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Title

Defining the NAM regulon for gene targets to biofortify crop iron, zinc, and protein concentrations

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Authors

Waters, Brian M.

Wang, Dong

Grusak, Michael

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Approximately half of the world population suffers from iron and/or zinc deficiency, and millions suffer from protein-energy malnutrition, primarily from reliance on plant based staple foods. These foods are low in iron, zinc, and protein density relative to animal based foods. Wheat is a major staple food in parts of central and western Asia, and can supply 50-70% of daily caloric intake in certain regions (Cakmak, 2008). We and others are interested in genetic improvement of plants to increase the nutritional value, a strategy termed biofortification. The average concentration of Zn in wheat grain ranges from 20-35 mg/kg (Cakmak, 2008). The research group HarvestPlus has set a target of increasing Zn in wheat (www.harvestplus.org) by 8 mg/kg.

In previous work, the *NAM* transcription factor genes of wheat were shown to regulate leaf senescence (Uauy et al., 2006) and iron, zinc, and nitrogen remobilization and translocation from vegetative tissues to grain (Waters et al., 2008). This work used comparisons between an RNAi *NAM* knockdown line and a control line (Uauy et al., 2006), with multiple time point sampling of mineral concentrations and contents in vegetative organs and grain. The results illustrated timing of nutrient content dynamics in different organs, and demonstrated that Fe, Zn, and N were not effectively translocated from vegetative tissues to grain when *NAM* transcripts were reduced. Thus, genes of the *NAM* transcription factor regulon are potential targets for increased expression to accomplish nutritional improvement of cereal or other seed crops.

As a first step to identify *NAM* regulated genes, we used the Affymetrix Wheat Genome microarray to profile genes that are differentially regulated in flag leaf tissue at mid-grain fill relative to anthesis, and that are also differentially regulated between control and *NAM* RNAi knockdown lines (germplasm generously provided by J. Dubcovsky and C. Uauy, UC-Davis). Over three hundred genes met the criteria to be potential *NAM* targets (Figure 1), several of which are annotated as coding for proteins that could be involved in nutrient transport or protein metabolism (Table 1).

A highly homologous *NAM* gene with developmentally regulated leaf expression similar to wheat *NAM* genes was cloned from *Sorghum bicolor*. Results of genome-wide bioinformatic and molecular screens to identify potential *NAM* regulated genes and putative *NAM* response elements in gene promoters will be presented.

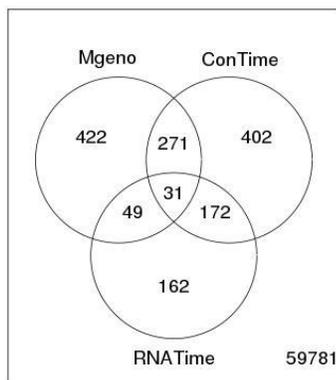


Figure 1. Venn diagram showing numbers of significantly differentially regulated genes (2-fold cutoff, $P < 0.05$, two biological replicates) on Affymetrix Wheat Genome microarrays. Figure labels: Mgeno, transcripts differentially regulated between control and RNAi flag leaves at mid-grain fill; ConTime, transcripts differentially regulated between anthesis and mid-grain fill in control line flag leaves;

RNAiTime, transcripts differentially regulated between anthesis and mid-grain fill in RNAi line flag leaves.

Table 1. Potential NAM regulated genes in wheat flag leaves that have relevance to metal transport or amino acid metabolism. Fold-change is presented for indicated pair-wise comparisons.

<u>Description</u>	Ctl vs. RNAi	Anth. vs mid-fill	
	<u>mid-fill</u>	<u>Control</u>	<u>RNAi</u>
Sugar transporter	3.4	6.6	
Metallothioneine type 2	6.9	4.1	
Metallothioneine type 3	2.6	2.8	
Peptide transporter	4.2	3.5	
POT transporter	2.2	14.7	
CDF transporter	8.2	9.8	
Potassium transporter	4.2	3.6	
Amino acid permease	-5.4		2.5
Serine			
carboxypeptidase	2.9	4.2	
Protease	4.4	2.9	
Aspartyl protease	3.2	4.4	
Aminotransferase	3.5	3.8	
Glutamine synthetase	3	2.1	

References

- Cakmak I (2008) Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? *Plant Soil* 302: 1-17
- Uauy C, Distelfeld A, Fahima T, et al. (2006) A *NAC* gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science* 314: 1298-1301
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