CHAPTER 17

Breeding for quantitative variables Part 4: Breeding for nutritional quality traits

Yusuf Genc, Julia M. Humphries, Graham H. Lyons and Robin D. Graham

17.1 INTRODUCTION

Malnutrition is the most important cause of mortality in the global human population. More than a quarter of children less than five years old suffer from protein-energy malnutrition, as determined by rates of stunting and underweight. Of these, 70 percent are in Asia, 26 percent in Africa and 4 percent in Latin America. Stunting in resource-poor populations is usually associated with reduced mental development (Stephenson, Latham and Otteson, 2000).

Dietary deficiencies of the micronutrients iron (Fe), zinc (Zn), vitamin A (in the form of pro-vitamin A carotenoids), selenium (Se) and iodine (I) are widespread globally, affecting well over half of the world's population, and often occur concurrently (WHO, 2003). These deficiencies increase the risk of severe disease in approximately 40 percent of the world's population (Graham, Welch and Bouis, 2001). Se, Fe, Zn and vitamins A, B and C have immunomodulating functions and thus influence the susceptibility of a host to infectious diseases and their courses and outcomes (Bhaskaram, 2002; Failla, 2003).

Most of the Fe in the body occurs in combination with proteins as the oxygen-carrying pigments haemoglobin in red blood cells and myoglobin in muscle cells. Deficiency results in reductions in haemoglobin (anaemia) and tissue Fe (myoglobin and Fe-containing enzymes), and in lethargy (Jones, 1997).

Zn is a component of over three hundred enzymes, involved in carbohydrate metabolism, DNA synthesis, protein synthesis and digestion, and bone metabolism. Deficiency can result in reduced growth rate, skin lesions and increased susceptibility to infection (Jones, 1997).

Vitamin A deficiency is a major cause of blindness, growth retardation and increased susceptibility to infection. It commonly occurs in association with protein and Zn deficiency (Wahlqvist, 1997). This chapter will deal with the plant precursors to vitamin A: carotenoids such as β-carotene, and the non-provitamin A carotenoids, including lutein and zeaxanthin. Carotenoids are responsible for many of the orange, red and yellow colours seen in plants and animals. A small proportion of the over 600 named carotenoids are precursors to vitamin A, and are essential for the prevention of vitamin A deficiency. β-carotene has the highest provitamin A activity. Carotenoids that are not precursors to vitamin A also have an important role in health and nutrition as antioxidants and in the maintenance of sight, and those most commonly found in staple foods are lutein and zeaxanthin. Lutein and zeaxanthin are abundant in maize, and lutein is the dominant carotenoid in both bread and pasta wheat.

Se is an integral component of at least three systems required for normal cell metabolism, and has antioxidant, anti-cancer and anti-viral effects (Arthur, 1999). I is involved in growth, development and metabolic regulation, through its role as a component of thyroid hormones (Hetzel and Pandav, 1996). Moreover, interactions between Se and I are important in the body. Both micronutrients are required for thyroid hormone synthesis, activation and metabolism, and the thyroid gland has the highest Se and I concentrations of all organs (Kohrle, 1999).

HarvestPlus is a Biofortification Global Challenge Program of the Consultative Group on International Agricultural Research (CGIAR). It is coordinated by the International Centre for Tropical Agriculture (CIAT) and the International Food Policy Research Institute (IFPRI). Genetic bio-fortification is a strategy of

breeding staple crops such as rice, wheat, barley, maize, cassava, potatoes and beans with the ability to fortify themselves with micronutrients. It offers a sustainable, cost-effective alternative to other strategies such as individual supplementation and fertilization, 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 (Graham, Welch and Bouis, 2001). Once a one-off investment is made to breed bio-fortified seed, recurrent costs are low and germplasm can be shared globally. Bio-fortification, commercial fortification and supplementation are complementary strategies for reaching malnourished populations. Furthermore, bio-fortification can increase farm productivity as certain micronutrients, such as Zn and Se, that improve human nutrition can help plants resist diseases and other environmental stresses (HarvestPlus, 2007).

Breeding criteria for micronutrient-enriched staple food crops have been reviewed recently (Welch and Graham, 2004). These criteria include (i) maintaining crop productivity, (ii) evidence for stability of micronutrient enrichment traits across various edaphic and climatic zones, (iii) demonstration of significant effects of enriched micronutrients on human health, (iv) demonstration of bio-availability of enriched micronutrients for human nutrition, and (v) consumer acceptance.

In this chapter, we will focus on genetic potential, genotype × environment interactions, screening protocols, breeding strategies for enhancing grain micronutrient accumulation. Physiological and molecular mechanisms of uptake, translocation and deposition of micronutrients in the grains or other edible parts of major staple food crops such as wheat, rice, maize, beans and cassava, which are consumed by billions of people in resource-poor nations will also be discussed. Sufficient genotypic variation in the trait to be selected is necessary for conventional breeding to be feasible, so we will discuss this as a first step, with reference to the five key micronutrients. Breeding principles discussed in this chapter are applicable to both traditional and participatory plant breeding.

17.2 GENOTYPIC VARIATION OF MICRONUTRIENT CONCENTRATION IN STAPLE FOOD CROPS

17.2.1 Fe & Zn

Over the last decade, there have been considerable efforts in several international research centres such as the International Maize and Wheat Improvement Centre (CIMMYT) in Mexico and the International Rice Research Institute (IRRI) in the Philippines, to identify wheat and rice germplasm with high Fe and Zn concentration, which has also been the subject of several reviews (Graham *et al.*, 1999; Rengel, Batten and Crowley, 1999; Cakmak *et al.*, 2000).

Wheat

At CIMMYT, in one study, 170 wheat lines selected out of 550 initially screened lines were grown in a replicated trial (Ortiz-Monasterio and Graham, 2000). This study identified three promising sources of high grain Fe and Zn concentration: wild species, landraces and breeding lines. Fe concentration was in the range of 25 to 56 mg/kg dry weight (DW), while Zn concentration varied from 25 to 65 mg/kg DW. In a second study, a group of 154 lines from the breeding programme were grown together. Lines were identified with up to 73 mg Fe/kg DW and 92 mg Zn/kg DW. Our group at the University of Adelaide also observed significant variation in grain Zn and Fe concentrations in commercial cultivars and advanced breeding lines

TABLE 17.1
Zn and Fe concentrations (mg/kg DW) in grains of durum and bread wheat genotypes grown in standard potting mix with all nutrients supplied adequately in a glasshouse

	No. of entries	Fe		Zn	
		Mean conc. (SD)	Range	Mean conc. (SD)	Range
Modern bread wheat (*T. aestivum*)	25	36 (6)	27–53	39 (8)	25–53
Synthetic hexaploid wheat (*T. aestivum*)	36	41 (8)	32–67	41 (9)	28–66
Durum wheat (*T. dicoccon*)	24	42 (7)	29–56	51 (6)	39–62
(*T turgidum*)	191	33 (8)	17–62	30 (12)	12–81

Note: Standard deviation, SD, is given in parentheses.
Source: Genc *et al*., unpublished.

