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

4
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15

16 **Highlights**

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

18 • **Selenite treatment results in higher selenium accumulation in grains than selenate**
19 **treatment**

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

22 • **For humans, selenite treatment increases bioavailable selenium in grains**

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

24

25 **Abstract**

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

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

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

38

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

41

42 1. INTRODUCTION

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

50 Selenium concentrations in food, including crops, depend not only on selenium
51 concentrations in agricultural soils (which vary considerably between countries and regions)
52 but also on selenium phytoaccessibility controlled by many abiotic and biotic factors such as
53 soil pH, redox conditions, organic matter content, microbial activities, irrigation and
54 compaction. In some countries or regions, low selenium levels in soil lead to low
55 concentrations in feed or forage, which in turn can result in selenium deficiency in livestock
56 and humans. For example, the average selenium intake is only 36 μg per day in France, 34 μg
57 per day in the UK and 35 μg per day in Sweden (Rayman, 2008); these levels are below the
58 recommended dietary allowance of 40 to 70 μg per day (World Health Organization et al.,
59 1996). To increase selenium levels in human and animal diets, several processes have been
60 developed including mineral supplementation, genetic biofortification (plant breeding) and
61 finally, the option chosen here, agronomic biofortification of food or forage.

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

67 metabolism are strongly dependent on the form of supplied selenium. The greater mobility of
68 selenate compared to selenite results in differences in the absorption, translocation and
69 metabolism of selenium within the plant. Indeed, when plants are exposed to selenite,
70 selenium accumulation is less than after selenate treatment (De Souza et al., 1998; Terry et al.,
71 2000; Ximenez-Embun et al., 2004; Zhang et al., 2003), with a greater reduction in biomass
72 production (Hopper et al., 1999; Ximenez-Embun et al., 2004). After selenate treatment,
73 selenium is almost entirely translocated to the leaves and weakly metabolized as selenoamino-
74 acids, with a selenate concentration in shoots (i.e. stems and leaves) representing more than
75 90% of the total shoot selenium (De Souza et al., 1998; Hopper et al., 1999; Li et al., 2008;
76 Mazej, Osvald & Stibilj, 2008; Terry et al., 2000; Ximenez-Embun et al., 2004; Zayed et al.,
77 1998; Zhang et al., 2003). In contrast, when supplied as selenite, selenium accumulates
78 principally in roots with little translocation, although selenoamino-acid production
79 (principally selenomethionine, selenocysteine and selenomethylselenocysteine) is greater (De
80 Souza et al., 1998; Hopper et al., 1999; Li et al., 2008; Liu & Gu, 2009; Terry et al., 2000;
81 Ximenez-Embun et al., 2004; Zayed et al., 1998) and the selenium volatilization rate is about
82 2-fold higher from those plants (De Souza et al., 1998).

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

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

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

137 2. MATERIALS AND METHODS

138 2.1. Seed germination and culture conditions

139 Three weeks after germination, *Zea mays sup.s.mays* (L.) corn seedlings were cultivated in
140 hydroponic conditions in 20 L plastic tanks filled with a modified Hoagland nutrient solution
141 consisting of KNO_3 (3 mmol.l⁻¹), $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (2.72 mmol.l⁻¹), NH_4NO_3 (2 mmol.l⁻¹), NaCl
142 (0.2 mmol.l⁻¹), KH_2PO_4 (0.98 mmol.l⁻¹), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.70 mmol.l⁻¹), $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$
143 (0.04 $\mu\text{mol.l}^{-1}$), H_3BO_3 (24 $\mu\text{mol.l}^{-1}$), MnSO_4 (13 $\mu\text{mol.l}^{-1}\text{M}$), ZnSO_4 (6 $\mu\text{mol.l}^{-1}$), CuSO_4 (1.5
144 $\mu\text{mol.l}^{-1}$) and FeEDDHA (6%) (4 $\mu\text{mol.l}^{-1}$). Two nutrient solutions were supplemented with
145 12 $\mu\text{mol.l}^{-1}$ selenium as either Na_2SeO_4 or Na_2SeO_3 (solutions $\text{Se}^{\text{VI-T}}$ and $\text{Se}^{\text{IV-T}}$),
146 respectively. Under control conditions (C-T), no selenium was added. Five corn seedlings
147 were transplanted in each tank and placed into a RUBIC5 plant growth chamber (Reactor
148 Used for Continental Isotopic Biogeochemistry), a 9 m³ sealed chamber (Servathin, France)
149 the atmospheric compositions of which are controlled. Lighting was provided by 15x400 watt
150 Philips Son-T Agro bulbs over an 8-hour photoperiod set at 600 $\mu\text{M.m}^{-2} \cdot \text{s}^{-1}$ photosynthetically
151 active radiation at plant height. Air temperature was set at 25°C during the day and 18°C at
152 night. Air humidity was controlled by a dew point condenser in order to maintain a set-point
153 of 70% relative humidity. Beyond this set point, excess water vapor was condensed and
154 collected using an Isco 3700 water sampler (so called “condensates”). The CO_2 concentration
155 was measured using a LI-COR (Lincoln, Nebraska USA) Li620 infrared gas analyzer set at
156 400 ppmv. The chamber had a slight positive pressure of +20 Pa to avoid entry of outside air.
157 Data were logged by a computer and averaged at 10 min intervals.

