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High-selenium wheat: Agronomic biofortification strategies to improve human nutrition

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Abstract

Selenium (Se) is an essential micronutrient for human and animal health, with antioxidant, anti-cancer and anti-viral properties, and wheat is a major dietary source of Se. Field trials on two soil types in South Australia showed that grain Se concentration increased progressively with applied Se, whether soil- or foliar-applied. Soil-applied Se increased grain Se concentration by 20- to 133-fold, and foliar by 6- to 20-fold. A maximal grain Se concentration of 12 mg kg^{-1} was achieved, which equates to a 10% recovery of applied Se. Grain yield and protein were not affected by applied Se ranging from 4-120 g ha^{-1}. In another trial, a relatively low rate of 30 kg ha^{-1} of S applied to the soil reduced grain Se concentration by 16%. It may be necessary to add Se to gypsum and high-S fertilisers, which are widely used in Australia, to maintain food crop Se concentrations. These trials did not reveal a significant effect of variety on grain Se concentration, perhaps because of the extreme variability in available soil Se concentration, that varied more than 100-fold between sites. Grain yield was not affected by variety or by the application of Se, S or N. Grain protein and S, but not Se, concentrations were increased by late-applied foliar urea. Fertilisation with sodium selenate is an inexpensive, practical method to produce wheat that contains concentrations of Se high enough to provide likely health benefits to consumers, including reduced cancer risk.

Key words: Selenium, health, wheat, biofortification, sulphur, cancer prevention.

Introduction

Se is an integral component of at least three metabolic systems essential for normal mammalian cell function[1]. Se deficiency is widespread and likely to be manifested in populations as increased risk of thyroid and immune dysfunction, serious sequelae of RNA viral infections, cancer and various inflammatory conditions[2,3]. Moreover, mounting evidence suggests that supranutritional Se intakes provide additional protection against disease, in particular, cancer[2,3,4]. Population Se status is sensitive to changes in the food supply, and where wheat (*Triticum aestivum* L.) is a major staple, to changes in wheat consumption and Se content[2, 5, 6].Wheat is an important source of bioavailable Se, and in Australia it may supply around half the Se intake of most people[2,7].Wheat grain, both globally and in Australia, is highly variable in Se concentration, which is determined mostly by the level of available soil Se[2]. In many soils Se is poorly available to plants, and of concern is a trend toward a reduction of Se in the global food chain, possibly caused by fossil fuel burning (with release of sulphur [S], a Se antagonist), acid rain, soil acidification and use of high-S fertilisers[8].

Exploiting the genetic variability in crop plants for micronutrient density (genetic biofortification) may be an effective method to improve the nutrition of entire human populations. Substantial variations in zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) density in wheat accessions grown together have been demonstrated.[9] Some studies have shown no evidence of genetic variability between wheat cultivars for Se density in grain,[10,11] while another showed higher concentrations of Se, Zn, lithium (Li), magnesium (Mg) and phosphorus (P) in hulled wheat (*Triticum spelta* L., *Triticum dicoccon* Schrank) grown together with modern wheats. However, it is not clear whether the Se values were significantly different.[12] Research is needed to determine whether sufficient genetic variability in grain Se density exists in wheat to enable selection for this trait, but in any case, selection remains problematical because of the high environmental (soil) effect on Se density of grain.

Agronomic approaches to micronutrient enhancement can be effective[13], and this is particularly so for Se. Although it is not considered to be an essential nutrient for higher plants[14], and most previous studies have shown no beneficial effect of Se on crop yield, an increase in wheat grain Se concentration represents a food system approach that would increase population Se intake, with consequent likely improvement in public health.

The use of Se as a soil amendment in fertiliser is practised mainly in Finland (by law from 1984)[15], where it is currently added to NPK fertiliser at a rate of 10 mg kg^{-1}, and in New Zealand (at an individual level, and generally on pastures).

Sodium selenate is the Se form generally used for crop and pasture fertilisation: it is weakly adsorbed on soil colloids and can bring about a rapid increase in plant Se level[16]. Studies of Se fertilisation of crops have been reviewed by Lyons et al [2]. There are no published studies of Se fertilisation of wheat in Australia. Australian conditions are different to those in Europe and Canada, where most studies have been conducted; in Australia, rainfall and yield are lower and temperatures higher.

As wheat is a major dietary source of Se, it is important to identify and, if necessary, modify agronomic practices that influence Se concentration in grain in order to maintain, if not enhance, the current Se level in Australian wheat. For example, gypsum (calcium sulphate) is commonly used to treat sodic soils, and S is also a component of superphosphate, ammonium sulphate and sulphate of potash, fertilisers widely used in Australia. Also, the practice of applying N in the form of urea after flowering to increase grain protein may influence the concentration of grain Se, which is protein-bound.

