CONTRIBUTION OF CEREALS AND BREAKFAST CEREALS TO MINERAL AVAILABILITY; PHYTATE CONCENTRATION AND EXPRESSION OF MINERAL TRANSPORTERS

Ana Rita B. Santos; Beatriz C.Ribeiro; Ana Gomes and Marta Vasconcelos

Escola Superior de Biotecnologia, Universidade Católica Portuguesa - Rua Dr. António Bernardino Almeida, 4200-072 Porto, Portugal

INTRODUCTION

In ideal scenery, daily food components should contain all the necessary macro and micronutrients for good health. However, in several countries, many nutritive deficiencies, such as of Fe, Ca, Zn and Cd have been identified. Understanding which genes are involved in the transport of these mineral nutrients to the edible plant parts is of great importance. Amongst the genes involved in this mechanism are the ferritins, the Zips and the phytosiderophores. Later, when a mineral is ingested, only a fraction is absorbed and used by the organism. Several compounds influence bioavailability, one of which is phytate, the dominant storage of phosphorus in cereals. This work aimed at evaluating the phytate concentration in daily diet cereals as well as to study *ferritin*, *ZIP* and *iron phytosiderophore* gene expression and conservation amongst cereal grains.

MATERIALS & METHODS

Cereal grains: *Avena sativa*, *Hordeum vulgare*, *Secale cereale*, *Lolium multiflorum*, *Zea mays* and *Triticum aestivum*.

Breakfast cereals: A (corn, wheat, oat), B (wheat), C (integral wheat, rice), D (wheat flour, hydrolyzed wheat), E (corn), F (wheat).

I: Optimization of Phytate Determination and WADE Extraction Method

- Each sample was weighed into individual tubes; 0,29 M HCl (2,4% HCl) was added and tubes were left to rest in a rotary shaker for 2 h at R.T., after vortexing.
- Two parameters were tested in order to increase phytate yield:
  1. HCl concentration was increased to 0,8 M (6,6% HCl)
  2. Digestion times were increased to 9 and 16 h.
- Samples were centrifuge and filter into new tubes.
- 20 % trichloroacetic acid was add to the filtered sample at a proportion of 6:1 and centrifuge in order to remove TCA-insoluble proteins.
- WADE reagent was add to the sample extract at a proportion of 3:1 (WADE:Sample) and read at 500 nm.

II: Gene Expression of Metal Transporters

- Seeds of. P cereal grains where germinated in fertile soil, and grown for 2 weeks at R.T.. Plantlets were watered every other day. Tissues were collected and conserved in liquid nitrogen.
- Isolation of total RNA from plant tissue.
- PCR.
- Electrophoresis.

III: Bioinformatic analysis using NCBI and BLASTP

CONCLUSIONS

I: Variable phytate concentrations (WADE 3:1): Rye, oat and corn have higher concentrations as well as D and E breakfast cereals.

II: Higher bioavailability in samples without pericarp.

III: Species similarity in terms of gene sequences but differential expression patterns.

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RESULTS & DISCUSSION

Phytate Extraction and Quantification

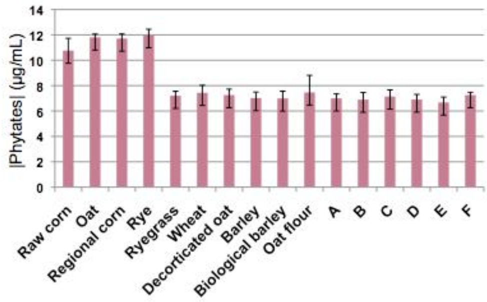

Figure 1. Phytate concentration in cereal grains and breakfast cereals, measured by the optimized method referred above. Black bars represent standard error, n=2.

Gene Expression of Metal Transporters

Table 1. Primer sequences utilized in the detection of *ferritin, ZIP* and *iron/phytosiderophore transporter genes through semiquantitative* reverse-transcriptase polymerase chain reaction (RT-PCR) and predicted size of the PCR products.

