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## Biofortification of UK food crops with selenium

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Se is an essential element for animals. In man low dietary Se intakes are associated with health disorders including oxidative stress-related conditions, reduced fertility and immune functions and an increased risk of cancers. Although the reference nutrient intakes for adult females and males in the UK are 60 and 75 µg Se/d respectively, dietary Se intakes in the UK have declined from >60 µg Se/d in the 1970s to 35 µg Se/d in the 1990s, with a concomitant decline in human Se status. This decline in Se intake and status has been attributed primarily to the replacement of milling wheat having high levels of grain Se and grown on high-Se soils in North America with UK-sourced wheat having low levels of grain Se and grown on low-Se soils. An immediate solution to low dietary Se intake and status is to enrich UK-grown food crops using Se fertilisers (agronomic biofortification). Such a strategy has been adopted with success in Finland. It may also be possible to enrich food crops in the longer term by selecting or breeding crop varieties with enhanced Se-accumulation characteristics (genetic biofortification). The present paper will review the potential for biofortification of UK food crops with Se.

**Agronomy: Diet: Fertilisers: Genetics: Plants: Selenium**

### Physical, chemical and biological properties of selenium

Se is a naturally-occurring oxygen-group (group VIA) element (for review, see Fordyce, 2005). Se has an atomic mass of approximately 79 and six natural isotopes exist, <sup>74</sup>Se, <sup>76</sup>Se, <sup>77</sup>Se, <sup>78</sup>Se, <sup>80</sup>Se and <sup>82</sup>Se. It is a chalcophile ('S-loving') element, replacing S in common sulfide minerals such as pyrite, chalcopyrite, pyrrhotite and sphaerite. It also forms several rare minerals including crookesite ((Cu, Tl, Ag)<sub>2</sub>Se), berzelianite (Cu<sub>2</sub>Se) and tiemannite (HgSe). Se exists in four main oxidation states, -2 (selenide), 0 (elemental Se), +4 (selenite) and +6 (selenate), and is highly mobile under oxidising conditions, although its mobility decreases with decreasing pH (Gondi *et al.* 1992). Se is immobile under reducing conditions; elemental Se or metal selenides will form in conditions of low

H<sup>+</sup> (pH) and electron (pe/Eh; a measure of the tendency of a chemical system to undergo redox reactions) activity. The solution chemistry of Se is principally (oxy)anionic, with selenite (SeO<sub>3</sub><sup>2-</sup>) and selenate (SeO<sub>4</sub><sup>2-</sup>) corresponding to sulfite and sulfate, although elemental Se is also stable over a wide pH range under reducing conditions (Brookins, 1987). Selenate is the major species in soil solution at high redox (pe+pH >15). In the medium redox range (pe+pH 7.5–15) selenite species predominate. Selenide species are stable only at low redox (pe+pH <7.5; Elrashidi *et al.* 1987, 1989). In contrast with S species, the lower redox state (+4; selenite) is more stable than the higher redox state (+6; selenate). Mobile selenate entering a drainage system is readily reduced to selenite if pe/Eh falls, whilst at lower pH levels selenite is likely to be strongly absorbed by hydrous secondary iron oxides and, to a lesser extent, by clays and organic matter.

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Abbreviations: HAST, high-affinity sulfate transporter; Sec, selenocysteine.

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Se is a biologically-active element that can form direct Se–C bonds, which occur in a range of organic compounds including selenoamino acids and selenoproteins. Selenoproteins have essential functional roles in a wide array of prokaryotes, archaeobacteria and eukaryotes (Driscoll & Copeland, 2003; Castellano *et al.* 2004). Se is incorporated into selenoproteins as the twenty-first amino acid selenocysteine (Sec), which is encoded by an UGA codon in the selenoprotein mRNA. Since UGA is normally read as a stop codon, the translation of selenoproteins requires several factors including a *cis*-acting Sec insertion sequence (frequently located in the 3' untranslated region of selenoprotein genes), a novel Sec-charged tRNA that contains the anticodon UCA on which Sec is universally synthesised and additional *trans*-acting factors that allow delivery of Sec-tRNA<sup>Sec</sup> to the ribosome (Driscoll & Copeland, 2003). About twenty-five to thirty Sec-containing proteins have been identified in eukaryotes, although these proteins are not distributed evenly amongst taxa; for example, selenoproteins have not yet been found in yeast and land plants. In organisms whose genomes lack Sec-containing genes selenoprotein homologues occur in which Sec is replaced by cysteine (Castellano *et al.* 2004).

#### Selenium inputs to soils

The Se concentrations of most soils in the world are low (normal range 0.01–2.0 mg Se/kg; mean 0.4 mg Se/kg), although concentrations  $\leq 1200$  mg Se/kg can occur in seleniferous soils (Fordyce, 2005). The Se content of most soils is primarily under geological control, and high-Se soils are associated with particular shales, sandstones, limestones and slate and coal series, including those formed in Cretaceous, Jurassic, Triassic, Carboniferous, Ordovician and Permian periods (Fordyce, 2005). Seleniferous soils are widespread in the Great Plains of the USA, Canada, South America, China and Russia. Other notable inputs of Se to soils include atmospheric deposition of Se originating from volcanic activity, weathering of rocks, sea spray and volatilisation–recycling from biota. All these factors contribute to global Se cycling. In the UK atmospheric Se deposition is approximately 2.2–6.5 g Se/ha per year (Fordyce, 2005). Anthropogenic sources of Se to soils arise from fossil fuel combustion, metal processing, applications of fertilisers, lime and manure, and disposal of sewage sludge (Fordyce, 2005). Se from fossil fuel combustion and metal processing is deposited to soils predominantly in rainwater, which contains 0.00001–0.001 mg Se/l (De Gregori *et al.* 2002). A correlation between the intensity of coal combustion and Se deposition has been observed in the Se content of historical plant samples (Haygarth *et al.* 1993). The use of fertilisers and irrigation water containing Se will also contribute to soil Se inputs in certain areas. For example, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> fertilisers contain  $\leq 36$  mg Se/kg, whilst phosphate rocks and single superphosphate can contain  $\leq 55$  mg Se/kg and  $\leq 25$  mg Se/kg respectively (White *et al.* 2004). Since single superphosphate has generally been replaced by triple superphosphate, which typically