TABLE 17.2
Zn and Fe concentrations (mg/kg DW) in grains of bread wheat genotypes in field trials in Australia

Location and year	No of entries	Fe		Zn	
		Mean conc. (SD)	Range	Mean conc. (SD)	Range
Birchip-2000	28	37 (3)	31–41	16 (2)	12–19
Birchip-1999	42	38 (2)	32–42	25 (3)	20–31
Birchip-1998	39	42 (4)	36–55	23 (2)	19–31
Horsham-1998	30	33 (3)	27–40	16 (1)	13–19
Bute-1997	42	32 (3)	27–38	18 (2)	15–21
Lameroo-1996	35	33 (3)	27–39	20 (2)	15–24

Note: Standard deviation, SD, is given in parentheses
Source: Graham *et al.,* unpublished.

of wheat in glasshouse and field trials conducted over a number of years in Australia (Tables 17.1 and 17.2). In general, grain Zn and Fe concentrations were higher in the glasshouse than in field studies, which can be attributed to better growing conditions (well-watered and fertilized) in the glasshouse than in the field. In the field studies, grain Zn concentration was in the range of 12 to 31 mg/kg DW, a narrower range than that found at CIMMYT. This narrower range and also lower values (<15 mg/kg DW) in grain Zn concentration are indicative of Zn deficiency in the field. Grain Fe concentration varied from 27 to 55 mg/kg DW. Recently, a large-scale screening by Cakmak *et al.* (2004) has identified wild wheat accessions with even higher Fe and Zn concentrations than those reported previously. In this comprehensive study of 825 accessions, including wild emmer wheat (*Triticum turgidum* subsp. *dicoccoides*), grain concentrations were 14 to 190 mg/kg DW and 15 to 109 mg/kg DW for Zn and Fe, respectively. In this study, no yield data were reported, thus we do not know whether high concentrations are associated with low yield. In the meantime, despite lower Fe and Zn concentrations than in wild species, more screening is needed of elite germplasm (modern wheat genotypes and advanced breeding lines) for high Fe and Zn concentration in the grain, as they have already improved agronomic performance (Graham *et al.*, 1999).

Rice

There also exists considerable genotypic variation for grain Zn and Fe concentration in rice. Researchers at IRRI and the

University of Adelaide (Australia) have evaluated a large set of brown rice varieties (1138), including breeding lines and wild rice and its derivates, and observed a wide range in Fe (6–24 mg/kg DW) and Zn (14–58 mg/kg DW) concentrations in the grain (Gregorio *et al.,* 2000). Some of these high-Fe and-Zn varieties were further tested alongside the two most popular cultivars in Asia, IR36 and IR64, in the same soil and year. The traditional variety, Jalmagna, had much higher grain Fe and Zn concentration than high-yielding IR36 (22 vs 12 mg Fe/kg DW; 32 vs 21 mg Zn/kg DW in Jalmagna and IR36, respectively). Moreover, aromatic rices are often reported to have higher grain Fe and Zn concentration than non-aromatic rices (Graham, Senadhira and Ortiz-Monasterio, 1997; Gregorio *et al.,* 2000).

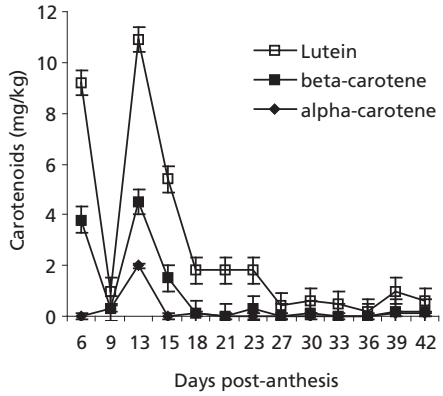
There is some evidence that breeding for either high Fe or Zn may also result in higher concentrations of other nutrients, as there is occasionally a significant positive correlation between Fe and Zn concentrations in the wheat and rice grain (Ortiz-Monasterio and Graham, 2000; Graham, Senadhira and Ortiz-Monesterio, 1997; Genc *et al.,* unpublished). This is also evident in a recent study by Vasconcelos *et al.* (2003), who introduced a soybean *ferritin* gene into *indica* rice, and found much higher Fe and Zn concentrations in the grains of transgenic plants compared with non-transgenic plants. However, as no seed weight data were provided, we do not know whether these high concentrations were associated with small seed size (concentration effect). Nevertheless, our calculations of their data established a significant positive correlation between grain Fe and Zn ($r^2 = 0.54$).

17.2.2 Vitamin A

The potential for finding genetic variation that can form the basis for breeding crops with increased carotenoid concentrations is great, given that all photosynthetic organisms have substantial concentrations of these compounds. However, in many staple crops it is necessary for the plant to store carotenoids in non-photosynthetic tissues, such as the tuber of the sweet potato, or in tissues that no longer have a photosynthetic capacity when harvested, as in wheat and maize. It is possible that the consumed portion of the crop that once had photosynthetic capacity may retain the carotenoids accumulated during the photosynthetic period post-degradation of the chlorophyll. However, in root crops, carotenoids must accumulate in non-photosynthetic tissues, and therefore need to be transported there from other photosynthetic tissues, or synthesized *de novo*.

A report by Graham and Rosser (2000) compared the synthesis patterns during maturation of both lutein and β-carotene in bread and durum wheat varieties (Figure 17.1). These results concur with an earlier report of Lacroix and Lier (1975), and indicate that a potential benefit may be gained by harvesting wheat at the immature (green) stage. However, appropriate storage and preservation methods are necessary in order for immature wheat to be stored for any period of time without spoilage. Such a method has been used for centuries in Middle Eastern countries, where wheat is harvested green and dry roasted to produce a product called *freekeh*. Substantial amounts of both β-carotene and lutein can be conserved from the photosynthetic stage in the roasted product by this method (Humphries and Khachik, 2003). This method of storage may be a valuable starting point for adoption into local cultures for

FIGURE 17.1
Time trend of synthesis and metabolism of various carotenoids in the caryopsis of wheat (var. Krichauff) from anthesis to maturity



Source: Graham and Rosser, 2000. Reprinted with permission from the UNU Food and Nutrition Bulletin (2000) 21: 404–409.

preservation of carotenoids present in immature wheat.

Selection against highly pigmented varieties in several staple foods has led to very little variation for this trait in modern cultivars. However, germplasm banks where landraces and old varieties are stored hold the key to retrieving genetic variation. These valuable resources can be used as a source of variation for the introduction of desirable traits back into commercially profitable and locally grown varieties, for nutritional benefit. Reports from screening of genetic resources obtained from germplasm banks indicate that many of the older varieties have substantial variation for carotenoid concentration.

Maize

White maize was previously highly desired for reasons of cleanliness and apparent purity, while maize varieties with high concentrations of pigmentation were used as stock feed. While people suffered from vitamin A deficiency, their stock remained healthy due to consumption of yellow maize (Brunsen and Quackenbush, 1962). Collaboration between nutritionists and agriculturalists resulted in the production of high-β-carotene maize adapted to local conditions, resulting in a reduction in vitamin A deficiency-associated diseases.

There have been several reports of genetic variation for carotenoids in maize. One of the first was that of Brunsen and Quackenbush (1962), who showed that the total concentration of carotenoids varied significantly between high- and low-carotene inbred lines, with an even greater variation between low and high for provitamin A carotenoids. Blessin *et al*. (1963) reported a range of 0.9 to 4.1 mg/kg for carotenes, and 18.6 to 48 mg/kg for xanthophylls in 39 maize inbreds. In the same year, Quackenbush *et al*. (1963) observed concentrations of up to 7.3 mg/kg carotenes, and a range of 2 to 33 mg/kg lutein in 125 inbred lines. The value of extensive screening for high-accumulation varieties was indicated in the report of Egesel (1997), who found a range of just 0.13 to 2.9 mg/kg β-carotene in 200 maize families. More recently, 16 yellow seeded maize lines were reported to have 143 to 278 mg/kg carotenoids (Maziya-Dixon *et al*., 2000). This study measured total carotenoid concentrations rather than defining provitamin and non-provitamin carotenoids, and although valuable for calculation of total carotenoid intake, gives no idea of the provitamin A potential of the cultivars.

Wheat

Wheat is another staple food that has been subjected to selection for colour, though with different outcomes depending on the end use, either bread or pasta. Bread wheat varieties (*Triticum aestivum*) have been

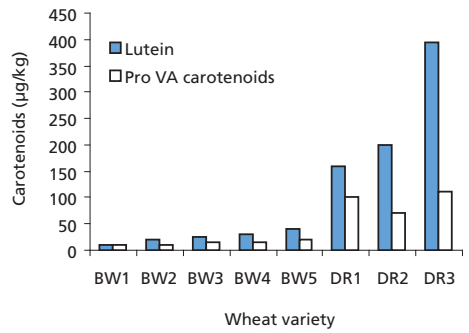
the subject of selection against pigmentation, though ironically, despite breeding efforts, it was still deemed necessary to use chemical bleaching rather than plant breeding alone to achieve the bright white colour demanded by bread wheat consumers. The process of bleaching has now been discontinued. However, the generations of selection against pigmentation has resulted in current cultivars containing very low concentrations of carotenoids. In Australia, there is an exception, a South Australian wheat by the name of Krichauff, which has relatively high concentrations of carotenoids in comparison with other current cultivars. It is also possible that the wheat used to make tortillas in South America may have substantial carotenoid concentrations that could be exploited for nutritional gain.

