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Research article

CHARACTERIZATION OF METAL HOMEOSTASIS RELATED RICE GENE ORTHOLOGS IN NUTRI- RICH MINOR MILLETS"

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ABSTRACT: Minor millets are nutritious food crops which bear potentials to serve as future food to combat the deep rooted malnutrition and nutritional insecurity prevailing in the developing world. The presented study focuses on characterizing different minor millet genotypes for grain micronutrient content along with the expression analysis of related genes. The study revealed higher level of iron and zinc content in millet grains as compared to major cereal crops. Fe content ranged from 25.65 µg/g in Kodo millet to 42.13 µg/g in Barnyard millet. In regard to grain zinc content, it ranged from 20.66 µg/g in Kodo millet to 54.34 µg/g in Barnyard millet. Expression analysis of sixteen metal homeostasis related rice gene orthologs depicted variable gene expression levels in seven genotypes of minor millets in flag leaf tissues at mid grain filling stage. VIT1 gene showed expression in all minor millets genotype with high level of expression in Sawa local, Melaghat-1, Melaghat-2, RLM-37, and TNAU-86. OsYSL2 and OsYSL18 genes depicted exceptionally high level of expression in flag leaf tissue in Kodo millet genotype TNAU-86. Similarly, NRAMP5 gene transcript showed extreme expression level in TNAU-86 followed by Melaghat-2 and RLM-37. Attempt to establish association between gene expression and grain Fe and Zn levels showed co linearity between these two parameters. Expression of three genes (YSL 2, NAS 1, NAS 3) was found to be correlated to high grain Fe contents, one gene (NAAT) to high grain Zn contents whereas expression of ten genes (IRT 1, NAS 2, ZIP 7, ZIP 5, ZIP 1, FER 1, FER 2, NAC 5, NRAMP 5, NRAMP 7) was found to be correlated to both high Fe and Zn contents.
Key words: Gene expression, Metal homeostasis, Micronutrient, Minor millets, Orthologs.

INTRODUCTION

Minor millets represent a collective term referring to a number of small-seeded annual grasses with short slender culm and small grains that are cultivated as cereal crops, primarily on marginal poor lands in dry areas of temperate, subtropical and tropical regions. Minor millets categorized as coarse cereals and are staple food for the tribal people where cultivation of major cereals like rice, wheat and maize is either not popular or fail to produce substantial yield [1]. Small millets are cheaper sources of nutritive food for poor and is in important fact as their health depends on the quality of food they consume.

Micronutrients are not only essential for plant growth and development but are also integral to human and animal health. In the last two decades, the concept of hidden hunger (deficiency of certain vitamins and micronutrient nutrients despite eating enough calories) has been well established [2]. As a result, the importance of micronutrient nutrition is increasing at a great pace. Screening of minor millets for grain nutritive traits by several workers has shown that these millets are highly nutritious food crops with higher fiber content along with quality protein and mineral composition which can serve as excellent dietary source for these elements [3]. They bear potentials to serve as future food to combat the deep rooted malnutrition and nutritional insecurity prevailing in the developing world [4,5]. Several researchers have also reported great variability existing among the collection of millets for grain nutritive traits and hence are amenable to employ efficient breeding strategies to improve these traits. Along with these facts, millets identified with high nutritive values can also be exploited for the identification and mining of genes/ alleles/ genomic regions governing these traits [6].

The short and easy way to approach food security fast is to explore diverse available resources which are not utilized yet or wild relatives having nutrition at par. Minor millets represent a unique biodiversity component in the agriculture and food security systems of millions of poor farmers. Deciphering the molecular and physiological roles of these metal homeostasis gene has become hottest topic of research since understanding the gene expression would enable researchers to develop elite varieties of crop plants with desired nutritive traits which will ensure global food security in the scenario of micronutrients malnutrition. This has resulted in gained momentum on minor millet research on important traits.

MATERIALS AND METHODS

Plant materials

The plant material used for the study included diverse millets genotype grown in different regions of Chhattisgarh along with few collections from Maharashtra. The plant material included seven diverse millets genotype belonging to three different groups of minor millets i.e. Barnyard millet, Kodo millet and Little millet (Table 1).

