Variations in the accumulation, localization and rate of metabolization of selenium in mature Zea mays 2 plants supplied with selenite or selenate

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Highlights

• Selenate decrease grain biomass whereas selenite decrease number of grains per plant

• Selenite treatment results in higher selenium accumulation in grains than selenate treatment

• For both selenite and selenate treatments, selenium in grains is exclusively organic selenium compounds

• For humans, selenite treatment increases bioavailable selenium in grains

• Selenate is the best supplement to enrich Zea mays forage for bioavailable selenium

Abstract

Quantification of selenium bioavailability from foods is a key challenge following the discovery of the antioxidant role of this micronutrient in human health. This study presents the uptake, accumulation and rate of metabolization in mature Zea mays plants grown in hydroponic solution supplemented with selenate or selenite.

Selenium content was lower in plants supplemented with selenate and accumulated mainly in the leaves compared with selenite-treated plants where the selenium was retained in the roots. Selenite-treated grains accumulated more selenium. Selenate was metabolized less than selenite in whole plants, but in grains selenium was present exclusively as organic selenium compounds.

For humans, the bioavailability of organic selenium was evaluated at 90% compared with only 50% for inorganic forms. Our results show that the potential for selenium bioavailability is increased with selenite treatment.

Keywords: Selenite, selenate, selenium bioavailability, enzymatic extraction, organo-selenium compounds, biofortification

1. INTRODUCTION

Selenium (Se) is an essential micronutrient in human and animal diets. More than 20 selenoproteins or selenoenzymes are involved in normal metabolism and selenium has also been proposed to lower the risk of cardiovascular diseases and cancer (Rayman, 2008; Thomson, 2004). Food is the principal route of selenium intake. Meat and seafood contain the highest amounts of selenium, with 0.4-1.5 µg per gram (Rayman, 2008), but cereals, fruits and vegetables are also good food sources. Selenium enters the food chain through plants and especially crops, which are part of the diet of both primary and secondary consumers.

Selenium concentrations in food, including crops, depend not only on selenium concentrations in agricultural soils (which vary considerably between countries and regions) but also on selenium phytoaccessibility controlled by many abiotic and biotic factors such as soil pH, redox conditions, organic matter content, microbial activities, irrigation and compaction. In some countries or regions, low selenium levels in soil lead to low concentrations in feed or forage, which in turn can result in selenium deficiency in livestock and humans. For example, the average selenium intake is only 36 µg per day in France, 34 µg per day in the UK and 35 µg per day in Sweden (Rayman, 2008); these levels are below the recommended dietary allowance of 40 to 70 µg per day (World Health Organization et al., 1996). To increase selenium levels in human and animal diets, several processes have been developed including mineral supplementation, genetic biofortification (plant breeding) and finally, the option chosen here, agronomic biofortification of food or forage.

In contrast to humans, the role of selenium for plants is more ambiguous, although studies on young plants have led to a better understanding of selenium pathways in higher plants (De Souza et al., 1998; Hopper & Parker, 1999; Li, McGrath & Zhao, 2008; Terry, Zayed, De Souza & Tarun, 2000; Ximenez-Embun, Alonso, Madrid-Albarran & Camara, 2004; Zayed, Lytle & Terry, 1998; Zhang, Pan, Chen & Hu, 2003). Plant development and selenium

metabolism are strongly dependent on the form of supplied selenium. The greater mobility of selenate compared to selenite results in differences in the absorption, translocation and metabolism of selenium within the plant. Indeed, when plants are exposed to selenite, selenium accumulation is less than after selenate treatment (De Souza et al., 1998; Terry et al., 2000; Ximenez-Embun et al., 2004; Zhang et al., 2003), with a greater reduction in biomass production (Hopper et al., 1999; Ximenez-Embun et al., 2004). After selenate treatment, selenium is almost entirely translocated to the leaves and weakly metabolized as selenoamino-acids, with a selenate concentration in shoots (i.e. stems and leaves) representing more than 90% of the total shoot selenium (De Souza et al., 1998; Hopper et al., 1999; Li et al., 2008; Mazej, Osvald & Stibilj, 2008; Terry et al., 2000; Ximenez-Embun et al., 2004; Zayed et al., 1998; Zhang et al., 2003). In contrast, when supplied as selenite, selenium accumulates principally in roots with little translocation, although selenoamino-acid production (principally selenomethionine, selenocysteine and selenomethylselenocysteine) is greater (De Souza et al., 1998; Hopper et al., 1999; Li et al., 2008; Liu & Gu, 2009; Terry et al., 2000; Ximenez-Embun et al., 2004; Zayed et al., 1998) and the selenium volatilization rate is about 2-fold higher from those plants (De Souza et al., 1998).

After ingestion by humans or animals, bioavailable selenium is the fraction that enters the systemic circulation (Thiry, Ruttens, De Temmerman, Schneider & Pussemier, 2012). As with other micronutrients, selenium bioavailability strongly depends on the chemical form of the element: organic forms (such as Se-methionine and Se-cysteine), mainly from plant and animal sources, have more bioavailability than inorganic forms (selenate and selenite), which are principally found in dietary mineral supplements. Experimental designs used to measure selenium bioavailability vary widely in the literature, making it difficult to compare the results (Knowles, Grace, Wurms & Lee, 1999; Nicholson, McQueen & Bush, 1991; Podoll, Bernard, Ullrey, Debar, Ku & Magee, 1992). According to Thomson (2004), the apparent

absorbed selenium (i.e. the difference between selenium ingested and selenium excreted in feces and urine) in humans was evaluated at about 90% for Se-met and Se-cys versus 50% for selenite or selenate supplements (Panel on Dietary Antioxidants and Related Compounds, Subcommittee on Upper Reference Levels of Nutrient, Subcommittee on Interpretation and Uses of DRIs, Standing Committee in the Evaluation of Dietary Reference Intakes of the Food and Nutrition Board, Institute of Medecine & the National Academies and Health Canada, 2000). However, due to a lack of data on the bioavailable fraction across all food products, the recommended daily dietary allowances of selenium for humans are based only on the total selenium concentration, without taking into account the speciation. The two percentages (90% and 50%) estimated by Thomson (2004) are a "pseudo reference" value used in the present study to evaluate the selenium bioavailability in our so-called "organic" and "inorganic" fractions in Zea mays plants.

