A roadmap towards the development of Zn-biofortified rice for Madagascar

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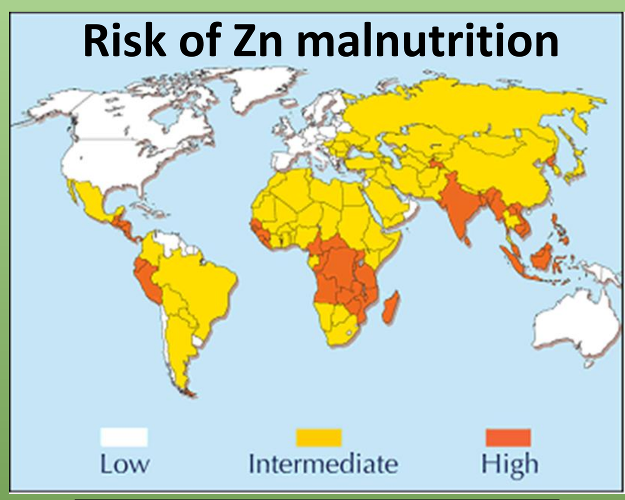
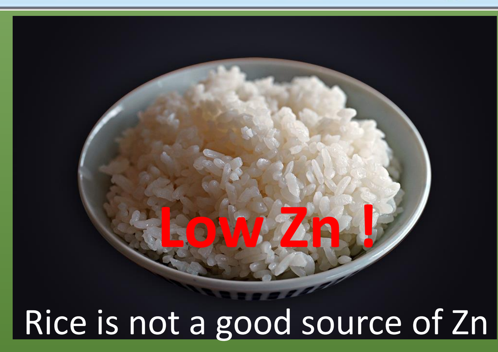
Introduction

One-third of the human population consumes insufficient quantities of zinc (Zn) to sustain a healthy life. Increasing Zn concentrations in edible parts of food crops, an approach termed Zn-biofortification, is one cost-effective option to address this problem. Especially infants in countries like Madagascar are at risk of Zn deficiency because their dominant food source, rice, contains insufficient Zn.



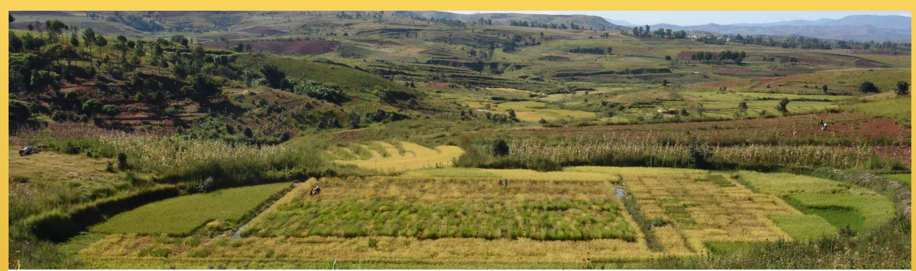


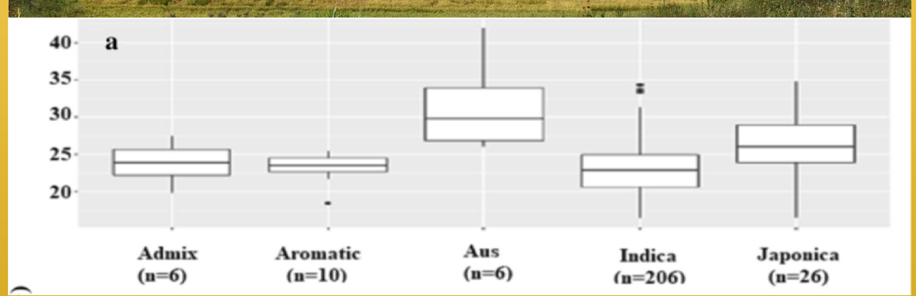
Rice is not a good source of Zn

Objectives

1. Explore genetic variation for grain Zn concentrations within the rice gene pool
2. Employ genomic tools to identify loci and donors for high grain Zn
3. Determine the stability of grain Zn concentrations across environments
4. Initiate Zn-biofortification breeding to raise grain Zn in local rice varieties from around 20 ppm to near target values of 30 ppm Zn

