Genome–Wide Analysis and Expression Pattern of the AP2/ERF Gene Family in Kiwifruit under Waterlogging Stress Treatment

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Abstract**—***APETALA2/ethylene response factor (AP2/ERF) transcription factors play important roles in the response to abiotic stresses. It is now possible to identify all of the AP2/ERF genes in the kiwifruit genome because the kiwifruit genome project has been completed. 183 AP2/ERF genes were identified and compared with AP2/ERF genes from Arabidopsis in this study. The 183 AP2/ERF kiwifruit genes were classified into four subfamilies: DREB (64), ERF (94), AP2 (19) and RAV (5), as well as one soloist. RNA-sequence and Quantitative RT-PCR (qRT-PCR) analysis results showed that 20 genes were responsive to waterlogging stress, suggesting that AP2/ERF transcription factors play important roles in the response to waterlogging stress in kiwifruit*

Keywords—AP2/ERF, kiwifruit, waterlogging stress.

I. INTRODUCTION

The APETALA2/ethylene response factor (AP2/ERF) superfamily is defined by the AP2/ERF domain which consists of about 60 to 70 amino acids, and can be classified into at least five subfamilies: AP2 (containing two repeated AP2/ERF domains), DREB (dehydration responsive element binding, containing a single AP2/ERF domain), ERF (containing a single AP2/ERF domain), RAV (containing a B3domain and a single AP2/ERF domain), and others(Zhang et al. 2012b). The ERF and DREB subfamilies can be further divided into six subfamilies: the B1–6 subfamilies and A1–6 subfamilies, respectively(Zhang et al. 2012b). It has been demonstrated that AP2/ERF play important roles in the plant cell cycle, growth and development, as well as the response to biotic and abiotic stresses (Zhang et al. 2012b).

Waterlogging is one of the most common stresses affecting plant growth and development. Many important crop plants are sensitive to waterlogging conditions caused by heavy rain. Waterlogging and submergence conditions impose a variety of challenges on the plants(Hinz et al. 2010). Previous research has shown that the AP2/ERF genes play an important role in the regulation of gene expression during waterlogging stress. RAP2.2 is induced in shoots by ethylene and functions in an ethylene-controlled signal transduction pathway and the overexpression of RAP2.2 resulted in improved plant survival under hypoxia (low-oxygen) stress, whereas lines containing T-DNA knockouts of the gene had poorer survival rates than the wild type(Hinz et al. 2010). The RAP2.2 gene plays a significant role in the metabolic adaptation to flooding stress in Arabidopsis(Hinz et al. 2010). The SUB1A-1 allele could reduce elongation growth and carbohydrate consumption, and to confer submergence tolerance (Fukao 2006; Xu et al. 2006; Jung et al. 2010). Flooding sensitive japonica cultivar over-expressing SUB1A -1 could increase ADH1 expression and flooding tolerance(Fukao 2006; Xu et al. 2006). Four ERF subfamily genes in Arabidopsis, namely RAP2.2 (At3g14230), RAP2.12 (At1g53910), HRE1 (At1g72360), and HRE2 (At2g47520) have been documented that play important role in the response to hypoxia (Hinz et al. 2010; Licausi et al. 2010). HRE1 over-expressing plants showed an increased activity in the fermentative enzymes pyruvate decarboxylase and alcohol dehydrogenase together with increased ethanol production under hypoxia, showed an improved tolerance of anoxia (Licausi et al. 2010). RAP2.2 was induced in shoots by ethylene and functions in an ethylene-controlled signal transduction pathway(Hinz et al. 2010). Overexpression of RAP2.2 resulted in improved plant survival under hypoxia stress, whereas lines containing T-DNA knockouts of the gene had poorer survival rates than the wild type(Hinz et al. 2010). Deepwater rice requires SNORKEL1 (SK1) and SK2 ERF transcription factors to elongate stem internodes and extend the hollow stems to the water surface for survival (Hattori et al. 2009). Du et al. reported (Du et al. 2014) that 38 of 184 AP2/ERF transcript factor genes were responsive to waterlogging stress and 25 genes were ERF subfamily.

