

# High-selenium wheat: biofortification for better health

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The metalloid Se is ubiquitous in soils, but exists mainly in insoluble forms in high-Fe, low-pH and certain leached soils, and hence is often of limited availability to plants. Consequently, it is often supplied by plants to animals and human consumers at levels too low for optimum health. Se deficiency and suboptimality are manifested in populations as increased rates of thyroid dysfunction, cancer, severe viral diseases, cardiovascular disease and various inflammatory conditions. Se deficiency probably affects at least a billion individuals. Optimal cancer protection appears to require a supra-nutritional Se intake, and involves several mechanisms, which include promotion of apoptosis and inhibition of neo-angiogenesis. Evidence suggests that in some regions Se is declining in the food chain, and new strategies to increase its intake are required. These could include education to increase consumption of higher-Se foods, individual supplementation, food fortification, supplementation of livestock, Se fertilisation of crops and plant breeding for enhanced Se accumulation. Se levels in Australian residents and wheat appear to be above the global estimated mean. Wheat is estimated to supply nearly half the Se utilised by most Australians. Increasing the Se content of wheat represents a food systems approach that would increase population intake, with consequent probable improvement in public health and large health cost savings. The strategies that show most promise to achieve this are biofortification by Se fertilisation and breeding wheat varieties that are more efficient at increasing grain Se density. Research is needed in Australia to determine the most cost-effective fertilisation methods, and to determine the extent of genetic variability for grain Se accumulation. Before recommending large-scale fortification of the food supply with Se, it will be necessary to await the results of current intervention studies with Se on cancer, HIV and AIDS, and asthma.

## Selenium: Biofortification: Wheat: Disease prevention

### Introduction

Ever since Se was recognised as an essential nutrient (Schwarz & Foltz, 1957), a voluminous literature has accumulated that describes the profound effect of this element on human health. The findings of recent human intervention trials (Clark *et al.* 1996; Yu *et al.* 1997) have stimulated interest in a cancer-preventive role for Se. In addition to its cancer preventive capacity, Se has an anti-viral effect (Beck *et al.* 1995; Baum *et al.* 1997; Yu *et al.* 1997, 1999). Given the high global incidences of HIV, hepatitis B and C, and other RNA viruses, including measles and influenza, the public-health implications of Se deficiency (estimated by Combs (2001) to affect more than a billion individuals) and suboptimality are enormous.

Several comprehensive reviews have examined Se and human health, including those of Reilly (1996), Rayman

(2000, 2002) and Combs (2001). Combs discusses Se in man within a food systems context and makes the distinction between Se's normal metabolic roles and its anti-carcinogenic activity at supra-nutritional levels.

It is important to note that the biological actions of Se are not properties of the element *per se*, but rather are properties of its various chemical forms. Inorganic Se forms (selenate, selenite) undergo reductive metabolism, yielding hydrogen selenide, which is incorporated into selenoproteins. Successive methylation of hydrogen selenide detoxifies excess Se. Selenomethionine can be incorporated non-specifically into proteins in place of methionine, and selenocysteine is catabolised to hydrogen selenide by a  $\beta$ -lyase (Combs, 2001).

The present review summarises briefly the roles of Se in soils, plants and animals. The importance of Se in human health is discussed, followed by Se intake by human con-

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**Abbreviations:** CIMMYT, Centro Internacional de Mejoramiento de Maiz y Trigo (International Maize and Wheat Improvement Centre); NPC, Nutritional Prevention of Cancer.

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sumers, with a focus on Australia. Strategies to increase Se intake are presented. The review then examines wheat as an important source of bioavailable Se, and discusses Se fertilisation and plant breeding, two strategies to increase Se density in wheat grain.

### Background

Soil Se is uneven in distribution and availability: concentrations range from less than 0.1 to more than 100 mg/kg; however, most soils contain between 1.0 and 1.5 mg/kg (Berrow & Ure, 1989). In general, total soil Se of 0.1–0.6 mg/kg is considered deficient. Soils in New Zealand, Denmark, Finland (pre-1984, before Se was added to fertilisers), central Siberia, and a belt from north-east to south-central China are notably Se-deficient and hence have suboptimal levels in their food systems (Gupta & Winter, 1975; Lag & Steinnes, 1978; Combs, 2001). Large areas of Africa, including much of Zaire, are also likely to be Se-deficient, but further mapping is required. On the other hand, parts of the Great Plains of the USA and Canada, Enshi County in China, and parts of Ireland, Colombia and Venezuela are seleniferous (Combs, 2001).

In acidic, poorly aerated soils, Se is relatively unavailable to plants and occurs mainly as insoluble selenides and elemental Se. In lateritic soils, which have a high Fe content, Se binds strongly to Fe to form poorly soluble ferric hydroxide–selenite complexes (Cary & Allaway, 1969). In wetter regions, selenate can be leached from the soil, resulting in Se-deficient areas, for example, New Zealand and Tasmania (Reilly, 1996). The availability of soil Se to crops can be affected by irrigation, aeration, liming and Se fertilisation (Gissel-Nielsen, 1998).

Australia has both high- and low-Se soils and large areas that have not been mapped for the element. Seleniferous soils occur in central Queensland and parts of Cape York Peninsula. Se deficiency in Australia usually occurs on acidic soils with more than 500 mm rain annually. Areas include the Central and Southern Tablelands and Slopes and the Northern Tablelands of New South Wales, the south-eastern coast of Queensland, south-west Western Australia; coastal and central regions of Victoria, much of Tasmania, and South Australia's Mount Lofty Ranges and Kangaroo Island (see Fig. 1) (Reuter, 1975; Reilly, 1996).

The Se contents of plants vary according to available soil Se and plant species. For example, wheat grown in Shaanxi Province, China may have 0.003 mg Se/kg in the grain, compared with 2.0 mg/kg for wheat from the North or South Dakota wheatlands (Combs, 2001). Wheat from highly seleniferous areas of South Dakota may contain more than 50 mg Se/kg (G Combs, personal communication), while *Astragalus* on the same soils may accumulate up to 15 000 mg/kg dry weight (Beath *et al.* 1937).

Although lower plants such as algae require Se for growth (Lindstrom, 1983), it is not considered to be an essential nutrient for higher plants (Terry *et al.* 2000), although previous studies to ascertain essentiality failed to account for volatile Se compounds (Broyer *et al.* 1966).