Genotypic variation in grain Zn





The IRRI genebank contains 3000 accessions with publicly available marker data. We phenotyped 254 of these in farmers' fields in Madagascar, followed by grain Zn analyses by ICP-MS

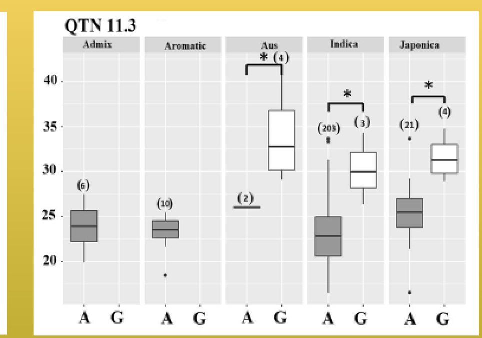
Grain Zn ranges from 17 - 43 ppm but is low (18 – 22 ppm) in most local varieties

The *aus* subspecies has highest grain Zn > 30 ppm

Genome-Wide Association (GWAS)

We conducted GWAS with 254 accessions to identify loci associated with elevated grain Zn

QTN	Chromosome	Position	R^2	MAF (%)	QTN effect ($\mu g\ Zn\ g^{-1}$)
2.1	2	18,697,369	5.5	45.9	1.2
4.1	4	20,025,747	5.9	20.6	1.2
8.1	8	26,505,039	3.0	10.7	1.9
10.1	10	14,217,374	3.5	5.5	1.5
11.1	11	26,546,816	4.9	13.0	1.2
11.2	11	27,604,708	3.2	15.6	− 1.4
11.3	11	28,757,650	15.4	4.4	3.7
12.1	12	7,184,806	2.1	9.7	0.9



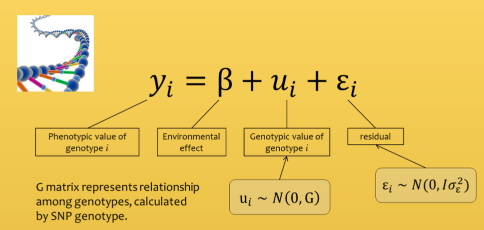
We identified 8 loci (QTN) associated with grain Zn but only QTN 11.3 has a large enough effect to be of interest, explaining 15% of the variation for grain Zn

A rare allele (G) at QTN 11.3 increases grain Zn in the ***aus***, ***indica*** and ***japonica*** subspecies

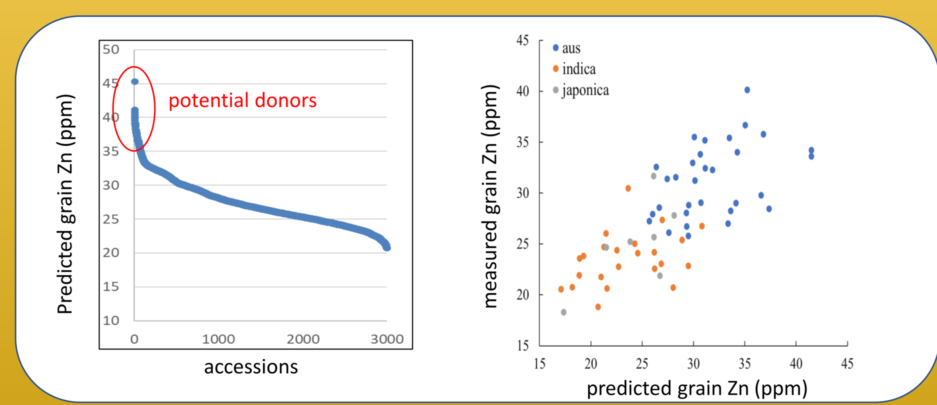
This locus should be followed up further to develop markers for marker-assisted selection

But generally it appears grain Zn is a highly polygenic trait with no single locus having a major effect

