Isolation and Sequence Analysis of Transcription Factor GmWRKY53 from Soybean (*Glycine max*)

BI Ying-dong[1,2], LI Wei[2], WANG Ping[3], LIU Ming[2], LI Wan[2], LIU Miao[2], XIAO Jia-lei[2], LAI Yong-cai[2]

(1. Heilongjiang Academy of Agricultural Sciences Postdoctoral Programme/Northeast Forestry University Postdoctoral Programme, Harbin 150086, China; 2. Crops Tillage and Cultivation Institute of Heilongjiang Academy of Agricultural Sciences, Harbin 150086, China; 3. Information Center of Heilongjiang Academy of Agricultural Sciences, Harbin 150086, China)

Abstract: The WRKY protein is a super family of transcription factors unique to plants which was so named as all family members contain a conserved amino acid sequence WRKYGQK and special zinc finger motif at its N-terminal end. WRKY protein may participate in regulating the expression of a wide range of genes involved in various biological processes such as development, metabolism and response to a range of abiotic and biotic stresses. We characterized a predicated transcription factor GmWRKY53 from soybean using the yeast one hybrid system with the bait vector which had W-box of the *AtNPR1* (*Arabidopsis thaliana*) promoter related with pathogen defense. It contained one conserved domain, and showed similarity with AtWRKY family members in both overall amino-acid sequences and the secondary structure arrangement within the DNA-binding motifs. In yeast one-hybrid system, GmWRKY53 can specially activate the genes fused with the promoter containing W-box of the *AtNPR1* promoter. The study on transcription factor WRKY of soybean is conduced to understand the regulation of various physiological programs of soybean, including pathogen defense, senescence and development.

Keywords: Transcription factor; WRKY; W-box; Soybean

大豆转录因子 GmWRKY53 的分离及序列分析

毕影东[1,2],李 炜[2],王 萍[3],刘 明[2],李 琬[2],刘 淼[2],肖佳雷[2],来永才[2]

(1. 黑龙江省农业科学院博士后工作站/东北林业大学博士后流动站,黑龙江 哈尔滨 150086; 2. 黑龙江省农业科学院 耕作栽培研究所,黑龙江 哈尔滨 150086; 3. 黑龙江省农业科学院 信息中心,黑龙江 哈尔滨 150086)

摘 要:WRKY 蛋白是只存在于植物中的一类转录因子家族,由 WRKY 蛋白 N 端高度保守的 WRKYGQK 氨基酸序列及特殊的锌指结构而命名。WRKY 除参与植物发育和代谢的调控外还与植物的抗逆反应有关。本研究利用抗病相关基因 *NPR1* 基因启动子区的 W-box 顺式元件采用酵母单杂交方法,以拟南芥 *AtNPR1* 启动子区域 W-box 元件构建诱饵载体,从大豆中分离到了一个转录因子 GmWRKY53,通过序列分析表明,GmWRKY53 具有与 AtWRKY 蛋白的保守氨基酸序列极相似的二级结构,在酵母单杂交系统中该蛋白能够与抗病相关基因 *AtNPR1* 基因启动子区的 W-box 特异结合并启动报道基因的表达。对大豆 WRKY 转录因子的研究有助于深入理解大豆抗病及发育调控机制。

关键词:转录因子;WRKY;W-box;大豆

中图分类号:S565.1 **文献标识码**:A **DOI**:10.11861/j.issn.1000-9841.2015.06.0972

1 Introduction

Transcriptional control of the stress-related genes is a crucial part of the plant development and response to stresses[1]. Transcription factors can interact with cis-regulatory sequences of target genes to regulate gene expression temporally and spatially[2]. Much more attentions had been paid to identify transcription factors that involved in plant development regulation and stresses response which related to agricultural production and quality[3]. WRKY transcription factors are one of the largest families of transcriptional regulators in plants and was so named because all family members contain a conserved amino acid sequence WRKYGQK at its N-terminal end. It contains zinc-finger-like motif which is able to bind specifically to the DNA sequence motif W-box "(T)(T)TGAC(T/C)"[4]. WRKY proteins often act as repressors or activators and play an

Received:2015-04-08

Foundation item: Special Financial Grant from China Postdoctral Science Foundation (2014T70373); 54 – class General Financial Grant from China Postdoctral Science Foundation (2013M541422); Department of Science and Technology of Heilongjiang Province (LC2011C30); Harbin Science and Technology Bureau(2011RFLYN019); Young Foundation Major Project of HAAS(2012ZD031); Department of Human Resource of Heilongjiang Province(2012-134).