Studies of crop and pasture plants show that increasing soil S level decreases Se uptake and transport[17-22]. The use of gypsum in Oregon increased the incidence and severity of white muscle disease, a Se-deficiency condition of sheep[21], and when added at the high rate of 11.2 t ha^{-1} resulted in substantial reduction in Se uptake by lucerne (*Medicago sativa* L.) grown on a coal fly-ash landfill site[17]. Research on N and Se interaction in plants is limited, but a high N level strongly increased the Se concentration in maize (*Zea mays* L.) roots exposed to selenite, but decreased translocation, and increased the proportion of free selenoamino acids to selenite in the leaves[23]. A pasture trial found that a high N application (320 kg ha^{-1} year^{-1}) increased total Se uptake at the first harvest by a factor of four, but had little effect on plant Se concentration[24]. A trial that tested the effect of applying N to wheat at 10 sites also found no effect on grain Se concentration[25]. In a survey of UK wheat and bread, Se level was found to be positively associated with protein level[26], and soft wheats were found to contain less Se (0.02-0.13 mg kg^{-1}) than hard wheats (0.05-1.09 mg kg^{-1}), probably because of the lower protein level of soft wheats[27].

The aims of this study are to investigate the effects of soil or foliar Se application at varying rates on wheat grain Se concentration and yield, and to examine the effects of S and N applied at commercial rates, and of variety on grain Se concentration, yield and protein concentration, using field trials.

Materials and Methods

Se Biofortification Studies

To test the effects of different rates and methods of application of Se on grain Se concentration and yield, two field trials were conducted in South Australia during 2002. As soil pH is an important determinant of Se availability[28], the two sites were chosen to provide a pH variation that encompasses most soils in South Australia's wheat-growing regions.

Table 1. Soil analysis of Charlick.

Texture	3.5	Reactive iron	773 mg kg^{-1}
Colour	Red-brown	Conductivity	0.0530 dS m^{-1}
Nitrate nitrogen	8 mg kg^{-1}	pH (CaCl$_2$)	5.7
Ammonium nitrogen	1 mg kg^{-1}	pH (H$_2$O)	6.6
Phosphorus Colwell	57 mg kg^{-1}	DTPA copper	0.39 mg kg^{-1}
Potassium Colwell	395 mg kg^{-1}	DTPA zinc	0.33 mg kg^{-1}
Sulphur	4.4 mg kg^{-1}	DTPA manganese	4.43 mg kg^{-1}
[1]Selenium	<0.2 mg kg^{-1}	DTPA iron	66.29 mg kg^{-1}
Organic carbon	13,300 mg kg^{-1}		

[1] Total soil Se determined by ICP mass spectrometry after digestion with nitric/perchloric acid.

***Charlick trial**:* This trial was conducted at the University of Adelaide's Charlick experimental farm near Strathalbyn, around 70 km south of Adelaide. Soil type was red-brown clay-loam over limestone with surface pH(H$_2$O) of 6.6 (Table 1).This was a 2x5x2 factorial trial with a split-split-plot design in four blocks. Two commercial bread wheat varieties, Krichauff (Australian Standard White grade) and Kukri (hard grade), were randomly allocated to the two whole plots within each block. The whole plots were then divided into five sub-plots and randomly assigned Se treatments of 0 (control), 10, 30, 100 and 300 g ha^{-1} of sodium selenate. These sub-plots were further divided into two sub-sub-plots and randomly allocated with soil or foliar Se treatments.

The sub-sub-plots comprised 6 rows with 15 cm spacings x 5 metres, with 35 cm between the sub-sub-plots; the final harvested area was 3.5 m^2. Basal fertiliser (N 220, P 120 g kg^{-1}) 110 kg ha^{-1} was applied at sowing on 18th June. Mean sowing rate was 72 kg ha^{-1} (190-200 seeds m^{-2} allowing for variation in seed size). Weed control comprised an application of 2,4-D amine at 1.5 L ha^{-1} for broadleaf weeds, and *Topik*®240EC (240 g L^{-1} clodinafop-propargyl; 60 g L^{-1} cloquintocet-mexyl) at 160 ml ha^{-1} for wild oats four weeks after emergence. The trial was harvested in mid December. The 2002 season was much drier than normal. Charlick recorded growing season (April to October) rainfall of 211 mm, with an annual total of 293 mm.

Treatment: Soil Se application: A solution of sodium selenate (technical grade, ACE Chemicals, Camden Park, South Australia) (250 ml per 5.5 m^2 plot, equivalent to 450 L ha^{-1}) was sprayed onto the soil surface just before sowing, using a 2-litre hand sprayer.

Foliar Se application: A solution of sodium selenate (80 ml per 3.5 m^2 plot, equivalent to 230 L ha^{-1}) plus a non-ionic surfactant (NuFarm *ChemWet 1000*® applied at 1.0 ml L^{-1} water) was applied using a two-wheeled plot sprayer specially designed to prevent spray drift, at the mid-milky dough stage (Zadok stage 76).