In higher plants, selenate is absorbed by roots via the

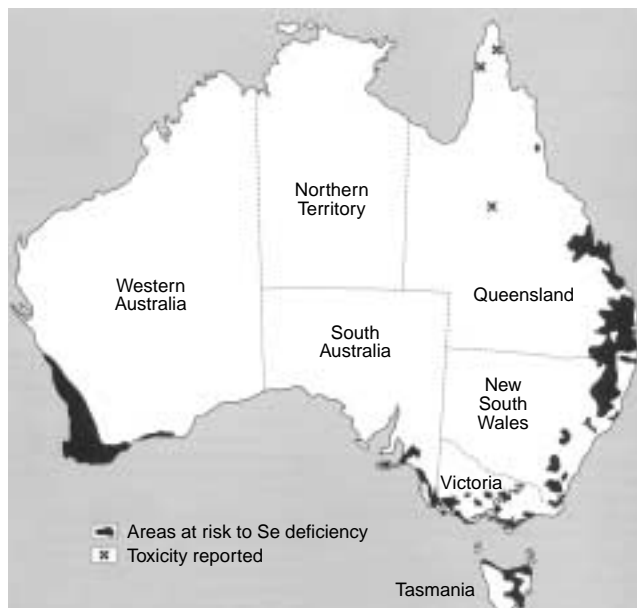


Fig. 1. Selenium in Australian soils (from Judson & Reuter, 1999).

sulfate transporter, a high-affinity permease. High soil sulfate level decreases selenate influx (Cary & Gissel-Nielsen, 1973). Se is transported via the xylem to chloroplasts in leaves where it is reduced and the Se converted to organic forms, some of which are volatile. Selenate is transported more easily from root to shoot than is selenite or organic Se (Terry *et al.* 2000).

Because Se is an essential nutrient, animals respond positively to it where their diet contains less than 0.1 mg Se/kg DM (Oldfield, 1993). Conditions that are related to Se deficiency, some of which occur on a wide scale in certain countries, include white muscle disease, exudative diathesis, pancreatic degeneration, liver necrosis, mulberry heart disease and ill-thrift. Se deficiency is usually not the only cause of these diseases (Reilly, 1996).

### Selenium: essential for human health

#### *Selenium-deficiency diseases*

In parts of China and eastern Siberia two overt Se-deficiency diseases occur; Keshan disease and Kaschin-Beck disease. Keshan disease occurs mainly in children and women of childbearing age and involves impairment of cardiac function, cardiac enlargement and arrhythmia (Reilly, 1996). The disease's aetiology is likely to be complex, involving Se and vitamin E deficiencies, and the presence of the Cocksackie B virus (Yang *et al.* 1994; Levander & Beck, 1999; Liu *et al.* 2002).

Kaschin-Beck disease is an osteoarthropy, which manifests as enlarged joints, shortened fingers and toes, and in severe cases dwarfism. Se and vitamin E deficiency (Reilly, 1996) and I deficiency (Neve, 1999) are likely to be predisposing factors, whereas fulvic acids in drinking water (Peng *et al.* 1999) or mycotoxins in food (Xiong *et al.* 1998) are probable causes.

*Antioxidant, anti-inflammatory, thyroid and immunity roles*

Selenocysteine, the twenty-first amino acid, is present in selenoproteins, which have important enzyme functions in man. Glutathione peroxidase (of which at least five forms exist) has an antioxidant role in reducing damaging  $H_2O_2$  and lipid or phospholipid hydroperoxides produced in eicosanoid synthesis by the lipoxygenase and cyclo-oxygenase pathways (Spallholz *et al.* 1990). This function reduces damage to lipids, lipoproteins and DNA, and hence reduces risk of cardiovascular disease and cancer (Diplock, 1994; Neve, 1996). Moreover, selenite inhibits tumour-necrosis factor- $\alpha$ -induced expression of adhesion molecules that promote inflammation (Zhang *et al.* 2002).

There is a growing body of evidence to suggest that Se (especially in the sodium selenite form) can alleviate conditions associated with high levels of oxidative stress or inflammation. These include asthma (Shaheen *et al.* 1999; Jahnova *et al.* 2002), diabetes (Kowluru *et al.* 2001), arthritis (Peretz *et al.* 2001), muscular dystrophy (Kurihara *et al.* 2000), cystic fibrosis (Kauf *et al.* 1994), acute pancreatitis (De las Heras Castano *et al.* 2000), osteoarthritis (Kurz *et al.* 2002), systemic inflammatory response syndrome (Angstwurm *et al.* 1999) and kwashiorkor (Ashour *et al.* 1999). In addition, Schrauzer (1998) discussed the application of selenite therapy to viral haemorrhagic fever, acute septicaemia and lymphoedema. Another group of selenoenzymes, the thioredoxin reductases, are involved in the reduction of nucleotides in DNA synthesis, the regeneration of antioxidant systems, and the maintenance of intracellular redox state (Allan *et al.* 1999).

The thyroid gland has the highest Se concentration of any human organ (Kohrle, 1999), and Se is involved in thyroid metabolism through the iodothyronine deiodinases, which catalyse the production of active thyroid hormone,  $T_3$ , from thyroxine,  $T_4$  (Beckett *et al.* 1987). The I deficiency diseases goitre and myxoedematous cretinism are more prevalent in central Africa in those regions that are deficient in both I and Se (Vanderpas *et al.* 1993), and in such areas supplementation with both nutrients is indicated.

Se has a role in many aspects of the immune response to infections. Se deficiency reduces immunocompetence, involving impairment of neutrophil, macrophage and polymorphonuclear leucocyte activity (Dimitrov *et al.* 1984; Boyne & Arthur, 1986; Spallholz *et al.* 1990). Se supplementation of even supposedly Se-replete individuals is immunostimulatory, and involves enhancement of natural-killer-cell and lymphocyte activity as well as enhancement of proliferation of activated T-cells (Kiremidjian-Schumacher *et al.* 1994).

*Cancer*

There is 'perhaps no more extensive body of evidence for the cancer preventive potential of a normal dietary component than there is for selenium.' (Combs & Gray, 1998). From the late 1960s, epidemiological studies have suggested an inverse association between human Se intake and cancer mortality (Combs & Gray, 1998). An extensive literature documents the numerous *in vitro* and animal studies that have been conducted during the past 35 years. Most

demonstrate that application or intakes of Se at supra-nutritional levels can inhibit tumorigenesis (El-Bayoumy, 1991; Combs & Gray, 1998; Ip, 1998). Prospective cohort and case-control studies that have involved as many as 34 000 subjects have generally shown an association between low Se status and a significantly higher risk of cancer incidence and mortality (Yoshizawa *et al.* 1998; Yu *et al.* 1999; Brooks *et al.* 2001).

Intervention studies using Se as a single chemopreventive agent include the Qidong trials in China, where selenite significantly reduced primary liver cancer (Yu *et al.* 1997). In the Nutritional Prevention of Cancer (NPC) trial in the USA, 200  $\mu\text{g}$  Se/d (as yeast) reduced total cancer mortality by 41 %, total cancer incidence by 25 % and prostate cancer incidence by 52 % in a cohort of 1300 subjects. The effect on total cancer was limited to male smokers (current or previous) with baseline Se levels below 113  $\mu\text{g}/\text{l}$ , although non-smoking males below this level are likely to have benefited from Se supplementation in terms of prostate and colon cancer protection (Duffield-Lillico *et al.* 2002).