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

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

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

165

166 **2.2. Total selenium analysis**

167 A suitable amount of powdered plant tissue (about 100 mg DW) was digested in 2 ml of
168 HNO₃ (70%) at 100°C for 24 hours in a closed digestion vessel. After cooling, 1 ml of H₂O₂
169 (30%) was added and the sample was heated again at 100°C for 24 hours. The selenium
170 concentration in the digested tissues samples was determined by inductively coupled plasma
171 atomic emission spectrometry (ICP-AES, JY 2000, LOD: 50 µg.l⁻¹). A blank and a reference
172 material (White clover, BCR402- IRMM) were included in each batch of samples. In the C-T
173 plants, the selenium concentrations fell below the detection threshold of ICP-AES.

174 Condensate selenium concentrations were determined by graphite furnace atomic absorption
175 spectrometry (GFAAS, UNICAM 989 QZ, LOD: 1.5 µg.l⁻¹). A certified reference material
176 (TMDA-64- Environment Canada) and a blank were included in each batch of samples.

177

178 **2.3. Separation and quantification analysis of selenate and selenite**

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

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

- 190 - mobile phase: $30\text{ mmol}\cdot\text{l}^{-1}$ KOH
- 191 - flow rate: isocratic at 1 ml/min
- 192 - injected sample volume: 200 μl
- 193 - column temperature: 30°C

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

199

200 **2.4. Calculation of leaf area**

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

$$204 \text{ Leaf area} = \sum_{i=1}^n (L_i * l_i * 0.75)$$

204

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

206

207 **2.5. Statistical analysis**

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

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

ACCEPTED MANUSCRIPT

216 3. RESULTS

217 3.1. Biomass production

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

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

227 Moreover, again for Se^{IV} -T, the biomass production of plants (**Figure 2.A**) as well as the
228 biomass production of shoots (data not shown) was about 70% less than C-T plants.
229 For grains, dry weights decreased by 60% and 80% in Se^{VI} -T and Se^{IV} -T plants, respectively,
230 compared to the control plants (**Figure 2.B**). The grain number produced by each plant
231 (**Figure 2.C**) decreased significantly (70%) with selenite. Biomass allocation was affected by
232 selenium; the ratio of grain dry weights to shoot dry weights was less with both selenium
233 treatments compared with C-T. In fact, grain biomass for the C-T plants represented 33% of
234 aerial biomass, but fell to 21% when selenium was present in the nutrient solution.