Minnipa trial: The second trial was conducted at the South Australia Research and Development Institute's Minnipa Agricultural Centre, 400 km north-west of Adelaide, on a red-brown calcareous sandy loam with surface pH(H$_2$O) of 8.6 (Table 2). This was a 5x2 factorial trial with a split-plot design in four blocks. Krichauff was the wheat variety used. Se application rates (same as used in the Charlick trial) were

Table 2. Soil analysis of Minnipa.

Texture	2	Reactive iron	293 mg kg^{-1}
Colour	Light-brown	Conductivity	0.111 dS m^{-1}
Nitrate nitrogen	4 mg kg^{-1}	pH (CaCl$_2$)	7.7
Ammonium nitrogen	4 mg kg^{-1}	pH (H$_2$O)	8.6
Phosphorus Colwell	22 mg kg^{-1}	DTPA copper	0.29 mg kg^{-1}
Potassium Colwell	467 mg kg^{-1}	DTPA zinc	0.52 mg kg^{-1}
Sulphur	2.4 mg kg^{-1}	DTPA manganese	4.39 mg kg^{-1}
Selenium	<0.2 mg kg^{-1}	DTPA iron	7.02 mg kg^{-1}
Organic carbon	7,800 mg kg^{-1}		

randomly allocated to the five whole plots within each block. The whole plots were then divided into two sub-plots and randomly allocated with soil or foliar Se treatments. Plot size was 1.6 m x 10 m. B asal fertiliser (N 220, P 100 g kg^{-1}) 50 kg ha^{-1} was applied at sowing in early June. Sowing rate was 60 kg ha^{-1}. Weed control comprised pre-emergent application of *Logran®* (triasulfuron) at 35 g ha^{-1}. The trial was harvested in late November. Minnipa had 219 mm of rain during the growing season, with an annual total of 278 mm.

Treatment: Soil Se application: Same selenate form and rates as used in the Charlick trial, applied pre-sowing in solution, with the equivalent of 300 L ha^{-1} of water, using a sprayer mounted on a 4-wheel motorbike.
Foliar Se application: Selenate was applied at the same rate as in the Charlick trial, with 1.0 L water per plot, equivalent to 625 L ha^{-1}, using a back-pack sprayer with hand-held mister. Surfactant was not used.

Trial sampling and analysis: In early September (at Zadok stage 48) leaf samples were taken from replicates 2 and 3 at Minnipa and replicates 2 and 3 (Kukri) at Charlick, and analysed by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent Technologies 7500c, Japan) for Se, and ICP optical emission spectrometry (ICP-OES) (Ciros, Spectro Analytical Instruments, Cleve, Germany) for S, Fe, Mn, B, Cu, Mo, Zn, Ca, Mg, Na, K, and P, in order to see whether Se treatment influenced the concentrations of any of these minerals in the leaves. After harvest, the grain from each trial was weighed to determine yield and a representative sample from each plot was analysed by ICP-MS (Se concentration below 1 mg kg^{-1}) or hydride ICP-OES (Se concentration above 1 mg kg^{-1}). In addition, selected samples were analysed by ICP-OES for S, Fe, Mn, B, Cu, Mo, Zn, Ca, Mg, Na, K, and P to see whether Se treatment influenced the concentrations of any of these minerals in the grain, and protein concentration was determined by Near Infrared analysis, using a Perten Instruments *DS 7000.* Furthermore, selected samples were tested for germination and root/shoot growth over 48 hours to evaluate the effect of grain Se concentration. Twenty seeds from each sample were placed on 11.4 cm ashless filter paper with 15 ml Milli-Q pure water in Petri dishes in an incubator at 25^{0}C. Germination was assessed, and shoot and total root length measured.

Statistical analysis: An ANOVA was performed for each response: grain Se concentration and grain yield. The analyses were performed in GenStat 6th Edition (Lawes Agricultural Trust, Rothamsted Experimental Station, UK)

Effects of S, N and variety on grain Se, yield and protein

A field trial was designed as a 5x3x3 factorial within a split-split-plot design with two blocks, and conducted at the Charlick site described above. Five wheat varieties, which included three popular commercial bread wheats (Krichauff; Kukri; Yitpi: hard grade) and two durum (pasta) wheats (Tamaroi, a popular commercial variety, and a boron (B)-tolerant University of Adelaide-Waite Campus breeder's line, WD 99006) were randomly allocated to the five whole plots within each block. The whole plots were divided into three sub-plots and randomly assigned high, medium or low N treatments. These sub-plots were further divided into three sub-sub-plots and randomly allocated with high, medium or low S treatments. The sub-sub-plots comprised 6 rows with 15 cm spacings x 5 metres, with 35 cm between the sub-sub-plots, with a final harvested area of 3.5 m^2. Mean sowing rate was 72 kg ha^{-1} (190-200 seeds m^{-2} allowing for variation in seed size).

