Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 6[10] September 2017 : 50-55

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Development of Genomic Resources Through Comparative Genomics Approach For Studying The Molecular Basis Of Heterosis in Sorghum And Other Cereals

I. Jaikishan, P. Rajendrakumar* and K. Hariprasanna
ICAR - Indian Institute of Millets Research, Hyderabad 500030, Telangana State, India

ABSTRACT

Hybrid technology offers one of the effective options to meet the future demand of many agricultural crops and more so in sorghum as demonstrated by the success of commercial hybrids. Prediction of heterosis and analysis of gene expression in relation to heterosis are the two important aspects leading to the development of superior hybrids in a short time. However, information on the heterosis-related genes and microsatellite markers present in such genes has not been reported in sorghum. The objective of this study was to identify the genes in sorghum that exhibit high homology with the heterosis-related genes of rice and maize available in the public domain and development of microsatellite markers. Homology search against sorghum genome resulted in the identification of 1900 genes of rice and maize, which exhibited ≥80% homology. About 40 genes with a higher order of homology were identified, which could be of immense utility for studying the differential gene expression in sorghum in relation to heterosis. A total of 1094 microsatellites were identified from heterosis-related genes of rice and maize, which exhibited >80% homology with sorghum. Di-nucleotide (49.5%) and tri-nucleotide (48.9%) repeat motifs were predominant microsatellite motifs. By considering only the Class I microsatellites, about 50 PCR-based markers were developed, which could be validated in sorghum. The set of genes identified and microsatellite markers developed in this study could be used in rice, maize and sorghum for gene expression analysis in relation to heterosis and prediction of heterosis.

Key words: *sorghum; heterosis; gene expression; microsatellite markers; comparative genomics*

Received 01.04.2017 Revised 23.05.2017 Accepted 29.07.2017

INTRODUCTION

Sorghum (*Sorghum bicolour* L. Moench) is one of the most important crops of semi-arid tropical regions of the world, mostly cultivated in harsh environments and marginal soils with low inputs. To meet the growing demands for food, feed, fodder and fuel, in the current scenario of depleting land and water resources, hybrid technology is the most suitable option that can contribute to higher as well as more stable yields. Discovery of cytoplasmic male sterility (CMS) and fertility restorer systems in sorghum [1] ushered in the development of superior hybrids covering a majority of the area under sorghum leading to doubling of grain yields. Even though heterosis has been successfully exploited for the past 70 decades, a clear understanding of its genetic and molecular basis is still lacking. A large number of transcriptomics studies in major cereal crops such as rice and maize by employing differential display analysis, cDNA-AFLP, and microarrays resulted in the identification and characterization of differentially expressed genes (DEGs) in relation to heterosis. With the advent of next-generation sequencing technologies, a large number of projects on transcriptome analysis targeting many economically important traits are being initiated in various crops of importance. However, in crops like sorghum, where transcriptomics studies related to heterosis is at infancy, the heterosis-related genes available in the public domain (Heterosis-related Gene Database; http://hrgd.big.ac.cn/index.html; [2]) acts as an invaluable resource to identify their orthologs in sorghum and analyze their expression in sorghum hybrids and their parents.

In the current decade, three heterotic trait loci (HTL) were reported in sorghum with gene effects that were synergistic and intra-locus on over-dominant heterosis for grain yield [3]. Prediction of heterosis using DNA markers is an important step in unravelling the molecular basis of heterosis, which will help in the identification of genomic regions and markers associated with heterosis expression. Studies on the prediction of heterosis gained momentum during the 2000s and very few reports were published in

sorghum using different types of DNA markers [4, 5]. Despite the availability of heterosis-related genes of major cereals such as rice, maize and wheat, microsatellites were not identified in these genes, which will be highly useful for the prediction of heterosis in these crops. The main objective of this study is to identify sorghum genes that exhibit high homology with the heterosis-related genes of rice and maize available in the public domain for use in the quantification of gene expression in relation to heterosis and development of microsatellite markers useful for the prediction of heterosis.

