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Genome-wide identification of soybean WRKY transcription factors in response to salt stress

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Abstract

Members of the large family of WRKY transcription factors are involved in a wide range of developmental and physiological processes, most particularly in the plant response to biotic and abiotic stress. Here, an analysis of the soybean genome sequence allowed the identification of the full complement of 188 soybean WRKY genes. Phylogenetic analysis revealed that soybean WRKY genes were classified into three major groups (I, II, III), with the second group further categorized into five subgroups (IIa–IIe). The soybean WRKYs from each group shared similar gene structures and motif compositions. The location of the *GmWRKYs* was dispersed over all 20 soybean chromosomes. The whole genome duplication appeared to have contributed significantly to the expansion of the family. Expression analysis by RNA-seq indicated that in soybean root, 66 of the genes responded rapidly and transiently to the imposition of salt stress, all but one being up-regulated. While in aerial part, 49 *GmWRKYs* responded, all but two being down-regulated. RT-qPCR analysis showed that in the whole soybean plant, 66 *GmWRKYs* exhibited distinct expression patterns in response to salt stress, of which 12 showed no significant change, 35 were decreased, while 19 were induced. The data present here provide critical clues for further functional studies of WRKY gene in soybean salt tolerance.

Keywords: WRKY, Soybean, Expression patterns, Salt stress

Background

Soybean (*Glycine max*) is a global cash crop. Apart from its major contribution to human and animal nutrition, the seed provides a feedstock for biodiesel production and represents a significant raw material for a number of pharmaceutical and industrial processes (Phang et al. 2008; Wang et al. 2010). In recent years, the demand for soybean is increasing rapidly, so it attracted more and more attention to improve soybean agronomic traits, such as stress tolerance. Soybean productivity is greatly compromised by soil salt. However, during the long period of evolution, soybean has evolved complex strategies to survive salt stress. These strategies are originated from the changes of various aspects, such as the genome, gene expression, metabolism and physiology (Phang et al. 2008). To present, functionally reported salt tolerance related genes in soybean are mainly categorized into several classes, including ion transporter coding genes (e.g. *GmHKT1*, *GmSALT3*, *GmNHX1*, *GmCAX1* and *GmCHX1*) (Chen et al. 2011; Guan et al. 2014; Li et al. 2006; Luo et al. 2005a; Qi et al. 2014), transcription factors (TFs) (e.g. GmNAC11/-20/-29, GmDREB1, GmMYB76/-92/-174/-177, GmbZIP44/-62/-78, GmWRKY27 and GmERF7) (Hao et al. 2011; Jin et al. 2010; Liao et al. 2008a, b; Wang et al. 2015; Zhai et al. 2013) and others genes (e.g. glutathione S-transferase gene *GsGST*, late embryogenesis abundant gene *GmLEA*, calcineurin B-like protein coding gene *GmCBL1* and flavone synthase gene *GmFNSII*) (Ji et al. 2010; Lan et al. 2005; Li et al. 2012; Phang et al. 2008; Wang et al. 2011; Yan et al. 2014; Zhou et al. 2010).

The product of a TF gene binds to a specific *cis*-regulatory sequence(s) in the promoter of its target gene. The WRKYs are among the largest class of plant TFs, and their promoter target (the W-box) has the sequence (T)

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(T)TGAC(C/T) (Rushton et al. 2010). WRKY TFs are recognized by the presence of a conserved DNA-binding region composed of about 60 residues (the "WRKY" domain), which harbors the WRKYGQK heptapeptide followed by a C_2H_2 or C_2HC zinc finger motif (Rushton et al. 2010). In some cases, the heptapeptide can take the form WRKYGKK or WRKYGEK (Rushton et al. 2010). WRKY TFs have been classified into three main groups: those possessing two heptapeptides are clustered into group I; both group I and II members harbor one C_2H_2 type zinc finger motif, while the group III members feature a C_2HC one. The large size of group II has been addressed by its division into five subgroups (IIa, IIb, IIc, IId and IIe), based on peptide sequence (Eulgem et al. 2000; Rushton et al. 2010).

Since the first reports of WRKY TFs (dePater et al. 1996; Ishiguro and Nakamura 1994; Rushton et al. 1995, 1996), considerable progress has been made in revealing the function of WRKY TFs. They are elucidated to be involved in various developmental and physiological processes (Rushton et al. 2010), such as seed development (Lagace and Matton 2004; Luo et al. 2005b), seed dormancy and germination (Xie et al. 2005, 2007; Zentella et al. 2007), senescence (Miao et al. 2004; Robatzek and Somssich 2002; Ulker et al. 2007) trichome morphogenesis (Johnson et al. 2002), metabolic pathways (Kato et al. 2007; Sun et al. 2003) and plant development (Cai et al. 2014; Devaiah et al. 2007; Guo et al. 2015; Yu et al. 2013, 2016). The particularly prominent roles of WRKY in plant appear to be the modulation of response to biotic and abiotic stresses (Chen et al. 2012; Eulgem and Somssich 2007; Pandey and Somssich 2009). In the root of the model plant *Arabidopsis*, 18 WRKY genes have been shown to be induced by exposure to salt stress (Jiang and Deyholos 2006); In rice, ten WRKY genes (of 13 analyzed) respond differentially to a range of abiotic stress treatment (Qiu et al. 2004), while in *Brachypodium distachyon*, over 60 % of a set of 86 WRKY genes assayed were up-regulated by heat and cold stress and over 50 % were down-regulated by salt, drought and/or oxidation stress (Wen et al. 2014). Among the soybean WRKY TFs, 25 out of 64 have been shown to be differentially expressed in response to at least one abiotic stress treatment (Zhou et al. 2008).

Base on the availability of the complete soybean genome sequence and several databases (PlantTFDB, SoyDB, SoyTFKB, NCBI and Phytozome), a previous study reported a genome-wide characterization of the WRKY family in soybean and a functional analysis of some genes involved in response to *Phakopsora pachyrhizi* (Bencke-Malato et al. 2014). However, in Phytozome (release v10), the new assembly (v2.0) replaces the Glyma1 assembly. The new database corrects several issues in pseudomolecule reconstruction in the Glyma1 assembly. According to the new database and a recent RNA-Seq result (Belamkar et al. 2014), Song et al. identified 176 GmWRKYs and analyzed their expression files in different tissues and in response to drought and salt stress (Song et al. 2016). However, beside Phytozome, there are many other soybean genome sequence databases. Furthermore, several other transcriptome experiment data sets of soybean under abiotic stress are provided by NCBI website. Here, integrating more databases and RNA-Seq results (Belamkar et al. 2014; Wei et al. 2015), we made a new genome-wide identification of soybean WRKYs and compared their response to salt stress in different tissues. In addition, we analyzed expression profiles of 66 *GmWRKYs* by quantitative RT-PCR (RT-qPCR). Our findings provide new clues for further investigation of WRKY gene in soybean salt tolerance.

Methods

Retrieval of GmWRKY sequences

A set of 185 *GmWRKY* sequences was recovered from Phytozome (http://phytozome.jgi.doe.gov/pz/portal.html, release 10.2) using the keyword PF03106 as a search term, along with three further *GmWRKY* sequences from the NCBI database (http://www.ncbi.nlm.nih.gov). The presence of a WRKY domain(s) in all 188 *GmWRKYs* was confirmed by running the SMART program (http://smart.embl-heidelberg.de) (Letunic et al. 2015). These *GmWRKY* genes were further checked in PlantTFDB (http://planttfdb.cbi.pku.edu.cn/, release 3.0) (Jin et al. 2014) and SoyTFKB (http://www.igece.org/Soybean_TF/).

Multiple sequence alignment and phylogenetic analysis

A multiple alignment of the *WRKY* sequences was performed using the ClustalW program implemented in MEGA v6.06 software package (http://www.megasoftware.net/) (Tamura et al. 2013). The sequences were also subjected to a phylogenetic analysis using the neighbor-joining method; the resulting tree was based on 1000 bootstrap replicates, the p-distance model and pairwise deletion.

Gene structure and conserved motifs analysis

The exon–intron structure of each gene was derived by comparing its coding sequence with the corresponding genomic DNA sequence, using the GSDS program (http://gsds.cbi.pku.edu.cn/) (Hu et al. 2015). The online program MEME v4.10.1 (http://meme-suite.org/tools/meme) was used to identify the conserved motifs present; the relevant parameters were: number of repetitions = any; maximum number of motifs = 16; optimum width of each motif = 6–70 residues.

Genomic location and gene duplication

Each WRKY gene was positioned in the genome by reference to the full genome sequence. The gene duplications in *GmWRKY* genes were identified based on the investigations described in previous study (Schmutz et al. 2010), and Circos software was used to provide a graphical representation of the position of homeologous chromosome segments (Krzywinski et al. 2009).

RNA-seq analysis

A transcriptomic analysis was based on archival RNA-seq data collected from a set of salt stress experiments, mounted on the NCBI GEO database (Belamkar et al. 2014). Experiments GSM1377923, -24 and -25 represented three independent replicates of plants sampled before any exposure to salt; GSM1377935, -36 and -37 related to plants sampled after a 1 h exposure to the stress; GSM1377938, -39 and -40 after a 6 h exposure; and GSM1377941, -42 and -43 after a 12 h exposure. The other transcriptomic analysis was also based on archival RNA-seq data derived from the NCBI GEO database (Wei et al. 2015), experiment GSE57960 was related to plants sampled after a 12 h exposure to salt stress, the aerial part of plants was used for sequencing. The reads per kilobase of exon model per million mapped reads (RPKM) algorithm was used for normalization and mean normalized values were used for the analysis. The transcription response was given in the form of fold changes relative to the 0 h control. Cluster v3.0 software (University of Tokyo, Human Genome Center) was used to perform hierarchical clustering, which was visualized using Java TreeView software (Saldanha 2004). The relevant parameters were: similarity measurement: correlation (uncentered); linkage method: average linkage method.

