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$_2$SeO$_3$ and Na$_2$SeO$_4$ 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.

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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-Se-methylSeCyst) (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 (Na₂SeO₄) 98% and 111.65 g L⁻¹ sodium selenite (Na₂SeO₃) 99% from Sigma (St Louis, USA). Sub boiling nitric acid (HNO₃) and hydrogen peroxide (H₂O₂) 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 (¹⁰⁶Rh) monoelemental 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 metabolism 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 HNO₃ and 0.5 mL 30% (v/v) H₂O₂. The decomposition of the samples was performed in a microwave oven (DGT, Proiecto 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, ¹⁰³Rh 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).

\[
TF = \frac{[\text{Se}]}{\text{leaves}} \div \frac{[\text{Se}]}{\text{roots}}
\]

**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
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 hyperaccumulators 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 H₂O₂ 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 (Puccielli 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; Puccielli 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\(_2\)SeO\(_4\) was tested as Se source. In onion, two doses of Se were applied, 127 \(\mu\)mol L\(^{-1}\) and 1270 \(\mu\)mol L\(^{-1}\) while in wheat, Se doses between 1 and 100 \(\mu\)mol 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; Haghhighi 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, \(^{61}\)Cu, \(^{47}\)PO, \(^{48}\)SO |

### Dynamic reaction cell operational conditions

| Element | Determined isotope/species | Reaction gas | Gas flow (L min\(^{-1}\)) | RP\(_q\) (V) | RP\(_a\) (V) |
|---------|---------------------------|--------------|---------------------------|-------------|-------------|
| Fe      | \(^{54}\)Fe, \(^{57}\)Fe   | Methane      | 1.20                      | 0.75        | 0.00        |
| Cu      | \(^{61}\)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        |

\(^1\)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%); Na\(_2\)SeO\(_4\) = 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.
Selenium biofortification on garlic growth and other nutrients accumulation

...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.

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\(^{-2}\) translocators due to the presence of SeO\(^{2-}\). 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 (Boldrini 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\(^{-2}\) translocators due to the presence of SeO\(^{2-}\), which does not affect the transport of S from the soil to the root in the presence of Se, as explained above (Boldrini 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).

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

| Se translocation factor |  
|-------------------------|
| September 4\(^{th}\) | 4.2a\(^2\)  
| October 16\(^{th}\) | 1.6a  
| December 5\(^{th}\) | 10.8b  
| LSD | 4.5  
| Se\(_1\) | 9.1b  
| Se\(_2\) | 4.0a  
| Se\(_3\) | 3.5a  
| LSD | 4.5  
| Se\(_1\) \times September 4\(^{th}\) | 5.0a  
| Se\(_2\) \times September 4\(^{th}\) | 2.5a  
| Se\(_3\) \times September 4\(^{th}\) | 19.8a  
| Se\(_1\) \times October 16\(^{th}\) | 4.3a  
| Se\(_2\) \times October 16\(^{th}\) | 1.5a  
| Se\(_3\) \times October 16\(^{th}\) | 6.1a  
| Se\(_1\) \times December 5\(^{th}\) | 3.3b  
| Se\(_2\) \times December 5\(^{th}\) | 0.8a  
| Se\(_3\) \times December 5\(^{th}\) | 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.

...
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 in sunflowers, corn and purslane where the Se effect in uptake and accumulation of Zn, Mg, Mn, Fe, Cu, P and S in garlic organs. On the other hand, Se doses and did not affect the growth and the profile of three vegetable species grown in selenium-enriched conditions. Critical Reviews in Food Science and Nutrition 56: 36-55.

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