The NPC trial was conducted in a region of the USA where Se intakes are estimated to be around 90  $\mu\text{g}/\text{d}$ , well above the level required for optimal selenoenzyme activity. This suggests additional mechanisms in Se's cancer-preventive role. While some cancer protection, particularly that through antioxidant activity, involves selenoenzymes, the anti-cancer effects of Se are likely to involve the production of specific anti-tumorigenic metabolites, such as methylselenol. Studies have suggested that Se provided in certain forms can neutralise carcinogens, enhance the immune system, alter gene (including p53) expression, inhibit tumour cell metabolism and neo-angiogenesis (blood vessel development around tumours), and promote apoptosis (programmed cell death) (Ip *et al.* 1991; Harrison *et al.* 1997; Combs & Gray, 1998; Jiang *et al.* 1999; Combs, 2000, 2001; Lu, 2000; Rayman, 2000; Finley & Davis, 2001; Seo *et al.* 2002).

According to this two-stage model of cancer prevention, which involves Se intakes that correct nutritional deficiency as well as much higher, supra-nutritional intakes, individuals with nutritionally adequate Se intakes may benefit from Se supplementation (Combs & Gray, 1998). Se's anti-cancer activities remain under intensive study worldwide.

*Viral and mycobacterial diseases*

Se deficiency is associated with increased virulence of a range of viral infections (Taylor, 1997). It is evident that in a Se-deficient host, normally harmless viruses can become virulent. For example, when Se-deficient mice are inoculated with benign Coxsackie B3 virus, the virus mutates into a virulent form that causes myocarditis similar to that seen in Keshan disease (Beck *et al.* 1995, 1998; Beck, 2001). Furthermore, Se-deficient mice develop severe pneumonitis when infected with a mild strain of influenza virus (Beck, 2001).

Se appears to be of particular importance for individuals with HIV. Se deficiency is a significant predictor of HIV-related mortality (Baum & Shor-Posner, 1998; Campa *et al.* 1999) and viral load (Baeten *et al.* 2001). A US study found

Se-deficient HIV patients to be twenty times more likely to die from HIV-related causes than those with adequate levels (Baum *et al.* 1997). The decline in blood Se levels occurs even in the early stages and is thus unlikely to be due to malnutrition or malabsorption (Look *et al.* 1997). Moreover, a study of HIV-1-seropositive drug users found low Se level to be a significant risk factor for developing mycobacterial disease, notably tuberculosis (Shor-Posner *et al.* 2002).

Se also appears to be protective in individuals infected with hepatitis B or C against progression to cirrhosis and liver cancer (Yu *et al.* 1997, 1999). Selenoproteins encoded by HIV, hepatitis C virus and the Ebola virus (which causes acute haemorrhage) have been discovered that consume the host's Se supply, thus reducing the immune response (Taylor & Nadimpalli, 1999; Zhao *et al.* 2000).

#### *Other health effects*

Low Se status has long been known to reduce fertility in livestock (Underwood, 1977), and this also appears to be the case for man. Low Se levels have been associated with male infertility (Behne *et al.* 1997) and spontaneous abortions (Barrington *et al.* 1996). In a Scottish study, supplementation of subfertile men with 100 µg Se/d for 3 months significantly increased sperm motility (Scott & MacPherson, 1998). Conversely, Hawkes & Turek (2001) found that a diet containing 300 µg Se/d caused a reduction in sperm motility in healthy men.

Se appears to be influential in the brain, and Rayman (2000) documented several studies that indicate that low Se levels are associated with cognitive impairment, depression, anxiety and hostility. These conditions can be alleviated in individuals with low baseline Se levels by Se supplementation. Recent studies suggest that selenoprotein P (Whanger, 2001), selenoprotein W (Jeong *et al.* 2002) and the newly discovered selenoprotein M (Korotkov *et al.* 2002) have important roles in the brain.

Se forms selenides with all metals, and detoxifies Hg, Cd, Pb, Ag, Tl and As. This effect can be enhanced by vitamin E (Frost, 1981). In the case of Cd and Hg, detoxification is achieved through the diversion in their binding from low- to high-molecular-weight proteins (Whanger, 1992).

### **Human selenium intake**

#### *Selenium intake: low and getting lower*

Se intake in human consumers is determined mainly by the level of available Se in the soil in which their food is grown, and by dietary composition. Se levels in major food classes usually occur within the following ranges: 0.10–0.60 mg/kg (fish); 0.05–0.60 (cereals); 0.05–0.30 (red meats); 0.002–0.08 (fruit and vegetables) (Combs, 2001). Bioavailability, which varies with the food source of Se, will be discussed on p. 52.

The absolute range of global daily Se intake by adults is between 7 (in Chinese Keshan disease areas) and 5000 µg/d (in Chinese selenosis areas). Estimates provided by Combs (2001) of Se intake for several countries include England (12–43), Belgium (45), Canada (98–224), USA (60–220),

Croatia (27), New Zealand (19–80), Japan (104–127) and Venezuela (200–350). In Australia few comprehensive studies have been conducted, but estimates of 63 and 96 µg/d have been provided, with a range of 23–204 µg/d (Fardy *et al.* 1989; Reilly, 1996). In view of the estimated mean plasma level of Se in Australian adults (see p. 49), a mean intake of around 75 µg/d appears likely for Australians.

The US recommended daily allowance, which is based on the Se levels considered to be necessary to maximise glutathione peroxidase activity, is 55 µg/d for both men and women, while in Australia it is 85 and 70 µg/d for men and women, respectively. The Third National Health and Nutrition Examination Survey in the USA (*n* 17 630) indicated that 99 % of the subjects were Se-replete (i.e. above 80 µg Se/l plasma) and thus supplementation is not recommended (Burk, 2002). However, referring to the study of Neve (2000), Rayman (2000) pointed out that if platelet, rather than plasma, saturation of glutathione peroxidase activity is used as the measure of Se repletion, a higher intake is required, in the range of 80–100 µg/d.

It is evident that many individuals do not consume enough Se to support maximum expression of selenoenzymes, let alone the level required for optimum prevention of cancer. Combs (2001) estimated the number of those who are Se-deficient in the world to be in the range of 500–1000 million. In addition, he considered that the vast majority of the world's population have suboptimal Se intakes, and hence are at increased risk of cancer, heart disease, viral diseases, and indeed any conditions that involve increased levels of oxidative stress.

Furthermore, evidence suggests that there is a trend toward a reduction of Se in the global food chain, caused by fossil fuel burning (with consequent S release), acid rain, soil acidification, the use of high-S fertilisers (Frost, 1987) and more intensive crop production (Gissel-Nielsen, 1998). Rayman (1997, 2000, 2002) and Giovannucci (1998) have observed that blood Se levels have decreased significantly in the UK during the decade 1984–1994, and current average Se intake in the UK may be as low as 34–39 µg/d (Barclay *et al.* 1995; Ministry of Agriculture, Fisheries and Food, 1997, 1999). These authors attribute this fall in part to the use of low-Se UK and European wheat in place of North American wheat. This highlights the sensitivity of Se intake and body levels to changes in the food supply. Both authors call for action to increase Se intake.

