J Plant Biotechnol (2018) 45:45–54
DOI:https://doi.org/10.5010/JPB.2018.45.1.045

ISSN 1229-2818 (Print)
ISSN 2384-1397 (Online)

Research Article

Transcriptome profiling of the coffee (*C. arabica* L.) seedlings under salt stress condition

Mesfin Haile · Won Hee Kang

Received: 3 November 2017 / Revised: 25 January 2018 / Revised: 22 February 2018 / Accepted: 22 February 2018

Abstract This research was conducted to study the gene expression of coffee (*Coffea arabica* L.) seedlings under salt stress condition. A solution of five percent (2.3 dS m^{-1}) deep sea water was used for the salt treatment, and it was thereby compared to normal irrigation water (0.2 dS m^{-1}) used for the control treatment. The mRNA was extracted from the leaves of the coffee seedlings for a comprehensive analysis. In this study, a total of 19,581 genes were identified and aligned to the reference sequences available in the coffee genome database. The gene ontology analysis was performed to estimate the number of genes associated with the identified biological processes, cellular components and molecular functions. Among the 19,581 genes, 7369 (37.64%) were associated with biological processes, 5909 (30.18%) with cellular components, and 5325 (27.19%) with molecular functions. The remaining 978 (4.99%) genes were therefore grouped as unclassified. A differential gene expression analysis was performed using the DESeq2 package to identify the genes that were differentially expressed between the treatments based on fold changes and p-values. Namely, a total of 611 differentially expressed genes were identified (treatment/control) in that case. Among these, 336 genes were up-regulated while 275 of the genes were down-regulated. Of the differentially expressed genes, 60 genes showed statistically significant ($p < 0.05$) expression, 44 of which were up-regulated and 16 which were down-regulated. We also identified 11 differentially expressed transcription factor genes, 6 of which were up-regulated and rest 5 genes were down-regulated. The data generated from this study will help in the continued interest and understanding of the responses of coffee seedlings genes associated with salinity stress, in particular. This study will also provide important resources for further functional genomics studies.

Keywords *Coffea arabica* L., Gene expression, Gene ontology

Introduction

The study from the Royal Botanic Garden implies that coffee is the second most widely traded commodity in world market exchanges (Davis et al. 2012). Among the coffee species, arabica coffee accommodates 70% of total worldwide production, with an estimated production of 8.5 million tons in 2015 (ICO 2015). Recently, climatic change has played a crucial role in the reduction and seasonal variation in coffee bean yields worldwide (Camargo 2010). The relationship between agricultural production and climatic parameters is complicated due to environmental factors influencing the growth and development of the coffee plant in various ways during the phenological stage (Camargo 2010).

Salinity is one of the important abiotic stress factors that limit plant growth and crop production (Shrivastava and Kumar 2015). The excessive accumulation of minerals (Na and Cl) in the plant shoot can lead to salt stress effects in various ways, such as ionic toxicity and imbalance nutrient uptake by plants and osmotic stress, thus, adversely affecting the growth and development of plants (Munns 2006). The development of stress-tolerant crops is vital to combat the stress effects primarily in areas where the agricultural lands are exposed to such stress conditions (Nakashima et al. 2012).

Recently, the advancement of plant genomic studies and techniques of molecular biology have played an important role in understanding the contributions of gene responses as the plants are exposed to a particular environmental stress factor (Joseph et al. 2011). Presently, RNA sequence analysis has been widely used to study the gene expression and profiling transcripts at the whole genome level in different organisms, which are used as a model and non-model organisms (Anna-

M. Haile · W. H. Kang (✉)
Department of Horticulture and Bio-system Engineering, Kangwon National University, Chuncheon 24341, Korea
e-mail: whkang@kangwon.ac.kr

durai et al. 2012). By using the *de-novo* short read assembly technology, the gene expression can be explored in a very comprehensive and successful way without a reference gene sequence (Huang et al. 2012).

Generally, transcriptomic analysis technology facilitates the identification of transcripts that are involved in stress response in a given organism and is used to analyze gene expression based on the absolute abundance of transcripts (Mortazavi et al. 2008). Transcriptome sequencing technology can produce a lot of data set to generate transcriptome map, quantifying gene expression, determining metabolic pathways, and discovering unidentified genes. Transcriptome sequence analysis has been used to examine molecular mechanisms of plants in response to different environmental stress factors for various crops such as maize (Lu et al. 2013), sunflower (Livaja et al. 2013), grape (Liu et al. 2012), and sorghum (Mizuno et al. 2012). There is limited information related to the gene responses of coffee plants to abiotic stress factor, particularly salt stress. Therefore, this experiment was conducted to estimate transcripts and study the gene expression of coffee seedlings under normal water and saline water irrigation conditions.

Materials and Methods

Plant material and salt stress treatment

In this experiment, 6 months old coffee (*C. arabica* L.) seedlings were used. The coffee seedlings were transplanted from the seedbed into plastic pot (12 cm diameter) that filled with soil and compost (2:1). The seedlings were grown under greenhouse conditions during 2016 from March-May at Kangwon National University, Gangwon Province, Korea. The treatment application was started one week later to ensure the establishment of the transplanted seedlings. Five percent (2.3 dS m^{-1}) deep sea water was used for salt treatment and compared to normal irrigation water (0.2 dS m^{-1}) used as control treatment. Application of irrigation was continued at every four days interval at the volume of 330 mL per seedling for three months. The experiment design was completely randomized with three replications.