Kiwifruit is a major fruit worldwide. However, the majority of currently growing kiwifruit cultivars, like ‘Hongyang’, are susceptible to waterlogging stress in East China. A thorough knowledge of kiwifruit resistance mechanisms will help to limit crop loss due to waterlogging stress, and to decrease the economic losses. It’s known to us that AP2/ERF transcript factor play important roles in the response to waterlogging stresses. It is possible to identify the AP2/ERF genes in many species as these plant genome projects have been completed. Previous studies showed that there are 147, 184, 132, 200, and 116 AP2/ERF genes in the Arabidopsis (Nakano 2006) , maize(Du et al. 2014), grapevine(Zhuang et al. 2009), poplar(Zhuang et

al. 2008), and Chinese plum (Du et al. 2013), respectively. However, few reports of the AP2/ERF superfamily are available in kiwifruit. Kiwifruit genome projects have been completed(Huang and al. 2013). In this article, AP2-like genes from kiwifruit were surveyed and comparatively analyzed. Here, 183 AP2/ERF transcription factors were identified from the kiwifruit genome database and the transcriptome sequencing database (Zhang et al., 2015). The expressions of kiwifruit AP2/ERF genes under waterlogged stress were performed. These analyses will be valuable to isolate and understand the molecular mechanism of AP2/ERF genes responded to waterlogging stress in kiwifruit.

II. MATERIAL AND METHOD

2.1 Plant Material and Treatment

The kiwifruit cultivars 'Jinkui' were obtained from the Institute of Botany, Jiangsu Province & Chinese Academy of Sciences, China. Plants were grown in pots containing a 2:1 mixture of garden soil and vermiculite without any added fertilizer, and were maintained in a plant growth chamber. The growth chamber conditions were: relative humidity of ~ 60%, 160μmol m^{-2} s^{-1} PAR, photoperiod of 12 h light/12 h dark for 24 h, and 25°C average temperature. Seedlings, already grown to the 8–10 node stage were selected for uniformity. The waterlogging treatment was performed as described previously (Yin et al. 2009a). The pots were flooded by standing in a 28 cm×14 cm×14 cm container filled with tap water to 2.5 cm above the level of the soil surface. The tap water had a pH of 7.3. The water temperature was held at ~25°C. Roots were sampled at 0, 24, 48, and 96 h after treatment, frozen in liquid nitrogen and stored at -80°C, which were later used for quantitative real time-PCR (qRT–PCR) for studying the expression profile of AP2 TF genes. Each treatment was repeated three times and there were 10 plants per treatment in every biological replication.

2.2 Database

Kiwifruit Genome Database (http://bioinfo.bti.cornell.edu/cgi-bin/kiwi/home.cgi) and transcriptome sequencing database (NCBI, Accession number: SRR2048539) were mined to identify members of the AP2/ERF. The amino acid sequence of the AP2/ERF domain (IYRGVRQRNSGKWVSEVREPNKKTRIWLGTFQTAEMAARAHDVAALALRGRSACLNF) from Arabidopsis AtCBF1 (also named AtDREB1B; accession number AT4G25490) was used as queries to search against the databases from Kiwifruit Genome Database Web site (http://bioinfo.bti.cornell.edu/cgi-bin/kiwi/home.cgi) using the BLASTP program at the e-value of 1e-3 to avoid false positives.

2.3 Phylogenetic Tree Construction

Phylogenetic trees of the aligned kiwifruit AP2/ERF protein sequences were constructed using MEGA version 5.0 (Tamura et al. 2011)(http://www.megasoftware.net) via the neighborjoining (NJ) method with the following parameters: Poisson correction, pairwise deletion, and bootstrap (1,000 replicates; randomseed).