MATERIALS AND METHODS

Heterosis-related genes exhibiting homology with sorghum

Heterosis-related genes from three cereal species have been chosen on the basis of their availability in Heterosis-related Genes Database (http://hrgd.big.ac.cn; [2]). This database contains information of differentially expressed genes (DEGs) among the rice hybrids and their parents from various tissues generated at Beijing Institute of Genomics and other major cereal crops like maize and wheat collected from published literature. A homology search of these DEGs was performed through BLAST analysis against the whole genome of sorghum with default algorithm parameters using maize and rice DEGs with identical bases of ≥400 nucleotides and homology of ≥80% and top hits (based on e-value) were identified. The genes with 9311 series denote rice genes. Wheat DEGs were not used for analysis because they were very few in number. The orthologous gene in sorghum and their physical position was confirmed through BLASTN analysis [6].

Development of microsatellite marker from heterosis-related genes

Perfect di-, tri-, tetra-, penta- and hexanucleotide repeats with the repeat motifs repeated ≥3 times resulting in a minimum repeat length of 6 (dinucleotide repeats) to 18 (hexanucleotide repeats) nucleotides were identified in the heterosis-related genes of maize and rice using the online resource Simple Sequence Repeat Identification Tool (SSRIT) [7]. The class I microsatellites (≥20 not repeat length) was used for designing primers using the web resource Primer3 (http://frodo.wi.mit.edu/primer3/).

RESULTS AND DISCUSSION

Even without a clear understanding of the genetic and molecular basis of heterosis, several hybrids developed in many crops have contributed to the dramatic increase in productivity. However, the success of hybrids in increasing the productivity further could be achieved if the information is available on the genetic and molecular factors contributing to the expression of heterosis. The study presented was designed to identify the sorghum genes that are orthologous to the heterosis-related genes of rice and maize available in the public domain and develop microsatellite markers from those genes.

Sorghum genes exhibiting homology with heterosis-related genes of maize and rice

Heterosis, an increase in vigor observed in progenies of crosses involving diverse parental lines is manifested by the cumulative effects of many genes with small effects. Homology search of DEGs of rice and maize related to heterosis available in the public domain (http://hrgd.big.ac.cn) against the complete genome of sorghum led to the identification of 1900 genes of rice and maize, which showed ≥80% homology with sorghum. However, none of the heterosis-related genes of rice and maize exhibited <70% homology with that of sorghum genome. This clearly indicated that the genes related to heterosis have been conserved among the cereals/grasses. A total of top 40 genes with maximum homology was short-listed (Table 1), which could be used as perfect target genes for analyzing the differential gene expression among sorghum hybrids and their parental lines through quantitative polymerase chain reaction (q-PCR) analysis. These genes could also be used for quantification of their expression in rice and maize in relation to heterosis. Among the short-listed genes, heterosis-related genes of maize exhibited higher homology (98.39-95.92%) with sorghum as compared to rice (94.41-91.06%), which is in confirmation of the fact that sorghum is more closely related to maize than rice [8, 9]. Moreover, the manifestation of heterosis is through the involvement of genes associated with the molecular pathways that associated with chromatin modification, transcriptional regulation, and protein synthesis, as well as interactions among plant development and biochemical pathways [10]. This is reflected by the annotation of heterosis-related genes of rice and maize exhibiting higher homology with sorghum in this study, where genes like nucleotide-sugar transporter family protein, oligopeptide transporter, photosystem II light-harvesting complex gene, ribosomal L29 family protein, glutamate synthase, and phosphoenolpyruvate carboxylase were identified. The short-listed genes may act as an invaluable genomics resource for sorghum, which could be used in the genes expression studies in relation to heterosis. The role of ribosomal proteins in increasing leaf area in the F_1 hybrid was reported in Chinese cabbage [11] while effective light harvesting followed by transfer of energy and photo-protection was reported in sorghum-sudan grass hybrid as compared to its parental lines [12].