#### *Human blood concentrations of selenium: the global view*

Blood Se levels are determined mainly by dietary intake, although gender, age, smoking and exposure to heavy metals can have an effect (Robberecht & Deelstra, 1994). Combs (2001) presented a comprehensive list of Se concentrations in plasma, serum or whole blood of healthy adults from sixty-nine countries. A sample of plasma or serum Se concentrations (µg/l) is as follows: Austria (67); Burundi (15); Canada (132); China, Keshan disease area (21); China, selenosis area (494); Finland pre-1984 (70); Finland post-1984 (92); Hungary (54); Japan (130); New Zealand (59); Norway (119); USA (119); Zaire (27) (Combs, 2001). Note that there are few data available from some of the most populous areas of the world, including

most of Africa, South America and central and south Asia. The mean value (calculated as the mean of the post-1990 means for a representative sample of forty-five countries) is 78 µg/l, with a range of means from 15 (Burundi) to 216 µg/l (Venezuela). However, this is likely to be optimistic as small studies of Se levels in much of Africa and central, south and South-East Asia indicate levels well below this.

This can be compared with the value of 70 µg/l, the WHO's reference level, which is the minimum level for maximisation of plasma or serum glutathione peroxidase activities (Neve, 1995). Rayman (1997) has quoted studies that show that a level of 100 µg/l is required for optimal expression of plasma glutathione peroxidase. The vast majority of the world's population would not reach this level of plasma or serum Se.

#### *Selenium levels in the Australian population*

An estimate of 94 µg/l of plasma or serum Se for Australia can be derived from the means determined from seventeen studies of blood Se levels. The studies (with mean plasma or serum Se levels in µg/l) are: Judson *et al.* (1978, 1982) (124, 114); Pearn & McCay (1979) (88); McOrist & Fardy (1989) (98, 86); Brock *et al.* (1991) (88); Cumming *et al.* (1992) (81); Lux & Naidoo (1995) (101); McGlashan *et al.* (1996) (80); Dhindsa *et al.* (1998) (92); Daniels *et al.* (2000) (77, 88); GJ Judson, (unpublished results from 1987 and 1988) (91, 92, 101, 95); G Lyons, GJ Judson, J Stangoulis, L Palmer and R Graham (unpublished results from 2002) (103). If post-1990 data only are used, the mean is 89 µg/l. Using either mean, Australia is above the estimated world mean reported Se level of 78 µg/l.

In Australia, relatively low blood Se levels have been reported in Adelaide infants. Daniels *et al.* (2000) found a plasma Se level of around 31 (SD 13) µg/l in a sample of newborn infants, a level comparable with that of New Zealand. Infant Se levels are typically half those of adults. These levels place the infants at increased risk of a range of conditions that involve oxidative stress and inflammation.

An apparent global decline in Se in the food chain was noted on p. 48. Se levels in South Australians may have fallen from 1977–1987. For instance, the mean whole-blood Se level for a 1977 sample of Adelaide health workers was 155 µg/l (Judson *et al.* 1978), whilst Kangaroo Island residents in 1979 recorded a mean of 143 µg/l (Judson *et al.* 1982). However, in later samples of healthy Adelaide adults from 1987–2002, mean whole-blood Se levels were 117, 126, 128, 118 (GJ Judson, unpublished results from 1987 and 1988) and 125 µg/l (G Lyons, GJ Judson, J Stangoulis, L Palmer and R Graham unpublished results from 2002), with Mount Gambier residents recording 121 µg/l in 1987 (GJ Judson, unpublished results from 1987), for a grand mean for the period 1987–2002 of 123 µg/l. The apparent decline in the early 1980s may be due to changes in dietary composition and/or a decrease in Se concentration in South Australian-grown wheat.

#### *Optimum selenium intake*

In the NPC trial in the USA, the protective effect of Se against cancer occurred in the lowest (relative risk 0.52;

95 % CI 0.33, 0.82) and middle (relative risk 0.64; 95 % CI 0.40, 0.97) tertiles, which included those individuals with plasma Se levels below 121 µg/l (Rayman & Clark, 2000). The latest analysis shows that the Se benefit was largely restricted to male smokers with baseline plasma Se levels below 113 µg/l. The strongest protective effect was against prostate cancer, with a hazard ratio of 0.48 (95 % CI 0.28, 0.80) (Duffield-Lillico *et al.* 2002). None of the subjects had plasma Se levels below 60 µg/l and very few were less than 80 µg/l; thus the cohort must be considered Se-adequate by current nutritional standards (Combs, 2000).

Although there is a risk in generalising results of individual epidemiological and intervention studies, this result would suggest, using the levels presented by Combs (2001) on p. 48, that the vast majority of the world's population (including that of Australia, with a probable mean plasma or serum level around 89 µg/l, and many populations in Europe (Rayman, 2000)) would be in the responsive range.

The NPC participants lived in a region where dietary Se intake is around 90 µg/d (Clark *et al.* 1996); thus with the addition of the 200 µg supplement, subjects in the treatment group would have received around 270–310 µg/d. Combs (2001) suggested that an Se intake of 200–300 µg/d may be required to significantly reduce cancer risk. This compares with an estimated Australian adult intake of 75 µg/d. Of course, as Rayman (2000) noted, Se requirement varies between individuals in the same population. Even Moyad (2002), who expressed doubts about the interpretations of certain Se studies, and considered some estimates of its cancer-protective effect to be optimistic, suggested that an intake of 200 µg Se/d and around 50 mg vitamin E/d may be beneficial, particularly for current or previous smokers. The results of the NPC trial (Duffield-Lillico *et al.* 2002) suggested that males may have a higher Se requirement than females. Further studies may find optimum adult Se intakes in the range 125–280 µg/d, with means of around 130 (for females) and 250 µg/d (for males). Pregnant females may have a higher Se requirement than non-pregnant females (Dylewski *et al.* 2002).

Chronic selenosis occurs in Enshi County, China, where coal-contaminated soil contains up to 8 mg Se/kg, and residents have consumed up to 7 mg/d. Common symptoms include nail thickening and cracking and hair loss, and some individuals exhibit skin lesions (Liu & Li, 1987; Yang & Zhou, 1994). The concern that the incorporation of selenomethionine into body proteins could increase Se to toxic levels appears unwarranted because a steady state is established, which prevents the uncontrolled accumulation of Se (Schrauzer, 2000).

Combs (2001) considered it probable that the WHO and European Union estimates of the upper safe limit of Se intake of 400 and 300 µg/adult per d, respectively, are too conservative. Under normal conditions, a Se intake of less than 1000 µg/d (or 15 µg/kg body weight) does not cause toxicity (Neve, 1991; Poirier, 1994; Whanger *et al.* 1996; Taylor, 1997). Those living in parts of China, the USA, Venezuela and Greenland have ingested Se at this level for their entire lives without ill effects (Taylor, 1997).

