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Identification and characterization of hemp WRKY transcription factors in response to abiotic stresses

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Abstract

Plant *WRKY* genes encode a complex and ancient class of zinc-finger transcription factors that are involved in multiple biological processes, especially in regulating defense against abiotic stresses. Despite a growing number of studies on the genomic organization of the *WRKY* gene family in various species, little information is available about this family in hemp (*Cannabis sativa* L.). In this study, based on the hemp genome sequence, 40 hemp *WRKY* (*CsWRKY*) genes were classified into three main groups and five subgroups according to their orthologs in *Arabidopsis*. Among these, 23, 15, and 14 *CsWRKY* genes were responsive to drought, NaCl, and Cd stress, respectively. Interestingly, the expressions of all of the 23 drought stress-responsive genes were up-regulated. Moreover, 18 *CsWRKY* genes were induced by abscisic acid (ABA) treatment. A total of six up-regulated genes related to all three stresses were identified. Among these, five were up-regulated, and one was down-regulated by ABA. These results indicate a diverse function of the *CsWRKY* genes, which provides a basis for future clarification of their function in hemp tolerance to abiotic stresses.

Additional key words: abscisic acid, cadmium, *Cannabis sativa*, drought, phylogenetic analysis, salinity.

Introduction

Plants have evolved unique strategies to cope with and adapt to adverse environmental conditions. Plant stress responses are mediated by complex signal transduction networks associated with distinct changes in gene expression controlled by transcription factors (Wang *et al.* 2014). Transcription factors exhibit sequence-specific DNA binding and can activate or inhibit transcription of downstream target genes. The WRKY protein family is one of the largest families of transcription regulators and is named after its characteristic protein sequence. In the WRKY domain, a conserved WRKYGQK hexapeptide sequence is usually followed by a C_2H_2- or C_2HC-type zinc finger motif (Eulgem *et al.* 2000).

In plants, WRKY proteins form a large family of transcription factors involved in responses to various abiotic stresses such as salinity, drought, and cold (Du and Chen 2000, Karam *et al.* 2002, Xie *et al.* 2006). Furthermore, WRKYs from rice (Rushton *et al.* 2010, Shimono *et al.* 2012), soybean (Zhou *et al.* 2008), chili pepper (Oh *et al.* 2008), and poplar (Levee *et al.* 2009) were shown to be positive and/or negative regulators in the defense against abiotic stresses. To date, the *WRKY* gene family has been analyzed in many plants, including *Arabidopsis thaliana* (Eulgem *et al.* 2000, Ulker and Somssich 2004), *Oryza sativa* (Wu *et al.* 2005, Ross *et al.* 2007, Ramamoorthy *et al.* 2008), *Cucumis sativus* (Ling *et al.* 2011), *Populus trichocarpa* (He *et al.* 2012), and *Arabidopsis lyrata* (Song and Gao 2014).

Hemp is an economically important plant used in the production of food, fiber, oil, and intoxicants (Gilmore and Peakall 2003). Drought stress is the main environmental factor that influences hemp growth and development (Schäfer and Honermeier 2006, Amaducci *et al.* 2008, Mihoc *et al.* 2012). Therefore, investigating the hemp regulation mechanism under drought and other

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Abbreviations: ABA - abscisic acid; PlantTFDB - plant transcription factor database; qPCR - quantitative polymerase chain reaction.
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stress conditions will have a very important role in breeding programs. In recent years, hemp genome sequencing (Bakel *et al.* 2011) and molecular marker development (Gao *et al.* 2014) have greatly advanced its molecular biological study. However, no hemp transcription factors have been identified until now. Therefore, in this study, hemp *WRKY* genes (*CsWRKY*) were selected and analyzed in detail including their classification, phylogeny, and conserved motif composition. Further, the expression of *CsWRKY* genes was identified under various abiotic stresses.

Materials and methods

Identification and phylogenetic analysis of the *WRKY* gene family: To identify WRKY proteins in hemp, amino acid sequences were obtained from the *Plant Transcription Factor Database v. 3.0* (*PlantTFDB*; Jin *et al.* 2014), and the genes were annotated using *The Cannabis Genome Browser* (http://genome.ccbr.utoronto.ca/). The *Arabidopsis* WRKY protein sequences were downloaded from *The Arabidopsis Information Resource* (*TAIR*), and the *WRKY* genes in hemp (*CsWRKY*) and *A. thaliana* (*AtWRKY*) were aligned using *Clustal X 2.1* (Larkin *et al.* 2007). Phylogenetic and molecular evolutionary analyses were conducted using *MEGA v. 6.0* (Tamura *et al.* 2013). An unrooted phylogenetic tree was constructed by the neighbor-joining method with a bootstrap test (1 000 replicates) using *MEGA 6.* The *CsWRKY* genes were classified into groups based on the *AtWRKY* classification. We used the *MEME v. 4.9.1* online program (Bailey *et al*. 2009) to identify conserved motifs of *CsWRKY* sequences.