Matus-Cadiz *et al.* (2003) reported that the lutein concentration of 79 diverse spring wheat varieties from Australia and Canada ranged between 1.8 and 3.7 mg/kg, and also reported genotype × environment (G×E) effects that will be discussed in the G×E section of this chapter. An extensive survey of bread wheat varieties (Humphries *et al.*, 2004) revealed considerable genetic variation for both provitamin and non-provitamin A carotenoid concentrations in germplasm from the CIMMYT germplasm bank. Results from the range of concentrations obtained from bread wheat combined with those for durum wheat varieties are given in Figure 17.2.

Alternatively, the importance of colour in durum wheat (*Triticum durum*) used for pasta, has led to extensive studies into carotenoid concentrations. Several of these studies are presented below, and together with other reports not included here, indicate that despite the apparent potential for a source of high concentrations of carotenoids in different cultivars, concentrations of provitamin A carotenoids are usually low. It appears that selection for the colour provided by lutein has led to varieties with an abundance of the enzymes responsible for hydroxylation of the provitamin A carotenoids, and consequently the durum wheats are not thought to be a useful source of genetic variation for increased provitamin A carotenoids.

Some of the first reports of the dominance of non-provitamin A carotenoids in durum wheat were published in 1935 (Markley and Bailey, 1935a, 1935b), and later it was confirmed that only a small proportion of carotenes were present in comparison to lutein (Munsey, 1938). This was followed up by Zechmeister and Cholnoky (1940) and Lepage and Sims (1968), who reported lutein ester concentration and no provitamin A carotenoids. A more recent evaluation of the carotenoid composition of durum wheat (Hentschel *et al.*, 2002) revealed a range of lutein concentrations from 1.5 to 4 mg/kg, and no carotenes.

FIGURE 17.2
Carotenoid concentrations of five bread wheat varieties (BW) and three durum wheat varieties (DR), representing the range of values found in collections from the germplasm banks in CIMMYT and ICARDA



Source: Graham and Rosser, 2000. Reprinted with permission from the UNU Food and Nutrition Bulletin (2000) 21: 404–409.

Crosses with barley

Tritordeum lines, which are a cross between the wild barley species *Hordeum chilense* and diploid, tetraploid and hexaploid wheat have consistently shown higher concentrations of carotenoids than wheat (Alvarez, Urbano and Martin, 1994), and are considered a useful source for increasing the carotenoid concentration of durum wheat. However, using this cross to increase concentrations is dependent on interactions between the genetics of the parents, and the final concentrations cannot be reliably predicted.

Ancient wheat

Einkorn (*T. monococcum*), an ancient diploid wheat, has been reported to have yellow coloration (D'Egidio, Nardi and Vallega, 1993; Abdel-Aal, Hucl and Sosulski, 1995; Borghi *et al*., 1996). When compared with other ancient wheat varieties, spelt (*T. aestivum* subsp. *spelta*), emmer (*T. turgidum* subsp. *dicoccum*), Kamut (*T. turgidum* subsp. *turanicum*) and Khorasan (*T. turgidum* subsp. *turanicum*) einkorn lines generally had higher concentrations of lutein (mean 8.1 ± 0.26 µg/g) (Abdel-Aal *et al*., 2002).

Cassava

The carotenoid concentration of cassava roots has been closely correlated to the intensity of the root colour. However, within groups of the same tuber colour, genotypic variation has also been reported, from 6 to 24 mg/kg fresh weight (FW) (Chavez *et al*., 2000). This variation within colour types necessitates individual analyses to determine individual concentrations, and colour alone cannot be relied upon to give accurate estimations of concentrations.

One of the largest reported analyses of cassava for carotenoid concentration was conducted by Iglesias *et al.* (1997), who screened a total of 632 accessions from the CIAT germplasm bank collection of 5500. The distribution of concentrations ranged from 1 to 24 mg β-carotene/kg FW. Those varieties with the deepest coloration towards orange had the greatest concentration of carotenoids, which is consistent with the report of Chavez *et al*., (2000).

The variability for carotene concentrations in cassava has been reported for accessions obtained from germplasm banks in India (Moorthy *et al*., 1990) and Brazil (Ortega-Florez, 1991). The highest concentrations were below 8 mg of β-carotene equivalents/kg FW, which is one-third the highest concentration reported by Iglesias *et al*. 1997. However, the potential for rapidly increasing carotene concentrations using recurrent selection was reported by Jos *et al*. (1990). Using this method, it is possible to increase the concentration by three times, from 4.2 to 13.8 mg/kg FW after 2 cycles of selection and recombination (Jos *et al*., 1990). It is therefore possible, in theory, to obtain concentrations of β-carotene up to 72 mg β-carotene/kg FW.

17.2.3 Se & I

To address dietary Se deficiency, agronomists and plant breeders have adopted complementary strategies to develop crops with higher Se content. The first is an agronomic (fertilizer) approach, discussed elsewhere (Lyons *et al.*, 2003, 2004; Broadley *et al.*, 2006). The second strategy is to develop varieties with improved Se accumulation and tolerance traits by either conventional breeding or genetic modification. To implement this approach, a comprehensive characterization of the interactions between Se and sulphur nutrition was conducted in *Arabidopsis* (White *et al.*, 2004). If sufficient genotypic variation exists in Se accumulation within a crop species, and if this variation is heritable, conventional plant breeding could

provide an alternative to agronomic biofortification and thus minimize the need for Se fertilizers (Broadley *et al.*, 2006).

Few data have been published on varietal differences for Se accumulation for most crop species. However, in *Lycopersicon* (tomatoes and related plants), four-fold differences in shoot Se accumulation have been found (Pezzarossa *et al.*, 1999), and in *Brassica* (broccoli) a significant genotype effect for Se concentration in heads was found in hybrids, but not inbreds. However, the effect of environment was around ten times stronger than that for genotype (Farnham *et al.*, 2007).

Wheat

In surveys and trials conducted by our group, involving diverse wheat germplasm and a total of eleven datasets in South Australia and Mexico, grain Se concentrations were in the range of 5 to 720 µg/kg DW, but much of this variation was associated with spatial variation in soil-available Se. South Australian soils are renowned for their microspatial variability, which makes detection of genotypic differences in grain Se density difficult. No significant genotypic variation in grain Se density among modern commercial bread or durum wheat varieties was detected in this study (Lyons *et al.*, 2005), which agrees with earlier research (Noble and Barry, 1982; Grela, 1996; Tveitnes, Singh and Ruud, 1996). However, the ancient diploid wheat, *Aegilops tauschii* L. and rye (*Secale cereale*) were found in our studies to be 42 percent and 35 percent higher ($p < 0.001$; $p = 0.03$), respectively, in grain Se concentration than other cereals in separate field trials, and in a hydroponic trial rye was 40 percent higher *(*$P < 0.001$) in foliar Se content than two wheat landraces. *Ae. tauschii* was also higher in Zn, Fe and Mn than other wheats in the trial. Other wild wheat relatives may also be efficient accumulators of Se and other minerals (Piergiovanni *et al.*, 1997).

Studies of genotypic variation of I in diverse plant species have yielded variable findings. A Japanese survey found no significant difference in leaf I concentration for plants grown on similar soils (Yuita *et al.*, 1982), while an earlier survey of different pasture species grown together in the field found a thirty-four-fold variation in leaf I concentration, with perennial ryegrass (*Lolium perenne* L.) the most efficient I accumulator (Johnson and Butler, 1957).

