Agronomic selenium biofortification of two-rowed barley under Mediterranean conditions

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ABSTRACT

In order to improve the nutritional value of two-rowed barley grain, two foliar selenium (Se) fertilizers (sodium selenate and sodium selenite) at four rates (0-10-20-40 g/ha) were applied during the growing seasons 2010/2011 and 2011/2012 in a field experiment conducted under semiarid Mediterranean conditions. The grain harvested in the 2010/2011 season accumulated a greater amount of total Se than the grain of the 2011/2012 season. Sodium selenate was much more effectively taken by plants than sodium selenite, and there was a strong and linear relationship between total Se concentration and Se rate in both sodium selenate and selenite. For each gram of Se fertilization, applied as sodium selenate or sodium selenite, the increases of total Se concentration in grain were 44 and 9 μg/kg dry weight, respectively. No increments in total or available Se were observed in soil after harvesting even at the highest doses of either fertilizer. It can be concluded that two-rowed barley would be a good candidate to be included in biofortification programs under Mediterranean conditions to increase Se in animal feeding and in the human diet through beer production.

Keywords: sodium selenite; sodium selenate; rainfed conditions; ICP-MS; cereals

Selenium (Se) is an important element associated with the enhancement of antioxidant activity in plants, animals and humans. Low dietary intakes of Se by humans can cause health disorders, including oxidative stress-related conditions, reduced fertility and immune function, and an increased risk of cancers (Reid et al. 2008). Globally, between 500 and 1000 million people from many countries, including Spain, may have an inadequate intake of Se (Arthur 2003). The European Recommended Dietary Allowance (RDA) of Se for humans is about 55 μg/day of Se (Elmadfa 2009). However, several clinical trials tested the effectiveness of a regular oral dose of 200 μg/day of Se in the reduction in the incidence of certain cancers, cardiomyopathy, free radical induced diseases and protection against HIV (Arthur 2003, Reid et al. 2008). As Se intake in Spain was estimated at 32.35 μg/day on average (Díaz-Alarcón et al. 1996), it would need to be highly increased to reach the recommended values. In addition, Se deficiency in livestock is common, causing diseases in animals such as white-muscle disease in cattle and sheep, hepatosis dietetica in pigs, pancreatic fibrosis or exudative diathesis (Hawkesford and Zhao 2007). Se requirements for sheep and cattle are 200–300 μg Se/kg dry food, respectively, to prevent Se deficiency diseases (National Research Council 1996).

Feeding is the main route of Se intake for animals and humans. Two-rowed barley (*Hordeum vulgare* L. ssp. *distichum*) is one of the most ancient and widely distributed crops. Nowadays, about two-thirds of barley crops are used for animal feed, approximately one-third for malting and about 2% for human food directly. Spain produces annually more than 8 million tons of barley grain and 32.5 million hectoliters of beer, being the fourth beer manufacturer in the EU (Magrama 2011).

Studies conducted in Israel (Yan et al. 2011) recorded a range of Se concentrations between 0–387 (47 on average) μg/kg dry weight (DW) in 92 genotypes of wild barley (*Hordeum spontaneum* C. Koch); similarly Bratakos and Ioannou (1989) found the Se values 160 μg/kg DW in 100 different locations in Greece. Very few studies dealt with Se biofortification in two-rowed barley (Gupta et al. 1993, Gupta and MacLeod 1994). So, many questions, such as the effectiveness of Se fertilizers in semiarid conditions, the amount of Se transferred to the grain, the bioavailability of Se in two-rowed barley grain etc., remain unknown. Therefore, the aim of the present study was to determine the effect of the type of foliar Se fertilizer and the application doses on the uptake and later accumulation of Se in barley grain under Mediterranean conditions. The influence of the fertilizer and its doses on the grain yield, 1000 grain weight and crude protein was also evaluated.

MATERIAL AND METHODS

The study was conducted in two different growing seasons, 2010/2011 and 2011/2012, in Badajoz, southern Spain (38°54'N, 6°44'W, 186 m a.s.l.), on a Xerofluvents soil under rainfed Mediterranean conditions. Weather-related parameters in this area for the study years, as well as for the average year obtained from a 30-year period, are shown in Figure 1.

