Gene Expression Analysis in Sorghum Hybrids and Their Parental Lines at Critical Developmental Stages in Relation to Grain Yield Heterosis by Exploiting Heterosis-Related Genes from Major Cereals

Inapakurti Jaikishan, Passoupathy Rajendrakumar, Krishna Hariprasanna, et al.

Plant Molecular Biology Reporter

ISSN 0735-9640
Volume 36
Number 3

Plant Mol Biol Rep (2018) 36:418-428
DOI 10.1007/s11105-018-1079-x

Springer

Plant Molecular Biology Reporter (2018) 36:418–428
https://doi.org/10.1007/s11105-018-1079-x

ORIGINAL PAPER

Gene Expression Analysis in Sorghum Hybrids and Their Parental Lines at Critical Developmental Stages in Relation to Grain Yield Heterosis by Exploiting Heterosis-Related Genes from Major Cereals

Inapakurti Jaikishan[1] · Passoupathy Rajendrakumar[1] · Krishna Hariprasanna[1] · B. Venkatesh Bhat[1]

Published online: 9 June 2018

Abstract

Relative expression levels of selected genes from the Heterosis-Related Gene Database exhibiting more than 90% homology with sorghum were studied in hybrids and their respective parental lines for a better understanding on the molecular basis of heterosis. A high (27A × RS 673) and a low heterotic hybrid (7A × CB 26) of sorghum along with their parental lines were used for this purpose. Twenty (15 maize and 5 rice) genes exhibiting more than 90% homology with that of sorghum were identified. The maize genes *ZmHG13*, *ZmHG16*, and *ZmhG19* exhibited more than fourfold increase over the male parent (RS 673) of high heterotic hybrid during booting stage, which started decreasing during flowering stage. Similarly, the rice genes *OsHG1* and *OsHG12* recorded > 2.5-fold increase. However, these genes recorded less than twofold increase during the same stage of the plant in the low heterotic hybrid. Notably, among the genes that exhibited higher expression in the highly heterotic hybrid were those coding for proteins, which were known to play crucial roles in the manifestation of heterosis in plants.

Keywords Sorghum · Grain yield heterosis · Gene expression · Comparative genomics · Real-time PCR · Molecular basis

Introduction

Sorghum (*Sorghum bicolor* L. Moench) is one of the most important cereals, grown in semi-arid tropical regions of Asia, Africa, and the world, mostly under marginal soils with minimum inputs. It is a climate resilient crop and an excellent C_4 model plant. Exploitation of heterosis or hybrid vigor was reported in sorghum as early as 1927 (Conner and Karper 1927), and the development of first sorghum hybrid dates back to the late 1940s through the use of genetic male sterility (Quinby 1974), achieving a yield advantage of 40% as compared to the cultivars available during that time (Klein et al. 2008). With the discovery of both cytoplasmic male sterility (CMS) and fertility restorer systems in 1952 (Stephens and Holland 1954), commercial seed production of CMS-based hybrids in sorghum began in 1956, resulting in the planting of 95% area with hybrids and the realization of doubling the grain yields (Quinby 1974; Smith and Frederiksen 2000). In spite of successful exploitation of heterosis in sorghum, the genetic and molecular basis of this phenomenon is not clearly understood. In this scenario, a clear understanding of the genes and mechanisms involved in the manifestation of heterosis will help the molecular breeders in the rapid and efficient identification of suitable parental combinations from a large genetic pool of parental lines as well as in broadening their genetic base.

In sorghum, heterosis for grain yield is manifested by more number of grains per panicle, mostly in the basal whorls (Blum 1970) and through a larger size of panicle (Miller and Kebede 1984). A larger apical dome observed in the sorghum hybrid as compared to its inbred parents at the onset of panicle initiation (Blum 1977) was not due to a prolonged vegetative period as indicated by a relatively shorter duration of the vegetative period in the hybrid than that in its parents. The larger apical dome in the hybrid could due to a larger seedling, indicating the determination of at least a part of the panicle size at

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11105-018-1079-x) contains supplementary material, which is available to authorized users.

✉ Passoupathy Rajendrakumar
rajendra@millets.res.in

[1] ICAR - Indian Institute of Millets Research, Hyderabad, Telangana State 500030, India

early growth stages, viz., seed embryo and emerging seedling. Based on this observation, Lippman and Zamir (2007) and Sanghera et al. (2011) concluded that yield heterosis in sorghum hybrid involves an early gain at the seed embryo and emerging seedling stages, followed by growth and differentiation of the apical meristem resulting in a bigger panicle with more number of grains than its parents within a short time. As per the genetic rules of heterosis in plants by Birchler (2013), it is assumed that one general mechanism manifests heterosis and the individual concepts are developed from different species. However, it is possible sometimes an increase in yield or other traits could be considered as heterosis, but in reality, the basis may be distinct from the canonical examples. Nevertheless, considering the similarity in the behavior of heterosis and inbreeding depression across plant species, a common principle underlying heterosis is likely and variants of different genes could be involved in triggering a basic molecular mechanism.

Even though a lot of head way has been made in sorghum through the identification of genomic regions associated with grain yield and its components (Srinivas et al. 2009; Nagaraja Reddy et al. 2013) and heterotic loci (Ben-Israel et al. 2012), the transcriptomic studies are very much essential to precisely understand heterosis since it is a genome-wide phenomenon involving a complex network of genes involved in various metabolic pathways. Several transcriptomic studies in maize and rice have highlighted the importance of certain key metabolic pathways in the manifestation of heterosis, however, they have also revealed the occurrence of common genes from those pathways in many hybrids suggesting a likelihood of regulatory mechanism(s) and their influence in most heterotic combinations (Baranwal et al. 2012). In the last two decades, differential gene expression studies in relation to heterosis have been performed in crop plants involving techniques such as differential display analysis (Zhang et al. 2012), cDNA-AFLP (Nie et al. 2015), and microarrays (Bassene et al. 2010). Several projects investigating the phenomenon of heterosis in different crops have contributed to identification and characterization of a number of heterosis-related differentially expressed genes (DEGs) (Zhang et al. 2008; Wei et al. 2009; He et al. 2010), and it has been reported that DEGs between a hybrid and their parental inbreds are correlated with heterosis (Guo et al. 2006; Thiemann et al. 2010).

Two of the three heterotic trait loci (HTL) identified in sorghum for grain yield heterosis with synergistic intra-locus effects by Ben-Israel et al. (2012) exhibited synteny with an earlier reported over-dominant QTL in maize for grain yield. Comparative genomics approaches help in the identification of orthologous genes of agronomic importance in related crop species. In this scenario, the availability of complete genome sequence of sorghum (Paterson et al. 2009) and the published Heterosis-Related Gene Database of rice and other crops (HRGD; http://hrgd.big.ac.cn/index.html; Song et al. 2009) have provided a perfect opportunity to select DEGs in relation to heterosis, identify those that exhibit maximum homology with sorghum, design primers, and validate their expression in sorghum hybrids and their parental lines. The present study used real-time PCR to analyze the expression of 20 heterosis-related genes (15 of maize and 5 of rice) available in the HRGD that exhibited > 90% homology with sorghum to reveal their relative expression levels between hybrids and their respective parental lines in order to identify genes that are likely to be involved in the manifestation of grain yield heterosis in sorghum.