In order to determine whether grain Se concentration of wheat grown in the trial was influenced by Se levels in the seed sown (which had been sourced from two South Australian sites), samples of the seed grain were analysed for Se concentration by hydride-ICP-OES with the following results (Se in µg kg^{-1}): Krichauff 160; Tamaroi 110; WD 99006 78; Kukri 53; Yitpi 33. It was subsequently found that Se concentration of grain grown in this trial was not associated with Se concentration in the seed sown.

Basal fertiliser (N 220, P 120 g kg^{-1}) 110 kg ha^{-1} applied at sowing equated to N 25 kg ha^{-1}. The trial was sown in late June. Weed control comprised an application of *Buctril MA®* (200 g L^{-1} bromoxynil + 200 g L^{-1} MCPA) for broadleaf weeds four weeks after emergence.

Treatments:
Sulphur:
Low: no S applied.
Medium: S 30 kg ha^{-1} (supplied as food-grade gypsum [$CaSO_4.2H_2O$], 990 g kg^{-1} pure, S 193 g kg^{-1}, pH 7.0, ACE Chemicals, Adelaide).
The gypsum was spread evenly on the soil surface of the relevant plots immediately after seeding, using a small spreader.
High: S 60 kg ha^{-1} (as gypsum). Applied to the soil surface immediately after seeding.
Nitrogen:
Low: N 25 kg N ha^{-1} (supplied as ammonium nitrate) applied at seeding.
Medium: N 50 kg N ha^{-1}, comprising N 25 kg ha^{-1} at seeding (as for the low N treatment), N 12.5 kg ha^{-1} as foliar urea (*Incitec Liquifert Lo-Bi®*urea: 2.1 kg per 20 L water, plus a non-ionic surfactant [NuFarm *ChemWet 1000®* applied at 100 ml per 100 L water] applied using the two-wheeled plot sprayer described earlier) immediately after anthesis (Zadok stage 69), and N 12.5 kg ha^{-1} applied as foliar urea at the mid-milky dough stage (Zadok stage 76).
High: N 75 kg ha^{-1}, comprising N 25 kg ha^{-1} at seeding (as for the low N treatment), N 25 kg ha^{-1} as foliar urea at Zadok stage 69, and N 25 kg ha^{-1} at Zadok stage 76.
To ensure that no Se was being inadvertently applied with the different treatments, the urea, gypsum and surfactant were analysed by hydride-ICPOES and no Se was detected (detection limit 1 µg kg^{-1}).

In the 2001 season, although the rainfall was little above average (total of 427 mm; growing season 307 mm), relatively heavy rainfall during October and November resulted in high yields. The plots were harvested in mid-December, the grain was weighed to determine yield and a representative sample from each plot was analysed by hydride-ICP-OES for Se and by ICP-OES for S, Fe, Mn, B, Cu, Mo, Zn, Ca, Mg, Na, K, and

P. Protein concentration was determined by Near Infrared analysis.

Statistical analysis: An ANOVA was performed for each response: grain Se concentration, grain S concentration, yield and protein, and regression analysis for S and Se, protein and Se, protein and S, and Se and yield. The analyses were performed in GenStat 6th Edition. ANOVA may not account for all spatial variation. Therefore, for Se concentration (which varied 3.3-fold in control plots), a spatial analysis was performed and the results compared to those produced from ANOVA. The spatial analysis of this response produced the same results as the ANOVA and so is not presented here.

Results and Discussion

Se biofortification studies:

Grain yield: Due to low rainfall at both sites (Charlick 289 mm, Minnipa 278), grain yields were below average, and well below those of 2001, a relatively wet year.

Yield was higher at Charlick (Krichauff 1.83 t ha^{-1}; Kukri 1.71 t ha^{-1}) than at Minnipa (Krichauff 1.42 t ha^{-1}). The yields of the two varieties grown at Charlick were not significantly different at the 5% level. Neither Se application rate nor method affected grain yield at either site, and in a small pilot trial at Charlick we were able to grow wheat with a grain Se concentration of 25 mg kg^{-1}, with no yield reduction, by applying selenate to the soil at seeding at 1000 g ha^{-1}.

In a Russian study comparing the response of several wheat cultivars to applied Se (in the form of sodium selenite seed treatment) yield of cv. Ivolga increased and that of cv. Moscow 35 decreased[29]. On the other hand, no yield reduction was found in a trial using Timothy Grass (*Phleum pratense* L.) even at the relatively high rate of Se 500 g ha^{-1} applied to the soil[30]. No correlation between grain Se concentration and yield have been reported in most studies [31,32], and with the addition of the findings of the current study we conclude that it is unlikely that soil or foliar application of sodium selenate at rates which provide Se up to 150 g ha^{-1} would affect grain yield or total dry matter yield under most conditions.

Seed viability: The germination and early growth trial of selected seed from the Charlick field trial yielded no significant difference in germination percentage, or root or shoot growth over 48 hours for seed lots with Se concentrations ranging from 0.04-9.2 mg kg^{-1}, a 230-fold variation. Indeed, the seed with the (numerically, but non-significantly) longest roots and shoots contained Se 9.2 mg kg^{-1}. However, the seed with the highest Se concentration, 25 mg kg^{-1}, exhibited Se toxicity, with reduced germination (65% compared to a mean of 93% for all other samples) and lower root and shoot growth.