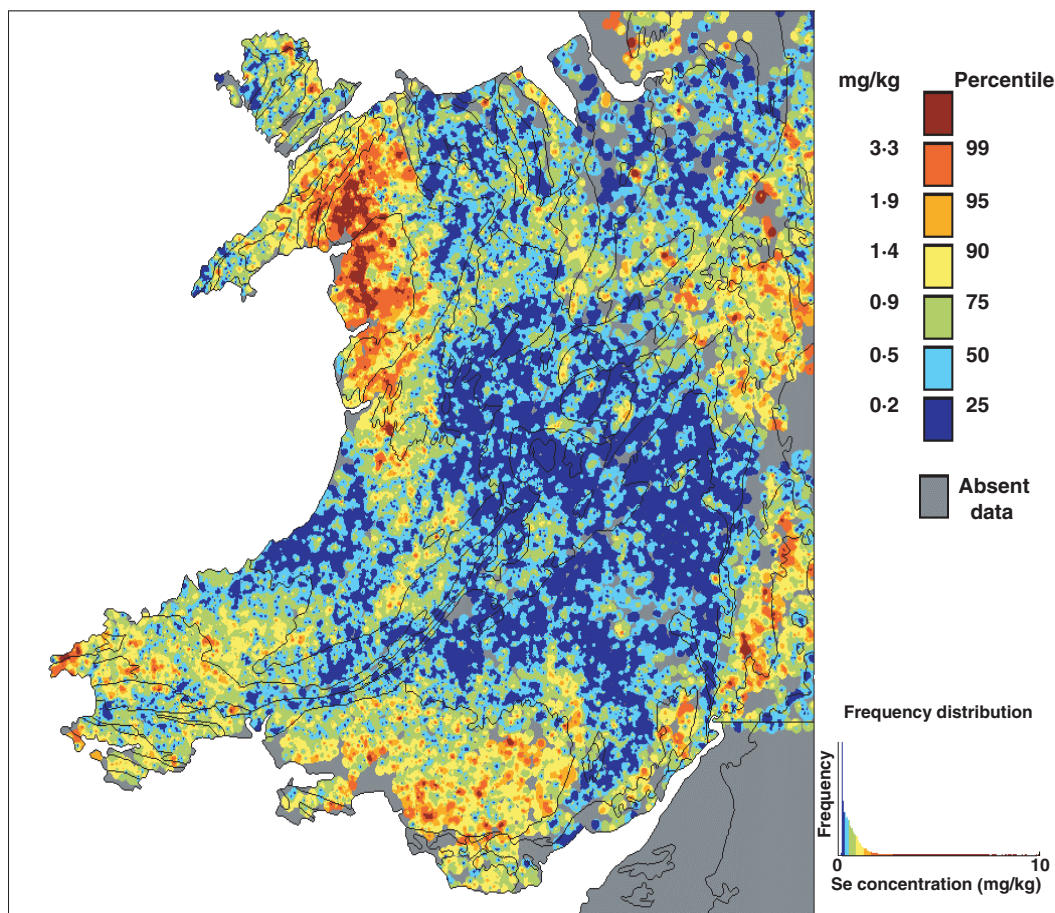
contains  $< 4$  mg Se/kg, fertiliser inputs of Se to soils have fallen in recent years in many parts of the world.

#### Selenium concentration of UK soils and stream sediments

The British Geological Survey has been surveying the baseline geochemistry of UK drainage sediments in streams, water samples and soils since the late 1960s (Geochemical Baseline Survey of the Environment; Johnson & Breward, 2004). For Se, soils and/or stream sediments in Wales and in the Midlands of England have been sampled at a density of one to two samples per km<sup>2</sup>. Samples of stream sediments and surface waters have been analysed for Se in Wales and the west Midlands, whilst in the Humber–Trent drainage basin and in the east Midlands soils, stream sediments and surface waters have been analysed for Se (Fig. 1). From these data it can be observed that total Se in soils and stream sediments in the UK range from 0.1 mg Se/kg to 4 mg Se/kg, with  $> 95\%$  of the UK soils containing  $< 1$  mg Se/kg. Despite the low abundances of this strongly chalcophile element there is a good resolution of Se-enriched features in restricted areas.

In Wales stream-sediment Se concentrations are primarily under geological control, including sulfide mineralisation in Snowdonia, with secondary mixed iron–manganese oxide enrichment in mid-Wales and industrial contamination in the south Wales coalfield and in the industrial west Midlands (Fig. 1(a)). Strong sulfate–selenate associations occur in the Triassic terrain of the Worcester and Stafford basins related to the evaporitic component of the Mercia Mudstone group. In Humber–Trent stream sediments and soils (Fig. 1(b,c)) Se concentrations are high over the basinal Visean deposits and lower parts of the Namurian deposits in the south-west of the region where there is a well-developed Black Shale sequence within the Widmerpool Formation. Se concentrations are also high over the area of the Trent Valley between Newark and Gainsborough, where the Quaternary alluvial sediments may contain material derived from the former area. One isolated Se anomaly is attributable to coal waste adjacent to a power station. Relatively high values also follow the Permian and Jurassic limestone outcrops. Very low Se concentrations are present over the marine alluvium of the coastal strip and the Fens, although natural stream coverage is poor in the latter area. In stream sediments and soils of the east Midlands (Fig. 1(d,e)) high Se levels are prominent at sites over river alluvium in the Dove, Derwent and Trent valleys. These high levels are almost certainly a result of material derived from the Black Shale lithology of the Widmerpool Formation and the hydrothermal sulfide mineralisation of the Peak District. Low Se concentrations occur over Triassic terrain generally, especially to the south of Birmingham and Coventry, with higher concentrations over the lower and middle Jurassic terrain. Elsewhere, high Se concentrations around Birmingham are likely to be industrial in origin, whilst a prominent high-Se feature extending from the north of Grantham to the south of Peterborough may be associated with peat deposits. High Se concentrations in the

(a)



(b)