2.4 Differentially expressed analysis of AP2/ERF family genes under waterlogging stress

We have previously conducted the transcriptome sequencing of kiwifruit under waterlogging. The number of reads per kilobase per exon region in a given gene per million mapped fragments (RPKM) was performed to identify AP2/ERF family genes are regulated by waterlogging stress(Chen et al. 2014). Differentially expressed AP2/ERF family genes of 0h and 96h were identified by the R program(Chen et al. 2014). We applied the Pearson's chi-squared test to assess the lane effect. The p-value was computed for each gene. The Benjamini–Hochberg false discovery rate (FDR) was then applied to correct the results for the q-value. The FDR method is generally used in deep-sequencing studies to identify over-representative AP2/ERF family genes (Junttila et al. 2013). If the FDR-adjusted q-value was ≤ 0.05, the AP2/ERF family genes were considered to be differentially expressed. Heatmap were performed using the software of MEV (Multi Experiment Viewer). Color scale represents reads per kilobase per million normalized log2 transformed counts where blue indicates low level and red indicate high level.

2.5 Quantitative RT-PCR (qRT-PCR) Analysis

Quantitative RT-PCR (qRT-PCR) was used to determine the expression of AP2/ERF family genes in kiwifruit under waterlogging. Total RNA isolation, DNase I treatment, First strand cDNA synthesise, and qRT-PCR assay were performed as described by Zhang et al (Zhang et al. 2012a). The relative levels of genes to control *AdActin* (Yin et al. 2009b; Yin et al. 2012) mRNAs were analysed using the ABI-7300 system software and the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). Gene-specific primers used in the qRT-PCR were listed in Table 1. Data analyses were conducted using SPSS version 17.0 statistical software. For all analyses, the level of significance between different time points was set at $P<0.05$. Heatmap were

performed using the software of MEV (Multi Experiment Viewer). Color scale represents $\log 2^{-\Delta\Delta Ct}$ counts where blue indicates low level and red indicate high level.

TABLE 1
PRIMERS FOR THIS PAPER

Gene Name	Forward primer	Reverse primer
RAV1	GCTTTTCCCGTTCAGGTCCAG	ACACCCAAATCCCAACATCTCC
RAV2	TCGGCGGGAAGAAACAATGC	GGTATCACCAGCCTGTTCAGC
RAV3	TTTGAAGGCGGGCGATGTC	ACTACTATCAACACCACCACTCAC
ERF1	CACCCCAACTTTGCCCCTAG	CCTCCTCTTCCGTGCTGAAC
ERF2	CTGGCTCGGAACATTTGATTCTG	CTCCTCACACCCATACTTCATCTC
ERF3	TGAAGGTGCCGAGCCAAAC	GCAGCGGAAGAATCAGTACAGG
ERF4	CGTTACAGAGGCGTGAGGAAG	TGGCGGTGGCAATGAGTTC
ERF5	GCCAGAGCAGCACCATCG	CGGAGGAAGAATCGGAGTCG
ERF6	GGAAAGTATGCGGCGGAAATC	GTACGCTCTCGCTGCTTCG
ERF7	GGGGTGACTTGCCGTTAAATTAC	GAGCCGTTGTCGTGGATGG
ERF8	AGGAAGCAGCAGGGGAAGAG	CAGCACCACAGCCGATGAC
ERF9	CGACCTCATCCGCCAACAC	GCAAGAACCGATTGATTCAGAGC
ERF10	CTACGATAGAGCCGCCTTCAAG	TCACCTAACCACACCTTCTTCAC
ERF11	CGTTTGATACGGCGGAGGAG	TCGGGCTCTGATTACAATGACTC
ERF12	TGGGAGATGGGTGGCTGAG	ATGAAATTCGTGCGAGTGTTGG
ERF13	GTTCTCGGCTTCTCTCTACGC	ATTCTCCTGTTTGTCTCCCTTCG
DREB1	AAGTGGGTTTGCGAGGTAAGAG	TCATTGGAATCCGTGGAAGCC
DREB2	GCTCCAACTCACTCTCGTCAAC	TCTGGGCTCTGGGTCTTGC
DREB3	CTGGCAAAATGGGGCAATCTG	TCAAGAAATCAAGACCGCAATCG
DREB4	ACGGAGGAAGGCGATAGAGG	ATGGCTTGAACCCAGAAGAAGG
AdActin	TGCATGAGCGATCAAGTTTCAAG	TGTCCCATGTCTGGTTGATGACT