Grain Se concentration: Although the yield in the Charlick trial was only half that of the previous year, the grain Se concentration was the same (61 µg kg^{-1} in 2001 v 63 µg kg^{-1} in 2002), so there was no dilution effect due to the higher yield in 2001. In contrast, a significant negative correlation between rainfall (and hence yield) and wheat grain Se concentration was found in two other Australian studies[10,33], while the opposite was found in two European studies[31,32]. It is likely that not only available soil Se concentration but also S concentration and the balance of different Se forms in the soil affect plant Se concentration as well as rainfall distribution and amount. From Table 3 it can be seen that, at both sites, Se application rate (p<0.001) and method (p<0.001) are significant determinants of grain Se concentration. In all cases, grain Se concentration increased progressively with applied Se.

Table 3. Effect of rate and application method of Se on wheat grain Se concentration (mg kg^{-1}) at two South Australian sites in 2002.

Se rate	Charlick		Minnipa	
g ha^{-1} selenate	Soil	Foliar	Soil	Foliar
0	0.05	0.07	0.60	0.65
10	0.21	0.15	1.05	0.88
30	0.36	0.35	0.94	1.13
100	2.13	0.60	2.73	1.78
300	8.33	1.24	11.95	3.58

Wheat cultivar: Krichauff
Means of four replicates
SE of differences in means: 0.40 (Charlick) and 0.60 (Minnipa)
LSD: 0.80 (Charlick) and 1.22 (Minnipa)

Application of sodium selenate to the soil at seeding was clearly more effective at increasing grain Se concentration than was post-anthesis foliar application of selenate (p<0.001). The highest grain Se concentration (15 mg kg^{-1}) was recorded in one of the Minnipa selenate 300 g ha^{-1} soil treatment plots.

The mobility of Se in soils depends upon soil pH, oxidation potential, calcium carbonate, cation exchange capacity[28], organic carbon, Fe and aluminium levels[34]. The Minnipa soil, when compared to the Charlick soil, is higher in pH and lower in S (which competes with Se for uptake), Fe and organic carbon (which bind Se).

In alkaline soils such as that at Minnipa, most Se is present as selenates, which are highly soluble and easily taken up by plants[35]. It is commonly stated in the literature that a total soil Se concentration of 0.1-0.6 mg kg^{-1} is considered deficient[36], however, this may need to be revised in view of the level of < 0.2 mg kg^{-1} found in the Minnipa soil, which produced wheat with Se 0.63 mg kg^{-1} in the grain, the highest our group has found in South Australia. We have found up to a 150-fold variation in available Se in soils analysed with total Se of < 0.2 mg kg^{-1} (Lyons et al. unpublished). Se deficiency, on the other hand, can be expected in acid soils derived from igneous parent materials[37].

The Minnipa soil had an available soil Se concentration around ten times that at Charlick, and for each application rate and method, the wheat in the Minnipa trial had a higher grain Se concentration than that grown at Charlick. However, due to the low baseline available soil Se concentration at Charlick (that produced control plot grain with 63 µg kg^{-1} Se), Se application had a proportionately larger effect at this site. Soil application of selenate 300 g ha^{-1}, for example, at Charlick increased grain Se concentration 133-fold, compared to 20-fold at Minnipa. For foliar application of selenate 300 g ha^{-1} the increase was 20-fold at Charlick and 6-fold at Minnipa. Also, given the large difference in soil characteristics of the two sites, it was expected that the grain Se concentrations for the foliar Se applications would have been closer for the two sites than those for the soil Se applications[38], but the opposite was the case.

Neither the concentrations of other minerals analysed in the grain nor protein were affected by the application of Se. In a study carried out in Finland the concentration of S (the mineral most likely to be influenced by Se fertilisation) was unaffected even by the application of 500 g ha^{-1} of Se as selenite or selenate[39]. However, in the current study the concentration of S in the leaves appears to have been increased by the highest soil-Se application rate (selenate 300 g ha^{-1}, or Se 120 g ha^{-1}) by 100% over the mean S concentration for all other soil-Se treatments and controls at Minnipa, and by 20% at Charlick. This effect has been observed by our group in trials using wheat, *Arabidopsis thaliana* L. Heynh., *Astragalus* species and radishes (*Raphanus sativum* L.) (Lyons et al. unpublished), and by others in *Arabidopsis,* where an increase in mRNA for the main S transporter, *Sultr 1;1* was observed. This effect was explained as a reduction in sulphate in the roots caused by selenate, which triggers the S deficiency response of *Sultr 1;1,* thus increasing the transport of sulphate from roots to leaves[40]. In the current trial, Se application did not affect the leaf concentrations of any other minerals analysed.