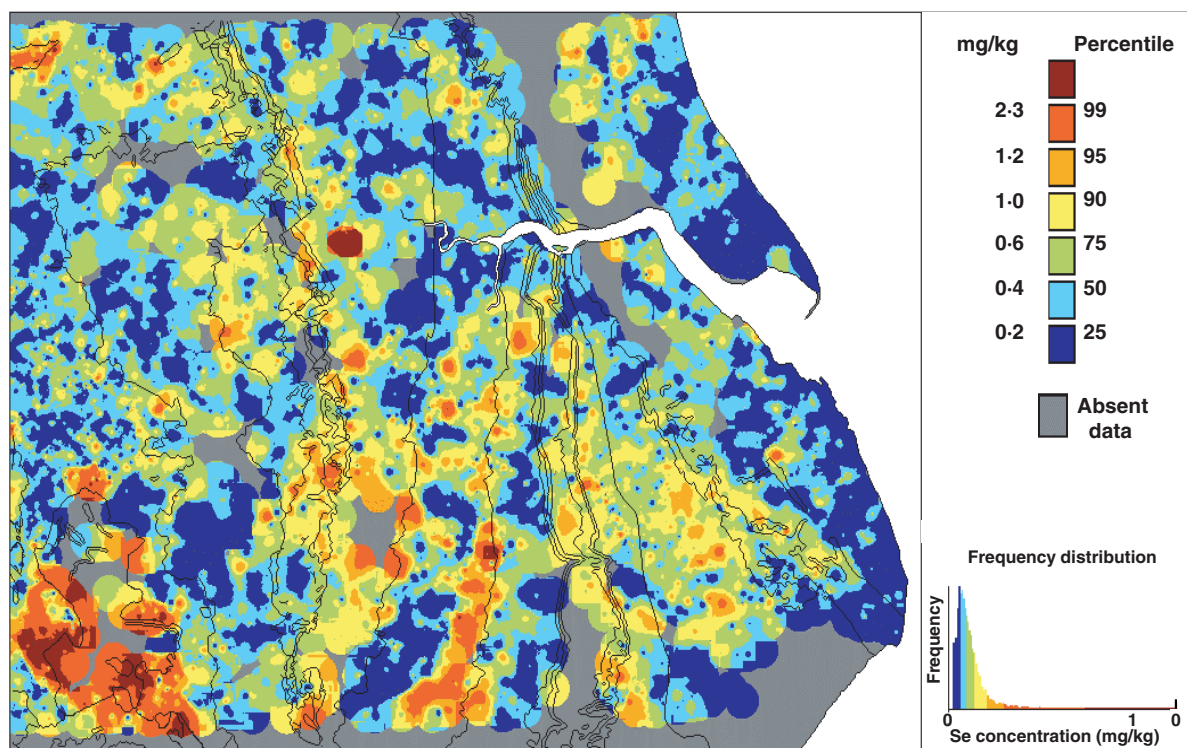


Fig. 1. (Cont.)

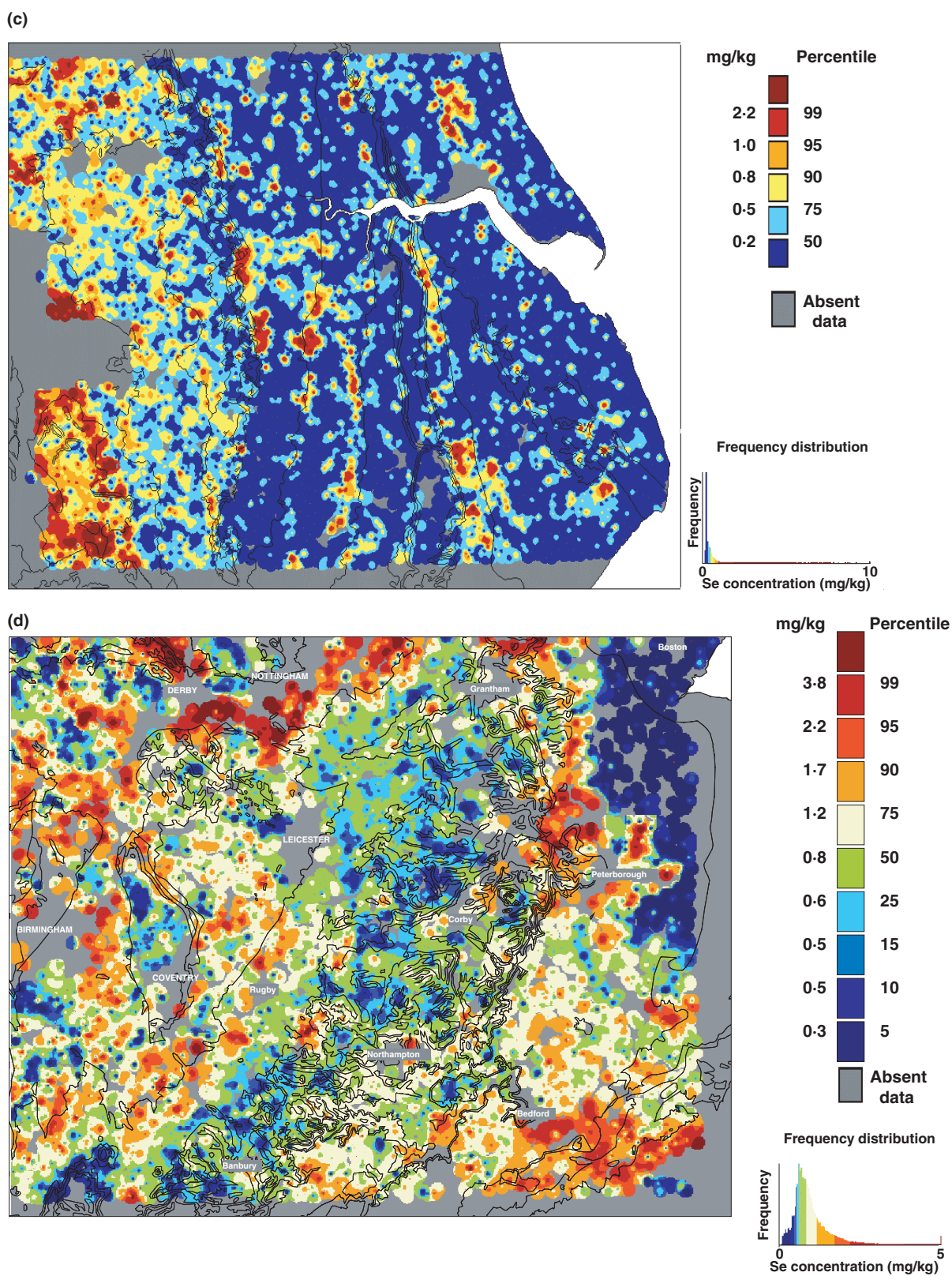
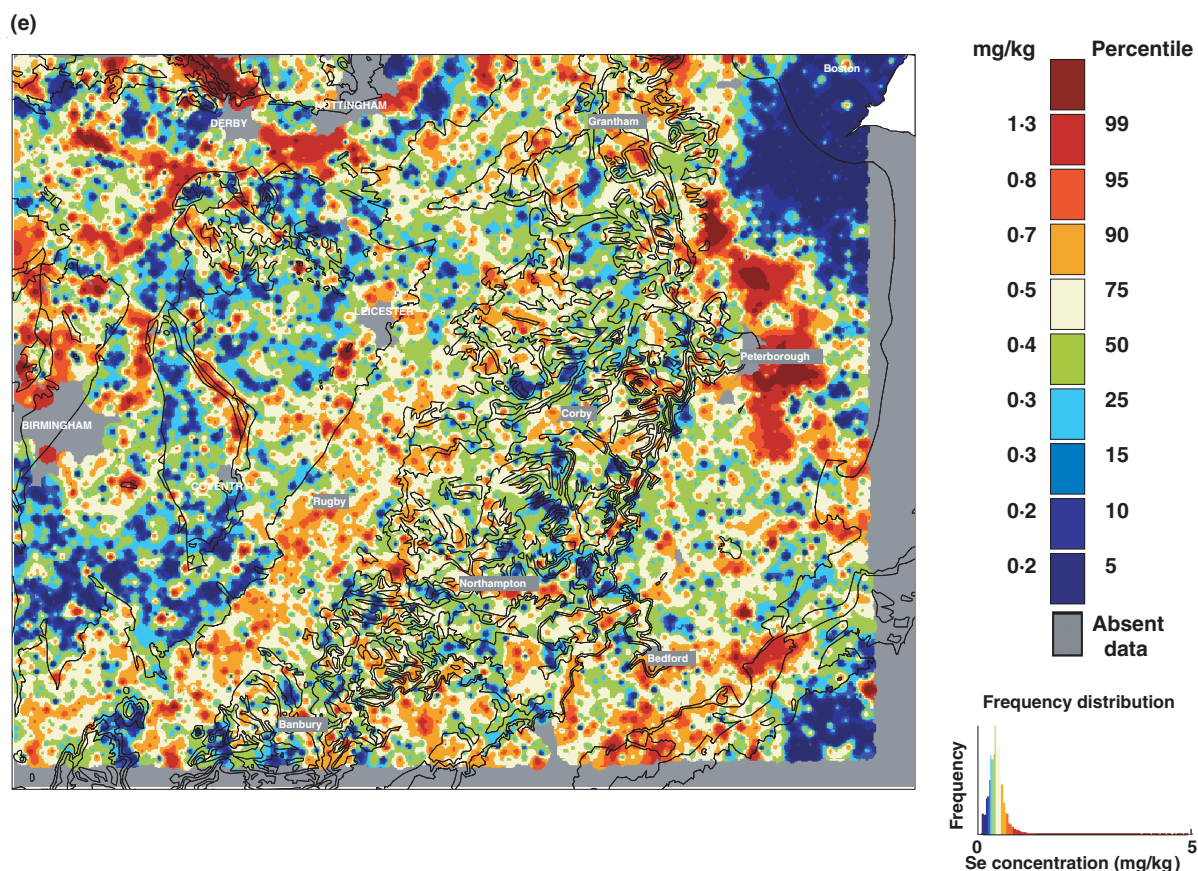