Gene	Forward primer	Reverse primer	Product size (bp)
ZIP Lm	GCGGCATACTCTTCTCAAGG	GAGGTTCTTGGGAGGGAAAC	270
IPS As	CATGCTGACAACCGAGAGAA	TAGGAATTGGCGAATCCAAG	230
Fer Ta	CTGAGAAAGGGGATGCTCTG	CCCTTTGCCGATTCTCATAA	212
Fer Zm	ACCACTCCCTCTTCGCCTAT	TGTGCAGCTTCTCATTGACC	179
Fer Hv	GGGATTTGCCAAGTTCTTCA	GCTAGAGCCAACTCCATTGC	180
ZIP Hv	TCATCAAGCTGCTGGAACAC	GCTGACCATGGTTGTTGTTG	136

ZIP Lm - Zip, *Lolium muiltiflorum, IPS As - iron/phytosiderophore transporter, Avena sativa, Fer Ta - Ferritin, Triticum aestivum, Fer Zm -* Ferritin, *Zea mays, Fer Hv - Ferritin, Hordeum vulgare, ZIP Hv - Zip, Hordeum vulgare.*

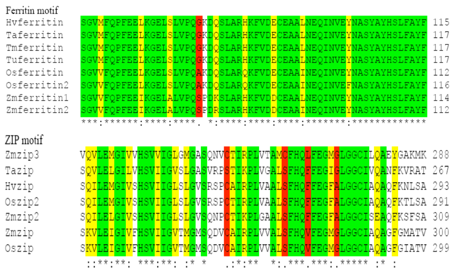

Figure 3. Amino acid sequence alignment of ferritin and zinc finger protein (ZIP) clade conserved motifs.

Two conserved motifs (ferritin and ZIP) were determined amongst some of the assayed cereals based on consensus of their sequences upon analysis by CLUSTAL method. Green-shaded areas represent consensus, red-shaded areas represent identical amino acids, and yellow-shaded areas represent similar amino acids. Asterisks represent fully conserved amino acids in cereals.

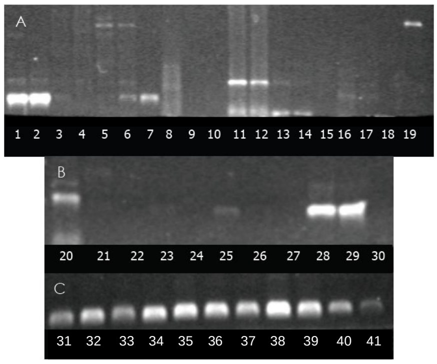

Figure 2 (A,B,C). Expression of ferritin (A) and zip genes (A and B) in cereal plant samples grown in greenhouse conditions for collection of tissues. 18S ribossomic subunit cDNA (C) was used as an internal control.

Lanes: 1, wheat stem; 2, wheat leaf; 3, ryegrass leaf; 4, ryegrass leaf; 5, oat stem; 6, oat leaf; 7, rye leaf; 8, corn; 9, barley stem; 10, barley leaf; 11, wheat stem; 12, wheat leaf; 13, barley stem; 14, barley leaf; 15, corn; 16, oat stem; 17, oat leaf; 18, rye leaf; 19, ryegrass stem; 20, ryegrass leaf; 21, wheat stem; 22, wheat leaf; 23, barley stem; 24, barley leaf; 25, corn; 26, rye leaf; 27, ryegrass stem; 28, ryegrass leaf; 29, oat stem; 30, oat leaf; 31, H2O; 32, rye leaf; 33, ryegrass leaf; 34, wheat leaf; 35, oat leaf; 36, barley leaf; 37, corn; 38, ryegrass stem; 39, barley stem; 40, oat stem; 41, wheat stem.

Rye, oat and corn showed to have higher phytate concentrations as well as Nestum Honey and Inflated Wheat with Honey, in case of breakfast cereals. There was greater similarity between ferritin sequences than between ZIP sequences, indicating that ZIPs suffered deeper evolutionary changes. Gene expression was tissue dependent.

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