Biography: Bi Yingdong(1974-), Male, PhD, Associate professor. Major in genetics and breeding in soybean. E-mail: ydbi308@163.com.

important role in the regulation of transcriptional reprogramming associated with plant stress responses[5-8]. Sometimes, they also exhibit extensive auto-regulation and cross-regulation in a dynamic process with built-in redundancy[5]. *Arabidopsis* WRKY18, WRKY40 and WRKY60 transcription factors interact both physically and functionally in plant defense responses. However, they may play different roles in modulating gene expression in plant abiotic stress response[9]. To date, many WRKY proteins have been identified which were involved in the defense against stress. Northern blotting analysis revealed that 10 of 13 *OsWRKY* genes responded to NaCl, PEG, cold or heat treatments differentially[10]. In wheat, overexpression of *TaWRKY*19 conferred tolerance to salt, drought and freezing stresses in transgenic plants. *TaWRKY*2 enhanced expression of STZ and RD29B, and bound to their promoters[11]. Berri et al[12] defined the existence of nine *OsWRKY* gene clusters comprising both phylogenetically related and unrelated genes that were significantly co-expressed, suggested that specific sets of *WRKY* genes might act in co-regulatory networks. *Arabidopsis*, dicot model organism, is commonly used to predict novel genes biological functions in plants[13]. *AtWRKY*40 and *AtWRKY*63 were particularly involved in regulating the expression of genes responding commonly to both mitochondrial and chloroplast dysfunction. This study establishes the role of WRKY transcription factors in the coordination of stress-responsive genes encoding mitochondrial and chloroplast proteins[14]. Soybean is one of the most important economic crops in the world, genome and transcriptome sequencing of soybean have been completed[15]. Moreover, a total of 133 *WRKY* members in the soybean genome were identified and could be classified into three groups(groups I, II and III) according to structural features of their encoded proteins. Some of these genes have been extensively studied and reported to be involved in many physiological and biochemical processes[16]. Zhou et al[17] used a transgenic approach to investigate three *WRKY* gnenes of soybean, the results indicated that they play differential roles in abiotic stress tolerance, moreover, one of them may have function in both lateral root development and the abiotic stress response. Lou et al[18] found that *GsWRKY*20, a gene of wild soybean, could enhance plant drought tolerance through regulating ABA signalling. Soybean is one of the most important crops for food production. However, frequent occurrences of drought or stress events have negatively impacts on soybean growth and production. Therefore, it is important to isolate or elucidate molecular mechanisms of cruial factors in soybean adaptation to stress. In this study, we characterized a predicated WRKY53 transcription factor from soybean using the yeast one hybrid system by the bait vector which had W-box in the *NPR*1 promoter related with pathogen defense. By applying current knowledge of stress-regulated WRKYs, their regulatory networks and crosstalk in abiotic stress responses were discussed.

2　Materials and methods

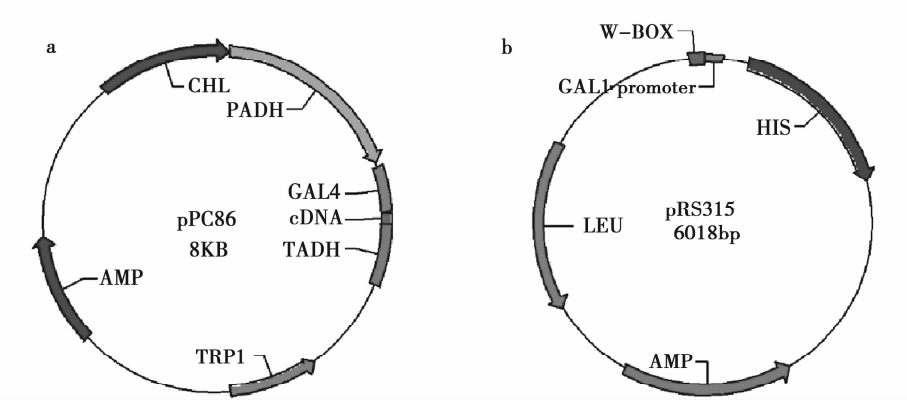
2.1　Materials

Soybean (*Glycine max*) *cv.* Williams 82 was used to construct soybean cDNAs library. Yeast strain *yWAM*2 (Leu −, His −, Trp −), *E. coli* Rosetta, Agrobacterium tumefaciens strain *LBA*4404, vectors pRS315His (Leu +), YepGAP(Trp +) were all reserved in our lab. The Y1H screening was performed following protocols as described previously[19].