Plant materials

Seed of *cv*. Williams 82 was germinated on a sheet of moist filter paper, and the seedlings were grown under a regime of 28/20 °C, 14 h photoperiod, light intensity 800 μmol m^{-2} s^{-1} and relative humidity 55 %. Two weeks old seedlings were exposed to 200 mM NaCl for either 0, 2, 6 or 24 h, after which the whole seedling was harvested, snap-frozen in liquid nitrogen and stored at −80 °C.

RT-qPCR analysis

Total RNA was extracted from the frozen plant material using the TRIzol reagent (Invitrogen, USA) following to the manufacturer's instructions. The resulting RNA was treated with RNase-free DNaseI (Promega, USA) to remove genomic DNA contamination, and the cDNA First strand was synthesized with 3 μg total RNA by TransScript One-step gDNA Removal and cDNA synthesis SuperMix (TransGen, China) following the manufacturer's protocol. The subsequent RT-qPCRs and data analysis were performed using a Bio-Rad Real-Time PCR detection system (Bio-Rad) based on the SYBR Green I master mix, as reported previously (Bustin et al. 2009; Seo et al. 2009). According to a previous study, *GmELF1b* was most stably expressed under salt stress, so it was used as a reference gene (Le et al. 2012). All reactions were carried out in triplicate, using samples harvested from independent plants. The relevant primer sequences are given in Additional file 1: Table S1.

Results and discussion

Identification of WRKY genes in soybean

The WRKYs represent one of the largest families of plant TFs. The acquisition of full genome sequences has simplified the enumeration of *WRKY* copy number, so that it is now clear that there are 81 *WRKY* copies in tomato (Huang et al. 2012), 55 in cucumber (Ling et al. 2011), 104 in poplar (He et al. 2012), 59 in grapevine (Wang et al. 2014), 116 in cotton (Dou et al. 2014) and 119 in maize (Wei et al. 2012). In a previous study, 182 putative WRKY gene models were identified (Bencke-Malato et al. 2014). In recent, phytozome updated the soybean assembly; the new assembly (v2.0) replaced the Glyma1 assembly. Therefore, we performed a comprehensive analysis of soybean WRKY sequences obtained from Phytozome, PlantTFDB and NCBI, and finally identified a total of 185 non-redundant putative WRKY genes. Compared with previous 182 WRKY genes, six genes *GmWRKY49* (*Glyma.05G203900*), *GmWRKY53* (*Glyma.06G061900*), *GmWRKY72* (*Glyma.07G161100*), *GmWRKY108* (*Glyma.10G171000*), *GmWRKY130* (*Glyma.14G085500*) and *GmWRKY131* (*Glyma.14G100100*) are novel ones, while three previous genes *GmWRKY17* (*Glyma06g06530*), *GmWRKY38* (*Glyma18g48460*) and *GmWRKY132* (*Glyma14g11440*) are considered obsolete according to the current version of the annotated genome. However, the three obsolete genes have been retained for further study. Thus, a total of 188 annotations of GmWRKYs were presented in this study (Table 1). All the 188 retrieved sequences were proved to contain WRKY domains using SMART analysis.

In previous genome-wide studies, the commonly accepted nomenclature for WRKY members was based on their location order on chromosomes (Dou et al. 2014; Ling et al. 2011). Identically, in the present study, *GmWRKYs* was designated from *GmWRKY1* to *GmWRKY188* based on their exact physical position from the top to the bottom on the soybean chromosomes 1–20 (Table 1). For genes producing more than one transcript, only the primary sequence was named. This nomenclature system was different from previous study

Table 1 The WRKY gene family in Soybean

Gene name	Gene ID[a]	Conserved heptapeptide[b]	Group	Chromosome	Amino acid
GmWRKY1	Glyma.01G043300	WRKYGQK	IIb	Chr1	509
GmWRKY2	Glyma.01G053800	WRKYGQK/WRKYGQK	I	Chr1	455
GmWRKY3	Glyma.01G056800	WRKYGQK	IIc	Chr1	297
GmWRKY4	Glyma.01G128100	WRKYGEK/WRKYGQK	I	Chr1	507
GmWRKY5	Glyma.01G189100	WRKYGQK	IId	Chr1	321
GmWRKY6	Glyma.01G222300	WRKYGQK	IIe	Chr1	245
GmWRKY7	Glyma.01G224800	WRKYGQK	III	Chr1	322
GmWRKY8	Glyma.02G007500	WRKYGQK	IIb	Chr2	484
GmWRKY9	Glyma.02G010900	WRKYGQK	IIc	Chr2	320
GmWRKY10	Glyma.02G020300	WRKYGQK	IIb	Chr2	480
GmWRKY11	Glyma.02G112100	WRKYGQK/WRKYGQK	I	Chr2	455
GmWRKY12	Glyma.02G115200	WRKYGQK	IIc	Chr2	293
GmWRKY13	Glyma.02G141000	WRKYGQK	IId	Chr2	355
GmWRKY14	Glyma.02G203800	WRKYGQK/WRKYGQK	I	Chr2	505
GmWRKY15	Glyma.02G232600	WRKYGQK/WRKYGQK	I	Chr2	580
GmWRKY16	Glyma.02G285900	WRKYGQK	IIc	Chr2	337
GmWRKY17	Glyma.02G293400	WRKYGQK	IIb	Chr2	401
GmWRKY18	Glyma.02G297400	WRKYGQK/WRKYGQK	I	Chr2	588
GmWRKY19	Glyma.02G306300	WRKYGQK/WRKYGQK	I	Chr2	507
GmWRKY20	Glyma.03G002300	Lost	III	Chr3	271
GmWRKY21	Glyma.03G042700	WRKYGEK/WRKYGQK	I	Chr3	507
GmWRKY22	Glyma.03G109100	WRKYGQK	IIc	Chr3	238
GmWRKY23	Glyma.03G159700	WRKYGQK	IId	Chr3	341
GmWRKY24	Glyma.03G176600	WRKYGQK/WRKYGQK	I	Chr3	448
GmWRKY25	Glyma.03G220100	WRKYGQK	IId	Chr3	253
GmWRKY26	Glyma.03G220800	WRKYGQK	IIc	Chr3	287
GmWRKY27	Glyma.03G224700	WRKYGQK	IIb	Chr3	541
GmWRKY28	Glyma.03G256700	WRKYGQK	III	Chr3	362
GmWRKY29	Glyma.04G054200	WRKYGKK	IIc	Chr4	161
GmWRKY30	Glyma.04G061300	WKKYGQK	IIa	Chr4	222
GmWRKY31	Glyma.04G061400	WRKYGQK	IIa	Chr4	220
GmWRKY32	Glyma.04G076200	WRKYGQK	IId	Chr4	279
GmWRKY33	Glyma.04G115500	WRKYGQK/WRKYGQK	I	Chr4	761
GmWRKY34	Glyma.04G173500	WRKYGQK	IIb	Chr4	531
GmWRKY35	Glyma.04G218400	WRKYGQK	IIc	Chr4	234
GmWRKY36	Glyma.04G218700	WRKYGKK	IIc	Chr4	196
GmWRKY37	Glyma.04G223200	WRKYGQK	III	Chr4	337
GmWRKY38	Glyma.04G223300	WRKYGQK	III	Chr4	317
GmWRKY39	Glyma.04G238300	WRKYGQK	III	Chr4	364
GmWRKY40	Glyma.05G029000	WRKYGQK	IIb	Chr5	594
GmWRKY41	Glyma.05G096500	WRKYGQK	IId	Chr5	334
GmWRKY42	Glyma.05G123000	WRKYGQK	IIb	Chr5	361
GmWRKY43	Glyma.05G123600	WRKYGQK	IIe	Chr5	430
GmWRKY44	Glyma.05G127600	WRKYGQK	IIc	Chr5	358
GmWRKY45	Glyma.05G160800	WRKYGQK	IIe	Chr5	255
GmWRKY46	Glyma.05G165800	WRKYGKR	III	Chr5	1355
GmWRKY47	Glyma.05G184500	WRKYGKK	IIc	Chr5	188
GmWRKY48	Glyma.05G185400	WRKYGQK	IIc	Chr5	216
GmWRKY49	Glyma.05G203900	Lost	IIc	Chr5	99