RNA extraction and sequencing

Total RNA was extracted for three biological replicates from the leaves of the coffee seedlings, which were irrigated with tap water (control), and 5% diluted deep sea water (DSW) following the Concert^{TM} (Invitrogen) method. The tissue was ground in liquid N_2, and 0.5 mL of Concert^{TM} was added to 100 mg of the ground tissue. The samples were homogenized and centrifuged for 2 minutes at 12,000 rpm at room temperature. Afterward, 100 μL of 5 M NaCl and 300 μL of chloroform were added to the supernatant, and the solution was exhaustively homogenized by inversion. The samples were centrifuged again for 10 minutes at 12,000 rpm at +4 °C, and the supernatant was transferred to a new tube. An equal volume of isopropanol was added, and the tubes were centrifuged as described before; the supernatant was then discarded. 1 mL of 75% ethanol was added to wash the pellet, and the tubes were then centrifuged for 1 minute at 12,000 rpm in room temperature, and the supernatant was again discarded. The tubes containing the precipitated RNA were left at room temperature to dry completely. The pellet was diluted with RNase free water. There were three biological and two technical replicates of each treatment, from which equal amounts of RNA were pooled for cDNA synthesis. The cDNA preparation was done according to the Illumina TruSeq Stranded protocol. The library was sequenced using the Illumina HiSeq－2000 platform. To obtain high-quality clean reads for de novo assembly, raw reads from mRNA-seq were filtered by discarding reads with adapter contamination and regions of low quality reads. The processed reads from both treatments were used for further analysis.

Gene ontology analysis

The sequences from the libraries were compared to *C. canephora* sequences available in the database (http://coffee-genome.org). The sequences were screened and compared to similar sequences by using the BLAST program. Blast2GO program was used to classify genes into GO categories, namely: biological processes, cellular components, and molecular function. A differential gene expression analysis was performed with the DESeq2 package to identify differentially expressed genes (salt treatment Vs. Control) based on fold change and p-value ($p < 0.05$).

Results

Sequencing statistics

RNA sequence analysis was used to study the gene expressions of coffee seedlings under tap water (control) and DSW (deep sea water) irrigation conditions. cDNA libraries were prepared from the coffee seedling leaves and subjected to RNA sequence analysis using the Illumina HiSeq 2000 platform. A total of 127.3 million and 143.6 million raw reads were obtained from the control and DSW (5%) treatment conditions, respectively

Table 1 Raw and trimmed data from both treatments

Reads	Treatments	Total read bases	Total reads	GC (%)	Q20 (%)	Q30 (%)
Raw	Control	12,863,176,853	127,358,187	46	99	97
	DSW (5%)	14,505,329,591	143,617,125	47	99	97
Clean	Control	12,550,884,035	125,080,102	46	99	98
	DSW (5%)	14,096,701,745	140,506,411	47	99	98

Note; Total read bases: total reads X read length
GC (%): GC (Guanine-cytosine) content
Q20 (%): phred quality score20, 99% certainty (1/100 chance of an incorrect base call)
Q30 (%): phred quality score30, 99.9% certainty (1/1,000 chance of an incorrect base call)

Table 2 Summary of Illumina transcriptome reads mapped to the reference genes

Reads mapping	Reads number (%)	
	Control	DSW (5%)
Processed reads	62,540,051	70,253,206
Total mapped reads	47,537,918 (76.02)	50,999,074 (72.68)
Unique match	46,136,502 (73.7)	48,830,704 (69.51)
Multiple position match	1,401,416 (2.95)	2,168,370 (4.23)
Total unmapped reads	15,002,133 (23.8)	19,254,132 (27.32)
Overall mapping ratio	76%	72.10%

Note: DSW (Deep Sea Water) was used as salt treatment).

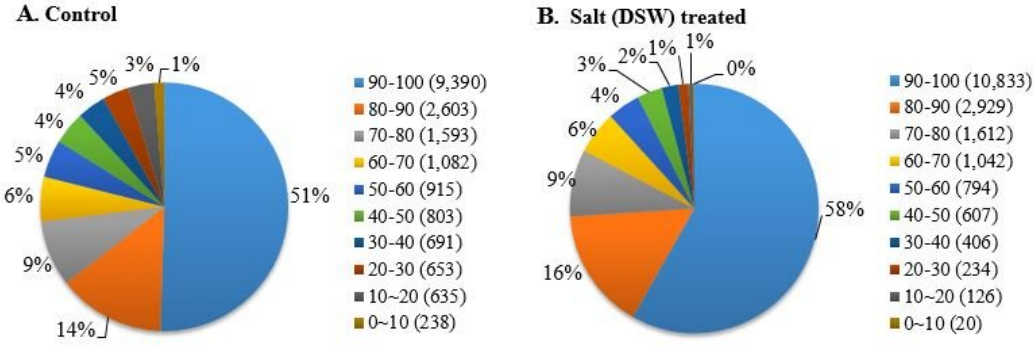

Fig. 1 The distribution of genes coverage in the leaves of the coffee seedling. The identified gene coverage is the percentage of a gene that is covered by reads and defined as the ratio of the number of bases in a gene covered by uniquely mapped reads to the number of total bases in the gene. The pie chart shows the percentage of the different gene coverage listing on the left of the pie chart (A. Control treatment. B. DSW treatment)

(Table 1). The trimmed (clean) reads were 125.0 million in the control treatment, and 140.5 million reads in DSW irrigated coffee seedling leaves sample. Guanine-cytosine (GC) content was estimated, and it accounted for 46% of the total read bases in control and 47% in diluted DSW (5%) treatment (Table 1). The Phred quality score (%) were checked (Q20 and Q30) to assess the sequence quality. Phred quality scores developed and used to identify repeated sequences and remove low-quality sequences, and estimate the sequence quality and quantification of an accurate consensus sequence.