Table 1: List of materials used

S. No	Crop/Genotypes	Details
a.	**Barnyard millet** (*Echinochloa frumentacea*)	
1	**Sawa local**	Selection from Baster region of C.G.
2	**Melghat-1**	High yielding variety released in Maharashtra
3	**Melghat -2**	High yielding variety released in Maharashtra
b.	**Kodo millet** (*Paspalum scrobiculatum*)	
1	**TNAU-86**	Advanced breeding line in the AVT trial
2	**RBK-155**	Characterized as nutria rich line with 25.86 ppm Fe & 23.50 ppm Zn (Chandel *et al*., 2014)
c.	**Little millet** (*Panicum sumatrense*)	
1	**JK-8**	Characterized as nutria-rich line with 31.82 ppm Fe & 33.00 ppm Zn (Chandel *et al*., 2014)
2	**RLM-37**	High Fe (32.20 ppm) & Zn (32.40 ppm), (Chandel *et al*., 2014)

Establishment of Plants on Pots

Seeds were surface sterilized with 0.1% Bavistin followed by rinsing with distilled water and placed on moist blotting paper at 37 ºC for germination. Germinated seedlings were transferred to pots with coco-peat as potting substrate. Plants were grown in controlled environment condition under a 16h light at 30ºC/ 8h dark at 25ºC regime. Plants were watered daily with nutrient solution containing 1mM KNO_3, 0.8mM $Ca(NO_3)_2$, 0.3mM KH_2PO_4, 1mM $MgSO_4$, 25µM $CaCl_2$, 25µM H_3BO_3, 2µM $MnSO_4$, 2µM $ZnSO_4$, 0.5µM $CuSO_4$, 0.5µM H_2MoO_4, 0.1µM $NiSO_4$, and 10µM Fe(III)HEDTA. Nutrient solution was added to each pot at rate of 650ml/pot, once in a week till maximum tillering stage and then 650ml/pot twice a week from maximum tillering to mid-grain filling stage (Fig. 1). Flag leaf samples were collected from each plant at mid grain filling stage. Tissues were frozen in liquid nitrogen immediately to be used for RNA extraction. For mineral analysis grains were harvested from the fully mature plants.

Extraction of RNA and semi-quantitative RT-PCR

Total RNA was extracted using Plant RNeasy kit from TRIzol® Reagent according to the manufacturer's instructions and quantified with NanoDrop Spectrophotometer ND-1000® (NanoDrop Technologies, USA). Total RNA extracted was utilized for cDNA synthesis using Verso cDNA Synthesis Kit as per manufacturer's instructions. Sequences of rice metal homeostasis related genes were retrieved from Rice Genome Annotation Project database (www.tigr.org.in). Gene specific primers were designed from cDNA of 16 metal homeostasis related candidate genes using Primer-3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) with particular specifications (Table 2).

Semi-quantitative RT-PCR reactions were carried out with 10 µl of the reaction solutions using gene specific primers and α tubulin gene primer as an internal control. Additional reaction components were: 10 mM polymerase buffer, 1 mM dNTPs, 0.1 units Taq polymerase and 1 µM specific primers. Amplified products were visualized on a 1% TAE agarose gel containing ethidium bromide. Bands were photographed using the Quantity One 4.5.1 Chemidoc EQTM Software System (Bio-Rad, CA, USA). The relative intensity of amplified fragments provided basis for quantification of level of expression of gene as high, moderate, low and negligible.

Table 2: Forward and Reverse Gene Specific Primers Used for Semi-Quantitative RT- PCR Amplifications

S.N.	Gene	Locus id	Forward primer	Reverse primer	Tm	Fragment size
1	OsYSL2	LOC_Os02g43370	TCTGCTGGCTTCTTTG CATTTTCTG	ACCATGTCGAACTCAG CATCCAGGA	60	2340
2	OsYS18	LOC_Os01g61390	TCGGCTTCTTCCTCCT CAAG	CGTAGAAGTGGTGGAA GACG	60	250
3	OsNRAMP5	LOC_Os07g15370	AAGATGCCAAGAAG CTCGACG	GCTAGCGACTGTATGA TAAGTGCGA	60	2050
4	OsNRAMP7	LOC_Os12g39180. 1	CGGGGCAGACTAGTA CCATAACG	CAGCAAGAGATAGCCA TTGATCG	58	2000
5	OsFER1	LOC_Os11g01530	TAGGCAAAAGTTCGT CGACGA	TCTTCTCAGCTGGGCG ACATAC	58	1200
6	OsFER2	LOC_Os12g01530	CTTGCTAGGCAAAAG TTCGTCG	CATTCACTCCATTGCTA CTGCGT	58	1150
7	OsZIP1	LOC_Os01g74110	AGAAGTCGATCGATC GATCGTG	CTCACTGACCTGGTTG TCGTAA	60	1520
8	OsZIP5	LOC_Os05g39560. 1	AGCAACAGTGATCAT GGCGAC	CGAGGAAGAGGGCGA GGTATAC	62	1450
9	OsZIP7	LOC_Os05g10840	CACTCGGTGATCATC GGATTG	GTATATTAGGATGCCG GCCGA	60	1800
10	OsIRT1	LOC_Os03g46470	TTGTAGTAGGTGAGC ATGAGCG	CAGAGTGTGATGCATC GTCAG	65	1400
11	OsNAS1	LOC_Os03g19436	GTTCCTGTACCCGAT CGTC	CTCTTCTGCCTTCTCAA CCGC	62	200
12	OsNAS2	LOC_Os03g19420	CTCTTCACCGACCTC GTCAC	ATCGACGATCGGGTAC AGG	58	510
13	OsNAS3	LOC_Os07g48980	AGGAGGAGGAGGTG ATCGAG	AAGTAGGGGAAGAGG GCGAG	62	298
14	OsNAC5	LOC_Os02g36880	AGCGAGAAGCAAGC AAGAAG	ATGCCCTGGATATCGT CGTA	56	600
15	OsNAAT1	LOC_Os02g20360	CATCTTCCTAACCGC TGGAG	CCTTTGGCAGAAGGAT TTGA	58	700
16	OsVIT1	LOC_Os04g38940	AAGAGCGAGGCAGA CCATTA	GGAATGGACGGTTTCC AGTA	56	980

