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Selenate-enriched urea granules are a highly effective fertilizer for selenium biofortification of paddy rice grain

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ABSTRACT

We examined the effects of applied selenium (Se) species, time of application, method of application and soil water management regime on accumulation of Se in rice plants. Plants were grown to maturity in a temperature- and humidity-controlled growth chamber using three water management methods: field capacity (FC), submerged until harvest, and submerged and drained two weeks before harvest; two Se species: selenate (SeO_4^{-2}) and selenite (SeO_3^{-2}) applied at a rate equivalent to 30 g ha^{-1}; and four application methods: i) Se applied at soil preparation, ii) Se-enriched urea granules applied to floodwater at heading iii) foliar Se applied at heading and iv) fluid fertilizer Se applied to soil or floodwater at heading. Total Se concentrations in rice grains, husks, leaves, culms and roots were measured, as well as Se speciation in grains from the Se-enriched urea granule treatment. Highest Se concentrations in the grain occurred with SeO_4^{-2} and with fertilizer applied at heading stage; SeO_4^{-2}-enriched urea granules applied at heading increased grain Se concentrations 5 to 6 fold (by 450-600 μg kg^{-1}) compared to the control (no fertilizer Se applied) in all water treatments. Under paddy conditions other Se fertilization strategies were much less effective. Drainage before harvesting caused Se to accumulate in/on rice roots, possibly through adsorption onto iron plaque on roots. Rice grains contained Se mainly in the organic form as selenomethionine (SeM) which comprised over 90 % of the total grain Se in treatments fertilized with SeO_4^{-2}-enriched urea granules. The results of this study clearly show of the fertilizer strategies tested that biofortification of Se in rice grains can best be achieved in

lowland rice by broadcast application of SeO_4^{-2}-enriched urea granules to floodwater at heading stage.

Keywords: *selenium, selenite, selenate, biofortification, fertilizer, rice, Se enriched urea*

INTRODUCTION

Selenium is an essential micronutrient for humans and animals (*1, 2*). Toxicity and deficiency of Se in humans and animals is separated by a very narrow margin compared to other nutrients (*3*). Low dietary intake of Se causes health problems including low immunity, oxidative stress related conditions, reduced fertility and cancer (*1*). Different strategies have been tested or implemented worldwide in order to achieve optimum Se concentrations in humans *(3)*, the most common strategies include the consumption of high-Se foods (e.g., Brazil nuts), individual supplementation, Se supplementation to livestock (*4*), and biofortification of food crops (*4*-*6*).

Biofortification is the increase in the bioavailable concentration of elements in edible portions of crop plants through either fertilization (agronomic biofortification) or crop selection and breeding (genetic biofortification) (*4*-*6*). Agronomic biofortification is an easy and cheap method to increase Se concentrations in edible portions of crops (*7*). Selenium fertilizer programs have successfully been implemented in Finland for forage and cereal crops and increased Se concentrations in animals and human population have been achieved (*5*). In Finland the Se intake per capita has increased from 25 µg d^{-1} to 124 µg d^{-1} (*5*). Increasing the Se concentration in foods such as wheat and rice is an appropriate target to increase human Se intake because they are the staples for most of the world’s population. Selenium fertilizer programs have been developed for wheat but no effective Se program has yet been developed for rice.

Rice can be grown in upland or lowland conditions, and different fertilization strategies may be needed for each scenario, both in terms of Se species used and method of fertilizer application (foliar or applied to soil). The availability of soil applications of Se are often much greater under upland conditions compared to flooded soils (*8, 9*), but the majority of rice crops around the world are lowland cultivated (*10*). In terms of the most effective Se species, for foliar applications SeO_4^{-2} has been found to be more effective than SeO_3^{-2} for Se biofortification of rice *(6)*. There are no data to evaluate the effectiveness of soil-applied SeO_3^{-2} or SeO_4^{-2} for biofortification of rice, but these species appear to be equally available to wheat plants under aerobic conditions in solution culture *(1)*. However, in aerobic soils addition of SeO_4^{-2} often leads to greater accumulation of Se in plants than SeO_3^{-2} (*11, 12*), likely due to greater retention of SeO_3^{-2} by soils (*11, 13, 14*). Developing a Se fertilization program for lowland rice is challenging because soil-applied Se availability depends on redox and pH conditions in submerged soils. Availability to rice of soil-applied SeO_4^{-2} fertilizer in flooded soils has been found to be low (*9, 15*), either due to reduction to SeO_3^{-2}, which is more strongly retained by soils than SeO_4^{-2} (*13, 16*), or to reduction of Se oxyanions to elemental Se(0) in anoxic soils (*17*). Hence, foliar Se fertilization of crops is often preferred (*18*) and has been tested on rice (*19, 20*). However, the disadvantages of foliar fertilization are the additional labour involved in separately applying Se from other broadcast granule applied nutrient, as well as the lack of any residual effect for subsequent crops. An effective alternative fertilization strategy for Se where macronutrients (nitrogen, phosphorus or potassium) are applied simultaneously with Se would therefore be advantageous.