Due to the essential function of selenium in staple foods, a number of recent studies on grains and seeds have been carried out not only in wheat, but also in sesame, buckwheat pumpkin and Zea mays (Broadley et al., 2010; Cubadda et al., 2010; Kapolna, Gergely, Dernovics, Illès & Fodor, 2007; Mbagwu, 1983; Moore et al., 2010; Smrkolj, Osvald, Osvald & Stibilj, 2007; Smrkolj, Stibilj, Kreft & Kapolina, 2005; Stibilj, Kreft & Smrkolj, 2004). In *Brassica rapa* (Lyons, Genc, Soole, Stangoulis, Liu & Graham, 2009), selenite fertilization increased seed number and weight produced by each plant. Regardless of the enrichment procedures employed in agricultural practice, the development and growth of plants and grains were not affected negatively by selenium supplementation (Broadley et al., 2010; Stibilj et al., 2004). Independently of the selenium concentration added as amendment, grains seem to be an ideal storage tissue, with selenium concentrations higher than in shoots or fruits (Cubadda et al., 2010; Mbagwu, 1983; Stibilj et al., 2004). It has previously been shown that the major selenium species in grains is selenomethionine accounting for 45% to 90% of total

117 selenium (Cubadda et al., 2010; Kapolna et al., 2007; Smrkolj et al., 2007; Smrkolj et al.,
118 2005), with only very low levels of selenate detected (Cubadda et al., 2010; Lyons, Genc,
119 Stangoulis, Palmer & Graham, 2005).

120 In the present study, we investigated selenium enrichment in Zea mays grains grown in a
121 hydroponic system. Cereal grains are rich in phytic acids, known for their antioxidant roles in
122 humans and which strongly bind mineral and trace elements (Hurrel, 2003). Zea mays grains
123 contain more of this compound than wheat grains (Egli, Davidsson, Juillerat, Bearclay &
124 Hurrell, 2003). Moreover, Zea mays is the most widely cultivated cereal in the world,
125 producing mainly forage and grains for animal feed but also grains as well as derived products
126 for human consumption. In Malawi, for instance, 50% of the diet is derived from Zea mays
127 (Chilimba et al., 2011). Consequently, the limited data available on selenium accumulation in
128 Zea mays grains has been obtained in specific locations (selenium-deficient (Chilimba et al.,
129 2011) or seleniferous areas) or for Se-supplemention, fly-ash for example (Mbagwu, 1983).
130 Furthermore, the influence of the chemical form of selenium in Zea mays plants, on
131 accumulation including location (i.e. roots, stems, shoots and grains), has not been widely
132 studied. The first objective of the present study was, therefore, to quantify the effects of those
133 two inorganic chemical forms (selenate and selenite) on Zea mays growth and seed
134 production. The second aim was to investigate the uptake, translocation and speciation of
135 selenium in different Zea mays tissues: roots, stems, leaves and grains.
136

2. MATERIALS AND METHODS

2.1. Seed germination and culture conditions

Three weeks after germination, *Zea mays sups.mays* (L.) corn seedlings were cultivated in hydroponic conditions in 20 L plastic tanks filled with a modified Hoagland nutrient solution consisting of KNO_3 (3 mmol.l^{-1}), $Ca(NO_3)_2.4H_20$ (2.72 mmol.l^{-1}), NH_4NO_3 (2 mmol.l^{-1}), NaCl (0.2 mmol.l^{-1}), KH_2PO_4 (0.98 mmol.l^{-1}), $MgSO_4.7H_20$ (0.70 mmol.l^{-1}), $(NH_4)_6Mo_7O_{24}.4H_2O$ (0.04 µmol.l^{-1}), H_3BO_3 (24 µmol.l^{-1}), $MnSO_4$ (13 µmol.l^{-1}M), $ZnSO_4$ (6 µmol.l^{-1}), $CuSO_4$ (1.5 µmol.l^{-1}) and FeEDDHA (6%) (4 µmol.l^{-1}). Two nutrient solutions were supplemented with 12 µmol.l^{-1} selenium as either Na_2SeO_4 or Na_2SeO_3 (solutions Se^{VI}-T and Se^{IV}-T), respectively. Under control conditions (C-T), no selenium was added. Five corn seedlings were transplanted in each tank and placed into a RUBIC5 plant growth chamber (Reactor Used for Continental Isotopic Biogeochemistry), a 9 m^3 sealed chamber (Servathin, France) the atmospheric compositions of which are controlled. Lighting was provided by 15x400 watt Philips Son-T Agro bulbs over an 8-hour photoperiod set at 600 µM.m^{-2}.s^{-1} photosynthetically active radiation at plant height. Air temperature was set at 25°C during the day and 18°C at night. Air humidity was controlled by a dew point condenser in order to maintain a set-point of 70% relative humidity. Beyond this set point, excess water vapor was condensed and collected using an Isco 3700 water sampler (so called “condensates”). The CO_2 concentration was measured using a LI-COR (Lincoln, Nebraska USA) Li620 infrared gas analyzer set at 400 ppmv. The chamber had a slight positive pressure of +20 Pa to avoid entry of outside air. Data were logged by a computer and averaged at 10 min intervals.

The change in aerial biomass production was followed by recording the leaf area five times during the experiments.

At maturity, plants (five for each treatment) were harvested and roots briefly rinsed in deionized water to remove traces of nutrient solution. The selenium concentration in this rinse

water fell below the detection threshold of CRC-ICP-MS. The leaves, stems, roots and grains were then separated. Plant samples were freeze-dried, ground with an automatic agate mortar, and dry weights (DW) were measured.

