn, ^{24}Mg, ^{55}Mn, ^{54}Fe, ^{57}Fe, ^{63}Cu, ^{47}PO, ^{48}SO

Dynamic reaction cell operational conditions					
Element	**Determined isotope/species**	**Reaction gas**	**Gas flow (L min^{-1})**	**RPq (V)**	**RPa (V)**
Fe	^{54}Fe, ^{57}Fe	Methane	1.20	0.75	0.00
Cu	^{63}Cu	Methane	0.70	0.70	0.00
Mn	^{55}Mn	Methane	0.70	0.70	0.00
Se	^{78}Se, ^{80}Se	Methane	0.70	0.80	0.00
P	^{47}PO	Oxygen	0.75	0.40	0.01
S	^{48}SO	Oxygen	0.75	0.40	0.00

Table 2. Total Se concentration in leaves, bulbs and root samples in different moments of garlic growth. Mendoza, INTA, 2014.

Se concentration (µg g^{-1} dry weight)[1]	
October 16th (vegetative growth stage)	57.4b
December 5th (final bulbing)	16.8a
LSD	9.4
Se_0	1.6a
Se_1	28.3b
Se_2	40.4c
Se_3	49.6c
LSD	12.0
Leaves	42.4b
Bulb	23.0a
Roots	24.6a
LSD	9.4
CV (%)	55.5

[1]The means are the result of 3 determinations on 3 replicates; [2]Means followed by same letters in the column do not differ statistically, Tukey test (5%); Se_0= 0 kg ha^{-1}; Se_1= 5 kg ha^{-1}; Se_2= 10 kg ha^{-1}; Se_3= 15 kg ha^{-1}; LSD= Least significant difference.

has also promoted plant growth of lettuce and potatoes (Xue *et al*., 2001; Turakainen *et al*., 2008). The Se doses tested were 1.0 and 0.9 mg kg^{-1} of soil, respectively. The growth-promoting effect of Se may be attributable to its antioxidant properties (Cheng *et al*., 2016). The negative or positive effect of Se over the plant growth depends on its concentration (Fargasova, 2004). Therefore, there is a Se concentration range where garlic can be enriched with this element without being affected by its toxicity.

Effect of Se biofortification on other nutrients of garlic plants

The effect of Se on the accumulation of other nutrients (Mg, Zn, Mn, Cu, Fe, P and S) was studied in the last stage of garlic growth to investigate possible changes in their accumulation and distribution in the plant (Table 4). The study was applied on garlic treated with the highest Se supplementation to evaluate the greatest change in accumulation.

Table 3. Se translocation factor (leaves Se concentration/root Se concentration) during garlic growth. Mendoza, INTA, 2014.

Se translocation factor[1]	
September 4th	4.2a [2]
October 16th	1.6a
December 5th	10.8b
LSD	4.5
Se_1	9.1b
Se_2	4.0a
Se_3	3.5a
LSD	4.5
Se_1 x September 4th	5.0a
Se_2 x September 4th	2.5a
Se_3 x September 4th	19.8a
Se_1 x October 16th	4.3a
Se_2 x October 16th	1.5a
Se_3 x October 16th	6.1a
Se_1 x December 5th	3.3b
Se_2 x December 5th	0.8a
Se_3 x December 5th	6.4a
LSD	7.9
CV (%)	82.9

[1]The means are the result of 3 determinations on 3 replicates; [2]Means followed by same letters in the column do not differ statistically, Tukey test (5%); Se_0= 0 kg ha^{-1}; Se_1= 5 kg ha^{-1}; Se_2= 10 kg ha^{-1}; Se_3= 15 kg ha^{-1}; LSD= Least significant difference.

The S accumulation (Table 4) was 1.3-folds higher in the leaves of garlic plants treated with Se than in the leaves of control plants, thus indicating that Se supplementation produces an increase in the translocation of S from roots to leaves. This phenomenon can be attributed to two different mechanisms. The first of them is mainly associated to the increase of the expression of SO_4^{-2} transporters due to the presence of SeO_4^{-2}. Se mimics S starvation to stimulate transporter expression, and hence, the flux of S assimilation pathway increases facilitating the increased of S and its redistribution (Boldrin *et al*., 2016). Likewise, another possible mechanism involved in S translocation due to Se in garlic, is related to the interference of Se on S for cysteine biosynthesis. This interference produces high amounts of o-acetylserine (OAS), which is an intermediate in the biosynthesis of cysteine and up-regulates S uptake. However, a reduction of S concentration in bulbs was observed and this could be due to a competition between S and Se to translocate into this organ. The S accumulation in the roots was not significantly affected by the presence of Se. This could be attributed to the increase of the expression of SO_4^{-2} transporters due to the presence of SeO_4^{-2}, which does not affect the transport of S from the soil to the root in the presence of Se, as explained above (Boldrin *et al*., 2016).

The effect that Se has over S accumulation and distribution is very important to the plant because S is replaced by Se in aminoacids like Cys and Met, thus altering the protein structure (Terry *et al*., 2000). Additionally, Se reduces the rate of protein synthesis because the substitution of SeMet for Met is less effective as a substrate for peptide bond formation during translocation (Eustice *et al*., 1981). On the other hand, the formation of Se-amino acids increases the ethylene production (Cheng *et al*., 2016). This phytohormone produces changes in the membrane lipid composition and increases membrane permeability that could affect the plant tissues (Xue *et al*., 2001).