Table 1 continued

Gene name	Gene ID[a]	Conserved heptapeptide[b]	Group	Chromosome	Amino acid
GmWRKY50	Glyma.05G211900	WRKYGQK	IIe	Chr5	288
GmWRKY51	Glyma.05G215900	WRKYGQK	III	Chr5	363
GmWRKY52	Glyma.06G054500	WRKYGKK	IIc	Chr6	175
GmWRKY53	Glyma.06G061900	WRKYGQK	IIa	Chr6	309
GmWRKY54	Glyma06g06530*	WRKYGQK	IIa	Chr6	294
GmWRKY55	Glyma.06G077400	WRKYGQK	IId	Chr6	300
GmWRKY56	Glyma.06G125600	WRKYGQK	III	Chr6	364
GmWRKY57	Glyma.06G142000	WRKYGQK	III	Chr6	319
GmWRKY58	Glyma.06G142100	WRKYGQK	III	Chr6	331
GmWRKY59	Glyma.06G147100	WRKYGKK	IIc	Chr6	196
GmWRKY60	Glyma.06G147500	WRKYGQK	IIc	Chr6	236
GmWRKY61	Glyma.06G168400	WRKYGKK	IIc	Chr6	160
GmWRKY62	Glyma.06G190800	WRKYGQK	IIb	Chr6	615
GmWRKY63	Glyma.06G212900	WKKYGQK	IIa	Chr6	242
GmWRKY64	Glyma.06G219800	WRKYGQK/WRKYGQK	I	Chr6	470
GmWRKY65	Glyma.06G242200	Lost/WRKYGQK	I	Chr6	176
GmWRKY66	Glyma.06G307700	WRKYGQK	IIb	Chr6	628
GmWRKY67	Glyma.06G320700	WRKYGQK/WRKYGQK	I	Chr6	776
GmWRKY68	Glyma.07G023300	WRKYGQK	IIa	Chr7	311
GmWRKY69	Glyma.07G057400	WRKYGQK	III	Chr7	369
GmWRKY70	Glyma.07G116300	WRKYGQK	IIc	Chr7	237
GmWRKY71	Glyma.07G133700	WRKYGQK	IId	Chr7	317
GmWRKY72	Glyma.07G161100	Lost/WRKYGQK	I	Chr7	252
GmWRKY73	Glyma.07G227200	WRKYGQK/WRKYGQK	I	Chr7	533
GmWRKY74	Glyma.07G238000	WRKYGQK	IIc	Chr7	391
GmWRKY75	Glyma.07G262700	WRKYGQK	IIb	Chr7	576
GmWRKY76	Glyma.08G011300	WRKYGEK	IIc	Chr8	147
GmWRKY77	Glyma.08G018300	WRKYGQK	IIe	Chr8	292
GmWRKY78	Glyma.08G021900	WRKYGQK	III	Chr8	359
GmWRKY79	Glyma.08G078100	WRKYGQK	IIb	Chr8	181
GmWRKY80	Glyma.08G078700	WRKYGQK	IIe	Chr8	429
GmWRKY81	Glyma.08G082400	WRKYGQK	IIc	Chr8	371
GmWRKY82	Glyma.08G118200	WRKYGQK	IIe	Chr8	261
GmWRKY83	Glyma.08G142400	WRKYGKK	IIc	Chr8	184
GmWRKY84	Glyma.08G143400	WRKYGQK	IIc	Chr8	235
GmWRKY85	Glyma.08G218600	WRKYGQK	IIa	Chr8	313
GmWRKY86	Glyma.08G240800	WRKYGQK/WRKYGQK	I	Chr8	523
GmWRKY87	Glyma.08G320200	WRKYGQK	IIb	Chr8	486
GmWRKY88	Glyma.08G325800	WRKYGQK/WRKYGQK	I	Chr8	577
GmWRKY89	Glyma.09G005700	WRKYGQK	IIb	Chr9	541
GmWRKY90	Glyma.09G029800	WRKYGQK	IIe	Chr9	506
GmWRKY91	Glyma.09G034300	WRKYGQK	IIc	Chr9	331
GmWRKY92	Glyma.09G061900	WRKYGQK	IId	Chr9	296
GmWRKY93	Glyma.09G080000	WRKYGQK	IIb	Chr9	458
GmWRKY94	Glyma.09G127100	WRKYGQK	IIb	Chr9	242
GmWRKY95	Glyma.09G129100	WRKYGQK	IIe	Chr9	372
GmWRKY96	Glyma.09G240000	WRKYGQK	IIb	Chr9	541
GmWRKY97	Glyma.09G244000	WRKYGQK	IIc	Chr9	238
GmWRKY98	Glyma.09G250500	WRKYGQK/WRKYGQK	I	Chr9	734

Table 1 continued

Gene name	Gene ID[a]	Conserved heptapeptide[b]	Group	Chromosome	Amino acid
GmWRKY99	Glyma.09G254400	WRKYGQK	IIc	Chr9	192
GmWRKY100	Glyma.09G254800	WRKYGQK	IIe	Chr9	348
GmWRKY101	Glyma.09G274000	WRKYGQK	III	Chr9	300
GmWRKY102	Glyma.09G280200	WIKYGQK/WRKYGQK	I	Chr9	543
GmWRKY103	Glyma.10G011300	WRKYGQK	IIc	Chr10	323
GmWRKY104	Glyma.10G032900	WRKYGQK	IId	Chr10	392
GmWRKY105	Glyma.10G111400	Lost	IIb	Chr10	305
GmWRKY106	Glyma.10G113800	WRKYGKK	IIa	Chr10	120
GmWRKY107	Glyma.10G138300	WRKYGQK	IIb	Chr10	482
GmWRKY108	Glyma.10G171000	WHQYGLK	IIc	Chr10	367
GmWRKY109	Glyma.10G171100	WRKYGQK	IIc	Chr10	192
GmWRKY110	Glyma.10G171200	WRKYGQK	IIc	Chr10	336
GmWRKY111	Glyma.10G230200	WRKYGQK	IIe	Chr10	297
GmWRKY112	Glyma.11G021200	WRKYGQK	IIe	Chr11	214
GmWRKY113	Glyma.11G053100	WRKYGQK	IId	Chr11	321
GmWRKY114	Glyma.11G163300	WRKYGQK/WRKYGQK	I	Chr11	548
GmWRKY115	Glyma.12G097100	WRKYGQK	IIb	Chr12	614
GmWRKY116	Glyma.12G152600	WRKYGQK/WRKYGQK	I	Chr12	467
GmWRKY117	Glyma.12G212300	WRKYGQK	IIe	Chr12	263
GmWRKY118	Glyma.13G102000	WRKYGQK	IId	Chr13	324
GmWRKY119	Glyma.13G117600	WRKYGQK	IIb	Chr13	383
GmWRKY120	Glyma.13G267400	WRKYGQK	III	Chr13	294
GmWRKY121	Glyma.13G267500	WRKYGQK	III	Chr13	296
GmWRKY122	Glyma.13G267600	WRKYGQK	III	Chr13	300
GmWRKY123	Glyma.13G267700	WRKYGQK	III	Chr13	270
GmWRKY124	Glyma.13G289400	WRKYGQK	IIe	Chr13	265
GmWRKY125	Glyma.13G310100	WRKYGQK	IIb	Chr13	614
GmWRKY126	Glyma.13G370100	WRKYGQK	IIa	Chr13	309
GmWRKY127	Glyma.14G006800	WRKYGQK/WRKYGQK	I	Chr14	508
GmWRKY128	Glyma.14G016200	WRKYGQK/WRKYGQK	I	Chr14	585
GmWRKY129	Glyma.14G028900	WRKYGQK	IIc	Chr14	335
GmWRKY130	Glyma.14G085500	Lost	IId	Chr14	276
GmWRKY131	Glyma.14G100100	WRKYGKK	IIc	Chr14	68
GmWRKY132	Glyma14g11440*	WRKYGKK	IIc	Chr14	137
GmWRKY133	Glyma.14G102900	WRKYGQK	IIa	Chr14	278
GmWRKY134	Glyma.14G103100	WRKYGQK	IIa	Chr14	282
GmWRKY135	Glyma.14G135400	WRKYGQK	IId	Chr14	316
GmWRKY136	Glyma.14G185800	WRKYGQK	III	Chr14	329
GmWRKY137	Glyma.14G186000	WRKYGQK	III	Chr14	303
GmWRKY138	Glyma.14G186100	WRKYGQK	III	Chr14	240
GmWRKY139	Glyma.14G199800	WRKYEDK	III	Chr14	332
GmWRKY140	Glyma.14G200200	WRKYGQK/WRKYGQK	I	Chr14	575
GmWRKY141	Glyma.15G003300	WRKYGQK	IIa	Chr15	330
GmWRKY142	Glyma.15G110300	WRKYGQK	IIb	Chr15	599
GmWRKY143	Glyma.15G135600	WRKYGQK	IIe	Chr15	523
GmWRKY144	Glyma.15G139000	WRKYGQK	IIc	Chr15	356
GmWRKY145	Glyma.15G168200	WRKYGQK	IId	Chr15	293
GmWRKY146	Glyma.15G186300	WRKYGQK	IIb	Chr15	451
GmWRKY147	Glyma.16G026400	WRKYGQK	III	Chr16	373

Table 1 continued

Gene name	Gene ID[a]	Conserved heptapeptide[b]	Group	Chromosome	Amino acid
GmWRKY148	Glyma.16G031400	WRKYGQK	IIc	Chr16	195
GmWRKY149	Glyma.16G031900	WRKYGQK	IIe	Chr16	335
GmWRKY150	Glyma.16G054400	WRKYGQK	IIc	Chr16	195
GmWRKY151	Glyma.16G176700	WRKYGQK	IIe	Chr16	274
GmWRKY152	Glyma.16G177000	WRKYGQK	IIe	Chr16	408
GmWRKY153	Glyma.16G219800	WRKYGQK	III	Chr16	265
GmWRKY154	Glyma.17G011400	WRKYGQK	IIb	Chr17	489
GmWRKY155	Glyma.17G035400	WRKYGQK	IIc	Chr17	398
GmWRKY156	Glyma.17G042300	WRKYGQK	IIb	Chr17	391
GmWRKY157	Glyma.17G057100	WRKYGQK	IId	Chr17	320
GmWRKY158	Glyma.17G074000	WRKYGQK/WRKYGQK	I	Chr17	505
GmWRKY159	Glyma.17G097900	WRKYGQK	IIb	Chr17	600
GmWRKY160	Glyma.17G168900	WRKYGQK	IId	Chr17	332
GmWRKY161	Glyma.17G197500	WRKYGQK	IId	Chr17	316
GmWRKY162	Glyma.17G222300	WRKYGQK	IIa	Chr17	312
GmWRKY163	Glyma.17G222500	WRKYGQK	IIa	Chr17	278
GmWRKY164	Glyma.17G224800	WRKYGKK	IIc	Chr17	164
GmWRKY165	Glyma.17G239200	WRKYGQK	IId	Chr17	278
GmWRKY166	Glyma.18G056600	WRKYGQK/WRKYGQK	I	Chr18	542
GmWRKY167	Glyma.18G081200	WRKYGQK/WRKYGQK	I	Chr18	577
GmWRKY168	Glyma.18G092200	WRKYGQK	IIb	Chr18	478
GmWRKY169	Glyma.18G124700	WRKYGQK	IIb	Chr18	529
GmWRKY170	Glyma.18G183100	WRKYGQK	IId	Chr18	308
GmWRKY171	Glyma.18G208800	WRKYGQK/WRKYGQK	I	Chr18	541
GmWRKY172	Glyma.18G213200	WRKYGQK	III	Chr18	299
GmWRKY173	Glyma.18G238200	WRKYGQK	IIe	Chr18	351
GmWRKY174	Glyma.18G238600	WRKYGQK	IIc	Chr18	192
GmWRKY175	Glyma.18G242000	WRKYGQK/WRKYGQK	I	Chr18	744
GmWRKY176	Glyma.18G256500	WRKYGQK	IIb	Chr18	541
GmWRKY177	Glyma.18G263400	WRKYGQK/WRKYGQK	I	Chr18	520
GmWRKY178	Glyma18g48460*	WRKYGQK	IIc	Chr18	225
GmWRKY179	Glyma.19G020600	WRKYGQK	IIb	Chr19	495
GmWRKY180	Glyma.19G094100	WRKYGQK	IIc	Chr19	188
GmWRKY181	Glyma.19G177400	WRKYGQK/WRKYGQK	I	Chr19	471
GmWRKY182	Glyma.19G217000	WRKYGQK	IId	Chr19	264
GmWRKY183	Glyma.19G217800	WRKYGQK	IIc	Chr19	290
GmWRKY184	Glyma.19G221700	WRKYGQK	IIb	Chr19	516
GmWRKY185	Glyma.19G254800	WRKYGQK	III	Chr19	362
GmWRKY186	Glyma.20G028000	WRKYGQK/WRKYGQK	I	Chr20	439
GmWRKY187	Glyma.20G030500	WRKYGQK	IIb	Chr20	163
GmWRKY188	Glyma.20G163200	WRKYGQK	IIe	Chr20	321