Fig. 1. (Cont.)



**Fig. 1.** Baseline selenium concentrations in UK drainage sediments in streams, water samples and soils from the Geochemical Baseline Survey of the Environment of the British Geological Survey (BGS; Johnson & Breward, 2004). Soils and/or stream sediments were sampled at a density of one to two samples per km<sup>2</sup> in Wales and in the Midlands of England. Samples of stream sediments were analysed for selenium in Wales and the west Midlands of England (a), whilst in the Humber–Trent drainage basin and in the east Midlands of England, stream sediments (b and d respectively) and soils (c and e respectively) were analysed. (Data are reproduced with permission of BGS.)

south-east of the map may be associated with the Bedfordshire brick industry, but this association is not yet certain (Fig. 1(e)).

#### Uptake, assimilation and accumulation of selenium by plants

Se has no proven function in plant nutrition. However, plants take up Se from the soil solution primarily as selenate, and to a much lesser extent as selenite. Selenate enters root cells through sulfate transporters in their plasma membranes (Terry *et al.* 2000; White *et al.* 2004). Sulfate transporters are encoded by a small family of genes in most plant species; these transporters are hydrophobic membrane proteins with twelve predicted membrane-spanning domains and few large extramembrane loops but, generally, with long N and C termini (Hawkesford, 2003, 2005). There are fourteen genes encoding sulfate transporters in the genome of *Arabidopsis thaliana* (L.) Heynh. and a similar number in groups of other plant species (Hawkesford, 2005). All sulfate transporters characterised to date can be placed into one of five groups based on

their protein sequences (Hawkesford, 2003, 2005), with each group having distinct functional characteristics. Group 1 transporters, such as AtSultr1:1, AtSultr1:2 and AtSultr1:3, are high-affinity sulfate transporters (HAST) that are thought to catalyse most selenate influx to plant cells. Sulfate uptake is regulated by gene transcription (Hawkesford, 2005); AtSultr1:1 and AtSultr1:2 catalyse sulfate influx to *Arabidopsis* roots and their expression is induced by S starvation (Takahashi *et al.* 2000; Yoshimoto *et al.* 2002; Maruyama-Nakashita *et al.* 2003). The expression of these HAST also appears to correlate well with plant Se uptake. For example, plants lacking group 1 HAST have reduced selenate uptake (Shibagaki *et al.* 2002), whilst the overexpression of genes encoding group 1 HAST in transgenic plants increases Se uptake (Terry *et al.* 2000). Interestingly, although the expression of genes encoding HAST is generally reduced when a plant has sufficient S, increasing the selenate concentration in the rhizosphere of S-replete plants can increase shoot S concentrations (Bell *et al.* 1992; White *et al.* 2004). This phenomenon has been interpreted as the consequence of either selenate or Se metabolites de-repressing the expression of genes encoding HAST (Takahashi *et al.* 2000;

White *et al.* 2004). Although several of the HAST will transport selenate, the molecular structure responsible for their anionic selectivity is not known.

Following uptake selenate is likely to be transported to the plastids, or may remain in the cytoplasm where it is assimilated via the S assimilation pathway (for reviews of Se assimilation in plants, see Terry *et al.* 2000; Ellis & Salt, 2003; for a review of S assimilation see Hawkesford, 2005). Briefly, selenate is activated by ATP sulphurylase to form adenosine 5'-phosphoselenate, which is reduced to selenite in the presence of adenosine 5'-phosphosulfate reductase, and subsequently to selenide via a non-enzymic step in the presence of glutathione. Selenide is assimilated into Sec and further into selenomethionine. These seleno-amino acids can be incorporated into proteins non-specifically, which can cause toxicity to the plant. Selenoamino acids can also be methylated; for example, Se-methylSec is a characteristic Se assimilation product within species in the genera *Allium* and *Brassica*, which includes numerous crop species of commercial importance including onions, leeks, garlic, oilseed rape and cabbages. Methylated selenoamino acids can be converted to methyl selenol (Lu *et al.* 1995; Ip *et al.* 2002) and ultimately to dimethylselenide and volatilised (Ellis & Salt, 2003). Se assimilation impacts directly on shoot Se accumulation. This relationship is supported by the observations that: (1) overexpression of ATP sulphurylase and/or genes involved in glutathione synthesis in transgenic *Brassica juncea* (L.) Czern. results in increased shoot Se accumulation (Pilon-Smits *et al.* 1999; Bañuelos *et al.* 2005); (2) overexpression of a Sec methyltransferase gene, which methylates Sec to methylSec in *Astragalus bisulcatus* (Hook.) A. Gray results in increased Se accumulation in transgenic *A. thaliana* and *B. juncea* (LeDuc *et al.* 2004); (3) expression of a Sec lyase, which breaks down Sec in mice, in the cytoplasm or in chloroplasts results in increased shoot Se concentration in transgenic *A. thaliana* (Pilon *et al.* 2003).