Genomic Prediction of grain Zn



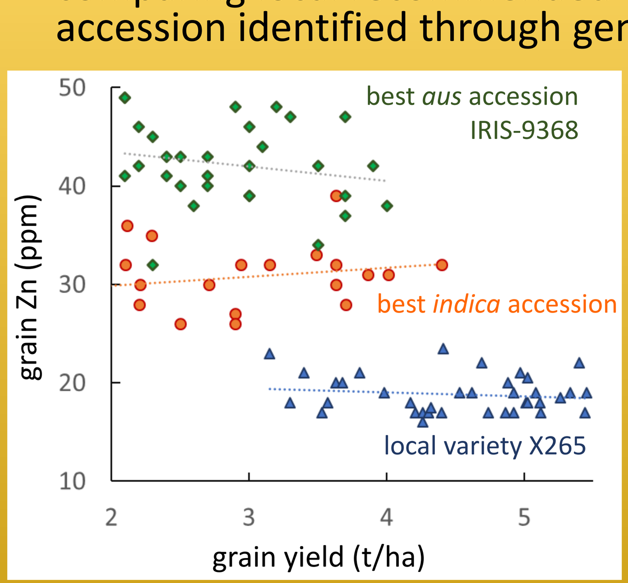
Based on the phenotype and marker data sets of 250 tested lines, we developed a genomic model that predicts the grain Zn in all the 3000 available accessions



The predicted grain Zn in the 3000 accessions ranged from 20 – 45 ppm. Among ***indica*** and ***japonica*** rice the maximum was 33 and 34 ppm, respectively, whereas many ***aus*** accessions were > 35 ppm. Their superiority was confirmed in subsequent field experiments in Madagascar and Japan

Stability of grain Zn across environments

Zn and yield data from multi-location trials in farmers' fields in Madagascar, comparing local recommended variety X265 to the best ***indica*** and ***aus*** accession identified through genomic prediction



Partition of variance for grain yield and grain Zn in fertilizer trials across 7 environments

	Grain yield			Grain Zn		
	Sum Sq	Eta^2	Pr >F	Sum Sq	Eta^2	Pr >F
Genotype (G)	30.2	11%	***	13553	78%	***
Environment (E)	85.9	31%	***	682	4%	***
Fertilizer (F)	33.2	12%	***	17.2	0%	ns
GxE	46.4	17%	***	1379	8%	***
GxF	7.3	3%	**	108	1%	*
ExF	23.6	8%	***	96.6	1%	*
GxExF	18.1	6%		475	3%	**

The environment strongly affected grain yield but genotypic effect were the dominant source of variation for grain Zn concentrations.

X265 and other local varieties had low Zn and were far below the biofortification target of 30 ppm Zn

Breeding of Zn-biofortified varieties for Madagascar

- Genetic resources from outside Madagascar need to be used to raise grain Zn towards target levels of Zn
- Donors from the ***aus*** subspecies identified here are one option, however, their adaptation to local conditions is poor, thus strong selection for adaptation and grain yield is needed
- Due to the stability of grain Zn across environments, selection for Zn can be centralized and ideally be done in early generations, whereas selection for yield and adaptation needs to be done in respective mega-environments
- Crosses with 2 ***aus*** donors have been made

A lucky break!

Our donor for P efficiency traits, DJ123, also belongs to the ***aus*** subspecies and tests showed it ranges from 35 – 40 ppm!

Subsequent evaluations in our DJ123-derived breeding lines identified a candidate variety with higher Zn compared to local upland rice variety Nerica4

This line will be released as P-efficient and high Zn variety Mavitrica in Madagascar

Mavitrica, our new P-efficient variety for Madagascar

	grain Zn concentrations (ppm)	
	brown rice	polished rice
Nerica4	27.9 ± 2.9 b	24.4 ± 3.1 b
Mavitrica	34.6 ± 2.3 a	28.8 ± 2.1 a

Rakotondramanana M, Tanaka R, Pariasca-Tanaka J, Stangoulis J, Grenier C, Wissuwa M (2022) Genomic prediction of zinc-biofortification potential in rice gene bank accessions. Theoretical and Applied Genetics (doi.org/10.1007/s00122-022-04110-2)

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