Enhancing Wheat Nutritional Quality: An Atlas of QTLs for Essential Minerals from the A.E. Watkins Landrace Collection Analysis

Petros Sigalas, PhD

Peter Shewry, Andrew Riche, Luzie Wingen, Ajay Siluveru, Noam Chayut, Amanda Burridge, March Castle, Saroj Parmar, Charlie Philp, David Steele, Michelle Leverington Waite, Shifeng Cheng, Simon Griffiths and Malcolm J. Hawkesford

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Nutrient Deficiencies

Humans require a range of minerals in their diets for physiological function.

- Major elements (calcium, magnesium, potassium, etc.)
- Micronutrients (iron, zinc, selenium, copper, manganese, molybdenum, etc.)

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Low intake of those mineral from the diet leads to deficiencies.

- Severe and widespread symptoms
- · Lack of Iron (Fe) is linked to anemia · Lack of Zinc (Zn) is associated with stunted

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- arowth in children Potassium (K) is important for the regulation of blood pressure
- Magnesium (Mg) deficiency is associated with cardiovascular diseases
- · Calcium (Ca) deficiency is linked to osteoporosis, hypertension, high serum cholesterol, and colorectal cancer

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Nutrient Deficiencies – A Global Burden

- Most widespread global deficiencies include Fe and Zn, with a more severe impact in developing countries.
- According to WHO 2015, 43% of children and 29% of women suffer from anemia, and approximately half of these cases are attributed to Fe deficiency.
- Globally, over 150 million children are affected by Zn deficiency.

In the UK:

- Up to 50% of adolescent have insufficient Fe intake and around 25% of adolescents have Zn intake below the lower reference intake (Bates et al., 2016).
- 27% of adult women in the UK have Fe intake below the average.
- A significant portion of adults in the UK have intakes below the recommended levels for magnesium, potassium, and selenium.



Bridging Gaps: The Promise of Wheat Biofortification



Up to 50% of calories in developing countries

20% of essential mineral intake in the UK

Improvement of wheat grain mineral content can have significant impact on human mineral uptake

WAYS TO INCREASE MINERAL CONTENT



Wheat Flor

Fortification

monitor in rural areas of developing countries.

Limitation: Difficult to implement and



Application of mineral as fertiliser. Limitation: Increased cost of crop production - problem in less developed countries.

Agronomic Biofortification



Genetic

Biofortification

Promising alternative for targeting rural populations.

Example: Development of high zinc wheat (Govindan *et al.*, 2022; Lowe *et al.*, 2022).

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Genetic Biofortification: Looking Beyond Elite Varieties

- Successful genetic biofortification relies on the availability of genetic variation.
- Elite/modern bread wheat varieties are less genetically diverse than older cultivars and landraces.
- □ Elite varieties: >20% loss of nucleotide diversity (Pont *et al.* 2019).
- Older cultivars and landraces, which have been less influenced by modern breeding practices, can serve as valuable reservoirs of genetic diversity.



Obtained from Pont et al. 2019





Exploiting diversity in the A.E Watkins Collection



- □ Collection of 827 landraces collected from 32 countries in 1920s and 1930s.
- Rich genetic and phenotypic diversity.
- □ Main resources of UK prebreeding programs.









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Grain Mineral Concentration in Elite Varieties and Landraces

- Wheat landraces exhibited greater genetic diversity in grain mineral content compared to elite varieties.
- Landraces demonstrated
 higher concentrations of most minerals in the grain.















Accounting for the Yield Dilution Effect



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GrnYld (t/ha)

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- However, wheat landraces are usually low yielding.
- Established concept of "yield dilution".
- Inverse correlation between concentrations of minerals in the grain and grain yield.
- Grain mineral contents were not only expressed as concentration, but also as µg/grain.
- ❑ Grain mineral deviation was also calculated: deviation from the negative regression between grain mineral concentration and yield as described by Monaghan *et al.* (2001).