However, it would be prudent at this stage to limit medium- to long-term Se intake to around the US reference dose, which has been set at 350 µg/d for a 70 kg human

adult (Schrauzer, 2000), for several reasons. First, Vinceti *et al.* (2001) have documented possible adverse effects of levels of supplemented Se around 300 µg/d on thyroid status. Second, a reduction in sperm motility in a group of eleven men supplemented with 300 µg Se/d for 3 months has been shown by Hawkes & Turek (2001). Third, there has been a surprising finding from the NPC trial of a non-significant increase in risk (based on small case numbers) of five cancer types (melanoma, lymphoma and leukaemia, breast, bladder and head and neck cancers) in the Se-supplemented group. Biofortification of cereals with Se at the rates discussed on p. 53 would be very unlikely, though, to place consumers at risk of any adverse health effects from Se.

The food systems of very few countries appear to deliver an optimum level of Se to their populations, and indeed the food systems of most populations do not even provide enough Se to maximise selenoenzyme expression. The impact of this deficiency and suboptimality in global health terms is difficult to quantify, but is likely to be enormous given the high prevalence of various cancers, cardiovascular diseases, viral diseases (including AIDS, hepatitis, measles and influenza), and exposure to environmental pollutants throughout much of the world. It is thus a matter of urgency that many countries begin to address this major public-health issue and develop effective, sustainable ways to increase Se intakes (Combs, 2001).

### Strategies to increase human selenium intake

Given that the populations of most countries would probably benefit from an increased Se intake, how could this be best achieved? Strategies to increase Se intake include increased consumption of higher-Se foods through education, individual supplementation, food fortification, supplementation of livestock, use of Se fertilisers, and plant breeding for enhanced Se accumulation. Each of these shall be discussed briefly.

#### *Increased consumption of higher-selenium foods through education*

Globally, wheat is one of the most important dietary sources of Se. Even in Europe, with its low levels of available soil Se, bread and cereals, being commonly consumed, are important Se sources. In the UK, for example, it is estimated that bread and cereals supply around 22 % of Se, second to meat, poultry and fish (36 %) (Ministry of Agriculture, Fisheries and Food, 1997).

Brazil nuts provide the most concentrated natural food source of Se. A study conducted in the UK found concentrations of 2–53 mg Se/kg (Thorn *et al.* 1976), while an Australian study found even higher levels; 0.5–150 mg/kg (Tinggi & Reilly, 2000).

#### *Individual supplementation*

In Western countries, many individuals currently consume Se supplements, which are available in both inorganic and organic forms. Sodium selenite, available in tablet or fluid form, is preferable to selenate (Chen *et al.* 2000; Finley & Davis, 2001). High-Se yeast includes several organic Se

forms, including selenomethionine and selenocysteine (Bird *et al.* 1997). Another form of individual supplementation is selective consumption of the fortified or 'functional foods' listed later.

Studies suggest that dietary sources of Se, vitamin E and β-carotene are preferable to supplements (Moyad, 2002). Moreover, a well-known drawback of individual supplementation as a population strategy to improve nutrition is that those who are most in need tend to be the least likely to take supplements.

#### *Food fortification*

This approach has been used successfully with folate-enriched breakfast cereals, Fe-enriched milk, iodised salt, carotene- and vitamin E-enriched margarine, as well as selenised salt in Se-deficient regions of China. Seleniferous areas can be considered resources for the production of Se-enriched plants; for example, in China an elixir is made from high-Se tea in Enshi County (Combs, 2001). These examples can be included in the functional foods category, along with high-Se broccoli (Finley, 1999), high-Se garlic (Ip & Lisk, 1994) and the high-Se yeast noted earlier.

#### *Selenium supplementation of livestock*

Supplementation strategies to increase dietary Se intake by livestock (and thus increased levels in meat and milk) include Se fertilisation of pastures, dietary supplements (for example, the now widespread practice of adding selenomethionine to the rations of livestock, including dairy cows during milking), and direct administration (drenches, slow-release reticulum–rumen 'bullets', injection).

In New Zealand, sodium selenate is supplied as a prill to pastures (Reilly, 1996). In a Western Australian study, slow-release barium selenate applied at 10 g/ha prevented subclinical Se deficiency in sheep for 4 years, whereas a single application of sodium selenate at the same rate was effective for only 15 months (Whelan & Barrow, 1994).

Supplementation of livestock with Se is unlikely to be an efficient strategy to increase Se level in the human population, however. In New Zealand, little increase in the Se content of human foods was observed after the introduction of Se supplementation for farm animals in the 1960s (Thomson & Robinson, 1980).

#### *Selenium fertilisation of crops*

The use of Se as a soil amendment in fertiliser is practised mainly in Finland (by law from 1984), where it is currently added to N, P and K fertiliser at a rate of 10 mg/kg (Euroala & Hietaniemi, 2000), and New Zealand (at an individual level, and generally on pastures).

The Finnish experiment has demonstrated the safety, effectiveness, ease and cost-efficiency of this approach to raise Se levels in a human population. Dietary Se intakes trebled and plasma Se concentrations nearly doubled within 3 years of the programme's commencement (Aro *et al.* 1995). However, it is difficult to isolate the effects of a single factor, such as dietary change, from other factors that can be involved in the aetiology of such conditions as can-

cer and cardiovascular disease. There have been significant decreases in the rates of cardiovascular disease and certain cancers in Finland since 1985, but with no controls for comparison, this cannot be ascribed to Se alone (Varo *et al.* 1994).

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 (Gupta & Watkinson, 1985). The enhancement of wheat-Se level by fertiliser will be discussed further on p. 52.

#### *Plant breeding for enhanced selenium accumulation*

Breeding for improved Se uptake and/or retention by plants may be an effective, sustainable strategy. Preliminary studies have found a 15-fold variation in Se-accumulating ability among brassica vegetables (Combs, 2001), and an Se-accumulating soyabean cultivar has been identified (Wei, 1996). Substantial variability exists within cereal crop varieties for Zn, Fe and other nutrients (Graham *et al.* 1999). These findings suggest that it should be possible to breed cultivars with enhanced Se uptake and/or retention, or to use genetic engineering to enhance Se levels (and even specific Se metabolites) in food crops.

In summary, each of these strategies could contribute to an enhanced delivery of Se to human populations through their food systems. As described by Welch & Graham (1999), a food systems paradigm encompasses an agriculture that aims not only at productivity and sustainability, but also at improved nutrition. In Australia, a programme that combines selection for enhanced grain Se content in wheat, strategic fertilisation of wheat and barley, education to encourage greater consumption of higher-Se foods, increased supplementation of livestock grazing on acid soils in high rainfall areas, and education and targeted supplementation of high-risk individuals, would be likely to significantly improve population health.

#### **Wheat: an important selenium source for human consumers**

Surveys indicate that wheat is the most efficient Se accumulator of the common cereal crops (wheat, rice, maize, barley, oats) and is one of the most important Se sources for human consumers. In a Russian survey, serum Se level was found to be highly correlated ( $r$  0.79) with Se level in wheat flour (Golubkina & Alfthan, 1999). Bread is the second most important source of Se in the USA (Schubert *et al.* 1987), and has been found to supply one-third of the daily Se intake of Australian children (Barrett *et al.* 1989). With the addition of Se supplied through breakfast cereals, cake and biscuits, and in view of its high bioavailability, wheat-Se probably supplies around half the Se utilised by Australians.