Although grain Se concentrations were not significantly different at the 0, 10 and 30 g ha^{-1} soil selenate applications and the 10, 30 and 100 g ha^{-1} foliar selenate applications due to large variances, nevertheless, the arithmetic means also differ by large factors. For example at Charlick soil-applied selenate at 10 g ha^{-1} raised grain Se concentration more than four-fold, from 50 to 210 µg kg^{-1}.

The grain Se concentrations obtained in this trial fall within the range reported in earlier studies in other countries. Generally, selenate application of 10 g ha^{-1} can increase Se of cereal crops[2] from a base grain concentration of 30-100 µg kg^{-1} to 300-500 µg kg^{-1}. Few studies have used rates as high as selenate 300 g ha^{-1} (Se 120 g ha^{-1}).

Sodium selenite was not used in this trial as most studies have shown that selenate, whether applied to the soil or the foliage, is much more effective[24,41-43]. For example, on a fine sandy loam of pH 6.0, selenate 10 g ha^{-1} applied to the soil raised barley grain Se from 33 to 234 µg kg^{-1}, while selenite 10g ha^{-1} caused no increase[42]. In many soils, selenite is readily adsorbed on clay colloids and becomes unavailable to plants. At both experimental sites in this study, basal Se application at seeding was more effective than post-anthesis foliar application at all rates except selenate 30 g ha^{-1}, where they were the same. In a study carried out in New Zealand, on the other hand, foliar selenate application at ear emergence was more effective than seed coating or prills. However, rainfall and grain yield were much higher than in our trial[44]. The relative effectiveness of soil or foliar application of Se depends on Se form, soil characteristics, rainfall, method of basal application, and time of foliar application. Yläranta[39] found basal and foliar selenate to be equally effective at the low rate (10 g ha^{-1}) but foliar application was more effective at 50 g ha^{-1}.

In a higher rainfall year it is likely that the foliar Se would have been more effective than observed in the current trial[32], as even at Zadok stage 75 (medium milk stage) extensive leaf yellowing was evident. This suggests that, for most of Australia's wheat-growing areas, which are characterised by soils within a pH(H_2O) range of 6.5-8.0 and moisture stress from flowering to harvest, basal Se application would generally be more effective. This method also precludes machinery damage to the crop. On the other hand, for soils of low pH with high Fe concentrations, foliar Se application would generally be preferable.

Table 4. Apparent Se recoveries. Total Se content of wheat grain expressed as a proportion of Se applied at different rates and application methods at two field sites (%).

	Se rate (selenate g ha^{-1})							
	10		30		100		300	
	soil	foliar	soil	foliar	soil	foliar	soil	foliar
Charlick[1]	6.5	3.8	4.5	3.0	9.5	2.5	12.8	1.8
Minnipa	13.5	9.3	3.8	6.0	7.4	4.2	13.5	3.5

[1]Data for cv. Krichauff only

Although the main effect of variety was not significant (p=0.17), Krichauff appeared to accumulate more Se at each treatment level. As Krichauff yielded numerically higher than Kukri in this trial (1.83 v 1.71 t ha^{-1}), the total grain Se content was substantially higher for Krichauff, for example, at the 300 g ha^{-1} soil application: Se 15.2 g ha^{-1} (Krichauff) v 10.5 g ha^{-1} (Kukri). Evidence for varietal variation in response to different levels of applied Se is lacking in the literature, apart from the results of a Russian study that suggested that wheat cultivars may vary in their response to applied Se, from growth stimulation to inhibition[29].

Se biofortification: recovery, economics, commercial application, and anti-cancer effect: From Table 4 an overall mean Se recovery (total grain Se content as a proportion of applied Se, taking into account the proportion of grain Se due to native soil Se, and the atomic weight of Se as 0.4 x the molecular weight of sodium selenate) can be determined, namely 6.6% (range 1.8-13.5%).

Soil application was more efficient than foliar (8.9% v 4.3%) and there was a location difference (Minnipa 7.7% v Charlick 5.6%). The most efficient application was Se applied to the soil at selenate 300 g ha^{-1} (Se 120 g ha^{-1}). In other studies where lower Se rates were used similar conversion efficiencies: 4.4% (foliar higher than soil-applied; site difference)[44] and 4.7-8.5% (soil-applied higher than foliar; site differences)[38] were reported. In these studies the mean recovery of applied Se in straw was 3.3%.

Our South Australian field trials confirm the findings in overseas studies that wheat grain Se concentration can be increased substantially by the application of relatively small amounts of selenate to different soil types. Table 5 shows the estimated cost of achieving target grain Se concentrations of 1 and 10 mg kg^{-1} at the two trial sites (yield around 1.6 t ha^{-1}).

At Minnipa, a site with a relatively high native soil available Se level, the material cost of increasing grain Se concentration to 1 mg kg^{-1} is very low. Moreover, if the Se was applied together with a pre-emergent herbicide there would be no additional labour and fuel costs. Given that the 300 g ha^{-1} soil selenate treatment provided the highest Se conversion

Table 5. Estimated rate and cost of soil-applied Se to achieve grain Se concentration targets of 1 and 10 mg kg^{-1} at two sites.

