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Selenium Enrichment of Paddy Rice Grains in Malaysia

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ABSTRACT

A greenhouse trial was carried out with the aim of assessing the Se concentration and Se uptake in different plant parts especially in rice grains and also the effect of Selenium on rice plant yield and some yield components. The experiment was carried out in a randomized complete block design with three replications. Three rice varieties (MR232, MR219 and MR253) and five rates of sodium selenite (Na_2SeO_3) including 0, 100, 300, 500 and 700 g ha^{-1} were selected. It was observed that leaf, culm and the grain of rice varieties as well as total Se uptake and grain uptake were affected by used Se rates. All the Se levels used in this study have been able to increase Se level in rice grains to be acceptable for human consumption but among the different Se levels supplied, the level of 500 g ha^{-1} of Se is recommended rate in order to increase Se concentration in grain. Plant dry matter yield, grain yield and yield components were not affected by the different supplied Se concentrations.

Key words: Selenium, rice, grain, yield, biofortification

INTRODUCTION

Selenium (Se) is one of the vital trace elements for human and animals because of some antioxidant effects (Fairweather-Tait *et al*., 2011). Unlike for plant Se is not considered essential, but its deficiency affects human health in different ways, such as immune function, viral infection, reproduction (especially male fertility), thyroid function, asthma and inflammatory conditions (Rayman, 2000). Epidemiological trials have shown that Se also plays a role in the prohibition of cardiovascular disease (Rayman, 2000; Stranges *et al*., 2006). A number of medical researches focusing on human have shown Se is very useful on decline of cancer (Whanger, 2004; Combs, 2005). The trial of US Nutritional Prevention of Cancer (NPC), one of the best recognized researches, showed the effects of a regular oral dose of 200 mg Se per day as enriched yeast on incidence of cancer 1312 American who were in high risk and found that, Se supplementation had no significant effect on the primary endpoint of non-melanoma skin cancer, but decreased the whole cancer morbidity significantly, whole cancer fatality and the morbidity of the prostate, lung and colon cancer (Clark *et al*., 1996; Whanger, 2004). Selenium concentrations in forage crops and foods can be improved with the addition of Se to soil crop systems, a practice termed as "agronomic biofortification" which is one of the safest strategies. The best example is adding sodium selenite in fertilizers in Finland since 1984 (Ylaranta, 1983; Eurola *et al*., 1991; Hartikainen, 2005). Initially, different levels of 16 and 6 mg Se/kg fertilizer were used for cereals and for hay crops and grass, respectively which resulted approximately 3 and 8 g Se ha^{-1} for cereals and grass, respectively (Eurola *et al.*, 1990). The amount of selenium in water, soils, animal products, humans and plants have been monitored repeatedly and the outcomes have been used for adjusting the amount of Se addition. The lower level (6 mg Se/kg fertilizer) was used for all crops between 1991 and 1997. From 1998, the Se concentration has been enhanced to 10 mg Se/kg fertilizer (Hartikainen, 2005). This method has considerably improved the concentration of Se in vegetables, crops, fruits and animal products (Eurola *et al.*, 1990). Consequently, in Finland the average Se consumption increased from 25 mg day^{-1} before Se fertilization to around 110 μg day^{-1} (Eurola *et al.*, 1991). Cereals contributions to the total Se intakes increased as well, from 9-26% (Eurola *et al.*, 1991). Agronomic biofortification has the advantage to direct Se supplementation as plants assimilated the inorganic Se into organic forms, such as selenomethionine, that is more bioavailable to humans. Furthermore, plants by acting as an effective buffer can prevent accidental excessive Se intake by humans that may occur with direct supplementation (Hartikainen, 2005). The minimum nutritional Se needs for the debarment of Keshan disease was found to be around 17 μg day^{-1} (Yang and Xia, 1995). Canadian and US Recommended Dietary Allowance (RDA) is resulting from this value by adding twice the coefficient of variation of 10% to give 55 μg Se/day (Thomson, 2004). The Population Reference Intake (PRI) for European is set at 55 μg day^{-1} as well. In Australia and New Zeland, the Recommended Dietary Intakes (RDI) for female and male adults are 60 and 70 μg day^{-1}, respectively (NHMRC, 2006). The reference nutrient intake in the UK, is set at 75 and 60 μg day^{-1} for male and female adults, respectively (Department of Health, 1991). Selenium poisoning (Selenosis), refers to excessive intake of Se, causes symptoms like, skin problems, loss of nails and hair, hepatomegaly, polyneuritis and gastrointestinal disturbances (Combs *et al.*, 2001).