Materials and Methods

Plant Materials

Two A_1 cytoplasm-based sorghum male sterile lines (7A and 27A) were crossed with five fertility restorer lines (C 43, RS 673, RS 627, CB 26, and CB 29) in a factorial design during post-rainy season 2012, and ten factorial F_1 hybrids were obtained. These parental lines are adapted to rainy season. Pedigree and salient features of the parental lines are given in Table 1.

Estimation of Grain Yield Heterosis

Seven parental lines and ten factorial hybrids were evaluated in a randomized complete block design with three replications in the Research farm at the ICAR – Indian Institute of Millets Research, Hyderabad, India (latitude 17° 19′ N, longitude 78° 23′ E, 542.3 m above mean sea level) during the rainy season of 2012. The crop was sown in 15 June and harvested during the third week of October. During this period, the average maximum temperature, minimum temperature, wind speed, and rainfall were 30.7 °C, 22.3 °C, 7.7 km h^{-1}, and 675 mm, respectively. Based on the estimated heterosis, two hybrids—one exhibiting a high heterosis for grain yield and the other exhibiting a low heterosis—were evaluated along with their parental lines during the rainy season of 2013. Days to flowering, plant height, panicle length, panicle weight, 100-grain weight, and grain yield per plant were measured for 30 plants per genotype. Heterosis over mid-parent (mid-parent heterosis) and better parent (heterobeltiosis) were estimated as per the formulae given by Alam et al. (2004):

$$\text{Mid-parent heterosis } (H_{mp}) = \left[\left(X_x - X_{mp}\right)/X_{mp}\right] \times 100$$

$$\text{Heterobeltiosis } (H_{bp}) = \left[\left(X_x - X_{bp}\right)/X_{bp}\right] \times 100$$

where "X_x" is the observed mean value of the hybrid, "X_{mp}" is the mean of mid-parent value, and "X_{bp}" is the mean of better parent.

Table 1 Details of parental lines used in this study

Parental line	Parentage	Salient features
27A	Derived from the cross 83B × 199B	Tan plant, green, broad and erect leaves, medium thick stem, well exerted, semi lax and cylindrical ear head, 130–135 cm plant height, short length glume, cream color, bold and round seed, light yellow and corneous endosperm
7A	Selection from AKMS 14A	Tan colored plant, pale green and drooping leaves, thick stem, exerted, semi-compact and elliptical shape ear head, straw colored glume, awns absent, early flowering (60–65 days), plant height 145–150 cm, photo-insensitive, freely threshable, medium size, corneous and round seed
C 43	Derived from the cross CS 3541 × IS 23549	Tan colored plant, green colored and semi erect leaves, thick stem, touching ear head exertion, compact and conical shape panicle, light red colored glume, 140–145 cm plant height, photo-insensitive and freely threshable grain, awn absent, white, round, lustrous and bold seed with corneous endosperm
RS 627	Derived from the cross RS 71 × M 35-1	Tan plant, enclosed inter-node, green colored drooping leaf, elliptical and semi-compact panicle, glume straw colored and short, freely threshable, medium size round and white seed
RS 673	Derived from the cross SPV 544 × K 24-1	Tan colored plant, green colored and drooping leaves, medium thick stem, well exerted ear head, semi-compact and conical shape panicle, straw colored glume, 176 cm plant height, photo-insensitive and partial threshable grain, awn absent, white, round, lustrous and medium bold seed with corneous endosperm
CB 26	Derived from the cross SB 1066 × SPV 775	Tan plant with drooping leaves, semi-compact panicle, straw colored glumes, medium height with white, round and bold seed
CB 29	Derived from the cross NSV 13 × SPV 475	Tan plant, medium tall and semi-compact panicle, light red colored glume, grains are white, bold and flat on one side and are easily threshable

Genes in Sorghum Exhibiting Homology to Heterosis-Related Genes in Public Domain

Heterosis-Related Gene Database (http://hrgd.big.ac.cn; Song et al. 2009) contains information of 3775 DEGs among the hybrid parent tissue panels from rice genomes generated at Beijing Institute of Genomics and other genomes of major cereal crops collected from published literatures. BLAST analysis was performed using the sequences (maize, rice, and wheat) in the database against the whole genome assembly of sorghum (version 2.1). Maize and rice DEGs of more than 400 nucleotides in length that exhibited homology of > 90% with sorghum were arranged in the decreasing order of homology. Primers were designed for 20 genes of maize and rice for further standardization and use in studying their expression in a high heterotic and a low heterotic hybrid in relation to their parental lines. These 20 genes comprised of 15 genes of maize and five genes of rice, and the selection of more genes of maize than rice was due to the fact that sorghum is closer to maize than rice. The selection of genes was done such that they are distributed across the sorghum genome since heterosis is a complex biological phenomenon involving a network of genes. Also, based on the reports published earlier, the genes associated with heterosis such as those coding for histones, UDP-D-glucuronate decarboxylase, chlorophyll a/b binding protein, ribosomal protein, PPR repeat family protein, etc., were selected. Gene-specific primers were designed using Primer Express® Software (Applied Biosystems, USA) for real-time PCR analysis using the maize and rice gene sequences as template. The T_m was set to 60–65 °C, with a T_m difference between primers less than 2 °C. The range of amplicon size selected was between 100 and 300 bp. The primers for these 20 genes were used for the standardization of PCR to get amplicons of expected size for the gene expression studies through real-time PCR.

Isolation of Total RNA and Synthesis of cDNA

The description of growth stages in sorghum on a scale of 0–9 was given by Vanderlip and Reeves (1972) while this was simplified into three growth stages, viz., planting to panicle initiation (GS1), panicle initiation to flowering (GS2), and flowering to physiological maturity (GS3) by Eastin (1972). In this study, the leaf samples were collected from four parental lines (7A, 27A, RS 673, and CB 26) and two hybrids (27A × RS 673 and 7A × CB 26) at vegetative, booting, and flowering stages representing GS1, GS2, and GS3 stages, frozen in liquid nitrogen immediately, and stored at – 80 °C. Total RNA was isolated using Trizol® (Invitrogen, USA) according to the manufacturer's instructions. Two independent RNA extractions per sample (uppermost leaves pooled from five plants) were performed for biological replicates. The quality of isolated RNA was checked in denaturing gel, and the quantity was determined by NanoDrop® 2000C (JH Bio Innovations).

cDNA was synthesized using the Verso cDNA synthesis kit (Thermo Fisher Scientific) as per manufacturer's instructions. Each 20-μL reaction mixture contained 3 μg total RNA, 50 mmol/L Tris-HCl (pH 8.3), 75 mmol/L KCl, 3 mmol/L $MgCl_2$, 10 mmol/L DTT (DTT, dithiothreitol), 50 mmol/L dNTPs, 50 pmol 3′ end anchored primer, and 200 U M-MLV reverse transcriptase. The reaction mixture was incubated at 37 °C for 2 h.

Real-time PCR Analysis

Efficiency of primers was estimated by qPCR assay with SYBR Green mix using 1-, 10^{-1}-, 10^{-2}-, 10^{-3}-, and 10^{-4}-fold dilutions of pooled cDNAs of three technical replicates for each gene including reference gene, and a standard curve was generated. Presence of unwanted amplicons during the PCR reaction was checked through a melt curve analysis. Fluorescence threshold (Ct) values were tabulated, and each mean Ct was plotted against the logarithm of the dilution factor of pooled cDNA. The slope of the linear regression of C_t versus logarithm of the cDNA dilution factor was used to determine the efficiency ($E = [10^{(1/-\text{slope})} - 1] \times 100\%$) for each primer. Primers used for heterosis-related genes as well as reference gene had efficiencies ranging between 92 and 96%.

