

***In Silico* identification of candidate genes involved for grain Fe and Zn concentration in sorghum using reported cereals gene homologs**

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Introduction

Sorghum is one of the top 10 crops that feed the world. It is a good source of energy, protein, carbohydrate, vitamins and minerals including the trace elements. It is one of the cheapest and sustainable options to combat the micronutrient malnutrition, particularly Fe and Zn in predominantly sorghum eating populations. Identification of genes governing grain Fe and Zn concentration in sorghum is of interest. Earlier studies on other cereals showed role of number of genes for grain Fe and Zn homeostasis and uptake, transport and loading, but so far no reports available on genomic regions/ QTLs and candidate genes governing sorghum grain Fe and Zn content in sorghum.

Material and Methods

A homology (*in-silico*) search of 91 candidate genes involved in governing grain Fe and Zn content in cereals (rice, wheat, maize and barley) was performed for sorghum genome. Fe and Zn gene sequences were downloaded from the public repository and BLAST searched against sorghum genome. Top hits (based on e-value) were identified and visualized in GBrowse which can be accessed at <http://10.4.19.64/cgi-bin/gb2/gbrowse/sorghum/?source=sorghum> .

Results and Discussion

Several genes responsible for metal cheating and phytosiderophore biosynthesis, uptake, transport, loading and storage have been identified in rice, barley, wheat and maize (Anuradha et al., 2012, Gross et al., 2003, Bashir et al.,2010, Sharma and Chauhan.,2008, noue et al., 2003, Banerjee and chandel et al 2011). Using 91 metal homeostasis candidate genes, homology (*in-silico*) search was performed on sorghum genome and a total of 77 genes having homology with sorghum genome were identified. The e - value was ranged from 0 to 1E-37 and 46 out of 77 hits found with Zero e – value (e- values of 0 mean that there's an exact match). Gene identical percentage (similarity of sequences) was ranged from 71.9 (*IDS3*) to 95.9 (*HMA*) with average of 86.4 (Table 2).

Table 1: Crop wise number of genes hits identified on Sorghum genome

| Species | No .of Genes used for blast | No. of top hits found on sorghum genome | % Homology |
|----------------------------------|------------------------------------|--|-------------------|
| <i>Oryza sativa (Rice)</i> | 44 | 36 | 82 |
| <i>Hordeum vulgare (Barley)</i> | 23 | 17 | 74 |
| <i>Zea mays (Maize)</i> | 15 | 15 | 100 |
| <i>Triticum aestivum (Wheat)</i> | 9 | 9 | 100 |
| Total | 91 | 77 | (Avg) 89 |

Maximum number of hits were found on chromosome 1 (24 numbers) and least on chromosome 8. Genes associated with grain Fe and Zn content from *maize* and *wheat* showed 100 percent homology for (gene) sequences in sorghum genome. On sorghum genome, highest number of hits were identified on chr 1 and

chr 6, from all the 4 crops. Out of 24 hits on chromosome 1, 10 hits (from genes of wheat, barley, rice, maize) pertaining to *Nicotianamine synthase (NAS)* gene were in same general genomic region around 61Mbp on chromosome 1; 9 out of 17 hits (from gene maize and rice) for *YSL (yellow stripe like)* gene were around 52 Mbp on chromosome 6; and 21 hits (from gene all 4 crops) for *Zip (Zn transporter protein)* were found on all chromosomes except chromosome 5 on sorghum genome. These could be putatively associated with Fe and Zn grain content in sorghum. *NAS*, *YSL* and *ZIP* genes are most important genes largely reported for Fe and Zn homeostasis in all cereal crops and over expression of single rice genes such as *OsNAS* has been reported to enhance the concentration of both iron and zinc (Johnson et al. 2011, Lee et al. 2011).

Table 2: List of reported candidate gene names involved for Fe and Zn grain content in cereals used for BLAST search sorghum genome and number of hits found and Gene Identical percentage

| S.no | List of reported candidate genes involved for Fe and Zn grain concentration in cereals | No. of hits found on sorghum genomic regions | Gene Identical (%) |
|------|--|--|--------------------|
| 1 | <i>DMAS (Deoxymugineic acid synthase)</i> | 3 | 88 |
| 2 | <i>FDH (Formate dehydrogenase)</i> | 1 | 89 |
| 3 | <i>FER (Ferritin)</i> | 3 | 86.6 |
| 4 | <i>HMA (Heavy metal-associated)</i> | 3 | 95.9 |
| 5 | <i>IDEF1(iron deficiency-responsive)</i> | 1 | 79.4 |
| 6 | <i>IDH1 (Acireductone dioxygenase)</i> | 1 | 93.5 |
| 7 | <i>IDS3 (iron deficiency-specific 3)</i> | 1 | 72 |
| 8 | <i>IRO2 (iron-related transcription factor)</i> | 1 | 79 |
| 9 | <i>IRT (Iron-regulated transporter)</i> | 2 | 91.5 |
| 10 | <i>NAAT (nicotianamine aminotransferase)</i> | 3 | 86.7 |
| 11 | <i>NAC (NAC domain-containing protein)</i> | 1 | 90.7 |
| 12 | <i>NAS (Nicotianamine synthase)</i> | 13 | 85.4 |
| 13 | <i>NRAMP (Natural resistance-associated macrophage protein)</i> | 5 | 89.6 |
| 14 | <i>VIT1.2 (Vacuolar iron transporter)</i> | 1 | 85.4 |
| 15 | <i>WRKY80 (WRKY transcription factor 80)</i> | 1 | 89.1 |
| 16 | <i>YSL (yellow stripe 1-like)</i> | 17 | 84.3 |
| 17 | <i>ZIP (Zinc transporter)</i> | 20 | 82.6 |
| | Total | 77 | 86.4 (avg) |

Conclusion

Earlier studies in cereals except sorghum, candidate genes were identified and characterized for grain Fe and Zn homeostasis and uptake, transport and loading. In the present study identified candidate genes (homologs) can then be used for the development of functional markers and QTL mapping of grain Fe and Zn concentration in Sorghum. High priority candidate gene(s) can be functionally characterized,

through expression profiling of identified candidate genes in different growth stages using Real Time PCR and through both overexpression and silencing approaches to understand their role in Fe Zn homeostasis.

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