Plants, growth, and treatments: The seeds of *Cannabis sativa* L. cv. Yunma 1 (a typical hemp cultivar in China) were germinated in pots with soil, and eight-week-old plants were subjected to three stress treatments: drought, salinity, and cadmium. The plants were exposed to drought stress by maintaining the relative soil water content at a maximum of 30 %. For salinity and abscisic acid (ABA) treatments, the plant roots were submerged in 100 mM NaCl and 50 μM ABA solutions, respectively. For heavy metal treatment, a solution of $CdCl_2$ was used to reach a final soil Cd content of 100 mg(Cd) kg^{-1}(soil). After 3 d (drought, NaCl, and Cd treatments) or 6 h (ABA treatment), whole hemp plants (three replicates for each treatment and the control) were sampled and immediately frozen in liquid nitrogen and stored separately at -80 °C.

Isolation of RNA, cDNA synthesis, and real-time quantitative PCR: The total RNA of each sample was isolated using an *EZNA* plant RNA kit (*Omega Bio-Tek*, Norcross, GA, USA). Syntheis of cDNA was carried out using *M-MLV* reverse transcriptase (*Fermentas*, Vilnius, Lithuania). Real time qPCR was conducted in an *iQ5 multicolor* real-time PCR system (*Bio-Rad*, Hercules, CA, USA). A reference gene (ID: 351134416) was used as an endogenous control. A list of primers used in real time qPCR is presented in Table 2 Suppl. Each treatment consisted of three biological replicates. The expression levels were calculated as the mean signal intensity across the three replicates.

Results

In total, 49 genes named *CsWRKY1* to *CsWRKY49* were identified from the amino acid sequences downloaded from *PlantTFDB*. Of those, *CsWRKY2*, *CsWRKY18*, and *CsWRKY26* were redundant, and *CsWRKY9*, *CsWRKY33*, *CsWRKY36*, *CsWRKY39*, *CsWRKY42*, and *CsWRKY46* did not contain an intact WRKY domain structure. The parameters that describe the *CsWRKY* proteins are listed in Table 1 Suppl., and they include the deduced protein length, relative molecular mass, and isoelectric point. The difference of this series of parameters means that different *CsWRKY* proteins may operate in different micro-environments.

The amino acid sequences of 40 *CsWRKYs* and 72 *AtWRKYs* were aligned in *Clustal X 2.1* (Larkin *et al*. 2007). On the basis of *AtWRKY* classification (Eulgem *et al.* 2000) and WRKY domain alignment, the *CsWRKYs* were classified into three major groups. Nine *CsWRKYs*, each of which contained two WRKY domains and the C_2H_2-type zinc-finger motif (C-X_4-C-X_{22-23}-H-X_1-H) were assigned to Group I. Twenty-seven *CsWRKY* proteins possessing a single WRKY domain were assigned to Group II, in which the C_2H_2-type zinc-finger structure was C-X_{4-5}-C-X_{23}-H-X_1-H. Group II was further divided into five subgroups (II-a to II-e) based on the primary amino-acid sequence. Finally, four *CsWRKYs*, each with a single WRKY domain, were assigned to Group III. Interestingly, only the *CsWRKY25* domain differed in one amino acid in the conserved WRKY signature (CRKYGQK) (Table 1 Suppl.).

An unrooted phylogenetic tree was constructed using the amino acid sequence alignment (Fig. 1). The N- and C-terminals of Group I WRKY domains were classified into two independent domains, I-N and I-C. The other clades were named Groups IIa, IIb, IIc, IId, IIe, and III. In

addition, Groups IId + IIe were closely related to Group III. The phylogenetic tree constructed from the WRKY domain of these genes resolved 20 distinct motifs (Fig. 2). Most of the closely related members in the phylogenetic tree showed a common motif composition. Motif 1 was shared by all members, motifs 10, 15, and 16 were dispersed only in Group IId, and subgroups IIa and IIb were closely related and shared motif 17. These results suggest that there were functional similarities among the WRKY proteins within the subgroups.