2.2. Total selenium analysis

A suitable amount of powdered plant tissue (about 100 mg DW) was digested in 2 ml of HNO_3 (70%) at 100°C for 24 hours in a closed digestion vessel. After cooling, 1 ml of H_2O_2 (30%) was added and the sample was heated again at 100°C for 24 hours. The selenium concentration in the digested tissues samples was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES, JY 2000, LOD: 50 $\mu g.l^{-1}$). A blank and a reference material (White clover, BCR402- IRMM) were included in each batch of samples. In the C-T plants, the selenium concentrations fell below the detection threshold of ICP-AES.

Condensate selenium concentrations were determined by graphite furnace atomic absorption spectrometry (GFAAS, UNICAM 989 QZ, LOD: 1.5 $\mu g.l^{-1}$). A certified reference material (TMDA-64- Environment Canada) and a blank were included in each batch of samples.

2.3. Separation and quantification analysis of selenate and selenite

For enzyme hydrolysis, a DW sample of about 100 mg was digested with 20 mg of Streptomyces protease (Protease Type XIV ≥ 3.5 units/mg solid from *Streptomyces griseus*, Sigma Aldrich, Saint-Quentin Fallavier, France), dissolved in 5 ml of 30 $mmol/l^{-1}$ Tris-HCl buffer (pH=7, Rockland) and heated at 37°C for 24 hours under regular agitation (DigiPREP Jr). Samples were then centrifuged at 4000 rpm for 30 min (Eppendorf 5810 centrifuge). The supernatants were filtered and stored at 4°C in 0.1% mercaptoethanol (β-mercaptoethanol molecular biology grade 99.8%, Calbiochem) to avoid oxidation. To determine the efficiency of enzymatic extraction, total selenium concentrations for each sample were determined by

ICP-AES (LOD: 10µg.l^{-1}). To identify selenate and selenite fractions, the two inorganic forms were separated by HPLC (Dionex, ICS 3000) using a high pressure pump and an anion exchange column (AS15, 4x250 mm) under the following conditions:

- mobile phase: 30 mmol.l^{-1} KOH
- flow rate: isocratic at 1 ml/min
- injected sample volume: 200 µl
- column temperature: 30°C

Standard solutions of the selenium species - Se(IV) (Sodium selenite 99%, Sigma Aldrich, Saint-Quentin Fallavier, France) and Se(VI) (Sodium selenate anhydrous , Sigma Aldrich) - were prepared at suitable concentrations. The chromatography system was off-line to GFAAS (LOD: 1.5 µg.l^{-1}) used for detection and quantification. The detection limits for each inorganic species were 5 µg.g^{-1} (i.e. 5% of total selenium) in plant tissues.

2.4. Calculation of leaf area

Measuring leaf area is a non-destructive method of monitoring plant growth during experimental studies. Leaf area was calculated using the following formula (Fakorede, Mulamba & Mock, 1977; Ruget, Bonhomme & Chartier, 1996):

$$Leaf\ area = \sum_{i=1}^{n} (Li * li * 0.75)$$

(L in m: length of leaf; l in m: width of leaf; n: leaf number per plant)

2.5. Statistical analysis

In our study, the number of samples was less than 30 (i.e. five plants). Non-parametric tests were used for the statistical analysis. The significance of the effect of treatment conditions was determined with a bilateral Mann-Whitney test (to compare 2 groups) or

Kruskal-Wallis test (to compare more than 2 groups), with an alpha risk equal to 0.05. These tests calculated the probability P of the difference between groups being random. P values less than 5% were considered statistically different. In the figures, the results of statistical tests are represented by the letters a, b and c. In this study, values are presented with the median (Q1; Q3).

3. RESULTS

3.1. Biomass production

Plant growth was monitored throughout the experiment by measuring leaf area, internode size and dry weights after harvest and drying.

Before day 20 and after day 75, there was no significant difference in the leaf areas between the three treatments (**Figure 1.A**). However, between days 30 and 70, the leaf area of Se^{IV}-T plants was on average 42% smaller than for control plants. At the end of experiment, the internode size of each plant was measured (**Figure 1.B**). For Se^{IV}-T, the internodes No. 3 to No. 9 were on average 2.3 (1.7; 2.5) times shorter than those of control plants. The largest difference was measured for the seventh internode, which was 2.9 times smaller in Se^{IV}-T plants versus controls.

Moreover, again for Se^{IV}-T, the biomass production of plants (**Figure 2.A)** as well as the biomass production of shoots (data not shown) was about 70% less than C-T plants.

For grains, dry weights decreased by 60% and 80% in Se^{VI}-T and Se^{IV}-T plants, respectively, compared to the control plants (**Figure 2.B)**. The grain number produced by each plant (**Figure 2.C)** decreased significantly (70%) with selenite. Biomass allocation was affected by selenium; the ratio of grain dry weights to shoot dry weights was less with both selenium treatments compared with C-T. In fact, grain biomass for the C-T plants represented 33% of aerial biomass, but fell to 21% when selenium was present in the nutrient solution.

3.2. Uptake and accumulation of total selenium in Zea mays

The total selenium concentration in the plant is the sum of the selenium concentration in each tissue **(Figure 3).** Supplementation with selenite versus selenate resulted in significant differences in the distribution of selenium in whole plants and between tissues: in whole plants, selenium concentration was 68% higher in Se^{IV}-T versus Se^{VI}-T plants, with selenium

241 concentrations of 210 µg.g^{-1} (156; 225) and 125 µg.g^{-1} (103; 126), respectively (**Figure 3A**).
242 Similarly but to a greater extent, selenium concentrations in roots were much higher (675%)
243 in Se^{IV}-T versus Se^{VI}-T plants (**Figure 3.B**); and selenium concentrations in grains were 1.7
244 times greater, i.e. 73%, in Se^{IV}-T versus Se^{VI}-T plants (**Figure 3.E**). Conversely, selenium
245 concentration in leaves was 73% lower in Se^{IV}-T compared to Se^{VI}-T plants (**Figure 3.D**).
246 Regardless of the inorganic form of selenium, the selenium concentrations in stems were
247 similar (**Figure 3.C**).