2.2　Construction of soybean cDNA library and yeast one-hybrid screening

Soybean cDNAs library were synthesized from 5 μg of soybean root mRNA using the Superscript Plasmid System, and ligated to the shuttle vector pPC86 (Fig. 1a). The vector, was composed of the class W-box sequence in the promoter of *AtNPR*1 assembled to vector pRS315H is as bait vector (Fig. 1b). The reporter constructs were prepared by the cloning of tandems containing 3 repeats of the cis-elements W-box sequence into the bait vector pRS315His (Leu +) *via Xba* I and *Bam*H I sites (Fig. 1b). 3 × W-box sequence were synthesized based on the *AtNPR*1 gene including the sense strand sequence: 5′-CTAGAGTTGACTTGACTTGGTTGACTTGACTTGGTTGACTTGACTTGGATC-3′, and the antisense: 5′-GATCCAAGTCAAGTCAACCAAGTCAAGTCAACCAAGTCAAGTCAACT-3′. The two oligo-nucleotides were annealed to form a double-stranded DNA. Similarly, 3 × mW-box (+): 5′-CTAGAGTCAGCTCAGCTTGGTCAGCTCAGCTTGGTCAG-CTCAGCTTGGATC-3′ and 3 × mW-box (−): 5′-GATCCAAGCTGAGCTGACCAAGCTGAGCTGACCAA-GCTGAGCTGACT-3′ were

synthesized and used for constructing of the rescue vector pRSmW-box (Leu +). Before yeast transformation, constructs with inserts were selected by PCR using primers derived from the vector primers and sequenced using the same primers. For the one-hybrid library screening, the cDNA library were cotransformed in competent yeast cells (pRSW-box contained) and the resulting cells were plated on the appropriate selective medium. 50 μL plasmid of pre-made cDNA library were transformated into yeast compenent cells (pRSW-box contained): 240 μL PEG (50% w/v), 36 μL 1.0M LiAc, 25 μL ss-DNA (2.0 mg·mL^{-1}). Incubated at 30℃ for 30 min, 42℃ for 45 min. After slow rotation (7 000 r·min^{-1}) 15 s, the yeast cells were harvested, resuspended in 1 mL dH_2O and spreaded on plates with selective media (Leu −, His −, Trp −) as well as media (Leu −, His −). Library plasmids were isolated from positive colonies which survived on selective media (Leu −, His −, Trp −) and the genes interacted with W-box were amplified.



a: Indicates schematic illustration of the vector used in cDNA library construction (pPC86-cDNA);
b: Indicates schematic illustration of the vector used in bait vector construction (pRS315-W-box).

Fig. 1 Schematic illustration of the vectors

2.3 Isolation of WRKY cDNA

According to BD SMART RACE protocols, 3 μg of total RNA from soybean were prepared for 3′and 5′ cDNA amplication. 3′ GSP1 primer (5′-GCGAAGTCCAAGCGGCAG-3′) and 5′ GSP1 primer (5′-CGC TGCCGCTTGGACTTCG-3′) were designed according to the cDNA fragments obtained by yeast one-hybrid screening. 5′-RACE and 3′-RACE were performed to achieve full-length cDNA using BD SMART RACE kit (Clontech, Japan). The nucleotide sequence was determined by overlapping clones and confirmed by a full-length cDNA clone.

2.4 Target DNA sequencing and phylogenetic analysis

DNA sequences were determined using the Taq Dye Primer Cycle Sequencing Kit (Amersham) and the ABI 373A automatic sequencer. The sequence was compared and analyzed with the non-redundant database at the National Centre of Biotechnology (NCBI) using BLAST program. Sequence alignment between WRKY and its plant homologs was performed by using ClustalW2 software (http://www.ebi.ac.uk/Tools/msa/clustalw2/). WRKY homologs in other higher plants were used for phylogenetic analysis by the neighbor-joining method with aid of MEGA v3.1 software.