To quantify the expression of heterosis-related genes in the parental lines of sorghum and their respective F_1 hybrids in relation to heterosis, real-time PCR was performed using Light Cycler® 480 Real-time PCR system (Roche) using Verso 1-step real-time PCR SYBR green mix (Thermo Fisher Scientific) according to manufacturer's instructions. The thermal cycling conditions were as follows: initial denaturation at 95 °C for 10 min, 40 cycles of denaturation at 95 °C for 20 s, primer annealing at 60–68 °C (depending on the primers) for 15 s, and primer extension at 72 °C for 30 s followed by melting curve analysis at 95 °C for 10 s, 65 °C for 60 s, and 97 °C for 1 s with a final cooling at 37 °C. Sorghum *Ubiquitin* gene (Sobic.001G311100) was used as the endogenous control to normalize the expression levels of target genes. Three technical replicates were used for each gene analyzed, and the normalization of results was done using the C_t (threshold cycle) obtained based on the expression of the control gene. C_t was determined based on the number of cycles in which the fluorescence generated within a reaction crossed the baseline (threshold). To calculate the mean relative expression levels, cDNAs from two independent biological samples in three technical replications each were used.

Mean values from two biological replicates were log-transformed to decrease the effects of outliers, equalized experimental averages and standard deviation, as suggested by Willems et al. (2008). Fold change in the expression of genes in sorghum hybrids in comparison to their parental and mid-parental value was estimated through ΔΔCt method. Significance for the fold change in gene expression was tested through Student's *t* test, and genes exhibiting more than twofold expression level were considered as significantly up-regulated.

Results

Heterosis for Grain Yield and Its Components

Results of the evaluation of ten factorial F_1 hybrids along with parental lines for grain yield and related traits in the field indicated that the estimated values of mid-parent heterosis and heterobeltiosis differed greatly among the hybrids for the seven component traits of grain yield. Grain yield/plant followed by panicle weight, primary branches/panicle, and 100-seed weight exhibited high levels of heterosis (Table 2). The mid-parent heterosis for grain yield/plant ranged from 7.10% (7A × CB 26) to 103.28% (27A × RS 673) while heterobeltiosis ranged between −6.17% (7A × CB 26) and 88.49% (27A × RS 627). With regard to panicle weight, the mid-parent heterosis ranged from 8.12% (7A × CB 26) to 87.08% (27A × RS 673) while heterobeltiosis ranged between −3.69% (7A × CB 26) and 70.05% (27A × RS 627). The range of mid-parent heterosis and heterobeltiosis for 100-seed weight was from 0.69% (27A × CB 29) to 48.52% (27A × RS 627) and from −1.33 (27A × CB 29) to 32.14% (7A × RS 673), respectively. The highest heterobeltiosis for grain yield/plant was recorded by the hybrid 27A × RS 673 (75.20%) while the same hybrid also recorded high mid-parent heterosis (103.28%).

Six hybrids (27A × RS 673, 27A × RS 627, 27A × CB 26, 7A × RS 627, 7A × RS 673, and 27A × CB 29) exhibited 50% or more mid-parent heterosis for grain yield/plant, of which four hybrids 27A × RS 673, 27A × RS 627, 27A × CB 26, and 7A × RS 627 also recorded heterobeltiosis above 50%. Based on this heterosis data, two hybrids—one exhibiting a high heterosis for grain yield (27A × RS 673) and the other exhibiting a low heterosis (7A × CB 26)—were selected for the gene expression studies through real-time PCR. These two hybrids were evaluated along with their parental lines during the rainy season of 2013, and the leaf samples were collected at critical developmental stages of the crop, viz., vegetative, booting, and flowering stages for the gene expression analysis through real-time PCR. The estimated mid-parent heterosis for grain yield was 91.16% for the hybrid 27A × RS 673 while it was 18.00% for 7A × CB 26. Similarly, heterobeltiosis for grain yield/plant was 72.02% for the hybrid 27A × RS 673 and −2.40% for the hybrid 7A × CB 26.

Table 2 Estimated heterosis for grain yield and its component traits

Hybrid	Days to flower		Plant height		Panicle length		Panicle weight		Primary branches/panicle		Grain yield/plant		100-seed weight	
	MH	HB	MH	HB	MH	HB	MH	HB	MH	HB	MH	HB	MH	HB
27A × C 43	−1.34	0.00	24.24	11.84	10.90	0.96	40.52	18.06	51.34	49.93	40.52	13.76	19.89	10.70
27A × RS 673	−4.91	−3.62	37.55	36.92	15.21	3.79	87.08	68.67	45.99	32.54	103.28	75.20	34.59	25.54
27A × RS 627	−6.15	0.00	33.67	24.55	17.84	2.83	75.80	70.05	5.11	−3.43	95.47	88.49	48.52	30.90
27A × CB 26	−1.58	−0.91	24.06	19.36	1.02	−4.70	73.07	60.37	27.48	20.64	85.32	69.10	13.87	9.78
27A × CB 29	−3.09	−0.46	26.69	26.11	5.53	−0.93	46.07	23.61	26.78	16.89	55.16	28.53	0.69	−1.13
7A × C 43	−7.66	−2.46	44.31	44.31	15.84	10.41	22.94	−0.22	9.63	4.76	22.99	−3.80	33.91	20.76
7A × RS 673	−7.66	−2.46	23.61	10.81	10.49	4.16	53.68	33.37	15.51	−0.11	57.26	30.51	38.23	32.14
7A × RS 627	−9.24	0.99	32.26	27.42	16.58	6.25	57.55	46.22	21.82	6.52	66.61	53.83	31.46	18.54
7A × CB 26	−8.88	−4.41	22.72	6.76	1.06	0.00	8.12	−3.69	−26.28	−33.72	7.10	−6.17	6.79	5.60
7A × CB 29	−9.84	−3.43	33.68	19.84	7.94	6.25	34.36	9.80	31.00	14.93	35.04	7.94	15.75	10.82

MH mid-parent heterosis, *HB* heterobeltiosis

Homology of Heterosis-Related Genes in Public Domain with Sorghum

BLAST analysis performed for heterosis-related genes of rice, maize, and wheat in the HRGD against the whole genome of sorghum resulted in the identification of 956, 944, and 12 genes of rice, maize, and wheat, respectively, which showed more than 90% homology with sorghum (Supplementary Table 1). Also, 401, 1453, and 9 genes of maize, rice, and wheat, respectively, showed 70–90% homology with sorghum while none of the genes of maize, rice, and wheat, respectively, showed < 70% homology with sorghum (Supplementary Table 2). The optimum thermal profiles for these 20 genes (15 of maize and 5 of rice) were standardized using the parental lines (7A, 27A, RS 673, and CB 26) to get clear and robust amplification and used for further analysis of their gene expression in a high (27A × RS 673) and a low heterotic hybrid (7A × CB 26) through real-time PCR. Clear and robust amplification in the case of maize genes as compared to rice genes may be due to the differences in the synteny among these crop species. The putative functions of the 20 selected genes were determined through BLASTX analysis with the sorghum genome. Details of gene-specific primers for 20 selected genes (15 of maize and 5 of rice) that were used for analysis of their expression through real-time PCR including their putative function are given in Table 3, and their distribution in the sorghum genome is shown in Fig. 1.