Previously we showed that the potential availability of soil-applied SeO_4^{-2}, SeO_3^{-2} and Se (0) differed markedly in both aerobic and anaerobic soils *(21)*. Application of Se fertilizers in any form during soil preparation was ineffective because Se availability (as determined by isotopic dilution) decreased rapidly after soil flooding (*22*). Pre-harvest oxidation of paddy soil is likely to release little available Se due to the slow oxidation of both Se(0) to SeO_3^{-2}, and also slow oxidation of SeO_3^{-2} to SeO_4^{-2} (*23*). Hence a more effective Se fertilization strategy is required.

Bioavailability of Se for humans and animals largely depends on the species of Se consumed rather than the total Se concentration. Organic Se species in the diet are more bioavailable than inorganic Se species (*24, 25*). Organic Se compounds such as methylselenocysteine (MeSeCys), selenomethionine (SeM) and γ-glutamyl-Se-methylselenocysteine (γ-glutamyl-MeSeCys) are effective chemo-protective agents which may prevent the development of breast, liver and prostate cancers (*24, 26*) but there is a paucity of studies on the organic and inorganic Se species present in rice grains following application of different Se fertilizers to plants or soils.

Here we report experiments to elucidate the effect of Se species, time of application, soil water regime and four Se application methods on the yield of rice and the accumulation of Se in grains, culms, leaves and roots under both upland and paddy growth conditions. The speciation of Se in grains from the SeO_4^{-2}-enriched urea granule treatment was further undertaken to examine the effect of increased Se accumulation on Se species present in grains.

MATERIALS AND METHODS

Standards and reagents

- All reagents and standards used were of trace metal grade and ultrapure deionised water (Milli-Q, Millipore) was used for all chemical preparations and dilutions. Sodium salts of sodium selenite and sodium selenate, SeM, and citric acid were purchased from Sigma (Australia). Individual stock solution of SeO_3^{-2} at 1000 mg Se L^{-1} was purchased from SPEX-Certiprep, USA. Selenomethionine selenoxide (SeOM) was prepared through the addition of excess hydrogen peroxide (0.1 mL of 30% H_2O_2) to 2 mL of SeM (100 mg of Se L^{-1}) (*27*).

Pot experiment 1

Plants were grown in 3.5 L black plastic pots lined with plastic bags and filled with 2 kg of the Hanwood loam (a Rodoxeralf (*37*)) collected near Griffith, a rice growing area of Australia. The soil had a pH 6.36 (1:5 soil: water suspension) (*28*), EC 141.2 µS cm^{-1} (*28*), total carbon 2% (*29*), cation exchange capacity (CEC) 21 cmol(+) kg^{-1} and a total Se concentration of 0.117 mg kg^{-1}. All pots received the equivalent of 150 kg N ha^{-1} (half applied at soil preparation and half at the heading stage), 25 kg P ha^{-1} and 100 kg K ha^{-1}, together with a micronutrient mix of 0.3 $ZnSO_4$, 0.3 $CuSO_4$, 0.1 H_3BO_3, 20 $CaSO_4$, 20 $MgSO_4$ and 0.01 $(NH_4)_6Mo_7O_24H_2O$ kg ha^{-1} (*30*) applied during soil preparation. Soils were either submerged or maintained at FC for 14 d before transplanting two eighteen day old healthy rice seedlings (*Oryza sativa* sp. Amaroo). Two weeks before harvest, submerged treatments were drained and moisture content was thereafter maintained at FC. Plants were grown until final harvest at physiological maturity.

Treatments and experiment design

The experiment was conducted in a light-, temperature-, and humidity-controlled growth chamber. Maximum and minimum temperatures inside the growth chamber were 30^0 C and 25^0 C and 12 h daylight cycle with high humidity (53 %). There were 81 pots in a randomized complete block design with a factorial arrangement of the treatments; two Se species; i.e. SeO_3^{-2} and SeO_4^{-2}, three water treatments; i.e. field capacity, submerged and submerged then drained; and four application methods (see below).