2.5 Activation analysis of *GmWRKY53*

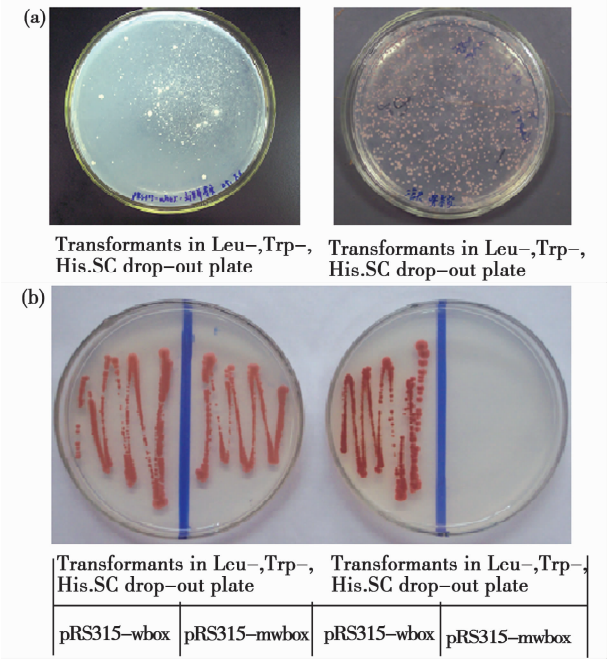
To detect activation of *GmWRKY53* in yeast, DNAs of pRSW-box and pPC86-*GmWRKY53* were co-transformed into the yeast cells, and cultured on the synthetic complete drop-out medium (Trp −, Leu −, His −) to determine the function of *GmWRKY53*.

3 Results and analysis

3.1 Isolation and characterization of WRKY protein from soybean

To isolate WRKY which binded with W-boxing in the promoter of *AtNPR1* gene, a soybean root cDNA library with 90% of recombinants was constructed. The results showed that the library contained more than 5.0 $\times 10^6$ clones and the inserts was over 1.2 kb on average. Based on the W-box motif of *AtNPR1* promoter, 3 × W-box were introduced into the bait vector pRS315His (Leu +) for screening the cDNA library. Four positive clones were isolated, which survived well on the synthetic complete drop-out medium (His −, Trp −, Leu −) (Fig. 2a).

Sequence analysis showed all of them contained WRKY domain and one of them was full-length gene containing 1 020 bp nucleotides encoding 340 amino acids. A BLAST search against GenBank revealed that this protein contained one conserved DNA binding domain of 56 amino acids that matched the probable WRKY transcription factor 53 of soybean named as GmWRKY53 (Fig. 3). Amino acid sequence analysis showed that GmWRKY53 had one WRKY domain with a FLYWCH-type zinc finger-containing protein. A homology search of GenBank databases showed that GmWRKY53 has 57.6% similarity with PtWRKY53 protein (*Populus trichocarpa*) as well as 56.4% similarity with PeWRKY41 (*Populus euphratica*). However, when compared with AtWRKYs, GmWRKY53 showed lower similarity of 41.8%. We also carried out a systematic phylogenetic analysis between GmWRKY53 and the other 8 WRKY proteins from different species using the ClustalW2 program. The 9 proteins were approximately divided into 3 groups (Fig. 3), suggesting that the functional of WRKY transcription factors manifestly diversited in evolution.



a: Indicates, grown on the drop-out plate (Trp –, Leu –) and the drop-out plate (His –, Trp –, Leu –) by screening; b: Indicates yeast cells carrying the wild type (W-box) or the mutated W-box (mW-box) sequence were transformed and grown on drop-out plate.

