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Determination of selenium in bread-wheat samples grown under a Se-supplementation regime in actual field conditions

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Abstract Selenium is an essential micronutrient for humans and animals, yet it is deficient in at least one billion people worldwide. Plants and plant-derived products transfer the soil-uptaken selenium to humans; therefore, the cultivation of plants enriched in selenium can be an effective way to improve the selenium status on humankind. This paper focuses on determining the ability of bread wheat to accumulate selenium after supplementation. One of the methods for supplementing this element in plants is foliar application with selenium solutions. These supplemented crop of wheat samples—bread wheat; *Triticum aestivum* L.—were used to determine if there is an increase of selenium content in cereal grains by comparing them with cereals cultivated in 2009 and harvested in 2010 with no supplementation. The experiments were done using sodium selenate and sodium selenite at three different selenium concentrations: 4, 20 and 100 g per hectare. Total Se is assessed by cyclic neutron activation analysis (CNAA), through short irradiations on the fast pneumatic system (SIPRA) of the Portuguese Research Reactor (RPI-ITN). The short-lived nuclide ^{77m}Se, that features a half-lifetime of 17.5 s, was used to determine the Se content in SIPRA. The experiment was successful, since the selenium concentration increased in the cropped grains and reached values up to 35 times the non-supplemented ones.

Keywords Bread wheat · Cereal biofortification · Growth stages · Foliar application · Selenium supplementation · CNAA

Introduction

The increasing attention paid to the role of selenium (Se) and selenoproteins in human health stems primarily from a similarly growing body of evidence about not only their actual (general) importance for a healthy immune system, but also for their protective (specific) effects against cardiovascular disease, asthma, male sterility, and, especially, certain forms of cancer [1–8]. These are not scattered observations or random effects. From about 100 selenoproteins that may exist in mammalian systems [9], more than 30 have been positively identified for Se through radiotracing [10], and at least 15 have been deemed essential in what concerns their biological function and physiological significance for major metabolic pathways [11–16].

Such an importance for human health has since been recognized by global organizations (FAO, WHO, IAEA, EU Scientific Committee on Food), leading to a paradigm that has suggested minimum Se dietary intakes of 40 and 30 μg/day for meeting the average requirements of male and female adults, respectively [17]. The current Recommended Dietary Allowance (RDA) for adult men and women regardless of age, by the Food and Nutrition Board of the Institute of Medicine (Washington DC,

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USA)—endorsed by Canadian regulators as well—is 55 μg/day [18]. Of course, between the minimum dietary intake for preventing severe conditions relating to Se deficiency, such as the Keshan disease (17 μg/day [19]), and a few higher normatives in countries like Australia, New Zealand or the United Kingdom (up to 75 μg/day for male adults [20, 21]) there is still a wide gap of debate on how to achieve optimal plasma or serum Se concentrations for, say, cancer prevention [22].

The Portuguese situation is difficult to assess due to scarce-to-null information [23] and lack of consistent research on the subject, save for two limited, cohort studies on potential Se intake based on actual diets [24, 25]. Still, it should not be that different from much of Europe, where falls in Se intake—and corresponding drops in human-blood indicators of Se status—have raised a widespread concern [5]. Cereals are the backbone of human diets worldwide [26, 27], and Portugal is no exception to such pattern, given that cereals and their derivatives (breads, breakfast blends, pastas, etc.) make up a sizeable share of the Portuguese food intake. As a major dietary source of Se [28], wheat seems an obvious candidate for biofortification strategies that may help enhance the Se status of an entire population [29–33].

In these terms, and within the framework of PTDC/QUI/65618/2006 (research contract by Fundação para a Ciência e a Tecnologia—FCT; Portugal), an Se-supplementation program of representative wheat varieties is being carried out in actual field conditions at the Elvas area (Alentejo; near the Spanish border). The present paper deals with the ability of bread wheat (*Triticum aestivum* L.) to accumulate selenium after being supplemented through foliar addition of selenate and selenite solutions in two different growth stages: booting, that is from the onset of flag leaf sheath extending until the first awns are visible (Feekes scale: 10.0–10.1; Zadoks scale: 40–49), and grain filling, that is from post-anthesis to physiological maturity or, on practical grounds, from medium milk to hard dough (Feekes scale: 11.1–11.4; Zadoks scale: 75–92) [34–36]. The results of these Se-supplemented samples are also compared with similar, non-supplemented crops from the 2010 harvest campaign at the same area.

Experimental

Triticum aestivum L. (Jordão cultivar), one of the most representative varieties of bread wheat in the country, was selected for selenium supplementation through foliar application. That cultivar was sown at Herdade da Comenda, Caia (Elvas), Portugal, in the end of November 2009, and 3 × 12 field plots (about 1.5 × 0.5 m each) were prepared to apply 12 different combinations of selenium supplements, in a 3-fold replication. Those combinations were done using (i) two different selenium compounds: sodium selenate and sodium selenite; (ii) supplementation in two different growth stages: booting and grain filling; and (iii) three selenium (area) concentrations: 4, 20 and 100 g ha^{-1} (equivalent to 0.2, 1 and 5 mg L^{-1}, respectively). Foliar application was carried out at the beginning of April 2010 (booting stage), and in June 2010 (grain-filling stage). For each application, 0.5 L of selenium solution was added to the wheat plants with sprayers of 1 L.