Significant changes in the accumulation and distribution of Zn, Mg, Mn, Cu and Fe were also observed in the present work (Table 4). The results showed that Se has an antagonist effect over Cu (LSD= 2.8), Mg (LSD= 19.4), Mn (LSD= 13.1), Fe (LSD= 932.4) and P (LSD= 458.8) and a positive effect over Zn (LSD= 906.2) uptake. The interaction between the Se treatment and the garlic organ was not significant for Zn. However, this interaction was significant for Cu, Mg, Mn, Fe and P.

These changes can be explained considering that Se might be preventing the over accumulation of nutrients such as Mg, Zn and Fe. Moreover, Se could generate changes in the distribution of these nutrients to maintain an optimal level of cellular metabolism, particularly in the parts containing plant chlorophyll that is beneficial for plants (Longchamp *et al*., 2015). However, these changes could be dangerous for the plant because Zn, Fe, Cu, and Mn act as co-factors in many antioxidant enzymes (Broadley *et al*., 2012). Therefore, if the activities of these enzymes decrease, the sensitivity of the plant to environmental stresses increases (Gurgul & Herman, 1994). Additionally, Mg and Fe are also related with the synthesis of pheophytin (chlorophyll molecule without Mg) and chlorophyll (Broadley *et al*., 2012). Therefore, significant changes in the concentration of these elements produce important problems in plants, such as chlorosis in leaves (Silva *et al*., 2011; Broadley *et al*., 2012).

In this research, P accumulation was observed to be significantly decreased by Se, mainly because SeO_3^{-2} and PO_4^{-2} compete for the same carriers (Zhang *et al*., 2017). This must be taken into special consideration because plants need P for roots development and plant photosynthesis (Pessarakli, 2005). The results also showed that P accumulation increased in leaves and decreased in bulbs and roots when garlic plants were treated with Se. These changes on the distribution of essential elements like P could be a mechanism to reactivate associated antioxidants and improve plant tolerance to stress (Feng *et al*.,

Table 4. Nutrient concentration (Zn, Mg, Fe, Cu, P and S) in different organs of garlic plants in the last stage of their growth. Mendoza, INTA, 2014.

	Nutrient accumulation (µg/g)[1]						
	Zn	**Mg**	**Mn**	**Fe**	**Cu**	**P**	**S**
Se_0	112.6a [2]	470.2b	78.4b	3864.6b	26.3b	2048.2b	6780.3b
Se_3	1160.0b	389.8a	51.6a	2393.0a	19.6a	1192.4a	6244.3a
LSD	906.2	19.4	13.1	932.4	2.8	458.8	420.1
Leaves	1075.0a	599.5c	31.8a	908.5a	20.2a	1918.8b	8996.6b
Bulb	510.4a	150.6a	13.1a	272.2a	20.8a	2260.3b	5401.9a
Root	323.5a	449.8b	150.1b	8205.2b	27.9b	681.9a	5288.4a
LSD	1167.3	88.8	16.1	1141.9	3.4	563.0	551.3
Se_0 x leaves	88.4a	547.3b,c	35.7a	990.8a	23.1a	1794.0a,b	7886.1c
Se_3 x leaves	2061.7a	651.7c	28.0a	826.2a	17.3a	2043.6a	10107.1d
Se_0 x bulb	71.5a	166.5a	16.7a	239.1a	23.2a	3172.6c	7043.2c
Se_3 x bulb	949.3a	134.6a	9.6a	305.3a	18.4a	1347.9a,b	3760.5a
Se_0 x root	177.9a	516.6b,c	182.8c	10363.7c	32.7b	1178.1a,b	5411.5b
Se_3 x root	469.1a	383.0b	117.3b	6047.4b	23.1a	185.8a	5165.3b
LSD	1701.6	26.6	22.7	1614.9	4.8	802.3	779.7
CV (%)	112.3	17.2	19.6	29.0	11.9	25.2	6.7

[1]The means are the result of 3 determinations on 3 replicates; [2]Means followed by same letters in the column do not differ statistically, Tukey test (5%); Se_0= 0 kg ha^{-1}; Se_1= 5 kg ha^{-1}; Se_2= 10 kg ha^{-1}; Se_3= 15 kg ha^{-1}; LSD= Least significant difference.

2013).

Finally, the obtained results confirm that the changes occurred on accumulation and distribution of the nutrients upon Se supplementation did not significantly affect garlic growth. Similar results were reported in sunflowers, corn and purslane where the Se effect in uptake and accumulation of other nutrients depends on the form of Se and the plant organs (Silva *et al*., 2011; Longchamp *et al*., 2015).In our research, the effects of Se fortification on garlic growth and the profile of main mineral nutrients were studied. Se accumulation was proportional to Se doses and did not affect the growth of garlic organs. On the other hand, Se supplementation made significant modifications on the accumulation and distribution of Zn, Mg, Mn, Fe, Cu, P and S. Therefore, successful biofortification of garlic plants with Se depends on the right choice of doses and the stage involved in their growth cycle.

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