Fig. 2　Yeast transformants growth on plates

```
TcWRKY30            QASYEKALSMLNYNTS------LAADQPQPSGLAIR-MSESPP-SRSGSPRSE-DSDRDF 97
RcWRKY              LSSYEKALSMLNLSR-------LITKEPLPTGVATNGMPESPPLSLSGSPRSDQDSDRDF 100
PtWRKY53            LASYERALLILNWGGS------MGQPQ---SVGVSAGVPESPI-SINGSSRSDEFDGGVK 96
PeWRKY41            LASYERALLILNWGGS------MGQPQ---SVGVSAGVPESPI-SINGSSRSDEFDGGVK 96
TcWRKY              LSSYEKALLILKMNRP------VGQPQQ--NVGATSGVPESPL-STNGSPRSDDLE---K 94
PREDICTEDWRKY53     LSSYEKALLILRCSNA------STSELQGMNQATPTLLPESPL-SVHGSPLREDVHGAIK 99
AtWRKY41            VSSFKKAILMLNGS---TTQHNPTIELAPDPLAHPGKVPGSPA-SITGNPRSEEFFNVRS 108
AlWRKY41            VSSLEKAILMLNGS---TTQHIPTTELALDLLANPGKVPESPA-SITESLRSEELFDVGS 106
AlWRKY53            VSSYERFLLLLNWSSSPTVQLIPTPATVVP-VANSGSVPESPA-SINGSPRSEEFADGGG 118
                     :* :: : :*. .                      :. **  *   .   :

TcWRKY30            K-----EQELKDASKKRKTLPRWTQLVRVTPGTALEGPLDDGFSWRKYGQKDILGAKYPR 152
RcWRKY              K-----DQDPKDVSRKRTSTARWTKQVRVNPGMGLEGPLDDGFSWRKYGQKDILGARYPR 155
PtWRKY53            D-----NQGYNEASKKRKTTPRWTDHVRVSPENGLEGPHDDGYSWRKYGQKDILGAKYPR 151
PeWRKY41            D-----NQGYNEASKKRKTTPRWTDHVRVSPENGLEGPHDDGYSWRKYGQKDILGAKYPR 151
TcWRKY              D-----NQDIRDVSKKRKMLPRWTDQVRVSSESLLEGPHDDGYSWRKYGQKDILGAKYPR 149
PREDICTEDWRKY53     D-----HHN----SKKRKITPKWMDRVRVSCESGLEGPHEDGYNWRKYGQKDILGAKYPR 150
AtWRKY41            -------KEFNLSSKKRKMLPKWTEQVRISPERGLEGPHDDIFSWRKYGQKDILGAKFPR 161
AlWRKY41            -------KDCNLSSKKRKMLPKWTEQVRISPERGLEGPHDGVFSWRKYGQKDILGAKYPR 159
AlWRKY53            SSESHHRQDYIFNPKKRKMLPKWSEKVRISPERGLEGPQDDVFSWRKYGQKDILGAKFPR 178
                           :     .:**.  .:* . **:.    **** :. :.***********::**

TcWRKY30            GYYRCTHRNVQGCLATKQVQRSDDDPTIFEITYRGRHTCTVASHVMPPSGPSESQEQ-GT 211
RcWRKY              GYYRCTHRIVQGCLATKQVQRSDEDPTIFEVTYRGRHTCTQMLHYNPQLPENINQETNSS 215
PtWRKY53            SYYRCTYRNTQNCWATKQVQRSDEDPTIFEITYRGTHTCAHGNQS--IPSPSSPEKQEKK 209
PeWRKY41            SYYRCTYRNTQNCWATKQVQRSDEDPTIFEITYRGTHTCAHGNQS--IPSPSSPEKQEKK 209
TcWRKY              SYYRCTYRNTQNCWATKQVQRSDEDPAIFEITYRGTHTCALGNQAA-VPPPASPEKQEHK 208
PREDICTEDWRKY53     SYYRCTFRSTQGCWATKQVQRSDEDPTMFDITYRGNHTCSQGNNA--VLPPKSPEKHEKP 208
AtWRKY41            SYYRCTFRNTQYCWATKQVQRSDGDPTIFEVTYRGTHTCSQG-----IPLP--------E 208
AlWRKY41            SYYRCTFRNTQYCWATKQVQRSDGDPTIFEVTYRGTHTCSQG-----IPPP--------K 206
AlWRKY53            SYYRCTHRSTQNCWATKQVQRSDSDATVFEVTYRGTHTCSQV-----ITPPPPPASPEKK 233
                    .*****.* .* * ********* *.::*::**** ***:
```

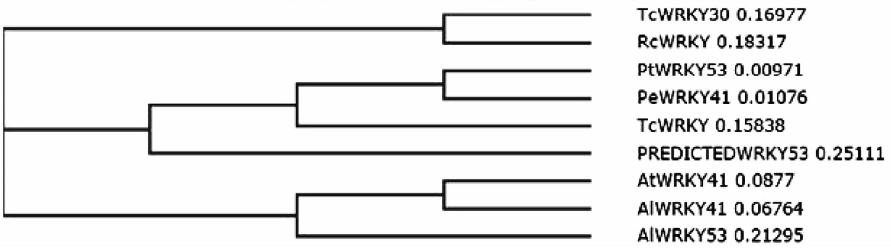


Fig. 3　The amino acid sequence of GmWRKY53 was aligned against WRKYs from different species with ClustalW2 software