Elemental analysis

The grains from fully mature plants were oven dried at 60ºC for 4 days and used for estimation of grain iron and zinc content using Atomic absorption spectrophotometer (AAS200, Perkin Elmer make) as per the method developed (Chandel *et. Al,* 2014) and practiced at Grain Nutritional Quality Lab, department of PMBB, IGKV Raipur

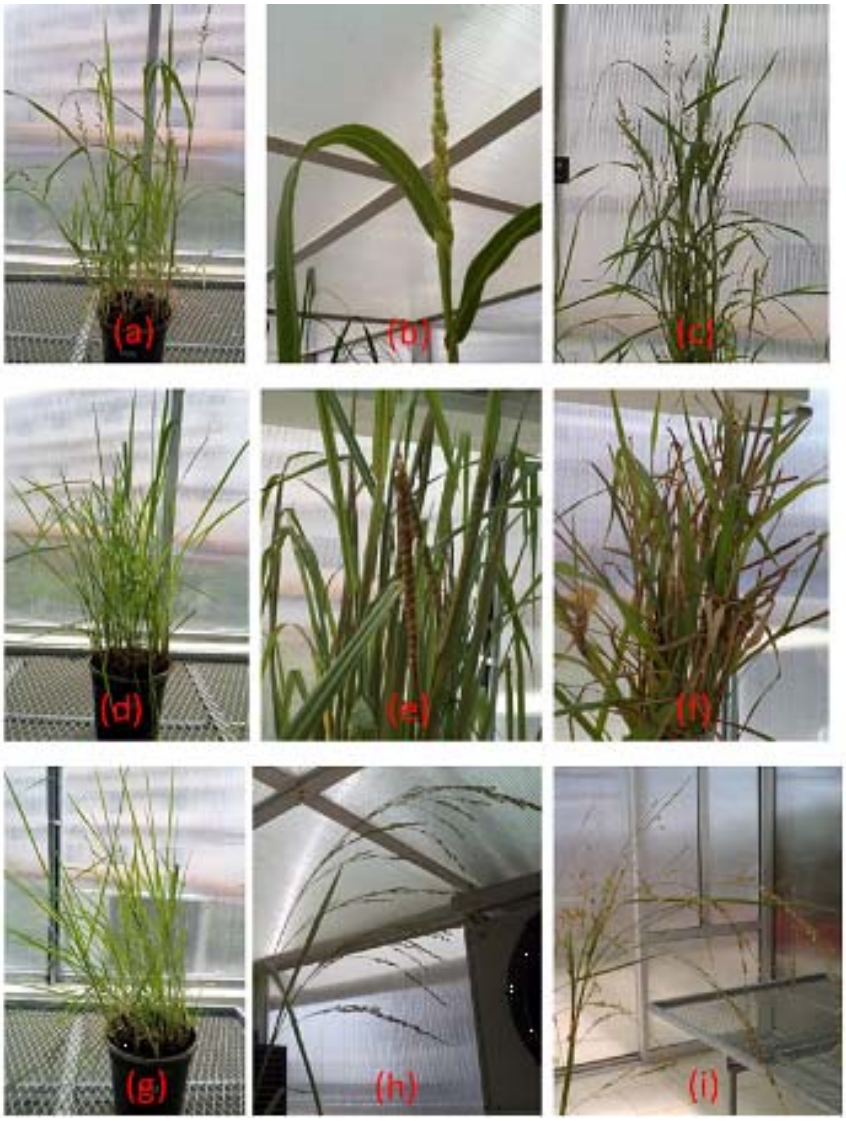

Figure 1: Plant morphology of selected genotypes of minor millets. (a) Sawa local at mid grain fill stage (b) panicle of melghat-1 at mid grain fill stage (c) tiller of melghat-2 at mid grain fill stage (d) TNAU-86 at formative stage (e) tiller of RBK-155 at mid grain fill stage (f) tiller of TNAU-86 at maturity stage (g) JK-8 at formative stage (h) tiller of JK-8 at mid grain fill stage (i) tiller of RLM-37 at mid grain fill stage.