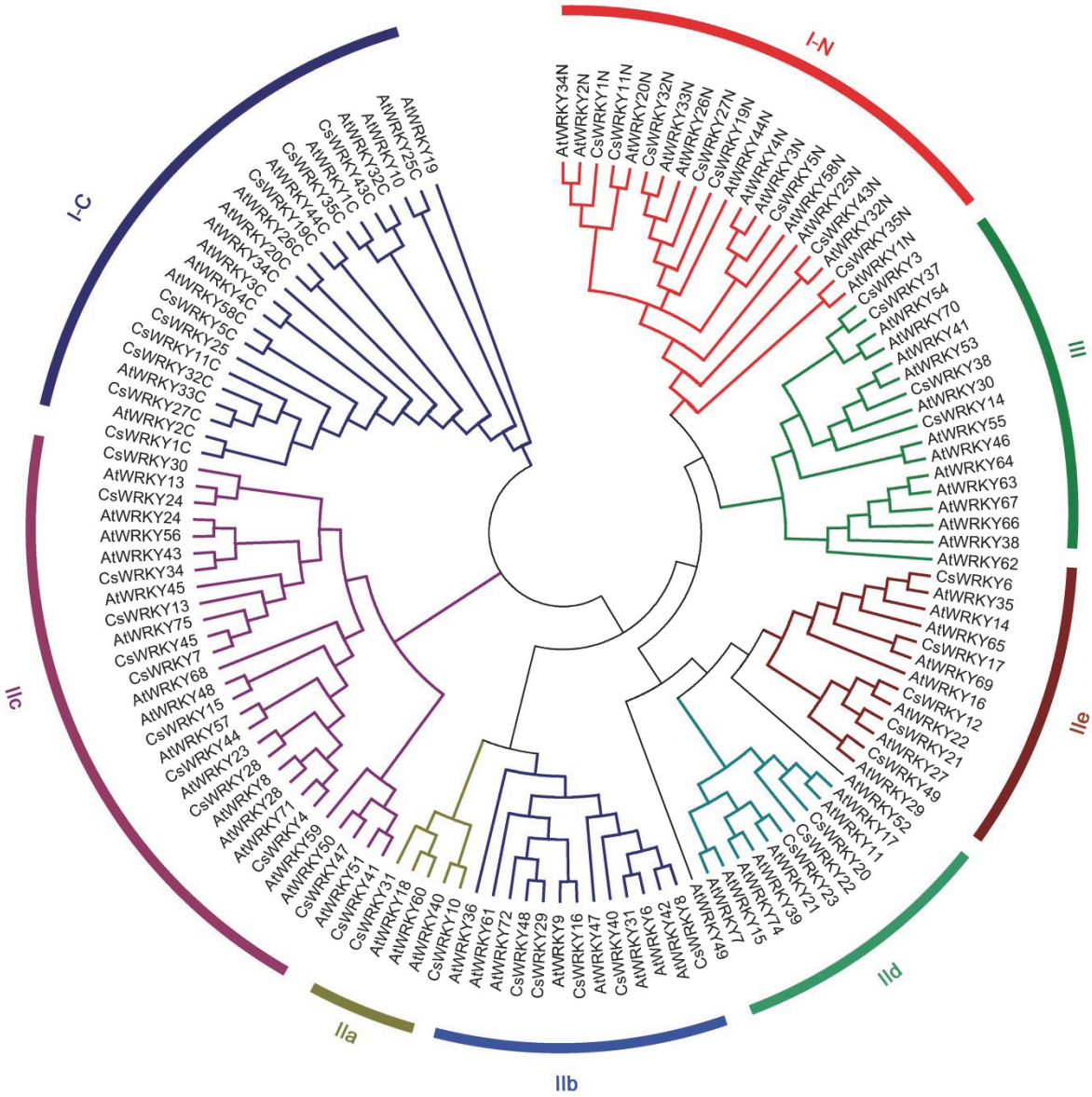

Fig. 1. An unrooted phylogenetic tree showing relationships among WRKY domains of hemp and *Arabidopsis*. The suffix "N" or "C" in Group I proteins indicates the N- or C-terminal WRKY domains.

