

Identification of SNPs Markers for Biofortification in Rice

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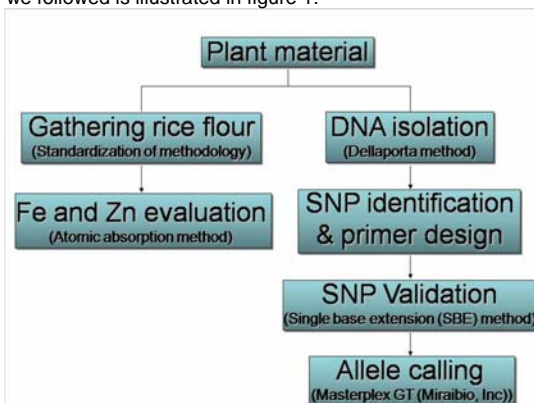


INTRODUCTION

Iron deficiency is probably the most widespread micronutrient deficiency in humans. Since micronutrients are effectively assimilated by humans through their daily diet, we aim to use staple foods in order to increase the levels of iron intake in people's diets. As rice is the main staple food for more than half of the world population it was chosen as our delivery vehicle. Various studies have evaluated the iron content in the rice grain including brown and milled rice. Results indicate that the iron content is rather low, especially in milled rice. Due to this fact several research groups have attempted to increase the iron content in rice; breeding, genetic engineering, biochemical and induced mutation methods have been applied. Our approach is aiming at a marker-assisted selection scheme; we have identified and validated SNPs located in 28 of 43 iron homeostasis candidate genes reported by Quintero et al, 2006. Rice lines have been genotyped and high iron content SNPs have been tagged. We are currently using data gathered to set up a marker-assisted selection breeding program.

MATERIALS Y METHODS

The process we followed is illustrated in figure 1:



RESULTS

Producing rice flour

This methodology successfully minimizes the contamination produced by metal agents during the milling process. The rice flour thus obtained does not present contamination. This methodology was implemented by the AgroSalud rice quality laboratory at CIAT, and this process is illustrated in figure 2.

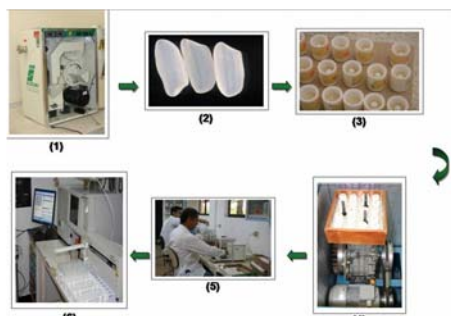


Figure2. Initially the rice is milled and polished for 18 seconds in Zatake grain mill, see (1) and (2) in the figure above. Then, 5 grams of polished rice grain (without husk and bran) are added to the Teflon chambers (3) in the figure above. A zirconium ball is then placed inside the Teflon chamber, which must be closed hermetically. The samples are then collected and placed inside wooden boxes; these boxes are shaken in a uniform manner for 7 minutes until a fine rice flour is obtained (4) in figure 2. In order to evaluate the content of Iron and Zinc the rice flour is sent to the Analytical Service Laboratory at CIAT (5) and (6) in figure 2. The atomic absorption method is used (Isaac and Kerber, 1971).

Content of Iron and Zinc in the plant material

Rice genotypes with a higher iron (≤ 5.0 mg/Kg) and zinc (≤ 9.0 mg/Kg) content were identified. Genotypes selected come from different sources: some from CIAT and IRR1 breeding programs, one wild rice species (*O.rufipogon*), and some from National Agricultural Research Systems. Table 1 presents the eleven genotypes selected.

Our initial analysis suggests the possibility to combine a high yield potential with a higher content of micronutrients. This is due, in part, to the existence of genetic variability in the rice germplasm kept in the germplasm banks.

Genotypes with high iron and zinc content in milled rice.

	Vegetal materia l	Fe (mg/Kg)	Zn (mg/Kg)
1	Bg90-2	5.1	9.6
2	FEDEARROZ 50	5.44	1.6
3	<i>O. rufipogon</i>	6.2	1.7
4	<i>Oryzica 1</i>	6.1	9.2
5	IR68144-2B-2-2-3-1-166	5.23	17.49
6	IR75862-206-2-8-3-B-B-B	5.43	21.43
7	CT18245-18-2-4-2-7	5.73	13.67
8	CT18247-12-8-2-2-3	5.14	14.29
9	IB51B3095-B	5.34	13.76
10	Tipo Juma 1	5.06	16.19
11	CT17334-2-1-1-2-4-5-M-1	6.68	17.38

Table 1. Average value of iron and zinc content for those 11 varieties tested at the analytical services laboratory in CIAT.

SNP validation and genotyping in the plant material

SNPs validation was carried out, in six rice genotypes used as controls: 3 belonged to *Oryza sativa* subsp. *japonica* (Koshihikari, Nipponbare and Caiapo), 2 to *indica* subsp. (93-11 and BG90) and the remaining to the African rice species *Oryza glaberrima*. Table 2 present the 44 markers evaluated according to their polymorphism in 47 samples of rice, having contrasting levels of iron and zinc in milled rice. Currently, we continue the genotyping of rice lines with high and low iron content to increase the number of individuals under study.

SNPs genotyping in the plant material.

Polymorphism	IUB Code ¹	SNP Type	Quantity
C/T o T/C	Y	Transitio n	30
A/C o C/A	M	Transversion	2
A/G o G/A	R	Transitio n	8
G/T o T/G	K	Transversion	1
C/G o G/C	S	transversio n	3

Table 2. SNP markers genotyped in 47 sample of rice.

¹International Union of pure and Applied chemistry.

Advantages of multiplex- PCR for SNP assays

Multiplex PCR, in which several markers are simultaneously amplified in the same reaction is used in this study to increase the amount of information generated per assay, and to reduce consumable and labour costs. To date, the analysis showed that SNPs present in two multiplexed PCR assays were successfully amplified (figure 3 and 4).

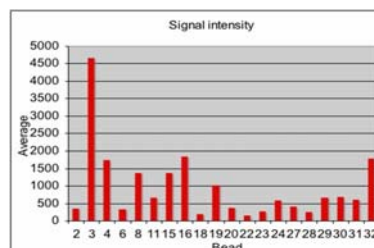


Figure3. 26 SNP primers were included in the first multiplexed PCR assay, and the SNP alleles had a signal intensity within the range of 152 – 4674.

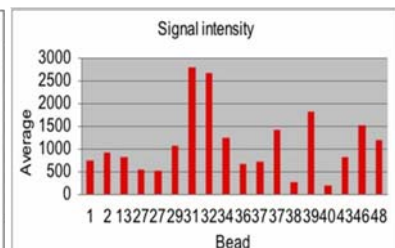


Figure4. 18 SNP primers were included in the second multiplexed PCR assay and the SNP alleles had a signal intensity within the range of 206 – 2788.

Preliminary results indicated a reliable capture of data and reproducibility of the single base extension method.

FUTURE WORK

- To continue the validation of the remaining SNPs from which PCR and SBE primers have been already designed.
- To continue the genotyping of rice lines with high and low iron content.
- To identify SNPs markers associated with some of the genes which may play major roles in the iron and zinc content in the grain of rice.

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