	Se 1 mg kg^{-1}		Se 10 mg kg^{-1}	
Site	Selenate g ha^{-1}	Cost \$ ha^{-1}	Selenate g ha^{-1}	Cost \$ ha^{-1}
Charlick	63	3.20	375	19.00
Minnipa	10	0.50	250	13.00

Sodium selenate cost: A\$50 kg^{-1}.

efficiency in these trials, it may be best to treat a relatively small area with a high Se rate, then blend this grain with average Se grain to achieve the desired Se concentration.

Commercial application of this research could be undertaken by food companies that would contract growers to apply Se at the required rate. The Se concentration of 1 mg kg^{-1} would be a desirable level in a "high-Se" breakfast cereal, flour, pasta or bread, as it is well within food regulatory guidelines and, based on average consumption, would significantly increase a consumer's daily Se intake. As the evidence for Se's importance in reducing risk of cancer and viral diseases builds[2-4], growers who produce high-Se wheat could eventually receive premium prices. It is reported that growers in South Dakota have been paid US$10-15 per bushel by European buyers for wheat with Se concentration around 10 mg kg^{-1}, while average wheat sells for around US$3 per bushel[45].

In a US trial that investigated the effect of different Se forms on the proliferation of colon cancer precursors in carcinogen-treated rats, high-Se wheat sourced from South Dakota was the most effective anti-cancer treatment. This wheat, which provided Se 2 mg kg^{-1} of diet, reduced the number of aberrant crypts by 37%, while sodium selenite had no effect[46]. Moreover, Se in wheat biofortified by either soil or foliar Se application was found to be more bioavailable (calculated as the mean of four criteria) than that from Dakota-grown wheat that was naturally high in Se[47]. Further animal and human trials to investigate the effect of high-Se wheat on different cancers are warranted.

Residual Se: Residual levels of Se following fertilisation were not investigated in this study; however, other studies using a range of soil types and pH have found the residual effect to be very low in the following year[24,34,41-43], even when Se was applied at the high rate[39] of 500 g ha^{-1}. In New Zealand, where Se fertilisation has been practised for over 30 years, no Se build-up has occurred, and positive responses continue to be obtained from its application[48]. It would appear that the risk of environmental Se accumulation due to responsible agricultural use is low.

Effects of S, N and variety on grain Se, S, yield and protein

Selenium: The main effect of S was the only significant response (p=0.02) in grain Se concentration in this 3-way factorial study in the field at Charlick in 2001 (Table 6). It can be concluded that Se is found in significantly higher concentrations, on average, in plots with no applied S. The numerically lowest Se concentration (13 µg kg^{-1}) occurred in the high S, low N Yitpi block 2 plot, while the highest Se concentration (130 µg kg^{-1}) occurred in the low S, medium N WD 99006 block 1 plot. There is no significant difference in Se concentration between plots with high (60 kg ha^{-1}) or medium (30 kg ha^{-1}) applications of S. The Se concentration in grain grown on control plots (no added S or N) was found to vary 3.3-fold (30-100 µg kg^{-1}). At other trial sites our group has found up to a 10-fold within-site, within-cultivar variation in background Se level.

Table 6. Effect of applied S on wheat grain Se concentration.

S (kg ha^{-1})	Grain Se (µg kg^{-1})
0	57
30	48
60	45

SE of difference of means: 4.2; LSD: 8.4.

The finding of a reduction in Se uptake due to S application is common in previous studies, regardless of soil type and pH. For example, Dhillon and Dhillon added gypsum (at higher rates than in the current study) to a seleniferous soil and found reductions of wheat grain Se concentration of 27%, 53% and 55% for application rates of gypsum 200, 800 and 3,200 kg ha^{-1}, respectively[18]. In Australia, S is applied in large amounts as superphosphate, potassium sulphate, sulphate of ammonia and, for sodic soils, gypsum. The current study shows that even a relatively low S application of 30 kg ha^{-1} can reduce grain Se concentration and content by 16%. A study in the UK[49] found an even greater reduction in Se concentration (33%) with the addition of just 20 kg ha^{-1}. Such losses (up to 54% with gypsum commonly applied at more than 1,000 kg ha^{-1}) in the food chain are likely to be medically important.

Although Australia probably does not need to follow Finland's example and legislate to add Se to all crop and pasture fertilisers, it may be necessary to add Se to S-containing fertilisers and gypsum in order to ensure that the Se concentration in wheat, in particular, and hence population intake, does not decline in future.