According to Table 2, the result showed that the processed reads from both treatments were 62.5 million in control treatment and 70.2 million reads in 5% DSW treated coffee seedling leaves. The total mapped reads were 47.5 million and 50.9 million reads in the control and 5% DSW treatments, respectively. The unique match, multiple position match, total unmapped reads and overall mapping ratio were estimated (Table 2). The percentage of gene coverage also estimated in both treatments (Fig. 1).

Gene Ontology (GO) analysis result

A total of 19,581 genes were aligned to the reference sequences avaliable in coffee genome hub (http://coffee-genome.org).

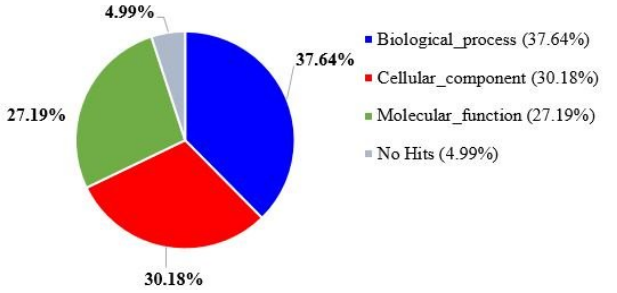

Fig. 2 Gene Ontology (GO) analysis result

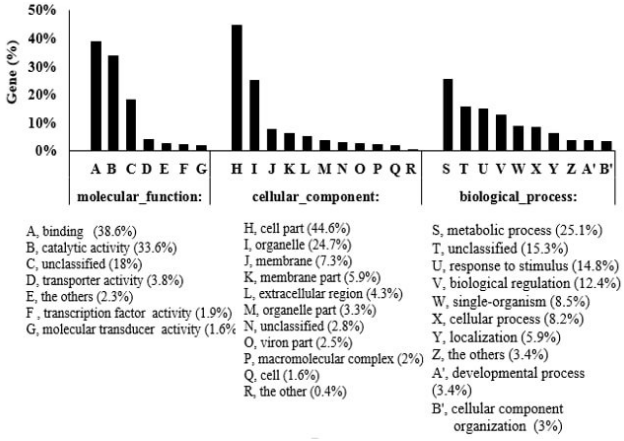

Fig. 3 The percentage of genes involved in different GO sub-categories

The genes were annotated by Blast2GO software to classify them into different GO term categories. Among the 19,581 genes; 37.64% of genes were classified at the biological process, 30.18% at the cellular level and 27.19% of genes were involved at the molecular level and 4.99% genes were categorized as no-hit (Fig. 2).

The biological process category was represented by a large number of genes. In the biological process category, metabolic process, response to stimulus and biological regulation were the most abundant processes and accounts, 25.1%, 14.8% and 12.4% respectively (Fig. 3). The other processes were single organism process (8.5%), cellular process (8.2%), localization (5.9%), developmental process (3.4%), cellular component organization (3%), unclassified (15.3%) and others (3.36%), (Fig. 3). Within the cellular component category, a large number of genes were involved in cell parts (44.6%), organelles (24.7) and membrane (7.3%) components. Binding and catalytic activities were the most abundant groups within the molecular function category and estimated 38.6% and 33.6%, respectively (Fig. 3).

Identification of differentially expressed genes

The number of sequences in each read counts were used to determine the differentially expressed genes between the libraries of comparison samples using the DEseq packages (Anders and Huber 2010). A total of 611 differentially expressed genes between the salt treated and control treatments were identified. Among them 336 genes were showed up-regulation and 275 genes were showed down-regulation. We identified 60 significantly ($p < 0.05$) differentially expressed genes. Of the significantly differentially expressed genes, 16 were down-regulated and 44 genes were up-regulated (Tables 4 and 5, respectively). We also found 15 significantly differentially expressed hypothetical genes, 3 that were down-regulated and the rest 12 genes were up-regulated (Tables 4 and 5).

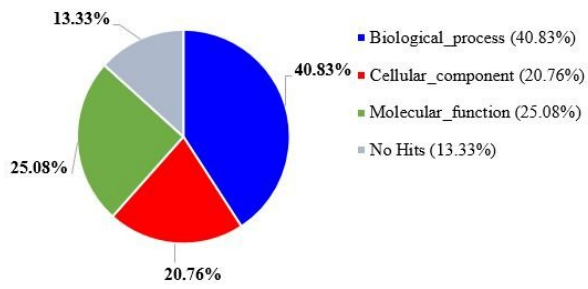

Fig. 4 Gene ontology (GO) enrichment analysis performed for significantly differentially expressed genes in salt-treated coffee seedlings

The gene ontology enrichment analysis was performed for significantly expressed genes. Sixty differentially expressed genes were classified into four categories. These groups are biological processes (40.83%), cellular components (20.76%), molecular functions (25.08%), and no-hit (13.33%) (Fig. 4). Among the significantly differentially expressed genes, a large number of genes were involved in biological processes. The up and down-regulated genes were further classified into several functional categories. Within the biological category, metabolic process, cellular process, single-organism process, response to a stimulus, biological regulation and localization comprised relatively a large number of up-regulated genes (20, 18, 17, 15, and 12 genes, respectively) (Fig. 5A). According to figure 5A, the down-regulated genes were relatively more involved in the metabolic process, cellular process, single-organism process, response to a stimulus, developmental process and biological regulations (7, 7, 7, 6, 5 and 5). Within the cellular component group, the number of up-regulated and down-regulated genes were classified as follows respectively; cell part (30 and 8), organelle (20 and 6), membrane part (10 and 1), membrane (9 and 2), extracellular region (8 and 0), cell (6 and 1), organelle part (3 and 1), and cell rejection (1 and 1) (Fig. 5B). Within the molecular function group, the number of up and down regulated genes were further classified into different activities accordingly such as binding (23 and 6), catalytic activity (11