Our Approach

- □ Three biparental segregating populations were developed by crossing Watkins Landraces x Paragon.
- □ Each population: 94 F4 recombinant inbred lines.
- □ The 35K Axiom Wheat Breeder array was used for population ParW292 and the 44K Axiom TaNG array for the other two populations.

Watkins parent	Country of origin	Ancestral group	Selected for	Year	N Fertilization Rate (Kg/ha)	
W160	Spain	C6	grain mineral	2018	200	
			content	2019	200	
			(P and Zn)	2020	200	
W239	Cyprus	C6	nitrogen use efficiency (NUE)	2015	50	
				2016	50, 200	
				2017	50, 200	
W292	Spain	С7	variation in height	2012	200	
				2013	200	
				2014	200	













Range of Concentrations in the Populations













Better Mineral Uptake or Better Use?



- To unravel the underlying mechanisms, the mineral content of the straw and the total biomass were also measured.
- Calculation of mineral content of the aboveground biomass and the mineral harvest index.
- The identification of co-located QTLs for the concentrations/total amounts of minerals in straw suggests a more efficient uptake of minerals by the plant.
- The absence of co-located QTLs for minerals in straw/biomass implies that the partitioning of minerals to the developing grain is more effective.













QTL Atlas of Essential Minerals

- 774 QTLs for minerals in grain, straw and calculated biomass were identified.
- Reduced to 23 strong robust QTLs for essential nutrients in grain by selecting for:
 - QTLs that were mapped in at least two sample sets.
 - with LOD* scores above 5 in at least one set.

Mineral	Chromosome	Beneficial Alleles		
Calcium	2B, 4A, 5A, 5D	P, W160, W239, W292		
Copper	4B, 5B, 7A, 7B, 7D	P, W239, W292		
Iron	2D, 3A, 5D, 6A	W160, W239		
Potassium	3D, 4B, 5A	P, W160, W239		
Magnesium	5A, 6A, 6D, 7A	P, W160, W239, W292		
Zinc	5A, 6A, 7A	W160, W239, W292		

*LOD (logarithm of the odds)











Gene Content Analysis and Genomic Comparison

- Strong QTLs were selected to determine the gene content within 5 Mb of DNA either side of the peak marker.
- Gene Functional Annotation.
- □ Knetminer was used to explore any association between genes and the traits of interest (Hassani-Pak *et al.* 2021).
- Whole genome sequence data (Watseq) were used to identify functional and copy number variations between Paragon and the Watkins lines.

Mineral	Са	Cu		Fe	к	Mg	Zn	
Chromosome	5A	4B	5B	2D	4B	7A	6A	7A
Gene number	127	52	102	120	29	128	40	91









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An ATPase Transporter as the Candidate Gene for Higher Ca Content

Based on the functional annotation, two candidate genes were identified in the region surrounding the 5A QTL peak:

- *TraesCS5A02G543300* which encodes a cation transporter/plasma membrane ATPase.
- *TraesCS5A02G542600* which encodes a major Facilitator Superfamily transporter.





TraesCS5A02G543300 TILLING mutants showed 10% higher Ca content in grain.

No difference in grain weight.

This suggests that *TraesCS5A02G543300* and its homoeologues could be manipulated to increase grain Ca content.



Focus on Ca-biofortified Wheat?

- □ Ca deficiency is a global concern, affecting up to half of the total population (Shlisky *et al.*, 2022).
- Insufficient Ca is linked to various health issues, including osteoporosis, hypertension, high serum cholesterol, colorectal cancer, and rickets (a pediatric bone disease).
- □ Adults aged 19 to 64 require a daily Ca intake of 700 mg.
- According to the National Diet and Nutrition Survey (NDNS), 15% of children (11-18) and 9% of women (19-74) have Ca intakes below the recommended levels.
- In the UK, adults (age 19-64) derive approximately 30% of their Ca from cereals, with around 15% coming from bread (NDNS).
- The increasing adoption of vegan diets necessitates obtaining Ca from alternative sources, such as cereals.
- In the UK, the fortification of white flour with Ca is mandatory, with an approximate content of 235-390 mg of Ca per 100 g of flour.





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Thank you for your attention!