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Transferability of Markers Specific to Micronutrient Transportation for Biofortification of Rice (*Oryza sativa* L.): A New Paradigm

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ABSTRACT

Enhancing micronutrients in staple crops to alleviate malnutrition is a current challenge among scientific fraternity. Metal transporters play major role in transporting micronutrients from root to grains. Rice, finger millet and Pearl millet were used to discern the variations present in their transporters. Among 12 gene specific markers for rice, four were amplified in finger millet and only one amplified in pearl millet. OsZIP3b is amplified in all three crops and hence, can be used in selecting the rice genotypes for higher grain micronutrient content. Information present in micronutrient rich crops can be used to biofortify staple food crops.

HIDDEN huger is a state where in spite of enough meals in a day,person's nutrition is not yet balanced (Rawat *et al.,* 2013). Question of food security is now beingredirected to nutrition security. Vitamin A, iron and zinc are the major three factors playing role in nutrition balance as deficiency of these three are considered as global threat.To address the problem of hidden hunger staple food crops are being targeted. Though rice is the staple food for more than half of the global population, it lacks micronutrients like iron and zinc.

Underutilized crops like finger millet (*Eleusinecoracana*) and pearl millet (*Pennisetumglaucum*) are inherently rich in micronutrients. When crops are grown onthe same soil, amount of iron and zinc accumulated in their grains varies immensely because of difference in their genetic ability to transport these micronutrients to grain. Metal transporters are the key players involved in bringing such differences (Palmer and Guerinot, 2009; Waters and Sankaran, 2011). Discerning the variation present in such transporters between crops which are rich in micronutrients and crops with lower micronutrient content would be a way out for micronutrient biofortification in ricesolving the problem of hidden hunger. Considering this possibility, an effort was made to understand the transferability of markers designed specific tometal transporters of rice in finger millet and pearl millet.

The experiment was conducted in *Kharif* 2015, with two genotypes each from three crops. Rice (varieties: ARB6 and Pokkali) was the target crop to enhance the micronutrient content.Presently, iron concentration in brown rice ranges between 2 and 15 mg $kg^{-1}$and zinc concentration between 5 and 20 mg kg^{-1}.Finger millet (varieties: MR6 and GPU28), an underutilized nutritious crop with higher iron content ranging between20 and 80 mg kg^{-1} and zinc content ranging between 20 and 30 mg kg^{-1},was selected along with pearl millet (Varieties: ICTP8203 and ICMV221), another underutilized crop with 40-120 mg kg^{-1}iron and 30-80mg kg^{-1}zinc (Shobana *et al.,* 2013).

DNA was isolated adopting CTAB method (Doyle and Doyle, 1990).PCR amplification of the markers was carried out in Mastercycler® Nexus Gradient, Eppendorf. Agarose electrophoresis was done with 3% gels to visualize the amplicons.

Metal transporters belonging to various familiesviz.,*OsZIP* and *OsYSL* which are involved in transportation of iron and zinc (Palmer and Guerinot, 2009; Waters andSankaran, 2011) were considered. Primers designed specific to these transporters by Hollaet *al.* (2014);PatilandShashidhar, (2016) were used to screen for transferability.

Among the twelve markers used for screening, as expected, all of them showed amplicons in both the genotypes of rice (Fig. 1). Size of amplicons was similar to the expected product size. However, only four primers namely OsZIP3b, OsZIP4b, OsYSL2_3 and OsYSL15_8showed amplification in finger millet. This indicates that the four transporter genes, namely *OsZIP3, OsZIP4, OsYSL2* and *OsYSL15* are operating efficiently in finger millet.