Evidence is scarce for significant genotypic variation in grain density of I in wheat (Shinonaga *et al.*, 2001), and no variation was detected in our South Australian trial, where varieties were grown at three locations, with three replications. I concentrations were low, typically less than 20 µg/kg (range 10–60 µg/kg DW) in whole grain (Lyons *et al.,* unpublished).

Maize

Little research has been conducted on genotypic variation in Se or I concentration in maize kernels. Our group has conducted a limited survey of diverse maize genotypes grown in the United States of America (Illinois) and Nigeria. No significant variation was detected for either micronutrient; however, the soils at both sites were low in available Se, resulting in kernel Se levels at the Nigerian site, for example, of just 5 µg/kg DW (range 3–10 µg/kg DW). Such low levels may have limited expression of possible varietal Se differences (Lyons *et al.*, unpublished).

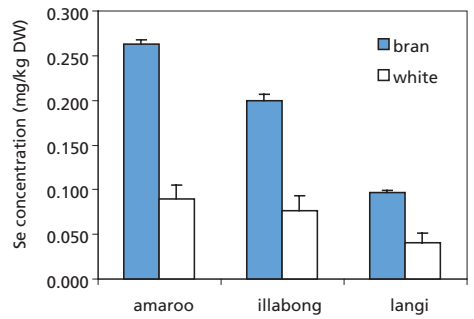
Rice

Rice appears to be a more promising cereal for genotypic variation in Se, with differences detected in other studies (Nan and

Han, 1993; Zhang *et al.*, 2004) as well as our own, which involved several varieties grown together in New South Wales, Australia. Three varieties differed in Se concentration in bran (means [SE] of 97 [12], 200 [17], 263 [14] µg/kg DW), while one was lower than the others in endosperm Se concentration (40 [2], 83 [5] µg/kg DW) ($P < 0.001$) (Lyons *et al.*, 2005b; Figure 17.3).

Genotypic variation in I concentration in rice bran was apparent in our Australian study, with all three cultivars different ($p = 0.02$). The mean I concentrations in the bran [SE] in µg/kg DW were 910 [50], 770 [10], and 500 [50]. There was no difference in I concentration in the endosperm for the three cultivars (Lyons *et al.*, unpublished).

FIGURE 17.3
Effect of genotype on Se concentration in rice bran and white (polished) rice. Bars represent means of three replications ± SE



Source: Lyons *et al.*, 2005b.

17.3 GENOTYPIC VARIATION IN STAPLE CROPS FOR DISTRIBUTION OF MICRONUTRIENTS WITHIN EDIBLE COMPONENTS

17.3.1 Fe & Zn

Wheat

Almost all studies to date have dealt with nutrient concentration in the whole grain, and there are few data available on the distribution of micronutrients in different grain fractions. Lyons *et al.* (2005c) studied distribution of grain Fe, Zn and other nutrients across the seed tissues of four wheat genotypes, and reported that the proportion of Zn in the grain (percent of total grain Zn content) was in the following order: endosperm (including aleurone layer) > embryo > bran (72–78, 11–27 and 2 percent, respectively). The proportion of Fe in the grain fractions followed the same order as Zn (79–91, 3–19 and 2–9 percent for endosperm, embryo and bran, respectively).

Rice

The highest proportion of Fe was located in pericarp, including aleurone layer (43 percent), followed by endosperm (42 percent) and embryo (10 percent) (Boyd *et al.*, 1972). The proportion of Zn was 60, 42 and 14 percent in pericarp (including aleurone layer), endosperm and embryo, respectively. It is interesting to see that when all proportions for Zn are added up, we get a value of over 100 percent, which may be due to contamination during dissection, processing or analytical errors associated with a very small sample size (Boyd *et al.*, 1972).

The distribution of nutrients in cereal grains is important for human nutrition. For obvious reasons, high concentration in the endosperm is desirable as endosperm makes up the majority of the grain. It is well known that a significant proportion of nutrients is lost in milling residue (Burk and Solomons, 1985). A further reduction in nutrient content occurs in the polishing process in rice (Graham *et al.*, 1999). Therefore, in breeding programmes, selection for higher Fe and Zn concentration in the endosperm would not only result in lower losses of Zn and Fe, but enhance bio-availability due to lower levels of phytate and fibre

components in endosperm than in bran (Lyons *et al.,* 2005c). Further research with a large number of genotypes is required to determine the extent of genotypic variation in distribution of Zn in the grain, and also to assess the potential for breeding for this trait.

17.3.2 Se & I

As noted above, genotypic variation was found by our group in rice bran and, to a lesser extent, endosperm for Se, but only in bran for I. Se concentration in rice bran was around three times that in endosperm, while I concentration in bran was around nine times that in endosperm (mean 730 vs 80 µg/kg DW) (Lyons *et al.,* 2005b). As most rice is eaten in the polished form, with the bran removed, selection for a higher proportion of grain Se and I stored in endosperm may be worthwhile, as noted for Zn and Fe above.

17.5 GENOTYPE × ENVIRONMENT INTERACTIONS AND THEIR EFFECTS ON MICRONUTRIENT TRAITS

G×E interaction is an important issue for plant breeders. A significant G×E interaction implies that rankings of genotypes differ with environment, indicating the need for testing at various sites or seasons. For example, a genotype may exhibit high-micronutrient-density traits in one environment, but not in others. A significant G×E interaction can be classified as either (i) non-crossover, where the ranking of genotypes remains consistent in different environments and it is the degree of accumulation that is affected; or (ii) crossover, where the rank of individual cultivars is affected by the environment (Baker and Kosmolak, 1977). G×E interactions and statistical methods for analysis and interpretation of G×E interactions have been reviewed elsewhere (Kang, 1990; Hill, Becker and Tigerstedt, 1998; Annicchiarico, Chapter 20 this volume). From a breeding point of view, it is important to understand the nature and extent of G×E interactions for designing breeding strategies and selection procedures (Eiseman, Cooper and Woodruff, 1990). Much effort has been directed to understanding G×E interactions in relation to yield, but little attention has been given to grain nutrient density. Graham *et al.* (1999) recognized that environment (soil type, fertilizer management and climate) can have a strong influence on nutrient density of grains; thus it is important to grow out the seed to be compared for at least one generation in the same environment to minimize the variation in nutrient density associated with previous growing conditions. Only then can valid comparisons of genetically controlled variation be made.

Fertilizer management can also influence micronutrient density in the grain. In studies with maize varieties grown at different nitrogen (N) and water levels, Feil *et al.* (2005) found that N fertilization reduced grain concentrations of Zn and Mn, which was attributed to higher grain yield as a result of N application. In contrast, continuous irrigation did not affect grain nutrient density. However, the rankings of varieties remained unchanged by water regime and N levels, pointing to stability of varietal differences in grain nutrient density over a range of N and water levels. At present, there is little information available on the effects of fertilization on grain micronutrient density in rice or wheat.

17.5.1 Fe & Zn

Wheat

From field trials conducted by our group in different locations and years

TABLE 17.3
Fe and Zn concentrations (mg/kg DW) in grains of wheat cultivars grown at different locations and years in Australia

Cultivar	Zn concentration						Fe concentration					
	Lameroo 1996	Bute 1997	Birchip 1998	Horsham 1998	Birchip 1999	Birchip 2000	Lameroo 1996	Bute 1997	Birchip 1998	Horsham 1999	Birchip 1999	Birchip 2000
Excalibur	22	18	21	17	25	18	37	32	44	35	40	37
Krichauff	20	19	25	18	24	18	36	34	46	38	39	38
Songlen	24	20	25	19	31	16	37	35	45	38	42	35
Trident	18	17	25	19	23	17	35	32	45	35	38	38
Yallaroi	20	17	22	16	24	14	32	32	45	36	40	37
Mean	21	18	24	18	26	17	35	33	45	36	40	37
Standard deviation	2	1	2	1	3	2	2	1	1	2	2	1

Source: Graham *et al.,* unpublished.