Application of selenium fertilizer

Rate of Se application was equivalent to 30 g ha^{-1} based on application rates used in previous studies (*31*) and assumptions regarding loss of Se by sorption to soil. Selenite and SeO_4^{-2} fertilizers were added to soil either at soil preparation or heading stage. Selenium applied as either SeO_4^{-2} or SeO_3^{-2} at soil preparation was sprayed as a dilute solution of sodium selenite or sodium selenate diluted in ultrapure deionised water (Milli-Q, Millipore) onto the soil and mixed thoroughly. At the heading stage either SeO_4^{-2} or SeO_3^{-2} was applied by 3 methods; SeO_4^{-2}, SeO_3^{-2}-enriched urea granules or fluid Se fertilizer was applied to the soil surface. The Se-enriched urea was prepared by spraying Se (sodium selenite or sodium selenate in high purity deionized water solution) onto the urea granules and letting the granules to dry at 30 ºC in an oven. The fluid Se fertilizer was a solution of either sodium selenite or sodium selenate in high purity deionised water. Foliar SeO_4^{-2} or SeO_3^{-2} fertilizer was of either sodium selenite or sodium selenate in high purity deionised water and was sprayed carefully onto leaves using aerosol sprayers at heading stage. Pots receiving the foliar fertilizer at heading were separated from other treatments to avoid contamination during spraying.

Rice sample preparation for analysis

At maturity, plants were harvested and shoots, roots and grains were separated. Roots were cleaned using reverse osmosis (RO) water, 1% sodium lauryl sulphate ($CH_3(CH_2)_{10}CH_2OSO_3Na$) (Sigma) and finally in high purity deionised water. Plant samples (grain, leaf, culm and root) were dried at 55°C to a constant weight and dry weight recorded (grain). Husks were removed from harvested grains using a laboratory-scale hand operated de-hulling machine. The plant tissues were ground using a laboratory seed grinder and sieved to < 500 µm.

Total Se analysis

The grain samples were digested using a closed vessel microwave procedure (Ethos E touch control, Milestone, North America) using a two-stage time program: 5 min at 300 W and 40 min at 500 W. Approximately 0.5 g of finely ground grain samples were weighed into a Teflon digestion vessels and 10 mL of concentrate HNO_3 acid (Aristar) added. After microwave digestion, the vessels were allowed to cool for 30 minutes at room temperature and then diluted to 50 mL with ultrapure deionised water (Milli-Q, Millipore). Digest solutions were filter to < 0.22 µm (Sartorius) and analysed for total Se concentrations by inductively coupled plasma-mass spectrometry (ICP-MS) (Agilent 7500ce ICP-MS with H_2 gas added to the collision cell at a flow rate of 4 mL min^{-1}). The accuracy of the digestion and ICP-MS analysis procedure were assessed through the analysis of certified reference materials NIST 1568a rice flour and NIST 1573a tomato leaves. The total Se concentrations determined in the rice and tomato certified reference material were in close agreement with the certified value (NIST 1568a - this study 0.38 ± 0.04 mg Se kg^{-1} (n = 3), certified value = 0.38 ± 0.04 mg Se kg^{-1} NIST 1573a - this study 0.054 ± 0.003 mg Se kg^{-1} (n = 3); certified value = 0.054 ± 0.006).

Enzymatic extraction of selenium species for chromatographic speciation

Approximately 0.2 g of ground grain tissue from the SeO_4^{-2}-enriched urea granule treatments were weighed into 15 mL Pyrex culture tubes with 20 mg protease XIV (Sigma) and 6 mL of ultrapure deionised water (Milli-Q, Millipore). The samples were shaken end over end at 37^0 C in an incubator for 24 hrs, centrifuged at 1200 g and filtered through a 0.22 µm filter. The resulting solutions were analyzed for Se species (OSeM, SeO_4^{-2}, SeM and SeO_3^{-2}) by high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) (*32*). The operating conditions for HPLC-ICP-MS are summarized in Table 1. The identification of Se species occurred through retention time comparisons with synthetic standards and concentrations were determined using peak areas..

Pot experiment 2

A second experiment was performed to examine uptake of Se from SeO_4^{-2}-enriched urea granules and pure seleno-urea (CH_4N_2Se). Environmental and soil conditions were identical to those outlined above for pot experiment 1, and treatments consisted of either SeO_4^{-2}-enriched urea granules, SeO_4^{-2}-enriched urea-ammonium nitrate (UAN) or pure seleno-urea. At rice heading stage, Se fertilizers were applied onto floodwater in a similar manner to the first experiment.

Floodwater samples were collected from 1-2 cm above the soil water interface at 1 and 10 d after Se fertilizer application. At each sampling time, 10 mL of floodwater was collected between two rice plants and filtered into centrifuge tubes using 0.22 µm filters (Millipore millex-GS). The floodwater solutions were acidified with 50 µL of 6 M HCl and analysed for total Se concentration using ICP-MS.