248 Based on the data for each tissue of Zea mays plants (**Figure 3**), we calculated the
249 selenium concentration in shoots (stems + leaves) and in tops (stems + leaves + grains). In
250 shoots and tops, selenium concentrations were 72 µg.g^{-1} (66; 80) and 86 µg.g^{-1} (86; 88) after
251 selenite treatment, and 151 µg.g^{-1} (114; 154) and 126 µg.g^{-1} (105; 130) after selenate
252 treatment, respectively.

253 Following root uptake, selenium can be redistributed to various degrees in the different plant
254 tissues. The translocation factor 'root-tops' (ratio of tops to roots concentrations) reflects the
255 capacity of selenium to be transferred to roots from aerial tissue. This ratio was lower with
256 selenite (0.13 (0.12; 0.13)) than with selenate (1.45 (1.16; 1.49)). The translocation factor
257 'shoot-grains' (ratio of grains to shoots concentrations) reflects the capacity of selenium to be
258 transferred to aerial vegetative tissue from grains. This ratio was higher with selenite (2.03
259 (1.66; 2.09)) than with selenate (0.51 (0.49; 0.53)).

260 Selenium amount (i.e. quantities in µg per plant or tissues) in tissues not only depends on
261 selenium concentrations but also on the biomass, which can vary considerably from one tissue
262 to another. Therefore, selenium amounts in each tissue provide important information about
263 selenium uptake by the plant. Selenium levels in whole plants were similar for selenite (4278
264 µg (4031; 5452)) and selenate (4813 µg (3833; 5418)). For selenate-treated plants, tops
265 accounted for more than 90% of the total selenium amount, with around 50% of total

266 selenium found in the leaves (**Figure 4**). After selenite treatment, selenium amounts in the
267 three tissues differed dramatically: selenium amount in tops was low (about 40% of total plant
268 selenium) whereas around 60% of total plant selenium was found in the roots. Regardless of
269 the form of selenium supplied in the nutrient solution, selenium amount in grains represented
270 15% of total plant selenium.
271

272 **3.3. Rate of selenium metabolization in Zea mays**

273 Concentrations of inorganic selenium species were determined after protease hydrolysis and
274 are presented in **Table 1**. The organic selenium fraction (i.e. pool of various organic selenium
275 species) was estimated as the difference between total selenium extracted by protease
276 hydrolysis and the sum of the inorganic species. This estimation is satisfactory for stems,
277 leaves and grains because the efficiency of enzyme hydrolyis is high, approximately 90%. In
278 roots, where the efficiency of enzyme hydrolyis is only about 35%, the fraction of non-
279 extractable selenium corresponds to chemically or physically sequestered organic selenium;
280 the percentage of inorganic selenium species is slightly over-estimated in this case.
281 After selenite treatment, neither selenate nor selenite was detected in any of the plant tissues.
282 Conversely, after selenate treatment, no trace of selenite was detected, but selenate was
283 identified in roots, stems and leaves, with a higher percentage in stems and leaves (54 ± 16%
284 and 39 ± 9%, respectively) than in roots (20 ± 5%). Finally, whatever the form of selenium
285 supplied, selenium was converted completely to organo-selenium compounds in grains.
286
287

4. DISCUSSION

4.1. Crop growth of Zea mays

Leaf area is used to monitor aerial biomass production throughout plant development. Changes in leaf area usually follow three successive stages also observed in the three treatments of our experiment: 1) a growing stage where aerial biomass production is exponential, 2) a reproductive stage where foliar development becomes weak or null, and 3) finally a shoots (i.e. stems and leaves) senescence stage when grains are mature (Gitelson, Vina, Arkebauer, Rundquist, Keydan & Leavitt, 2003).

Based on the dry weights of plants or tissues and the leaf areas, supplementation with inorganic selenium at high concentration (12 µM) was harmful to Zea mays growth. In selenite-treated plants, the development of all tissues was affected, with a decrease in dry forage biomass as well as quantity (number) and quality (dry weight) of grains. A decrease in the leaf area of plants treated with selenite was observed only during the reproductive stage, i.e. between days 30 and 70. The internodes that were affected by selenite treatment corresponded to those developed during the vegetative stages. Selenite toxicity has already been observed in white lupine and in sunflower, for example, with a biomass reduction of 20% and 40%, respectively (with 12 $\mu mol.l^{-1}$ selenite) (Ximenez-Embun et al., 2004). These results contrast with data on *Brassica rapa* (Lyons et al., 2009), which showed that at very low selenium concentrations (0.05 $\mu mol.l^{-1}$ selenite) in hydroponic solution, plant biomass and dry weight of each grain were not affected, and moreover grain number increased by 43% for each plant. In selenate-treated plants, vegetative tissues of Zea mays were not affected, according to literature data on different varieties of crops (such as Zea mays and wheat) or other plants (such as pumpkin, buckwheat, dry beans) fertilized with different techniques (foliar application of selenate, selenate liquid or solid addition in soils, or fly-ash amendment) (Broadley et al., 2010; Cubadda et al., 2010; Mbagwu, 1983; Smrkolj et al., 2005; Stibilj et