The number of *CsWRKY* genes belonging to each group was compared with that in other plant species (Table 1). The number of genes in Group I of the WRKY family in hemp was similar to those recorded for *Arabidopsis*, grapevine, and castor bean (Ulker and Somssich 2004, Li *et al.* 2012, Wang *et al.* 2014) but differed greatly from the one in poplar (He *et al.* 2012). Similar to the groups observed in poplar and tomato (He *et al.* 2012, Huang *et al.* 2012), subgroup IIc was the most abundant subgroup of Group II. The number of *CsWRKYs* genes in Group III in hemp was less than that in rice, bread wheat, and barley (Xie *et al.* 2006, Mangelsen *et al.* 2008, Okay *et al.* 2014). A similar number of *CsWRKY* genes was found in castor bean, poplar, tomato, cucumber, and grapevine (Ling *et al.* 2011, He *et al.* 2012, Huang *et al.* 2012, Li *et al.* 2012,

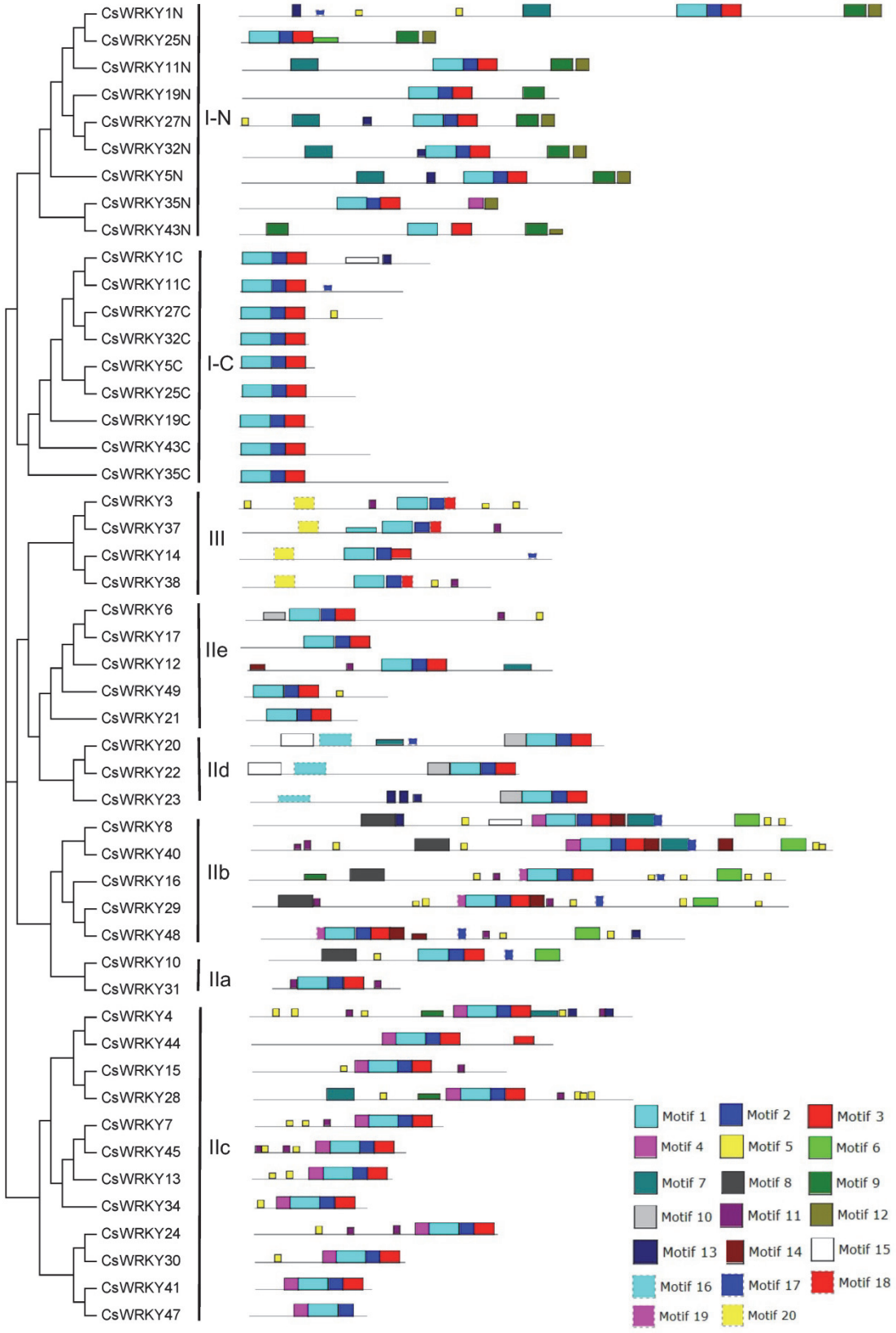

Fig. 2. A phylogenetic tree of deduced *CsWRKY* domains associated with the motif composition in the amino-acid sequences.