Plant sap sample collection was undertaken following the method used by Li et al. (*33*). Shoots were cut from 3 cm above the water level. The cut surface was washed using ultrapure deionised water and blot dried with clean tissues before sap samples were collected for two hours. Total Se concentrations in sap samples were determined using ICP-MS.

Statistical analysis

The significance of fertilizer applied Se species, application time, application method, and soil water management on grain dry yield (grain) and Se concentrations in rice tissues (grain, leaves, culm, and roots) were determined using analysis of variance (ANOVA) in Genstat software (Genstat 10th ed, VSN International, Hempstead, UK). Least significant differences (LSD) were used for comparison of the treatment means.

RESULTS AND DISCUSSION

Treatment effects on grain yield

Rice plants growing in aerobic soil (FC) had lower grain dry weights than the other water treatments ($p \leq 0.05$) with little or no effect of Se species or application method (Supplementary information, Table 1). The reason for higher DW in grains from submerged and submerged-drained treatments could be the availability of some nutrients in FC soils being lower than submerged soils (Supplementary information, Table 2). We did not measure nutrients in flood water for this study, however as other studies have reported elsewhere, stabilization of pH around neutrality in submerged rice soils has some implications for the availability of nutrients (*34*). In addition, the ability of the rice plant to grow under aerobic conditions is often less (*22*). In particular, soil solution concentrations of P,and K, normally increase with submergence (*10*). These are essential nutrients for root development and tillering (*10*) which ultimately determine final yield. Method of Se application had little consistent effect on grain yields, except in FC treatments where the control was significantly lower than Se fertilizer treatments.

Treatment effects on Se accumulation in rice plants

Grain and husk selenium accumulation

Concentrations of Se in rice grains and husks under different treatments are shown in Table 2. The largest part of the variance in Se concentrations was explained by application method and Se species, and their interaction (Table 3). Overall, SeO_4^{-2} treatments led to highest Se concentrations in grains while SeO_3^{-2} treatments led to the lowest grain concentrations. However, the 3-way interaction of Se application method × applied Se species × water treatment was significant for both grain and husk Se concentrations ($p \leq 0.001$). Selenium applied at the heading stage led to higher grain Se than Se applied at soil preparation. Perhaps, at heading stage, plants are more physiologically active and mobilizing nutrients to fill the grains with photosynthetic products faster. Also, by heading stage, plant roots were well developed and well distributed ready for nutrient uptake. Furthermore, Se applied at soil preparation had two weeks without plants and, by the time plants were introduced into the pots, Se added as SeO_3^{-2} would have been sorbed onto/into soil colloids/minerals and also SeO_4^{-2} may have been reduced (*22*). By the time the root system of transplanted rice was ready for nutrient uptake, most of the added Se may have been converted to unavailable forms such as selenide (Se^{-2}) or elemental selenium (Se (0)).

The highest grain Se concentration was recorded for SeO_4^{-2}-enriched urea granules applied at heading stage for all water treatments. In FC soils, fluid SeO_4^{-2} applied at heading also had a statistically similar effect on accumulating Se in grains. It was expected to find higher grain Se concentrations in the fluid SeO_4^{-2} treatment applied at heading in FC treatments because SeO_4^{-2} has been shown to be highly available in aerobic soils (*35*-*38*). In addition, studies on upland crops such as wheat and barley have also recorded higher grain Se concentrations with soil-applied SeO_4^{-2} than SeO_3^{-2} (*4, 39*).

When Se was applied to soil at planting, drainage of floodwater before harvest had no effect on increasing Se concentrations in grain, suggesting that oxidation of any reduced Se species in soil to SeO_4^{-2} was too slow to influence crop Se accumulation.

Foliar application at the heading stage gave the highest grain Se concentration (0.27 mg kg^{-1}) for those plants received SeO_3^{-2} in FC soils. In a study conducted in China examining foliar application of Se in paddy rice, researchers reported Se concentrations of 0.355 and 0.411 mg kg^{-1} for two different varieties given SeO_3^{-2} at a rate of 18 g ha^{-1} (*40*). In another study, a rate of 20 g ha^{-1} of SeO_3^{-2} led to a grain Se concentration of 0.471 mg kg^{-1} (*19*). However in our study, grain Se concentration in submerged pots with foliar SeO_3^{-2} was far below to those values (Table 2). In another submerged field trial, testing for SeO_3^{-2} foliar spray showed that in order to achieve 40-75 μg kg^{-1} Se in rice grains, the solution should contain 20-30 μg Se L^{-1} (*41*). These values cannot be compared with our data because there was no information given about how much solution was sprayed per plant or area and also no information on whether they considered the Se concentrations in control pots not receiving Se fertilizer. Possible reasons for the different Se values among different researches could be varietal differences (*7*) and time remaining until harvest after fertilizer application. For instance, in the study undertaken by Hu et al. (*40*), plants had ~3 months after fertilizer application at heading until harvest, at which stage the plants had a long time to transfer Se from leaf to grains. In our study, the elapsed time was a little over one month from heading to harvest.