Microsatellites in heterosis-related genes

In order to identify microsatellites, the rice and maize heterosis-related genes that exhibited >80% homology with sorghum were considered. A total of 1094 microsatellites were identified comprising of 176, 559 and 359 Class I, Class II and Class III microsatellites, respectively. Among the microsatellites, di-nucleotide (49.5%) and tri-nucleotide (48.9%) repeat motifs the most abundant followed by tetra- (1.1%), hexa- (0.3%) and penta-nucleotide (0.2%) repeat motifs. Among the di-nucleotide repeat motifs, AG/GA/CT/TC motif was the most common (19.93%) similar to that observed in other crop species [13, 14] followed by AT/TA (14.90%) and AC/CA/TG/GT (8.78%) motifs, whereas GC/CG motif was the least common (5.94%) (**Fig. 1**). The very low frequency of CG/GC motif observed in this study was similar to that observed in ESTs/genes of many other monocot species [15]. The frequency of di-nucleotide GA/CT motifs is high in ESTs/genes since a di-nucleotide can signify multiple codons based on the reading frame and get translated into different amino acids. Among the tri-nucleotide repeat motifs, GCG/CGC/CGG/GGC/GGA were the most abundant (21.29%) followed by the motifs CCG/GCC/CCT/GCT (9.05%), AGC/CGA/GAC/CGT/GTC/TCG (5.94%) and AAC/CCA/CAC/GGT/TGG (2.47%) whereas the motifs AAG/AGA/GAA/CTT/TTC/TCT (1.46%) and ATG/TGA/GAT/TCA were the least common (1.01%) (**Fig. 2**). The abundance of tri-nucleotide motifs highlights the fact that these motifs in coding regions of a gene would not lead to a frame-shift mutation that could cause gene silencing but would lead to variation in the number of amino acid residues [16]. Overall there were only 11, 3 and 2 different tetra-, penta- and hexanucleotide motifs, respectively. A low proportion of tetra-, penta- and hexanucleotide repeats was observed similar to other crops.

In order to convert microsatellites into PCR-based markers, genes of ≥ 500 nucleotides length possessing Class I microsatellites were considered for primer designing and a total of 50 markers were developed, which were distributed across the sorghum genome (Table 3). The number of microsatellite markers developed ranged from 2 (chromosome 5) to 9 (chromosome 1). Many of these microsatellite markers were developed from the genes coding for zinc finger family protein, transcription factors, ion transporters, putative uncharacterized protein, etc., which are reported to be involved in the manifestation of heterosis in crop plants. Microsatellites were recently employed in sorghum for the prediction of grain yield heterosis. A significant and positive correlation between parental polymorphism using SSR markers with mid-parent (r = 0.48*) and better parent heterosis (r = 0.65*) for grain yield was reported [17]. An average correlation (r = 0.65) between marker value (yield-related QTL) and yield heterosis was reported [5] in which dominance and additive effects were accounted individually. Selection of appropriate markers such as those developed in this study will help in improving the power of prediction of heterosis in rice, maize and sorghum. The microsatellite markers reported in this study could be validated in sorghum for their transferability for further use in genotyping.

CONCLUSION

In conclusion, the present study resulted in the identification of 40 heterosis-related genes of rice and maize that showed the higher order of homology with sorghum and about 50 microsatellite markers were developed through *in silico* analysis. A combined set of these genes and microsatellite markers can be used as a vital genomic resource for analyzing the gene expression in relation to heterosis and prediction of heterosis, respectively in rice, maize as well as sorghum. Such studies using the markers and genes reported in this study will help in the dissection of the genetic and molecular basis of heterosis.

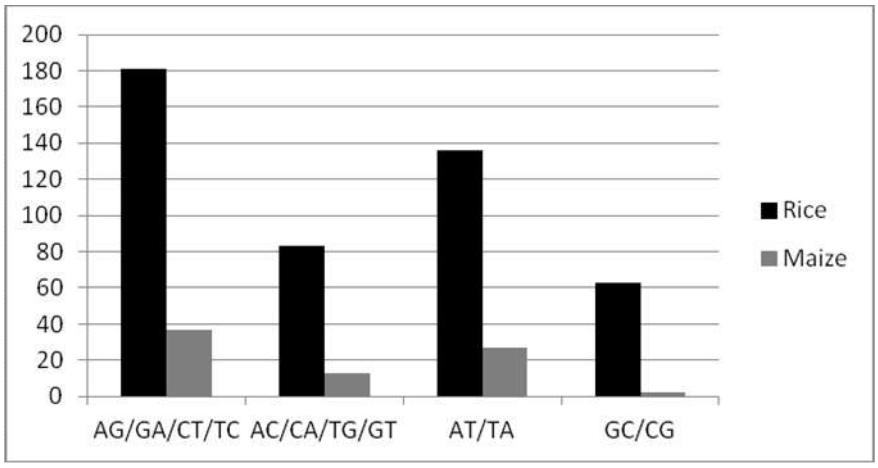

Fig. 1 Frequency of di-nucleotide repeat motifs in heterosis-related genes of rice and maize.