Flowering plant (angiosperm) species differ in their ability to assimilate and accumulate Se (Rosenfeld & Beath, 1964; Dhillon & Dhillon, 2003; Ellis & Salt, 2003; White *et al.* 2004). These species can be divided into three groups: non-accumulators; Se indicators; Se accumulators. Non-accumulator plants rarely contain >100 µg Se/g DM, Se-indicator plants can contain ≤1000 µg Se/g DM and Se-accumulator plants can contain ≤40 000 µg Se/g DM when sampled from Se-rich environments, e.g. in areas of western USA where soils have been derived from seleniferous shale and sedimentary materials. Also, non-accumulator plants have a lower Se:S in their shoot tissues than Se-accumulator plants (Bell *et al.* 1992; Feist & Parker, 2001). Remarkably, there is often no correlation between the shoot Se and S concentrations of different plant species (or even genotypes of the same species) growing in the same environment (Feist & Parker, 2001; White *et al.* 2004). This finding suggests that the transporters responsible for the uptake or translocation of Se are selective for either sulfate (in non-accumulator plants whose shoot Se:S is lower than that in the rhizosphere solution) or selenate (in Se-accumulator plants whose shoot Se:S ratio is higher than that in the rhizosphere

solution). A selective advantage associated with plant Se accumulation has been suggested, since increased Se concentration protects *B. juncea* against infection with *Fusarium* and *Alternaria* and against herbivory by aphids and caterpillars but not snails (Hanson *et al.* 2003, 2004). Examples of Se hyperaccumulators include members of the Fabaceae (*Astragalus bisulcatus*, *A. racemosus* Pursh), Asteraceae (*Aster occidentalis* (Nutt.) Torr. & A. Gray, *Machaeranthera ramosa* A. Nelson), and Brassicaceae (*Stanleya pinnata* (Pursh) Britton). In Se accumulators Se predominantly occurs as non-protein-amino acid forms, Se-methylSec, and in a conjugated form as γ-glutamyl-methylSec, but also as selenocystathione, selenohomocysteine, γ-glutamyl-selenocystathione, methyl selenol and selenate (Pickering *et al.* 2000, 2003). Extreme Se accumulation (as high as 22 g/kg DM) has been reported in the fruit of some species within the Lecythidaceae family. This neotropical family of trees comprises 325 species within ten genera (Morton *et al.* 1998), including the familiar edible Brazil nut (*Bertholletia excelsa* Humb. & Bonpl.) and less-familiar edible nuts such as the Paradise nut (*Lecythis zabucaja* Aubl.). However, the ingestion of certain other *Lecythis* species, including Coco de Mono (*L. ollaria* Loeffl.) and Sapucaia nut (*L. elliptica* Kunth), can induce acute selenosis in human subjects (Kerdell-Vegas, 1966; Dickson, 1969), characterised by symptoms of hair and nail loss, alongside other dermatological, neurological and gastric disorders (Fordyce 2005). In *B. excelsa* the dominant form of Se is selenomethionine (Vonderheide *et al.* 2002; Kannamkumarath *et al.* 2005).

#### Selenium is an essential element for man

Se was identified as an essential element for mammals in the 1950s (Schwarz & Foltz, 1957). There are approximately thirty mammalian selenoproteins, about half of which have been characterised (Rayman, 2002). These proteins include those with functional roles as antioxidants (e.g. glutathione peroxidase) and those that contribute to protein stability, transcription of mRNA and other biochemical functions. Given these functions it is not surprising that Se is important for human health, and Se deficiency in human subjects has been linked to a plethora of physiological disorders (Rayman, 2000, 2002; Jackson *et al.* 2004). For example, low Se status causes Keshan disease (a cardiomyopathy) and Kashin-Beck disease (an osteoarthritis disorder) in parts of China where dietary Se intakes are extremely low (Fordyce, 2005). As a result of its role as an antioxidant, low Se status has also been linked to CVD, pancreatitis, asthma and inflammatory response syndrome (Rayman, 2000, 2002). Further, there is evidence that low Se status impacts on immune system functioning, response to viral infection, female (e.g. reduced rates of miscarriage) and male (e.g. sperm development and function) fertility and thyroid functioning if Se and I status are both deficient (Rayman, 2002).

There is substantial evidence that Se is a potent anti-carcinogen when it is present at levels above those required for the maximal expression of selenoproteins, i.e. well above those levels associated with incipient Se deficiency

(Combs, 2005). Indeed, both organic and inorganic forms of Se consistently inhibit cell growth and stimulate programmed cell death *in vitro*, whilst there is supporting (albeit inconclusive) epidemiological evidence of inverse relationships between Se status and the incidence of cancer (Combs, 2005). For example, in a randomised clinical trial 200 µg Se/d has been shown to reduce total cancer incidences by a factor of 0.63 and to reduce incidences of carcinomas of the prostate and colon–rectum by 0.51 and 0.46 respectively (Clark *et al.* 1996, 1998; Combs, 2005).

### Selenium intake and status have declined in the UK

Individual Se intakes range from 3 to 7000 µg Se/d worldwide, although most intakes are at the lower end of this distribution (Fordyce, 2005). Se intake in the UK has declined from >60 µg Se/d in 1974 to 29–39 µg Se/d (for reviews, see Rayman, 1997, 2000, 2002, 2004). In several other EU countries Se intakes are less than half the UK reference nutrient intakes of 60 and 75 µg Se/d for females and males respectively (Rayman, 2004). A concomitant decline in Se status based on analysis of blood and serum has been reported, and thus the UK population may be at risk from an increased prevalence of certain health disorders (Rayman, 1997, 2000, 2002, 2004). Cereals represent a major source of Se in most UK diets, and the decline in Se intake and status in the UK has been attributed to changes in the sourcing of the wheat used for flour production from grains that are high in Se concentration and grown on high-Se soils in North America to UK-sourced wheat that has been grown on low-Se soils and has a low grain Se content. The use of UK wheat in grists has risen from 15% in the 1950s to >80% in 2005 (Lea, 2005).

The baseline Se concentration of wheat grain used in UK bread has been analysed in samples taken in 1982, 1992 and 1998 (Adams *et al.* 2002), and a minimal difference in mean grain Se concentration between these samples has been found (0.025, 0.033 and 0.025 mg Se/kg respectively, with interquartile ranges varying from 0.015 to 0.019 mg Se/kg). By comparison, mean values of 0.370 and 0.457 mg Se/kg have been reported for US wheat grain and 0.760 mg Se/kg for Canadian grain (Adams *et al.* 2002). Worldwide, the Se content of wheat grain ranges from 0.001 to 30 mg/kg, being predominantly within the range 0.020–0.600 mg Se/kg (Lyons *et al.* 2005a). It is important to emphasise that the primary reason for higher concentrations of Se in US and Canadian wheat grain than in wheat grain from the UK is a result of differences in the underlying geology and consequent higher Se concentrations in the North American soils. It is less related to differences in historical or current agronomic practices or to soil nutrient depletion. Consistent with the hypothesis of a link between Se content of wheat and dietary Se intakes and status is the observation that in New Zealand Se intakes and status increased when Australian wheat containing higher levels of Se were imported (Watkinson, 1981; Thomson & Robinson, 1996). In addition to changes in the sourcing of wheat grain, levels of Se in UK diets may also have declined in recent years because of changes

in fertiliser practices (e.g. replacing single superphosphate with triple superphosphate; White *et al.* 2004), or dilution in the crop as a result of improved yields (Adams *et al.* 2002). Further, since Se and pyritic S concentrations are correlated in coal (Spears *et al.* 1999), declines in Se inputs may also be related to a decline in the intensity of coal combustion following the Clean Air Act of 1956 (Ministry of Housing and Local Government, 1956; Haygarth *et al.* 1993) and/or the use of low-S coal or desulphurisation combustion technologies.