For each study year, the experiment was designed as a split plot arrangement with four repetitions, including each Se form (sodium selenate (Na_2SeO_4) and sodium selenite (Na_2SeO_3)) in each plot, and within the plot, four application doses (0-10-20-40 g/ha of diluted in 3 L of water) were randomly distributed. The crop area for each treatment was 15 m^2 (3 m × 5 m). Fertilizers were applied at the end of tillering EC-39, on sunny days as foliar application. The experimental area used each year had not been previously fertilized with Se, therefore a potential residual effect of Se in the soil can be ruled out. The two-rowed barley cultivar used in the experiments was Quench. Conventional tillage treatment was used to prepare a proper seedbed before sowing. Seeding rate was 170 kg/ha, in 20 cm wide rows. Sowing took place in late December both years. An N-P-K fertilizer (8-15-15) was applied before sowing at a 200 kg/ha dose in all plots.

Each year, before sowing, four representative soil samples of 30 cm depth were taken from the experimental site. Additionally, in 2011/2012, at harvesting, one soil sample per treatment was taken to study Se accumulation in the soils. Soil samples were air dried and sieved to < 2 mm using a roller mill. Texture was determined gravimetrically, soil pH was determined using a calibrated pH meter (Alella, Spain) (ratio 10 g soil:25 mL deionized H_2O) and soil organic matter was determined by oxidation with potassium dichromate.

Total Se was determined as follows: a portion of each soil was finely ground (< 0.45 mm) using an agate ball mill (PM 400 mill, Retsch, Haan, Germany); 1 g was digested with ultrapure concentrated nitric acid (2 mL) and 30% w/v hydrogen peroxide (2 mL) using a closed-vessel microwave digestion protocol (Mars X, CEM Corp, Matthews, USA), and diluted to 25 mL with ultra-pure water (Adams et al. 2002). Sample vessels were thoroughly acid-washed before use. For quality assurance, a blank and a standard (tomato leaf material, NIST

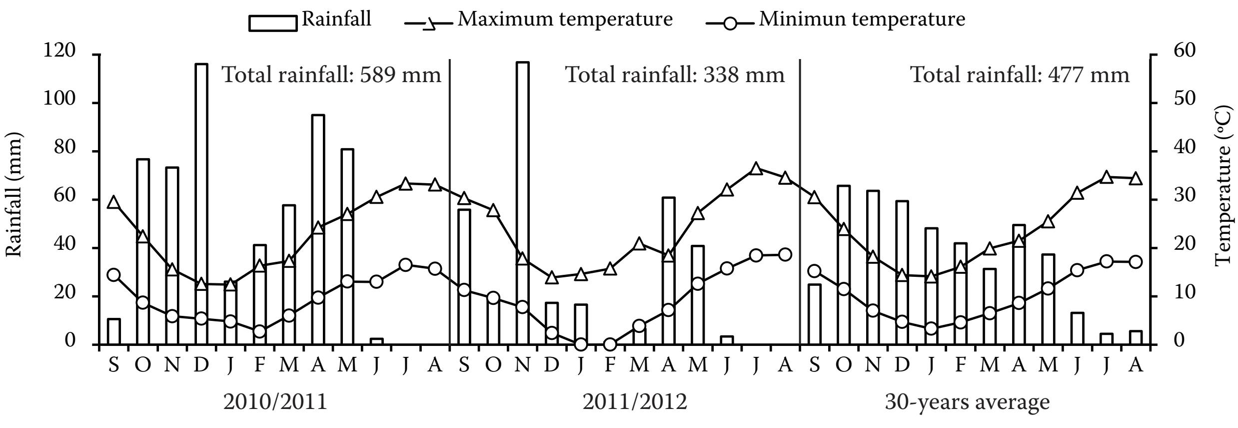

Figure 1. Monthly and annual rainfall and mean maximum and minimum temperatures in 2010/2011, 2011/2012 and in an average year from a 30-year period at Badajoz (Spain)

1573a) were included in each batch of sample for quality control. Repeated analysis (n = 8) of the NIST 1573a showed mean values of 53.8 ± 0.8 µg Se/kg, which are in good agreement with the certified value of 54 µg Se/kg. Concentrations of Se were determined using an inductively-coupled plasma mass spectrometer (ICP-MS) (Agilent 7500ce, Agilent Technologies, Santa Clara, USA) operating in the hydrogen gas mode (the analytical method was developed by the Elemental and Molecular Analysis Service of the University of Extremadura). All results were reported on a dry weight (DW) basis. Available Se in the soil samples were determined using KH_2PO_4 (0.016 mmol, pH 4.8) extractions (ratio 10 dry weight soil:30 mL KH_2PO_4 w/v) (Zhao and McGrath 1994), and the Se concentration was determined by the ICP-MS as described above.