RESULTS AND DISSCUSSION

Iron content in millet grains- Wide and significant genetic variation for grain Fe levels were recorded among the different crops and genotypes of each of the minor millets used in this study. The grain Fe content varied from 25.65 µg/g in Kodo millet to 42.13 µg/g in Barnyard millet genotypes. The observed values are higher than the average iron content present in major cereal crops such as rice, wheat, maize, etc. as well as other millets like sorghum and pearl millet. The iron content in barnyard millet ranged from 29.8 µg/g to 42.13 µg/g with the variety Sawa local showed the highest iron content of 42.13 µg/g followed by Melaghat-2 (35.87 µg/g) and Melaghat-1 (29.8 µg/g). Among the two different genotypes of Kodo millet genotype, TNAU-86 showed the high Fe (29.42 µg/g) followed by RBK-155 (25.65 µg/g) and in Little millet genotype RLM-37 (39.88 µg/g) showed the high amount of Fe followed by JK-8 (31.97 µg/g). Sawa local variety of Barnyard millet showed highest concentration of iron with 42.13µg/g (Table 3, Fig. 4.3). Highest concentration of iron (40.2 ppm) in Barnyard millet followed by Finger millet with 34.15 ppm, Little millet with 32.71 ppm, Kodo millet with 32.275 ppm and Foxtail millet with 27.19 ppm iron content in grain and also reported the significant Fe variability among the minor millet genotypes [6].

Zinc content in millet grains- Similarly significant variations in the zinc content in mature seed were also recorded for the seven minor millet genotypes analyzed. The zinc content ranged in whole grain of minor millets from 20.66 µg/g in Kodo millet to 54.34 µg/g in Barnyard millet. Zinc content in Barnyard millet ranged from 43.73 µg/g to 54.34 µg/g with the variety Melghat-2 showing the highest Zn content of 54.34 µg/g followed by Melghat-1 (46.44 µg/g), Sawa local (43.73 µg/g). Among the two different genotypes of Kodo millet genotype RBK-155 showed 20.70 µg/g of Zn and TNAU-86 was found to show 20.66 µg/g of Zn. Both genotype of Kodo millet showed similar Zn content. The Zinc content in the genotype of Little millet showed 33.45 µg/g in JK-8 and 30.62 µg/g in RLM-37. Genotype Melghat-2 of Barnyard millet showed highest Zn content of 54.34 µg/g among the all tasted minor millet genotypes (Table 3, Fig. 2). Significant Zn variability among the minor millet genotypes in this study is also supported by earlier observation [6].

Table 3: Whole grain Fe and Zn concentration in µg/g of seven selected genotype of minor millets

S. No.	Crop/Genotypes	Zinc (µg/g)				Iron (µg/g)			
		R1	R2	R3	Mean ± SE	R1	R2	R3	Mean (SE)
a.	**Barnyard millet** (*Echinochloa frumentacea*)								
1	**Sawa local**	44.05	44.40	42.74	43.73 ± 0.50^{b}	43.66	40.10	42.64	42.13± 1.05^{a}
2	**Melghat-1**	49.45	47.60	42.28	46.44 ± 2.14^{b}	28.79	29.80	30.85	29.81± 0.59^{c}
3	**Melghat -2**	54.81	53.72	54.50	54.34 ± 0.32^{a}	36.31	34.16	37.16	35.87± 0.89^{b}
b.	**Kodo millet** (*Paspalum scrobiculatum*)								
1	**TNAU-86**	21.20	20.02	20.76	20.66 ± 0.34^{d}	31.95	28.34	27.98	29.42± 1.26^{c}
2	**RBK-155**	19.83	19.79	22.49	20.70 ± 0.89^{d}	26.20	25.90	24.87	25.65± 0.40^{d}
c.	**Little millet** (*Panicum sumatrense*)								
1	**JK-8**	33.74	35.12	31.50	33.45 ± 1.05^{c}	32.36	32.77	30.79	31.97± 0.60^{c}
2	**RLM-37**	32.05	28.88	30.93	30.62± 0.92^{c}	41.92	35.27	42.45	39.88± 2.31^{a}

Note: Values are three independent replicates data and their means. For each trait, means followed by different letters are significantly different from each other for "genotype x Zn and Fe content" (LSD test, $P < 0.05$)