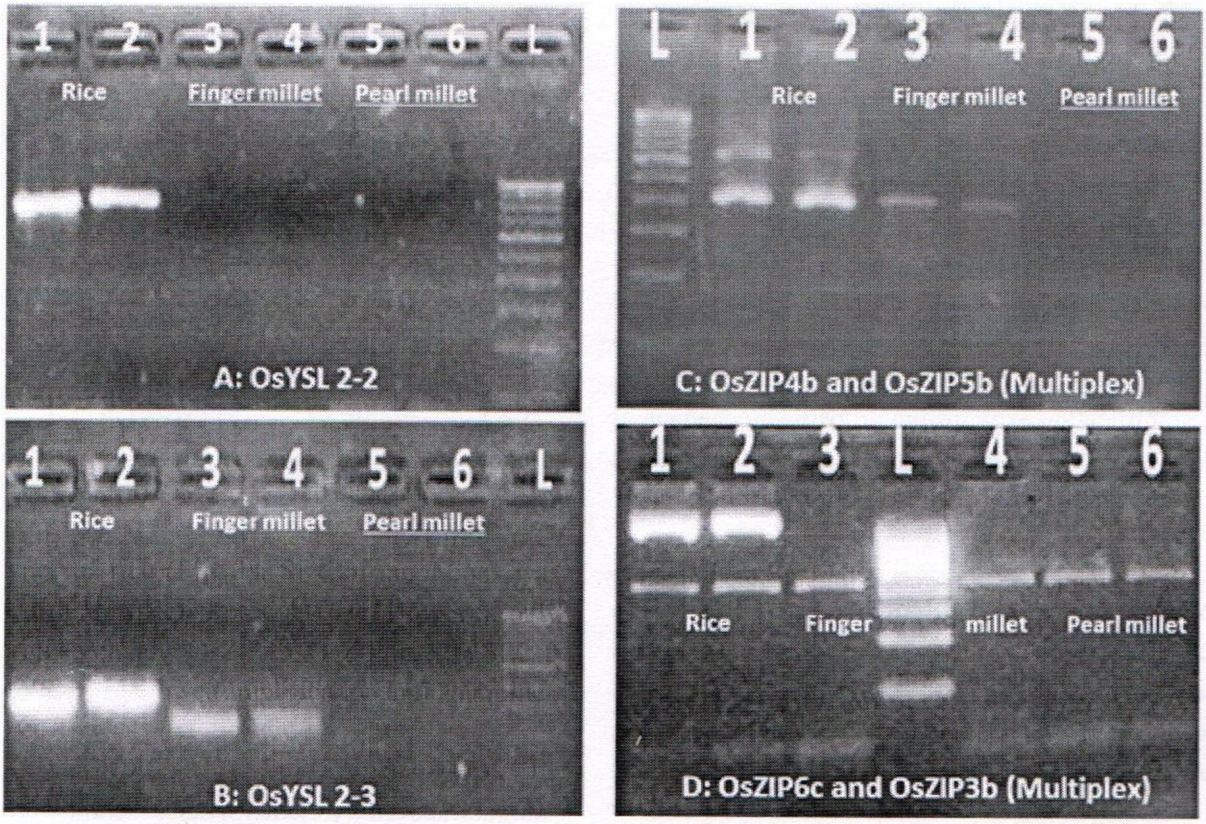

Fig.1: Molecular profile of various markers used in the study (Crops underlined were with null alleles for that marker)
Legends : 1) ARB6, 2) POKALI, 3) MR 6, 4) GPU28, 5) ICTP8203, 6) ICMV221, L: Ladder (100 bp)

TABLE I

Transferability of markers used in the study

Primer name	Specific to transporter	Amplicon size (bp)		
		Rice	Finger millet	Pearl millet
OsZIP3b	OsZIP3	500	500	500
OsZIP4b	OsZIP4	400	400	-
OsYSL2_3	OsYSL2	350	450	-
OsYSL15_8	OsYSL15	600	600	-
OsZIP6c	OsZIP6	1000	-	-
OsYSL2_1	OsYSL2	752	-	-
OsZIP1b	OsZIP1	850	-	-
OsZIP5b	OsZIP5	600	-	-
OsYSL2_2	OsYSL2	1000	-	-
OsYSL16_5	OsYSL16	650	-	-
OsYSL16_6	OsYSL16	400	-	-
OsYSL4_1	OsYSL4	777	-	-
Multiplex OsZIP4b &OsZIP5b	OsZIP4b & OsZIP5b	400 & 600	400 & 600	-
Multiplex OsZIP3b & OsZIP6c	OsZIP3b &OsZIP6c	500 & 1000	500 & 1000	500 & 1000