[a] Genes that are not annotated in the new assembly (v2.0) are marked with the star symbol

[b] The variants of conserved WRKYGQK peptide are shown in red color and some conserved WRKYGQK sequences are lost in several members

(Bencke-Malato et al. 2014). A full comparison of currently known WRKY genes is given in Additional file 2: Table S2.

In silico mapping revealed that the WRKY genes were distributed over all 20 soybean chromosomes. Chromosome 7 harbored the highest number of *GmWRKY* genes (16, 8.51 %), while chromosome 11, 12 and 20 harbored only three (1.60 %). The largest WRKY product was encoded by *GmWRKY46* (1355 residues), and the shortest was GmWRKY131 (68 residues) (Fig. 1; Table 1).

Classification of WRKY genes in soybean

As described previously, WRKY family is typically categorized into three main groups defined by the number of WRKY domains present and the configuration of their zinc finger (Rushton et al. 2010). The 188 soybean WRKY genes were also categorized into the three main groups (Additional file 3: Fig. S1). The group I members numbered 32 (GmWRKY65 and -72 harbored a single N-terminal WRKY domain); there were 130 sequences assigned to group II, sub-divided into subgroup IIa (14 members), IIb (33 members), IIc (42 members), IId (21 members) and IIe (20 members); the remaining 26 sequences belonged to group III (Table 1; Fig. 1). In *Arabidopsis* and poplar, group I houses the largest number of WRKYs, while in rice, group III is the largest (He et al. 2012). However, the largest group in soybean is group II, implying that this group had experienced more gene duplications during the evolutionary course.

Although most of the sequences harbored the well conserved WRKYGQK motif, variants were present in 24 of the sequences: WRKYGKK in 11, WRKYGEK in three, WKKYGQK in two, and WRKYGKR, WRKYEDK, WIKYGQK and WHQYGLK each in one. Strikingly, A WRKYGQK-like stretch was lacking in GmWRKY20, -49, -105 and -130, while the group I members WRKY65 and -72 had both lost their N terminal WRKYGQK-like stretch (Table 1; Additional file 3: Fig. S1). The largest number of variants belonged to group IIc, 11 out of 24. The WRKYGQK sequence was highly conserved in subgroups IIb, IId and IIe, as well as in the C terminal WRKY domain of group I members (Table 1; Additional file 3: Fig. S1). This is consistent with the implication that this group experienced more gene duplications. There was also some variation in the zinc finger motif (including its complete absence) in 11 of the sequences (WRKY6, -42, -52, -65, -72, -79, -94, -106, -112, -139 and -165) (Additional file 3: Fig. S1).

Gene duplication of soybean WRKY genes

Duplication events contribute not only to functional redundancy, but also generate functional novelty (Moore and Purugganan 2005). The modern soybean genome has undergone two whole genome duplication (WGD) events, the first, associated with the evolution of the legume clade occurred ~59 million years ago (Lavin et al. 2005), while the second, which was responsible for the creation of the *Glycine* genus, occurred ~13 million years ago (Schmutz et al. 2010). To investigate whether the expansion of *GmWRKY* genes had primarily happened during both WGD events, we mapped the *GmWRKYs* to the duplicated blocks (Fig. 2). Consistent with previous study (Schmutz et al. 2010), the blocks between chromosomes involved more than just two chromosomes. Of 188 *GmWRKYs*, 180 (95.7 %) genes were located in the blocks (the exceptions were *GmWRKY46*, *-63*, *-65*, *-72*, *-94*, *-105*, *-106*, and *-139*) (Fig. 2), indicating that WGD was the primary reason for the expansion of *GmWRKYs* (Fig. 2).

Besides WGD, tandem duplication event is the other approach for gene expansion. Precise mapping analysis showed the presence of 14 adjacent genes possibly due to tandem duplication (Fig. 2; Additional file 4: Fig. S2a). These 14 WRKY genes were localized in 6 distinct tandem duplicate gene clusters, with four clusters containing two tandem genes (*GmWRKY120/123*, *GmWRKY121/122*, *GmWRKY131/132* and *GmWRKY151/152*) and two clusters possessing three ones (*GmWRKY108/109/110* and *GmWRKY136/137/138*). All the 14 tandem duplicated WRKY genes were mapped onto the duplicated blocks, implying that local duplications occurred earlier than the WGD.

Gene structure and conserved motifs of *GmWRKYs*

Gene structural diversity may reflect the evolution of multigene families (Hu et al. 2010). In order to look into the structural diversity of *GmWRKY* genes, we first