### Biofortifying crops in the UK

The decline in Se intake and status in the UK can be rectified by dietary diversification, mineral supplementation of human subjects or livestock, food fortification during processing or through crop biofortification (Rayman, 2002, 2004). Since foods such as Brazil nuts, offal and crab meat, which naturally contain high levels of Se, are not eaten in great quantities, the potential for dietary diversification to increase Se delivery in the UK is limited (Rayman, 2002). The use of high-Se-containing supplements, including yeast-based formulations, appears to be an effective and a safe option for human subjects (Rayman, 2004). However, supplements are relatively expensive and only a small proportion of the population are likely to take such personal intervention measures, particularly since recent EU legislation restricts the sale of such supplements. During flour processing in the UK statutory nutrients are currently added to flour using a pre-blend that contains CaCO<sub>3</sub>, Fe, thiamin and nicotinamide. Although Se could be fortified through this route, alterations to this blend would require a change in legislation. There is also a food safety issue associated with storing concentrated Se compounds in a mill or a baking environment. The present review will therefore focus on the strategy of biofortification, defined as increasing the bioavailable concentrations of essential elements in edible portions of crop plants through the use of fertilisers (agronomic biofortification) or through crop selection or breeding (genetic biofortification; Graham *et al.* 2001; Bouis, 2003; Bouis *et al.* 2003; Lyons *et al.* 2003).

### Agronomic biofortification

The potential for using Se-containing fertilisers to increase crop Se concentrations, and thus dietary Se intakes, in the UK has been proposed previously (Adams *et al.* 2002; Rayman, 2002; Arthur, 2003). Possible strategies for Se fertilisation in the UK based on data obtained from other parts of the world will be reviewed. Strategies based on fertilising both pasture or forage crops for consumption by livestock and crops intended for direct human consumption will be considered.

Biofortification of pastures or forages using Se fertilisers has been widely demonstrated (for reviews, see Gissel-Nielsen, 1998; Gupta & Gupta, 2002). The primary driver for supplying pastures or forages with increased Se has been to prevent disorders amongst grazing livestock. For example, the muscular dystrophy disorder, white muscle disease, is associated with low-Se-status soils, notably in



Australia, New Zealand and parts of North America (Wichtel, 1998; Lee *et al.* 1999; Fordyce, 2005). In early studies it was demonstrated that  $\text{Na}_2\text{SeO}_4$  or  $\text{K}_2\text{SeO}_4$  is more available for immediate uptake by pasture crops than selenite (Gissel-Nielsen, 1998). However, in the years following Se application selenite or less-soluble forms of selenate (e.g.  $\text{BaSeO}_4$ ) were found to provide longer-lasting effects. For example, in Australia the application of prilled Se fertilisers (i.e. a granulated compound form) to pastures grazed by sheep was shown to increase crop Se concentration and consequently whole-blood and plasma Se concentrations, thereby increasing wool yield and live weights (Whelan *et al.* 1994a,b). In these studies it was found that 3–5 g Se/ha per year supplied as Selcote<sup>®</sup> (comprising 10 g Se/kg as  $\text{Na}_2\text{SeO}_4$ ) provides an adequate Se supply to sheep for 1 year, whilst 10 g Se/ha per year, supplied as slow-release Selcote Two Year<sup>®</sup> (10 g Se/kg as 1:1  $\text{Na}_2\text{SeO}_4$ : $\text{BaSeO}_4$ ; 10 g Se/kg), provides adequate cover for 3 years. In Canada it has been shown that Selcote Ultra<sup>®</sup> (10 g Se/kg as 1:3  $\text{Na}_2\text{SeO}_4$ : $\text{BaSeO}_4$ ) increases Se uptake by lucerne (*Medicago sativa* L.; Gupta, 1995). Crop Se concentration is increased from 0.067 to 0.187 and 0.220 at 5 and 10 g Se/ha per year respectively in the first cut and in the first year after application. Similarly, Se concentration in the first cut of Italian ryegrass (*Lolium multiflorum* Lam.) is increased from 0.067 to 0.231 and 0.292 at 5 and 10 g Se/ha per year respectively. In subsequent years crops grown on soils that have initially received Se fertilisation contain more Se than non-fertilised control crops.

Elsewhere, similar increases in the Se concentration of pasture and forage crops in response to applications of low levels of Se have been reported. For example, in Canada an increase in Se concentrations has been reported for red clover (*Trifolium pratense* L.), timothy (*Phleum pratense* L.) and Italian ryegrass (Gupta & MacLeod, 1994). In the USA the Se concentration of bahia grass (*Paspalum notatum* Flüggé) sprayed with Selcote Ultra<sup>®</sup> at 5 g Se/ha per year has been shown to increase, as has that of fescue (*Festuca* spp.) top-dressed at the same rate (Valle *et al.* 2002). In Ireland Se uptake by ryegrass (*Lolium perenne* L.) has been found to increase from 0.10 and 0.13 Se mg Se/kg dry weight in control plots to 0.62 and 0.19 Se mg Se/kg at first and second cuts respectively in response to 76 g Se as  $\text{Na}_2\text{SeO}_3$ /ha per year (Murphy & Quirke, 1997). Numerous other studies have demonstrated the ease of increasing the Se concentrations of pasture or forage crops using small quantities of Se fertilisers applied directly to the soil or applied as foliar sprays (Peterson & Butler, 1962; Davies & Watkinson, 1966; Cary *et al.* 1967; Watkinson & Davies, 1967; Cary & Allaway, 1969, 1973; Gissel-Nielsen & Bisbjerg, 1970; Gissel-Nielsen, 1977, 1984, 1986; Gupta & Winter, 1981, 1989; Gupta *et al.* 1982; Watkinson, 1983; Gissel-Nielsen *et al.* 1984; Van Dorst & Peterson, 1984; Ylärinta, 1984c; Whelan, 1989; Coutts *et al.* 1990; Rimmer *et al.* 1990; Shand *et al.* 1992; Jukola *et al.* 1996; MacLeod *et al.* 1998; Gupta & Gupta, 2002; Valle *et al.* 2002).