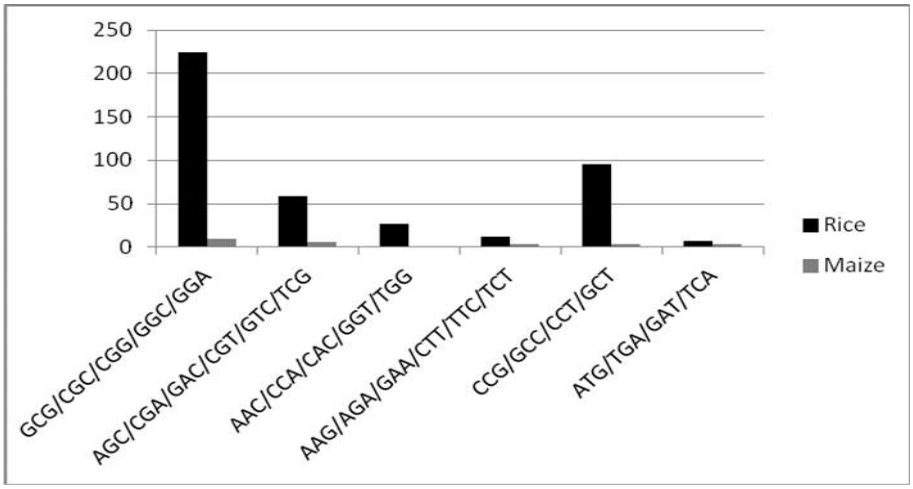

Fig. 2 Frequency of tri-nucleotide repeat motifs in heterosis-related genes of rice and maize.

Table 1 Heterosis-related genes of rice and maize exhibiting higher homology with sorghum

Heterosis-related genes	Sorghum Ortholog	Homology (%)	Annotation in Sorghum
9311_Chr03_1401	Sobic.001G409900.1	93.69	Nucleotide-sugar transporter family protein
9311_Chr03_1240	Sobic.001G425400.2	93.09	high cyclic electron flow 1
9311_Chr03_3769	Sobic.001G087700.1	92.34	oligopeptide transporter
9311_Chr10_1782	Sobic.001G193600.1	92.32	methionine gamma-lyase
9311_Chr03_2721	Sobic.001G177000.1	92.30	photosystem II light harvesting complex gene
CB885421*	Sobic.001G201700.1	98.39	NAD-dependent malic enzyme 2
CB411252*	Sobic.001G537300.1	97.59	Homeobox-leucine zipper family protein
DV622317*	Sobic.001G073150.1	96.21	flavodoxin family protein
CD058715*	Sobic.001G335300.1	96.20	
9311_Chr07_1917	Sobic.002G339200.2	92.03	light-harvesting chlorophyll B-binding protein 3
CD568678*	Sobic.002G402700.1	96.31	glutamate synthase 1
9311_Chr01_4871	Sobic.003G401000.1	92.54	Adaptin family protein
9311_Chr01_1709	Sobic.003G153800.1	91.85	Plant neutral invertase family protein
9311_Chr01_1407	Sobic.003G135400.1	91.49	beta-6 tubulin
DV622327*	Sobic.003G168200.1	96.93	
DV622487*	Sobic.003G330000.1	96.41	embryo sac development arrest 14
9311_Chr02_3478	Sobic.004G238500.1	93.26	Pyridine nucleotide-disulphide oxidoreductase family protein
9311_Chr02_0135	Sobic.004G011700.3	92.95	Heat shock protein 70 (Hsp 70) family protein
9311_Chr02_3839	Sobic.004G331500.1	91.76	H(+)-ATPase 8
9311_Chr02_2726	Sobic.004G220400.1	91.49	PHE ammonia lyase 1
CB604128*	Sobic.004G106900.1	97.03	phosphoenolpyruvate carboxylase 3
CD661753*	Sobic.004G152400.1	96.26	Ribosomal L29 family protein
9311_Chr11_1177	Sobic.005G112800.1	92.24	S-adenosyl-L-homocysteine hydrolase
CB815632*	Sobic.005G050600.1	97.89	Alba DNA/RNA-binding protein
9311_Chr06_0201	Sobic.006G134900.2	94.41	Ferritin/ribonucleotide reductase-like family protein
9311_Chr04_2403	Sobic.006G172000.2	93.85	Pectin lyase-like superfamily protein
9311_Chr04_1685	Sobic.006G105900.5	92.28	glyceraldehyde 3-phosphate dehydrogenase A subunit 2
9311_Chr04_1663	Sobic.006G264201.1	91.82	light harvesting complex photosystem II subunit 6
CB833932*	Sobic.006G029800.2	97.84	lactoylglutathione lyase family protein
DV550664*	Sobic.006G265600.1	97.56	staurosporin and temperature sensitive 3-like b
CB816420*	Sobic.007G126200.1	96.67	mRNA splicing factor, thioredoxin-like U5 snRNP
CB616981*	Sobic.001G112600.1	96.64	actin-11
DV621347*	Sobic.007G088900.1	96.41	
CB885492*	Sobic.007G025400.2	96.19	glyceraldehyde-3-phosphate dehydrogenase C subunit 1
9311_Chr12_1663	Sobic.008G135000.1	91.87	Ribosomal protein L2 family
DV494969*	Sobic.008G098900.1	96.74	ubiquitin-specific protease 12
9311_Chr05_2567	Sobic.009G201500.1	91.94	heat shock protein 101
CD572992*	Sobic.009G087000.1	96.96	minichromosome maintenance (MCM2/3/5) family protein
CB816337*	Sobic.010G173100.1	97.26	Translation elongation factor EF1B, gamma chain
CD527826*	Sobic.010G089500.1	97.04	annexin 7