235

236 3.2. Uptake and accumulation of total selenium in *Zea mays*

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

241 concentrations of $210 \mu\text{g}\cdot\text{g}^{-1}$ (156; 225) and $125 \mu\text{g}\cdot\text{g}^{-1}$ (103; 126), respectively (**Figure 3A**).
242 Similarly but to a greater extent, selenium concentrations in roots were much higher (675%)
243 in $\text{Se}^{\text{IV}}\text{-T}$ versus $\text{Se}^{\text{VI}}\text{-T}$ plants (**Figure 3.B**); and selenium concentrations in grains were 1.7
244 times greater, i.e. 73%, in $\text{Se}^{\text{IV}}\text{-T}$ versus $\text{Se}^{\text{VI}}\text{-T}$ plants (**Figure 3.E**). Conversely, selenium
245 concentration in leaves was 73% lower in $\text{Se}^{\text{IV}}\text{-T}$ compared to $\text{Se}^{\text{VI}}\text{-T}$ 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 $72 \mu\text{g}\cdot\text{g}^{-1}$ (66; 80) and $86 \mu\text{g}\cdot\text{g}^{-1}$ (86; 88) after
251 selenite treatment, and $151 \mu\text{g}\cdot\text{g}^{-1}$ (114; 154) and $126 \mu\text{g}\cdot\text{g}^{-1}$ (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 metabolism 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 hydrolysis is high, approximately 90%. In
278 roots, where the efficiency of enzyme hydrolysis 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 ($54 \pm 16\%$
284 and $39 \pm 9\%$, respectively) than in roots ($20 \pm 5\%$). Finally, whatever the form of selenium
285 supplied, selenium was converted completely to organo-selenium compounds in grains.

286

287

288 **4. DISCUSSION**289 **4.1. Crop growth of *Zea mays***

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

296 Based on the dry weights of plants or tissues and the leaf areas, supplementation with
297 inorganic selenium at high concentration (12 μM) was harmful to *Zea mays* growth. In
298 selenite-treated plants, the development of all tissues was affected, with a decrease in dry
299 forage biomass as well as quantity (number) and quality (dry weight) of grains. A decrease in
300 the leaf area of plants treated with selenite was observed only during the reproductive stage,
301 i.e. between days 30 and 70. The internodes that were affected by selenite treatment
302 corresponded to those developed during the vegetative stages. Selenite toxicity has already
303 been observed in white lupine and in sunflower, for example, with a biomass reduction of
304 20% and 40%, respectively (with 12 $\mu\text{mol.l}^{-1}$ selenite) (Ximenez-Embun et al., 2004). These
305 results contrast with data on *Brassica rapa* (Lyons et al., 2009), which showed that at very
306 low selenium concentrations (0.05 $\mu\text{mol.l}^{-1}$ selenite) in hydroponic solution, plant biomass
307 and dry weight of each grain were not affected, and moreover grain number increased by 43%
308 for each plant. In selenate-treated plants, vegetative tissues of *Zea mays* were not affected,
309 according to literature data on different varieties of crops (such as *Zea mays* and wheat) or
310 other plants (such as pumpkin, buckwheat, dry beans) fertilized with different techniques
311 (foliar application of selenate, selenate liquid or solid addition in soils, or fly-ash amendment)
312 (Broadley et al., 2010; Cubadda et al., 2010; Mbagwu, 1983; Smrkoj 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 volatilize 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 $12\mu\text{mol.l}^{-1}$).
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 ($0.05\mu\text{mol.l}^{-1}$ selenium), i.e. 240-fold lower than in our experiments.

