

18. Expression profiling of metal homeostasis related candidate genes in rice (*Oryza spp.*) using semi quantitative RT-PCR analysis.

G. CHANDEL^{1)*}, S. BANERJEE¹⁾ and S. B. VERULKAR²⁾

1)Department of Biotechnology

2)Department of Plant Breeding and Genetics

College of Agriculture Krishak Nager Indira Gandhi Agricultural University Raipur 492 06 C. G. INDIA

Tel: 91-771-2442069, 094063-82169 (m)

Fax: 91-771-2442131

Email: girishchandel@yahoo.com

* Author for correspondence

Improving grain iron and zinc content in rice, the major staple food crop is the most recent and promising approach to combat micronutrient malnutrition widespread among the rice eating populations in Asia. Rice absorbs metal ions from soil by roots in the form of divalent metal ions chelated with phytosiderophores which is transported to leaves along with xylem sap and remobilized to sink tissues with phloem sap (developing seeds). Grain micronutrient content is thus a function of plethora of processes governed by genes encoding metal-chelate transporter proteins as well as phytosiderophore biosynthesis pathway related enzymes (Grusak, 2002 Narayanan et al., 2007). Several genes responsible for metal transport, sequestration and phytosiderophore biosynthesis have been identified in rice, Arabidopsis, Barley, Wheat and Maize (Gross et al., 2003 Bughio et al., 2002 Guerinot et al., 2000 Koike et al., 2004 Inoue et al., 2003). Characterization of expression of metal homeostasis related genes in rice leaves and root tissues and analysis of any correlation between level of expression of gene in a tissue with grain Fe and Zn content will help identify the role of candidate genes in iron and zinc uptake, transport and accumulation in sink i.e. seed. Expression of 21 rice genes belonging to 5 gene families (OsNRAMPs, OsFROs, OsZIPs, OsFERs and OsYSLs) and 4 non rice gene homologues (OsNAAT1, OsNAS2, OsNAC, OsVIT1) was analyzed in root and leaf tissue of 12 diverse rice genotypes at maximum tillering and mid grain fill stages by semi quantitative RT-PCR analysis. Since flag leaf is considered as major source of remobilized photo-assimilates to developing grains flag leaf transcriptome was analyzed at mid-grain fill stage. For expression analysis twelve rice genotypes belonging to six categories of cultivated rice described by Garris et al., (2005) as temperate *japonica*, *tropical japonica*, *indica*, *aus*, aromatic, submerged and three wild rice (*O. nivara*, *O. officinalis*, *O. latifolia*) were grown in controlled environment in pots with coco-peat as potting substrate and nutrient supplied in solution (as described by Grusak, 2002), with 10 μ M iron as Fe (III)-HEDTA. Grain iron and zinc contents were estimated using Atomic Absorption Spectrophotometer (AAS200) as per the methods described in HP protocol (*HarvestPlus*, 2006).

Differential level of expression was recorded for the 25 candidate genes among the tissue type, stages and genotypes. Root transcriptome analysis revealed that 5 genes (OsFER2, OsNAS2, OsNRAMP6, OsZIP5 and OsZIP10) showed lower or negligible expression, 3 genes (OsFER1, OsVIT1 and OsFRO2) expressed at maximum tillering stage only, 7 genes (OsIRT1, OsFER2, OsZIP7, OsZIP8, OsZIP9, OsNRAMP4, OsNRAMP6 and OsYSL12) expressed at mid-grain filling stage only and 10 genes (OsZIP4, OsZIP11, OsNRAMP5, OsNRAMP7, OsYSL2, OsYSL4, OsYSL6, OsYSL9, OsNAAT1 and OsNAC) expressed at both the stages. Leaf transcriptome analysis at maximum tillering stage indicated that 5 genes (OsFER2, OsNAS2, OsZIP10, OsYSL7 and OsYSL12) showed no expression while 4 genes (OsNRAMP4, OsNRAMP6, OsZIP5, and OsZIP9) expressed at very low level in all 12 rice genotypes while remaining 16 genes showed variable level of expression among genotypes. In flag leaf tissue all 25 genes showed expression in at least one genotype, with uniform high level of expression of 5 genes (OsFER1, OsZIP5, OsYSL2, OsYSL6 and OsYSL12) in all 12 rice genotypes. To pursue role of metal homeostasis related genes in influencing grain iron and zinc contents (Table 1), the level of expression of genes was compared with the level of Fe/Zn in grains. The correlation analysis suggested that 10 genes (OsFER1, OsFRO2, OsNRAMP5, OsIRT1, OsZIP7, OsZIP8, OsYSL2, OsYSL6, OsYSL9, OsVIT1 and OsNAAT1) showed higher expression in leaf tissue of high zinc rice genotypes (R-RF-31, R 1033-968-2-1, BAS370, Nagina22, wild rice lines and Jaldoobi) while 3 genes (OsYSL6, OsFRO2 and OsNAC) expressed at high level in high iron rice lines at maximum tillering stage (lane 7, 8 in Fig. 1c). Root transcriptome analysis revealed higher level of expression of 6 genes (OsFRO2, OsZIP8,

OsYSL4, OsNAAT1, OsNRAMP5 and OsVIT1) in high zinc rice genotypes (lane 7, 8, 9, 10 11 in Fig. 1a) and OsZIP10 gene in high iron rice genotypes at maximum tillering stage (lane 2, 5, 6 in Fig. 1a). Association of level of expression of these genes in leaf tissue and root tissue at maximum tillering stage with high grain Fe and Zn contents indicated their participation in iron /zinc uptake and transport which might contribute to enhance the level of grain micronutrients.