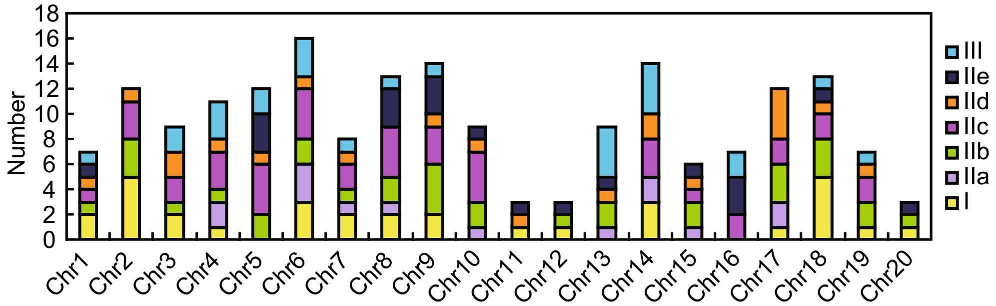

Fig. 1 The distribution of WRKY genes on the 20 chromosomes of soybean

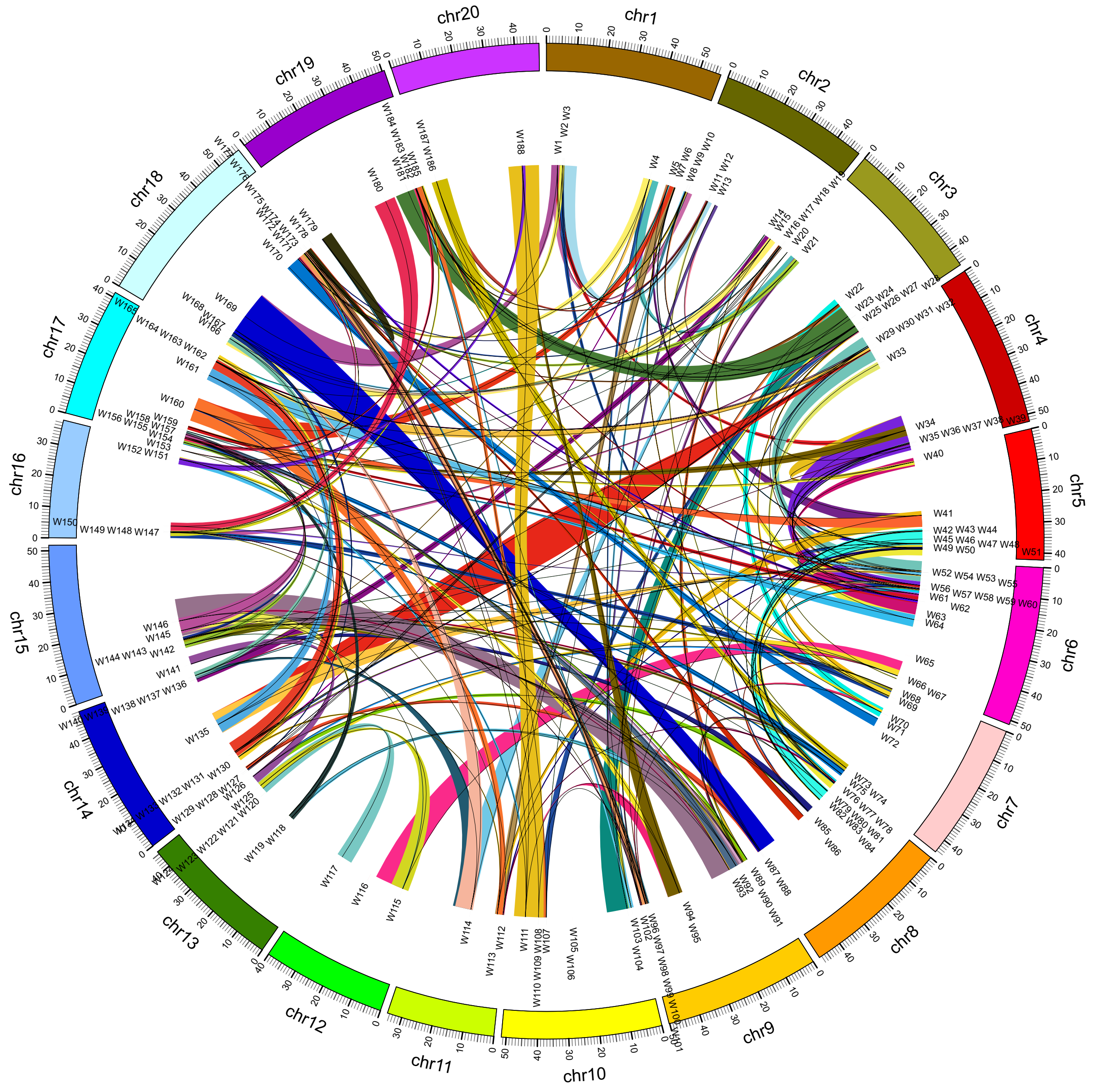

Fig. 2 Chromosomal location of the soybean WRKY genes. The illustrated genome-wide chromosome organization caused by whole genome duplication events is accomplished using the Circos software based on the duplication coordinates defined in the current genome assembly v2.0. Segmental duplicated blocks are color coded. Paralogous pairs are connected with lines

constructed a phylogenetic tree based on the full-length GmWRKY polypeptide sequences, and they were also categorized into seven subfamiles as above (Additional file 4: Fig. S2a). From the tree, we could find that each clade consists of two to four genes, which well matched the two WGD events and confirmed that the expansion of GmWRKY happened during both WGD events. We then analyzed the exon–intron organization in the coding sequences of each soybean WRKY genes HD-ZIP genes (Additional file 4: Fig. S2b). Previous study showed that most *Populus* WRKY genes contain two to four introns (He et al. 2012). Similarly, the majority of soybean WRKY members harbored two to four introns. For instance, over 60 % members of subgroups IIc (26/42), IId (17/21), IIe (14/20) and III (23/26) harbored two introns; over 60 % group I members (17/32) harbored four; most members in group IIa harbored three (7/14) or four (5/14) (Additional file 4: Fig. S2b). In contrast, the gene structure appeared to be more variable in groups IIb, the number of introns in this group varied from one to six (Additional file 4: Fig. S2b). In *Populus*, although there were only eight members in group IIb, the numbers of introns varied from three to six (He et al. 2012). These results indicated that WRKY genes in different species were relatively conserved during the evolution. Furthermore, genes shared similar exon–intron organization within the same subgroup, while they were strikingly distinct in the gene structure among different groups, suggesting that they were not only conserved, but diverged during the evolution.

To better understand the conservation and diversification of WRKY genes in soybean, putative motifs of GmWRKYs were predicted using MEME software and finally 16 distinct motifs were identified (Additional file 4: Fig. S2c). As expected, most of the closely related members in the phylogenetic tree shared common motif compositions, suggesting that the WRKY proteins within the same subfamily might be of similar functions. However, like putative motifs predicted in ZmWRKYs (Gao et al. 2014), the biological significance of most of the putative motifs in GmWRKYs was also unclear because they did not have homologs when searching against Pfam (http://pfam.sanger.ac.uk/search) and SMART (Simple Modular Architecture Research Tool) databases. The same phenomenon also existed in *Populus* NAC and HD-ZIP proteins (Hu et al. 2010, 2012). According to previous study, WRKY proteins harbor typical WRKY domains and zinc-finger motifs (Eulgem et al. 2000; Rushton et al. 2010). Here, motif 1, 2 and 9 comprised the WRKY domain, motif 3 and 10 were the partial zinc-finger motifs followed motif 2 and 9 (Additional file 5: Table S3). The product size of the group I and subgroup IIb genes was larger than that of members of the other groups (Table 1), consistent with their harboring a greater number of motifs (Additional file 4: Fig. S2c). In contrast, although subgroup IIc possessed the largest number of members, they harbored the least number of motifs (one to three). Even though the C-terminal regions of GmWRKYs were highly divergent, we could also identify several conserved motifs which were present in GmWRKYs from specific subgroups, for example, motifs 3, 5 and 6 in group I, motif 14 in subgroup IIb, and motif 13 in subgroups IIa and IIb (Additional file 4: Fig. S2c). Whether these motifs play functional roles remained to be further elucidated.

Expression profiles of *GmWRKYs* in response to salt stress

The WRKY gene family is heavily implicated in the plant response to abiotic stress (Chen et al. 2012), as indicated by a number of microarray-based transcriptomic data sets. Several studies have reported the influence of abiotic stress on WRKY genes based on these data sets (Dou et al. 2014; Satapathy et al. 2014; Wei et al. 2012). In soybean (*cv.* Kefeng No. 1), the response of a set of 64 WRKY genes following the plant's exposure to salt stress has been described (Zhou et al. 2008), but this number represents only about one-third of the total WRKY genes. In order to give insight to the function of GmWRKYs in plant response to salt tolerance, we analyzed the soybean (*cv.* Williams 82) gene expression profiles under salt stress (Belamkar et al. 2014). Finally, 66 of the 188 *GmWRKY* genes were transcriptionally regulated under salt stress (Fig. 3a; Additional file 6: Table S4). 65 genes were up-regulated, with only *WRKY71* being down-regulated (Fig. 3a). The response of WRKY was typically quite rapid (Eulgem et al. 2000), most notably in the case of *GmWRKY20*, *-47*, *-76*, *-126*, *-134*, *-153*, *-164*, which responded by an at least five fold rise in transcript abundance after a 1 h exposure to the stress. In some cases, the response was transient: some examples were the genes *WRKY44*, *-51*, *-54*, *-78*, *-81*, *-85*, *-102* and *-107*, for which transcript abundance peaked after a 6 h exposure and then fell away (Fig. 3a; Additional file 6: Table S4). The most responsive gene (*WRKY134*) belonged to subgroup IIa, which was increased to ~226 fold after a 6 h exposure (Fig. 3a; Additional file 6: Table S4). By contrast, the expression of *GmWRKY71*, a member of group IId was down-regulated in response to salt stress (Fig. 3a).

In soybean, a notable number of responsive genes belonged to subgroup IIb (18 of 33), although their level of induction by salt treatment was only modest (Fig. 3b). In addition, most of subgroup IIc (14/42) and group III (14/26) members were significantly induced and these genes tended to be dramatically up-regulated after a 6 h exposure (Fig. 3c, d). In *Arabidopsis* root, the 18 salt induced members belong to group I (4/18), II (11/18) and III (3/18), respectively (Jiang and Deyholos 2006). These data indicated that either in soybean or *Arabidopsis*, group II members made major contribution in salt response, suggesting that WRKY functions in response to salt stress in different organisms appeared to be conserved during evolution.

The material used in the above RNA-seq was soybean root which was not able to represent other parts. We then analyzed the other RNA-seq data which were derived from the aerial part of soybean plants (*cv.* SuiNong 28) (Wei et al. 2015). A total of 49 *GmWRKY* genes were transcriptionally regulated under salt stress (Fig. 3e; Additional file 7: Table S5). 47 genes were down-regulated, with only two (*WRKY155* and *WRKY183*) being up-regulated (Fig. 3e). These results were quite different with the above RNA-seq analysis, indicating that *GmWRKY* genes showed distinct response profiles in different tissues.