Harvesting took place at maturity in early June. In addition to grain yield, 1000 grain weight was also determined. Total N content was determined using the Dumas combustion method (FP-428 N analyzer, Leco Corporation, St. Joseph, USA). Grain protein was determined by multiplying the grain N and 5.7 as conversion factor. Total Se contained in the grain was determined by an ICP-MS as described above for soil samples after milling the grain with a corundum mill (Wolfgang Mock, Munich, Germany).

Data of total Se in grain, grain yield, 1000 grain weight and crude protein were subjected to a 3-way ANOVA, including year, Se form, Se doses, and their interactions in the model. For a better interpretation of the data additional 2-way ANOVA was carried out for each growing season separately. When significant differences were found in ANOVA, means were compared using the Fisher's protected least significant difference (*LSD*) test at $P \leq 0.05$. Pearson's correlation tests were performed between total Se and all the studied quality and quantity parameters. All the analyses were performed with the Statistics v. 8.10 package (Tallehessee, USA).

RESULTS AND DISCUSSION

The analysis of the samples showed that the soil of the field experiment had a loamy texture, a pH of 7.3 ± 0.10 (mean ± standard error (SE)), and a soil organic matter of 11 ± 0.14 g/kg. The Se content of the soil is the major influencing factor on final grain Se uptake and later accumulation. Before Se application, total Se in soil was 134.4 ± 16.3 µg/kg (mean ± SE) in 2010/2011 and 123.8 ± 15.4 µg/kg in 2011/2012. So, according to the classification by Hawkesford and Zhao (2007), the soils can be considered as deficient-marginal in total Se. Díaz-Alarcón et al. (1996) found higher values, 210 µg/kg in agricultural soils from southeast Spain, and Moreno Rodríguez et al. (2005) recorded values ranging from 200 to 4380 µg/kg in soils from central Spain. However, although total Se is the most commonly used parameter, it is not always a good indicator of the Se available for plants (Moreno Rodríguez et al. 2005). In the present study only over 3.6% of total Se (4.8 ± 0.19 µg/kg) the first year, and over 3.5% (4.3 ± 0.18 µg/kg) the second year was available. These values are lower than 27 µg/kg of available Se, minimum concentration required to produce crops with sufficient Se for human nutrition (Stroud et al. 2010). After the Se fertilization, the concentrations of total Se and available Se (150 µg/kg and 9.8 µg/kg, respectively) did not increased significantly. Therefore, at working doses, a potential accumulation of Se in the soil could be ruled out.

The 3-way ANOVA carried out on the quality and quantity grain parameters showed a significant effect of the study year on the yield, crude protein and 1000 grain weight (df = 1; P < 0.001). The study year affected also the influence of the Se form on the crude protein content (as the interaction year × Se form was significant, df = 1; P < 0.05) and that of the Se dose on the grain yield (as the interaction year × Se dose was significant, df = 3; P < 0.05). Higher crude protein and 1000 grain weight were obtained in 2010/2011, with 10.4% versus 9.1% and 45.7 g versus 40.4 g, respectively. In contrast, grain yield was higher in 2011/2012 (2039 kg/ha versus 1810 kg/ha). All the production parameters and nutritive values were in line with other works carried out in the same area with the same barley cultivar. The 2-way ANOVA performed for each study year separately (Table 1) showed a significant effect of the Se form on the grain yield and on the 1000 grain weight in 2010/2011, and on the crude protein in 2011/2012. In all of these significant cases, selenite produced the highest values (Table 1). The Se dose only had a significant effect on the grain yield in 2011/2012. In such case, the highest value was obtained with a dose of 10 g/ha (Table 1). These results may indicate that, at least up to 40 g/ha,

Table 1. Mean grain protein, grain yield and 1000 grain weight as affected by Se form and Se dose for each study year