III. RESULTS AND DISCUSSION

3.1 Identification and Prediction of Kiwifruit AP2/ERF Transcription Factors

A total 180 kiwifruit AP2/ERF genes were downloaded from Kiwifruit Genome Database, and 91 kiwifruit AP2/ERF genes were obtained from the results of transcriptome sequencing. However, 88 of the 91 AP2/ERF genes were the same with those from kiwifruit genome database. So, a total of 183 kiwifruit AP2/ERF genes were obtained.

183 kiwifruit AP2/ERF genes were classified into the DREB, ERF, AP2 and RAV subfamilies, and one soloist based on alignment of the AP2/ERF domain from kiwifruit and *Arabidopsis* (Table 2). We compared the AP2/ERF genes from kiwifruit, grapevine, *Arabidopsis*, rice and maize (Table 2). The number of AP2/ERF genes of kiwifruit, grapevine, *Arabidopsis*, maize and rice were 183, 132, 147, 184, and 164, respectively. In kiwifruit, 64 AP2/ERF genes were classified into the DREB subfamily, compared with 36, 57, 51 and 52 in grapevine, *Arabidopsis*, rice and maize, respectively; 94 kiwifruit AP2/ERF genes were classified into the ERF subfamily, compared with 73, 65, 107 and 79 in grapevine, *Arabidopsis*, rice and maize respectively. 19 kiwifruit AP2/ERF genes were predicted to encode proteins with two AP2/ERF domains, and were classified into the AP2 subfamily. In comparison, grapevine, Arabidopsis, rice and maize contain 18, 18, 22 and 26, respectively. Five kiwifruit AP2/ERF genes were predicted to encode proteins with an AP2/ERF domain and a B3 domain, and were classified into the RAV subfamily, compared to 4, 6, 3 and 7 in grapevine, *Arabidopsis*, rice and maize,

respectively. The remaining kiwifruit AP2/ERF gene (Achn205451) has a low homology with other AP2/ERF genes. Therefore, this gene was designated as a soloist.

TABLE 2
SUMMARY OF THE AP2/ERF FAMILY AMONG KIWIFRUIT, GRAPEVINE, ARABIDOPSIS, RICE AND MAIZE

AP2/ERF family	Group	Eudicot			Monocot	
Classification		Kiwifruit	Grapevine	Arabidopsis	maize	Rice
DREB subfamily	A1	10	7	6	10	10
	A2	9	4	8	5	4
	A3	0	0	1	1	1
	A4	27	13	16	11	15
	A5	14	7	16	12	13
	A6	4	5	10	12	9
	Total	64	36	57	51	52
ERF subfamily	B1	19	7	15	31	16
	B2	7	3	5	20	16
	B3	27	37	18	18	18
	B4	4	4	7	10	9
	B5	12	4	8	6	6
	B6	25	18	12	22	14
	Total	94	73	65	107	79
AP2 subfamily		19	18	18	22	26
RAV subfamily		5	4	6	3	7
Solosist		1	1	1	1	0
Total AP2/ERF genes		183	132	147	184	164
Total putative genes		39040	30434	26819	39656	38000
The percentage of AP2/ERF family genes (%)		0.46875	0.4337	0.5481	0.4640	0.4315
Genome size (Mb)		616	487	125	2500	430
The average number of AP2/ERF family genes per Mb		0.297	0.271	1.176	0.0736	0.3814

At the whole-genome level, the average number of AP2/ERF genes per Mb in kiwifruit is 0.297, which is more than in grapevine (0.271) and maize (0.0736), but less than in rice (0.3814) and Arabidopsis (1.176). The percentage of the AP2/ERF gene family in kiwifruit is 0.46875 %, which is more than in grapevine (0.4337 %), rice (0.4315 %) and maize (0.4640%), but less than in Arabidopsis (0.581 %, Table 2).