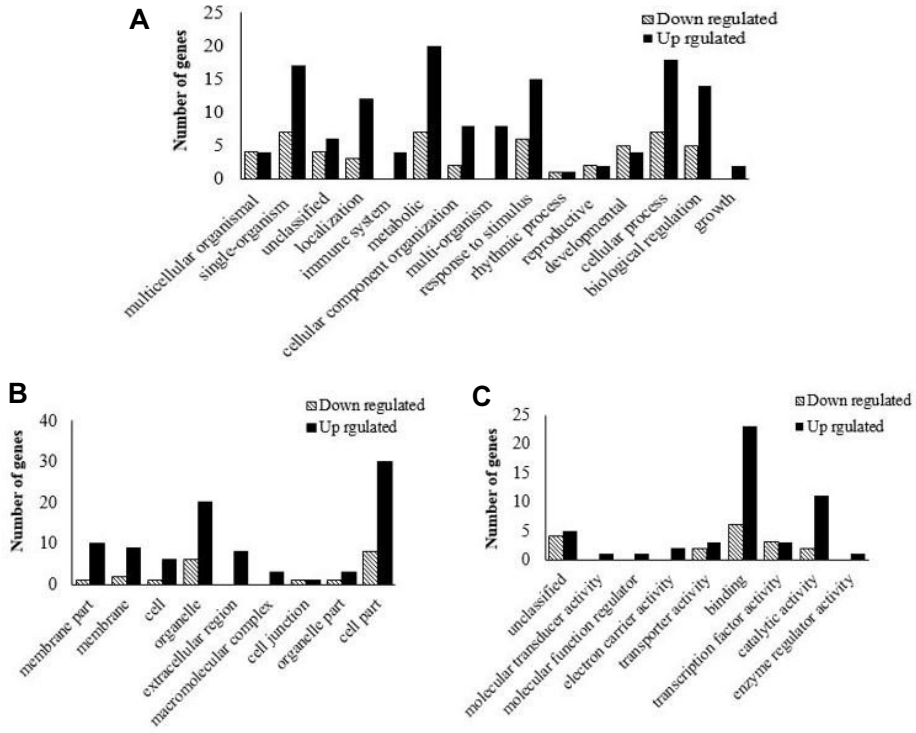

Fig. 5 Significantly ($p < 0.05$) differentially expressed genes in salt-treated coffee seedlings were grouped in different sub-categories (A, Biological process; B, Cellular component; C, Molecular function)

Table 3 List of identified differentially expressed transcription factor genes in coffee seedlings under salt stress condition

	Locus_Tag	Gene Description	Fold change	E-Value
Up-regulated	Cc00_g13890	Double WRKY type transfactor	2.1	2.00E-124
	Cc10_g04710	Ethylene-responsive transcription factor ERF011	2.0	9.00E-49
	Cc02_g14240	Pathogenesis-related genes transcriptional activator PTI5	2.4	1.00E-25
	Cc04_g05080	Probable WRKY transcription factor 40	2.2	7.00E-90
	Cc08_g11060	Putative Probable WRKY transcription factor 50	2.4	1.00E-43
	Cc06_g01240	Trihelix transcription factor GT-3a	2.1	2.00E-61
Down-regulated	Cc05_g16570	Myb family transcription factor APL	-2.5	9E-99
	Cc02_g10740	Putative transcription elongation factor SPT5 homolog 1	-2.3	0
	Cc06_g21410	Putative Transcription elongation factor SPT6	-2.0	0
	Cc02_g17440	Putative Transcription factor bHLH63	-2.4	3E-52
	Cc07_g03240	Transcription factor bHLH135	-2.4	2E-23

and 2), transcription factor activity (3 and 3), transporter activity (3 and 2), electron carrier activity (2 and 0), molecular function regulator (1 and 0), molecular transducer activity (1 and 0), enzyme regulator activity (1 and 0), and unclassified (5 and 4) (Fig. 5C).

Discussion

Abiotic stress is one of the serious constraints that limits agricultural productions and caused severe yield reduction, such as salinity and drought (Bray et al. 2000). However several plants have developed various mechanisms to tolerate these effects (Munns 2002). A previously conducted research has indicated that the transcription factor genes are expected to have a crucial role in regulating gene and a group of two or more genes (Nakashima et al. 2009). In the present study a total of 11 differential expressed transcription factor genes were identified (Table 3). Among differentially expressed transcription factor genes, 5 genes were showed down-regulation, and the other 6 genes were showed upregulation in salt treated coffee seedlings compared to control treatment (Table 3).

Table 4 List of significantly ($p < 0.05$) differentially expressed down regulated genes under salt stress condition

Accession ID	Locus_Tag	Description	Fold change
ID77370	Cc01_g02340	Hypothetical protein	-9.8
ID216235	Cc04_g06680	Putative Probable S-adenosylmethionine	-6.4
ID238605	Cc05_g07560	Hypothetical protein	-5.4
ID198486	Cc03_g10470	Putative disease resistance protein RGA4	-4.3
ID133785	Cc02_g20110	Amino acid permease 6	-2.9
ID221155	Cc04_g10190	Hypothetical protein	-2.7
ID251461	Cc05_g16570	Myb family transcription factor APL	-2.5
ID208311	Cc04_g01570	Putative NADH dehydrogenase	-2.5
ID412256	Cc00_g11240	Putative Protein of unknown function	-2.4
ID289967	Cc07_g03240	Transcription factor bHLH135	-2.4
ID296423	Cc07_g07990	Putative unknown protein	-2.4
ID228137	Cc04_g15750	Probable peptide/nitrate transporter	-2.2
ID220611	Cc04_g09840	Putative NAC domain-containing protein 68	-2.2
ID321865	Cc08_g05640	ABC transporter G family member 14	-2.1
ID378315	Cc11_g02080	Acetylornithine aminotransferase, chloroplastic/mitochondrial	-2.1
ID349824	Cc09_g08740	Auxin response factor 6	-2.1