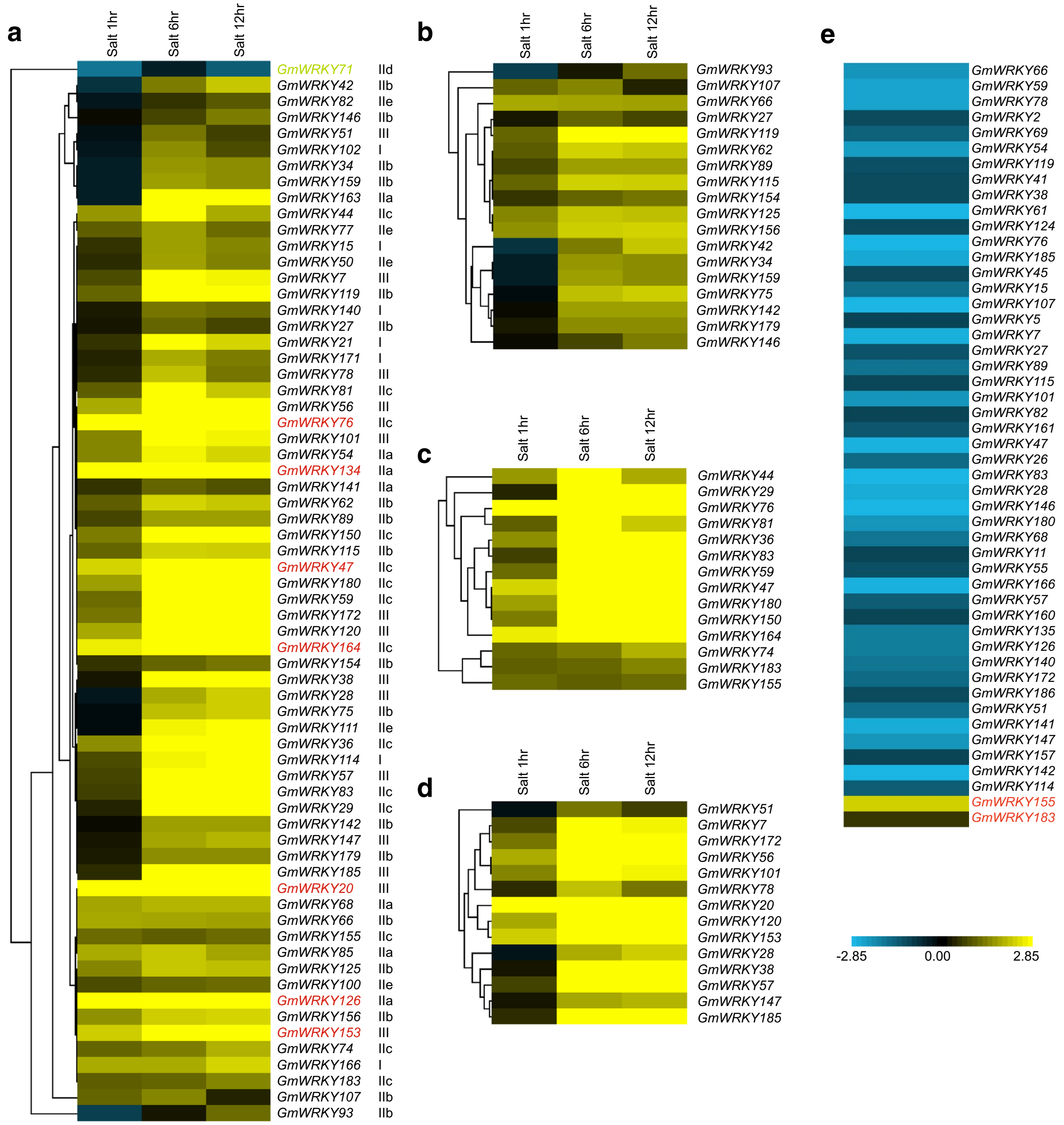

Fig. 3 The transcription response of the WRKY genes in response to salt stress. Transcript abundance levels have been normalized and hierarchical clustered. *Blue colored* blocks indicate a decreased and *yellow ones* an increased level of transcription relative to the control. **a** The set of 66 genes transcriptionally altered in soybean root by the stress. Genes with remarkable changed expressions are labeled in *red*. **b**–**d** The transcription profiles of genes belonging to subgroups **b** IIb and **c** IIc and to **d** group III. *hr* number of hours of exposure to the stress. **e** The set of 49 genes transcriptionally altered in the aerial part of soybean by the stress

Investigation of *Gm*WRKY gene expressions by RT-qPCR

The transcriptional profiles we analyzed above could provide clues for revealing the function of GmWRKYs in plant response to salt tolerance. However, the material used in RNA-seq was soybean root or aerial part which was not able to represent the entire plant. To shed light on the expression profiles of *GmWRKY* genes, 2 weeks old soybean seedlings (*cv*. Williams 82) were exposed to

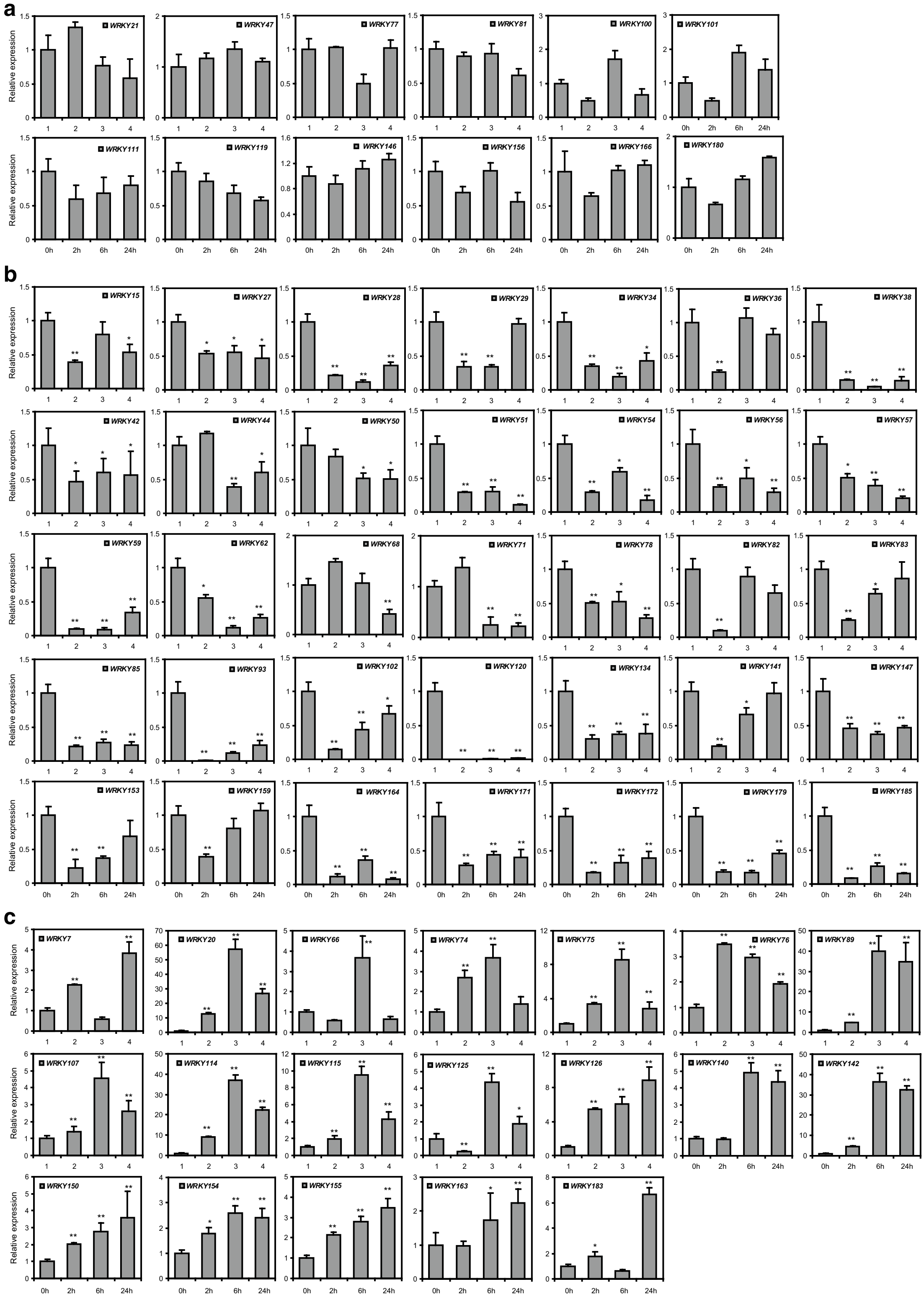

Fig. 4 RT-qPCR-based transcription profiling of 66 WRKY genes. **a** Unaffected WRKY genes. **b** Salt-inhibited WRKY genes. **c** Salt-inducible WRKY genes. *Error bars* represent SD (n = 3). (*t* test, *P < 0.05; **P < 0.01)

200 mM NaCl for 0, 2, 6 or 24 h, respectively, and then the total RNA of the whole plant was isolated used for RT-qPCR analysis. 66 *GmWRKY* genes were tested and exhibited distinct expression patterns in response to salt stress, of which 12 showed no significant change (Fig. 4a), 35 were decreased (Fig. 4b), while 19 were induced (Fig. 4c). *GmWRKY38*, *-120* and *-185* were substantially decreased, especially *GmWRKY120*. In contrast, *GmWRKY20*, *-89*, *-114* and *-142* were remarkably induced. These expression patterns were different with the above RNA-seq analysis, indicating that *GmWRKY* genes showed distinct response profiles in the whole plants compared to different tissues. The response of WRKY to abiotic stresses was generally rapid and transient (Eulgem et al. 2000). Likely, most of the *GmWRKY* genes responded rapidly, their expressions were decreased (31/35) or induced (16/19) after only a 2 h exposure (Fig. 4b, c). In addition, the response of *GmWRKYs* was transient, such as *GmWRKY36*, *-82*, *-83*, *-141*, *-153*, *-159* and *-66* (Fig. 4b, c).

In tomato and cucumber, most *WRKYs* are up-regulated by salt stress (Huang et al. 2012; Ling et al. 2011). In contrast, the majority of *Brachypodium distachyon WRKYs* are down-regulated by the stress (Wen et al. 2014). In soybean, most *WRKYs* were up-regulated in root, while down-regulated in the aerial part by the stress (Fig. 4). Species differences presumably reflected a major degree of functional divergence in the WRKY gene family.

Conclusion

The present study has taken a genome-wide view of the soybean WRKY gene family, and characterized their transcriptional response to salt stress. An analysis of their phylogeny, chromosomal location, gene structure and content of conserved motifs has allowed the genes to be classified into the standard set of groups. The expansion in copy number of the *GmWRKYs* has occurred largely as a result of the two well recognized ancient whole genome duplication events. To date, only three *GmWRKY* genes have been functionally investigated (Jiang and Deyholos 2009), leaving unknown the function of the remaining more than 180. The responsiveness to salt stress of about one-third of the *GmWRKY* complement confirms the potential of gene manipulation within this gene family as means of improving the salt tolerance of important crop species.

Additional files

Additional file 1: Table S1. Primers used in this study.

Additional file 2: Table S2. A comparison between the WRKY genes identified in the current study and those described previously (Bencke-Malato et al. 2014).

Additional file 3: Figure S1. Multiple alignment of the conserved WRKY domain sequences.

Additional file 4: Figure S2. The phylogeny, gene structure and conserved motifs of the soybean WRKY gene family. (**a**) A multiple sequence alignment of 188 full length polypeptide sequences. The seven groups/subgroups (I, IIa-e and III) are depicted by different colors. (**b**) Exon–intron structures. (**c**) The 16 conserved motifs as identified by MEME software. Detailed sequence information given in Additional file 4: Table S3.

Additional file 5: Table S3. Conserved WRKY motif sequences as predicted by MEME software.