#### *Selenium concentrations in wheat grain*

*The global view.* There is wide variation in wheat-grain Se level between and within countries. Published values range from 0.001 mg/kg in south-west Western Australia (White *et al.* 1981) to 30 mg/kg in highly seleniferous areas

of South Dakota (University of California, 1988), but most of the world's wheat falls within the 0.020–0.600 mg/kg range (Alfthan & Neve, 1996). Canada and the USA have relatively high levels, usually in the 0.2–0.6 mg/kg range (Reilly, 1996). New Zealand and Eastern Europe generally have low levels, for example, the 0.028 mg/kg average found by Mihailovic *et al.* (1996) for Serbia.

Some countries, including China (with a range of 0.01–0.23 mg/kg; Alfthan & Neve, 1996), Canada and the USA, have highly variable wheat-Se levels, even within states. Se concentrations in wheat grain from twelve locations in Manitoba, Canada in 1986–1988 ranged from 0.06–3.06 mg/kg, and levels varied between years within a location (Boila *et al.* 1993). A US survey, also in 1986–1988, analysed major brands of white bread in nine different geographical regions. The overall range was 0.06–0.74 mg/kg, while a single brand of bread collected from different bakeries in Boston alone had a range of 0.24–0.92 mg/kg (CV 41%), with a mean of 0.60 mg/kg (Holden *et al.* 1991).

*Selenium in Australian wheat.* Four published surveys of Se concentrations in Australian wheat have been identified. The Queensland study (Noble & Barry, 1982) found a mean value for wheat-grain-Se of 0.150 (range 0.020–0.800) mg/kg for wheat grown between 1974 and 1978. Similar levels were found for sorghum and soyabean. Site differences accounted for a 40-fold difference between wheat-Se values, and a 110-fold difference for sorghum.

Very low Se concentrations were found in wheat surveyed in south-west Western Australia in 1975; mean Se concentration in grain was 0.023 (SE 0.006) mg/kg, with a range of 0.001–0.117 mg/kg (White *et al.* 1981). The value of 0.001 mg/kg is the lowest level reported globally for Se in wheat. The lowest levels were found on soils derived from Archaean granite, which are also associated with a high prevalence of white muscle disease in lambs (Godwin, 1975).

Watkinson (1981) compared Se concentrations in New Zealand-grown wheat with wheat imported from Australia, grown in 1978 and 1979. The Australian wheats were higher in Se (mean 0.123 (SD 0.026) mg/kg, range 0.043–0.224 mg/kg) than the New Zealand wheats (mean 0.028, (SD 0.010) mg/kg, range 0.011–0.086 mg/kg).

The South Australian study (Babidge, 1990) used pooled wheat and barley samples from 107 and 100 silos, respectively, across the state in the 1981 season for wheat and the 1981 and 1982 seasons for barley. There were significant differences ( $P < 0.001$ ) between regions; for example, the Upper Eyre Peninsula region had a wheat-Se median value of 0.229 (range 0.120–0.316) mg/kg, compared with the South East with 0.118 (range 0.047–0.240) mg/kg. Wheat and barley showed the same regional trends, but wheat levels were around 30% higher.

A recent targeted survey of wheat grown in South Australia in the 2000 and 2001 seasons yielded a range of 0.005–0.700 mg Se/kg, with values typically 0.080–0.160 mg/kg, and an estimated grand mean of 0.120 mg/kg (G Lyons, L Palmer, J Stangoulis and R Graham, unpublished results).

The findings of these studies (means of 0.150 (Queensland), 0.023 (Western Australia), 0.150 (South

Australia), 0.091 (New South Wales), and South Australia median of 0.170 mg/kg), together with the findings of the human blood surveys discussed on p. 48, suggest that Australian wheat-grain Se concentrations are above the global average. They are well above the New Zealand, UK and Eastern European levels, but generally lower than those of Canada and the USA.

#### *High bioavailability of wheat-selenium*

In human nutrition terms, bioavailability can be defined as the amount of a nutrient in a meal that is absorbable and utilisable by the individual eating the meal (Van Campen & Glahn, 1999). Se is well absorbed (generally 73–93 %) from most sources, with selenomethionine and selenate usually absorbed more efficiently than selenite (Raghib *et al.* 1986; Stewart *et al.* 1987; Moser-Veillon *et al.* 1992; Van Dael *et al.* 2002).

However, the assessment of bioavailability of food micronutrients in general (House, 1999; Graham *et al.* 2001) and different forms of dietary Se is not straightforward (Reilly, 1996). Several measures can be used to determine bioavailability and they are subject to a range of variables. If tissue retention is used as a measure, naturally occurring food Se is by far the most available form of the element, although there are significant differences between different types of food.

Se form is important; selenomethionine, the form in which Se mainly occurs in cereals (Olson *et al.* 1970), beans, mushrooms and yeast (Reilly, 1996), enters the general protein pool and is well retained. However, it must be released from the protein pool and be catabolised to hydrogen selenide before it can support selenoenzyme expression. Selenocysteine, on the other hand, cannot be incorporated directly into proteins, but is catabolised directly to hydrogen selenide. It is thus better utilised for the selenoenzymes but not retained as well as selenomethionine (Deagen *et al.* 1987; Levander & Burk, 1990; Combs, 2001).

Se is generally more bioavailable from plant forms than from animal foodstuffs (Combs, 1988; Bugel *et al.* 2002), and wheat-Se is one of the most bioavailable forms (Jaakkola *et al.* 1983; Laws *et al.* 1986; Hakkarainen, 1993). The importance of imported North American wheat as a former Se source for the British population was noted on p. 48. This is reinforced by a Scottish study which found that the Se content of wheat harvested in 1989, which was used for bread making in Scotland, ranged from 0.028 mg/kg for home-grown wheat to 0.518 mg/kg for Canadian wheat, and was significantly correlated ( $P < 0.001$ ) with protein content (Barclay & MacPherson, 1992). Norway's population, despite a modest total Se intake, has the highest serum Se level in Europe at 119 µg/l. The probable explanation is that their major Se source is North American wheat. In a Norwegian study, Meltzer *et al.* (1992) demonstrated the high bioavailability of wheat-Se by feeding trial participants Se-rich bread providing 100, 200 or 300 µg Se daily for 6 weeks. Serum Se increased in a dose–response manner by 20, 37 and 53 µg/l, respectively, in the three groups ( $P < 0.001$ ).

Wheat enriched with Se by foliar application was found

to be highly effective in raising plasma Se (53 % increase after 6 weeks) in a Serbian study. Glutathione peroxidase activity in blood increased and oxidative stress parameters decreased (Djujic *et al.* 2000b). A follow-up study found that Se-enriched wheat increased levels of Cu, Fe and Zn in erythrocytes, compared with individuals consuming low-Se wheat (Djujic *et al.* 2000a). This is the first time such interactions have been reported, and further studies are warranted in view of the billions in the human population who are Fe- and/or Zn-deficient.