The products of several differentially expressed genes have been known for protecting plant cells from injury by producing various enzymes for the synthesis of osmolytes and enzymes to avoid reactive oxygen species and dehydrins (Bartels et al. 2005). Transcription factor genes have been widely involved in regulating the productions of functional proteins, which have a key role in plant defense mechanism (Rahaie et al. 2010 and Singh et al. 2002). Within the up regulated transcription factor genes, a significantly differential expressed WRKY genes were identified in salt-stressed coffee seedlings (Table. 3). These genes are Cc08_g11060 (Putative Probable WRKY transcription factor 50) and Cc00_g13890 (Double WRKY type transfactor). The WRKY genes are frequently reported to be involved in various stress responses. In salt-stressed roots of cotton plants, several WRKY genes showed a significant expression, such as WRKY6, WRKY33, WRKY40, and WRKY53 (Yao et al. 2011). The Cc06_g01240, (Trihelix transcription factor GT-3a) gene showed overexpression in salt-stressed coffee seedlings (Table 3). Trihelix transcription factors gene play an essential role in controlling the developmental process and response to abiotic and biotic stress factors (Wang et al. 2016). The GT-1 clade, GT-3a, and GT-3b have been shown to respond to salt stress in Arabidopsis (Park et al. 2004). Ethylene is an important stress hormone because its synthesis is induced under different oxidative environments. In the present study, the ethylene-responsive transcription factor ERF011 gene (Cc10_g04710) was significantly expressed in salt stressed coffee seedlings and it was up regulated (Table 3). Down regulated MYB family transcription factor APL gene (Cc05_g16570) was found in salt stressed coffee seedlings. Previously published studies indicated that MYB proteins are involved in many significant physiological and biochemical processes, including the regulation of primary and secondary metabolism, the control of cell development and the cell cycle, the participation in defense and response to various biotic and abiotic stresses, and hormone synthesis and signal transduction (Dubos et al. 2010 and Zhang et al. 2011). The bHLH superfamily is the second largest TF family in plants (Feller et al., 2011). Results from previous studies showed, bHLH-coding genes have suggested that they are involved in regulating a diverse array of biological and biochemical processes, such as light signaling (Roig-Villanova et al. 2007 and Leivar et al. 2008), and abiotic stress responses (Chinnusamy et al. 2003 and Kiribuchi et al. 2004). Under salt stressed coffee seedlings, down regulated bHLH transcription factors genes existed (Table 3). These genes are putative transcription factor bHLH63 (Cc05_g16570) and transcription factor bHLH135 (Cc07_g03240). According to Mao et al. (2017), under salt stress condition some special bHLH TFs are activated and bind to the promoter of the key genes involved in various signaling pathways and regulate the stress tolerance of plants by regulating the transcription level of these target genes. In this experiment, two down regulated STP transcription factor genes were significantly expressed under salt stress condition. These genes are putative transcription elongation factor SPT5 homolog 1 (Cc02_g10740) and putative transcription elongation factor SPT6 (Cc06_g21410) (Table 3).

The genes that were previously studied and known to be

Table 5 List of significantly ($p < 0.05$) differentially expressed upregulated genes under salt stress condition

Accession ID	Locus_Tag	Description	Fold change
ID196801	Cc03_g08920	Hypothetical protein	160.3
ID197373	Cc03_g09460	Hypothetical protein	27.5
ID262669	Cc06_g07480	Hypothetical protein	12.8
ID197396	Cc03_g09490	Hypothetical protein	8.7
ID128885	Cc02_g16600	Snakin-2	5
ID312625	Cc07_g19850	Bifunctional monodehydroascorbate reductase	4.4
ID217289	Cc04_g07360	Putative Protein aspartic protease in guard cell 1	4.3
ID412790	Cc00_g11630	Probable pre-mRNA-splicing factor	4.1
ID94194	Cc01_g14620	Putative Probable LRR receptor	3.9
ID140324	Cc02_g24340	Hypothetical protein	3.8
ID238942	Cc05_g07810	Glutaredoxin-C9	3.6
ID130154	Cc02_g17510	Hypothetical protein	3.3
ID241357	Cc05_g09770	Putative uncharacterized protein	3.2
ID432820	Cc00_g30460	Putative RING/U-box superfamily protein (PUB)	3.2
ID143867	Cc02_g26780	Putative uncharacterized protein	3.1
ID425123	Cc00_g22460	COBRA-like protein 1	3.1
ID161846	Cc02_g39350	Cytochrome b561/ferric reductase transmembrane	3
ID349096	Cc09_g08190	Putative UDP-glycosyltransferase 85A2	3
ID217284	Cc04_g07350	Putative Protein aspartic protease in guard cell 1	2.9
ID220977	Cc04_g10090	Putative unknown protein	2.9
ID213561	Cc04_g05040	Putative Bifunctional dihydroflavonol 4-reductase	2.9
ID216639	Cc04_g06970	Calmodulin binding protein 60	2.7
ID111904	Cc02_g04570	Hexose carrier protein HEX6	2.7
ID421434	Cc00_g19080	Hypothetical protein	2.6
ID329413	Cc08_g11350	Hypothetical protein	2.6
ID241348	Cc05_g09760	Putative uncharacterized protein	2.6
ID271405	Cc06_g13370	4-coumarate--CoA ligase 1	2.4
ID328966	Cc08_g11060	Putative Probable WRKY transcription factor 50	2.4
ID324636	Cc08_g07970	Hypothetical protein	2.2
ID371323	Cc10_g13630	Putative Probable calcium-binding protein CML44	2.2
ID271443	Cc06_g13410	Xyloglucan endotransglucosylase	2.2
ID133388	Cc02_g19820	Putative Uncharacterized protein	2.2
ID421361	Cc00_g19040	Hypothetical protein	2.2
ID431183	Cc00_g28570	Putative Urease accessory protein	2.2
ID198445	Cc03_g10440	Cytokinin riboside 5'-monophosphate phosphoribohydrolase	2.1
ID213871	Cc04_g05230	Hydroxycinnamoyl-Coenzyme A	2.1
ID415458	Cc00_g13890	Double WRKY type transfactor	2.1
ID404570	Cc00_g05030	Putative Probably inactive leucine-rich repeat receptor-	2.1
ID127357	Cc02_g15570	Putative uncharacterized protein	2.1
ID338558	Cc09_g00710	Auxin-responsive family protein	2.1
ID214818	Cc04_g05850	Protein Transporter, Pam16	2.1
ID244479	Cc05_g11900	Putative Early nodulin-like protein 2	2.1
ID313389	Cc07_g20430	Hypothetical protein	2
ID156312	Cc02_g35670	Hypothetical protein	2