3.2 Phylogenetic Relationships Analysis

Phylogenetic trees of the DREB (Fig. 1) and ERF (Fig. 2) subfamilies in kiwifruit were constructed. A total of 64 DREB subfamily genes distributed into the A1–A2 and A4-A6 groups in kiwifruit; A1, A2, A4, A5 and A6 contain 10, 9, 27, 14 and 4 genes, respectively (Table 1). 94 genes belonging to the ERF subfamily in kiwifruit distributed into the B1–B6 groups; B1, B2, B3, B4, B5 and B6 contain 19, 7, 27, 4, 12, 25 genes (Table 1), respectively. Lastly, 19 genes were classified into the AP2 subfamily (Fig. 3) and five genes into the RAV subfamily (Fig. 4).

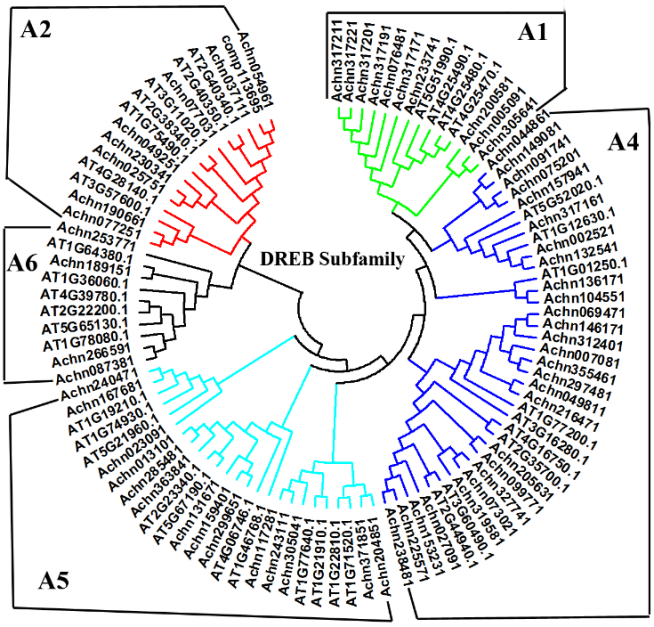

FIG. 1. THE PHYLOGENETIC TREE OF DREB SUBFAMILY GENES FROM KIWIFRUIT AND ARABIDOPSIS.