338 With selenite treatment, most of the selenium accumulated in roots. In Li *et al.* (2008),
339 Terry *et al.* (2000), De Souza *et al.* (1998) and Ximenez-Embun *et al.* (2004), the 'roots-tops'
340 translocation factor (0.5) was greater than that suggested by our result (0.13), but similar to
341 that of Lyons *et al.* (2009) (0.08). These two very similar results are the only data obtained
342 from experiments carried out up to the reproductive stage in a hydroponic system. The
343 developmental stage of the plant seems to influence the root storage capacity of selenium:
344 root uptake and accumulation appear to increase as the plants mature. Moreover, with selenite
345 treatment, organo-selenium compounds were produced to a greater extent than with selenate
346 treatment: in whole plants, no traces of inorganic selenium were detected. In several papers,
347 traces of selenite were detected in roots or shoots, but always less than 7%, indicating that
348 selenium in plants is overwhelmingly organoselenium compounds (Ximenez-Embun *et al.*,
349 2004). With selenate treatment, most of the selenium taken up by *Zea mays* was translocated
350 and accumulated in the tops of plants, especially in the leaves; much less accumulated in roots
351 (Pickering, Prince, Salt & Georges, 2000). In our study, the 'roots-tops' translocation factor
352 (1.45) was the same order of magnitude as published data (1.5-17) (De Souza *et al.*, 1998; Li
353 *et al.*, 2008; Terry *et al.*, 2000). Selenate was metabolized less than selenite in whole plants.
354 This finding is coherent with the fact that reduction of selenate into selenite is the rate-
355 limiting step in selenate metabolism in plants (De Souza *et al.*, 1998; Li *et al.*, 2008; Terry *et*
356 *al.*, 2000). Selenate absorbed by roots is metabolized to organic selenium compounds (that
357 represent only 20% of total selenium in roots) and/or is quickly translocated to the tops of
358 plants. The percentage of selenate in leaves (39%) is less than in stems (54%), which seems to
359 indicate that selenate is also metabolized in leaves. Mazej *et al.* (2008) and Li *et al.* (2008)
360 showed that on average, 60 to 100% of selenium in leaves and roots is selenate. In the results
361 presented by Ximenez *et al.* (2004) in India mustard, the selenate form represents 30% in
362 roots and 90% in shoots; moreover in sunflower, selenate in leaves (35%) is similar to that in

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

370 In the literature, selenium accumulation in grains has been studied mainly in wheat or rice
371 (Broadley et al., 2010; Cubadda et al., 2010; Eurola, Ekholm, Ylinen, Koivistoinen & Varo,
372 1991; Lyons et al., 2005), but to date, few data exist for selenium accumulation in Zea mays
373 grains, except in Chilimba *et al.* (2011) and Mbagwu (1983), for example. Moreover, studies
374 on grains or seeds are usually carried out in soil (pot or yield), naturally or manually enriched
375 with Se-supplementation, but with little information on water-soluble selenium bioavailable
376 for plants (Broadley et al., 2010; Chilimba et al., 2011; Cubadda et al., 2010; Eurola et al.,
377 1991; Kapolna et al., 2007; Lyons et al., 2005; Mbagwu, 1983; Smrkolj et al., 2007; Smrkolj
378 et al., 2005; Stibilj et al., 2004). Thus, our results can only be compared with Zea mays grown
379 in soils or other plant species. In our hydroponic system, selenium concentrations obtained in
380 Zea mays grains ($93 - 226 \mu\text{g}\cdot\text{g}^{-1}$) were much higher than in the majority of studies (Eurola et
381 al., 1991; Lyons et al., 2009; Lyons et al., 2005; Mbagwu, 1983; Smrkolj et al., 2005; Stibilj
382 et al., 2004). For example, in Zea mays grains in Malawi, selenium concentrations were only
383 45 to $500 \text{ ng}\cdot\text{g}^{-1}$ (Chilimba et al., 2011), while winter wheat grains (Broadley et al., 2010) can
384 accumulate up to $2.6 \mu\text{g}\cdot\text{g}^{-1}$. Only grains of wheat harvested in the Nawanshahr-Hosshiarpur
385 region of India had selenium concentrations similar to ours with 29 and $185 \mu\text{g}\cdot\text{g}^{-1}$ (Cubadda
386 et al., 2010). These differences are probably due to the growing conditions, specifically the
387 soils or hydroponic solution. In our study, the hydroponic experiments allowed us to study the

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

392

393 **4.3. Selenium enrichment of Zea mays to improve the quality of human and livestock** 394 **food**

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

398 With selenate treatment, Zea mays grains with the lowest selenium concentrations
399 accumulated less selenium than other Zea mays tissues. However, with granular fertilization
400 of selenate, Gissel-Nielsen (1986) found that selenium concentrations in barley grains were
401 equal to or slightly higher than in barley straw. On the other hand, with selenite treatment and
402 according to the literature (Cubadda et al., 2010; Mbagwu, 1983), selenium concentrations are
403 higher in grains than in shoots. In our study, grains contained twice as much selenium as
404 shoots, which is slightly higher than the levels reported by Cubadda et al. (2010) and Mbagwu
405 (1983) (around 1.15 times). Similarly, in field experiments and with different methods of
406 selenium application (granular fertilization and foliar application), selenium concentrations in
407 barley grains treated with selenite were on average 1.6 times higher than in straw (Gissel-
408 Nielsen, 1986). Thus, with selenite supplementation, grains appear to be a secondary tissue
409 for selenium storage after the roots.