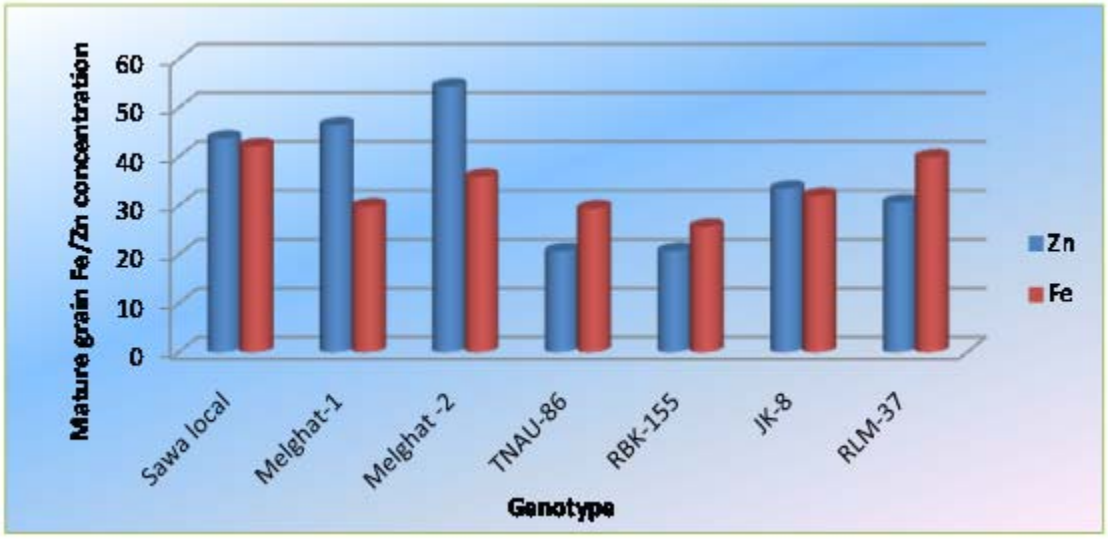

Figure-2: Fe and Zn content level in different minor millet genotype.

Semi-quantitative RT-PCR Analysis:

The semi-quantitative PCR analysis of 16 Fe and Zn homeostasis related rice genes ortholog, including 10 genes belonging to OsZIPs, OsNRAMPs, OsFERs and OsYSLs families and 6 non-rice genes (OsNAAT1, OSNAC, OSNAS1, OSNAS2, OSNAS3 and OsVIT1), was performed in flag leaf transcriptome at mid-grain filling stages. Expression profiling of flag leaf tissues revealed differential expression of metal homeostasis related candidate genes among genotypes.

OsFER Gene Family: Ferritin gene family is known to encode seed iron storage protein, its expression analysis revealed mid-grain filling stage specific expression of OsFER1 gene and showed uniform level of expression in Flag leaf tissue in all three genotypes of Barnyard millet (Sawa local, Melaghat-1 and Melaghat- 2). Its high expression indicated that higher amount of OsFER1 gene is synthesized in tissues during grain fill stage. However grain Fe content of theses genotype showed slight variation. In little millet, RLM-37 showed low level of expression while another genotype (JK-8) showed negligible expression and was found to be correlated to high Fe content in RLM-37 than JK-8. Similarly in Kodo millet, TNAU-86 showed low level of expression and RBK-155 showed no expression and correlated to high Fe than another (RBK-155). Similarly OsFER 2 gene showed negligible expression in Barnyard millets and Kodo millets. While in little millets, both genotype (RLM-37, JK-8) showed no genotypic variation and expressed with moderate level of expression (Fig. 3 (b)). Expression of OsFER1 gene in flag leaf with variation in level of expression was found among four rice cultivars [7].

OsYSL Gene Family: The OsYSLs genes are known as components of strategy II of metal transport found in cereals [8,9]. The expression of two OsYSL genes (OsYSL2 and OsYSL18) was analyzed in flag leaf tissues of seven minor millet genotypes at mid grain fill stage. In Barnyard millet OsYSL2 expressed at low level in Sawa local and remaining two genotype (Melghat-2 and Melghat-1) showed negligible expression. OsYSL2 transcript depicted high level of expression in flag leaf tissue in Kodo millet (TNAU-86) and negligible in RBK-155 genotype while no variation found in grain Fe & Zn content. In Little millets OsYSL2 was not expressed. High level of expression of YSL2 was recorded in flag leaf tissues of all tested genotypes at mid-grain filling stage [10]. Similarly, OsYSL18 genes showed expression in flag leaf tissue in barnyard millet with slightly variation, high level of expression in melghat-2 and moderate level of expression in sawa local and melghat-1. In little millets RLM-37 showed moderately low level of expression and JK-8 showed no expression. While in kodo millets, OsYSL18 showed genotype variation in expression, TNAU-86 showed high level of expression and RBK-155 showed low level of expression. But further it was not correlated to grain Fe & Zn variation (Fig. 3 (c)).