## **Strategies to increase selenium in wheat**

### *Background*

The foregoing evidence suggests that for many countries an increase in Se concentration in wheat would be the most effective and efficient way to increase the Se intake of the human population, with consequent probable improvement in public health, and also to reverse the trend of declining Se levels in food systems.

It is clear that Se fertilisation of wheat is an effective means to increase grain Se concentration (Gupta & Gupta, 2000; Combs, 2001), and this will be examined later. Education is also important to encourage the consumption of appropriate amounts and proportions of different classes of healthy foods, including whole-grain cereal products.

However, in developing countries those individuals who are most at risk of nutrient deficiencies (frequently women and children) often rely on one staple food, for example wheat, rice, cassava or maize, for most of their energy and nutrient requirements, and lack the money to improve their diet. Hence the high prevalence of Fe, Zn, vitamin A, I and Se deficiencies, and the high incidence and prevalence of infectious diseases in these countries (Graham & Welch, 1996; Graham *et al.* 2001). For certain soil types in developing countries, the most sustainable, cost-effective approach may be to breed wheat cultivars that are better at accumulating grain-Se.

### *Selenium fertilisation*

To overcome the low Se levels in food crops in certain areas, different methods of Se fertilisation have been investigated for more than 30 years. Of particular interest are the experiments of Ylaranta (Finland), Gissel-Nielsen (Denmark) and Gupta (Canada). It is a very inexpensive method to increase Se intake by human consumers; the material cost of applying 10 g selenate/ha is around US \$1.15/ha (€1.15/ha). Its effectiveness is illustrated by the increase in blood Se levels in Finland post-1984. Moreover, Mutanen *et al.* (1987) found that the bioavailability (calculated as the mean of four criteria) of wheat-Se was higher for Se-fertilised (both soil-applied and foliar) wheat than for American wheat, which was naturally high in Se.

The Finland Se programme was discussed on p. 50. The Se level in all domestic cereal grains in Finland pre-1984 was 0.01 mg/kg or less; now spring wheats typically contain around 0.25 mg/kg, and for the less-fertilised winter wheat, around 0.05 mg/kg (Eurola *et al.* 1990). Current



application rate is 5–10 g selenate/ha, and the practice appears to be safe.

The concern that the continuing use of Se-amended fertilisers might eventually lead to accumulation of toxic levels in the environment appears to be unfounded (Vuori *et al.* 1994; Oldfield, 1999). The residual effect of Se treatments was found to be low to negligible in the following year, even when it had been applied at the high rate of 500 g/ha (Ylaranta, 1983*a,b*, 1984; Singh, 1991; Shand *et al.* 1992; Gupta *et al.* 1993). Furthermore, the bioavailability of residual Se is lowered by the reducing action of microorganisms in the soil and rumen. A Californian study (Norman *et al.* 1992) found that long-term Se supplementation of cattle at maximum permitted levels did not result in Se contamination in streams that received runoff from feeding areas. However, a Californian study of a deer forest range treated with seleniferous fertiliser found significant Se accumulation in aquatic biota in streams in the same area (Maier *et al.* 1998), a not unexpected finding. 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 Se application (Oldfield, 1999). It would appear that the risk of environmental Se accumulation due to responsible agricultural use is low.

Most studies have shown selenate, whether applied to the soil or as a foliar fertiliser, to be much more effective than selenite (Ylaranta 1983*a,b*, 1984; Singh, 1991; Shand *et al.* 1992; Gupta *et al.* 1993). For example, a grain Se content of 100–200 µg/kg in barley was obtained by applying 10–20 g selenate/ha, but over 100 g selenite/ha was required to reach this level (Ylaranta, 1983*b*). On a fine sandy loam of pH 6.0, 10 g selenate/ha applied to the soil raised barley grain from 33 (control) to 234 µg/kg, while 10 g selenite/ha caused no increase (Gupta *et al.* 1993). In many soils, selenite is readily adsorbed on clay colloids and becomes unavailable to plants.

The relative effectiveness of soil or foliar application of Se depends on Se form, soil characteristics, method of basal application and time of foliar application. Ylaranta (1983*b*) found basal and foliar selenate to be equally effective at the low (10 g/ha) rate, foliar better at 50 g/ha, and both equal at the high rate of 500 g/ha. In further trials, foliar selenate applied at the three to four leaf stage was found to be more effective than basal application on clay soil of pH 6.3, of similar effectiveness on high-humus, fine sandy soil of pH 4.6, and slightly less effective than basal fertiliser on a fine sandy soil of pH 5.0. Foliar selenate, at the level of 10 g/ha, using a wetting agent, raised the wheat-grain Se level from 16 to 168 µg/kg on the clay soil, while 9 g basally applied raised it to just 77 µg/kg. Overall, foliar application was the more effective method, except where growth was poor due to low rainfall (Ylaranta, 1984). This suggests that, for most of Australia's wheat-growing areas, basal application may be preferable, especially where foliar N (to which Se could be added) to boost growth or protein is not used.

At the Se levels used for fertilisation of wheat in the field (5–100 g/ha), it is unlikely that there would be any phytotoxic effects, including growth inhibition.

The question arises as to what is a desirable target level for Se in wheat grain. From the studies discussed earlier, it

is evident that 10 g selenate/ha can raise wheat crops from a base level of 30–100 to 200–500 µg grain-Se/kg. Is this as high as required? It could be considered a minimum target level in view of the estimated 300 µg Se level of a loaf of wholemeal bread made from this wheat. The consumption of this bread over a week would substantially increase an individual's Se intake.

There are no published studies of Se fertilisation of wheat in Australia. Conditions are very different from those in Europe and Canada, where most studies have been conducted; in Australia, rainfall and yield are lower and temperatures higher. Our group is currently conducting field trials to assess different application methods and rates of selenate on wheat in South Australia.

#### *Wheat breeding to increase grain selenium density*

*The extent of human micronutrient malnourishment.* Around half of the world's population is malnourished. More than two billion individuals consume diets that are less diverse than 30 years ago, leading to deficiencies in micronutrients, especially Fe, Zn, I and Se, and also vitamin A. In some regions, almost everyone suffers from some form of 'hidden hunger'. In South-East Asia, for example, it is estimated that Fe deficiency affects 98.2% (around 1.4 billion) of the population (World Health Organization, 1999).

Cereals are generally low in micronutrients compared with other food crops, thus cereal-dominated food systems are low in micronutrients. The individuals most at risk are resource-poor women, infants and children. Graham *et al.* (2001) concluded that a new agricultural paradigm is needed to address global micronutrient malnutrition: '... an agriculture which aims not only for productivity and sustainability, but also for balanced nutrition, or what we have called the *productive, sustainable, nutritious food systems paradigm*.' (Graham *et al.* 2001, p. 91).