Quantification of Expression of Heterosis-Related Genes in Relation to Grain Yield Heterosis

Among the 15 maize genes that were analyzed for their expression in a high (27A × RS 673) and a low heterotic (7A × CB 26) sorghum hybrid, a total of eight genes (*ZmHG2*, *ZmHG10*, *ZmHG11*, *ZmHG13*, *ZmHG14*, *ZmHG16*, *ZmHG17*, and *ZmHG19*) exhibited more than twofold increase in their expression as compared to their parents or mid-parent value (Supplementary Fig. 1). Specifically, the expression of the genes *ZmHG13* (4.67 ± 0.09), *ZmHG16* (4.19 ± 0.12), and *ZmHG19* (5.05 ± 0.19) reached a peak of more than fourfold increase over the male parent (RS 673) in the samples collected from high heterotic hybrid (27A × RS 673) during the booting stage, which started decreasing when the plant reached flowering stage. However, these genes recorded less than twofold increase (Fig. 2a–c) during the same stage of the plant in the low heterotic hybrid (7A × CB 26). Moreover, the genes *ZmHG13* (1.88 ± 0.05) and *ZmHG16* (2.09 ± 0.09) exhibited up to twofold increase over the mid-parent. These three genes code for protein similar to histone H2B.3, histone H2B.5, and UDP-D-glucuronate decarboxylase. Analysis of expression of five heterosis-related genes of rice in a high (27A × RS 673) and a low heterotic (7A × CB 26) sorghum hybrid revealed that three genes (*OsHG1*, *OsHG8*, and *OsHG12*) recorded an expression of more than twofold increase as compared to their parents or mid-parent value (Supplementary Fig. 1). Among these three genes, *OsHG1* and *OsHG12* recorded a peak of > 2.5-fold increase in the high heterotic hybrid (27A × RS 673) during the booting stage over both the parents and mid-parent value, whose expression started declining towards zero during flowering stage (Fig. 3a, b). The genes mentioned above code for proteins similar to Os02g0208100 (ADP/ATP carrier) protein, lipase-like protein, and chlorophyll a/b binding protein.

The maize genes *ZmHG2*, *ZmHG10*, *ZmHG11*, *ZmHG14*, and *ZmHG17* exhibited up to 3.5-fold increase in their expression over the male parent (RS 673) in the samples collected from high heterotic hybrid (27A × RS 673) during the booting stage whereas they recorded less than twofold increase during the same stage of the plant in the low heterotic hybrid (7A ×

Table 3 Details of primers used for RT-PCR and real-time PCR analysis

Gene	Chr. no. (PP in Mb)	Putative function in sorghum	Primer sequence (5′–3′)	AT (°C)
ZmHG1	super_59	Unknown	F: gacgttatgcttgcttttggtactc R: gaacccatatgtcgtcttaccatga	68
ZmHG2	3 (28.86)	Unknown	F: tgattgtattgagccttggtatgg R: aagggatttggctcaggattg	64
ZmHG3	1 (8.88)	Similar to actin-1	F: aaggagaagctcgcctacattg R: gtccgtcaggcagctcgta	64
ZmHG6	10 (50.56)	Similar to elongation factor 1-gamma 3	F: tccgtggccaggatgttc R: tccttctgggcctcatcaga	60
ZmHG7	1 (3.41)	Unknown	F: cccaagaaggtgctgcagtt R: gcagtcgaaggttgccttga	60
ZmHG8	10 (51.87)	Similar to elongation factor 1-alpha	F: gctccagttgggtccttcttc R: ctcagtatcctcctcttggtcgat	68
ZmHG10	8 (48.22)	PPR repeat family (PPR_2)/pentatricopeptide repeat domain (PPR_3)	F: tgctcgcactgtctttgatga R: ccatgttgccacacttgca	60
ZmHG11	super_3009	50S ribosomal protein L32, chloroplastic	F: gcttccccggtttagttggt R: atgcctttatgcctatcgtcgta	62
ZmHG12	1 (17.90)	Similar to actin-1	F: gtggattcctggagcttcca R: gcttgatgggcaagtgatca	60
ZmHG13	7 (56.83)	Similar to histone H2B.3	F: tgaaggagttcatgatggacatg R: agcgtggagacctacaagatctaca	68
ZmHG14	1 (62.70)	Similar to histone H2B.3	F: gacatcggcatctcctccaa R: gttgtaccgcgcgagctt	60
ZmHG15	9 (3.08)	Similar to S-adenosylmethionine synthetase 1	F: tgagggtggtgccatggt R: cttcaggtcagcagcgatctc	62
ZmHG16	4 (60.35)	Similar to histone H2B.5	F: agaagagcgtggagacctacaaga R: tgaaggagttcatgatggacatg	68
ZmHG17	4 (60.35)	Similar to histone H2B.5	F: cggacggaggtctggatct R: atcaacgacatcttcgagaagct	64
ZmHG19	1 (62.78)	Similar to UDP-D-glucuronate decarboxylase	F: gctcccccgtggatattctt R: tggtgctcatggaggatgatt	60
OsHG1	4 (7.45)	Similar to Os02g0208100 (ADP/ATP carrier) protein	F: gtggagacgaagacgctgaag R: cgtcttgaggaacgggatga	60
OsHG8	2 (72.40)	Similar to lipase-like protein	F: ccatcccttcgaagctagca R: gcatttttcacagccatgaatg	60
OsHG11	8 (49.00)	Similar to 60S ribosomal protein L8-1	F: gatgctcaaggctggaaacg R: gcaccttaggccagcagttc	60
OsHG12	2 (70.79)	Chlorophyll A/B binding protein	F: cgccgagctcaaggtcaa R: aagaagccgaacatggagaaca	60
OsHG13	1 (1.58)	Similar to 40S ribosomal protein S4	F: attgtgcattccgttacttttgag R: cagaccgaaccttgcaaagc	62
SbUbi	1 (59.81)	Sobic.001G311100	F: ggttcgggaggtggcataggt R: agcatgtacattcccagcggt	60

Values in parenthesis indicate the physical position in Mb

PP physical position, *AT* annealing temperature

CB 26). Interestingly, the gene *ZmHG2* exhibited higher fold increase in all the critical stages (vegetative, booting, and flowering) of the high heterotic hybrid while the genes *ZmHG10*, *ZmHG11*, and *ZmHG14* exhibited higher fold increase only in the booting stage. However, the expression of these genes was less than twofold (Supplementary Fig. 1) during the same stage of the plant in the low heterotic hybrid (7A × CB 26). The abovementioned genes code for protein similar to PPR repeat family (PPR_2)/pentatricopeptide repeat domain (PPR_3), 50S chloroplastic ribosomal protein L32, histone H2B.3, and histone H2B.5. Among the rice genes, three of them (*OsHG1*, *OsHG11*, and *OsHG12*) exhibited greater than twofold increase in expression in the high heterotic hybrid as compared to its male parent (RS 673) than in the low heterotic hybrid (Supplementary Fig. 1). From the results, it is clear that majority of the genes showed increased fold change in their expression during the vegetative and booting stages, which covers the critical period of panicle initiation. One of the most interesting observations made in this study is that the expression of the maize genes *ZmHG12* (4.37 ± 0.16) and *ZmHG15* (1.96 ± 0.11) was higher in vegetative stage of the low heterotic hybrid as compared to that of the high heterotic hybrid. Similarly, the expression *OsHG8* (1.61 ± 0.09) was higher in vegetative stage of the low heterotic hybrid as compared to that of the high heterotic hybrid.