In Enshi County in central China, chronic selenosis has been reported among the residents, where locally produced foods grown on their soils and water leached through seleniferous coal seams contain unusually high levels of Se (Yang and Xia, 1995; Combs *et al.*, 2001). Studies in Enshi, China revealed that the toxic dietary Se intake that cause selenosis symptoms was approximately 1600 μg day^{-1} (Yang and Xia, 1995).

Rice is one of the important Se source for humans, also it is a staple food in Asia, especially in poor countries (Abilgos-Ramos *et al.*, 2007). However, rice products are generally low in Se in comparison with other crops, to combat with the Se deficiency, improving Se levels in rice can have great influences on human Se status (Hu *et al.*, 2002). Thus, enriching rice products with Se should be a safe and effective way of providing Se and avoiding Se deficiency among those habitually consuming a rice-based diet but also reducing the risk of cancer in these and other populations (Wang *et al.*, 2013).

MATERIALS AND METHODS

The experiment was conducted at the glasshouse complex at University Putra Malaysia. The experimental site was at latitude of N 3°0' 32", longitude E 101°42' 19" and altitude of 64 m above the sea level.

The three Malaysian rice varieties namely MR219, MR232 and MR253 and five different range of Se including (0, 100, 300, 500 and 700 g ha^{-1}) were selected as treatment variables for this experiment. A total of 3 germinated seedlings were transplanted and inserted to a depth of 1-2 cm in each pot with 16 cm diameter and 30 cm height containing approximately 10 kg of top soil (Table 1) on 7th February 2013. The experiment was laid out in a Randomized Complete Block Design (RCBD) in 3 replications. Plants were fed with nitrogen, phosphorus and potassium

Table 1: Characteristics of top soil (serdang series) used in the experiment

Soil variables	Values	Soil variables	Values
Clay (%)	44.98	Calcium (mg kg^{-1})	150.24
Silt (%)	41.50	Magnesium (mg kg^{-1})	106.77
Sand (%)	13.38	Potassium (mg kg^{-1})	40.00
pH	5.30	phosphorons (mg kg^{-1})	279.00
Carbon (%)	1.45	Sodium (mg g^{-1})	0.25
Nitrogen (%)	0.15	EC (dS m^{-1})	0.29
C/N ratio	9.60	CEC (cmol(+) kg^{-1})	5.58

Table 2: Analysis of variance for the effects of selenium applications and varieties on grain, culm, leaf, total uptake, grain uptake, grain yield, total shoot dry weight and 1000 grain weight

Parameters	Grain Se	Culm Se	Leaf Se	Total Se uptake	Grain uptake	Grain yield	Total shoot dry weight	1000 grain weight
Block	0.0042*	0.0009ns	0.0005ns	0.00000444*	0.00000110ns	10.028ns	33.569ns	0.605ns
Variety (V)	0.0001ns	0.0911*	0.011*	0.00003714*	0.00000051ns	22.399*	30.277ns	3.534ns
Selenium (Se) level	0.1290*	0.1171*	0.124*	0.00016103*	0.00001701*	2.387ns	4.133ns	0.932ns
Se×V	0.0008ns	0.0110*	0.002*	0.00000532*	0.00000019ns	5.0184ns	15.283ns	0.214ns
Error	0.0006	0.00080	0.0007	0.00000079	0.00000035	4.331	9.841	1.227

ns: Non-significance, *Significantat at 0.01 probability levels

fertilizers as urea (46% N), single superphosphate (18% P_2O_5) and muriate of potash (60% K_2O) during the plant vegetative stage with fertilizer rate of 50 kg N ha^{-1} and 50 kg K ha^{-1} and 100 kg P ha^{-1}. Hence, a total of 0.705 g urea and 0.54 g MOP were given for each pot by using pocket application. Different rates of selenium were applied to all plants on the day of 90 in a heading time in order to prevent more Se fixation by the clay minerals of the soil. Watering of the plants were controlled every day in order to ensure sufficient amount of water on top of the soil and all the rice plants were kept at saturation situation. Each rice plant was harvested by cutting the plants in the pot at the soil level manually when every plant in the pot reached 120 days of growth in order to have uniform seedling establishment. All three plants from each variety were taken and used as samples for measurements of other agronomic traits. The dry weight of roots, shoots and grains were recorded by drying in the oven for 48 h at 70°C. About 1000 grain counted and the weights of 1000 grain were determined. The wet-acid digestion method with H_2SO_4 and HNO_3 acid was used to determine the Se in tissue. All data was statistically analyzed using ANOVA procedure in SAS 9.2 (SAS, 2007). Mean separation test between treatments was performed using Duncan Multiple Range Test (DMRT) and p-value of ≤ 0.05 was regarded as significant.