involved in response to salt stress and existed in our study are described below. The Cc04_g06970 (Calmodulin binding protein 60) gene expression was higher in diluted deep sea water (salt water) irrigated treatments (Table 5). Recent research studies have indicated that calmodulin binding protein 60 (CBP60) family associated in response to both biotic and abiotic stresses (Wan et al. 2012). Some members of calmodulin binding protein (for example, Q8H6T7) are identified to be involved in plant defense mechanisms against stress conditions (Ali et al. 2003). The researcher also found a significantly expressed

up-regulated auxin-responsive protein coding gene (Cc09_g00710) in salt stress condition (Table 5). Subsequently, significantly expressed down regulated auxin response factor gene (Cc09_g08740) was identified. The response of plants to various environmental stress factors at the molecular level associated with the expression of many genes involved in different pathways. Plant hormones have been associated with several abiotic and biotic stress factors (abscisic acid, ethylene, salicylic acid and jasmonic acid). Some recent research suggests that auxin is also linked to abiotic and biotic stress signaling pathways (Wang et al. 2003). The result of our experiment is in line with the finding of Jain and Khurana (2009) who reported that during various abiotic stress conditions, several auxin-responsive genes showed differential expression, which indicated a crosstalk between auxin and abiotic stress signaling. The Cc00_g22460 (COBRA-like protein 1), gene was significantly expressed in salt stressed coffee seedlings. The cell wall plays important functions in establishing the morphology of the plant cell, defense response to biotic and abiotic stresses, and mechanical properties of organs. The COBRA gene encodes a putative glycosylphosphatidylinositol (GPI)-anchored protein that controls the ability to change cellulose deposition and determine cell development in the plant cell (Gao et al. 2013).

Plants use different mechanisms to tolerate salinity stress. Among them, accumulation of lignin or modification of the monomeric composition of lignin in the cell wall is one of the major mechanisms (Neves et al. 2010). The expression of Cc04_g05230 (Hydroxycinnamoyl-Coenzyme A) gene was significant in salt treated coffee seedlings (Table 5). The accumulation of hydroxycinnamoyl-CoA, shikimate hydroxycinnamoyl transferase, acid peroxidase, and cysteine protein, is associated with lignification and was induced by salt stress in xylem sap of *Brassica oleracea* (Fernandez-Garcia et al. 2009).

A significantly differentially expressed two aspartic protease coding genes (putative aspartic protease in guard cell 1) were identified. The expression of putative aspartic protease coding genes (Cc04_g07350 and Cc04_g07360) were up-regulated in salt stressed coffee seedlings (Table 5). Some research studies indicated that genes encoding plant aspartic proteases have been identified from different plant species (Mutlu and Gal 1999 and Murakami et al. 2000). Several studies have reported the functions of aspartic proteases in different physiological processes during plant development such as seed germination (Belozersky et al. 1989 and Dunaevsky et al. 1989), leaf senescence (Kato et al. 2004), the immunity response (Xia, et al. 2004), cell death (Ge, et al., 2005) and reproduction (Chen et al. 2008), little is known that aspartic proteases involving in abiotic stress responses (Yao et al. 2012). The result from this experiment also can be an additional supporting information regarding the involvement of aspartic protease gene in response to salt stress.

Up regulated putative RING/U-box superfamily protein (PUB) coding gene was identified in salt treated coffee seedlings and its expression was significant ($p < 0.05$) (Table 5). This result is similar to the finding of Banzai et al. (2002), who reported that a study in mangrove (*Brugueira gymnorhiza*) showed an indication of a plant U-box protein coding gene to be highly expressed under salt stress condition. Mangroves are able to survive under high salt stress conditions; this became interesting for scientists to study and identify salinity tolerance genes in its genome. Although a PUB gene (BG55) showed a temporary increase in expression in response to salt treatment (Banzai et al. 2002). As mentioned by Parida and Jha (2010), salinity tolerance of mangroves is known to be tightly associated with the regulation of gene expression. Various consistent observation in a number of AtPUB genes expressing under hormonal action served as a preliminary clue in determining other stressed related regulation by plant U-box (PUB) proteins in the plant (Sharma et al. 2013). Many of the U-box genes in Arabidopsis such as AtPUB23 and AtPUB24 were known to show strong upregulation in the roots under salt and drought stress conditions (Cho et al. 2008). The genes that were significantly expressed in salt stressed coffee seedlings are listed (Tables 4 and 5) and can be used for future studies as a reference to cross-check the expression of those genes. In general the result of this study will provide an information for further detail investigation of salt responsive genes in coffee plant.