Table 1. The size of the WRKY groups and subgroups in different plant species. NG - non-grouped.

	Group I	Group II					Group III	NG	Total	Reference
		IIa	IIb	IIc	IId	IIe				
Hemp	9	2	5	12	3	5	4		40	
Canola	12	2	5	8	7	6	3		43	Yang *et al*. (2009)
Barley	8	4	1	11	5	3	13		45	Mangelsen *et al*. (2008)
Castor bean	9	3	10	12	3	5	5		47	Li *et al*. (2012)
Cucumber	10	4	4	16	8	7	6		55	Ling *et al*. (2011)
Grapevine	12	3	8	15	6	7	6	2	59	Wang *et al*. (2014)
Arabidopsis	16	3	8	17	7	8	14	1	74	Ulker and Somssich (2004)
Tomato	15	5	8	16	6	17	11	3	81	Huang *et al*. (2012)
Rice	15	4	8	15	7	11	36		96	He *et al*. (2012)
Poplar	50	5	9	13	13	4	10		104	Xie *et al*. (2006)
Bread wheat	20	16	3	34	17	11	57	2	160	Okay *et al*. (2014)

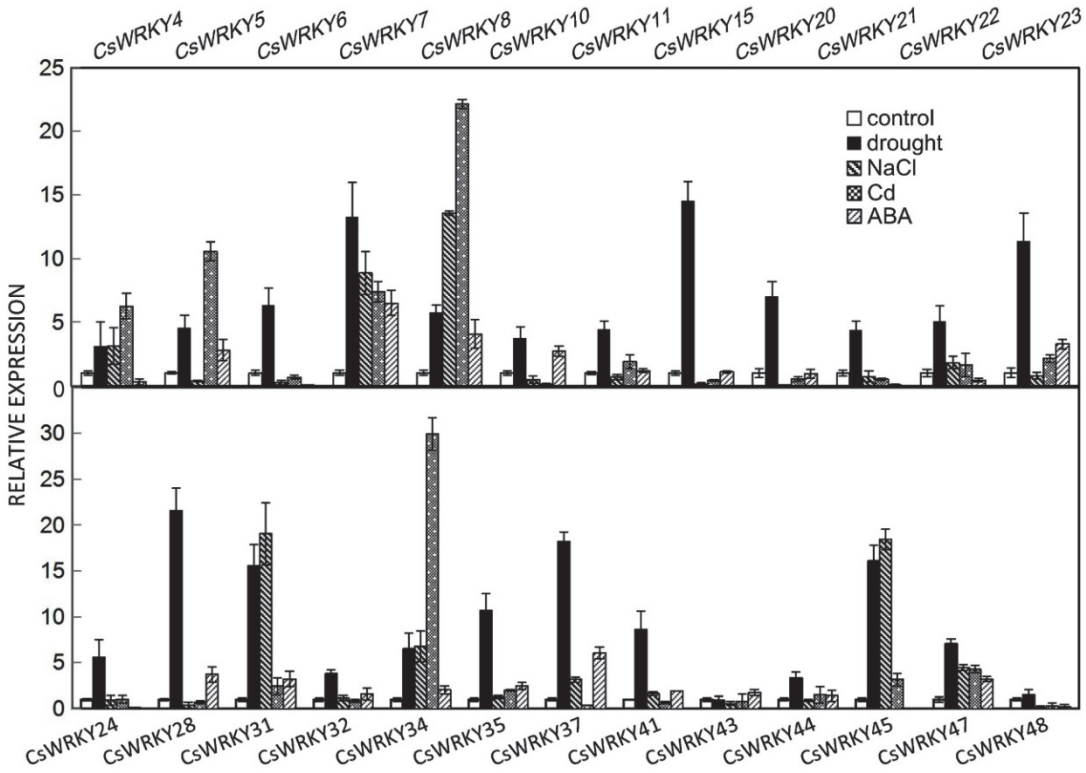

Fig. 3. The expression pattern of stress-responsive *CsWRKY* genes. The x-axis shows the control, drought-stress, NaCl-stress, Cd-stress, and ABA treatment. The y-axis shows the relative expression level under stress-treatment to the expression of the control, which was designed as one unit. The *bars* represent standard errors of three independent assays of real time qPCR.