OsZIP Gene Family: The ZIP family genes including OsIRT1 are known to participate in divalent metal transport in plants [11, 12, 13,14]. Studies conducted so far to characterize leaf and flag leaf transcriptome in rice have revealed high level of expression of OsZIP5, OsZIP7, OsZIP8 and OsZIP10 genes in both leaf and flag leaf tissues at mid-grain fill stage [7]. Expression of four (IRT1, ZIP1, ZIP5, ZIP7) of the OsZIP family genes was analyzed in the present study (Fig. 3 (d)). IRT1 gene expressed at variable level in Barnyard millet with low level of expression in Sawa local, moderately low level of expression in Melghat-2 and poor expression in Melghat-1. In Little millet it was not expressed. While in Kodo millet only TNAU-86 showed expression at moderate level. The OsIRT1 gene expressed at high level in root and flag leaf tissues of 12 rice genotypes at MGF stage [10]. Expression of OsZIP1& ZIP5 transcripts were observed with low level of expression in flag leaf tissues of minor millet genotypes. In Barnyard millets only one genotype Melghat-2 showed ZIP1 gene transcript at low level. Little millet showed no transcript and in Kodo millet one genotype TNAU-86 showed low level of expression however no transcript were detected in RBK-155. OsZIP5 also showed similar pattern of expression like as OsZIP1, difference was found only in TNAU-86 genotype of Kodo millet, ZIP5 expressed at moderate level in TNAU-86. OsZIP7 gene transcript was found in Barnyard millet with low level of expression in Melghat-1 and Melghat-2 and Sawa local showed poor expression. No expression was analyzed in Little millet and among Kodo millet genotype only TNAU-86 showed high level of gene expression.

OsNRAMP family- NRAMP genes are considered to take part in high affinity transport of iron across cell membranes through transmembrane proteins encoded by these genes [15,16]. NRAMP5 gene transcript was observed in Barnyard millets with high level of expression in Melghat-2 and moderate level of expression in Melghat-1 and Sawa local genotype showed negligible expression. In Little millet RLM-37 genotype showed high level of expression while JK-8 showed no expression. Similarly in Kodo millet, NRAMP5 expressed at extremely high level and was not expressed in RBK-155. NRAMP7 gene showed expression in Barnyard millet with low level of expression in Melghat-2 and moderately low level of expression in Melghat-1 and negligible expression in Sawa local. It was not expressed in Little millet and in Kodo millet only TNAU-86 showed expression at low level (Fig. 3 (a)). Variation of these gene (NRAMP5 & NRAMP7) among genotype is also showed in previous studies in rice in flag leaf tissue at mid grain fill stage [10].