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

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

419

420 To improve dietary intake of selenium, the amount of selenium ingested is important but
421 the quality and quantity of bioavailable selenium are also key factors. According to Thomson
422 (2004), the bioavailability of organic selenium is 90% compared with 50% for selenite or
423 selenate. Based on these data, we calculated selenium bioavailability for humans and animals
424 in *Zea mays* in our experiment (**Table 2**).

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

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

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

457

458 **5. CONCLUSIONS**

459 Our data suggest ways to improve agronomic biofortification of *Zea mays* with selenium.
460 The absorption, accumulation, distribution and metabolization of selenium in mature *Zea*
461 *mays* plants depend on the form of selenium supplied. Despite a decrease in grain biomass in
462 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

474 **ABBREVIATIONS USED**

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

476 ICP-AES: Inductively coupled plasma atomic emission spectrometry;

477 GFAAS: graphite furnace atomic absorption spectrometry;

478 DW: Dry weight;

479 LOD: Limit of detection;

480 SD: Standard deviation;

481 FeEDDHA: Iron- Ethylenediaminedi-Q-hydroxyphenylacetic acid;

482 IRMM : Institute for Reference Materials and Measurements

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- 593
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595 **LIST OF FIGURES**

596 **Figure 1.** Change in leaf area (cm²) (A) and internode length (cm) (B) of Zea mays plants
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
602 tissues of Zea mays plants with the two different treatments (B. roots, C. stems, D. leaves and
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
605 roots, stems, leaves, and grains treated with Se^{VI}-T (left) or Se^{IV}-T (right).

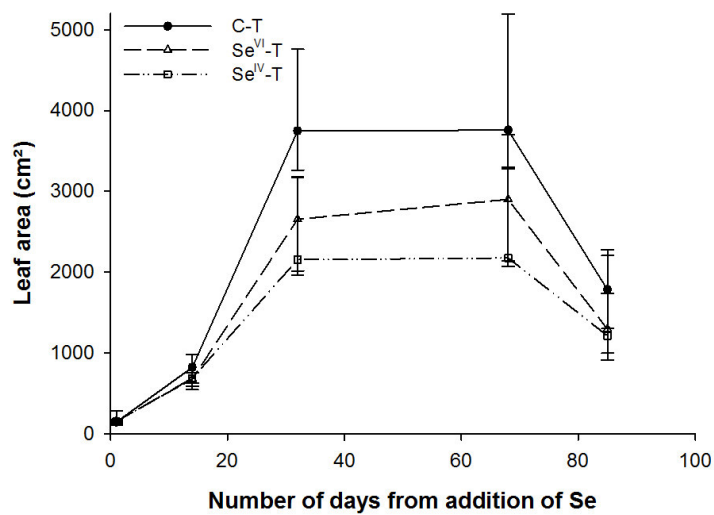
606 **Table 1.** Selenium species in Zea mays after enzyme hydrolysis.

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.

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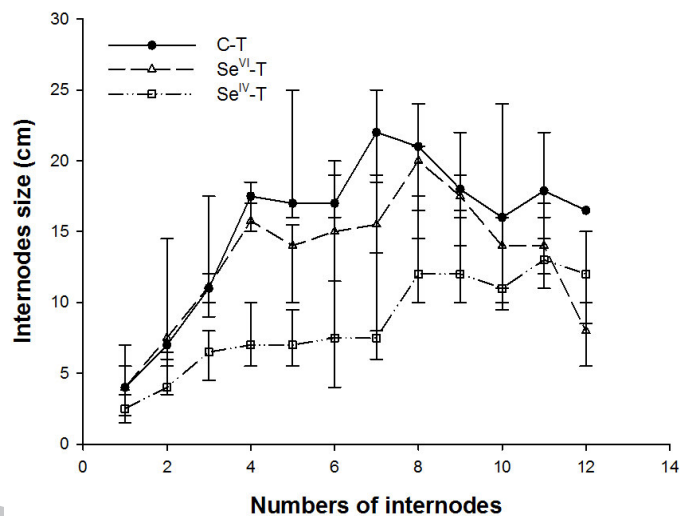
611 **Figure 1.** Change in leaf area (cm²) (A) and internode length (cm) (B) of Zea mays plants
 612 with the three different treatments.