Wang *et al*. 2014).

The expression of *CsWRKY* genes was altered significantly (fold change > 2) in the plants exposed to drought, NaCl, and Cd. Among the 40 predicted genes, 23, 15, and 14 genes were responsive to the drought, NaCl, and Cd, respectively (Fig. 3). Furthermore, the number of genes with more than 10-fold increase in response to the drought, NaCl, and Cd stress was 8, 3, and 3, respectively, compared to the control plants. Importantly, six genes were identified related to all the three abiotic stresses (Table 1 Suppl.).

To further investigate the response of *CsWRKY* genes to ABA, real time qPCR was performed after the ABA treatment. The results indicate that 18 genes were affected significantly by ABA. Among these genes, 17, 10, and 12 genes were related to the drought, NaCl, and Cd, respectively. Of the six genes related to all the three abiotic stresses, five were up-regulated and one was down-regulated by the ABA treatment.

Table 2. The expressions of *CsWRKY* genes under different treatments (drought, NaCl, and Cd). Some genes up-regulated or down-regulated by those treatments were also up-regulated or down-regulated by the ABA treatment.

Treatments	Up-regulated	Down-regulated	Total	Up-regulated by ABA	Down-regulated by ABA	Total
Drought	23	0	23	11	6	17
NaCl	8	7	15	6	4	10
Cd	8	6	14	8	4	12

Discussion

WRKY proteins are classical transcription factors that are ubiquitous in plants and have been surveyed in several species. In this study, 40 *WRKY* genes were identified for the first time in hemp and classified into three major groups indicating their close evolutionary relationship. Group I of *CsWRKYs* contained two WRKY domains and accounted for 22.5 % of the entire *CsWRKY* family. This proportion was similar to that in *Arabidopsis* (21.6 %), grapevine (20.34 %), and castor bean (19.15 %), whereas Group I has undergone significant expansion in poplar (48.08 %) (Table 1). The close relationship between subgroups IIa and IIb and between subgroups IId and IIe suggests that they may belong to similar genes (Kumar *et al.* 2011). Group III *CsWRKYs* contained the motif 20 (Fig. 2), and there were fewer these proteins in hemp than in rice, bread wheat, and barley (only 10 % of the entire *CsWRKY* family). Similar proportions were detected in castor bean, poplar, tomato, cucumber, and grapevine. These findings show that there are large differences in the size and distribution of *WRKY* genes between species.

The involvement of transcription factors in regulation of plant responses to abiotic stress has been described in many species (Liu *et al*. 2015, Song *et al*. 2015). In this study, 25 *CsWRKY* genes showed a differential expression in response to at least one abiotic stress indicating that these genes may play an important role in stress responses in hemp. Drought stress strongly influences hemp growth and production (Schäfer and Honermeier 2006, Amaducci *et al.* 2008, Mihoc *et al.* 2012), which was also suggested in our finding that 23 *CsWRKY* genes were up-regulated under the drought stress. In plants, responses to drought involve multiple metabolic pathways (Zhou *et al.* 2008). The up- and down-regulation of *CsWRKY* genes under the NaCl and Cd stresses (Table 2) reveal that the *CsWRKY* genes may be involved in different regulatory networks. Research on rice *WRKY* genes has confirmed the presence of two co-regulated networks in abiotic stress responses (Berri *et al.* 2009). Here, six *CsWRKY* genes were up-regulated by all the three abiotic stresses indicating that they play an important role in stress response in hemp.

Signal networks involve the binding of signaling molecules and ligands, triggering events inside the cell. Abscisic acid mediates abiotic stress responses in plants. Among the genes responsive to ABA treatment, 64.7 % (11/17), 60.0 % (6/10), and 66.7 % (8/12) were up-regulated under the drought, NaCl, and Cd stresses, respectively (Table 2). These results indicate that ABA plays an important role in the response of hemp to abiotic stresses. The up-regulation of numerous genes in combination with each of the imposed stresses may reflect crosstalk and redundancy intrinsic to abiotic stress (Rushton *et al.* 2010).

In conclusion, we identified 40 hemp *WRKY* genes for the first time and analyzed the expressions of these genes in response to the abiotic stresses. Twenty-five *CsWRKY* genes were identified associated to the abiotic stresses and six *CsWRKY* genes were selected in response to all the three abiotic stresses. These results provide a platform for further investigations of the function of the *WRKY* gene family and improvement of hemp tolerance to abiotic stresses in the future.