3.2 GmWRKY53 specifically binds to W-box

In order to validate the specific combination of protein-DNA interactions, yeast reporter cells were transformed with pPC86-*GmWRKY*53 and pRSW-box. The cells survived well on the synthetic complete drop-out medium (His -, Trp -, Leu -) indicating that the GmWRKY53 protein could bound to W-box and activate expression of HIS3. For further confirmation of the association between GmWRKY53 and W-box, the mutated version (3 × mW-box) were cloned into the bait vector pRS315His (Leu +). As a result, yeast reporter cells contained GmWRK53 and 3 × mW-box could not survive on the drop-out medium (His -, Trp -, Leu -) (Fig. 2b, right panel), indicating that GmWRKY53 could bind to the W-box element in yeast specifically.

4 Conclusion and discussion

Many previous studies have shown that WRKY TFs are involved in process of responsing to biotic and abiotic stresses in soybean. Researches demonstrated that GmWRKY13, GmWRKY21, GmWRKY54[17] and GsWRKY20[18], participated in salt stress, cold stress and drought tolerance or other biotic stress responses. This will not only bring an exciting foreground for plant genetic engineering of stress tolerance, but also provide new gene targets for marker-assisted selection. To isolate *WRKY* genes from soybean, yeast one-hybrid method was used with 3 × W-box as a bait and one full-length gene was obtained from a soybean root cDNA library, four positive clones containing WRKY domain have been fetched and *GmWRKY*53 have been cloned. The results showed *GmWRKY*53 coding a WRKY protein with a FLYWCH-type zinc finger domain. A homology search of GenBank databases showed that GmWRKY53 has 40.49% similarity with AlWRKY53 protein (*Arabidopsis lyrata* subsp. *lyrata*). Hu et al[20] found that *AtWRKY*53 accompanied with *AtWRKY*46, *AtWRKY*70 involved in SA-signaling pathway and demonstrated negative cross-regulation resistance to *P. syringae* among these three genes. These results indicate that WRKY protein plays overlapping and synergetic roles in plant basal defense. To further confirm the binding specificity between GmWRKY53 and W-box, the W-box sequence was modified and cloned into the bait vector pRS315His. As a result, yeast reporter cells could not survive on the drop-out medium (His -, Trp -, Leu -) (Fig. 1b, right panel). NPR1 is a key transcriptional co-regulator in plant defense responses. It promotes efficient expression of defense response genes following infection and prevents spurious activation of defensive responses in the absence of infection[21]. A large body of evidence indicates that WRKY transcription factors increase the expression levels of the *NPR*1 gene in the defense response[22]. Considering the W-box was refered to the *AtNPR*1 promoter, we speculate it might play an important role in the AtNPR1-regulated pathway. However, more experiments are needed to test this hypothesis. Furthermore, Tripathi et al[23] found that NaCl stress led to inducibility of responsive soybean *GmWRKY*53 promoter as well as ABA and JA treatments utilizing tobacco Bright Yellow 2 (BY-2) cell system. Taken together, these results indicated that *GmWRKY*53 gene may play an important role in plants during abiotic stress. However, the regulation mechanism of the defense response mediated by WRKY transcription factors is fairly complex, and further research is needed.

Reference

[1] Kasuga M, Liu Q, Miura S, et al. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor [J]. Nature Biotechnology, 1999, 17(3): 287-291.

[2] Riechmann J L, Ratcliffe O J. A genomic perspective on plant transcription factors [J]. Current Opinion in Plant Biology, 2000, 3(5): 423-434.

[3] Singh K B, Foley R C, Oñate-Sánchez L. Transcription factors in plant defense and stress responses [J]. Current Opinion in Plant Biology, 2002, 5(5): 430-436.

[4] Zhang Y, Wang L. The WRKY transcription factor superfamily: Its origin in eukaryotes and expansion in plants [J]. BMC Evolutionary Biology, 2005, 5(1): 1.

[5] Rushton P J, Somssich I E, Ringler P, et al. WRKY transcription factors [J]. Trends in Plant Science, 2010, 15(5): 247-258.