RESULTS AND DISCUSSION

Treatment effect on Se concentration in rice grain: From the analysis of variance (ANOVA) for the treatment effects of Se accumulation in rice grain of three rice varieties (MR232, MR219 and MR253), variety and interaction between varieties and rates were found to be not significant but different Se rates were significantly different at $p \leq 0.01$ (Table 2).

The Se rates of 500 and 700 g ha^{-1} showed significantly higher grain Se than the other three Se rates. Figure 1 showed a clear trend of increasing Se in grain from control to 500 g ha^{-1} and remains almost constant when increased to 700 g ha^{-1}.

A possible explanation for this might be the adsorption of SeO_3^{2-} onto soil colloids and minerals and decrease the availability to plants.

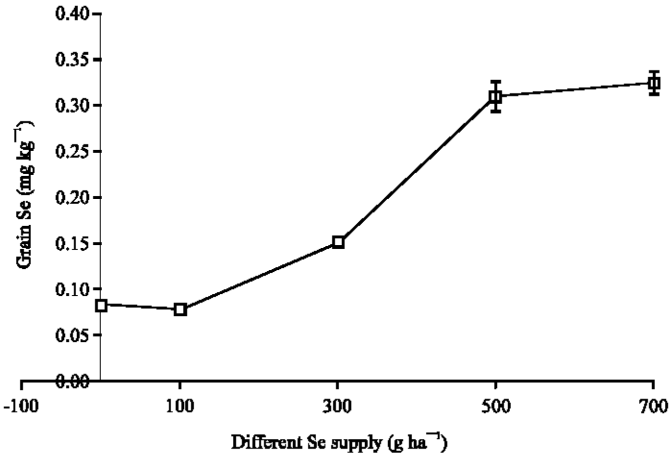

Fig. 1: Effect of different Se supply on grain Se concentration

Table 3: Effect of different Se supply on leaf Se concentration (mg kg^{-1})

	Se in leaf of different varieties (mg kg^{-1})		
Se supply (g ha^{-1})	MR232	MR219	MR253
0	0.102^{d}	0.076^{c}	0.0816^{d}
100	0.087^{d}	0.096^{c}	0.091^{d}
300	0.166^{c}	0.190^{eb}	0.126^{c}
500	0.413^{a}	0.350^{a}	0.283^{a}
700	0.301^{b}	0.320^{a}	0.231^{b}

Mean followed by the same superscript letters in the same column are not significant at p = 0.05

Lyons *et al.* (2003) measured different sodium selenate rates of 0, 10, 30, 100 and 300 g ha^{-1} on wheat and found that Se biofortification on grain Se concentration increased progressively with applied Se. Hu *et al.* (2002) reported that compared to the control, the Se concentration in rice was significantly increased by Se treatment.

These findings suggest that among this various Se rates, 500 g ha^{-1} could be an acceptable rate in order to increase grain Se concentration. This experiment also did not detect any evidence of differences between varieties and Se concentration in grain, but similar responses were seemed. However, a further study with more focus on different rice varieties is therefore suggested.

Treatment effect on Se concentration in leaf and culm: There are significant differences between rice varieties and Se rates in both leaf and culm and the interaction between these factors (Table 2).

Each variety showed different values of Se concentration in leaf, which means there were significant differences between them (Table 3 and 4). It can be seen from Table 3 that for Se concentration in leaf, all three varieties at 500 g ha^{-1} Se supply recorded the highest value of 0.413, 0.35 and 0.283 mg kg^{-1}, respectively for MR232, MR219 and MR253, which was followed by 0.301, 0.320 and 0.231 mg kg^{-1} at Se supply of 700 g ha^{-1}, respectively in MR232, MR219 and MR253. Whereas in MR232, the lowest Se concentration in leaf of 0.087 and 0.102 mg kg^{-1} were attained in Se supply of 100 and 0 g ha^{-1}, respectively. In MR219 and MR253, the lowest leaf Se concentration of 0.076 and 0.081 mg kg^{-1} was observed in control.

