Plant Physiology and Biochemistry 83 (2014) 300–307

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

# Dual effects of different selenium species on wheat

B. Guerrero [a, *], M. Llugany [b], O. Palacios [c], M. Valiente [a]

[a] Universitat Autònoma de Barcelona, Centre GTS, Department of Chemistry, 08193 Bellaterra, Barcelona, Spain
[b] Universitat Autònoma de Barcelona, Laboratory of Plant Physiology, 08193 Bellaterra, Barcelona, Spain
[c] Universitat Autònoma de Barcelona, Inorganic Chemistry Unit, Department of Chemistry, 08193 Bellaterra, Barcelona, Spain

ARTICLE INFO

*Article history:*
Received 17 June 2014
Accepted 11 August 2014
Available online 21 August 2014

*Keywords:*
ICP-MS
Selenate
Selenite
Selenium
Toxicity
Wheat

ABSTRACT

Wheat (*Triticum aestivum*) and its derivative products account for a major source of dietary intake of selenium (Se) in humans and animals, because of its essentiality due to its presence in vital enzymes. Se antioxidant role has resulted in the popularity of agronomic biofortification practises in Se deficient areas. Controlling Se uptake, metabolism, translocation and accumulation in plants will be important to decrease healthy risk of toxicity and deficiency and to help selecting adequate methods for biofortification.

Selenate and selenite are the two main inorganic Se forms available in soil and in most of the studies are given separately. That study reveals that both Se species behave differently but combined the prevalent pattern is that of selenite; so it is taken up faster and it seems that interferes with selenate uptake and transport. Selenium has dual effects on wheat plants; at low concentrations it acts as growth stimulant whereas at high concentrations it reduces root elongation and biomass production and alters uptake and translocation of several essential nutrients.



## 1. Introduction

Selenium (Se) has received considerable scientific attention during the past century. It is an essential trace element for humans and animals mainly due to its presence in several vital enzymes, such as glutathione peroxidase (Deagen et al., 1991). Its biological importance and its putative anticancer activity have resulted in the popularity of food supplements (Navarro-Alarcon and Cabrera-Vique, 2008). The narrow margin between toxicity, essentiality and deficiency grows up the interest in Se because of both health and environmental impacts (Pyrzynska, 2002).

The most important human source of Se is food; therefore plants play an important role in Se supplementation (Finley, 2005). Plants synthesize selenoproteins from Se containing soils; hence Se content in crops depends on soil Se richness. Se bioassimilation processes are important to better understand growth parameters changes and nutrient uptake in correlation with Se uptake.

Cereal grains are poor sources of key mineral nutrients. As a result, the world's poorest people subsisting on a monotonous cereal diet are also those most vulnerable to mineral deficiencies. In this study we select wheat (*Triticum aestivum*) because its derivative products (breads, cakes, cereals, pasta…) are an important source of Se for human diet (Lyons et al., 2003). Wheat is a non-Se-accumulator plant and the threshold Se-toxicity concentration is dependent on the form of Se accumulated (Terry et al., 2000). On the other hand, Se concentration in wheat shows great variations between countries and regions (Zhu et al., 2009).

With respect to Se determination in plant samples and hydroponic solutions, ICP-MS has been applied as one of the most powerful tools for trace level concentration analysis using $H_2$ gas collision cells (Huerta et al., 2003).

The particular chemical form of an element determines its toxicity, biological activity, bioavailability and environmental impact. The relative availability of selenate and selenite in nutrient solutions depend on the presence of competing ions (Hopper and Parker, 1999). Several studies report that selenate competes with sulfate transporters for uptake by plants, however little is known about selenite uptake which seems to have a great passive component and a minor active one mediated by phosphate transporters (Asher et al., 1983; Arvy, 1993; Ellis and Salt, 2003; Li et al., 2007; Govasmark et al., 2008; Feng et al., 2009). In our study, selenite in nutrient solution at pH 6.0 exists primarily as $HSeO_3^-$ (Geochem, data not shown) and behaves as a weak acid that can compete with phosphate uptake. Hopper and Parker (Hopper and

* Corresponding author. Tel.: +34 937882300; fax: +34 937891906.
*E-mail addresses:* bea.guerrero.lopez@gmail.com, bguerrero@leitat.org (B. Guerrero), merce.llugany@uab.cat (M. Llugany), oscar.palacios@uab.cat (O. Palacios), manuel.valiente@uab.cat (M. Valiente).

http://dx.doi.org/10.1016/j.plaphy.2014.08.009
0981-9428/

Parker, 1999) demonstrated that the inhibition of sulfate by selenate is stronger than that of phosphate by selenite. Taking into account these findings in our experiments, levels of both nutrients have been lowered to avoid part of this competition (White et al., 2004).

Selenium oxidation state depends on pH and redox potential of soil and those parameters are strongly dependent on season factors: soil composition, water content, organic matter and bacteria activity. Even thus, it has been observed that in acidic grounds selenite is the main Se form while in basic ones selenate, much more soluble than selenite, is the most abundant form (Masscheleyn et al., 1990), so the soil coexistence of both forms can be easily found. Considering that selenite exhibits higher toxicity than selenate, but selenate is more soluble, and thus available for plants, Se toxicity can be also related with soil redox potential. For these reasons, the effect of both selenium forms, separately and together, must be considered in order to assess the mechanisms which prevent or promote Se plant toxicity. It must be also considered that selenium concentrations ranging from 3 to 330 μg $L^{-1}$ have been detected in shallow post mining grad water from coal mines (Martin et al., 1988) and its accumulation in soils can be high enough to be hazardous for animals and plants.