Jordão wheat was sampled during the harvest season, in July 2010. The wheat plants were cut with the help of pruning shears, all the spikes in each plot were collected. The grains were separated from the spikes using a combine machine HEGE, available at the INRB/INIA-Elvas. Whole grains were cleaned, weighed and stored in a dry room. All grain samples were ground to a fine powder in a Waring Blender HGB50E2, heat-sealed in polyethylene vials (1.2 mL), and then placed in medium-sized irradiation vials (7 mL). The analyses were performed upon three replicates per sample, around 800 mg each.

All samples were irradiated on the fast pneumatic system (SIPRA) at the Portuguese Research Reactor (RPI-ITN; Sacavém), at a thermal-neutron flux density of 1.7×10^{12} n cm^{-2} s^{-1}. Gamma spectra were acquired with a liquid-N_2 cooled, high-purity Ge, coaxial detector (1.85 keV resolution at 1.33 MeV; relative efficiency: 25%), and an advanced digital gamma-ray spectrometer DSPEC Pro from ORTEC®, to correct for the dead-time losses. The samples were put through cyclic neutron activation analysis (CNAA). The number of cycles was 10; in each cycle, irradiation and counting times were 20 s, and the decay time 5 s. Elemental concentrations were assessed through the relative method, using NIST-SRM 1567a (wheat flour) and NIST-SRM 1568a (rice flour).

Results and discussion

The quality control of the method resulted in 1.3 ± 0.2 mg kg^{-1} of the selenium, which is in agreement with the NIST-SRM 1567a certified value of 1.1 ± 0.2 mg kg^{-1}, both at a 95% confidence level.

Selenium concentration in soil of the experimental fields was 118 ± 6 μg kg^{-1} [37], and selenium content in samples without supplementation was 59 ± 10 μg kg^{-1}. Wheat plants supplemented in the booting stage with selenium solutions equivalent to 4, 20 and 100 g ha^{-1} showed concentrations of Se in grains of 75 μg kg^{-1} (selenite) and 150 μg kg^{-1} (selenate), 120 μg kg^{-1} (selenite) and 570 μg kg^{-1} (selenate), and 450 μg kg^{-1} (selenite) and 2100 μg kg^{-1} (selenate), respectively. Wheat plants supplemented in

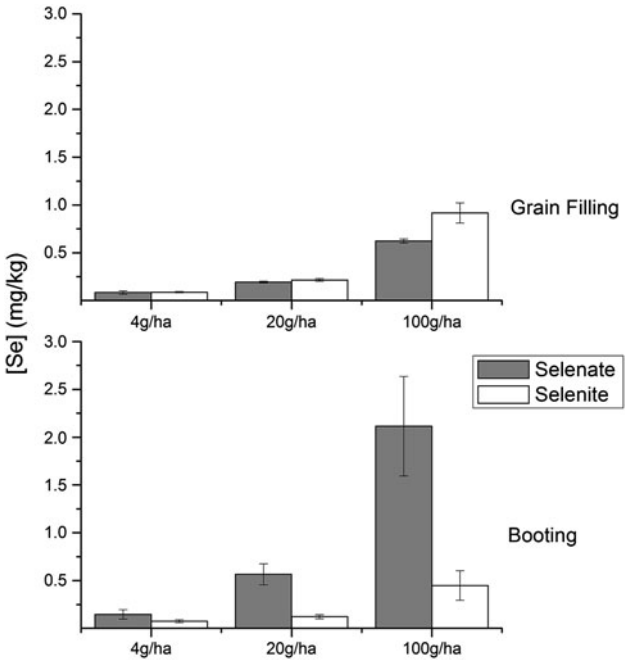

Fig. 1 Selenium concentration (fresh weigh) in wheat grains supplemented with different concentrations of selenium, as selenate and selenite forms, at two different growth stages: booting and grain filling

the grain-filling stage with selenium solutions equivalent to 4, 20 and 100 g ha^{-1} showed concentrations of Se in grains of 85 μg kg^{-1} (selenate) and 90 μg kg^{-1} (selenite), 200 μg kg^{-1} (selenate) and 220 μg kg^{-1} (selenite), and 620 μg kg^{-1} (selenate) and 920 μg kg^{-1} (selenite), respectively. An overview of the differences between acting upon the two growth stages is given in Fig. 1.