Conclusions

Coffee is one of the most important commercial crops worldwide. Currently, there are a lot of constraints that decline its production including biotic and abiotic factors. From this research, we can suggest there are some genes that involve in different abiotic stress factor including salt stress. Detail investigations of genomic study are crucial to figuring out salt-responsive genes in coffee. The data generated in this study will help in understanding the response of coffee seedlings at the genomic level associated with abiotic stresses in general, salt stress in particular. This study will also provide resources for functional genomic studies.

Acknowledgment

The study was supported by 2015 research grant from Kangwon National University (No. 520150118).

References

Ali GS, Reddy VS, Lindgren PB, Jakobek JL, Reddy ASN (2003) Differential expression of genes encoding calmodulin-binding proteins in response to bacterial pathogens and inducers of defense responses. Plant Mol Biol 51:803-815

Anders S, Wolfgang H (2010) Differential expression analysis for sequence count data. Genome Biol 11:106

Annadurai RS, Jayakumar V, Mugasimangalam RC, Katta MA, Anand S, Gopinathan S, Rao SN (2012) Next generation sequencing and de novo transcriptome analysis of *Costus pictus* D. Don, a non-model plant with potent anti-diabetic properties. BMC Genomics 13:663

Banzai T, Hershkovits G, Katcoff DJ, Hanagata N, Dubinsky Z, Karube I (2002) Identification and characterization of mRNA transcripts differentially expressed in response to high salinity by means of differential display in the mangrove, *Bruguiera gymnorrhiza*. Plant Sci 162:499-505

Bartels D, Sunkar R (2005) Drought and salt tolerance in plants. Crit Rev Plant Sci 24:23-58

Belozersky MA, Sarbakanova ST, Dunaevsky YE (1989) Aspartic proteinase from wheat seeds: isolation, properties and action on gliadin. Planta 177:321-326

Bray EA, Bailey-Serres J, Weretilnyk E (2000) Responses to abiotic stresses. Biochem Mol Biol Plants 1158-1249

Camargo MBP (2010) The impact of climatic variability and climate change on arabica coffee crop in Brazil. Bragantia 69:239-247

Chen J, Ding J, Ouyang Y, Du H, Yang J, Cheng K, Liu K (2008) A triallelic system of S5 is a major regulator of the reproductive barrier and compatibility of indica-japonica hybrids in rice. Proc Natl Acad of Sci USA 105:11436-11441

Chinnusamy V, Ohta M, Kanrar S, Lee BH, Hong X, Agarwal M, Zhu JK (2003) ICE1: a regulator of cold-induced transcriptome and freezing tolerance in Arabidopsis. Genes Dev 17:1043-1054

Cho SK, Ryu MY, Song C, Kwak JM, Kim WT (2008) Arabidopsis PUB22 and PUB23 are homologous U-Box E3 ubiquitin ligases that play combinatory roles in response to drought stress. Plant Cell 20:1899-1914

Davis AP, Gole TW, Baena S, Moat J (2012) The impact of climate change on indigenous arabica coffee (*Coffea arabica* L.): predicting future trends and identifying priorities. PLoS One 7:e47981

Dubos C, Stracke R, Grotewold E, Weisshaar B, Martin C, Lepiniec L (2010) MYB transcription factors in Arabidopsis. Trends Plant Sci 15:573-581

Dunaevsky YE, Sarbakanova ST, Belozersky MA (1989) Wheat seed carboxypeptidase and joint action on gliadin of proteases from dry and germinating seeds. J Exp Bot 40:1323-1329

Fernandez-Garcia N, Lopez-Perez L, Hernandez M, Olmos E (2009) Role of phi cells and the endodermis under salt stress in *Brassica oleracea*. New Phytol 181:347-360

Gao Y, Bian L, Shi J, Xu J, Xi M, Wang G (2013) Expression of a conifer COBRA-like gene ClCOBL1 from Chinese fir (*Cunninghamia lanceolata*) alters the leaf architecture in tobacco. Plant Physiol Biochem 70:483-491

Ge X, Dietrich C, Matsuno M, Li G, Berg H, Xia Y (2005) An Arabidopsis aspartic protease functions as an anti-cell-death component in reproduction and embryogenesis. EMBO Rep 6:282-288

Huang HH, Xu LL, Tong ZK, Lin EP, Liu QP, Cheng LJ, Zhu MY (2012) De novo characterization of the Chinese fir (*Cunninghamia lanceolata*) transcriptome and analysis of candidate genes involved in cellulose and lignin biosynthesis. BMC Genomics 13:648

ICO (2015) Coffee Statistics International Coffee Organization. Trade Statistics of Total Production of Exporting Countries. Retrieved from: http:// www.ico.org/International Coffee Organization.

Jain M, Khurana JP (2009) Transcript profiling reveals diverse roles of auxin-responsive genes during reproductive development and abiotic stress in rice. FEBS J 276:3148-3162

Joseph B, Jini D, Sujatha S (2011) Development of salt stress-tolerant plants by gene manipulation of antioxidant enzymes. Asian J Agric Res 5:17-27

Kato Y, Murakami S, Yamamoto Y, Chatani H, Kondo Y, Nakano T, Sato F (2004) The DNA-binding protease, CND41, and the degradation of ribulose-1, 5-bisphosphate carboxylase/oxygenase in senescent leaves of tobacco. Planta 220:97-104

Kiribuchi K, Sugimori M, Takeda M, Otani T, Okada K, Onodera H, Minami E (2004) RERJ1, a jasmonic acid-responsive gene from rice, encodes a basic helix-loop-helix protein. Biochem Biophys Res Commun 325:857-863