Table 4: Effect of different Se rates on culm Se concentration

	Se in culm of different varieties (mg kg^{-1})		
Se supply (g ha^{-1})	MR232	MR219	MR253
0	0.0657^{c}	0.0917^{b}	0.027^{c}
100	0.0917^{c}	0.0827^{b}	0.035^{c}
300	0.188^{b}	0.1013^{b}	0.061^{b}
500	0.4233^{a}	0.2933^{a}	0.1417
700	0.3933^{a}	0.34^{a}	0.1323^{a}

Mean followed by the same superscript letters in the same column are not significant at $p = 0.05$

For Se concentration in culm, the highest value of 0.423 and 0.1417 mg kg^{-1} was recorded at 500 g ha^{-1} Se supply in MR232 and MR253, respectively while in MR219 rice variety the highest value of 0.34 mg kg^{-1} was belonged to 700 g ha^{-1} Se supply. The lowest value of 0.027 mg kg^{-1} Se concentration in culm was recorded at 0 g ha^{-1} Se supply in MR253 and the value of 0.065 and 0.08 mg kg^{-1} Se in culm belonged to 0 and 100 g ha^{-1} Se supply in MR232 and MR219, respectively (Table 4).

The results of culm and leaf showed significant increase in Se concentration with different varieties. The finding showed that although the Se concentration in grain was not different in MR232, MR219 and MR253 varieties, the effect of Se in each variety was different in both leaf and culm. Alifar *et al.* (2013) reported that MR232, MR219 and MR253 varieties were different in the concentration of Se in culm and also in root in solution culture, at maturity they also showed the same response in terms of Se concentration in leaf and culm.

It was observed by Chen *et al.* (2013) that when Se solution was sprayed in rice plant, the Se concentration in rice increased significantly with increasing Se treatment level. However, more research on the Se concentration in leaf and culm of different variety needs to be undertaken to clearly understand the association between Se rate and variety.

Grain yield: Rice grain yield were affected by the different variety while application of different Se levels and their interaction were not significant at $p \leq 0.01$ (Table 1).

From the data in Fig. 2, it is apparent that different rice varieties showed various grain yields. A comparison of the results revealed that MR232 and MR219 varieties with the value of 11.75 and 11.72 g plant^{-1}, respectively reported significantly more grain yield than MR253 with the value of 9.57 g plant^{-1}.

In contrast to yield result, Chen *et al.* (2013) reported that when Se was supplied via foliar-spray, the rice yield was generally improved.

In an Australia study comparing two wheat varieties, the yield respond to applied selenium had shown no significant difference at 5% level and also no correlation between grain Se concentration and yield have been reported in most studies (Tveitnes *et al.*, 1995; Lyons *et al.*, 2004). Hu *et al.* (2002) reported that foliar application of selenite or selenite mixed fertilizers induced no apparent changes on rice yield. Whereas Wang *et al.* (2013) presented that the optimised foliar fertilization increased rice yield by up to 1.29 times, from 2.1 in control to 2.7 g plant^{-1} under treatment of 21 g Se ha^{-1} applied as sodium selenite.

Total se uptake: There is significant interaction between Se rates and rice variety on the total Se uptake in plants. Significant differences were also found between variety and Se rates (Table 2).

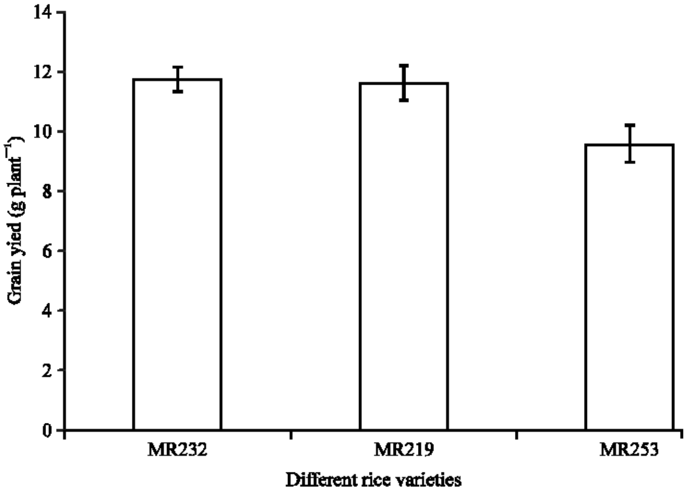

Fig. 2: Effects of different Se rates on grain yield of different rice varieties

Table 5: Effect of different levels of Se on Se total uptake

	Total Se uptake in different varieties (mg kg^{-1})		
Se supply (g ha^{-1})	MR232	MR219	MR253
0	0.0029^{c}	0.0029^{c}	0.0024^{c}
100	0.0031^{c}	0.0034^{c}	0.0021^{c}
300	0.0059^{b}	0.0051^{b}	0.0039^{b}
500	0.0141^{a}	0.0121^{a}	0.0079^{a}
700	0.0126^{a}	0.0121^{a}	0.0076^{a}

