ARTICLE IN PRESS

Field Crops Research xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Field Crops Research

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

Difference of selenium uptake and distribution in the plant and selenium form in the grains of rice with foliar spray of selenite or selenate at different stages

Xiaofang Deng[a,b], Kezhi Liu[c], Mingfeng Li[a,b], Wei Zhang[a,b], Xiaohu Zhao[a,b], Zhuqing Zhao[a,b], Xinwei Liu[a,b,*]

[a] *Microelement Research Center, Huazhong Agricultural University, Wuhan 430070, China*
[b] *Hubei Provincial Engineering Laboratory for New-Type Fertilizer, Wuhan 430070, China*
[c] *Soil and Fertilizer Workstation of Qianjiang City, Qianjiang 433199, Hubei, China*

ARTICLE INFO

Keywords:
Selenium species
Growth stage
Selenium distribution ratio
Organic selenium
Protein selenium

ABSTRACT

Foliar spray of selenium (Se) has increasingly been applied to improve Se concentrations in crops. There is a lack of systematic and in-depth study comparing the effects of foliar spray with different Se sources at different growth stages. In this study, selenite or selenate (75 g Se ha^{-1}) was sprayed to the foliage of rice plants at the late tillering or full heading stage. Se concentrations in different parts of rice plants were determined to assess the effects of foliar spray with different Se sources at different growth stages on Se distribution in the plant, Se metabolism in the grains, and Se recovery efficiency in the brown rice. At the same spraying stages, total Se, organic Se, and protein Se concentrations in brown rice obtained with selenate were ~2-fold those obtained with selenite. With the same Se sources, total Se, organic Se, and protein Se concentrations in brown rice treated at full heading were 2-fold higher than those treated at late tillering. The delay of spraying stage decreased the distribution ratio of Se in straw and roots, while it increased the distribution ratio of Se in grain; this trend was more evident with selenate. The proportions of organic Se and protein Se in brown rice showed no differences between selenite and selenate treatments at the same stages. The delay of spraying stage decreased the proportions of organic Se and protein Se in brown rice (still up to 80% and 44%, respectively), whereas the recovery efficiency of Se was improved in brown rice by more than 1-fold. In conclusion, appropriately delaying the spraying stage and selecting selenate as the Se source can be more efficient for producing Se-enriched rice.

1. Introduction

Selenium (Se) is an essential micronutrient in human body and a non-essential element for plants. Although the human demand for Se is not high, the abundance of Se is very low in the Earth's crust. Thus, Se deficiency is still recognized as a global health problem that needs to be addressed (Valdiglesias et al., 2010). Increasing the production of Se-enriched dietary sources is necessary, because Se supplementation from natural food sources is considered safer than directly ingesting inorganic Se (Liu et al., 2012). Rice is a staple food in at least 33 countries and it provides ~80% of the daily caloric intake to 3 billion people (Boldrin et al., 2013). The average Se concentration of rice is only 95 μg kg^{-1} in the major rice production areas of the world (Williams et al., 2009). Therefore, increasing Se concentrations in rice by application of Se fertilizer has great implications for improving Se nutrition in human beings (Giacosa et al., 2014; Hu et al., 2014).

Selenite and selenate are the two major types of exogenous Se fertilizer. When applied into the soil, selenite is less bioavailable due to adsorption onto ferric soil minerals or accumulation in plant tissue that is not part of human diet (Carey et al., 2012a; Keskinen et al., 2011; Li et al., 2015b). More than 80% of the Se applied as selenite exists in the forms of Fe-Mn oxide-bound Se, organic bound Se, and residual Se within a month, which are difficult for uptake and utilization by crops in the current season (Liu et al., 2015). Soil application of selenate is potentially a wasteful method of Se biofortification, as 80–95% of the Se added as selenate may be leached out by irrigation or rainfall (Keskinen et al., 2011). In addition, the antagonistic effect between sulfur and Se in soil can significantly reduce Se concentrations in crops (Liu et al., 2015; Liu et al., 2017). In particular, rice grows in flooded and anaerobic conditions for a long term, which could accelerate selenite fixation and selenate leaching. Therefore, soil application of Se fertilizer is considered disadvantageous for increasing Se