Selenium accumulation in husks was generally lower than that of grain. Selenate-enriched urea granules and fluid SeO_4^{-2} applied at heading had similar husk Se concentrations in aerobic soils, but SeO_4^{-2}-enriched urea granule treatment was much more effective than all other treatments in accumulating Se in rice husks in submerged and submerged/drained treatments (Table 2). Overall, SeO_4^{-2}-enriched fertilizers were more effective than SeO_3^{-2}-enriched fertilizers in increasing husk Se concentrations.

Selenium accumulation in leaves and culms

The three way interaction among applied Se species, application time, and method of application were statistically significant ($p \leq 0.001$). However, interaction effects between Se application methods × applied Se species had significant and major effects on leaf Se concentration ($p \leq 0.001$). Highest leaf Se concentrations were for foliar applied Se in all soils (Table 4), likely due to retention of foliarly applied Se to leaf surfaces. As observed for grain Se data, SeO_4^{-2}-enriched urea granule treatments also had high Se concentrations in leaves and culms, and in this case the Se must have derived from root uptake.

Selenium accumulation in/on roots

By far the greatest proportion of the variation in Se concentrations in roots was explained by water management method with FC soils having the lowest root Se concentrations and submerged/drained soils the highest (Table 5). Even though the roots were thoroughly cleaned before analysis, we cannot be certain whether this accumulation occurred inside the roots or on the root surface, as we observed iron plaque on the roots of submerged plants.

Iron plaque is known to strongly sorb oxyanions (*42, 43*) and it is therefore highly likely that SeO_3^{-2} and/or SeO_4^{-2} sorbed to the iron plaque.

Selenium application method

Application of fertilizer Se to soil prior to planting was ineffective possibly due to reactions of Se in submerged soils that reduced the availability of both SeO_3^{-2} and SeO_4^{-2} (*21*). Application of Se at heading to the floodwater appears to be the most effective biofortification strategy, and SeO_4^{-2} was generally more effective than SeO_3^{-2} in this regard. There was no consistent advantage of foliar Se over fluid Se fertilizer applied to the floodwater. However, it is evident from the results that the application of SeO_4^{-2}-enriched urea granules to flood water at heading is a very promising way to deliver Se to paddy rice to stimulate Se biofortification of grains. There are several possible mechanisms to explain the efficiency of this treatment. The presence of N fertilizer (urea, mostly in the form of NH_4^+ in rice soils) may have influenced absorption of Se from the root mat at the soil surface, as after application to flood water the Se-coated urea granules sank onto the exposed rot mat at the interface of the soil and floodwater. Plant roots exposed to high NH_4^+ or urea have the potential to absorb more anions (*44, 45*). Alternatively, there may be more efficient translocation of Se inside the plant when Se is applied with urea. Efficient translocation of Fe in the presence of N fertilizer in wheat plants has been shown previously (*46*). Higher leaf Se concentrations in the submerged treatments receiving Se-enriched urea granules, compared to those receiving fluid Se, lends support to the idea of efficient translocation of Se when Se is co-applied/co-located with urea. It should be noted however, that the fluid Se treatments also received urea, although in this case the Se and urea were not co-located. A third hypothesis was that Se applied with urea may have reacted to form selenourea (CH_4N_2Se) in the granules or at the soil/floodwater interface, and this enhanced the absorption of Se by the roots. Very little is known on the formation and reactions of selenourea in soils. Sorption of selenourea to iron hydroxides is much less than that of selenite (*47*) and it is know that selenourea forms in reduced environments (*48*). However, it is readily oxidised (*49*) and we believe it is unlikely to be stable in rice flood waters or in the oxidised rhizosphere of rice roots. We tested the hypothesis that selenourea could be taken up by rice plants in experiment 2, where pure selenourea was applied to flood waters and persistence in floodwater determined, as well as translocation to the xylem of rice plants growing under submerged conditions. While addition of selenourea resulted in higher concentrations of Se in floodwater (compared to SeO_4^{-2}-enriched UAN and urea) 1 day after application, it did not persist and Se was not detectable in floodwater 10 days after fertilizer application (Table 6). Concentrations of Se in rice xylem sap was highest with selenourea 1 day after fertilizer application, but 9 days later Se concentrations in rice xylem sap were highest with SeO_4^{-2}-enriched urea granule treatment (Table 5).