313 al., 2004). However, contrary to Mbagwu (1983) and Broadley et al. (2010), grain biomass
314 decreased in our study when plants were supplied with high selenate concentrations, although
315 there was only a small decrease in the number of grains. Selenate does not appear to influence
316 the quantity of grains but seems to inhibit their normal filling.
317
318 **4.2. Uptake, accumulation and speciation of selenium in Zea mays**
319 To control for possible volatilization of selenium from the plant tissues, which could also
320 result in a decrease in selenium content, condensate samples were collected throughout the
321 experiments. Selenium concentrations measured in those condensate samples (data not
322 shown) indicated that Zea mays does not significantly volatize selenium, which is why this
323 factor is not taken into account in the remainder of the discussion.
324 Although selenate is the most mobile form of selenium, the total selenium concentration in
325 Zea mays was higher in the presence of selenite (selenium concentration 12µmol.l^{-1}).
326 However, as significant toxicity was manifested as a reduction in biomass production, the
327 accumulated selenium in whole plants was similar with both selenate and selenite treatments.
328 Ours results differ from a majority of studies concluding that accumulation is higher after
329 supplementation with selenate compared to selenite (De Souza et al., 1998; Terry et al., 2000;
330 Ximenez-Embun et al., 2004; Zayed et al., 1998). However, some studies on rice, wheat or
331 soybean show that selenite can accumulate as much as (Li et al., 2008; Zayed et al., 1998) or
332 even more (Zhang et al., 2003) than selenate. It should be noted that our results cannot be
333 compared directly to any previous data because our experiments were carried out on the
334 mature plant, unlike previous experiments conducted in young plants. Among those studies,
335 Lyons *et al.* (2009) measured total selenium concentrations in roots, shoots and also seeds of
336 *Brassica rapa* grown in hydroponic conditions, but the sodium selenite concentration in this
337 study was very low (0.05 µmol.l^{-1} selenium), i.e. 240-fold lower than in our experiments.

With selenite treatment, most of the selenium accumulated in roots. In Li *et al.* (2008), Terry e*t al.* (2000), De Souza *et al.* (1998) and Ximenez-Embun *et al.* (2004), the 'roots-tops' translocation factor (0.5) was greater than that suggested by our result (0.13), but similar to that of Lyons *et al.* (2009) (0.08). These two very similar results are the only data obtained from experiments carried out up to the reproductive stage in a hydroponic system. The developmental stage of the plant seems to influence the root storage capacity of selenium: root uptake and accumulation appear to increase as the plants mature. Moreover, with selenite treatment, organo-selenium compounds were produced to a greater extent than with selenate treatment: in whole plants, no traces of inorganic selenium were detected. In several papers, traces of selenite were detected in roots or shoots, but always less than 7%, indicating that selenium in plants is overwhelmingly organoselenium compounds (Ximenez-Embun et al., 2004). With selenate treatment, most of the selenium taken up by Zea mays was translocated and accumulated in the tops of plants, especially in the leaves; much less accumulated in roots (Pickering, Prince, Salt & Georges, 2000). In our study, the 'roots-tops' translocation factor (1.45) was the same order of magnitude as published data (1.5-17) (De Souza et al., 1998; Li et al., 2008; Terry et al., 2000). Selenate was metabolized less than selenite in whole plants. This finding is coherent with the fact that reduction of selenate into selenite is the rate-limiting step in selenate metabolism in plants (De Souza et al., 1998; Li et al., 2008; Terry et al., 2000). Selenate absorbed by roots is metabolized to organic selenium compounds (that represent only 20% of total selenium in roots) and/or is quickly translocated to the tops of plants. The percentage of selenate in leaves (39%) is less than in stems (54%), which seems to indicate that selenate is also metabolized in leaves. Mazej *et al.* (2008) and Li *et al.* (2008) showed that on average, 60 to 100% of selenium in leaves and roots is selenate. In the results presented by Ximenez *et al.* (2004) in India mustard, the selenate form represents 30% in roots and 90% in shoots; moreover in sunflower, selenate in leaves (35%) is similar to that in

Zea mays, and is also less than in the stems (97%). Thus, in our study, the metabolization rate, which is higher than in the literature, can probably be attributed to the difference in the developmental stage, which was more advanced in our case. This increased metabolization of selenate in fully developed mature plants could be explained by 1) increased enzymatic generation (increase in the amount synthesized or in the activity rate of enzyme) and/or 2) a decrease in selenate absorption at the reproductive stage involving a larger proportion of selenate metabolized.

In the literature, selenium accumulation in grains has been studied mainly in wheat or rice (Broadley et al., 2010; Cubadda et al., 2010; Eurola, Ekholm, Ylinen, Koivistoinen & Varo, 1991; Lyons et al., 2005), but to date, few data exist for selenium accumulation in Zea mays grains, except in Chilimba *et al*. (2011) and Mbagwu (1983), for example. Moreover, studies on grains or seeds are usually carried out in soil (pot or yield), naturally or manually enriched with Se-supplementation , but with little information on water-soluble selenium bioavailable for plants (Broadley et al., 2010; Chilimba et al., 2011; Cubadda et al., 2010; Eurola et al., 1991; Kapolna et al., 2007; Lyons et al., 2005; Mbagwu, 1983; Smrkolj et al., 2007; Smrkolj et al., 2005; Stibilj et al., 2004). Thus, our results can only be compared with Zea mays grown in soils or other plant species. In our hydroponic system, selenium concentrations obtained in Zea mays grains (93 - 226 $\mu g.g^{-1}$) were much higher than in the majority of studies (Eurola et al., 1991; Lyons et al., 2009; Lyons et al., 2005; Mbagwu, 1983; Smrkolj et al., 2005; Stibilj et al., 2004). For example, in Zea mays grains in Malawi, selenium concentrations were only 45 to 500 $ng.g^{-1}$ (Chilimba et al., 2011), while winter wheat grains (Broadley et al., 2010) can accumulate up to 2.6 $\mu g.g^{-1}$. Only grains of wheat harvested in the Nawanshahr-Hosshiarpur region of India had selenium concentrations similar to ours with 29 and 185 $\mu g.g^{-1}$ (Cubadda et al., 2010). These differences are probably due to the growing conditions, specifically the soils or hydroponic solution. In our study, the hydroponic experiments allowed us to study the

process of uptake in roots and translocation to shoots, which cannot be clearly identified in soil due to its complex composition. Another explanation may be that phytic acid (a chelating compound for trace elements) concentrations are higher in Zea mays grains than, for example, in wheat grains (Egli et al., 2003), causing Zea mays to accumulate more selenium. .

4.3. Selenium enrichment of Zea mays to improve the quality of human and livestock food

Unlike many studies, we were able to compare both selenate and selenite uptake in Zea mays under the same experimental conditions: variations in the inorganic chemical form of supplied selenium greatly influenced the ability of grains to accumulate selenium.