* Corresponding author at: College of Resources and Environment, Huazhong Agricultural University, NO.1 Shizishan Ave., Hongshan District, Wuhan, 430070, Hubei, China.
E-mail address: lxw2016@mail.hzau.edu.cn (X. Liu).

http://dx.doi.org/10.1016/j.fcr.2017.06.008
Received 7 April 2017; Received in revised form 12 June 2017; Accepted 12 June 2017
0378-4290/

Please cite this article as: Deng, X., Field Crops Research (2017), http://dx.doi.org/10.1016/j.fcr.2017.06.008

concentrations in crops.

Numerous studies have shown that foliar spray of Se significantly improves Se concentrations in crops such as rice, wheat, maize, lentil, and table grape (Boldrin et al., 2013; Nawaz et al., 2015a; Rahman et al., 2015; Wang et al., 2013a; Zhu et al., 2017). However, the choice of the Se form supplied to plants strongly influences the amount of bioavailable Se in human and animal foodstuffs (Longchamp et al., 2015). For instance, there exists a huge difference in the uptake of selenite and selenate by crop roots. Selenate can migrate into the roots and immediately translocate to the shoots via high-affinity sulfate transporters, whereas selenite is mostly assimilated into organic Se in the roots and unlikely to translocate to the shoots (Sors et al., 2005). What are the differences in the translocation and distribution of Se in various parts of crops with foliar spray compared to soil application of Se? Do these differences vary with the spraying stage or not, and what are the differences in the results of foliar spray with different Se forms, such as selenite or selenate? A systematic report regarding these questions is still lacking.

The bioavailability of Se for humans and animals largely depends on the forms of Se in the edible parts rather than the total Se concentration in the plant (Premarathna et al., 2012), because the anti-cancer effects of selenium depend on its speciation (Carey et al., 2012b). Compared with inorganic Se, organic Se is more bioavailable for human health (Longchamp et al., 2015). Organic Se generally constitutes more than 80% of the total Se in crop grains (Eiche et al., 2015). Protein Se is the major form of organic Se in crops. It has been reported that organic Se accounts for more than 90% of the total Se in wheat grains, while protein Se accounts for more than 70% of the total Se (Cubadda et al., 2010). In potato tubers, 49–65% of the Se is present in protein components (Turakainen et al., 2006). The above results of Se analysis are mainly obtained from crops grown in Se-rich soils. However, the process of Se metabolism and assimilation by the roots differs from that by the leaves. Additionally, after Se uptake by the roots, there is sufficient time to assimilate inorganic Se into organic Se within the growth period of crops (Premarathna et al., 2012). Then, are there any differences in the assimilative capacity of plants for Se with foliar spray compared to soil application of Se? What are the differences in the assimilative capacity of plants for Se after foliar spray with different Se sources at different stages? Addressing these two questions is of great importance for safe production of Se-enriched rice.

Assuming foliar spray of Se is an effective approach to increase Se concentrations in crops and mitigate the environmental risks, then, elucidating the effect of foliar spray with different Se sources at different stages on the recovery efficiency of Se in rice is critical for commercial operations of Se-enriched rice in implementing efficient production. To this end, we sprayed selenite or selenate to the foliage of rice plants at the late tillering or full heading stage in the actual field conditions. The objectives were to determine the effects of time of foliar application and form of Se on 1) Se accumulation and distribution in rice plants; 2) organic and protein Se concentrations in brown rice; and 3) Se recovery efficiencies in the whole plant and brown rice.