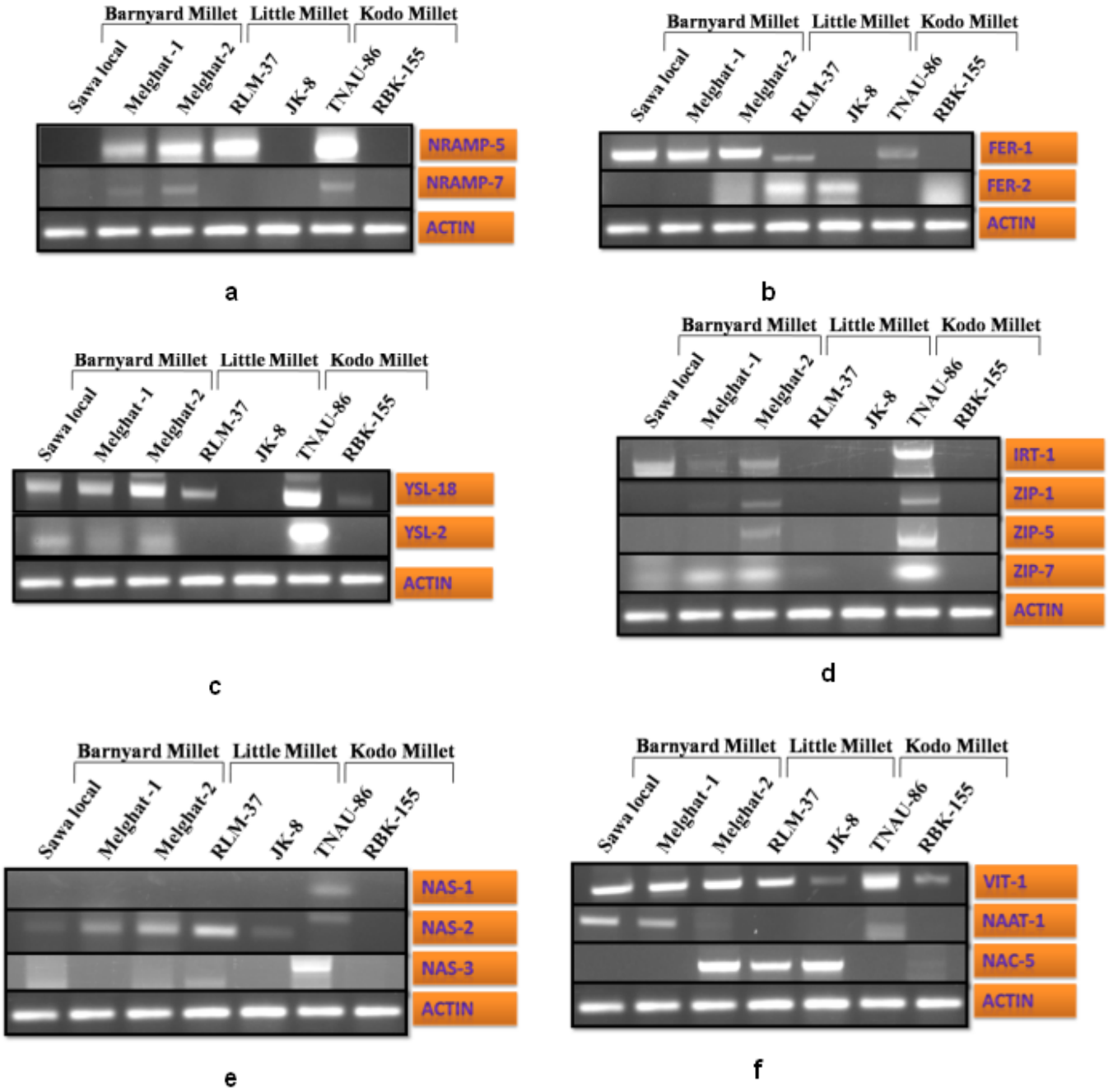

Figure 3 Semi-quantitative RT-PCR profile of metal homeostasis related candidate genes in seven diverse millet genotypes at mid grain fill stage in flag leaves tissue (a) OsNRAMP family gene (b) OsFER family gene (c) OsYSL family gene (d) OsZIP family gene (e) OsNAS gene (f) other genes (VIT-1, NAAT, NAC-5)

Other Metal Homeostasis Related Genes.

OsNAS Gene- Three genes NAS1, NAS2, NAS3 were selected for transcriptome analysis from flag leaf tissue at mid grain filling stage. NAS1 gene transcript was observed in only one genotype TNAU-86 of Kodo millet. NAS2 gene expressed at variable level in Barnyard millets with moderately low level of expression in Melghat-2 and low level of expression in Melghat-1, whereas poor expression in Sawa local. In Little millet, RLM-37 showed moderate level of expression and JK-8 showed poor expression. NAS2 gene showed negligible expression in Kodo millet. NAS3 gene showed expression with moderate level only in TNAU-86 genotype of Kodo millet and rest of the genotypes showed poor expression (Fig. 3 (f)). In an earlier observation [10] in rice, expression analysis revealed MGF stage specific expression of the OsNAS2 gene whereas no expression was recorded in MTS in leaf and root tissue.

OsNAAT gene- The OsNAAT1 gene is speculated to participate in biosynthesis of phytosiderohores related to Strategy I of metal uptake in cereals Two genotype of Barnyard millet, Sawa local and Melghat-1 showed uniformly low level of expression and Melghat-2 showed negligible expression and no transcript was found in Little millet. In Kodo millet poor expression was analyzed in TNAU-86 and in RBK-155 genotype no transcript was analyzed (Fig. 3 (f)). NAAT1 gene expressed at varied level among the tested rice genotypes in both the stages but its expression in MGF flag leaf and root tissues was found to be correlated to high grain Zn content [10].

OsNAC Gene- As per the expression of NAC gene reported during reproductive stage and senescence in wheat [17], the OsNAC gene also showed high level of expression in MGF root and flag leaf tissues of all twelve rice genotypes [10]. In Barnyard millets NAC5 were expressed with high level of expression in Melghat-2, and no expression were depicted in Sawa local and Melghat-1. NAC5 gene showed uniform high level of expression in both genotype of Little millet and in Kodo millet no expression was found (Fig. 3 (f)). OsNAC gene was found to express in root and flag leaf tissues at MGF stage at uniform high level but the gene showed genotype specific variation in MTS leaf tissue [10].