[6] Ren X, Chen Z, Liu Y, et al. ABO3, a WRKY transcription factor, mediates plant responses to abscisic acid and drought tolerance in *Arabidopsis* [J]. The Plant Journal, 2010, 63(3): 417-429.

[7] Lippok B, Birkenbihl R P, Rivory G, et al. Expression of *AtWRKY*33 encoding a pathogen-or PAMP-responsive WRKY transcription factor is regulated by a composite DNA motif containing W box elements [J]. Molecular Plant-microbe Interactions, 2007, 20(4): 420-429.

[8] Rushton D L, Tripathi P, Rabara R C, et al. WRKY transcription factors: Key components in abscisic acid signalling [J]. Plant Biotechnology Journal, 2012, 10(1): 2-11.

[9] Chen H, Lai Z, Shi J, et al. Roles of Arabidopsis WRKY18, WRKY40 and WRKY60 transcription factors in plant responses to abscisic acid and abiotic stress [J]. BMC Plant Biology, 2010, 10(1): 281.

[10] Qiu Y, Jing S, Fu J, et al. Cloning and analysis of expression profile of 13 *WRKY* genes in rice [J]. Chinese Science Bulletin, 2004, 49(20): 2159-2168.

(下转第 981 页)

点开发和性状的分子标记选择提供了重要信息,为分子标记辅助育种奠定基础。

参考文献

[1] Corder E H, Saunders A M, Risch N J, et al. Protective effect of polio protein E type 2 allele for late on set Alzheimer disease [J]. Nature Genetics,1994,7:180-184.

[2] Chapman A, Pantalone V R, Ustun A, et al. Quantitative trait loci for agronomic and seed quality traits in an F_2 and $F_{4:6}$ soybean population[J]. Euphytica, 2003, 129: 387-393.

[3] Meksem K, Njiti V N, Banz W J, et al. Genomic regions that underlie soybean seed isoflavone content[J]. Journal of Biomedicine and Biotechnology, 2001, 1:38-45.

[4] Jun T, van K, Kim M Y, et al. Association analysis using SSR markers to find QTL for seed protein content in soybean [J]. Euphytica, 2008,162:179-191.

[5] Shi A, Chen P, Zhang B, et al. Genetic diversity and association analysis of protein and oil content in food-grade soybeans from Asia and the United States [J]. Plant Breeding, 2010, 3 (129): 250-256.

[6] 齐照明, 侯萌, 韩雪, 等. 东北地区大豆主栽品种油分蛋白含量的关联分析 [J]. 中国油料作物学报, 2014,36(2):168-174. (Qi Z M, Hou M, Han X, et al. Correlation analysis in northeast soybean cultivars oil protein content[J]. Chinese Journal of Oil Crop Science, 2014,36(2): 168-174.)

[7] Department of Human Genetics University of Chicago./http://pritch. bsd. uchicago. edu/structure. html/2010-2-2.

[8] Edward Buckler Lab. http://www. maizegenetics. net/bioinformatics/ta-ssel,2011-10-22.

[9] 文自翔, 赵团结, 郑永战, 等. 中国栽培和野生大豆农艺品质性状与 SSR 标记的关联分析:1. 群体结构及关联标记[J]. 作物学报, 2008, 34(7): 1169-1178. (Wen Z X, Zhao T J, Zheng Y Z, et al. Association analysis of agronomic and quality traits with SSR markers in *Glycine max* and *Glycine soja* in China: I. Population structure and associated markers[J]. Acta Agronomica Sinica, 2008, 34(7): 1169-1178.)

[10] Reinprecht Y, Poysa V, Yu K, et al. Seed and agronomic QTL in low linolenic acid, lipoxygenase-free soybean (*Glycine max* (L.) Merrill) germplasm[J]. Genome, 2006, 49(12):1510-1527.

[11] 张军, 赵团结, 盖钧镒. 中国东北大豆育成品种遗传多样性和群体遗传结构分析 [J]. 作物学报, 2008, 34(9): 1529-1536. (Zhang J, Zhao T J, Gai J Y. Genetic diversity and genetic structure of soybean cultivar population released in Northeast China [J]. Acta Agronomica Sinca, 2008, 34(9): 1529-1536.

[12] Panthee D R, Pantalone V R, West D R, et al. Quantitative trait loci for seed protein and oil concentration, and seed size in soybean[J]. Crop Science, 2005, 445(5):2015-2022.