TABLE 17.4
Fe and Zn concentrations (mg/kg DW) in grains of wheat cultivars grown at two levels of Zn fertilization at Lameroo (1996) and Bute (1997) in South Australia

Cultivar	Zn concentration						Fe concentration					
	Lameroo			Bute			Lameroo			Bute		
	Nil	+Zn	Mean*	Nil	+Zn	*Mean	Nil	+Zn	*Mean	Nil	+Zn	*Mean
Barunga	14	22	18	15	20	17	38	36	37	33	31	32
Cascades	14	21	17	11	20	15	38	35	36	35	31	33
Excalibur	14	22	18	11	18	14	36	37	36	34	32	33
Frame	12	19	15	12	19	15	32	31	32	35	34	35
Halberd	13	23	18	12	17	14	35	33	34	36	35	36
Janz	12	18	15	11	16	13	29	29	29	29	27	28
Krichauff	12	20	16	12	19	15	38	36	37	37	34	35
RAC750	12	22	17	13	21	17	33	31	32	31	31	31
RAC809	11	19	15	10	16	13	36	35	35	34	30	32
RAC812	11	17	14	12	20	16	33	31	32	35	34	34
RAC820	15	22	18	10	15	13	33	33	33	34	32	33
RAC826	13	21	17	12	18	15	33	34	34	36	33	34
RAC832	13	21	17	14	24	19	30	31	31	38	35	37
RH911996	15	21	17	10	16	13	32	33	32	31	31	31
RH912025	14	20	17	11	18	14	36	31	34	33	35	34
Songlen	14	24	19	11	20	15	36	37	37	36	35	35
Tammin	14	20	17	10	17	14	37	35	36	34	31	32
Trident	12	18	15	11	17	14	35	35	35	35	32	33
WI334	11	17	14	11	16	14	33	33	33	35	33	34
WI94063	11	18	15	13	19	16	31	30	30	29	30	30
WI94091	10	14	12	12	18	15	29	27	28	32	29	31
Yallaroi	17	21	19	12	17	15	34	32	33	32	32	32
Yanac	11	17	14	11	16	13	31	30	31	35	31	33
Mean	13	20		12	18		34	33		34	32	
Range	10–17	14–24		10–15	18–24		29–38	27–37		29–38	27–35	

NOTES: Genotype × Zn fertilization interaction for grain Zn and Fe concentrations was non-significant, while genotype × location interaction was significant ($LSD_{0.05}$=3 for Zn and Fe). +Zn treatment received granular zinc (zinc oxysulphate, 32 percent Zn) at a rate of 7 kg/ha at seeding and a foliar spray (Zincsol, 16.7 percent Zn) at a rate of 2 L/ha at the early growth stage.
Source: Graham *et al.,* unpublished.

in Australia, Zn application resulted in an increase in grain Zn concentration in all varieties and sites. However, few genotypes in these trials were retained year after year, thus G×E interactions could not be analysed for all environments. However, when we analysed grain Zn and Fe data for the five genotypes tested in 6 environments (Table 17.3) or 23 genotypes tested in two environments (Table 17.4), responses of genotypes differed with environments (locations and years), indicating the presence of G×E interactions. When we subjected the data for grain Zn concentration (adequate Zn only) in Table 17.5 to Spearman's Rank Correlation Test (r_s), we found a non-significant correlation between rankings of genotypes in two different environments (r_s = 0.223), suggesting that rankings of genotypes differ with environment.

Most recently, significant G×E interactions were also reported for grain Zn and Fe concentrations, which would make direct selection for these traits difficult (Oury *et al.*, 2006). The ranges in grain Zn and Fe concentrations of adapted material in this study (15–35 and 20–60 mg/kg DW for Zn and Fe, respectively) were wider than those found in our field study (Table 17.5), which might be attributed to differences in genotypes and environments between the two studies. These limited studies suggest that there is a need for further field trials at different locations and years to determine or confirm the extent and nature of G×E interactions and their effects on grain Zn and Fe concentrations in wheat.

Rice

It was reported that high Fe and Zn traits were expressed in all rice environments (Graham *et al.*, 1999) and G×E interactions were sufficiently moderate (Gregorio *et al.*, 2000), suggesting that breeding for high Fe and Zn traits is a worthwhile effort. However, there was some evidence of G×E interaction in extreme environments. Although these limited studies are encouraging, there is clearly a need for further studies in this area.

17.5.2 Vitamin A

No G×E effect on lutein concentration was reported by Matus-Cadiz *et al.* (2003) when they investigated the effect of genotype, year and location in Australian and Canadian wheat varieties. However, further statistical analysis of the data revealed that 12 of the 79 cultivars showed significant crossover genotype-by-year interactions, indicating that in different years those cultivars reported changed in lutein concentrations that affected their rank.

17.5.3 Se

While genotypic differences may exist in modern wheat varieties, they are likely to be small in comparison with background soil variation. Soil Se is uneven in distribution and availability, with total Se concentrations ranging from less than 0.1 to more than 100 mg/kg DW (Berrow and Ure, 1989). Areas that are notably low in Se include parts of China, Siberia, central Africa, eastern Europe and New Zealand (Combs, 2001). In studies of grain Se concentration in wheat grown in South Australia, our group has found substantial microspatial (that is, metre-to-metre) variation in levels of available Se in soils. For example, at one trial site near Bordertown, south-east of Adelaide, we found a six-fold variation in grain Se concentration in four replicates of one wheat cultivar, grown together in the same field (Lyons *et al.*, 2004). Hence

the detection of what may be relatively small (for example, 10 percent) genotypic variations in Se uptake efficiency between wheat cultivars under these field conditions is virtually impossible. Background soil variation in available I has also been found to be substantial at the South Australian sites we have used, although less so than for Se. This large microspatial variation in soils makes it difficult, if not impossible, to accurately assess genotypic differences across environments for Se and I. This and narrow genotypic variation reported so far may be the reasons why to date there have been no studies reported on G×E interactions for Se and I.

17.6.SCREENING AND ANALYTICAL METHODS FOR MICRONUTRIENTS IN FOOD CROPS

Where should screening be carried out: field or greenhouse? The principles of both controlled environment and field screening are reviewed elsewhere (Graham, 1984), and therefore will not be dealt with in detail here.

17.6.1 Fe & Zn

The data presented in Table 17.4 suggest that screening for grain Zn concentration should be carried out in optimal growing conditions, as variation in grain Zn concentration under Zn deficient conditions is rather narrow. Unlike traits such as agronomic Zn efficiency, screening at the early growth stage does not appear to be suitable for detecting or identifying genotypes with the ability to load more Zn into the grain, due probably to the overriding importance of re-mobilization of Zn from leaves into grain occurring towards maturity. The evidence for this comes from a study in barley (Lonergan, 2001) in which two of the four chromosomal regions (also known as Quantitative Trait Loci, QTL) identified were found to co-segregate for grain Zn accumulation and vegetative Zn accumulation at anthesis, indicating little prospect for screening for grain Zn accumulation even as early as anthesis. Moreover, the only QTL detected for shoot Zn concentration or content (chromosome 4) did not co-segregate with Zn concentration or content at either anthesis or maturity, suggesting that screening for grain Zn accumulation at the early stage will not be reliable or relevant to grain Zn accumulation.

Inductively Coupled Plasma Optical Emission Spectrometry (ICPOES) is commonly used to determine mineral nutrient concentrations in plant tissues, and allows the determination of interactions among the various essential nutrients, effects that can be large and important. This method is fast and reliable, but can be costly for breeding programmes in both developed and developing countries, where tens of thousands of samples are handled each year. Are there alternative and cheaper methods to ICPOES? The researchers at the University of Adelaide (Australia) have developed a rapid, cheap and user-friendly assay for determination of Fe in the grain of rice or wheat (Choi, Graham and Stangoulis, 2007). This new cost-effective assay consists of two phases. In phase one, the assay is used to identify high grain-Fe lines from thousands of samples, while in phase two, the high-Fe lines identified in phase one are confirmed by ICPOES.