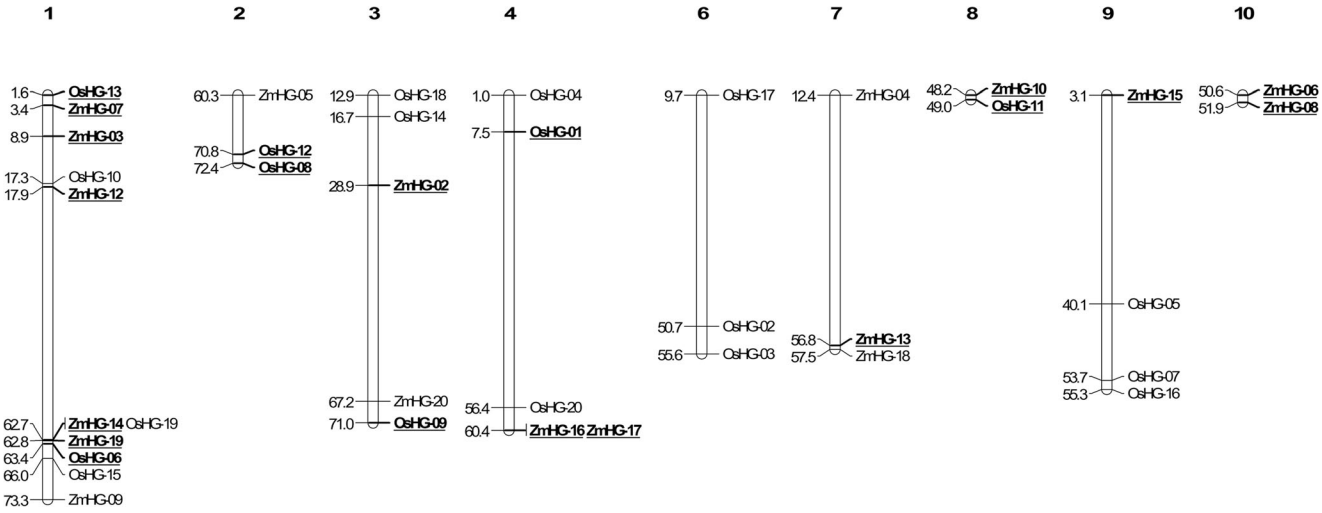

Fig. 1 Physical map of heterosis-related genes of maize and rice showing maximum homology to sorghum selected for this study (genes underlined and bold are used for expression analysis in sorghum hybrids and their parental lines through real-time PCR)

Discussion

Deciphering of the genetic and molecular mechanisms involved in heterosis by the way of increasing and/or improving the knowledge at genetic, molecular, and physiological level will help in a better understanding of the complex biological phenomenon of heterosis in crop plants. Although several studies have been reported in *Arabidopsis*, rice, and maize that contributed to a better understanding of heterosis at the genetic as well as molecular level (Meyer et al. 2012; Nie et al. 2015; He et al. 2010; Song et al. 2010), very little effort has been made in sorghum towards this end (Ben-Israel et al. 2012; Lu et al. 2014). The study presented was designed to analyze the DEGs related to heterosis that have been reported in maize and rice, in sorghum hybrids, and in their parental lines, thereby revealing the potential mode of action of these genes contributing to heterosis.

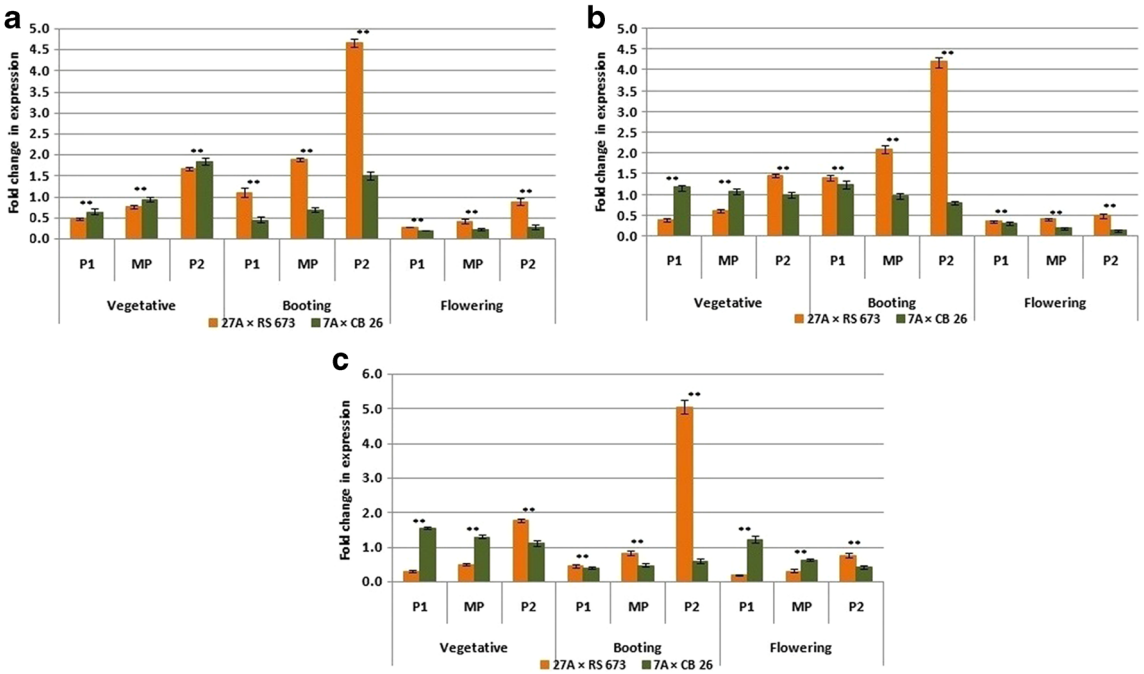

Fig. 2 **a** Fold change of *ZmHG13* gene expression of F_1 hybrids over their parents and mid-parent value. Single and double asterisks indicate significant differences in fold change at $P < 0.05$ and $P < 0.01$, respectively. (P1, female parent; P2, male parent; MP, mid-parent). **b** Fold change of *ZmHG16* gene expression of F_1 hybrids over their parents and mid-parent value. Single and double asterisks indicate significant differences in fold change at $P < 0.05$ and $P < 0.01$, respectively. (P1, female parent; P2, male parent; MP, mid-parent). **c** Fold change of *ZmHG19* gene expression of F_1 hybrids over their parents and mid-parent value. Single and double asterisks indicate significant differences in fold change at $P < 0.05$ and $P < 0.01$, respectively. (P1, female parent; P2, male parent; MP, mid-parent)