*refers to maize genes

Table 2 Microsatellite markers developed from heterosis-related genes of rice and maize

Marker	Sequence ID	Repeat Motif	Physical position (Mb)	Forward Primer (5' – 3')	Reverse Primer (5' – 3')	Expected size (bp)
OsHRGM01-0.3	9311_Chr03_4565	$(ct)_{11}$	0.3	GTAGGAAGAAATGTCGTCTTGG	ACATTCAGAGAATGCATACGG	229
OsHRGM01-6.6	9311_Chr03_3790	$(tc)_{19}$	6.6	AAGAACCTGCGTATCAAGACC	CACACGGAAGCAGAATTAGC	168
OsHRGM01-8.2	9311_Chr03_0467	$(ttc)_{9}$	8.2	GCTTGTACAGCGTCAGAAAGTC	CTTCACAACTCGGGTCAATAAC	164
OsHRGM01-17.2	9311_Chr10_1782	$(gca)_{7}$	17.2	CTACTACGAGACCCTCATGTCG	GTCACACCTTGCAGTACTTGG	230
OsHRGM01-17.3	9311_Chr10_1778	$(tc)_{22}$	17.3	GTGCCCTAAGAATCATCTCTCC	ACAACAGCTACAGACACACAGC	238
OsHRGM01-51.0	9311_Chr10_2054	$(ct)_{10}$	51.0	GGCGCTAATTTACTCATATTGC	GAGTATGCCTGCAATGAAGC	229
OsHRGM01-54.8	9311_Chr03_2507	$(at)_{10}$	54.8	GTTGGTATTGATTCTGCTACGG	AAGCTCAAGCATAACATCTTGC	226
ZmHRGM01-66.6	BM073678	$(gcg)_{10}$	66.6	GAATGGAGGTACATCTCAGAGC	CGAGGCTTCTAGTAGACCAAGG	223
OsHRGM01-69.7	9311_Chr03_0467	$(ttc)_{9}$	69.7	GTCTACAATTATGGGGAAATCG	ACAAAAGAACCGGATAAAGAGG	199
OsHRGM02-57.1	9311_Chr09_0828	$(ga)_{15}$	57.1	GAATTCCCTTCTTGCTAGTGC	ACCTCAATAAACCTGACATTGC	206
OsHRGM02-62.3	9311_Chr09_1381	$(agc)_{7}$	62.3	CAAGAACGCGATCACCAC	GTCTGGAGAAGGCTGGAGA	186
OsHRGM02-69.6	9311_Chr07_1714	$(ct)_{12}$	69.6	CCTCGTCTCGAGTAAGTTGC	GACTAGATTGCCAACCCTTACC	289
OsHRGM02-73.3	9311_Chr07_2275	$(ctc)_{7}$	73.3	AGGTGAAGAAGCTGCAGGAG	CAGTTGAGGAACCTGTCGAG	172
OsHRGM02-75.5	9311_Chr07_2586	$(tc)_{12}$	75.5	CGCGGGAGTACTACGACTA	TCCGCCTAGAAACACTGAA	225
ZmHRGM03-13.2	DV491284	$(tc)_{13}$	13.2	ACGTATATCGCGTAGATGATCC	ATCGTCATCATTCGAGCAGA	206