*Breeding for higher nutrient density in staple crops.* Programmes that include fortification, education and supplementation have been successful in countering micronutrient deficiencies in certain cases and will continue to play a role. However, they tend to be expensive, require ongoing inputs and often fail to reach all individuals at risk. Furthermore, such programmes themselves are at risk from economical, political and logistical impacts (Gibson, 1994; Graham & Welch, 1996).

On the other hand, a strategy of breeding staple crops with enhanced ability to fortify themselves with micronutrients offers a sustainable, cost-effective alternative, which is more likely to reach those most in need and has the added advantage of requiring no change in current consumer behaviour to be effective. It represents a strategy of 'tailoring the plant to fit the soil' rather than the opposite, which is afforded by the soil fertilisation approach (Graham *et al.* 2001).

Exploiting the genetic variation in crop plants for micronutrient density is likely to be an effective method to improve the nutrition of entire populations. A four- to five-fold variation was found between the lowest and highest grain Fe and Zn concentrations among wheat accessions

studied at Centro Internacional de Mejoramiento de Maiz y Trigo (International Maize and Wheat Improvement Centre) (CIMMYT), and the highest concentrations were double those of popular modern varieties (Graham *et al.* 2001). Moreover, wild, small-seeded relatives of modern bread wheats have been found with 50 % more Fe and Zn than the highest CIMMYT germ plasm studied (Ortiz-Monasterio, 1998). If, for example, a wheat variety were identified, which was both high-yielding and produced twice the grain Se density of most other varieties (i.e. 100 instead of 50 µg Se/kg on a relatively low-Se soil), it could, assuming wheat supplies 50 % of available Se ingested in Australia, result in an increase in Se intake from the current estimate of 75 µg/adult per d to 113 µg/adult per d, with significant health benefits.

Graham *et al.* (2001) emphasised the importance of combining nutrient-density traits with high yield. There is unlikely to be a premium paid for a higher quality product by resource-poor consumers, so new, high-nutrient varieties must also be attractive to farmers in terms of yield. In all the crops examined so far, it is possible to combine high micronutrient density with high yield. These authors also stress the importance of bioavailability; will the added nutrients be sufficient to have a significant effect on human nutrient status? As discussed on p. 52, wheat-Se is highly bioavailable.

A benefit–cost analysis of a breeding approach to increase wheat-grain Zn density in Turkey, using very conservative assumptions and current dollar values, estimated that costs of US \$13 million would produce benefits of US \$274 million (economic only, with no account taken of improved health and quality of life), a favourable benefit : cost ratio of 21 (Graham *et al.* 2001). In contrast, conventional fortification requires yearly funding, and if the investment is not sustained, the benefits cease.

*Screening and selection: the importance of genotype–environment interaction.* In the screening phase of a breeding programme, genotype–environment interaction needs to be relatively low for a breeding approach to be viable. Field trials where different genotypes are grown at the same site in the same season should enable comparison of genotypes. Ideally, trials should be conducted for 2 years at the same site, as different grain nutrient levels of genotypes grown on different soils can even be reflected in the first-year grain harvested. Furthermore, the variability in soil availability for micronutrients is generally much greater than that for macronutrients (Graham, 1991), hence field trials need to be of limited area to reduce spatial variability; paired-plots, where each replicate comprises adjacent treatment and control plots, can be a useful technique.

A major gene together with several minor additive genes are likely to control the uptake of nutrients into crop plants, and another group of genes, also featuring a major gene, appear to control grain loading (Epstein, 1972; Ripperger & Schreiber, 1982; Graham, 1984). In selecting for grain Se density, both high uptake from the soil and high capacity to move Se from the vegetative tissues to the grain are required. However, as Se is not known to be essential for higher plants, agronomic efficiency may not

be involved. Nevertheless, an increase in loading efficiency into the grain is desirable. This could be achieved by the endogenous chelator of Se in the phloem which increases Se transport to the inflorescence, or by more transporters at the plasma membrane at unloading or uptake into the grain.

Gene technology, including the use of molecular markers (cDNA, restriction fragment length polymorphisms, amplified fragment length polymorphisms and randomly amplified polymorphic DNA) for the mRNA expressed by efficiency and grain-loading genes, provides an alternative to conventional plant breeding as a means to enhance micronutrient density in cereals (Graham *et al.* 1997). The introduction, for example, of a gene that facilitates expression of the permease sulfate transporter would be likely to increase uptake and transport of selenate.

Surveys of Se level in grains have suggested that environment may be more important than genotype in determining grain Se density. The Queensland study of Noble & Barry (1982), discussed on p. 51, found differences in Se concentration in wheat of up to 100-fold at different sites, but little difference between wheat varieties. The maximum variation for both species and varieties at any site was five-fold. A Japanese study found that Se levels in rice grown in different parts of the country varied from 11 to 182 µg/kg. A significant difference was found between levels in rice from different districts, but not between different rice varieties grown on the same soil (Yoshida & Yasumoto, 1987). Our group has found up to a six-fold variation in grain Se concentration in one wheat cultivar at a trial site in South Australia.

Genotypic differences were apparent, however, in a study of Se in hulled (spelt and emmer accessions) and modern bread and durum wheats, grown together. The hulled wheats had higher concentrations of Se, Li, Mg, P and Zn. Five spelt accessions had twice the Se concentrations of emmer, and from two to eight times those of normal wheat (Piergiorganni *et al.* 1997). This is in contrast to the findings of Grella (1996), who observed no Se differences between spelt and wheat. It is not clear at this stage whether sufficient genetic variability for grain Se density exists between wheat cultivars to enable selection for this trait, and further research is proceeding.

## Conclusion

Research results continue to illustrate the importance of Se in human health, in particular its anti-inflammatory, anti-cancer and anti-viral activities. It is evident, due mainly to its poor availability in many soils, that as many as one billion individuals may be Se-deficient. The vast majority of the world's population would receive well below the level needed to maximise cancer prevention, which is likely to be within the range of 125–280 µg/adult per d, depending on gender, pregnancy and exposure to oxidative stress. The average Australian adult would ingest around 75 µg Se/d.

Se levels in Australian wheat are generally moderate, but due to widespread wheat consumption and the high bioavailability of Se in wheat, this source probably accounts for nearly half the Se utilised by Australians. An increase in the Se content of wheat grain is likely to be the most cost-effective method to increase Se levels in the

human population. A substantial increase in population Se intake may result in decreased rates of several important cancers, cardiovascular disease, viral disease sequelae, and a range of other conditions that involve oxidative stress and inflammation, with resultant reductions in health costs.

The most promising strategies to increase Se in wheat appear to be biofortification by Se fertilisation and breeding wheat varieties with superior ability to accumulate Se in the grain. Studies in Europe and North America have shown that the addition of as little as 10 g Se/ha can increase grain Se level by up to 0.40 mg/kg. However, studies are needed to determine the most efficient methods for Australian conditions, and to determine the extent of genetic variability for grain Se accumulation.

Furthermore, before recommending large-scale fortification of the food supply with Se, it will be necessary to await the results of current intervention studies with Se on cancer (including the SELECT and PRECISE trials), asthma, and HIV and AIDS.

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