Additional file 6: Table S4. Normalized transcript levels of 66 GmWRKY genes in root under salinity stress conditions.

Additional file 7: Table S5. Normalized transcript levels of 49 GmWRKY genes in aerial part under salinity stress conditions.

Abbreviations

NCBI: National Center for Biotechnology Information; PlantTFDB: Plant Transcription Factor Database; SoyDB: Database of Soybean Transcription Factors; SoyTFKB: Soybean Transcription Factor Knowledge Base; SMART: Simple Modular Architecture Research Tool; GSDS: Gene Structure Display Server; MEME: Multiple Em for Motif Elicitation.

Authors' contributions

YY were involved in designing the research. YY and RH collected and analyzed the data. YY and NW performed the experiments. YY and FX wrote the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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References

Belamkar V, Weeks NT, Bharti AK, Farmer AD, Graham MA, Cannon SB (2014) Comprehensive characterization and RNA-Seq profiling of the HD-Zip transcription factor family in soybean (*Glycine max*) during dehydration and salt stress. BMC Genom 15:950

Bencke-Malato M, Cabreira C, Wiebke-Strohm B, Bucker-Neto L, Mancini E, Osorio MB, Homrich MS, Turchetto-Zolet AC, De Carvalho MCCG, Stolf R, Weber RLM, Westergaard G, Castagnaro AP, Abdelnoor RV, Marcelino-Guimaraes FC, Margis-Pinheiro M, Bodanese-Zanettini MH (2014) Genome-wide annotation of the soybean WRKY family and functional characterization of genes involved in response to *Phakopsora pachyrhizi* infection. BMC Plant Biol 14:236

Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT (2009) The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem 55:611–622

Cai YH, Chen XJ, Xie K, Xing QK, Wu YW, Li J, Du CH, Sun ZX, Guo ZJ (2014) Dlf1, a WRKY transcription factor, is involved in the control of flowering time and plant height in rice. PLoS ONE 9:e102529

Chen H, He H, Yu D (2011) Overexpression of a novel soybean gene modulating Na^+ and K^+ transport enhances salt tolerance in transgenic tobacco plants. Physiol Plantarum 141:11–18

Chen LG, Song Y, Li SJ, Zhang LP, Zou CS, Yu DQ (2012) The role of WRKY transcription factors in plant abiotic stresses. BBA-Gene Regul Mech 1819:120–128

dePater S, Greco V, Pham K, Memelink J, Kijne J (1996) Characterization of a zinc-dependent transcriptional activator from *Arabidopsis*. Nucleic Acids Res 24:4624–4631

Devaiah BN, Karthikeyan AS, Raghothama KG (2007) WRKY75 transcription factor is a modulator of phosphate acquisition and root development in Arabidopsis. Plant Physiol 143:1789–1801

Dou LL, Zhang XH, Pang CY, Song MZ, Wei HL, Fan SL, Yu SX (2014) Genome-wide analysis of the WRKY gene family in cotton. Mol Genet Genomics 289:1103–1121

Eulgem T, Somssich IE (2007) Networks of WRKY transcription factors in defense signaling. Curr Opin Plant Biol 10:366–371

Eulgem T, Rushton PJ, Robatzek S, Somssich IE (2000) The WRKY superfamily of plant transcription factors. Trends Plant Sci 5:199–206

Gao J, Peng H, He XJ, Luo M, Chen Z, Lin HJ, Ding HP, Pan GT, Zhang ZM (2014) Molecular phylogenetic characterization and analysis of the WRKY transcription factor family responsive to Rhizoctonia solani in maize. Maydica 59:32–41

Guan RX, Qu Y, Guo Y, Yu LL, Liu Y, Jiang JH, Chen JG, Ren YL, Liu GY, Tian L, Jin LG, Liu ZX, Hong HL, Chang RZ, Gilliham M, Qiu LJ (2014) Salinity tolerance in soybean is modulated by natural variation in *GmSALT3*. Plant J 80:937–950

Guo DS, Zhang JZ, Wang XL, Han X, Wei BY, Wang JQ, Li BX, Yu H, Huang QP, Gu HY, Qu LJ, Qin GJ (2015) The WRKY transcription factor WRKY71/EXB1 controls shoot branching by transcriptionally regulating RAX genes in Arabidopsis. Plant Cell 27:3112–3127

Hao YJ, Wei W, Song QX, Chen HW, Zhang YQ, Wang F, Zou HF, Lei G, Tian AG, Zhang WK, Ma B, Zhang JS, Chen SY (2011) Soybean NAC transcription factors promote abiotic stress tolerance and lateral root formation in transgenic plants. Plant J 68:302–313

He HS, Dong Q, Shao YH, Jiang HY, Zhu SW, Cheng BJ, Xiang Y (2012) Genome-wide survey and characterization of the WRKY gene family in *Populus trichocarpa*. Plant Cell Rep 31:1199–1217

Hu RB, Qi G, Kong YZ, Kong DJ, Gao QA, Zhou GK (2010) Comprehensive analysis of NAC domain transcription factor gene family in *Populus trichocarpa*. BMC Plant Biol 10:145

Hu RB, Chi X, Chai G, Kong Y, He G, Wang X, Shi D, Zhang D, Zhou G (2012) Genome-wide identification, evolutionary expansion, and expression profile of homeodomain-leucine zipper gene family in poplar (Populus trichocarpa). PLoS One 7(2):e31149

Hu B, Jin JP, Guo AY, Zhang H, Luo JC, Gao G (2015) GSDS 2.0: an upgraded gene feature visualization server. Bioinformatics 31:1296–1297

Huang SX, Gao YF, Liu JK, Peng XL, Niu XL, Fei ZJ, Cao SQ, Liu YS (2012) Genome-wide analysis of WRKY transcription factors in *Solanum lycopersicum*. Mol Genet Genomics 287:495–513

Ishiguro S, Nakamura K (1994) Characterization of a cDNA encoding a novel DNA-binding protein, SPF1, that recognizes SP8 sequences in the 5′ upstream regions of genes coding for sporamin and β-amylase from sweet potato. Mol Gen Genet 244:563–571

Ji W, Zhu YM, Li Y, Yang LA, Zhao XW, Cai H, Bai X (2010) Over-expression of a glutathione S-transferase gene, GsGST, from wild soybean (*Glycine soja*) enhances drought and salt tolerance in transgenic tobacco. Biotechnol Lett 32:1173–1179

Jiang YQ, Deyholos MK (2006) Comprehensive transcriptional profiling of NaCl-stressed Arabidopsis roots reveals novel classes of responsive genes. BMC Plant Biol 6:25

Jiang YQ, Deyholos MK (2009) Functional characterization of Arabidopsis NaCl-inducible *WRKY25* and *WRKY33* transcription factors in abiotic stresses. Plant Mol Biol 69:91–105

Jin TC, Chang Q, Li WF, Yin DX, Li ZJ, Wang DL, Liu B, Liu LX (2010) Stress-inducible expression of GmDREB1 conferred salt tolerance in transgenic alfalfa. Plant Cell Tiss Org 100:219–227

Jin JP, Zhang H, Kong L, Gao G, Luo JC (2014) PlantTFDB 3.0: a portal for the functional and evolutionary study of plant transcription factors. Nucleic Acids Res 42:D1182–D1187

Johnson CS, Kolevski B, Smyth DR (2002) *TRANSPARENT TESTA GLABRA2*, a trichome and seed coat development gene of Arabidopsis, encodes a WRKY transcription factor. Plant Cell 14:1359–1375

Kato N, Dubouzet E, Kokabu Y, Yoshida S, Taniguchi Y, Dubouzet JG, Yazaki K, Sato F (2007) Identification of a WRKY protein as a transcriptional regulator of benzylisoquinoline alkaloid biosynthesis in *Coptis japonica*. Plant Cell Physiol 48:8–18

Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D, Jones SJ, Marra MA (2009) Circos: an information aesthetic for comparative genomics. Genome Res 19:1639–1645

Lagace M, Matton DP (2004) Characterization of a WRKY transcription factor expressed in late torpedo-stage embryos of *Solanum chacoense*. Planta 219:185–189

Lan Y, Cai D, Zheng YZ (2005) Expression in *Escherichia coli* of three different soybean late embryogenesis abundant (*LEA*) genes to investigate enhanced stress tolerance. J Integr Plant Biol 47:613–621

Lavin M, Herendeen PS, Wojciechowski MF (2005) Evolutionary rates analysis of Leguminosae implicates a rapid diversification of lineages during the tertiary. Syst Biol 54:575–594

Le DT, Aldrich DL, Valliyodan B, Watanabe Y, Van Ha C, Nishiyama R, Guttikonda SK, Quach TN, Gutierrez-Gonzalez JJ, Tran LSP, Nguyen HT (2012) Evaluation of candidate reference genes for normalization of quantitative RT-PCR in soybean tissues under various abiotic stress conditions. PLoS One 7(9):e46487

Letunic I, Doerks T, Bork P (2015) SMART: recent updates, new developments and status in 2015. Nucleic Acids Res 43:D257–D260

Li WYF, Wong FL, Tsai SN, Phang TH, Shao GH, Lam HM (2006) Tonoplast-located *GmCLC1* and *GmNHX1* from soybean enhance NaCl tolerance in transgenic bright yellow (BY)-2 cells. Plant, Cell Environ 29:1122–1137

Li ZY, Xu ZS, He GY, Yang GX, Chen M, Li LC, Ma YZ (2012) Overexpression of soybean *GmCBL1* enhances abiotic stress tolerance and promotes hypocotyl elongation in *Arabidopsis*. Biochem Biophys Res Commun 427:731–736

Liao Y, Zou HF, Wang HW, Zhang WK, Ma B, Zhang JS, Chen SY (2008a) Soybean *GmMYB76*, *GmMYB92*, and *GmMYB177* genes confer stress tolerance in transgenic *Arabidopsis* plants. Cell Res 18:1047–1060