With selenate treatment, Zea mays grains with the lowest selenium concentrations accumulated less selenium than other Zea mays tissues. However, with granular fertilization of selenate, Gissel-Nielsen (1986) found that selenium concentrations in barley grains were equal to or slightly higher than in barley straw. On the other hand, with selenite treatment and according to the literature (Cubadda et al., 2010; Mbagwu, 1983), selenium concentrations are higher in grains than in shoots. In our study, grains contained twice as much selenium as shoots, which is slightly higher than the levels reported by Cubadda et al. (2010) and Mbagwu (1983) (around 1.15 times). Similarly, in field experiments and with different methods of selenium application (granular fertilization and foliar application), selenium concentrations in barley grains treated with selenite were on average 1.6 times higher than in straw (Gissel-Nielsen, 1986). Thus, with selenite supplementation, grains appear to be a secondary tissue for selenium storage after the roots.

These findings suggest that the use of selenite fertilizer could be attractive because (i) after roots, selenium accumulates principally in grains; (ii) selenite is less mobile than selenate, thereby enriching the soil in selenium at each fertilization, meaning that in the long term,

plants grown on this soil will be enriched in selenium without the use of Se-fertilizers; and (iii) the low mobility of selenite also limits selenium dispersion in the surrounding environment. Moreover, another technique involving foliar application of selenite was found to be more effective than granular fertilization for soybeans (Yang, Chen, Hu & Pan, 2003). Although not confirmed for rice (Hu, Chen, Xu, Zhang & Pan, 2002), it would be interesting to test this technology with Zea mays.

To improve dietary intake of selenium, the amount of selenium ingested is important but the quality and quantity of bioavailable selenium are also key factors. According to Thomson (2004), the bioavailability of organic selenium is 90% compared with 50% for selenite or selenate. Based on these data, we calculated selenium bioavailability for humans and animals in Zea mays in our experiment (**Table 2**).

Despite growth in highly variable conditions (species, concentrations and techniques of selenium supplementation), all previous published results (Cubadda et al., 2010; Kapolna et al., 2007; Smrkolj et al., 2007; Smrkolj et al., 2005), as well as the present data, show that selenium in grains is, over-whelmingly, present as organo-selenium compounds. Furthermore, Kalpona *et al.* (2007), Cubadda *et al*. (2010) and Smrkolj *et al*. (2005) showed that selenomethionine represents around 80% of the total selenium in grains of sesame, wheat and pumpkin, respectively. Consequently, the evaluation of bioavailable selenium for humans is straightforward because bioavailable organic selenium accounts for 90% of total selenium in grains. In our study, the bioavailable selenium per plant did not differ according to the form of inorganic selenium supplied (**Table 2**). However, despite a decrease in grain biomass for the selenite treatment at 12 µM, the bioavailable selenium concentration in grains was higher than with selenate. In fields with granular selenium fertilization, selenium concentrations in wheat or barley grains were higher with selenate than with selenite fertilizer (Gupta & Winter, 1989;

Singh, 1991). This difference is probably due to the fact that, in soil, selenite has lower mobility and so is less bioavailable for plants compared to selenate. However, selenite can enrich soil over the long term and avoid environmental pollution. Our results show that, at equal ratios (i.e equal grain mass), and despite an observed decrease in grain biomass production, the grains treated with selenite supply 73% more bioavailable selenium than those treated with selenate (**Table 2**). Selenite is, therefore, the best treatment to enrich grains with bioavailable selenium for animals and humans.

All aerial parts of plants (stems + leaves + grains = tops) are used as forage for livestock. To evaluate the bioavailable selenium for animals in our Zea mays plants, it was necessary to take into account not only the selenium amount or concentration, but also the selenium speciation. With selenite treatment, the biomass production of tops decreased, but 95% of the selenium was organo-selenium whereas, with selenate, the biomass production of tops was greater but the selenium was present as both organo-selenium compounds or selenate (less bioavailable). So, based on selenium amount and speciation in tops, we conclude that plants treated with selenate supply 148% more bioavailable selenium per plant compared to those treated with selenite (**Table 2**). However, at equal ratios (i.e equal tops mass), tops treated with selenite or selenate supply the same amount of bioavailable selenium (**Table 2**). Thus, since selenate treatment does not affect shoot biomass, it is the best supplement to enrich forage in bioavailable selenium for animals.

5. CONCLUSIONS

Our data suggest ways to improve agronomic biofortification of Zea mays with selenium. The absorption, accumulation, distribution and metabolization of selenium in mature Zea mays plants depend on the form of selenium supplied. Despite a decrease in grain biomass in the presence of selenite or selenate in the nutrient solution, selenium is present mainly as

463 organo-selenium compounds in grains. The choice of the form of selenium supplied strongly
464 influences the amount of bioavailable selenium in human and animal foodstuffs: to obtain the
465 highest selenium content for consumers (human or animal), selenate should be used for
466 animal feed and selenite for human food. Because health benefits associated with selenium as
467 well as its toxicity, the creation of dietary recommendations is a key challenge for human and
468 animal health. Nonetheless, data on selenium bioavailability in food are scarce in the
469 literature. Despite our specific experimental conditions (i.e. hydroponic), and although cereals
470 are considered non-accumulators, they do accumulate and metabolize selenium to organo-
471 selenium compounds: this study estimated, for the first time, selenium bioavailability in
472 edible parts for human and animals of an important cereal in the diet, Zea mays.
473

ABBREVIATIONS USED

CRC-ICP-MS: Collision/reaction cell - Inductively coupled plasma mass spectrometry;

ICP-AES: Inductively coupled plasma atomic emission spectrometry;

GFAAS: graphite furnace atomic absorption spectrometry;

DW: Dry weight;

LOD: Limit of detection;

SD: Standard deviation;

FeEDDHA: Iron- Ethylenediaminedi-Q-hydroxyphenylacetic acid;

IRMM : Institute for Reference Materials and Mesurements

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594

595 **LIST OF FIGURES**

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597 with the three different treatments.