The reduction in plant Se level due to increased S supply is largely due to competitive inhibition of the sulphate transporter[14,20,50]. However, this effect is not limited to selenate. In another study it was found that increasing sulphate from 0.25 mM to 10 mM inhibited selenite and selenomethionine uptake by 33% and 15-25%, respectively, as well as inhibiting selenate uptake by 90%, in broccoli, Indian mustard, sugarbeet and rice[51]. This suggests that other mechanisms are involved in the inhibition of Se uptake by S. Moreover, field trials have showed that sulphate increases leaching of selenate, also reducing its uptake by plants[52]. A lack of effect of N or variety on grain Se concentration as found in our study has been found also in previous studies[25,53]. Others have reported significant differences due to wheat variety[29], and while we believe genotypic differences in uptake efficiency for Se must exist, our concern is that at least in Australia, the metre to metre soil variation already mentioned is too great to allow selection for moderate differences due to genetic control of uptake.

Sulphur: While applied S did not affect grain S concentration, both late-applied N and variety did. N at both rates strongly increased grain S concentration (p= 0.001), a finding supported by other studies[54,55]. A large proportion of grain S is protein-bound, and S and protein were positively correlated (r=0.55). Variety was also found to affect S concentration (p=0.005), with the bread wheats (mean S 1386 mg kg^{-1}) higher than the durum wheats (1293 mg kg^{-1}). Although variety did not appear to be a significant determinant of grain Se concentration (see above), the durum wheats were numerically higher in Se (mean Se 59 µg kg^{-1}) than the bread wheats (44 µg kg^{-1}), a finding consistent with the mechanism of Se substitution for S in the amino acids methionine and cysteine. It is possible, provided the concentrations of S-containing amino acids are the same for bread and durum wheats, that durum wheats could incorporate a higher proportion of Se in these amino acids than bread wheats, but this remains to be tested, and in surveys of Se in South Australian-grown wheat, such differences have not been apparent (Lyons et al. unpublished), and in this trial grain Se and grain S were not associated.

Yield: The mean yield for this trial was 3.7 t ha^{-1}, which is well above average for this region, due to relatively high late-season rainfall. Neither S, N nor variety affected grain yield in this trial. N was applied after flowering in order to increase grain protein. Had it been applied early in the season we would have expected a yield response[24]. There was no association between grain Se concentration and yield, which has been found previously[31].

Protein: Protein concentrations ranged from 91,000-138,000 mg kg^{-1}, with a grand mean of 115,000 mg kg^{-1}. The main effect of N is highly significant (p=0.001) (Table 7). In this trial, N applied as foliar urea after flowering was an effective way to increase grain protein concentration. There was no association between grain Se concentration and protein.

Table 7. Effect of applied N on wheat grain protein.

N(kg ha^{-1})	Grain protein(mg kg^{-1})
[1]25	106,000
[2]50	117,000
[3]75	123,000

SE of difference of means: 0.14; LSD: 0.27
[1]Applied to soil at seeding as ammonium nitrate
[2]Basal application plus 25 kg N ha^{-1} applied as foliar urea after flowering
[3]Basal application plus 50 kg N ha^{-1} applied as foliar urea after flowering

Conclusions

In the Se biofortification trials grain Se concentration increased progressively with applied Se, whether added to the soil or to the leaves. Se applied to the soil as sodium selenate at seeding was more effective than post-anthesis foliar application at both trial sites. Although the sites had similar total soil Se levels they varied 10-fold in available Se concentration, as evidenced by grain Se concentration. Due to low baseline available soil Se concentration at the lower-pH site, Se application at this site had a proportionately greater effect. The conversion of applied Se to grain Se was higher for soil application and for the heavier application rates. In Australia's wheat-growing areas, most of which have soils with surface pH(H_20) above 6.5 and crops subject to late-season moisture stress, selenate applied to the soil at seeding is likely to be more effective than foliar application after flowering.

S applied as gypsum to the soil at seeding, even at relatively low rates, can reduce Se concentration in wheat grain. Application of gypsum and high-S fertilisers such as single superphosphate, potassium sulphate and sulphate of ammonia at commercial rates is likely to reduce Se concentration in crops, and it may be necessary in future to add Se to such fertilisers to prevent this.

These trials did not reveal a significant effect of variety on grain Se concentration, although bread wheats were higher in grain S concentration than durum wheats. Although genotypic differences in Se uptake efficiency no doubt exist in wheat, they are likely to be small in comparison with background soil variation.

Grain yield was not affected in these trials by variety or by the application of Se, S or N. Grain protein and S concentration, but not Se concentration, were increased by late-applied urea. No associations were found between grain Se concentration and grain S concentration, protein or yield, or between grain S concentration and protein. Moreover, the concentrations of Fe, Zn, Cu, Mn, B, Ca, Mg, Na, P and K in the grain were not affected by the application of Se, S or N.

The material cost of Se biofortification of a wheat crop is low and the application methods simple. Even a high level of 10 mg kg^{-1} of grain Se, which lowered cancer risk in animal trials[46], could be achieved relatively inexpensively on most soils. Food companies could contract growers to supply high-Se wheat to be used in special breakfast cereal, flour, bread and pasta lines. If the current large clinical trials using Se against cancer, HIV/AIDS and asthma provide further evidence for its effectiveness, such products are likely to be popular.

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