Leivar P, Monte E, Al-Sady B, Carle C, Storer A, Alonso JM, Quail PH (2008) The Arabidopsis phytochrome-interacting factor PIF7, together with PIF3 and PIF4, regulates responses to prolonged red light by modulating phyB levels. Plant Cell 20:337-352

Liu GT, Wang JF, Cramer G, Dai ZW, Duan W, Xu HG, Li SH (2012) Transcriptomic analysis of grape (*Vitis vinifera* L.) leaves during and after recovery from heat stress. BMC Plant Biol 12:174

Livaja M, Wang Y, Wieckhorst S, Haseneyer G, Seidel M, Hahn V, Bauer E (2013) BSTA: a targeted approach combines bulked segregant analysis with next-generation sequencing and de novo transcriptome assembly for SNP discovery in sunflower. BMC Genomics 14:628

Lu X, Chen D, Shu D, Zhang Z, Wang W, Klukas C, Zhang C (2013) The differential transcription network between embryo and endosperm in the early developing maize seed. Plant Physiol 162:440-455

Mao K, Dong Q, Li C, Liu C, Ma F (2017) Genome wide identification and characterization of apple bHLH transcription

factors and expression analysis in response to drought and salt stress. Front Plant Sci 8

Mizuno H, Kawahigashi H, Kawahara Y, Kanamori H, Ogata J, Minami H, Matsumoto T (2012) Global transcriptome analysis reveals distinct expression among duplicated genes during sorghum-*Bipolaris sorghicola* interaction. BMC Plant Biol 12:121

Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B (2008) Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nature Methods 5:621-628

Munns R, James RA, Läuchli A (2006) Approaches to increasing the salt tolerance of wheat and other cereals. J Exp Bot 57:1025-1043

Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Enviro 25:239-250

Murakami S, Kondo Y, Nakano T, Sato F (2000) Protease activity of CND41, a chloroplast nucleoid DNA-binding protein, isolated from cultured tobacco cells. FEBS Letters 468:15-18

Mutlu A, Gal S (1999) Plant aspartic proteinases: enzymes on the way to a function. Physiol Plant 105:569-576

Nakashima K, Ito Y, Yamaguchi-Shinozaki K (2009) Transcriptional regulatory networks in response to abiotic stresses in Arabidopsis and grasses. Plant Physiol 149:88-95

Nakashima K, Takasaki H, Mizoi J, Shinozaki K, Yamaguchi-Shinozaki K (2012) NAC transcription factors in plant abiotic stress responses. Biochim Biophys Acta 1819:97-103

Neves GYS, Marchiosi R, Ferrarese MLL, Siqueira-Soares RC, Ferrarese-Filho O (2010) Root growth inhibition and lignification induced by salt stress in soybean. J Agron Crop Sci 196: 467-473

Parida AK, Jha B (2010) Salt tolerance mechanisms in mangroves: a review. Trees 24:199-217

Park HC, Kim ML, Kang YH, Jeon JM, Yoo JH, Kim MC, Yoon HW (2004) Pathogen-and NaCl-induced expression of the SCaM-4 promoter is mediated in part by a GT-1 box that interacts with a GT-1-like transcription factor. Plant Physiol 135:2150-2161

Rahaie M, Xue GP, Naghavi MR, Alizadeh H, Schenk PM (2010) A MYB gene from wheat (*Triticum aestivum* L.) is up-regulated during salt and drought stresses and differentially regulated between salt-tolerant and sensitive genotypes. Plant Cell Rep 29:835-844

Roig-Villanova I, Bou-Torrent J, Galstyan A, Carretero-Paulet L, Portolés S, Rodríguez-Concepción M, Martínez-García JF (2007) Interaction of shade avoidance and auxin responses: a role for two novel atypical bHLH proteins. EMBO J 26: 4756-4767

Sharma M, Pandey A, Pandey GK (2013) Role of Plant U-BOX (PUB) Protein in Stress and Development. Plant Stress 7:1-9

Shrivastava P, Kumar R (2015) Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi J Biol Sci 22:123-131

Singh KB, Foley RC, Oñate-Sánchez L (2002) Transcription factors in plant defense and stress responses. Curr Opin Plant Biol 5:430-436

Wan D, Li R, Zou B, Zhang X, Cong J, Wang R, Li G (2012) Calmodulin-binding protein CBP60g is a positive regulator of both disease resistance and drought tolerance in Arabidopsis. Plant Cell Rep 31:1269-1281

Wang H, Miyazaki S, Kawai K, Deyholos M, Galbraith DW, Bohnert HJ (2003) Temporal progression of gene expression responses to salt shock in maize roots. Plant Mol Biol 52:873-891

Wang Z, Liu Q, Wang H, Zhang H, Xu X, Li C, Yang C (2016) Comprehensive analysis of trihelix genes and their expression under biotic and abiotic stresses in *Populus trichocarpa*. Sci Rep 6

Xia Y, Suzuki H, Borevitz J, Blount J, Guo Z, Patel K, Lamb C (2004) An extracellular aspartic protease functions in Arabidopsis disease resistance signaling. EMBO J 23:980-988

Yao D, Zhang X, Zhao X, Liu C, Wang C, Zhang Z, Li F (2011) Transcriptome analysis reveals salt-stress-regulated biological processes and key pathways in roots of cotton (*Gossypium hirsutum* L.). Genomics 98:47-55

Yao X, Xiong W, Ye T, Wu Y (2012) Overexpression of the aspartic protease ASPG1 gene confers drought avoidance in Arabidopsis. J Exp Bot 63:2579-2593

Zhang L, Zhao G, Jia J, Liu X, Kong X (2011) Molecular characterization of 60 isolated wheat MYB genes and analysis of their expression during abiotic stress. J Exp Bot 63:203-214