High level of expression of 7 genes (OsYSL4, OsYSL6, OsZIP7, OsNRAMP5, OsNRAMP6, OsNRAMP7 and OsNAAT1) was observed in high grain Fe rice genotypes at mid grain filling stage in root tissue, while only OsNRAMP4 expressed at high level in high zinc rice genotypes (lane 7, 8 in Fig. 1b and Table 1). In flag leaf transcriptome 8 genes (OsFER2, OsFRO2, OsZIP7, OsZIP9, OsZIP10, OsYSL4, OsNRAMP5 and OsVIT1) showed higher level of expression in rice genotypes having higher grain Fe concentration and 3 genes (OsZIP7, OsZIP8 and OsNAAT1) showed high level of expression in high Zn rice genotypes (lane 2, 5 and 6 in Fig. 1d and Table 1), whereas the 12 genes (OsFER1, OsIRT1, OsZIP4, OsZIP11, OsYSL6, OsYSL9, OsYSL12, OsNRAMP4, OsNRAMP6, OsNRAMP7, OsNAC and OsNAS2) expressed at high level in both high and low iron and zinc containing rice genotypes. Based on the root and leaf transcriptome analysis at two stages we report critical association of 3 genes (OsZIP8, OsFER1 and OsNAAT1) with high grain zinc, 1 gene (OsZIP10) with high grain Fe and 8 genes (OsFRO2, OsNRAMP5, OsNRAMP7, OsYSL9 OsYSL2, OsNAC OsVIT1, OsZIP7) for both high grain Fe and Zn contents (Fig. 2). Our findings suggested existence of three different types of metal transporters/metal transport systems in rice viz. one each for Fe and Zn and one common system for both the metal ions. The genes showing association specifically to grain Fe and Zn concentration were identified as potential candidate genes involved in grain accumulation of Fe and/or Zn. Our analysis also suggested that higher activity of metal transporters at maximum tillering stage (both tissues) was associated to high grain Zn content while more activity of these transporters at mid-grain fill is related to high grain Fe content. We can thus conclude that grain Zn content is more influenced by rate of zinc absorbed and transported during early growth phase of plant while grain iron content is affected more by iron absorbed and transported through out plant growth, even during grain development stage.

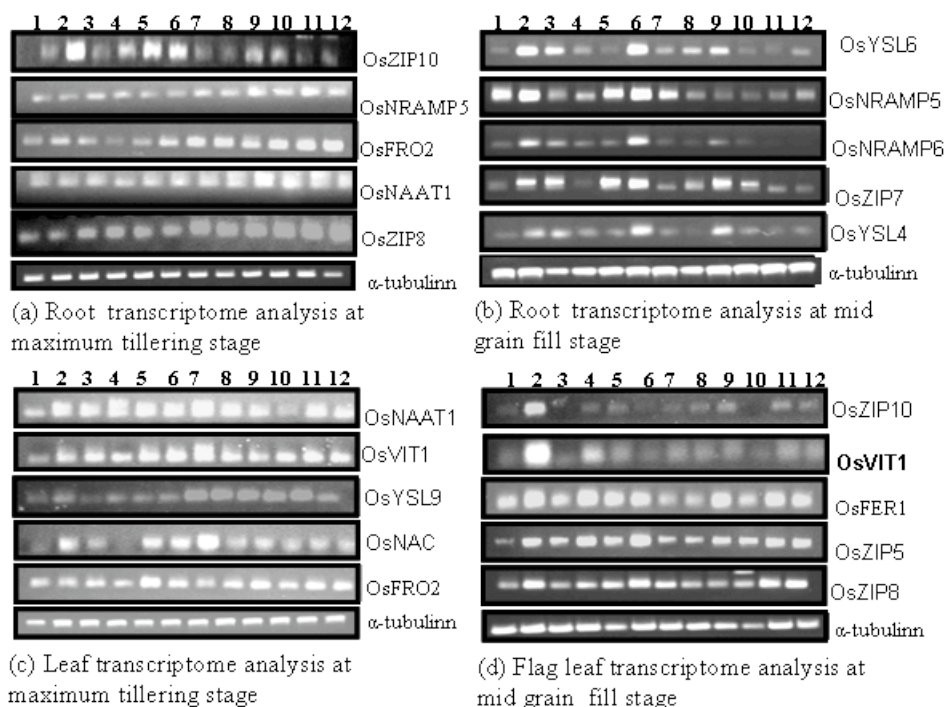


Fig. 1 Semi Quantitative RT-PCR analysis of metal related genes in root (1a and 1b), leaf (1c) and flag leaf (1d) tissues in twelve diverse cultivars. Lane 1: Nipponbare, 2: Moroberekan, 3: Nagina22, 4: Swarna, 5: Basmati 370, 6: IR 68144-3B, 7: R-RF 31,8-R1033-968-2-1, 9: *O. officinalis*, 10: *O. latifolia*, 11: *O. nivara* and 12: Jaldooobi.