OsHRGM03-15.8	9311_Chr01_1604	$(tatg)_{5}$	15.8	GGTAGAGATTTCGTTACCTTTGC	ATACCCATACCTGTGATTAGGG	181
OsHRGM03-16.6	9311_Chr01_1709	$(tc)_{10}$	16.6	ACTTTGGAGAGTCAGCTATTGG	GGTTTGCACATCTACTCTTTCC	214
OsHRGM03-50.9	9311_Chr01_2420	$(ctt)_{7}$	50.9	GAATCCCGAGTAGTAAGTCTCC	ACTAACTACCACCACCCACCTC	250
OsHRGM03-55.8	9311_Chr10_0470	$(ct)_{10}$	55.8	TGTATGATGGTTACCAGAAAGG	CCATCCACCTGTAACATTTAGG	218
OsHRGM03-59.8	9311_Chr01_3309	$(ta)_{34}$	59.8	ATCCAAACCAGCCTTTTACTTC	ATATGTCCCACAGCTATTACGG	229
OsHRGM04-1.9	9311_Chr02_0229	$(tc)_{15}$	1.9	ATAACTTGTGACACTAGGTTGTCC	ACAAACCTTGGCAATAACTGG	296
OsHRGM04-5.6	9311_Chr02_0701	$(ct)_{14}$	5.6	ATCTCCGAAGGTGAGCAGAG	CGTAAAAATCCGATCATGTACC	175
OsHRGM04-50.4	9311_Chr02_2052	$(gt)_{10}$	50.4	GGTGATTGTGATGTGCTAAAGG	ACTCACACTGATCTGGACAGG	223
OsHRGM04-50.5	9311_Chr02_2063	$(cgg)_{7}$	50.5	ATGAAGGCCGACTCCAAGT	AGACGGAGGAGAAGCCATC	283
OsHRGM04-66.4	9311_Chr02_3932	$(ct)_{15}$	66.4	TCCTTCACTCTTTTTCTCTTGC	AAGAACTCTTCTAGGTGCATCG	232
OsHRGM04-66.9	9311_Chr02_4011	$(ct)_{16}$	66.9	CACAATTCACAAGCCTAAAACC	GCAATTGAGACGATGAGTAGC	207
ZmHRGM05-44.1	BM333756	$(at)_{12}$	44.1	GTTCTAATCCAACAACAGATCG	ACCTTCTACGTCTCCAAGTCC	217
OsHRGM05-60.8	9311_Chr06_1234	$(at)_{19}$	60.8	ATCTTCCCAAGCTTATTGTCG	ATCTTCCCAAGCTTATTGTCG	257
OsHRGM06-47.6	9311_Chr04_1606	$(ttg)_{7}$	47.6	TGTTCTTTGTTGATGTGCTACG	TCCTTATTAGCATGGCTGACC	172
OsHRGM06-47.9	9311_Chr09_0597	$(ta)_{41}$	47.9	TAGGATGATCCAACTACAGACG	AGGTTTTCTGTCAAAAGCTAGG	278
OsHRGM06-48.5	9311_Chr04_1689	$(ct)_{10}$	48.5	TTCTAGCAGTAAGTTGGCATCC	AGGCACCATCTATGGAGAGG	167
OsHRGM06-49.7	9311_Chr04_1861	$(ac)_{14}$	49.7	CAGATGGCTTGATTCTAAGAGC	CACCCTGACAGTTAAACACTCC	248
OsHRGM06-50.6	9311_Chr04_1999	$(gaaa)_{5}$	50.6	GAGATCTGTGATTAGGTGTAGGC	CTTCTGCTTCTTCTTGAACTCC	245