It is clearly possible to increase the Se concentration of pasture and forage crops by Se fertilisation, and commercial products are available for supplying low levels

of Se. Selcote Ultra<sup>®</sup> (Nufarm NZ, Auckland, New Zealand) was first released in 1989–90 to replace previous formulations Selcote<sup>®</sup> and Selcote Two Year<sup>®</sup>, which were released in the early 1980s (M Shirer (AgBioResearch Ltd, Richmond, New Zealand), personal communication; there have been several changes in the ownership of Selcote<sup>®</sup> products, and companies cited in scientific publications include Lime and Marble Ltd, Agtech Developments Ltd, ICI Rural Division, ICI Crop Care, Crop Care, Nufarm NZ and AgBioResearch Ltd, which has been an international distributor of Selcote Ultra<sup>®</sup> since the late 1990s). In New Zealand Se can be applied to a maximum of 10 g Se/ha per year (M Shirer, personal communication). In 1999 Top Stock<sup>®</sup> (Yara UK, Immingham, Lincs., UK), containing 0.012 g Se/kg, was released to the UK market. Recommended application rates for Top Stock<sup>®</sup> are 6.6 g Se/ha, and >25 000 ha grassland in the UK are currently treated with this product.

Biofortification of food crops for human consumption is a more direct strategy to increase dietary Se intake (Gissel-Nielsen, 1998; Gupta & Gupta, 2002). For example, the Se concentrations of wheat grain and flour can easily be raised by applying low concentrations of Se to soils (Euroala *et al.* 1989; Stephen *et al.* 1989; Singh, 1994; Lyons *et al.* 2003, 2004, 2005a,b). This strategy is also applicable to other cereal and grain crops (Gupta & Gupta, 2002); for example, in field-grown rice the foliar application of 20 g Se/ha increases the Se concentrations of rice grain from a control level of 0.071 (SD 0.002) to 0.471 (SD 0.134) and 0.640 (SD 0.191) when Se is supplied as selenite and selenate respectively (Chen *et al.* 2002).

The best example of biofortification of food crops for human consumption using Se fertilisers comes from Finland. As a consequence of low dietary Se intakes the Finnish Ministry of Agriculture and Forestry decided in 1983 that Se would be incorporated into all multinutrient fertilisers used in agriculture from 1 July 1984 onwards (for reviews of the Finnish experience, including the primary literature sources for the Finnish Ministry directives, see Ylärinta, 1984a,b,c; Varo *et al.* 1988; Euroala *et al.* 1989, 1991, 2004; Aro *et al.* 1995; Venäläinen *et al.* 1997; Rayman, 2002; see also references cited therein). The primary aim of the Finnish policy was a 10-fold increase in cereal-grain Se concentration (Venäläinen *et al.* 1997). For grain production and horticulture 16 mg Se/kg was added to multinutrient fertiliser formulations, whilst for fodder crop and hay production 6 mg Se/kg was added. However, since >10-fold increases in shoot Se concentration were reported in the years following initial Se applications, a new directive came into force from 16 June 1990 onwards, and fertilisers containing 16 mg Se/kg were removed from the market and a single level of supplementation of 6 mg Se/kg was used (Venäläinen *et al.* 1997). In 1998 Se supplementation was increased from 6 to 10 mg Se/kg fertiliser for all crops.

The effect of adding Se to fertilisers for crops in Finland has been marked; for example, Se concentrations have increased in 125 indigenous food items (Euroala *et al.* 1991). Notably, the Se concentration of wheat bran has increased 10-fold from 0.03 to 0.35 mg Se/kg DM. The Se content of all fruit and vegetables was <0.01 mg Se/kg DM

before Se supplementation (except lettuce (*Lactuca sativa* L.) and spinach (*Spinacia oleracea* L.), for which Se contents were 0.01 and 0.02 mg/kg DM respectively; Eurola *et al.* 1989). After Se fertilisation  $\geq 10$ -fold (and  $\leq 100$ -fold) increases in the Se concentration of vegetable and fruit crops were reported (Eurola *et al.* 1989). Although the Se concentration of all crops has increased, more subtle effects have also been noted. For example, Se fertilisation has increased the Se concentration of oats (*Avena sativa* L.; Eurola *et al.* 2004). Not surprisingly, the grain Se concentration is greater in crops supplied with fertilisers incorporating Se at 10 mg/kg than at 6 mg/kg, and it is lower in grain from organic production systems in which no fertilisers have been added. The Se concentration of oat grain is higher in seasons of low precipitation, and cultivar differences have been detected in variety trials. The Se concentrations of meat, meat products, fish, dairy products and processed foods have all increased following the introduction of Se fertilisation (Eurola *et al.* 1991); for example, for pig muscle meat and liver the Se concentrations were found to have increased from 0.08 and 0.49 mg/kg respectively in 1985 to a peak of 0.30 and 0.73 mg/kg respectively in 1989 (Venäläinen *et al.* 1997). Similarly, the Se concentrations for cattle muscle and liver increased from 0.07 and 0.28 mg/kg respectively in 1985 to 0.21 and 0.51 mg/kg respectively in 1989.

Based on average Finnish diets, it has been reported (Eurola *et al.* 1991) that there was an increase in Se intake from 25  $\mu\text{g/d per capita}$  in 1975–6 to 124  $\mu\text{g/d per capita}$  in 1989, and an increase in the consumption of Se from all foodstuffs. Notably, for cereals the increase in Se consumption was from 9 to 30  $\mu\text{g/d per capita}$  between 1975–6 and 1989. Over the same period the increase in the Se consumption ( $\mu\text{g/d per capita}$ ) was from 0.4 to 4 for vegetables and fruit, from 7.7 to 45 for meat, from 7.9 to 12 for fish and from 5.4 to 32 for dairy products and eggs (Eurola *et al.* 1991). Furthermore, a concomitant increase in blood (Varo *et al.* 1988) and serum (Wang *et al.* 1998) Se concentrations has been reported. For example, for Finnish children aged <15 years of age, the increase in mean serum Se was from 0.87 (range 0.54–1.44)  $\mu\text{mol/l}$  in 1985 to 1.26 (range 0.96–1.57)  $\mu\text{mol/l}$  in 1986 (Wang *et al.* 1998). Similarly, in adults, serum Se increased from 1.04 (range 0.62–1.35)  $\mu\text{mol/l}$  in 1985 to 1.30 (range 0.87–1.72)  $\mu\text{mol/l}$  in 1986. These increases have been subsequently maintained. Increases in the Se status of Finnish human milk between 1987 and 1995 have also been noted (Kantola & Vartiainen, 2001). Thus, the use of Se fertilisers in Finland has increased the crop Se content, increased dietary Se intakes and increased the Se status of the Finnish population (Rayman, 2002).

### Genetic biofortification

Although it may be possible to increase the consumption of crop species that are genetically predisposed to accumulate more Se, dietary diversification is not always feasible (Rayman, 2002). However, within-species genetic variation could be used to increase the delivery of Se to human diets, i.e. through genetic biofortification (Lyons *et al.*

2004). In the first instance, it may be possible to simply select existing varieties of crops that accumulate more Se. In the longer term, it may be possible to breed crops for increased Se concentration.