Selenium speciation in rice grain

In this study, the SeO_4^{-2}-enriched urea treatment had significantly higher concentrations of Se in grains and husks than the other treatments. Therefore, rice grain samples from this treatment were used for speciation studies by HPLC-ICP-MS. Enzymatic hydrolysis using Protease XIV extracted 93 ± 7 % of the total Se present in the grain samples. The results of the speciation analysis showed that SeM was the predominant species in grains of the SeO_4^{-2}-enriched urea treatment (Figure 1). Quantitative data from the HPLC-ICP-MS analysis (column recovery ~90-100 %) indicate that SeM comprised over 90 % and SeOM ~ 9% of the total extracted grain Se. Similar results have been reported elsewhere (*50*). Our results clearly show that applied inorganic Se (SeO_4^{-2}) accumulates in the rice grains as organic Se in the form of SeM which is more bio-available for humans than inorganic Se species (*9*). The SeO_4^{-2}-enriched urea treatment did not cause the accumulation of different Se species than those expected with a foliar or fluid SeO_4^{-2} fertilizer.

In summary, differences in Se accumulation in rice plants were a function of the species of applied Se, time of application, method of application and soil moisture regime. More Se accumulated in rice plants when SeO_4^{-2} was the Se species used in fertilizer in all water regimes. This study confirmed previous findings that Se application pre-planting is not an effective method of enhancing accumulation of Se in rice plants. Heading was the best time for Se application. For both aerobic and submerged rice pots, Se fertilizer applied at the heading stage, as SeO_4^{-2}-enriched urea was extremely effective as an agronomic biofortification strategy. Selenium accumulation in rice plants decreased from rice roots in the order grains> leaves> culms and husks. Drainage pre-harvest caused increased Se accumulation in/on rice roots, possibly through adsorption onto iron plaque. Selenium in the rice grains accumulated from application of SeO_4^{-2}-enriched urea was mainly in the form of SeM which is highly bioavailable. Coating or incorporation of SeO_4^{-2} onto urea is simple and inexpensive, and as farmers often apply a side dressing of urea to floodwaters during crop growth, the practice is a simple and extremely effective way to supply Se to crops and to biofortify grains with bioavailable Se. Further studies are needed to confirm the effectiveness of this fertilizer strategy under field conditions and to understand the mechanisms responsible for the enhancement of Se uptake observed with this fertilizer combination.

Abbreviations Used

FC- field capacity

DW- dry weight

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Figure captions

Figure 1. Selenium speciation in protease extracted rice grains of plants growing on pots treated with SeO_4^{2-}-enriched urea granules applied at heading.

Tables

Table 1. Operating conditions for HPLC-ICP-MS

Isocratic chromatographic parameters	
Column	Hamilton PRP-X100 anion exchange column (Phenomenex) (250 x 4.6 mm, 10 um)
Mobile phase	10 mM Citric acid buffer 2%(v/v) methanol
pH	5.5 pH was adjusted using NH_3 solution
Column temperature (^{o}C)	25
Flow rate (ml min^{-1})	1.0
Injection volume (µl)	50
ICP-MS parameters (Agilent 7500ce)	
Isotopes monitored	^{76}Se, ^{77}Se, ^{78}Se and ^{82}Se,
Total analysis time(s)	900

Table 2. Effect of applied Se species, method of application and water management on grain and husk Se concentrations (n = 3)#

Moisture treatment	Se application method	Grain Se concentration (mg kg^{-1})#			Husk Se concentration (mg kg^{-1})#		
		SeO_3^{-2}	SeO_4^{-2}	control	SeO_3^{-2}	SeO_4^{-2}	control
FC	At soil preparation	0.059^{a}	0.079^{abcd}	0.076^{abc}	0.025^{a}	0.029^{ab}	0.030^{abc}
	Se -enriched urea at heading	0.063^{ab}	0.405^{lm}		0.030^{abc}	0.230^{kl}	
	Foliar at heading	0.273^{kl}	0.150^{hij}		0.157^{k}	0.050^{defg}	
	Fluid at heading	0.094^{bcdefg}	0.407^{m}		0.055^{defgh}	0.208^{kl}	
Submerged	At soil preparation	0.085^{abcde}	0.092^{bcdef}	0.086^{abcde}	0.091^{j}	0.056^{defghi}	0.036^{abcd}
	Se -enriched urea at heading	0.117^{defghi}	0.590^{m}		0.077^{ghij}	0.299^{l}	
	Foliar at heading	0.122^{efghi}	0.105^{cdefgh}		0.090^{lmno}	0.084^{hij}	
	Fluid at heading	0.089^{abcde}	0.166^{ij}		0.046^{cdef}	0.071^{fghij}	
Submerged/ drained	At soil preparation	0.082^{abcde}	0.092^{bcdef}	0.097^{cdefg}	0.046^{cdef}	0.046^{cdef}	0.043^{bcde}
	Se -enriched urea at heading	0.138^{ghij}	0.485^{m}		0.063^{efghij}	0.295^{l}	
	Foliar at heading	0.136^{fghij}	0.176^{ij}		0.068^{fghij}	0.080^{hij}	
	Fluid at heading	0.109^{cdefgh}	0.189^{jk}		0.056^{defghi}	0.086^{ij}	