References

Amaducci, S., Zatta, A., Pelatti, F., Venturi, G.: Influence of agronomic factors on yield and quality of hemp (*Cannabis sativa* L.) fiber and implication for an innovative production system. - Field Crop Res. **107**: 161-169, 2008.

Bailey, T.L., Boden, M., Buske, FA., Frith, M., Grant, C.E., Clementi, L., Ren, J., Li, W.W., Noble, W.S.: MEME SUITE: tools for motif discovery and searching. - Nucl. Acids Res. **37** (Web server issue): W202-W208, 2009.

Bakel, H.V., Stout, J.M., Cote, A.G., Tallon, C.M., Sharpe, A.G., Hughes, T.R., Page, J.E.: The draft genome and transcriptome of *Cannabis sati*va. - Genome Biol. **12**: R102, 2011.

Berri, S., Abbruscato, P., Rampant, O.F., Brasileiro, A.C., Fumasoni, I., Satoh, K., Kikuchi, S., Mizzi, L., Morandini, P., Pe, M.E., Piffanelli, P.: Characterization of WRKY co-regulatory networks in rice and *Arabidopsis*. - BMC Plant Biol. **9**: 120, 2009.

Du, L., Chen, Z.: Identification of genes encoding receptor like protein kinases as possible targets of pathogen- and salicylic acid-induced WRKY DNA-binding proteins in *Arabidopsis*.

- Plant J. **24**: 837-847, 2000.

Eulgem, T., Rushton, P.J., Robatzek, S., Somssich, I.E.: The WRKY superfamily of plant transcription factors. - Trends Plant Sci. **5**: 199-206, 2000.

Gao, C., Xin, P., Cheng, C., Tang, Q., Chen, P., Wang, C., Zang, G., Zhao, L.: Diversity analysis in *Cannabis sativa* based on large-scale development of expressed sequence tag-derived simple sequence repeat markers. - PLoS ONE **9**: e110638, 2014.

Gilmore, S., Peakall, R.: Isolation of microsatellite markers in *Cannabis sativa* L. (marijuana). - Mol. Ecol. Notes **3**: 105-107, 2003.

He, H., Dong, Q., Shao, Y., Jiang, H., Zhu, S., Cheng, B., Xiang, Y.: Genome-wide survey and characterization of the WRKY gene family in *Populus trichocarpa*. - Plant Cell Rep. **31**: 1199-1217, 2012.

Huang, S., Gao, Y., Liu, J., Peng, X., Niu, X., Fei, Z., Cao, S., Liu, Y.: Genome-wide analysis of WRKY transcription factors in *Solanumlycopersicum*. - Mol. Genet. Genomics **287**: 495-513, 2012.

Jin, J., Zhang, H., Kong, L., Gao, G., Luo, J.: *PlantTFDB 3.0*: a portal for the functional and evolutionary study of plant transcription factors. - Nucl. Acids Res. **42**: D1182-D1187, 2014.

Karam, B.S., Rhonda, C.F., Luis, O.S.: Transcription factors in plant defense and stress response. - Curr. Opin. Plant Biol. **5**:430-436, 2002.

Kumar, R., Tyagi, A.K., Sharma, A.K.: Genome-wide analysis of auxin response factor (ARF) gene family from tomato and analysis of their role in flower and fruit development. - Mol. Genet. Genomics **285**: 245-260, 2011.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G.: *Clustal W* and *Clustal X version 2.0*. - Bioinformatics **23**: 2947-2948, 2007.

Levee, V., Major, I., Levasseur, C., Tremblay, L., MacKay, J., Seguin, A.: Expression profiling and functional analysis of *Populus* WRKY23 reveals a regulatory role in defense. - New Phytol. **184**: 48-70, 2009.

Li, H.L., Zhang, L.B., Guo, D., Li, C.Z., Peng, S.Q.: Identification and expression profiles of the WRKY transcription factor family in *Ricinus communis*. - Gene **503**: 248-253, 2012.

Ling, J., Jiang, W.J., Zhang, Y., Yu, H.J., Mao, Z.C., Gu, X.F., Huang, S.W., Xie, B.Y.: Genome-wide analysis of WRKY gene family in *Cucumis sativus*. - BMC Genomics **12**: 471, 2011.

Liu, X.Q., Zhu, J.J., Wei, C.J., Guo, Q., Bian, C.K., Xiang, Z.H., Zhao, A.C.: Genome-wide identification and characterization of the DREB transcription factor gene family in mulberry. - Biol. Plant. **59**: 253-265, 2015.