There are few data on varietal differences in Se accumulation for most crop species. However, in the most detailed study reported to date Lyons *et al.* (2005a) have surveyed grain Se concentrations in commercial and advanced breeding lines of wheat, in ancestral diploid (*Aegilops tauschii* (Coss.) Schmal.) and durum wheats (*Triticum dicoccum* (Schrank) Schubl.) and their progeny, in wheat landrace accessions, in wheat recombinant inbred and doubled haploid mapping populations and in other cereal grains from barley (*Hordeum vulgare* L.), triticale (*X Triticosecale* Wittmack ex A. Camus.) and rye (*Secale cereale* L.). Grain Se concentrations vary from 0.005 to 0.720 mg Se/kg, although most of the variation in grain Se from these species is attributed to soil factors. However, *A. tauschii* accumulates most Se in the grain and there is therefore the potential to breed for increased grain Se concentration in wheat (Lyons *et al.* 2005a).

In other crops, 4-fold differences in shoot Se accumulation between four *Lycopersicon* cultivars (tomatoes and relatives) grown at low sulfate levels have been reported (Pezzarossa *et al.* 1999). Differences in short-term net Se uptake and shoot Se concentration have also been noted amongst eight *Lycopersicon esculentum* L. cultivars and six other *Lycopersicon* species, with greater variation in shoot Se concentration between wild *Lycopersicon* taxa than between cultivated *Lycopersicon* taxa (Shennan *et al.* 1990). Variation in Se accumulation has also been reported for soyabean (*Glycine max* (L.) Merr.; Yang *et al.* 2003; Zhang *et al.* 2003) and onion (*Allium cepa* L.; Kopsell & Randle, 1997), whilst Se accumulation in rapid-cycling *Brassica oleracea* L. has been shown to be moderately heritable, with expected gains of 5% per cycle of recurrent selection (Kopsell & Randle, 2001).

In non-crop species shoot Se concentrations have been found to vary amongst sixteen populations of *Stanleya pinnata* by 1.4-fold–3.6-fold and shoot Se concentrations correlate with the Se concentration of the soil from which the populations were originally collected (Feist & Parker, 2001).

For a given crop species, if sufficient genetic variation exists in Se accumulation, and if this variation is heritable, traditional breeding programmes could be developed that would provide an alternative to agronomic biofortification and thus minimise the need to use Se fertilisers except at the lowest soil Se concentrations (Lyons *et al.* 2004, 2005a).

In addition to identifying suitable germplasm for breeding, accelerated breeding strategies to increase Se concentration in crops may also be possible. For example, Se accumulation can be quantified in different accessions of model crop or non-crop plant species for which genetic maps are available. Inbred progeny from crosses between these two accessions can then be used to map Se accumulation to specific chromosomal loci, i.e. quantitative trait loci analyses (Vreugdenhil *et al.* 2005; Wissuwa, 2005). Following the identification of quantitative trait loci impacting on shoot Se accumulation, for example, candidate

genes or loci could be resolved through fine mapping, and sequence information could be used directly, for gene-based selection or marker-assisted breeding. An advantage of this strategy is that knowledge of the genes and/or chromosomal loci controlling Se accumulation in one plant species could be used in a different target crop species. It is also possible to devise breeding or selection strategies (conventional or through genetic modification) for increasing shoot Se concentration based on existing knowledge of Se (and S) uptake and assimilation. For example, overexpressing ATP sulphurylase and/or genes involved in glutathione synthesis increases shoot Se accumulation in transgenic *B. juncea* (Pilon-Smits *et al.* 1999; Bañuelos *et al.* 2005), whilst overexpressing a Sec methyltransferase gene that methylates Sec to methylSec in *A. bisulcatus* increases Se accumulation in transgenic *A. thaliana* and *B. juncea* (LeDuc *et al.* 2004).

### Perspective

Se intake and status in the UK population can be increased in the short term through agronomic biofortification. The pasture and forage sector already uses commercial Se fertilisers and an increased usage in this sector could increase dietary Se intakes through meat and dairy products. However, in order to provide adequate quantities and forms of Se for the widest possible number in the population Se fertilisation of crops for direct human consumption is likely to be needed. Such a strategy has been adopted in Finland, and there is substantial evidence to demonstrate that this strategy is biologically safe and environmentally acceptable (Wang *et al.* 1994; 1995; Mäkelä *et al.* 1995). From a crop perspective, antagonistic and synergistic interactions between Se and S will occur during plant uptake that may affect crop quality (White *et al.* 2004). These interactions will depend on the prevailing relative concentration of each of the elements in the soil. However, at low external S, and under low rainfall conditions, Se fertiliser application rates of  $\leq 200$  g Se/ha have not been found to induce toxic effects or to retard growth in a low-yielding wheat crop (Lyons *et al.* 2005b).

In cereals selenomethionine is the dominant organic form of Se. Wheat is the most efficient accumulator of Se within the common cereal crops (wheat>rice>maize>barley>oats) and it is one of the most important Se sources for human subjects in the UK (Lyons *et al.* 2003). Thus, wheat is an obvious target crop for agronomic biofortification to increase the dietary Se intake, and thus the Se status, of the UK population. Currently, the average Se level in the grain of UK wheat is 25–33  $\mu\text{g Se/kg}$  (Adams *et al.* 2002). Since the application of 10 g Se/ha applied as  $\text{Na}_2\text{SeO}_4$  to the soil or as a foliar feed is likely to increase grain concentrations by 10-fold, a Se-fertilisation strategy to rectify dietary Se deficiency for large sections of the population would require only a small addition of Se through fertilisation. In contrast, vegetables and fruit deliver small proportions of minerals to the diet (White & Broadley, 2005). Thus, altering Se concentrations of these crops will have a minimal effect on dietary Se intakes. However, some vegetables contain organic forms of Se that make them attractive complimentary targets for

biofortification. For example, Se-methylSec occurring in *Allium* and *Brassica* crops can be converted directly into methyl selenol, a bioactive substance that may protect against cancer (Lu *et al.* 1995; Ip *et al.* 2002; Whanger, 2004).

In addition to providing adequate quantities and forms of Se, an effective fertilisation strategy must be demonstrably safe to the environment. Although >95% UK soils for which geochemical data are available contain  $<1.0 \mu\text{g Se/g}$ , elevated soil Se levels are associated with restricted geological, alluvial and industrial features (Fig. 1) and thus Se-fertilisation strategies must be devised and monitored appropriately. In the UK the quality and quantity of baseline geochemical data for soils, stream sediments and surface waters will enable such monitoring to be undertaken with confidence. However, total Se concentration in soil does not necessarily indicate its bioavailability, and thus factors influencing Se bioavailability must also be considered. In the longer term, it may be possible to exploit genotypic variation in Se accumulation in crops to select or breed varieties with increased Se (Lyons *et al.* 2004, 2005a), thereby minimising the need to use Se fertilisers in all but the lowest soil Se situations. This strategy may have economic advantages over a strategy based entirely on fertilisation.

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