613 **A**



614

615 **B**



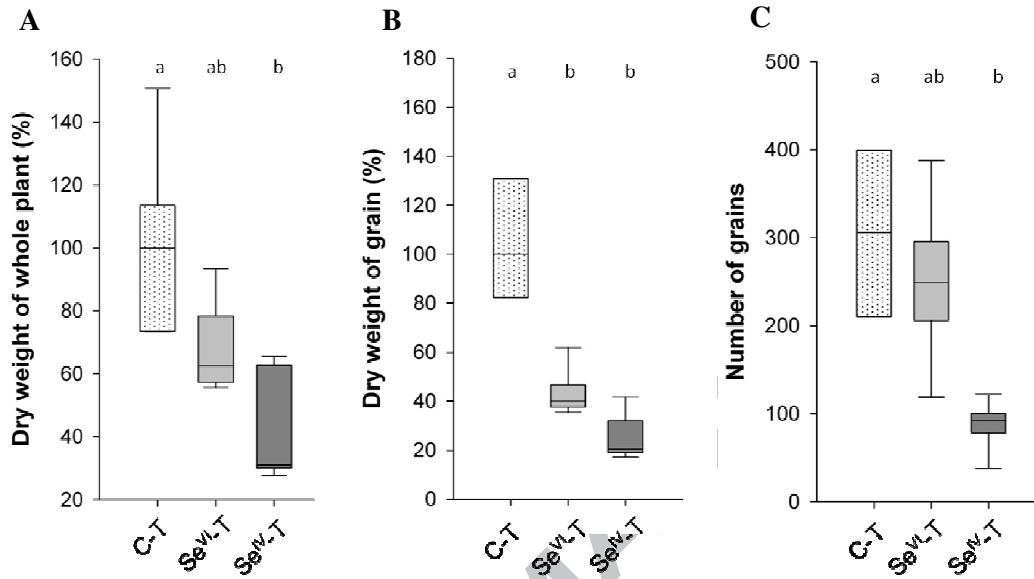
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618 Values are medians (lower: 10th percentile; upper: 90th percentile)

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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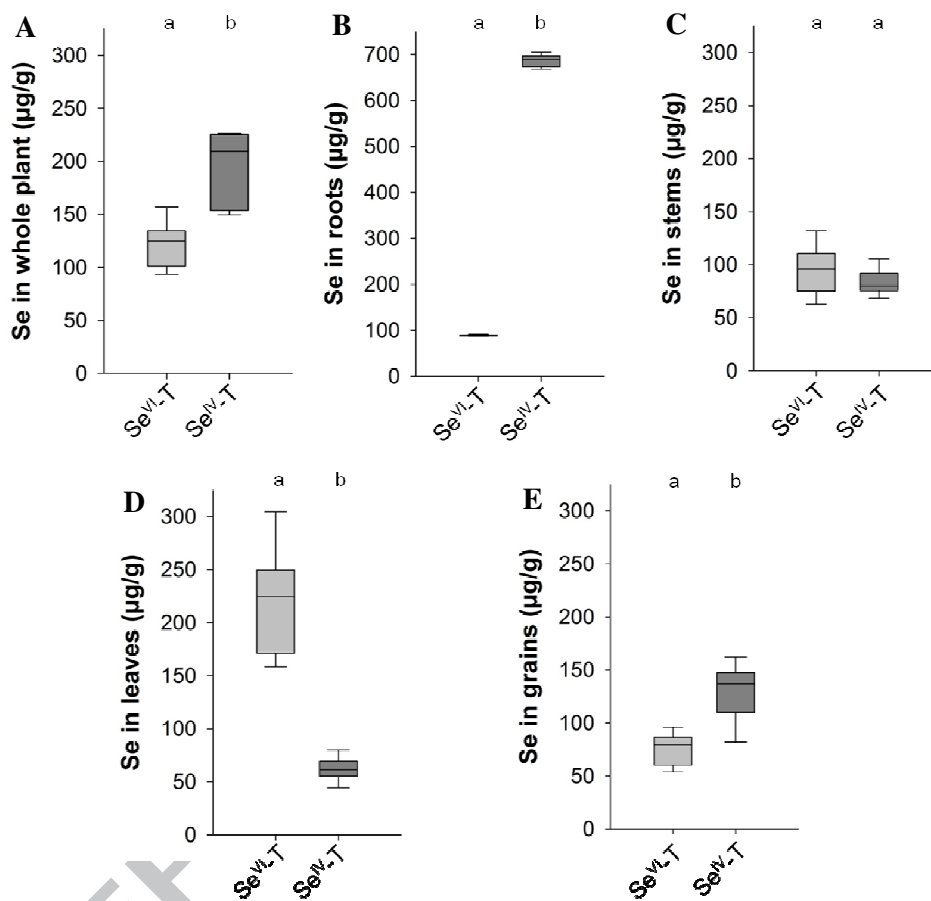