Year	Se dose (g/ha)	Grain protein (%)			Grain yield (kg/ha)			1000 grain weight (g)		
		selenate	selenite	mean	selenate	selenite	mean	selenate	selenite	mean
2010/2011	0	10.5 ± 0.1	10.5 ± 0.4	10.5 ± 0.2	1634 ± 163	1685 ± 120	1660 ± 87	46.0 ± 1.0	46.4 ± 0.6	46.2 ± 0.5
	10	10.3 ± 0.2	10.5 ± 0.3	10.4 ± 0.2	1555 ± 176	1555 ± 122	1554 ± 91	44.2 ± 0.4	46.1 ± 1.2	45.1 ± 0.7
	20	10.6 ± 0.4	10.1 ± 0.4	10.3 ± 0.3	1705 ± 206	1812 ± 179	1759 ± 118	44.2 ± 0.9	47.2 ± 0.7	45.7 ± 0.8
	40	10.6 ± 0.6	10.3 ± 0.3	10.4 ± 0.3	1619 ± 42	1940 ± 200	1779 ± 108	44.9 ± 0.7	46.2 ± 0.8	45.5 ± 0.5
	mean	10.5 ± 0.2	10.4 ± 0.2	–	1628 ± 65^b	1748 ± 74^a	–	44.8 ± 0.4^b	46.5 ± 0.4^a	–
2011/2012	0	9.2 ± 0.2	9.4 ± 0.3	9.3 ± 0.1	1757 ± 79	2027 ± 83	1892 ± 73^B	39.3 ± 0.1	39.1 ± 1.4	39.2 ± 0.6
	10	8.9 ± 0.2	9.8 ± 0.3	9.3 ± 0.2	1973 ± 96	2282 ± 175	2128 ± 105^A	40.0 ± 1.8	40.7 ± 1.6	40.4 ± 1.0
	20	8.5 ± 0.4	9.4 ± 0.3	8.9 ± 0.3	1697 ± 159	2075 ± 128	1886 ± 116^B	39.1 ± 2.0	43.8 ± 1.1	41.4 ± 1.4
	40	8.6 ± 0.1	9.2 ± 0.4	8.9 ± 0.2	1739 ± 150	1813 ± 145	1777 ± 91^B	40.6 ± 1.2	39.8 ± 0.5	40.2 ± 0.6
	mean	8.8 ± 0.1^b	9.4 ± 0.1^a	–	1791 ± 58	2050 ± 71	–	39.8 ± 0.6	40.9 ± 0.7	–

For each studied parameter, averages in the same row with the same lowercase letter were not significantly different ($P < 0.05$) according to the *LSD* test. Averages in the same column, with the same uppercase letters were not significantly different ($P < 0.05$) according to the *LSD* test. When letters do not appear in rows or columns, the corresponding variables (Se form and Se dose) did not have any significant effect ($P > 0.05$) on the studied parameter according to the 2-way ANOVA carried out for each year separately

the Se application may not cause a reduction in the productive parameters which are usually used by the industry to pay farmers. This is an important point, as it would be impossible to successfully implement a potential biofortification program in the future, if the main agents of the process, i.e. farmers, obtained lower income gains as a consequence of Se application.

According to the 3-way ANOVA, total Se in two rowed barley grain was significantly affected ($P \leq 0.001$) by all the three variables (year, Se form and Se dose) as well as by all the possible interactions. Se accumulation was much more important in selenate. The total Se amount in the barley grain in 2010/2011 ranged between 69 µg/kg DW at doses 0, and 520–2336 µg/kg DW at doses 40 g/ha of selenite and selenate, respectively. In 2011/2012 the range was between 60 µg/kg and 316–1347 µg/kg DW, respectively (Figure 2). Previous studies showed that selenate is more available for imme-