598 **Figure 2.** Dry biomass production (%) in Zea mays plants (A) or in grains (B) and number of
599 grains per plant (C) with the three different treatments: C-T (dots), Se^{VI}-T (light gray) and
600 Se^{IV}-T (dark gray).

601 **Figure 3.** Selenium concentrations (µg/g DW) in whole Zea mays plants (A) or in different
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603 E. grains): Se^{VI}-T (light gray) and Se^{IV}-T (dark gray).

604 **Figure 4.** Schematic representation of a Zea mays plant showing the selenium amount (%) in
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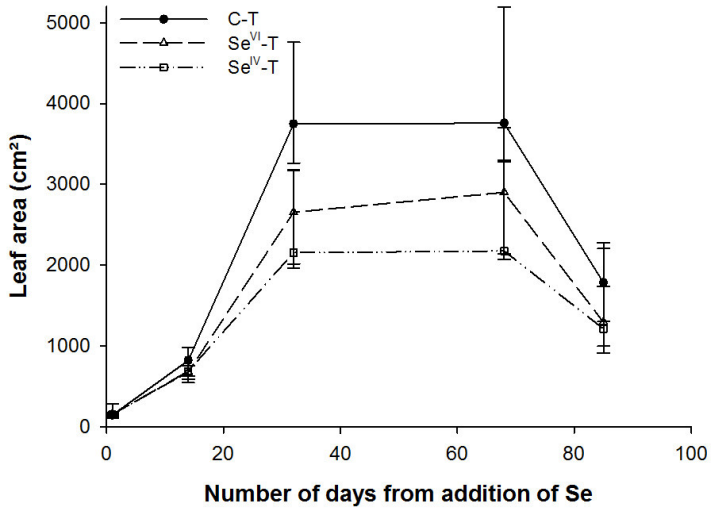
607 **Table 2.** Estimated concentration (µg/g) and amount (µg/plant) of selenium bioavailable for
608 animals (tops of plant) or humans (grains of plant) with the two selenium treatments: Se^{VI}-T
609 and Se^{IV}-T.

610

611 **Figure 1.** Change in leaf area (cm²) (A) and internode length (cm) (B) of Zea mays plants

612 with the three different treatments.

613 **A**



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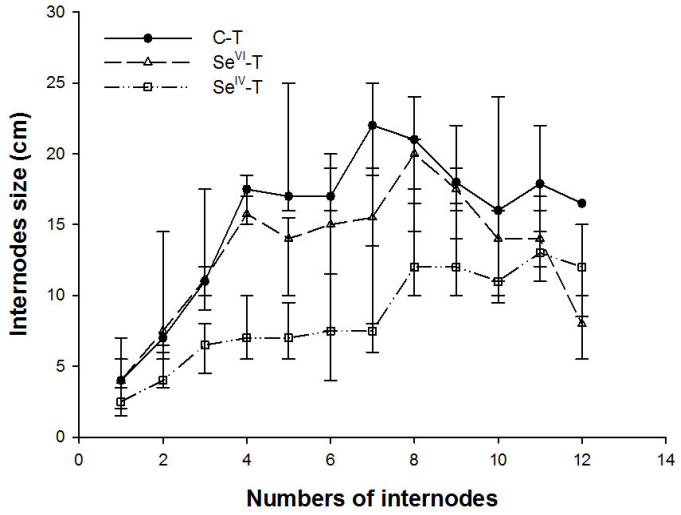
615 **B**



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618 *Values are medians (lower: 10th percentile; upper: 90th percentile)*

619 **Figure 2.** (A) Dry biomass production (%) of Zea mays plants (A) or of grains (B) and
620 number of grains per plant (C) with the three different treatments: C-T (dots), Se^{VI}-T (light
621 gray) and Se^{IV}-T (dark gray).

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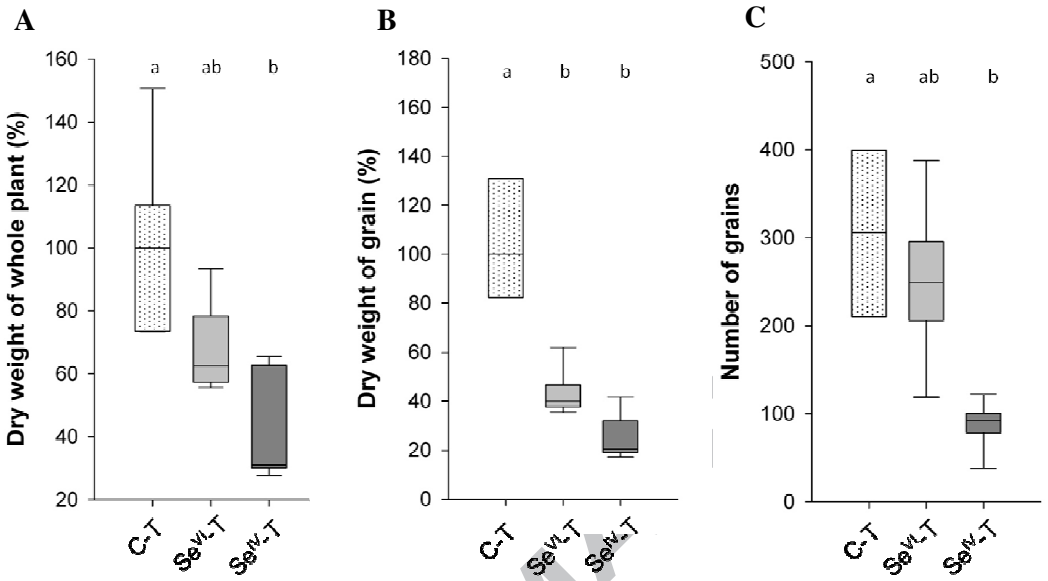