OsHRGM06-51.1	9311_Chr04_2076	$(at)_{24}$	51.1	GTGAAATACCAGCACATGACG	ATCAAGAGAGATCTGCATACCC	264
OsHRGM06-58.6	9311_Chr02_0649	$(ct)_{15}$	58.6	CTCCCCTTAATTTTGACATGC	GTGTGAAGCCACATATGATTCC	209
OsHRGM07-13.0	9311_Chr08_0981	$(cgg)_{11}$	13.0	TGGGTCGTCACCAAGAAG	CGCCTTGCTCTTCTTCAC	234
OsHRGM07-24.5	9311_Chr08_1411	$(cct)_{7}$	24.5	ATCCACATCGTGCGACTC	GGCCGCCATTAAATTATCC	244
OsHRGM07-37.9	9311_Chr08_1462	$(agc)_{7}$	37.9	GCGCGTTCTTTAATTCTTGC	ATCCCAACTTACGACAAGAGG	172
OsHRGM07-59.4	9311_Chr08_2752	$(ccat)_{5}$	59.4	TTGATTTGTGCAGGGATACG	TGACGTACTCTGAGGAAAGAGG	244
OsHRGM07-62.3	9311_Chr08_2434	$(at)_{10}$	62.3	TTCAACTGTAATCATCCTGTGG	AATAAAACCCTTACAGGCTTGC	170
OsHRGM08-14.6	9311_Chr12_0741	$(ga)_{22}$	14.6	TGTACCAGCAATTCTCTGTCC	AAGAGAGACAAGGGAGAAGACG	190
OsHRGM08-48.5	9311_Chr12_1644	$(taaa)_{5}$	48.5	AGTGCTACAAGCAGGATTGG	CAAGAACCTAGTGCTTAAACAACG	189
OsHRGM08-49.1	9311_Chr12_1680	$(ag)_{19}$	49.1	ATCGTCATTCAGACTTGAGAGG	ACGGGTACATAGGTAGAGATGG	297
OsHRGM09-42.1	9311_Chr05_1480	$(ct)_{26}$	42.1	ATTGTTGACATCTGTGAGAAGG	CAGATACACATGTTGGAAAAGC	234
OsHRGM09-49.5	9311_Chr05_1875	$(ta)_{16}$	49.5	TTTTAACTAGGGCTGGAAAAGG	TCAGTCCCTTGATAGAAAATGC	248
OsHRGM09-52.7	9311_Chr05_2982	$(taa)_{26}$	52.7	CACGGAACAGTACTAAGGTTGG	GTTCATACAAGTGAAACTCTCATCC	237
OsHRGM10-4.5	9311_Chr06_0597	$(gcc)_{7}$	4.5	CTCCAGCAGCAGATGATGA	CATATGAGCCCCGTGTAGC	206
OsHRGM10-9.4	9311_Chr06_1092	$(agg)_{7}$	9.4	ACGACGACGGGGAGAATA	ACGAAGTCCTGGAAGATGC	229
OsHRGM10-51.4	9311_Chr06_2270	$(ct)_{10}$	51.4	CTCCAGGAAACCCTGTAAGC	TATGAAGGACCAGACGAAGC	249
OsHRGM10-55.6	9311_Chr06_2703	$(tg)_{14}$	55.6	GTCTGCTGTCTGATGTCAACC	CAGGACTGTTCCTACTTTAAATGC	244