623

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

625 **Figure 3.** Selenium concentrations ($\mu\text{g/g}$ 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): $\text{Se}^{\text{VI}}\text{-T}$ (light gray) and $\text{Se}^{\text{IV}}\text{-T}$ (dark gray).

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629

630 *a, b: results of Mann and Whitney test*

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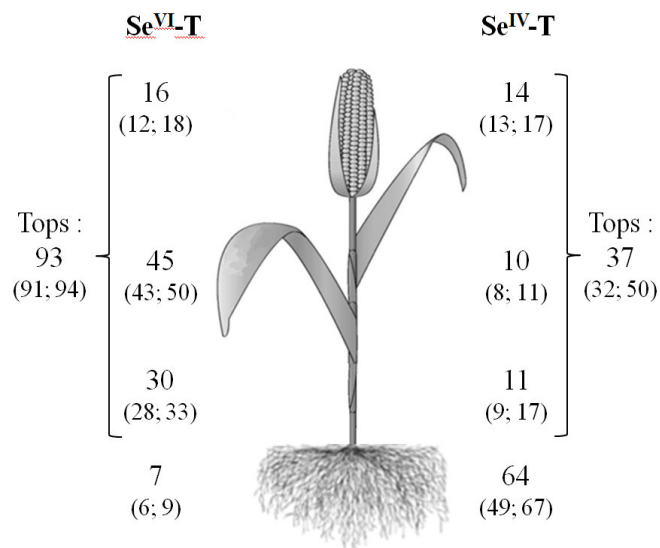
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637 **Figure 4.** Schematic representation of a *Zea mays* plant showing the selenium amount (%) in
 638 roots, stems, leaves, and grains treated with $\text{Se}^{\text{VI}}\text{-T}$ (left) or $\text{Se}^{\text{IV}}\text{-T}$ (right).

639



641 *Values are medians (Q1; Q3)*

642

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

644

645

	Efficiency of enzyme hydrolysis (%)	Se^{VI}-T			Se^{IV}-T		
		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 hydrolysis648 ^b difference between total *Se* extracted by protease hydrolysis and sum of inorganic species

649 Values are means ± SD

650 **Table 2.** Estimated concentration ($\mu\text{g/g}$) and amount ($\mu\text{g/plant}$) of selenium bioavailable for
 651 animals (tops of plant) or humans (grains of plant) with the two selenium treatments: $\text{Se}^{\text{VI}}\text{-T}$
 652 and $\text{Se}^{\text{IV}}\text{-T}$.

		$\text{Se}^{\text{VI}}\text{-T}$	$\text{Se}^{\text{IV}}\text{-T}$
654 655	Grains	71^{a} (61; 78)	126^{b} (119; 133)
		669^{a} (541; 760)	520^{a} (482; 723)
656 657	Tops	$97^{\text{a}\#}$ (84; 100)	$72^{\text{a}\#}$ (71; 82)
		$3023^{\text{a}*}$ (2593; 3903)	$1362^{\text{b}*}$ (1104; 2288)

658
 659 *Values are medians (Q1; Q3)*

660 *a, b: results of Mann-Whitney test*

661