OsVIT Gene- Vacuolar sequestration is another important mechanism in regulating Fe homeostasis, and could also serve as a safe Fe storage strategy. Results obtained in present study showed that the OsVIT1 gene is expressed in flag leaf tissues at mid-grain filling stage in minor millets. In Barnyard millet all three genotype Sawa local, Melaghat-1, Melaghat-2, showed high level of expression with no variation in expression. In Little millet VIT1 expressed at moderate level in RLM-37 and low level of expression in JK-8. In Kodo millet TNAU-86 showed high level of expression, however RBK-155 showed low level of expression (Fig. 3 (f)). The expression of vacuolar iron transporter protein encoding AtVIT1 gene is known to be induced during seed development stage [18,19] under Fe deficiency. OsVIT1 gene, rice homologue of AtVIT1 gene, expressed in flag leaf tissues at mid-grain filling tissue showing genotypic variation in level of expression [10].

Association with grain Fe & Zn contents:

The level of expression of the genes were analysed for any correlation with grain micronutrient concentrations to understand the mechanism of iron and zinc homeostasis in Minor millets. The genes showed distinct variation in level of expression among genotypes in flag leaf tissue at mid-grain filling stage. Expression of three genes (YSL 2, NAS 1, NAS 3) was found to be correlated to high grain Fe contents, one gene (NAAT) to high grain Zn contents whereas expression of ten genes (IRT 1, NAS 2, ZIP 7, ZIP 5, ZIP 1, FER 1, FER 2, NAC 5, NRAMP 5, NRAMP 7) was found to be correlated to both high Fe and Zn contents. Where, the two genes viz. VIT-1 and YSL-18 showed no correlation with the grain Fe & Zn contents. But grossly it was observed that expression of selected genes was found to be in line with the grain micronutrients levels (Fig. 4).

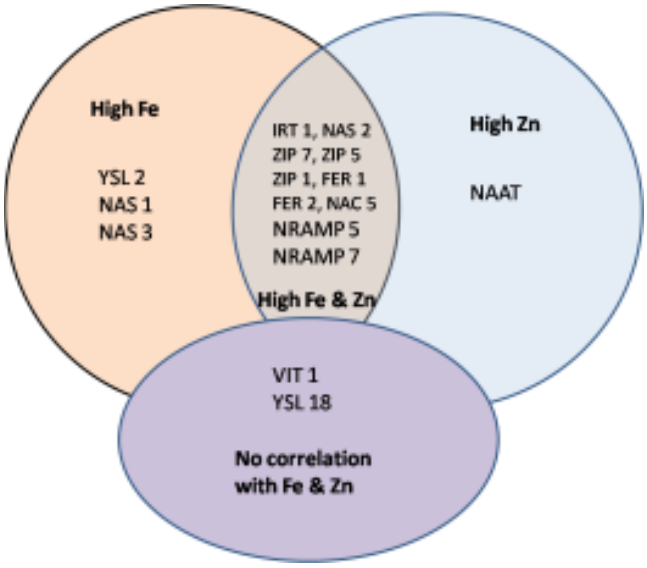

Figure-4: Schemetic representation of candidate gene expression with grain micronutrients level among selected genotypes.

The expression analysis of OsIRT1, OsZIP1, OsZIP5, OsZIP8, OsYS15, OsYS16, OsYS17, OsYS18, OsNRAMP2, OsNRAMP4 and OsNRAMP7 shows higher level of expression in non-flag and flag leaves of cultivar IR68144 having higher grain iron (~21µg/g) concentration [6]. The expression level of 9 genes (OsYSL6, OsYSL8, OsYSL14, OsNRAMP1, OsNRAMP7, OsNRAMP8, OsNAS1, OsFRO1 and OsNAC5) in flag leaves exhibited significant correlations with Fe and/or Zn concentrations in the seeds [20]. The expression pattern of metal related candidate genes in reference to grain Fe/Zn contents of twelve rice genotypes indicated differential expression of metal transporter genes at mid grain filling stage [10].

CONCLUSION

Due to richness of important micronutrients like iron and zinc along with previously discovered health benefits, minor millets can be one of the magic cereal foods to combat micronutrient malnutrition. Based on the finding of this study, it can be concluded that minor millet are superior cereal grain with good nutrient profile and hence will be worthy addition to one's diet. Enhancing micronutrient in the diet by using minor millet cereal foods in the diet to ensure adequate attainment of iron and zinc seems to be most suitable strategy to combat micronutrient malnutrition. Additionally, owing to their high nutritive value, minor millets can also serve as source of new genes or more valuable allels of existing genes, which can be further utilized by transgenic program to improve micronutrient content in the dominated foods.

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