17.6.2 Vitamin A

Several standard procedures for extraction and identification of carotenoids from plant material have been used since the discovery and naming of carotene in the early 19th century. Separation of carotenoids initially involved a two-step chromatographic

method involving open-column and thin-layer chromatography (TLC). These two methods have been combined in high performance liquid chromatography (HPLC), which is now the preferred method for carotenoid analysis.

Spectrophotometric analysis, TLC and HPLC all require extensive extraction procedures using organic solvents that are both costly and toxic. While there is no doubt that these methods are necessary for elucidation of specific isomers and absolute quantitative analysis, this reduces the scope for identification of high-carotenoid parent lines. Given the participatory focus of this book, a fast and accurate method of identifying high carotenoid concentrations would vastly increase the number of lines that could be screened to identify suitable parents by persons with little organic chemistry background.

Spectrophotometric determination of wheat grain xanthophyll concentration following extraction of flour or meal with water-saturated butanol is well established (AACC, 1983). Similarly, reflectance spectrophotometric measurement of flour colour is commonly used (Oliver, Blakeney and Allen, 1992), as is the relationship between Commission Internationale l'Eclairage (CIE) b* and extractable yellow pigments (Mares and Campbell, 2001). Colour determined by CIE classifies colour in three dimensions: L*, brightness; a* red to green colour; and b* yellow to blue colour. CIE colour is influenced by inherent genotypic characteristics, environmental conditions and stresses during grain production, the milling procedure and by the size of flour particles and bran flakes, which is caused by differences in grain hardness and moisture content of the grain at milling. Variation in L* affects the measurement of b* and potentially could result in errors in estimating carotenoid content (Mares and Campbell, 2001).

Current methods for the identification of wheat genotypes high in specific carotenoids involve HPLC and are slow, costly and highly labour intensive. The chemical structure of carotenoids indicates that a correlation with colour is likely and it is therefore possible that divergent selection for colour in bread and pasta wheat has influenced the carotenoid content of these species. Determination of a correlation between a fast and accurate colour measurement, such as that obtained from the Minolta Chroma Meter, and carotenoid concentration determined by HPLC, could vastly increase the number of samples that could be screened in a given period.

In a recent report (Humphries *et al.*, 2004), whole-meal wheat, including both bread and durum varieties, and triticale samples were analysed for their carotenoid content by HPLC, and also for colour using reflectance spectrophotometry (CIE L*a*b*). A positive correlation between CIE b* (yellowness) and lutein concentration was shown in all wheat groups, but was strongest in the durums. There was little correlation between CIE L* (lightness) or CIE a* (redness) and lutein, α- or β-carotene. By contrast, the b* value correlated well with the concentration of α- and β-carotene, and therefore the vitamin A activity, though those wheat groups that did not have a strong correlation were those with the lowest CIE b* values. The durum wheat had the highest CIE b* value and the highest lutein concentration, but a relatively low concentration of β-carotene.

17.6.3 Se & I

Because of the high soil variation in available Se and I, screening for higher

Se and I traits in cereals needs to include hydroponic trials and pot trials using a standardized growth medium, backed up by field studies conducted on soils that are relatively uniform in Se and I. Selenate is the most mobile Se form and the dominant available Se form in well-aerated, neutral to alkaline soils (Cary and Allaway, 1969), while selenite is the major form taken up by rice in flooded paddy soils of lower pH (Wang and Gao, 2001). Thus the composition of hydroponic culture media needs to be tailored to the relevant field situation. Using solution culture containing selenite as the dominant available Se form, Zhang *et al.* (2004) have found genotypic variation in Se concentration in the leaves of rice seedlings, and the levels are well correlated with those in grain.

Genotypic variation in I uptake in rice may be explained by differences in the oxidising power of the roots, which can oxidise the iodide ion to form molecular I, which is then absorbed more readily. A significant correlation was found between the oxidising power of rice roots and the uptake of I (Yamada *et al.,* 2005), hence this may prove to be a suitable screening method.

Commonly used methods of Se analysis include hydride ICPOES, ICP mass spectrometry, and fluorimetry. Sample preparation for hydride ICPOES involves digestion with a nitric+perchloric acid mixture, followed by hydrochloric acid digestion, then treatment with sodium borohydride (Tracy and Moller, 1990). ICP mass spectrometry, in which plasma is used as the ionization source, is a highly sensitive method for Se and I analyses (Hieftje and Vickers, 1989). Another commonly used (and time-honoured) method for both Se and I analysis is the fluorimetric method. This is based on the reaction of 2,3-diaminonaphthalene (DAN) with Se (IV) to form a fluorescent Se-DAN complex, piazselenol (Koh and Benson, 1983). Samples for I analysis are typically prepared using tetramethyl ammonium hydroxide (TMAH) extraction.

17.7 INHERITANCE OF MICRONUTRIENT ACCUMULATION IN FOOD CROPS

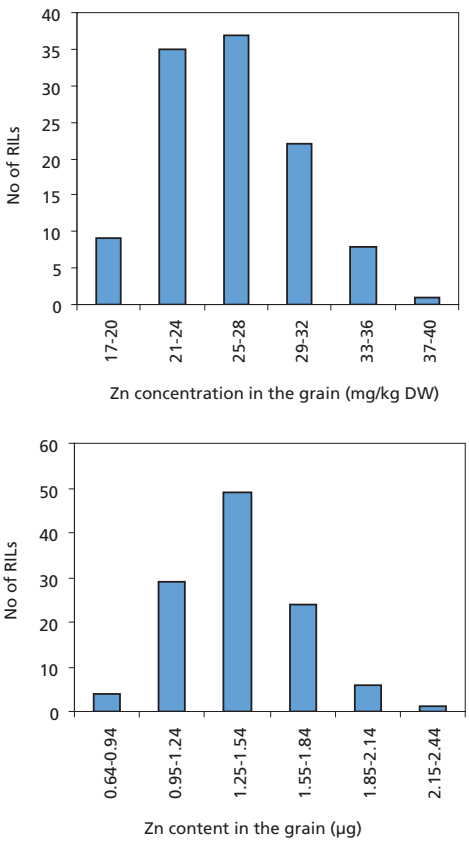
Apart from the existence of genetic variation, breeding for enhanced grain nutrient content also requires knowledge of genetic control mechanisms.

17.7.1 Fe & Zn

Wheat

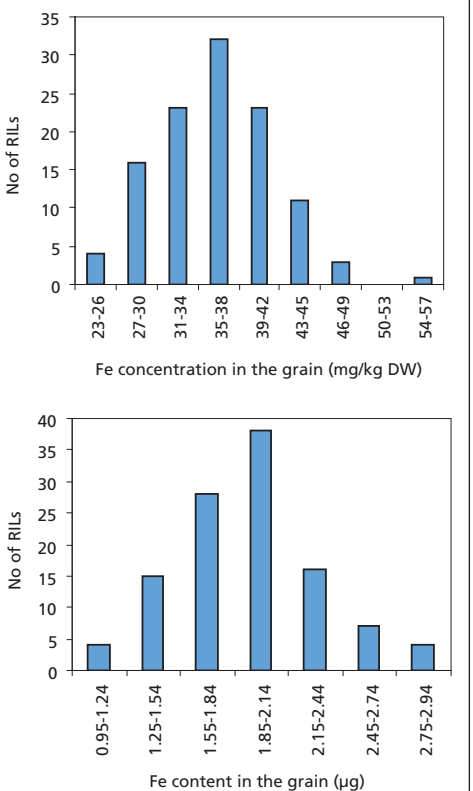
At present, there is little or no information available on genetics of Zn and Fe accumulation in wheat grain. The continuous variation in grain Zn and Fe concentration and content within Recombinant Inbred Lines (RIL) (n=113) derived from Opata × Synthetic cross (Figures 17.4 and 17.5) indicates that the two traits are quantitative and controlled by several genes (Genc *et al.,* unpublished). It is interesting to note that some RILs had higher Zn and Fe concentration and content than either of the parents, suggesting a transgressive segregation, which is probably due to these lines carrying favourable allele combinations from both parents. This multi-gene control hypothesis is supported by recent studies that identified several chromosomal regions associated with grain Zn concentration (Shi *et al*., 2007; Genc *et al*., 2009) and content (Shi *et al*., 2007). However, these field studies were conducted at a single location and QTLs identified were mapped to either different chromosomes or to different regions of the same chromosome. Therefore, there is a need to test mapping populations at multiple sites and years to validate the QTLs and also to determine the extent of GE interactions on grain Zn and Fe

FIGURE 17.4
Frequency distribution of grain Zn concentration and content in Recombinant Inbred Line population of Opata × Synthetic grown in standard potting mix in a glasshouse (Zn concentration, 24 and 20 mg/kg DW; Zn content, 1.29 and 1.08 µg/grain for Opata and Synthetic, respectively)



Source: Genc *et al.*, unpublished.