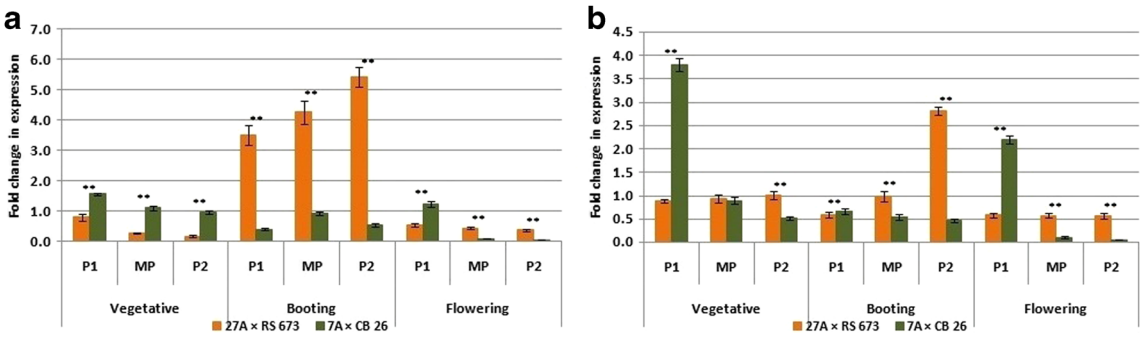

Fig. 3 **a** Fold change of *OsHG1* gene expression of F_1 hybrids over their parents and mid-parent value. Single and double asterisks indicate significant differences in fold change at $P < 0.05$ and $P < 0.01$, respectively. (P1, female parent; P2, male parent; MP, mid-parent). **b** Fold change of *OsHG12* gene expression of F_1 hybrids over their parents and mid-parent value. Single and double asterisks indicate significant differences in fold change at $P < 0.05$ and $P < 0.01$, respectively. (P1, female parent; P2, male parent; MP, mid-parent)

An increase or decrease in mid-parent heterosis and heterobeltiosis estimated in percentage indicates their true potential (Hochholdinger and Hoeckenger 2007). However, heterobeltiosis is considered as the more realistic and practical estimate since it accounts for the performance of the hybrid against the best parent unlike the average heterosis that compares the hybrid against the mean of two parental lines (Lamkey and Edwards 1999). A wide range of mid-parent heterosis and heterobeltiosis was observed in the present study, which was similar to that reported in sorghum by various researchers (Haussmann et al. 2000; Hemlata and Vithal 2006; Ringo et al. 2015). Significant variation among the hybrids was observed for mid-parent heterosis (7.10 to 103.28%) and heterobeltiosis (− 3.80 to 88.49%) for grain yield, which indicated the presence of sufficient genetic divergence among the parental lines. Wide range of heterobeltiosis for grain yield/plant in sorghum was reported in the past by Elwafa et al. (2005). The extent of mid-parent heterosis in the present study was higher than 88 and 81.90% reported by Haussmann et al. (2000) and Ringo et al. (2015), respectively. Similarly, the heterobeltiosis was more than 69.52 and 77.18% reported by Hemlata and Vithal (2006) and Ringo et al. (2015), respectively. However, a similar higher heterobeltiosis for grain yield/plant of 103.09% had been reported by Hovny (2000). Majority of the hybrids that exhibited positive and significant heterosis for grain yield/plant also showed similar trend for most of the component traits contributing to grain yield as reported by Jain and Patel (2013) and Ringo et al. (2015).

Various heterosis projects on crop species, especially rice, wheat, and maize that were executed globally, have resulted in the accumulation of a large number of heterosis-related DEGs. This information is organized in Heterosis-Related Gene Database (http://hrgd.big.ac.cn; Song et al. 2009). BLAST analysis performed for heterosis-related genes of rice and maize in the HRGD against the whole genome of sorghum revealed that 39.38 and 70.45% of genes of rice and maize, respectively, exhibited more than 90% homology with sorghum (Supplementary Table 1). This observation is in line with the synteny relationships among these crops revealed from the fact that sorghum is more closely related to genomes of maize and sugarcane than rice (Gaut et al. 1997; Swigonova et al. 2004; Paterson et al. 2004). Primers were designed for 20 genes (15 of maize and 5 of rice), standardized, and used for further analysis.

Previous studies in crops like maize and rice indicated that the DEGs between a hybrid and its parents are likely to play a vital role in the expression of heterosis (Bao et al. 2005; Swanson-Wagner et al. 2006; Zhang et al. 2008). Majority of the DEGs identified in rice, maize, and wheat by various research groups are being maintained in HRGD, and further investigation on the expression of these genes through real-time PCR could help in understanding of the molecular mechanism of heterosis. The fold change of the expression of 20 selected heterosis-related genes in the sorghum hybrids was determined over both the parents as well as the mid-parent value through real-time PCR. Three maize genes *ZmHG13*, *ZmHG16*, and *ZmHG19* that exhibited more than fourfold increase over the male parent (RS 673) in the high heterotic hybrid (27A × RS 673) during the booting stage code for protein similar to histone H2B.3, histone H2B.5, and UDP-D-glucuronate decarboxylase. The histone genes may be important since it was reported by many researchers that the modifications in histones are associated with heterosis (Ni et al. 2009; He et al. 2010; Li et al. 2011). The enzyme UDP-D-glucuronate decarboxylase is reported to be of central importance due to its involvement in sugar-nucleotide interconversion leading to cell wall biosynthesis in plants (Seifert 2004). Three genes (*OsHG1*, *OsHG8*, and *OsHG12*) that exhibited more than twofold increase in expression as compared to their parents or mid-parent value code for proteins similar to Os02g0208100 (ADP/ATP carrier) protein, lipase-like protein, and chlorophyll a/b binding protein. The expression of non-additive and up-regulation of additive chlorophyll a/b-binding proteins reported by Han et al. (2015) through proteomic analysis indicated a stronger ability to harvest light, transfer energy, and photo-protection in the sorghum–sudan

grass hybrid than its parents. A higher expression of chlorophyll a/b-binding protein in the F_1 hybrid of Chinese cabbage than the parental lines was observed by Saeki et al. (2016), which could be a prerequisite for the continuous increase in cell size as well as number of photosynthetic cells in the early stages of plant development. The role of lipases in the regulation of plant development, morphogenesis, synthesis of secondary metabolites, and defense response was reported by many researchers. The above results clearly indicate that heterosis is predominantly governed by non-additive gene action for majority of the genes with the involvement of additive gene action only for two genes analyzed in this study. Such type of expression patterns indicate the role of regulatory interactions among parental alleles at an early stage of development similar to that reported in maize by Meyer et al. (2007). Several studies in various plant species have reported the prevalence of non-additive gene action in the hybrid in *Arabidopsis*, rice, and maize (Pea et al. 2008; Zhang et al. 2008; Fujimoto et al. 2012; Meyer et al. 2012). The involvement of additive gene action was also reported in maize and rice by various research groups (Guo et al. 2006; Meyer et al. 2007; Zhang et al. 2008; Thiemann et al. 2014). The maize genes *ZmHG2*, *ZmHG10*, *ZmHG11*, and *ZmHG14* that exhibited higher fold increase in the high heterotic hybrid as compared to the low heterotic hybrid code for protein similar to PPR repeat family (PPR_2)/pentatricopeptide repeat domain (PPR_3), 50S chloroplastic ribosomal protein L32, histone H2B.3, and histone H2B.5. The fertility restorer PPR proteins prevent the translation or accumulation of mitochondrial transcripts that induce cytoplasmic male sterility in crop plants (Dahan and Mireau 2013). The contribution of ribosomal proteins towards increasing leaf area in the F_1 hybrid was reported recently by Saeki et al. (2016). Similar case of heterosis-related gene expression during the early stages of the plant was also reported by various research groups working on different plant species (Zhang et al. 2008; He et al. 2010; Meyer et al. 2012; Fujimoto et al. 2012).