Wheat plants supplemented in the booting stage with selenium in the form of selenate produced grains with higher concentration of selenium than those with selenium in the form of selenite. This may be explained because selenate is more soluble and mobile than selenite in soil, and, therefore, more bioavailable to plants [33]. Selenium supplementation at the grain-filling stage was shown to be independent from chemical form of selenium. Accumulation of selenium in wheat grains increases with the concentration of supplemented selenium 100 g ha^{-1} > 20 g ha^{-1} > 4 g ha^{-1}, for both booting and grain-filling stages (Fig. 2). Booting shows much more effectiveness in accumulating supplemented selenium than grain filling. Average selenium values (dry weight) for European countries range from 21 to 200 μg kg^{-1}; for U.S., the mean value is 490 μg kg^{-1}, and for Egypt is 340 μg kg^{-1} [38]. Kabata-Pendias and Pendias [38] have also reported that wheat grains from Australia had 23 μg kg^{-1} of selenium, which are values of the same order of magnitude of ours without supplementation (59 ± 10 μg kg^{-1}, within the low tail of the European range).

We may also compare our selenium values with supplementation with the ones reported by Lyons et al. [39, 40], for Australia. According to the former authors [39], after foliar application of sodium selenate at the flowering (anthesis) stage of *Triticum aestivum* L., in two different soils, the addition of 4, 20 and 100 g ha^{-1} resulted respectively in 100 and 700 μg kg^{-1}, 200 and 1000 μg kg^{-1}, and 600 and 1800 μg kg^{-1} in grains. Using selenate, the maximum values we got in booting stage were 150 ± 50, 600 ± 100 and 2100 ± 500 μg kg^{-1} for an addition of 4, 20 and 100 g ha^{-1}, respectively, which are in the same order of magnitude of the previously reported [39].

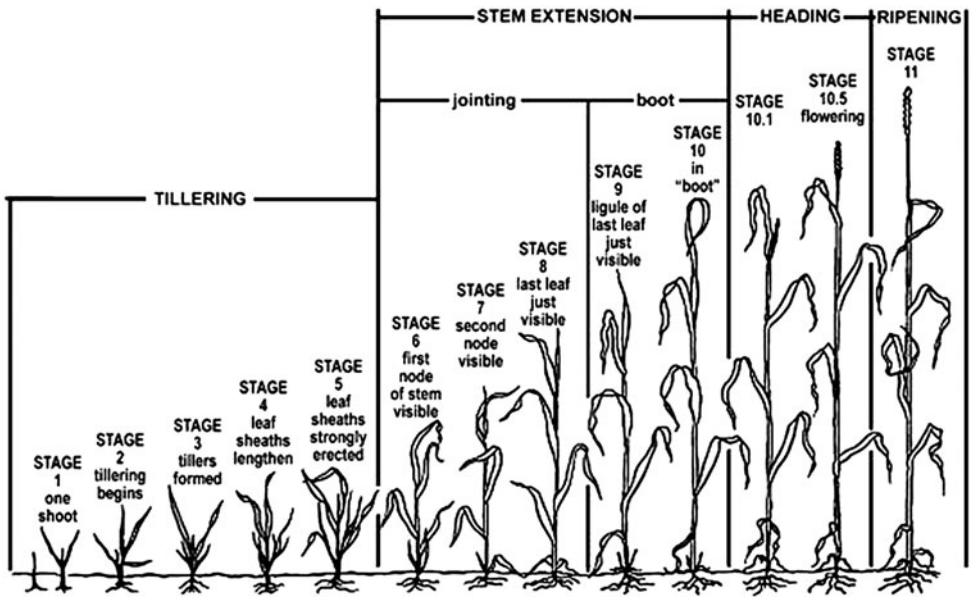

Fig. 2 Growth stages of wheat: stages 10 and 11 correspond to booting and grain filling, respectively [42]

The addition of 4, 20 and 100 g ha^{-1} in the booting stage increased the selenium contents in grains by factors of 2.5, 10 and 35, respectively.

Considering that, as an average, each Portuguese ingests around 60 g of wheat bread per day [41], the success of this supplementation case-study demonstrates that, acting upon foliar supplementation of the wheat plants, the selenium daily needs can be effectively met. Using non-supplemented wheat, the contribution of bread to the recommended dietary allowance (RDA) is 6%; using the biofortified ones, we get a contribution of 16, 60 and 230%, for a supplementation rate of 4, 20 and 100 g ha^{-1}, respectively. The highest rate (100 g ha^{-1}) might not be adequate though, because it exceeds the RDA more than two times.

Sodium concentration in samples supplemented at the booting stage was on average 5.2 ± 0.5 mg kg^{-1}. Sodium concentration in samples supplemented at the grain-filling stage was on average 5.2 ± 0.8 mg kg^{-1}. Sodium content of samples without supplementation was 7 ± 1 mg kg^{-1}. These data show that there is no increase of sodium after supplementation of plants with sodium selenate or sodium selenite.

Conclusions

Accumulation of selenium in wheat grains increases with the concentration of supplemented selenium for both booting and grain-filling stages. The booting stage shows much more effectiveness in accumulating selenium than the grain-filling stage. It is suggested that the foliar application of a selenate solution equivalent to a field-supplementation rate of 20 g of Se per ha, at the booting stage, might be enough to increase the selenium intake of Portuguese people up to a level close to the RDA for this element. Cyclic neutron activation analysis proved to be an adequate and fast methodology to determine selenium concentrations in all wheat samples.

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