SOURCE OF FUNDING
This study was funded by the grant received from Department of Biotechnology (DBT), Government of India under Rapid Grant for Young Investigators (Grant No. BT/PR/13256/GBD/27/240/2009).

ACKNOWLEDGEMENT
The authors thank Director, ICAR – Indian Institute of Millets Research, Hyderabad for constant support and encouragement.

CONFLICT OF INTEREST- None.

REFERENCES

1. Stephens, J.C. & Holland, R.F. (1954). Cytoplasmic male sterility for hybrid sorghum seed production. Agron. J. 46; 20-23.
2. Song, S., Huang, Y., Wang, X. Wei, G., Qu, H., Wang, W., Ge, X., Hu, S., Liu, G., Liang, Y. & Yu, J. (2009). HRGD: a database for mining potential heterosis-related genes in plants. Plant Mol. Biol. 69(3); 255-260.
3. Ben-Israel, I., Kilian, B., Nida, H. & Fridman, E. (2012). Heterotic trait locus (HTL) mapping identifies intra-locus interactions that underlie reproductive hybrid vigor in *Sorghum bicolor*. PLoS One 7(6); e38993.
4. Jordan, D.R., Tao, Y., Godwin, I.D., Henzell, R.G., Cooper, M. & McIntyre, C.L. (2003). Prediction of hybrid performance in grain sorghum using RFLP markers. Theor. Appl. Genet. 106(3); 559-567.
5. Lu, X.P., Liu, D.D., Wang, S.Y., Mi, F.G., Han, P.A. & Lu, E.S. (2014). Genetic effects and heterosis prediction model of *Sorghum bicolor* × *S. sudanense* grass. Acta Agron. Sin. 40(3); 466-475.
6. Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D.J. (1997). Gapped BLAST and PSIBLAST: A new generation of protein database search programs. Nucl. Acids Res. 25(17); 3389-3402.
7. Temnykh, S., DeClerck, G., Lukashova, A., Lipovich, L., Cartinhour, S. & McCouch, S. (2001). Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): frequency, length variation, transposon associations, and genetic marker potential. Genome Res. 11(8); 1441-1452.
8. Gaut, B.S., Clark, L.G., Wendel, J.F. & Muse, S.V. (1997). Comparisons of the molecular evolutionary process at *rbcL* and *ndhF* in the grass family (Poaceae). Mol. Biol. Evol. 14(7); 769-777.
9. Paterson, A.H., Bowers, J.E. & Chapman, B.A. (2004). Ancient polyploidization predating divergence of the cereals, and its consequences for comparative genomics. Proc. Natl. Acad. Sci. USA 101(26); 9903-9908.
10. Kaeppler, S. (2012). Heterosis: Many genes, many mechanisms - End the search for an undiscovered unifying theory. ISRN Botany 2012; Article ID 682824.
11. Saeki, N., Kawanabe, T., Ying, H., Shimizu, M., Kojima, M., Abe, H., Okazaki, K., Kaji, M., Taylor, J.M., Sakakibara, H., Peacock, W.J., Dennis, E.S. & Fujimoto, R. (2016). Molecular and cellular characteristics of hybrid vigour in a commercial hybrid of Chinese cabbage. BMC Plant Biol. 16; 45.
12. Han, P., Lu, X., Mi, F., Dong, J., Xue, C., Li, J., Han, B. & Zhang, X. (2015). Proteomic analysis of heterosis in the leaves of sorghum - sudangrass hybrids. Acta Biochim. Biophys. Sin. 48(2); 161-173.
13. Temnykh, S., Park, W.D., Ayres, N., Cartinhour, S., Hauck, N., Lipovich, L., Cho, Y.G., Ishii, T. & McCouch, S.R. (2000). Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). Theor. Appl. Genet. 100(5); 697-712.
14. Poncet, V., Rondeau, M., Tranchant, C., Cayrel, A., Hamon, S., Kochko, A. & Perla, H. (2006). SSR mining in coffee tree EST databases: potential use of EST–SSRs as markers for the *Coffea* genus. Mol. Gen. Genomics 276(5); 436-449.
15. Kantety, R.V., Rota, M.L., Matthews, D.E. & Sorrells, M.E. (2002). Data mining for simple sequence repeats in expressed sequence tags from barley, maize, rice, sorghum and wheat. Plant Mol. Biol. 48; 501-510.
16. Cloutier, S., Niu, Z., Datla, R. & Duguid, S. (2009). Development and analysis of EST-SSRs for flax (*Linum usitatissimum* L.). Theor. Appl. Genet. 119(1); 53-63.
17. Rajendrakumar, P., Hariprasanna, K., Jaikishan, I., Madhusudhana, R. & Patil, J.V. (2013). Potential of microsatellite marker polymorphism in the prediction of grain yield heterosis in sorghum [*Sorghum bicolor* (L.) Moench]. (Eds. Rakshit, S., Das, I.K., Shyamprasad, G., Mishra, J.S., Ratnavathi, C.V., Chapke, R.R., Tonapi, V.A., Dayakar Rao, B. and Patil, J.V.), Compendium of papers and abstracts: Global consultation on millets promotion for health and nutritional security, 18-20 December, 2013. Society of Millets Research, Hyderabad, p 329.

CITATION OF THIS ARTICLE
I. Jaikishan, P. Rajendrakumar and K. Hariprasanna. Development Of Genomic Resources Through Comparative Genomics Approach For Studying The Molecular Basis Of Heterosis In Sorghum And Other Cereals . Bull. Env. Pharmacol. Life Sci., Vol 6[10] September 2017: 50-55