In conclusion, the present study analyzed the expression patterns of selected heterosis-related genes of maize and rice among a highly heterotic sorghum hybrid and its parents as well as a low heterotic sorghum hybrid. The study identified the genes that exhibited higher expression in the highly heterotic sorghum hybrid as compared to their parental lines, but recoding a reduced expression in the low heterotic hybrid. Although the role of more number of DEGs related to the complex biological phenomenon of heterosis needs to be further investigated, this study provided important information on the expression pattern of a set of potential genes involved in the manifestation of grain yield heterosis and the stages of their differential expression for the first time in sorghum. These set of genes will act as specific targets for the plant breeders to validate them in a different set of hybrids and their parental lines under different environments, thereby helping to understand the association between the differential expression pattern of specific genes and the phenotypic expression of heterosis.

Acknowledgements The authors thank the Director of ICAR – Indian Institute of Millets Research, Hyderabad, for providing the facilities. IJ thanks the Osmania University, Hyderabad for permitting him to register for Ph.D. to complete his dissertation work.

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

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Alam MF, Khan MR, Nuruzzaman M, Parvez S, Swaraz AM, Alam I, Ahsan N (2004) Genetic basis of heterosis and inbreeding depression in rice (*Oryza sativa* L). J Zhejiang Univ SCI 5(4):406–411

Bao JY, Lee S, Chen C, Zhang XQ, Zhang Y, Liu SQ, Clark T, Wang J, Cao ML, Yang HM, Wang SM, Yu J (2005) Serial analysis of gene expression study of a hybrid rice strain (LYP9) and its parental cultivars. Plant Physiol 138:1216–1231

Baranwal VK, Venugopal M, Barwale UZ, Tyagi AK, Kapoor S (2012) Heterosis: emerging ideas about hybrid vigour. J Exp Bot 63(18): 6309–6314

Bassene JB, Froelicher Y, Dubois C, Ferrer RM, Navarro L, Ollitrault P, Ancillo G (2010) Non-additive gene regulation in a citrus allotetraploid somatic hybrid between *C. reticulata* Blanco and *C. limon* (L.) Burm. Heredity 105:299–308

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:e38993

Birchler JA (2013) Genetic rules of heterosis in plants. In: Chen ZJ, Birchler JA (eds) Polyploid and hybrid genomics. John Wiley & Sons, Inc., Hoboken, pp 313–321

Blum A (1970) Nature of heterosis in grain production by the sorghum panicle. Crop Sci 10:28–31

Blum A (1977) The basis of heterosis in the differentiating sorghum panicle. Crop Sci 17:880–882

Conner AB, Karper RE (1927) Hybrid vigor in sorghum. In: Texas Agricultural Experimental Station Bulletin 359. Texas A & M University, College Station

Dahan J, Mireau H (2013) The Rf and Rf-like PPR in higher plants, a fast-evolving subclass of PPR genes. RNA Biol 10(9):1469–1476

Eastin JD (1972) Photosynthesis and translocation in relation to plant development. In: Rao NGP, House LR (eds) Sorghum in seventies. Oxford and IBH Publishing Co., New Delhi, pp 214–246

Elwafa AA, Ahmed TA, Hassaballa EA, Sayed MA (2005) Heterosis and line × tester analysis of combining ability in grain sorghum [*Sorghum bicolor* (L.) Moench]. Assiut J Agric Sci 36(1):159–175

Fujimoto R, Taylor JM, Shirasawa S, Peacock WJ, Dennis ES (2012) Heterosis of *Arabidopsis* hybrids between C24 and Col is associated

with increased photosynthesis capacity. Proc Natl Acad Sci U S A 109:7109–7114

Gaut BS, Clark LG, Wendel JF, Muse SV (1997) Comparisons of the molecular evolutionary process at *rbcL* and *ndhF* in the grass family (Poaceae). Mol Biol Evol 14(7):769–777

Guo M, Rupe MA, Yang X, Crasta O, Zinselmeier C, Smith OS, Bowen B (2006) Genome-wide transcript analysis of maize hybrids: allelic additive gene expression and yield heterosis. Theor Appl Genet 113: 831–845

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

Haussmann BI, Obilana AB, Ayiecho PO, Blum AA, Schipprack W, Geiger HH (2000) Yield and yield stability of four population types of grain sorghum in a semi-arid area of Kenya. Crop Sci 40:319–329

He GM, Zhu XP, Elling AA, Chen LB, Wang XF, Guo L, Liang MZ, He H, Zhang HY, Chen FF, Qi Y, Chen R, Deng XW (2010) Global epigenetic and transcriptional trends among two rice subspecies and their reciprocal hybrids. Plant Cell 22:17–33

Hemlata S, Vithal S (2006) Heterosis in Sorghum (*Sorghum bicolor* L. Moench). Agr Sci Digest 26(4):245–248

Hochholdinger F, Hoeckenger N (2007) Towards the molecular basis of heterosis. Trends Plant Sci 12:427–432

Hovny MRA (2000) Heterosis and combining ability in grain sorghum [*Sorghum bicolor* (L.) Moench]. Assiut J Agric Sci 31(3):17–30

Jain SK, Patel PR (2013) Heterosis studies for yield and its attributing traits in sorghum [*Sorghum bicolor* (L.) Moench]. Forage Res 39(3): 114–117

Klein RR, Mullet JE, Jordan DR, Miller FR, Rooney WL, Menz MM, Franks CD, Klein PE (2008) The effect of tropical sorghum conversion and inbred development on genome diversity as revealed by high-resolution genotyping. Crop Sci 48(S1):S12–S26

Lamkey KR, Edwards JW (1999) The quantitative genetics of heterosis. In: Coors JG, Pandey S (eds) Proceedings of the International Symposium on the Genetics and Exploitation of Heterosis in Crops, CIMMYT, Mexico City, Mexico, 17–22 Aug. 1997. ASA, CSSA, and SSSA, Madison, pp 31–48

Li C, Huang L, Xu C, Zhao Y, Zhou DX (2011) Altered levels of histone deacetylase OsHDT1 affect differential gene expression patterns in hybrid rice. PLoS One 6:e21789

Lippman ZB, Zamir D (2007) Heterosis: revisiting the magic. Trends Genet 23:60–66

Lu XP, Liu DD, Wang SY, Mi FG, Han PA, Lu ES (2014) Genetic effects and heterosis prediction model of *Sorghum bicolor* × *S. sudanense* grass. Acta Agron Sin 40(3):466–475

Meyer S, Pospisil H, Scholten S (2007) Heterosis associated gene expression in maize embryos 6 days after fertilization exhibits additive, dominant and overdominant pattern. Plant Mol Biol 63:381–391