[13] Qi Z M, Wu Q, Han X, et al. Soybean oil content QTL mapping and integrating with meta-analysis method for mining genes[J]. Euphytica, 2011, 179:499-514.

[14] Rossi M E, Orf J, Liu L J, et al. Genetic basis of soybean adaptation to North American *vs.* Asian mega-environments in two independent populations from Canadian × Chinese crosses [J]. Theoretical and Applied Genetics, 2013, 126(7):1809-1823.

[15] Kim H K, Kim Y C, Kim S T, et al. Analysis of quantitative trait loci (QTLs) for seed size and fatty acid composition using recombinant inbred lines in soybean[J]. Life Science, 2010, 20(8): 1186-1192.

[16] Chen Q S, Zhang Z C, Liu C Y, et al. QTL analysis of major agronomic traits in soybean [J]. Agricultural Sciences in China, 2007 (4):399-405.

[17] Lu W, Wen Z, Li H, et al. Identification of the quantitative trait loci (QTL) underlying water soluble protein content in soybean [J]. Theoretical and Applied Genetics, 2012, 126(2). Doi: 10. 1007/s00122-012-1990-8.

(上接第 976 页)

[11] Niu C F, Wei W, Zhou Q Y, et al. Wheat WRKY genes *TaWRKY2* and *TaWRKY19* regulate abiotic stress tolerance in transgenic *Arabidopsis* plants [J]. Plant, Cell & Environment, 2012, 35(6):1156-1170.

[12] Berri S, Abbruscato P, Faivre-Rampant O, et al. Characterization of WRKY co-regulatory networks in rice and *Arabidopsis*[J]. BMC Plant Biology,2009, 9(1):120.

[13] Wang Q, Wang M, Zhang X, et al. WRKY gene family evolution in *Arabidopsis thaliana*[J]. Genetica,2011, 139(8):973-983.

[14] van Aken O, Zhang B, Law S, et al. *AtWRKY40* and *AtWRKY63* modulate the expression of stress-responsive nuclear genes encoding mitochondrial and chloroplast proteins [J]. Plant Physiology, 2013, 162(1):254-271.

[15] Shultz J L, Kurunam D, Shopinski K, et al. The Soybean Genome Database (SoyGD): A browser for display of duplicated, polyploid, regions and sequence tagged sites on the integrated physical and genetic maps of *Glycine max* [J]. Nucleic Acids Research, 2006, 34(Suppl 1):D758-D765.

[16] Yin G, Xu H, Xiao S, et al. The large soybean (*Glycine max*) WRKY TF family expanded by segmental duplication events and subsequent divergent selection among subgroups [J]. BMC Plant Biology,2013, 13(1):148.

[17] Zhou Q Y, Tian A G, Zou H F, et al. Soybean WRKY-type transcription factor genes, *GmWRKY13*, *GmWRKY21*, and *GmWRKY54*, confer differential tolerance to abiotic stresses in transgenic Arabidopsis plants [J]. Plant Biotechnology Journal,2008, 6(5):486-503.

[18] Luo X, Bai X, Sun X, et al. Expression of wild soybean WRKY20 in *Arabidopsis* enhances drought tolerance and regulates ABA signalling [J]. Journal of Experimental Botany, 2013, 8 (64):2155-2169.

[19] Zhang L, Wang X, Bi Y, et al. Isolation and functional analysis of transcription factor GmWRKY57B from soybean [J]. Chinese Science Bulletin,2008, 53(22):3538-3545.

[20] Hu Y, Dong Q, Yu D. *Arabidopsis* WRKY46 coordinates with WRKY70 and WRKY53 in basal resistance against pathogen *Pseudomonas syringae*[J]. Plant Science,2012, 185:288-297.

[21] Mukhtar M S, Nishimura M T, Dangl J. NPR1 in plant defense: It's not over'til it's turned over [J]. Cell, 2009, 137(5): 804-806.

[22] Fountain J C, Raruang Y, Luo M, et al. Potential roles of WRKY transcription factors in regulating host defense responses during *Aspergillus flavus* infection of immature maize kernels [J]. Physiological and Molecular Plant Pathology,2015, 89:31-40.

[23] Tripathi P, Rabara R C, Lin J, et al. *GmWRKY53*, a water-and salt-inducible soybean gene for rapid dissection of regulatory elements in BY-2 cell culture [J]. Plant Signaling & Behavior,2013, 8(5): e24097-e24102.