Table 1 Mean grain iron and zinc contents of twelve diverse rice genotypes

S. No.	Name of the rice genotype	Grain iron content* ($\mu\text{g/g}$)	Grain Zinc content* ($\mu\text{g/g}$)
1	Nipponbare (Temperate japonica)	12.51 \pm 0.70	17.07 \pm 0.48
2	Moroberekan (Tropical japonica)	12.72 \pm 0.34	19.52 \pm 1.37
3	Nagina22 (Aus type)	12.65 \pm 1.08	26.84 \pm 1.47
4	Swarna (Indica)	8.50 \pm 0.29	13.95 \pm 1.75
5	Bas370 (Aromatic)	20.51 \pm 1.07	25.58 \pm 1.00
6	IR681444-3B (Indica- Int check)	12.23 \pm 0.30	21.79 \pm 0.75
7	R-RF-31 (indica high zinc)	8.96 \pm 0.35	27.64 \pm 1.05
8	R 1033-968-2-1 (indica high zinc)	10.60 \pm 0.36	30.06 \pm 2.06
9	<i>O. officinalis</i> (WR1)	17.52 \pm 1.33	39.32 \pm 0.75
10	<i>O. latifolia</i> (WR2)	18.61 \pm 1.01	36.90 \pm 0.79
11	<i>O. nivara</i> (WR3)	12.53 \pm 1.64	32.47 \pm 1.16
12	Jaldoobi (Submerged tolerance)	11.40 \pm 1.86	23.67 \pm 0.55

*Dehusked brown grains were used for estimation of Fe/Zn.

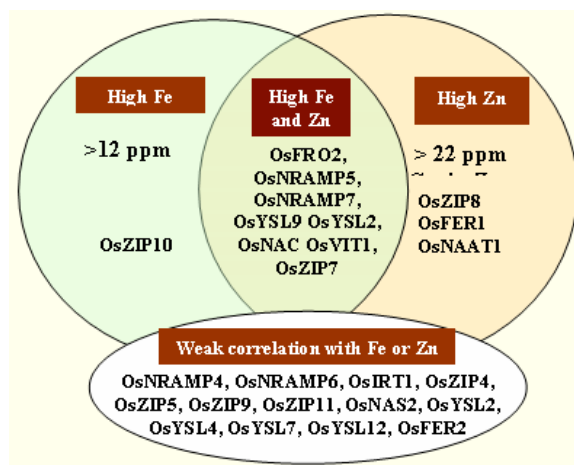


Fig. 2 Identification of potential metal homeostasis related genes associated with high grain Fe/Zn contents in rice based on root/shoot transcriptome analysis at maximum tillering and mid grain fill stages.

Acknowledgements

This study was supported by a grant from the Rice Bio-fortification project of the Department of Biotechnology, Ministry of Science and Technology, Govt of India, New Delhi.

References

- Bughio N., H. Yamaguchi, N. K. Nishizawa, H. Nakanishi and S. Mori, 2002. Cloning an iron-regulated metal transporter from rice. *J. Exp. Bot.* 53: 1677–1682.
- Garris A., J. Tai, T. H. Coburn, J. Kresovich and S. McCouch, 2005. Genetic structure and diversity in *Oryza sativa* L. *Genetics* 169: 1631–1638.
- Gross J., R. J. Stein, A. G. Fett-Neto and J. P. Fett, 2003. Iron homeostasis related genes in rice. *Genet. Mol. Biol.* 26: 477–497.
- Grusak M. A., 2002. Enhancing mineral content in plant food products. *J. Am. Coll. Nutr.* 21: 178-183.

- Guerinot M. L., 2000. The ZIP family of metal transporters. *Biochim. Biophys. Acta.* 1465: 190–198.
- HarvestPlus* (2006) Harvest Plus; Rice Processing protocol www.harvesplus.org.
- Inoue H., K. Higuchi, M. Takahashi, H. Nakanishi, S. Mori and N. K. Nishizawa, 2003. Three rice nicotianamine synthase genes, OsNAS1, OsNAS2 and OsNAS3 are expressed in cells involved in long-distance transport of iron and differentially regulated by iron. *Plant J.* 36: 366–381.
- Koike S, H. Inoue, D. Mizuno, M. Takahashi, H. Nakanishi, S. Mori and N. K. Nishizawa, 2004. OsYSL2 is a rice metal-nicotianamine transporter that is regulated by iron and expressed in the phloem. *The Plant Journal* 39: 415–424.
- Narayanan N. N., M. W. Vasconcelos and A. M. Grusak, 2007. Expression profiling of *Oryza sativa* metal homeostasis genes in different rice cultivars using a cDNA macroarray. *Plant physiology and Biochemistry* 45: 277-286.