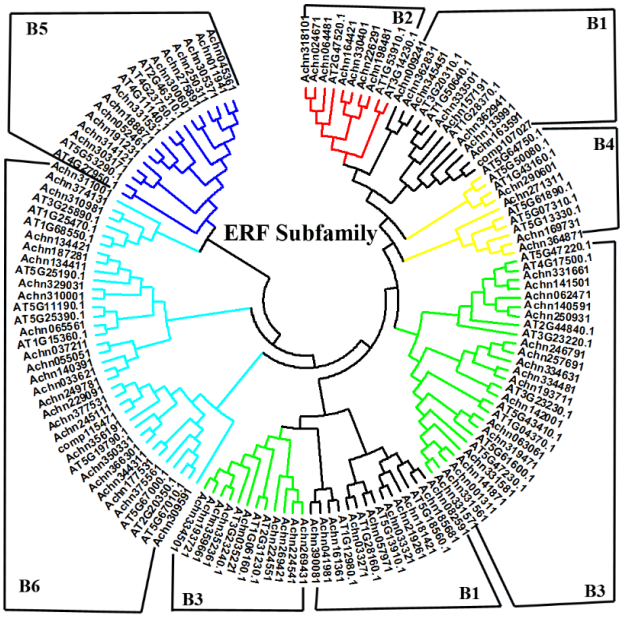

FIG. 2. THE PHYLOGENETIC TREE OF ERF SUBFAMILY GENES FROM KIWIFRUIT AND ARABIDOPSIS

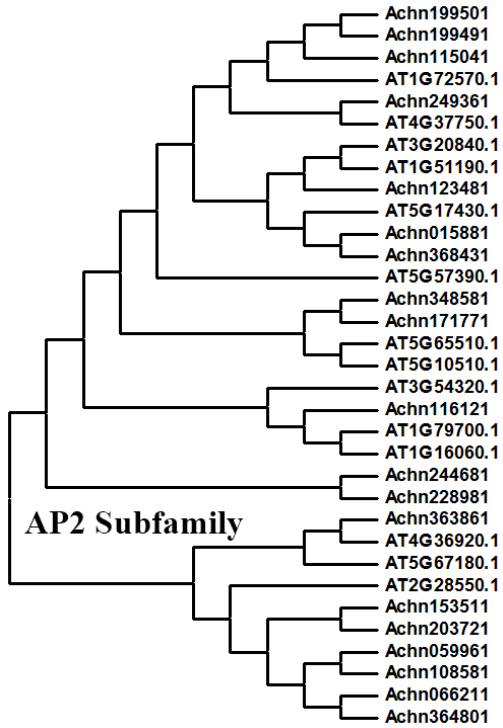

FIG. 3. THE PHYLOGENETIC TREE OF AP2 SUBFAMILY GENES FROM KIWIFRUIT AND ARABIDOPSIS

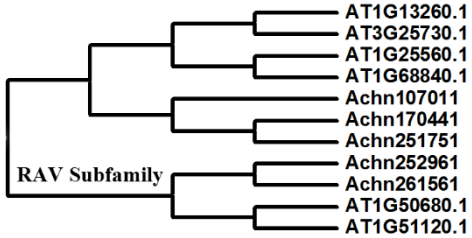

FIG. 4. THE PHYLOGENETIC TREE OF RAV SUBFAMILY GENES FROM KIWIFRUIT AND ARABIDOPSIS

3.3 Expression Analysis of AP2/ERF Family Genes under Waterlogging Stress

RNA-sequencing was conducted previously (Data not show) and the results showed that the expression levels of 20 AP2/ERF genes were changed (Table 3, Fig. 5A). RAV subfamily were 3, named RAV1, RAV2 and RAV3. 13 AP2/ERF genes (named ERF1 to ERF13) in ERF subfamily were responsive to waterlogging stress. B1, B2, B3, and B6 subgroups were 4, 2, 4, and 3, respectively. DREB subfamily was 4 (named DREB1 to DREB4). A1, A2, A3, and A4 subgroups had 1, respective (Table 3, Fig. 5A). The expression levels of 20 AP2/ERF waterlogging stress responsive genes are diverse. There

are 9 genes and their expression levels are decreased under 96 h waterlogging condition. There are 11 genes and their expression levels are increased under 96 h waterlogging treatment.