FIGURE 17.5
Frequency distribution of grain Fe concentration and content in Recombinant Inbred Line population of Opata × Synthetic grown in standard potting mix in a glasshouse (Fe concentration, 33 and 30 mg/kg DW; Fe content, 1.74 and 1.63 µg/grain for Opata and Synthetic, respectively)



Source: Genc *et al.*, unpublished.

traits. Identification and validation of QTLs associated with high grain Fe and Zn traits will accelerate breeding for these complex traits. Marker-assisted selection is discussed in Chapter 19 of this volume.

Rice

Genetic analysis of grain Fe concentration using four traditional varieties, three advanced lines and three IRRI-released varieties revealed the presence of a large genetic effect (additive and non-additive gene action) and small environmental effects (Gregorio *et al.*, 2000). Narrow-sense and broad-sense heritabilities were 44 percent and 88 percent, respectively. This study suggested that selection for a high grain-Fe trait should be delayed as late

as F_5 generation where dominance effect is not evident. This study also identified three chromosomal regions associated with a high grain-Fe trait (chromosomes 7, 8 and 9), providing evidence for multi-gene control for this trait, in which case, selection as late as F_5 may mean that lines bearing the fullest expression of high Zn concentration may be few in number and so lost in earlier generations unless populations are large.

17.7.2 Vitamin A

Maize

It is important to be aware of reciprocal differences in their contribution to kernel content of carotenoids, as this will affect the inheritance of the traits. Several studies have shown that the pollen parent affects carotenoid concentrations of the F_1 seed of reciprocal crosses (Mangelsdorf and Fraps, 1931; Johnson and Miller, 1938; Randolf and Hand, 1940; Grogan *et al.*, 1963)

However, a study by Egesel (2001) found that the female parent had the greatest influence on carotenoid concentrations in open pollinated kernels. Another study reported broad sense heritability of 33 percent for β-carotene and 47 percent for β-cryptoxanthin (another carotenoid with provitamin A activity) (Wong, 1999).

To produce colour in the maize kernel, numerous genes are necessary for structural and regulatory mechanisms. For carotenoid production, three genes have been reported to be relevant to carotenoid concentration. The *Y1* (yellow 1) gene on chromosome 6 encodes for phytoene synthase (Buckner *et al.*, 1996), an essential enzyme in the carotenoid pathway; the *VP9* (viviparous 9) gene on chromosome 7 is associated with ζ-carotene desaturase; and the *VP5* gene encodes for phytoene desaturase (Wong *et al.*, 2004). Carotenoids are produced in the starchy endosperm, and because of this, the yellow or white colours are only seen when the aleurone layer is colourless. The endosperm is triploid in nature, two from the maternal parent and one from the paternal parent. Thus the maternal parent can give a good indication of the expected carotenoid concentration (Egesel *et al.*, 2003). This results in a heterozygote containing two dominant or two recessive alleles, resulting in phenotypic differences (Table 17.5). For example, in the monohybrid cross for *Y1*, the endosperm can be one of four genotypes, which produce variation in colour (Symcox, Shadley and Weber, 1987). The genotypic differences correlate to the colour of the endosperm, and to the carotenoid concentration.

TABLE 17.5
Genotype and phenotype of maize heterozygote, and contributions from paternal and maternal parents

Endosperm genotype	Maternal contribution	Paternal contribution	Phenotype
Y1Y1Y1	Y1Y1	Y1	Yellow
Y1Y1*y*1	Y1Y1	*y*1	Light yellow
*y*1*y*1Y1	*y*1*y*1	Y1	Pale yellow
*y*1*y*1*y*1	*y*1*y*1	*y*1	White

Cassava

It was initially reported that the inheritance of root colour was simple and that a single dominant gene was responsible for yellow colour (Hershey and Ocampo, 1989). However, since that report, inheritance for carotenoid concentration has been found to be under the control of two genes, one responsible for transport to the non-photosynthetic roots, the other for accumulation within these storage organs (Chavez *et al.*, 2000). These two genes do not function independently of each other, rather each affects the expression of the other, though the mechanisms behind this are as yet unreported. The two major genes

are also combined with other genes with smaller effects that affect accumulation.

Wheat

A study into the genetic origin of an increase in carotenoid pigments in the cross between a wild barley (*Hordeum chilense*) and durum wheat located this trait to the α-arm of chromosome 7H^{ch} (Alvarez, Martin and Martin, 1998). Screening of 35 lines with various Tritordeum lines revealed that although the presence of the H^{ch} genome is responsible for increased carotenoid concentrations in these lines it is difficult to predict the effect of the interaction between the barley and wheat genetics. *H. chilense* is therefore a useful but not entirely reliable source of increased carotenoid concentrations for *T. durum* (Alvarez, Martin and Martin, 1999).

17.8 PHYSIOLOGICAL AND MOLECULAR MECHANISMS OF MICRONUTRIENT UPTAKE, TRANSLOCATION, RE-MOBILIZATION AND ACCUMULATION

17.8.1 Fe & Zn

For a breeding programme to be successful, it is important to understand the processes leading to accumulation of nutrients in the grain. Obviously an increase in accumulation of Fe and Zn in the grain of any plant species will require higher uptake, translocation or re-mobilization from source (leaves) to sink (grain). The role of these processes in relation to accumulation of Fe, Zn and other micronutrients has been reviewed recently (Grusak, Pearson and Marentes, 1999); thus it will not be discussed in detail here. It is interesting to note that Fe has been the most studied micronutrient in rice, while Zn has been researched to a larger extent in wheat. There is some suggestion that increasing the levels of Fe-chelating agents (phytosiderophores, nicotianamine, organic acids), reducing agents (ferric reductase), enzymes and transport proteins in the root cells could enhance Fe uptake and transport (Grusak, Pearson and Marentes, 1999). However, higher uptake and transport does not necessarily imply higher accumulation in the grain (phloem loading). For example, a pea mutant of cultivar Sparkle (*brz*) accumulated 36-fold higher Fe in the leaves compared to Sparkle, but did not have higher Fe in the seeds (Grusak, 1994). The author concluded that the rate limitation to phloem Fe loading was due to an unidentified ligand species that would complex with Fe prior to phloem loading rather than the availability of Fe as substrate. This study was followed by the study of Marentes and Grusak (1998), who demonstrated that a second mutant of cultivar Sparkle (*dgl*) had 2.5-fold higher Fe concentration in the embryo compared to Sparkle (163 and 65 mg/kg DW, respectively). This mutant also had higher Fe concentration in the seed coat. The authors used radiotracer ^{59}Fe to determine the movement of Fe in the seed coat, and found that Fe was symplastically phloem loaded. They further suggested that Fe resided within the non-vascular seed coat cells, and that the cells at the inner surface of the seed coat may facilitate the release of Fe to the embryo apoplast. The form of Fe in the seed coat or embryo is still not known at present.