Meyer RC, Witucka-Wall H, Becher M, Blacha A, Boudichevskaia A, Dörmann P, Fiehn O, Friedel S, von Korff M, Lisec J, Melzer M, Repsilber D, Schmidt R, Scholz M, Selbig J, Willmitzer L, Altmann T (2012) Heterosis manifestation during early *Arabidopsis* seedling development is characterized by intermediate gene expression and enhanced metabolic activity in the hybrids. Plant J 71:669–683

Miller FR, Kebede Y (1984) Genetic contributions to yield gains in sorghum: 1950 to 1980. In: Fehr WR (ed) Genetic contributions to yield gains of five major crop plants, vol 7. Crop Science Society of America, Madison, pp 1–14

Nagaraja Reddy R, Madhusudhana R, Murali Mohan S, Chakravarthi DV, Mehtre SP, Seetharama N, Patil JV (2013) Mapping QTL for grain yield and other agronomic traits in post-rainy sorghum [*Sorghum bicolor* (L.) Moench]. Theor Appl Genet 126:1921–1939

Ni Z, Kim ED, Ha M, Lackey E, Liu J, Zhang Y, Sun Q, Chen ZJ (2009) Altered circadian rhythms regulate growth vigour in hybrids and allopolyploids. Nature 457:327–331

Nie HS, Li SP, Shan XH, Wu Y, Su SZ, Liu HK, Han JY, Yuan YP (2015) Analysis of gene expression patterns and levels in maize hybrids and their parents. Genet Mol Res 14(4):15399–15411

Paterson AH, Bowers JE, Chapman BA (2004) Ancient polyploidization predating divergence of the cereals, and its consequences for comparative genomics. Proc Natl Acad Sci U S A 101(26):9903–9908

Paterson AH, Bowers JE, Bruggmann R, Dubchak I, Grimwood J, Gundlach H, Haberer G, Hellsten U, Mitros T, Poliakov A, Schmutz J, Spannagl M, Tang H, Wang X, Wicker T, Bharti AK, Chapman J, Feltus FA, Gowik U, Grigoriev IV, Lyons E, Maher CA, Martis M, Narechania A, Otillar RP, Penning BW, Salamov AA, Wang Y, Zhang L, Carpita NC, Freeling M, Gingle AR, Hash CT, Keller B, Klein P, Kresovich S, McCann MC, Ming R, Peterson DG, Mehboob-ur-Rahman, Ware D, Westhoff P, Mayer KF, Messing J, Rokhsar DS (2009) The *Sorghum bicolor* genome and the diversification of grasses. Nature 457:551–556

Pea G, Ferron S, Gianfranceschi L, Krajewski P, Pe ME (2008) Gene expression non-additivity in immature ears of a heterotic F_1 maize hybrid. Plant Sci 174:17–24

Quinby JR (1974) Sorghum improvement and the genetics of growth. Texas A & M Univ. Press, College Station

Ringo J, Onkware A, Mgonja M, Deshpande S, Rathore A, Mneney E, Gudu S (2015) Heterosis for yield and its components in sorghum (*Sorghum bicolor* L. Moench) hybrids in dry lands and sub-humid environments of East Africa. Aust J Crop Sci 9(1):9–13

Saeki N, Kawanabe T, Ying H, Shimizu M, Kojima M, Abe H, Okazaki K, Kaji M, Taylor JM, Sakakibara H, Peacock WJ, Dennis ES, Fujimoto R (2016) Molecular and cellular characteristics of hybrid vigour in a commercial hybrid of Chinese cabbage. BMC Plant Biol 16:45

Sanghera GS, Wani SH, Hussain W, Shafi W, Haribhushan A, Singh NB (2011) The magic of heterosis: new tools and complexities. Nat Sci 9:42–53

Seifert GJ (2004) Nucleotide sugar inter-conversions and cell wall biosynthesis: how to bring the inside to the outside. Curr Opin Plant Biol 7:277–284

Smith CW, Frederiksen RA (2000) Sorghum—origin, history, technology and production. John Wiley & Sons, Inc, New York

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 69:255–260

Song GS, Zhai HL, Peng YG, Zhang L, Wei G, Chen XY, Xiao YG, Wang L, Chen YJ, Wu B, Chen B, Zhang Y, Chen H, Feng XJ, Gong WK, Liu Y, Yin ZJ, Wang F, Liu GZ, Xu HL, Wei XL, Zhao XL, Ouwerkerk PB, Hankemeier T, Reijmers T, van der Heijden R, Lu CM, Wang M, van der Greef J, Zhu Z (2010) Comparative transcriptional profiling and preliminary study on heterosis mechanism of super-hybrid rice. Mol Plant 3:1012–1025

Srinivas G, Satish K, Madhusudhana R, Nagaraja Reddy R, Murali Mohan S, Seetharama N (2009) Identification of quantitative trait loci for agronomically important traits and their association with genic-microsatellite markers in sorghum. Theor Appl Genet 118: 1439–1454

Stephens JC, Holland RF (1954) Cytoplasmic male sterility for hybrid sorghum seed production. Agron J 46:20–23

Swanson-Wagner RA, Jia Y, DeCook R, Borsuk LA, Nettleton D, Schnable PS (2006) All possible modes of gene action are observed in a global comparison of gene expression in a maize F_1 hybrid and its inbred parents. Proc Natl Acad Sci U S A 103(18):6801–6810

Swigonova Z, Lai J, Ma J, Ramakrishna W, Llaca V, Bennetzen JL, Messing J (2004) Close split of sorghum and maize genome progenitors. Genome Res 14(10):1916–1923

Thiemann A, Fu J, Schrag TA, Melchinger AE, Frisch M, Scholten S (2010) Correlation between parental transcriptome and field data for the characterization of heterosis in *Zea mays* L. Theor Appl Genet 120:401–413

Thiemann A, Fu J, Seifert F, Grant-Downton RT, Schrag TA, Pospisil H, Frisch M, Melchinger AE, Scholten S (2014) Genome-wide meta-analysis of maize heterosis reveals the potential role of additive gene expression at pericentromeric loci. BMC Plant Biol 14:88

Vanderlip RL, Reeves HE (1972) Growth stages of sorghum. Agron J 64: 13–16

Wei G, Tao Y, Liu G, Chen C, Luo R, Xia H, Gan Q, Zeng H, Lu Z, Han Y, Li X, Song G, Zhai H, Peng Y, Li D, Xu H, Wei X, Cao M, Deng H, Xin Y, Fu X, Yuan L, Yu J, Zhu Z, Zhu L (2009) A transcriptomic analysis of superhybrid rice LYP9 and its parents. Proc Natl Acad Sci U S A 106:7695–7701

Willems E, Leyns L, Vandesompele J (2008) Standardization of real-time PCR gene expression data from independent biological replicates. Anal Biochem 379:127–129

Zhang HY, He H, Chen LB, Li L, Liang MZ, Wang XF, Liu XG, He GM, Chen RS, Ma LG, Deng XW (2008) A genome-wide transcription analysis reveals a close correlation of promoter INDEL polymorphism and heterotic gene expression in rice hybrids. Mol Plant 1(5): 720–731

Zhang XM, Xiao N, Zhang HX, Feng YX, Liu ZX, Gao Y, Dai ZY, Chen JM (2012) Analysis of the relationship between differential expression of rice gene and heterosis. China Agric Sci 45(7):1235–1245