TABLE 3

EXPRESSION OF AP2/ERF TRANSCRIPT FACTOR GENES IN KIWIFRUIT UNDER WATER LOGGING TREATMENT

Name id for transcription	Name id for genome*	Sample CK	Sample T4	Fold_change (log2(T4/ck))	p_value	q_value	Identity (%)	Category	Name id
comp100639_c0_seq2	achn251751	11.16	73.01	2.710	7.70E-11	5.47E-09	98	RAV	RAV1
comp100786_c0_seq3	achn170441	17.79	40.54	1.188	6.10E-03	9.18E-02	100	RAV	RAV2
comp104656_c0_seq4	achn107011	26.91	2.99	-3.170	1.98E-05	6.22E-04	99	RAV	RAV3
comp81900_c0_seq1	achn219261	5.87	10.68	0.863	3.79E-01	1.00E+00	99	ERF-B1	ERF1
comp1127686_c0_seq1	achn352361	0.76	0.26	-1.547	1.00E+00	1.00E+00	96	ERF-B3	ERF2
comp249351_c0_seq1	achn064481	1.7	33.2	4.288	3.74E-07	1.64E-05	100	ERF-B2	ERF3
comp107027_c0_seq1	/	345.07	80.48	-2.100	5.41E-39	1.70E-36	/	ERF-B1	ERF4
comp110156_c0_seq1	achn133991	249.75	102.14	-1.290	6.77E-16	7.40E-14	99	ERF-B1	ERF5
comp79023_c0_seq1	achn140391	15.57	65	2.062	1.35E-07	6.38E-06	96	ERF-B6	ERF6
comp102502_c0_seq1	achn140591	14.61	34.05	1.221	1.09E-02	1.47E-01	96	ERF-B3	ERF7
comp67160_c0_seq1	achn318101	2.62	762.96	8.186	3.01E-161	4.26E-158	99	ERF-B2	ERF8
comp110147_c0_seq3	achn319471	130.03	18.1	-2.845	1.65E-20	2.45E-18	97	ERF-B3	ERF9
comp104372_c0_seq3	achn331571	14.07	2.09	-2.751	5.32E-03	8.21E-02	96	ERF-B3	ERF10
comp87832_c0_seq1	achn362941	29.67	206.84	2.801	2.50E-29	5.65E-27	96	ERF-B1	ERF11
comp117167_c1_seq1	achn366301	31.84	1.08	-4.882	1.39E-07	6.55E-06	97	ERF-B6	ERF12
comp115471_c0_seq1	/	13.14	0.32	-5.360	1.07E-03	2.15E-02	/	ERF-B6	ERF13
comp48535_c0_seq1	achn317191	3.4	12.49	1.877	4.83E-02	4.61E-01	90	DREB-A1	DREB1
comp95477_c0_seq1	achn007081	8.38	1.03	-3.024	3.46E-02	3.60E-01	99	DREB-A4	DREB2
comp113695_c0_seq3	/	13.35	111.17	3.058	1.56E-17	1.88E-15	/	DREB-A2	DREB3
comp95463_c0_seq2	achn363841	4.52	18.98	2.070	6.73E-03	9.97E-02	92	DREB-A5	DREB4

http://bioinfo.bti.cornell.edu/cgi-bin/kiwi/home.cgi

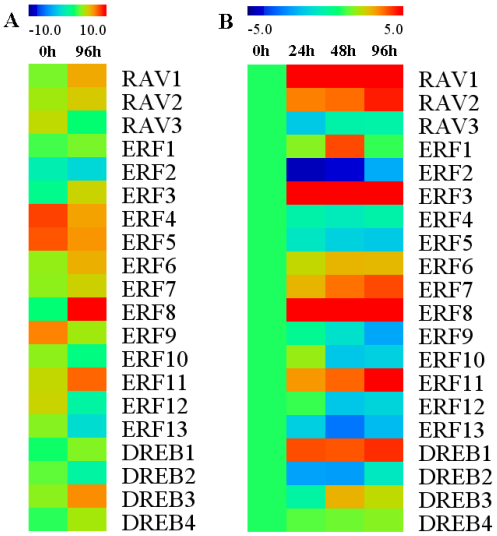

FIG. 5. Heatmap show expression level of 20 AP2/ERF genes in the root of kiwifruit after waterlogging stress. A: The expression was measured by RNA-seq analysis under 0 and 96 h waterlogging stress, respectively. Color scale represents reads per kilobase per million normalized log2 transformed counts where blue indicates low level and red indicate high level. B: The expression was measured by qRT-PCR. Color scale represents $log2^{-\Delta\Delta Ct}$ counts where blue indicates low level and red indicate high level.