*OsZIP3*was reported to be expressed in roots of rice indicating their role in transporting zinc and iron from root to shoot. Amplification of *OsZIP3* in finger millet shows that it is an effective transporter involved in accumulation of micronutrients in shoot which will later be accumulated in grains. Amplification of *OsZIP4*(reported to be expressed in shoots and grains of rice)in both rice and finger millet explains that micronutrients which are transported by *OsZIP3*from root to shoot are further transported to grains by *OsZIP4* transporters. *OsYSL2* and *OsYSL15*(which were also amplified in both crops)were reported to be involved in remobilising the micronutrient stored in leaves into the grains (Bashir*et al*., 2012). This pattern of amplification explains the probable mechanism of efficient transportation and accumulation of high amount of iron in the grains of finger millet.

In case of pearl millet, onlyOsZIP3bwas amplified(Table 1). This indicates that *OsZIP3*was a very important micronutrient transporter which is active in all three crops studied. As *OsZIP3*was reported to be expressed in both shoot and grains of the rice plant, its expression in case of pearl millet would be a reason for increased accumulation of both iron and zinc in its grains.

Other eight markers were not amplified in finger millet and 11 markers were not amplified in pearl millet even after replicating the experiment thrice. The markers with amplicons only in rice as well as in rice and finger millet were multiplexed with OsZIP3b. OsZIP3b was a marker with amplicons in all three crops. Hence, absence of bands is true absence of that particular DNA sequence in the genomic DNA of the respective crop and not due to any kinds of technical error. The transporters like *OsZIP6, OsZIP1* and *OsYSL4*, which were having no amplicons in both finger millet and pearl millet,can be considered not so important since these crops are still managing higher micronutrient status in their absence.

OsZIP6 was reported to be expressed in root of rice and *OsZIP1* was reported to be expressed in root and shoot of rice (Banerjee *et al.,* 2010). Root forms the important part of the plant for nutrient accumulation since it is the part which comes in contact with soil to absorb these nutrients and hence, contain higher concentration of these nutrients. Any crop which can effectively transport the accumulated micronutrients into the grains would be rich in micronutrients. Hence, transporters which work in root are of most importance and should be more efficient. *OsZIP6* and *OsZIP1* may have efficient substitutes in case of pearl millet and finger millet making them more powerful in accumulating higher micronutrients in their grains.

Interestingly, in case of OsYSL 2_3 there was a difference in the amplicon size between rice and finger millet. *OsYSL2*was reported to be expressing in shoot of monocot plants (Palmer and Guerinot, 2009). This variation indicated that,although it is present in both the crops, there is a variation in length of the DNA sequence. This would probably be contributing to the difference that was exhibited in these two crops.

The presence of polymorphism also leads to a question about the variation in the sequences with similar amplicon length. As reported in earlier study (Holla*et al.,*2014) any monomorphic marker has similar information as that of a polymorphic marker. Hence, assuming that 'crops with amplicons of same size have same efficiency in transporting the micronutrients to grain' would be misleading. Dissecting the variation present in the transporters at nucleotide level would shed a light on the reason behind the differential accumulation of micronutrient in the grains of these crops belonging to same family.

Results indicated that among twelve markers screened, four were amplified in finger millet and one was amplified in pearl millet. As OsZIP3b was amplified in all the three crops, it can be used in selecting the rice genotypes for higher grain micronutrient content. Further, information regarding the transporters working in finger millet and pearl millet are of immense importance as the same information can be used to enhance the micronutrient status of the rice. This study concludes that discerning the variations present among the crops for micronutrient transporters paves a new dimension in biofortification of crops like rice.

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