Mean followed by the same superscript letters in the same column are not significant at p = 0.05

From the data in Table 5, it is apparent that for the total Se uptake in different rice varieties, no significant differences were found between 500 and 700 g ha^{-1} Se rates. The highest value of 0.0141, 0.0123 and 0.0079 mg plant^{-1} were recorded in MR232, MR219 and MR253, respectively. There was not a significant difference between the two Se levels of 0 and 100 g ha^{-1}.

From this data, we can see that 0 and 100 g ha^{-1} Se levels resulted in the lowest value of 0.0024 mg plant^{-1} in MR253 and the value of 0.0029 mg plant^{-1} in both MR219 and MR253 after Se rates of 300 g ha^{-1}. This resulted in the value of 0.0059, 0.0051 and 0.0034 mg plant^{-1} in MR232, MR219 and MR253, respectively.

The finding showed that total Se uptake in all three varieties was varied, whereas grain Se uptake was not related with these rice varieties because as mentioned above, only Se concentration in culm and shoot was affected by varieties.

The result of Johnsson (1991) on Se uptake in a pot experiment with spring wheat and winter rape revealed no significant differences in grain Se uptake between the two crops, was obtained. Nothstein *et al.* (2013) reported that when rice plants are treated with Se after a period of Se-free growth, uptake of the element is strongly dependent on the plant's vitality, which can be deduced from plant dry mass, leaf colour and plant heights.

Grain se uptake: The effect of Se rates on Se uptake in rice grain of three different rice varieties and interaction between varieties and rates were found to be not significant but different Se rates were significantly different at $p \leq 0.01$ (Table 2). Different Se supply showed different effects on Se concentration on rice grain (Fig. 3).

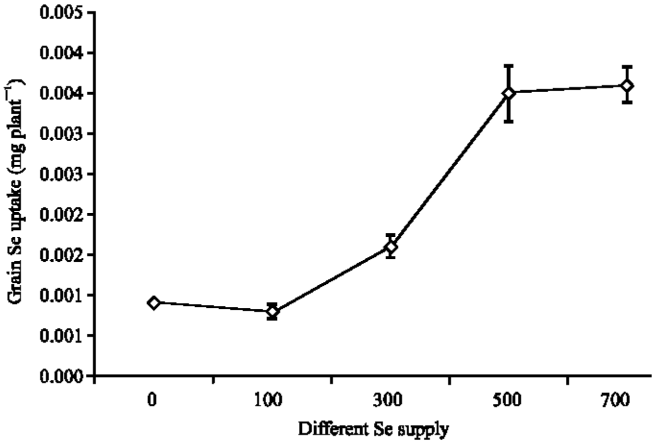

Fig. 3: Effect of different Se rates on grain Se uptake

The Se rates of 500 and 700 g ha^{-1} reported significantly more Se grains than the other three Se rates. In Fig. 3, there is a clear trend of increasing Se in grain from control to 500 g ha^{-1} while it almost remains constant when the rates were increased to 700 g ha^{-1}.

The result of Johnsson (1991) on Se uptake in a pot experiment with spring wheat and winter rape revealed no significant difference in grain Se uptake between the two crops. The mean Se content for wheat grain and rape seeds was 560 and 602 ng Se g^{-1}, respectively.

1000 grain weight and total shoot dry weight (grain, culm, leaf): The main effect of Se and varieties and their interaction on 1000 grain weight and total shoot dry weight were not significant (Table 2).

Yao *et al.* (2013) attained the result that Se application significantly increased flower dry weight at vigorous growth, flower bud differentiation and bud stages. However, Se supplies at seedling and flower stages did not affect flower biomass accumulation, indicating that the effects of Se on flower biomass depends on application stage.

CONCLUSION

In this study, it was observed that leaf, culm and the grain of rice varieties as well as total Se uptake and grain uptake were affected by different selenium rates. All the Se levels use in this study have been able to increase Se level in rice grain to be acceptable for human consumption but among the different Se levels supplied, the level of 500 g ha^{-1} of Se is recommended rate in order to increase Se concentration in grains. Rice yield, grain yield and yield components were not affected by different Se supply. Different rates were not toxic and did not have any toxic effect for plant growth.

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