A recent study in rice suggests that nicotianamine (NA) and nicotianamine synthase (NAS) genes (OsNAS1, OsNAS2 and OsNAS3) are also involved in long-distance transport of Fe (Inoue *et al.*, 2003), apart from their roles in the release of low-molecular weight compounds, phytosiderophores, from the roots of graminaceous plants. This release of phytosiderophores solubilizes rhizospheric

Fe(III) thus increasing plant uptake of Fe, for example in rice (Takagi, 1976; Takahashi *et al.,* 2001). Most recently, a rice metal-NA transporter, OsYLS2, has been linked to phloem transport and translocation to the grain of Fe (Koike *et al.,* 2004). Increasing the expression of transporters such as OsYSL2 and OsNASs in rice and other species can enhance Fe, and to some extent Zn, accumulation in the grains. However, further studies are required to determine the extent of genotypic variation in NA concentrations and its relative contribution to Fe and Zn accumulation in several varieties with low and high Fe and Zn in the grain.

In contrast to Fe, there is little information available on Zn translocation in the plant and transport to the grain. An earlier study (Longnecker and Robson, 1993) suggested that organic complexes with citrate and malate may be important in re-mobilization of Zn in the phloem. A recent study indicates that this may not be the case, as no relationship could be found between the presence of complexes or ligands and loading of Zn into the wheat grain (Pearson *et al.,* 1996). However, this result does not rule out the possibility of other endogenous chelates that may play a role in the long-distance transport of micronutrients (Welch, 1995). At the same time, transport to the grain is thought to occur predominantly via the phloem (Pearson *et al.,* 1995), and is well regulated (Herren and Feller, 1997). This regulation of Zn transport has been reported by Pearson, Rengel and Graham (1999), who suggested that low-Zn grain was not a strong sink for Zn, while in the case of high-Zn grain, there may be a barrier preventing excessive accumulation in the grain. It has also been suggested that phytosiderophores and nicotianamine may facilitate Zn uptake and transport in the plant (Scholz, Seifert and Grun, 1987; Treeby, Marschner and Romheld, 1989; Zhang, 1991; Cakmak *et al.,* 1996), but this needs to be established in future studies.

The positive correlations between Fe and Zn concentrations in cereal grains provide evidence that both nutrients may be taken up and translocated to the grain through the same process. However, as the correlation coefficient is never 1, there must be other mechanisms specific to Fe or Zn uptake or translocation to the grain, which warrants further investigation.

Having briefly discussed uptake and translocation of Fe and Zn to the grain, two questions arise: "How much of the Fe and Zn in the plant ends up in the grain?" and "Where are these nutrients deposited in the grain?" Contrary to earlier suggestions of poor re-mobilization of Fe from vegetative tissues to the grain (4 percent in rice, Marr *et al.,* 1995; 20 percent in wheat, Miller *et al.,* 1994), a recent growth-room study with wheat reported that 77 percent of total shoot Fe was re-mobilized to the grain at maturity (Garnett and Graham, 2005). The lower re-mobilization of Fe in the earlier studies involving field-grown plants were attributed to (i) precipitation of Fe in the apoplasm (inactive Fe) at high concentrations, which may result in non-re-mobilization, (ii) saturation of either the grain loading or phloem loading, and (iii) contamination of plant tissues by soil (references in Garnett and Graham, 2005). Miller *et al.* (1994) reported that, in wheat, 70 percent of Zn in the leaves was re-mobilized to the grains, while only 42 percent of shoot Zn re-mobilized to the grain in the study of Garnett and Graham (2005). The differences in the amounts of Zn re-mobilized in these two studies may be due to differences in genotypes and experimental conditions. It has been suggested that,

in wheat, relatively large amounts of Zn are transported into crease or inner pericarp tissues via the crease phloem, and translocation to the embryo and endosperm continues throughout grain development (Pearson *et al*., 1998). As Zn status of the grain improves, more Zn is distributed to the inner pericarp and less Zn to the endosperm, outer pericarp and embryo (Pearson, Rengel and Graham, 1999).

17.8.2 Se & I

In most plants, uptake, transport and assimilation of selenate is the same as for sulphate, and leads to synthesis of selenocysteine and selenomethionine; selenocysteine is then incorporated into proteins (Lauchli, 1993). Hence, the transfer of sulphate/selenate transporter genes from a Se accumulator like *Astragalus bisulcatus* may be useful for phytoremediation of high-Se areas (Goodson *et al.*, 2003). However, this strategy may not assist with Se uptake on soils with low Se availability, where most Se is present as selenite, selenide and elemental Se forms (Cary and Allaway, 1969). Selenite absorbed by the roots undergoes a series of reduction reactions, including conversion to selenide, and finally a reaction with O-acetylserine to form selenocysteine (Tsang and Schiff, 1978). Because of shared transporters, sulphate in growth media inhibits uptake of selenate (Ferrari and Renosto, 1972), and sulphite may inhibit uptake of selenite, but further studies are required to confirm this.

Iodine species of lower oxidative state and molecular weight (iodide, -1 and 116, respectively) are absorbed more readily than the heavier, higher valency forms (iodate, +5 and 214, respectively) (Umaly and Poel, 1971). I is transported mostly in the xylem, hence little is re-translocated from the leaves into the grain, where most is stored in the bran layers and lost during milling or polishing (Muramatsu *et al.*, 1989). To date, little has been reported on the physiology of I in the plant system and further studies are needed, especially on the forms in which I is transported and stored.

17.9 CONCLUSIONS

17.9.1 Fe & Zn

There is substantial evidence for genotypic variation to justify breeding efforts towards developing high grain-Fe and -Zn varieties. However, our knowledge of genetics, physiological mechanisms responsible for high grain Fe and Zn trait and G×E interactions is very limited, and now it is time to focus on these areas. One important point we should mention is that these proposed studies should be supplemented by bio-availability studies in animals and humans. There have been some concerns with respect to poor bio-availability of these nutrients due to naturally occurring high phytate concentrations in the grains. However, a study in rats reported that bio-availability of Fe and Zn remained constant in low- and high-density genotypes of cereals and beans (Welch *et al.,* 2000). So it is a reasonable argument that there will be an increase in absorption of these nutrients as their concentrations increase in the grain, despite their low bio-availability compared to animal food sources, as observed in the Philippine rice study (Haas *et al.*, 2005), though in that study, the varieties differed simultaneously in Fe and Zn, giving rise to potential interactions in the gut. We believe that breeding for these traits is a worthwhile approach given the impact the small increment in absorption of these nutrients will have on the lives of billions of people who are reliant on staple food crops such as rice and wheat for their dietary requirements of Fe and Zn. Finally and importantly, if

breeding for high grain-Fe and -Zn traits is to be successful and the varieties adopted by farmers, the high grain-Fe and -Zn traits must be linked to high yield. This has been achieved in rice (Gregorio, 2002), and results from wheat trials are also encouraging (R.M. Trethowan, pers. comm.).

17.9.2 Vitamin A

Despite extensive selection against pigmentation in several staple foods, genetic variation for carotenoid concentration can still be revealed by screening varieties available from germplasm banks, as illustrated in this chapter. Even within those staple crops that have substantial concentrations of carotenoids, the value of screening ancient varieties for sources of higher accumulation is obvious. The time consuming and expensive nature of carotenoid analysis still remains a significant restriction, though recent progress in the development of fast screening methods for wheat will expedite mass screenings for this staple crop. Although much work has been done in elucidating sources of increased carotenoid concentrations in staple foods there is still much to do before we obtain concentrations that can alleviate vitamin A deficiencies. In addition, it is not merely enough to develop lines with high carotenoid concentrations; they must be adapted to local conditions, and also be culturally acceptable and the carotenoids bio-available.

17.9.3 Se & I

The limited investigations carried out to date suggest that rice may be the most promising of the major cereals for breeding to improve grain Se and I density, although further screening of all the major cereals may reveal more germplasm that can enhance these traits. For rice, in particular, further pot trials and field trials conducted at sites with different soil types and including a wide range of germplasm grown together are needed to confirm whether sufficient genetic variability exists to enable selection for uptake and grain loading efficiency of Se and I. Previous studies suggest that Se and I delivered through bio-fortified cereals are highly bio-available (Jiang, Cao and Jiang, 1997; Lyons *et al*., 2003).

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