In this work, wheat plants were exposed to Se in the form of either sodium selenate, sodium selenite or a mixture of both, in order to study the effect of chemical speciation on Se bioavailability and to better understand Se-mechanisms of uptake, transport, metabolism, nutrient interaction and tolerance. Although, there are some studies about the effect of Se on the uptake of essential nutrients in plants (Feng et al., 2009; Arvy et al., 1995), interaction between Se and essential nutrients is not still well understood, while the effect of both Se species together on nutrient uptake has been poorly studied.

## 2. Methods

### 2.1. Plant material and culture conditions

*T. aestivum* cv. Pinzón (Semillas Fitó S.A., Spain) was used in this study as soft wheat well-known for its high flour quality. Seeds were germinated on moist filter paper for 3 days at 25 °C. Seedlings were then transferred to 1 L plastic pots and were allowed to acclimate and grow for a week with ¼ strength Hoagland's nutrient solution. Afterwards plants were exposed to 5 days-long under different Se treatments.

Each plastic pot contained three plants and each Se treatment was duplicated. In addition, the complete hydroponics culture experiment was performed twice. The composition of the nutrient solution was: 1.0 mM $KNO_3$, 1.0 mM $Ca(NO_3)_2$, 0.25 mM $MgSO_4$, 0.5 mM $K_2HPO_4$, 2.0 μM $MnCl_2$, 3 μM $H_3BO_3$, 0.1 μM $(NH_4)_6Mo_7O_{24}$, 2 μM $ZnSO_4$, 1 μM $CuSO_4$ and 60 μM FeNa-EDTA. The pH of this solution was buffered at 6.0 with 2 mM MES (2-morpholinoethanesulphonic acid, pH adjusted with KOH). The solution was aerated continuously and renewed twice in the middle of growth period. The culture was carried out in a controlled culture chamber with the following conditions: a photoperiod of 16 h day/8 h night, a temperature of 24 °C day/18 °C night and a light intensity of 320 μE $m^{-2}$ $s^{-1}$.

### 2.2. Selenium treatments

Selenium treatments were done by adding Se to the nutrient solution cultures in the form of sodium selenite ($Na_2SeO_3 \cdot 5H_2O$, Fluka) and sodium selenate ($Na_2SeO_4$, Fluka) separately at four different concentrations: 1 μM, 10 μM, 50 μM and 100 μM. Additionally, the same Se concentrations were reached by mixing both Se species at equal quantities. These treatments with mixed Se species are abbreviated as 0.5 + 0.5, 5 + 5, 25 + 25 and 50 + 50.

### 2.3. Root elongation growth, absorbed solution and sample preparation

After 5 days of incubation in Se enriched media, plants were harvested and roots desorbed with $CaCl_2$ solution to remove Se in root apoplast. Aliquots of nutrient solutions were taken for analysis of total Se.

After desorption, the length of the longest root of each plant was measured and root elongation was calculated as the difference between the final and the initial length corresponding to the length of the longest root before and after the treatments, respectively. Then plants were divided into roots and shoots, weighed and frozen until further processing. Absorbed solution was measured as the difference in volume between the volume at the beginning of the experiment and the final volume at harvesting.

### 2.4. Total Se and nutrient elements determination

Part of the plant material was dried and acid digested in closed HP500 PFA vessels with $HNO_3$:$H_2O$ (7:3) in a microwave digestion system (Mars 5, CEM, USA) under EPA 3052. Total Se in the digests was measured by ICP-MS (PQExCell, Thermo Elemental, UK) using a standard solution of 1000 mg $L^{-1}$ Se (Aldrich, USA) for the calibration procedure and SELM-1 CRM (Selenium Enriched Yeast Certified Reference Material, NRC, Canada) The analysed Se content (1980 ± 41 mg $kg^{-1}$ expressed as average ± SD, $n = 4$) was within the range of the values determined by NRC (2059 ± 64 mg $kg^{-1}$) (Mester et al., 2006).

Nutrient elements concentrations were analysed by ICP-OES (Optima 3200RL, Perkin Elmer, USA). Corresponding standard 1000 mg $L^{-1}$ solutions purchased from High Purity Standards (USA) were used for the calibration procedure.

### 2.5. Data analysis

Statistical analysis for comparison of means between different treatments was done by a two sample *t*-test at a significant level of 0.05 with SPSS software package. Data from root elongation and fresh weight are mean of 12 replicates. It is noteworthy that roots and shoots from the same pot, separately, were mixed together to form 1 pooled sample for the nutrient analysis, thus results are expressed as means of 4 replicates with corresponding standard errors.

## 3. Results

Results, expressed in terms of wheat growth parameters, Se distribution and concentration in wheat and nutrient uptake effects, have been analysed to determine various effects of Se uptake.

### 3.1. Growth parameters

To check the effect of Se on plant growth several parameters were used: root elongation, shoot and root fresh weight, and nutrient solution absorbed, connected with normal plant growth needs.

Our results showed that Se exposure up to 10 μM Se caused a slightly higher root elongation for selenate treatment (Fig. 1a). By contrast, selenite and mixture treatments at the same range showed no significant differences in root elongation in comparison with the control.