#Different letters in the table for grain and husk Se concentrations separately are significantly different for the three way interaction of Se application method × applied Se species × water management.

Table 3. ANOVA table for the statistical analysis of grain Se concentrations

Source of variance	DF	SS	MS	v.r	F.prob
Se application method (A)	4	2.17033	0.54258	46.16	<0.001
Water management (B)	2	0.03245	0.01622	1.38	0.260
Applied Se species (C)	2	1.27325	0.63663	54.17	<0.001
A × B	8	0.53626	0.06703	5.70	<0.001
A × C	2	1.53315	0.76658	65.22	<0.001
B × C	4	0.03292	0.00823	0.70	0.595
A × B × C	4	0.27580	0.06895	5.87	<0.001
Residual	54	0.63468	0.01175		
Total	80	6.48885			

Table 4. Effect of applied Se species, method of application and water management on leaf and culm Se concentrations (n = 3)

Water treatment	Se application method	Leaf Se concentration (mg kg^{-1})$^{\#}$			Culm Se concentration (mg kg^{-1})$^{\#}$		
		SeO$_3^{-2}$	SeO$_4^{-2}$	control	SeO$_3^{-2}$	SeO$_4^{-2}$	control
FC	At soil preparation	0.043^{a}	0.045^{a}	0.046^{a}	0.046^{abc}	0.042^{ab}	0.060^{bcdef}
	Se -enriched urea at heading	0.053^{ab}	0.269^{gh}		0.032^{a}	0.200^{klmn}	
	Foliar at heading	0.427^{hi}	0.458^{i}		0.216^{lmno}	0.170^{jklm}	
	Fluid at heading	0.065^{ab}	0.313^{ghi}		0.052^{abcde}	0.235^{lmnop}	
Submerged	At soil preparation	0.106^{cde}	0.113^{cde}	0.077^{bc}	0.046^{abcd}	0.105^{ghij}	0.080^{defgh}
	Se -enriched urea at heading	0.084^{bcd}	0.351^{hi}		0.076^{cdefg}	0.289^{nop}	
	Foliar at heading	0.393^{hi}	0.318^{ghi}		0.391^{p}	0.252^{mnop}	
	Fluid at heading	0.110^{cde}	0.129^{de}		0.150^{ghij}	0.092^{fghi}	
Submerged/ drained	At soil preparation	0.125^{de}	0.136^{ef}	0.157^{ef}	0.105^{ghij}	0.120^{ghij}	0.109^{ghij}
	Se -enriched urea at heading	0.150^{ef}	0.379^{hi}		0.145^{ijkl}	0.240^{lmnop}	
	Foliar at heading	0.327^{ghi}	0.294^{ghi}		0.221^{klmno}	0.248^{mnop}	
	Fluid at heading	0.153^{ef}	0.206^{fg}		0.132^{hijk}	0.137^{ijk}	

$^{\#}$Different letters in the table for leaf and culm and concentrations separately are significantly different for the three way interaction of Se application method × applied Se species × water management.

Table 5. Effect of applied Se species, method of application and water management on root Se concentrations (n = 3)

Water management	Se application method	Root Se concentration (mg kg^{-1})[#]		
		SeO_3^{-2}	SeO_4^{-2}	Controll
FC	At soil preparation	0.208^{ab}	0.143^{a}	0.173^{a}
	Se -enriched urea at heading	0.193^{a}	0.429^{def}	
	Foliar at heading	0.163^{a}	0.175^{a}	
	Fluid at heading	0.356^{cde}	0.416^{cdef}	
Submerged	At soil preparation	0.441^{ef}	0.387^{cdef}	0.311^{bc}
	Se -enriched urea at heading	0.496^{f}	0.499^{f}	
	Foliar at heading	0.317^{bcd}	0.371^{cde}	
	Fluid at heading	0.432^{ef}	0.428^{def}	
Submerged/drained	At soil preparation	0.780^{gh}	0.768^{gh}	0.219^{ab}
	Se -enriched urea at heading	1.008^{j}	0.863^{hi}	
	Foliar at heading	0.678^{g}	0.812^{h}	
	Fluid at heading	0.940^{ij}	0.853^{hi}	