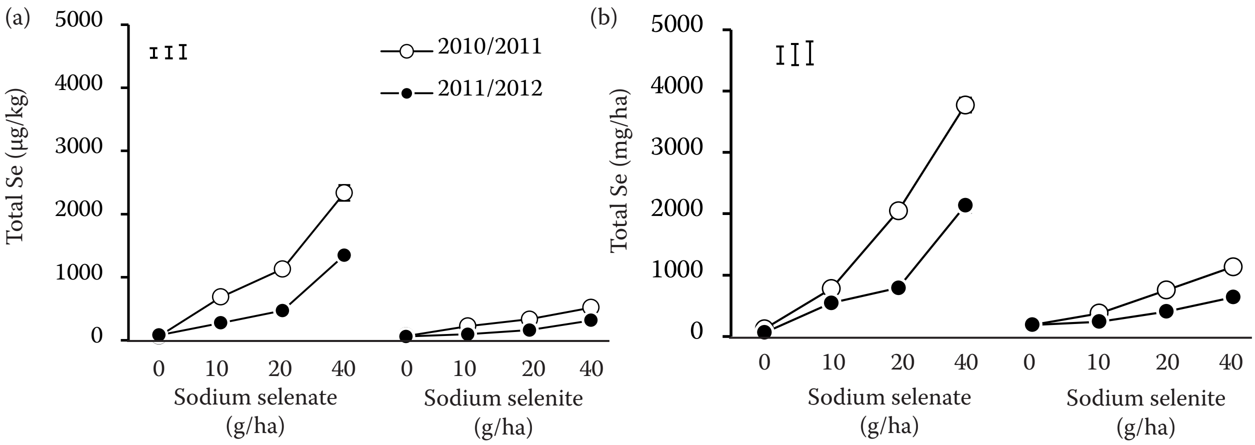

Figure 2. Total Se in grain as influenced by the interaction year × Se form × Se dose. Vertical bars on the markers represent ± standard errors. Vertical bars on the left corner represent *LSD* ($P < 0.05$): the one located more to the left, for the same level of year and Se form; the one located in the middle, for the same level of year; and the other for a different level of year

diate uptake than selenite (Moreno Rodriguez et al. 2005, Stroud et al. 2010). The concentration of total Se in the non-treated grains was in consonance with the values recorded in Spain in other winter cereals (Díaz-Alarcón et al. 1996), was lower than the Greek samples analyzed by Bratakos and Ioannou (1989) in non-deficient soils and considerably higher than the average found by Yan et al. (2011) in China. Selenium concentration was also investigated by the atomic absorption spectrometry with hydride generation technique in emmer, einkorn and spring wheat varieties (Lachman et al. 2012). Higher Se concentration in grains was related to emmer (59–68 μg/kg DM) and einkorn (50–55 μg/kg DM) varieties, in spring varieties Se concentration ranged from 30 to 40 μg/kg DM.

The highly significant linear relationship between the total Se concentration in grain and the Se doses regardless of the Se application form (Table 2) is consistent with many previous studies performed since the 1970s (reviewed by Lyons et al. 2003). The Se accumulated in two-rowed barley grain increased by 55–33 μg/kg DW and 10–6 μg/kg DW for each g/ha of Se applied as sodium selenate and sodium selenite in 2010/2011 and 2011/2012, respectively (Table 2). This may mean that the application of selenate, at a dose even lower by 10 g/ha, regardless of the climatic conditions of the growing season, may provide one kg of barley with grain Se concentrations higher than the 200–300 μg Se/kg DM that sheep and cattle require as minimum concentration to prevent Se deficiency diseases (National Research Council 1996). If the total Se is referred to hecte ares (multiplying the total Se in μg/kg of Se by the grain yield) the year did not affect the amount of accumulated Se at the lower dose of the fertilizer (Figure 2b). This would mean that the amount of Se that the crop is able to accumulate in the grain per hectare is quite stable and independent of the climatic conditions, depending mainly on the Se form included in the fertilizer and especially on the applied doses. With regard to the human diet, as the Spanish beer consumption is around 48.3 L per year and person (Magrama 2011), it could become an important source of Se for adult people. On the other hand, information in the scientific literature on this is still scarce and very few references have been recovered; Fantozzi et al. (1998) found a Se content of 8 ± 4.0 μg/L in Italian beers, and Bamforth (2002) recorded a range between < 0.4 and 7.2 μg/L. Therefore it would be very interesting to carry out further experiments to evaluate how much of the Se accumulated in the grain is able to remain in the beer after the manufacturing process and so to achieve a 'Se enriched beer'.

Table 2. Total Se accumulation in grain (y) in μg/kg expressed as a linear relationship of the Se dose (x) in g/ha in each Se form (sodium selenate and sodium selenite)

Se form	Year	Expression	R^2
Sodium selenate	2010/2011	$y = 55.391x + 93.92$	0.997**
	2011/2012	$y = 32.88x + 42.77$	0.966*
Sodium selenite	2010/2011	$y = 10.375x + 113.78$	0.995**
	2011/2012	$y = 6.09x + 58.11$	0.951*

*$P < 0.01$; **$P < 0.001$

In conclusion, the present study affirms that two-rowed barley could be a potentially good way of introducing Se into animal feeding and potentially into the human diet throughout beer, and therefore may be a very good candidate to be included in Se biofortification programs. It is important to indicate that sodium selenate was much more effective than sodium selenite, and that a dose of 10 g/ha of Se applied as sodium selenate at the end of tillering was able to increase Se concentrations in the grain close to those recommended. At this dose, the content of Se in the grain per hectare was stable and independent of the weather conditions.

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