Mangelsen, E., Kilian, J., Berendzen, K.W., Kolukisaoglu, U.H., Harter, K., Jansson, C., Wanke, D.: Phylogenetic and comparative gene expression analysis of barley (*Hordeum vulgare*) WRKY transcription factor family reveals putatively retained functions between monocots and dicots. - BMC Genomics **9**: 194, 2008.

Mihoc, M., Pop, G., Alexa, E., Radulov, I.: Nutritive quality of Romanian hemp varieties (*Cannabis sativa* L.) with special focus on oil and metal contents of seeds. - Chem. Cent. J. **6**: 122, 2012.

Oh, S.K., Baek, K.H., Park, J.M., Yi, S.Y., Yu, S.H., Kamoun, S., Choi, D.: *Capsicum annuum* WRKY protein CaWRKY1 is a negative regulator of pathogen defense. - New Phytol. **177**: 977-989, 2008.

Okay, S., Derelli, E., Unver, T.: Transcriptome-wide identification of bread wheat WRKY transcription factors in response to drought stress. - Mol. Genet. Genomics **289**: 765-781, 2014.

Ramamoorthy, R., Jiang, S.Y., Kumar, N., Venkatesh, P.N., Ramachandran, S.: A comprehensive transcriptional profiling of the WRKY gene family in rice under various abiotic and phytohormone treatments. - Plant Cell Physiol. **49**: 865-879, 2008.

Ross, C.A., Liu, Y., Shen, Q.J.: The WRKY gene family in rice (*Oryza sativa*). - J. integr. Plant Biol. **49**: 827-842, 2007.

Rushton, P.J., Somssich, I.E., Ringler, P., Shen, Q.J.: WRKY transcription factors. - Trends Plant Sci. **15**: 247-258, 2010.

Schäfer, T., Honermeier, B.: Effect of sowing date and plant density on the cell morphology of hemp (*Cannabis sativa* L.). - Ind. Crop Prod. **23**: 88-98, 2006.

Shimono, M., Koga, H., Akagi, A.Y., Hayashi, N., Goto, S., Sawada, M., Kurihara, T., Matsushita, A., Sugano, S., Jiang, C.J., Kaku, H., Inoue, H., Takatsuji, H.: Rice WRKY45 plays important roles in fungal and bacterial disease resistance. - Mol. Plant Pathol. **13**: 83-94, 2012.

Song, Y., Gao, J.: Genome-wide analysis of WRKY gene family in *Arabidopsis lyrata* and comparison with *Arabidopsis thaliana* and *Populus trichocarpa*. - Chin. Sci. Bull. **59**: 754-765, 2014.

Song, Z.Z, Yang, Y., Ma, R.J., Xu, J.L., Yu, M.L.: Transcription of potassium transporter genes of KT/HAK/KUP family in peach seedlings and responses to abiotic stresses. - Biol. Plant. **59**: 65-73, 2015.

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

Ulker, B., Somssich, I.E.: WRKY transcription factors: from DNA binding towards biological function. - Curr. Opin. Plant Biol. **7**: 491-498, 2004.

Wang, M., Vannozzi, A., Wang, G., Liang, Y., Tornielli, G.B., Zenoni, S., Cavallini, E., Pezzotti, M., Cheng, Z.M.: Genome and transcriptome analysis of the grapevine (*Vitis vinifera* L.) WRKY gene family. - Hort. Res. **1**: 16, 2014.

Wu, K.L., Guo, Z.J., Wang, H.H., Li, J.: The WRKY family of transcription factors in rice and *Arabidopsis* and their origins. - DNA Res. **12**: 9-26, 2005.

Xie, Z., Zhang, Z.L., Zou, X., Yang, G., Komatsu, S., Shen, Q.J.: Interactions of two abscisic-acid induced WRKY genes in repressing gibberellin signaling in aleurone cells. - Plant J. **46**: 231-242, 2006.

Zhou, Q.Y., Tian, A.G., Zou, H.F., Xie, Z.M., Lei, G., Huang, J., Wang, C.M., Wang, H.W., Zhang, J.S., Chen, S.Y.: Soybean WRKY-type transcription factor genes, *GmWRKY13*, *GmWRKY21*, and *GmWRKY54*, confer differential tolerance to abiotic stress in transgenic *Arabidopsis* plants. - Plant Biotechnol. J. **6**: 486-503, 2008.