2. Materials and methods

2.1. Experimental site

A replicated field experiment was conducted for two seasons (2015 and 2016) in the Shekou village of Qianjiang, Hubei Province, China (30°33′17″ N, 112°53′23″ E). The annual precipitation was ~1113 mm. The soil was fluvo-aquic soil with the following properties: pH, 8.19 (soil/water ratio = 1:2.5); organic matter, 28.39 g kg^{-1}; available nitrogen, 91.07 mg kg^{-1}; Olsen phosphorous, 6.14 mg kg^{-1}; available potassium, 83.00 mg kg^{-1}; and total Se, 0.35 mg kg^{-1}. The seeds of rice (*Oryza sativa* L.) variety 'Liangyoulongzhan' were purchased from Hubei Provincial Seed Group Co., Ltd. (Wuhan, Hubei, China)

2.2. Experimental design

Foliar spray of Se was applied at 75 g ha^{-1} as sodium selenite (Na_2SeO_3) or sodium selenate (Na_2SeO_4). The two Se sources were prepared into 75 mg L^{-1} Se solutions and applied to the foliage of rice plants at the late tillering (LT) or full heading (FH) stage. The treatments were fully orthogonal. The Se solution was substituted with distilled water as the control (CK). A total of five treatments were included in each experiment. In every year, the experiment was performed as a randomized complete block design with three replicates. The plot area was 20 m^2 each, with 15 plots in total. Other fertilizers included nitrogen (N, 225 kg ha^{-1}), phosphorus pentoxide (P_2O_5, 90 kg ha^{-1}), potassium oxide (K_2O, 120 kg ha^{-1}), and Zinc (Zn, 1.5 kg ha^{-1}), which were applied in the form of $CO(NH_2)_2$, KH_2PO_4, KCl, and $ZnSO_4 \cdot 7H_2O$, respectively. Specifically, 60% of N was applied as basal fertilizer and the remaining 40% as tillering fertilizer; P_2O_5 and Zn were applied as basal fertilizers; 80% of K_2O was applied as basal fertilizer and the remaining 20% as tillering fertilizer. Unified management was implemented during the growth period of rice. The transplanting dates were June 6, 2015 and June 4, 2016; the harvest dates were September 10, 2015 and September 13, 2016.

2.3. Sample preparation

Eight whole plants per plot were randomly sampled at harvest. The plants were divided into roots, straw, spike axis, and grains, then each part was carefully washed with tap water to remove attached soil and other contaminants, followed by rinsing at least three times in deionized water. The grain was further divided into husk and brown rice using a motorized dehusker (JLGJ4.5, TZYQ, Zhejiang, China). Each part of rice plants was oven-dried at 65 °C for 72 h to constant weight, then the dried samples were ground into powder to pass through a sieve of 0.15 mm and sealed in ziplock bags before Se analysis. After whole-plot harvesting and threshing, the grains were air-dried and weighed to calculate the empirical yield.

2.4. Determination of soil properties

Soil organic matter content was determined using the wet dichromate digestion method (Walkley and Black, 1934). Soil pH and the concentrations of available soil nutrients were measured according to the Soil Physicochemical Analysis Handbook (Bao, 2000). Briefly, soil pH was determined potentiometrically in 1:2.5 soil/distilled water suspensions after shaking. Soil available nitrogen was determine using the alkaline hydrolysis diffusion method. Soil available phosphorous was determined using Olsen's method. Soil available potasssium was extracted with 1 mol L^{-1} NH_4OAC and determined by flame photometry (FP6410, INESA, China).

2.5. Determination of Se concentrations

The total Se concentration of various rice parts were determined by adding HNO_3–$HClO_4$ (4:1) for digestion, during which the temperature was maintained at approximately 180 °C.Then, the digestion solution was restored to volume with 6 mol L^{-1} HCl, cooled and filtered at a set volume. The Se concentration in the filtrate was measured by hydride generation-atomic fluorescence spectrometry (HG-AFS-8220, Titan Instruments Co., Beijing, China).

For determining inorganic Se and organic Se concentrations, brown rice flour (1.00xx g) was weighed accurately into a glass Erlenmeyer flask, followed by addition of 30 mL of ultra-pure water. After 30 min of sonication, the extract was centrifuged at 5000 r min^{-1} for 10 min. Liquid supernatant was collected and transferred into a glass separating funnel. A total of 5 mL of cyclohexane was added to the liquid supernatant, and the water phase collected into a beaker 4 h later. This solution, which contained inorganic Se, was heated for 2–3 min after