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Abstract

Millets as a group of crops are represented by sorghum (Jowar), pearl millet (Bajra), finger millet (Ragi), and minor millets like banyard millet, proso millet, kodo millet and foxtail millet. Millets assume importance as climate-smart crops to usher in food and nutritional security across semi-arid tropics under changing climate scenario. Being excellent source of essential nutrients to the millions of rural poor in India, these are also called as 'nutritious cereals'. In fact millets are the staple food for the world's poorest and most food insecure people across the semi-arid tropics. The Directorate of Sorghum Research, All India Coordinated Pearl Millet Improvement Project and All India Coordinated Small Millet Improvement Project under the aegis of Indian Council of Agricultural Research, and other institutions in both public and private sector are working to improve livelihood security of dryland farmers through millet improvement. The Global meet on Millets will discuss the most pertinent issues to chalk out a road-map to make millets highly sustainable and profitable to the farmers and industry. It will also deliberate on policy issues to support the cause of millets; to devise novel processes for value addition; industrial utilization as food, feed, fodder and bio-fuel to usher in livelihood, nutrition, livestock, biofuel and health security - as millets are the potent sources to enable these envisaged targets. This publication is a compendium of papers and abstracts from national and international experts on millets crop.

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FP03: In *Silico* identification of candidate genes involved for grain Fe and Zn concentration in sorghum using reported cereals gene homologs

Kotla Anuradha, Bhanu Prakash, Punna Ramu, Trushar Shah, A Ashok Kumar and Santosh P Deshpande

Abstract

Sorghum is among 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 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 concentration. A homology (In-silico) search of 91 candidate genes involved in governing grain Fe and Zn concentration in cereals (rice, wheat, maize and barley) is performed on sorghum genome. Blast is performed against the sorghum genome database and downloaded Fe and Zn gene sequences as query. Total 77 hits are found on sorghum genomic regions, which are involved in Fe and Zn homeostasis. Highest number of genes found on chromosome 1 (24 blast hits) and least on chromosome 8. Genes associated with grain Fe and Zn concentration from maize and wheat showed 100 % homology on sorghum genome. On sorghum genome, highest number of hits are identified on chr 1 and 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 (N AS) gene are in same general genomic region around 61Mbp on sorghum chromosome 1. Blast hits on sorghum chromosomes from genes of wheat, barley, rice, maize pertaining to Nicotianamine synthase (NAS), Zip (Zn transporter protein) and YSL (yellow stripe like) gene are shown higher % homology. These could be putatively associated with Fe and Zn grain concentration in sorghum. Candidate genes (Homologs) identified in this study can be used for the development of functional markers for improving grain Fe and Zn concentration in sorghum.

Key words: Candidate gene, Fe and Zn content, Sorghum

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) is performed for sorghum genome. Fe and Zn gene sequences are downloaded from the public repository and BLAST searched against sorghum genome. Top hits (based on e-value) are identified and visualized in GBrowse which can be ac-

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cessed 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 *et al.* 2011). Using 91 metal homeostasis candidate genes, homology (in-silico) search is performed, on sorghum genome and a total of 77 genes having homology with sorghum genome are identified. The e - value is 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 %age (similarity of sequences) is ranged from 71.9 (IDS3) to 95.9 (HMA) with average of 86.4 (Table 2).

Maximum number of hits are 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 % homology for (gene) sequences in sorghum genome. On sorghum genome, highest number of hits are 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 Nicotianamide synthase (NAS) gene are 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 are around 52 Mbp on chromosome 6; and 21 hits (from gene all 4 crops) for Zip (Zn transporter protein) are 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).

Earlier studies in cereals except sorghum, candidate genes are 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 over expression and silencing approaches to understand their role in Fe Zn homeostasis.

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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
Oryza sativa (Rice)	44	36	82
Hordeum vulgare (Barley)	23	17	74
Zea mays (Maize)	15	15	100
Triticum aestivum (Wheat)	9	9	100
Total	91	77	89

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

List of reported candidate genes involved for Fe and Zn grain concentration in cereals	No. Of hits found on sorghum genomic regions	Gene Identical (%)
DMAS (Deoxymugineic acid synthase)	3	88
FDH (Formate dehydrogenase)	1	89
FER (Ferritin)	3	86.6
HMA (Heavy metal-associated)	3	95.9
IDEF1 (iron deficiency-responsive)	1	79.4
IDI1 (Acireductone dioxygenase)	1	93.5
IDS3 (iron deficiency-specific 3)	1	72
IRO2 (iron-related transcription factor)	1	79
IRT (Iron-regulated transporter)	2	91.5
NAAT (nicotianamine aminotransferase)	3	86.7
NAC (NAC domain-containing protein)	1	90.7
NAS (Nicotianamine synthase)	13	85.4
NRAMP (Natural resistance-associated macrophage protein)	5	89.6
VIT1. 2 (Vacuolar iron transporter)	1	85.4
WRKY80 (WRKY transcription factor 80)	1	89. 1
YSL (yellow stripe 1-like)	17	84.3
ZIP (Zinc transporter)	20	82.6
Total	77	86.4