623
624 *a, b: results of Kruskal-Wallis test*

625 **Figure 3.** Selenium concentrations (µg/g DW) in whole Zea mays plants (A) or in different
626 tissues of Zea mays plants with the two different treatments (B. roots, C. stems, D. leaves and
627 E. grains): Se^{VI}-T (light gray) and Se^{IV}-T (dark gray).
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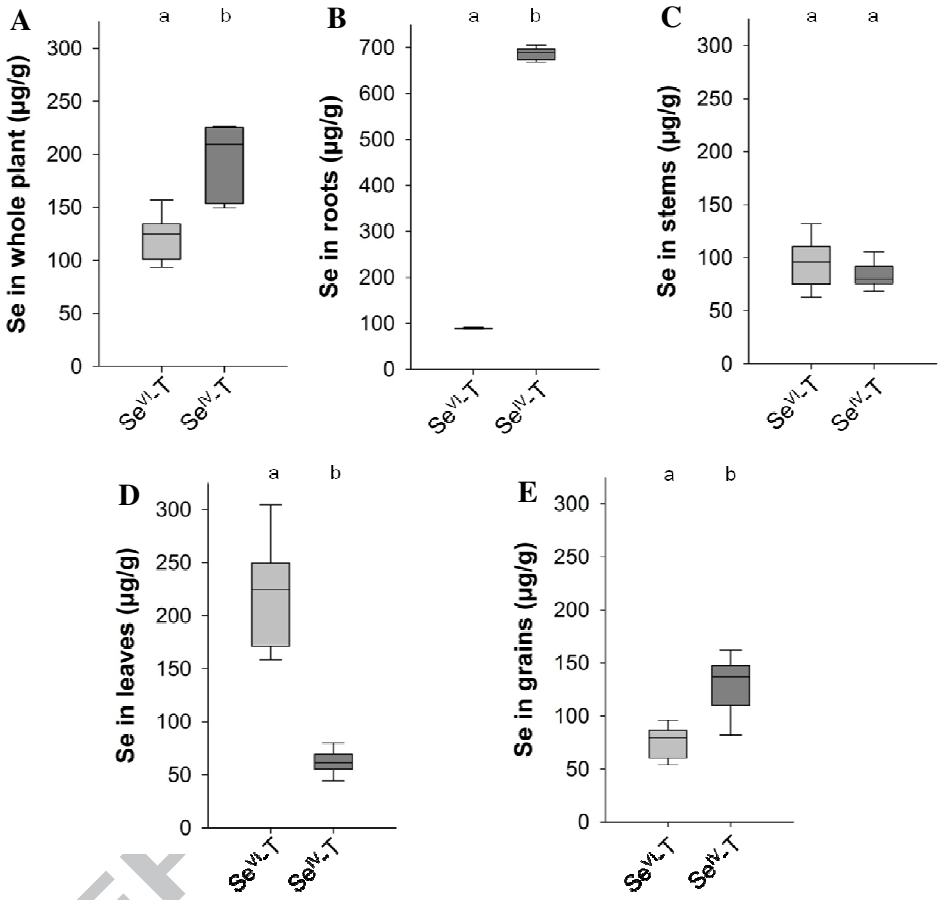

629
630 *a, b: results of Mann and Whitney test*
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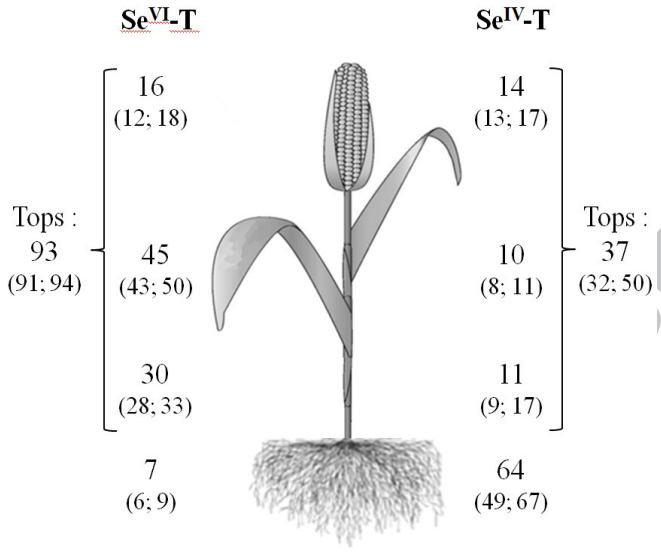
Figure 4. Schematic representation of a Zea mays plant showing the selenium amount (%) in roots, stems, leaves, and grains treated with Se^{VI}-T (left) or Se^{IV}-T (right).



Values are medians (Q1; Q3)

643 **Table 1**. Selenium species in Zea mays after enzyme hydrolysis.

644

645

		Se^{VI}-T			Se^{IV}-T		
	Efficiency of enzyme hydrolyis (%)	Se fraction (%)[a]			Se fraction (%)[a]		
		Selenate	Selenite	Se-organic[b]	Selenate	Selenite	Se-organic[b]
Roots	36 ± 8	20 ± 5	ND	80	ND	ND	≥ 95
Stems	89 ± 13	54 ± 16	ND	46	ND	ND	≥ 95
Leaves	93 ± 6	39 ± 9	ND	61	ND	ND	≥ 95
Grains	104 ± 7	ND	ND	≥ 95	ND	ND	≥ 95

646

647 [a] *% of Se species after protease hydrolysis*

648 [b] *difference between total Se extracted by protease hydrolysis and sum of inorganic species*

649 *Values are means ± SD*

Table 2. Estimated concentration (µg/g) and amount (µg/plant) of selenium bioavailable for animals (tops of plant) or humans (grains of plant) with the two selenium treatments: Se^{VI}-T and Se^{IV}-T.

		Se^{VI}-T	Se^{IV}-T
Grains	**µg/g**	71[a] (61; 78)	126[b] (119; 133)
	µg/plant	669[a'] (541; 760)	520[a'] (482; 723)
Tops	**µg/g**	97[a#] (84; 100)	72[a#] (71;82)
	µg/plant	3023[a*] (2593; 3903)	1362[b*] (1104; 2288)

Values are medians (Q1; Q3)

a, b: results of Mann-Whitney test