Liao Y, Zou HF, Wei W, Hao YJ, Tian AG, Huang J, Liu YF, Zhang JS, Chen SY (2008b) Soybean *GmbZIP44*, *GmbZIP62* and *GmbZIP78* genes function as negative regulator of ABA signaling and confer salt and freezing tolerance in transgenic *Arabidopsis*. Planta 228:225–240

Ling J, Jiang WJ, Zhang Y, Yu HJ, Mao ZC, Gu XF, Huang SW, Xie BY (2011) Genome-wide analysis of WRKY gene family in *Cucumis sativus*. BMC Genom 12:471

Luo GZ, Wang HW, Huang J, Tian AG, Wang YJ, Zhang JS, Chen SY (2005a) A putative plasma membrane cation/proton antiporter from soybean confers salt tolerance in *Arabidopsis*. Plant Mol Biol 59:809–820

Luo M, Dennis ES, Berger F, Peacock WJ, Chaudhury A (2005b) *MINISEED3* (*MINI3*), a *WRKY* family gene, and *HAIKU2* (*IKU2*), a leucine-rich repeat (*LRR*) *KINASE* gene, are regulators of seed size in *Arabidopsis*. Proc Natl Acad Sci USA 102:17531–17536

Miao Y, Laun T, Zimmermann P, Zentgraf U (2004) Targets of the WRKY53 transcription factor and its role during leaf senescence in *Arabidopsis*. Plant Mol Biol 55:853–867

Moore RC, Purugganan MD (2005) The evolutionary dynamics of plant duplicate genes. Curr Opin Plant Biol 8:122–128

Pandey SP, Somssich IE (2009) The role of WRKY transcription factors in plant immunity. Plant Physiol 150:1648–1655

Phang TH, Shao G, Lam HM (2008) Salt tolerance in soybean. J Integr Plant Biol 50:1196–1212

Qi XP, Li MW, Xie M, Liu X, Ni M, Shao GH, Song C, Yim AKY, Tao Y, Wong FL, Isobe S, Wong CF, Wong KS, Xu CY, Li CQ, Wang Y, Guan R, Sun FM, Fan GY, Xiao ZX, Zhou F, Phang TH, Liu X, Tong SW, Chan TF, Yiu SM, Tabata S, Wang J, Xu X, Lam HM (2014) Identification of a novel salt tolerance gene in wild soybean by whole-genome sequencing. Nat Commun 5:4340

Qiu YP, Jing SJ, Fu J, Li L, Yu DQ (2004) Cloning and analysis of expression profile of 13 *WRKY* genes in rice. Chin Sci Bull 49:2159–2168

Robatzek S, Somssich IE (2002) Targets of *At*WRKY6 regulation during plant senescence and pathogen defense. Genes Dev 16:1139–1149

Rushton PJ, Macdonald H, Huttly AK, Lazarus CM, Hooley R (1995) Members of a new family of DNA-binding proteins bind to a conserved *cis*-element in the promoters of *α-Amy2* genes. Plant Mol Biol 29:691–702

Rushton PJ, Torres JT, Parniske M, Wernert P, Hahlbrock K, Somssich IE (1996) Interaction of elicitor-induced DNA-binding proteins with elicitor response elements in the promoters of parsley PR1 genes. EMBO J 15:5690–5700

Rushton PJ, Somssich IE, Ringler P, Shen QJ (2010) WRKY transcription factors. Trends Plant Sci 15:247–258

Saldanha AJ (2004) Java Treeview-extensible visualization of microarray data. Bioinformatics 20:3246–3248

Satapathy L, Singh D, Ranjan P, Kumar D, Kumar M, Prabhu KV, Mukhopadhyay K (2014) Transcriptome-wide analysis of WRKY transcription factors in wheat and their leaf rust responsive expression profiling. Mol Genet Genomics 289:1289–1306

Schmutz J, Cannon SB, Schlueter J, Ma JX, Mitros T, Nelson W, Hyten DL, Song QJ, Thelen JJ, Cheng JL, Xu D, Hellsten U, May GD, Yu Y, Sakurai T, Umezawa T, Bhattacharyya MK, Sandhu D, Valliyodan B, Lindquist E, Peto M, Grant D, Shu SQ, Goodstein D, Barry K, Futrell-Griggs M, Abernathy B, Du JC, Tian ZX, Zhu LC et al (2010) Genome sequence of the palaeopolyploid soybean. Nature 463:178–183

Seo PJ, Xiang FN, Qiao M, Park JY, Lee YN, Kim SG, Lee YH, Park WJ, Park CM (2009) The MYB96 transcription factor mediates abscisic acid signaling during drought stress response in Arabidopsis. Plant Physiol 151:275–289

Song H, Wang P, Hou L, Zhao S, Zhao C, Xia H, Li P, Zhang Y, Bian X, Wang X (2016) Global analysis of WRKY genes and their response to dehydration and salt stress in soybean. Front Plant Sci. doi:10.3389/fpls.2016.00009

Sun CX, Palmqvist S, Olsson H, Boren M, Ahlandsberg S, Jansson C (2003) A novel WRKY transcription factor, SUSIBA2, participates in sugar signaling in barley by binding to the sugar-responsive elements of the *iso1* promoter. Plant Cell 15:2076–2092

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Ulker B, Mukhtar MS, Somssich IE (2007) The WRKY70 transcription factor of *Arabidopsis* influences both the plant senescence and defense signaling pathways. Planta 226:125–137

Wang Z, Libault M, Joshi T, Valliyodan B, Nguyen HT, Xu D, Stacey G, Cheng JL (2010) SoyDB: a knowledge database of soybean transcription factors. BMC Plant Biol 10:14

Wang YX, Suo HC, Zhuang CX, Ma H, Yan XL (2011) Overexpression of the soybean *GmWNK1* altered the sensitivity to salt and osmotic stress in *Arabidopsis*. J Plant Physiol 168:2260–2267

Wang LN, Zhu W, Fang LC, Sun XM, Su LY, Liang ZC, Wang N, Londo JP, Li SH, Xin HP (2014) Genome-wide identification of *WRKY* family genes and their response to cold stress in *Vitis vinifera*. BMC Plant Biol 14:103

Wang FF, Chen HW, Li QT, Wei W, Li W, Zhang WK, Ma B, Bi YD, Lai YC, Liu XL, Man WQ, Zhang JS, Chen SY (2015) GmWRKY27 interacts with GmMYB174 to reduce expression of GmNAC29 for stress tolerance in soybean plants. Plant J 83:224–236

Wei KF, Chen J, Chen YF, Wu LJ, Xie DX (2012) Molecular phylogenetic and expression analysis of the complete WRKY transcription factor family in Maize. DNA Res 19:153–164

Wei W, Li QT, Chu YN, Reiter RJ, Yu XM, Zhu DH, Zhang WK, Ma B, Lin Q, Zhang JS, Chen SY (2015) Melatonin enhances plant growth and abiotic stress tolerance in soybean plants. J Exp Bot 66:695–707

Wen F, Zhu H, Li P, Jiang M, Mao WQ, Ong C, Chu ZQ (2014) Genome-wide evolutionary characterization and expression analyses of WRKY family genes in *Brachypodium distachyon*. DNA Res 21:327–339

Xie Z, Zhang ZL, Zou XL, Huang J, Ruas P, Thompson D, Shen QJ (2005) Annotations and functional analyses of the rice *WRKY* gene superfamily reveal positive and negative regulators of abscisic acid signaling in aleurone cells. Plant Physiol 137:176–189

Xie Z, Zhang ZL, Hanzlik S, Cook E, Shen QXJ (2007) Salicylic acid inhibits gibberellin-induced alpha-amylase expression and seed germination via a pathway involving an abscisic-acid-inducible *WRKY* gene. Plant Mol Biol 64:293–303

Yan JH, Wang BA, Jiang YN, Cheng LJ, Wu TL (2014) *GmFNSII*-controlled soybean flavone metabolism responds to abiotic stresses and regulates plant salt tolerance. Plant Cell Physiol 55:74–86

Yu YC, Hu RB, Wang HM, Cao YP, He G, Fu CX, Zhou GK (2013) MlWRKY12, a novel *Miscanthus* transcription factor, participates in pith secondary cell wall formation and promotes flowering. Plant Sci 212:1–9

Yu YC, Liu ZH, Wang L, Kim SG, Seo PJ, Qiao M, Wang N, Li S, Cao XF, Park CM, Xiang FN (2016) WRKY71 accelerates flowering via the direct activation of FLOWERING LOCUS T and LEAFY in Arabidopsis thaliana. Plant J 85:96–106

Zentella R, Zhang ZL, Park M, Thomas SG, Endo A, Murase K, Fleet CM, Jikumaru Y, Nambara E, Kamiya Y, Sun TP (2007) Global analysis of DELLA direct targets in early gibberellin signaling in *Arabidopsis*. Plant Cell 19:3037–3057

Zhai Y, Wang Y, Li Y, Lei T, Yan F, Su L, Li X, Zhao Y, Sun X, Li J, Wang Q (2013) Isolation and molecular characterization of GmERF7, a soybean ethylene-response factor that increases salt stress tolerance in tobacco. Gene 513:174–183

Zhou QY, Tian AG, Zou HF, Xie ZM, Lei G, Huang J, Wang CM, Wang HW, Zhang JS, Chen SY (2008) Soybean WRKY-type transcription factor genes, *GmWRKY13*, *GmWRKY21*, and *GmWRKY54*, confer differential tolerance to abiotic stresses in transgenic *Arabidopsis* plants. Plant Biotechnol J 6:486–503

Zhou GA, Chang RZ, Qiu LJ (2010) Overexpression of soybean ubiquitin-conjugating enzyme gene *GmUBC2* confers enhanced drought and salt tolerance through modulating abiotic stress-responsive gene expression in *Arabidopsis*. Plant Mol Biol 72:357–367