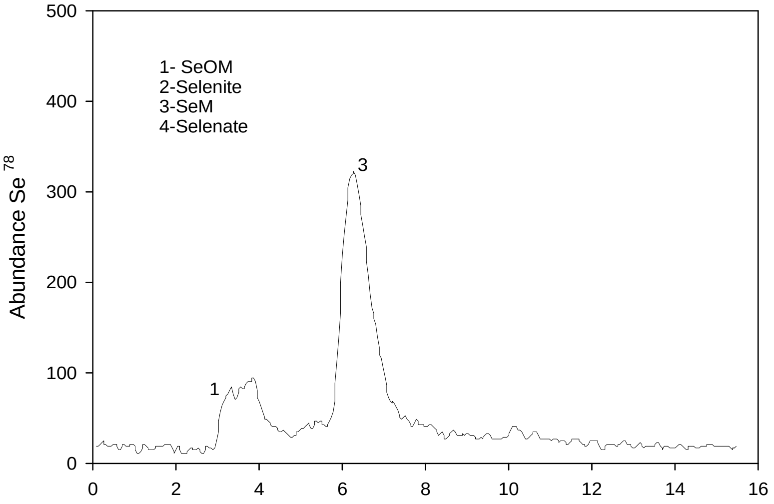
[#] Different letters in the table for root concentrations are significantly different for the three-way interaction of the Se application method × applied Se species × water management treatment.

Table 6. Selenium concentration in flood water and plant xylem sap collected at two times during the second experiment

Selenium treatment[#]	Sample location	1 d after Se application	10 d after Se application
Se -enriched urea 10 µg kg^{-1}	Flood water	0.33 ± 0.05	<0.20
Pure seleno-urea 10 µg kg^{-1}		1.47 ± 0.05	<0.20
UAN+ Se 10 µg kg^{-1}		0.60 ± 0.16	<0.20
Control		<0.20	<0.20
Se -enriched urea 10 µg kg^{-1}	Xylem sap	2.00 ± 0.0	8.40 ± 0.85
Pure seleno-urea 10 µg kg^{-1}		3.33 ± 1.09	5.00 ± 1.41
UAN+ Se 10 µg kg^{-1}		2.00 ± 0.0	<0.2
Control		<0.20	<0.2

[#] UAN- urea ammonium nitrate solution

Figure 1


1- SeOM
2-Selenite
3-SeM
4-Selenate
Abundance Se 78
0
100
200
300
400
500
0
2
4
6
8
10
12
14
16
1
3

Supplementary information

Table 1. Grain dry weights as a function of method of Se application and water management (n = 3)

Application method	Water Management[#]		
	FC	Submerged	Submerged then drained
Control	7.27^{a}	$14.86^{hijklmno}$	13.13^{cdefg}
Se applied at soil preparation	10.69^{bc}	$13.44^{defghijkl}$	12.12^{bcdef}
Se enriched urea at heading	12.03^{bcde}	$14.81^{hijklmn}$	$13.20^{cdefghi}$
Fluid Se at heading stage	10.35^{b}	$14.09^{defghijkl}$	13.19^{cdefgh}
Foliar Se at heading stage	11.72^{bcd}	$14.31^{defghijklm}$	$13.72^{defghijk}$

[#] Different superscript letters in the table are significantly different ($p \leq 0.05$) for the two way interaction of the effect of water management × Se application method.

Table 2. Nutrient contents in leaf and grain tissues from rice plants grown in control pots in submerged and field capacity soils

Nutrient	Leaf element concentration (mg kg^{-1})		Grain element concentration (mg kg^{-1})	
	submerged	field capacity	submerged	field capacity
Ca	5388.0±2.9	3681.9±2.5	107.2±0.2	116.3±0.3
Fe	151.3±0.0	53.2±0.1	38.5±0.4	32.8±0.1
K	25903.8±37.6	24922.8±7.9	2432.7±5.0	2877.6±5.9
Mg	1888.4±4.5	1994.8±0.8	1230.2±2.7	1144.5±2.7
Mn	694.1±0.8	668.8±0.6	31.5±0.0	47.0±0.1
Na	98.3±0.1	64.8±0.0	5.0±0.0	12.6±0.0
P	1532.5±1.3	941.0±0.6	2996.2±5.9	3297.5±7.2
S	1135.9±3.3	944.1±0.5	675.6±0.7	1132.4±1.4
Zn	44.7±0.0	25.1±0.0	22.2±0.0	46.1±0.2