At the same time, qRT-PCR analyses of 20 AP2/ERF genes were performed in kiwifruit roots treated by waterlogging at 0h, 24h, 48h and 96h. The results indicated that all of 20 AP2/ERF genes exhibited similar expression kinetics to those obtained from the RNA sequencing analysis (Fig. 5B) at 96 h after waterlogging treatment. During the first 96 h after waterlogging treatment, the expression levels of RAV2, ERF7, ERF8, ERF11, DREB1 and DREB2 were strongly induced (Fig. 5B). Expression levels of RAV3, ERF2, ERF4, ERF5, ERF13, and DREB2 were decreased during the first 96 h after

waterlogging treatment. ERF9, ERF10 and ERF12 genes expression were induced at 24h after treatment and then decreased, and the expression levels of those genes were lower than those base levels between 48 h and 96 h after waterlogging treatment (Fig. 5B). Expression of RAV1, ERF1, ERF3, ERF6, and DREB3 genes rapid increased, peaking 48h after treatment with waterlogging in roots of kiwifruit, and then decreased, and the expression levels of those genes were higher than those base levels (Fig. 5B).

IV. DISCUSSION

AP2/ERF transcript factor is a superfamily (Zhuang et al. 2008; Zhuang et al. 2009), and participate in plant developmental processes (Nilsson et al. 2006; El Ouakfaoui et al. 2010; Wang et al. 2008) as well as biotic and abiotic stress signaling (Krishnaswamy et al. 2010; Hong et al. 2009; Abogadallah et al. 2011; Zhang et al. 2009; Licausi et al. 2010; Hinz et al. 2010). The growth and productivity of kiwifruit are greatly affected by abiotic stresses, especially flooding. Previous studied showed that the AP2/ERF transcript factor involved in waterlogging stress response (Du et al. 2014; Hinz et al. 2010; Licausi et al. 2010). Du *et al.* reported (Du et al. 2014) that 38 of 184 AP2/ERF transcript factor genes were responsive to waterlogging stress and 25 genes were ERF subfamily. In present study, 183 kiwifruit AP2/ERF genes were identified, classified. The results of qRT-PCR analysis have confirmed that 20 of 183 genes were responsive to waterlogging stress and 13 genes were ERF subfamily. 25 maize waterlogging responsive ERF subfamily genes belonged to B1, B2, B3, and B6 subgroups(Du et al. 2014). 13 kiwifruit waterlogging responsive ERF subfamily genes were also belonged to B1, B2, B3, and B6 subgroups. These results showed that those of AP2/ERF family genes might play a key role in waterlogging stress.

Previous studies showed that genes in the ERF-B2 subfamily play a key role in waterlogging tolerance in Arabidopsis and rice. There are five ERF-B2 subfamily genes in Arabidopsis, namely RAP2.2 (At3g14230), RAP2.12 (At1g53910), RAP2.3 (At3g16770), HRE1 (At1g72360), and HRE2 (At2g47520). RAP2.2, RAP2.12, HRE1, and HRE2 genes have been reported that play important role in the response to hypoxia (Licausi et al. 2010; Hinz et al. 2010). SubA1, which was a water tolerance gene, restricts rice elongation at the rice seedling stage during flash floods(Fukao 2006; Xu et al. 2006). Du *et al.* reported (Du et al. 2014) that There are 20 ERF-B2 subfamily genes in maize genome, and 9 of 20 are response to waterlogging stress. In our study, there are 7 ERF-B2 subfamily genes in kiwifruit genome (Table 2), and 2 of 7 are responsive to waterlogging stress (Fig. 5). Further studies about ERF subfamily responsive genes are ongoing, and will be reported in future.

V. CONCLUSION

In our study, 183 AP2/ERF genes were identified and compared with AP2/ERF genes from *Arabidopsis*. The 183 AP2/ERF kiwifruit genes were classified into five subfamilies: DREB (64), ERF (94), AP2 (19) and RAV (5), as well as one soloist. RNA-sequence and Quantitative RT-PCR (qRT-PCR) analysis results showed that 20 genes were responsive to waterlogging stress, suggesting that AP2/ERF transcription factor play important roles in the response to waterlogging stress in kiwifruit.

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