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Molecular Networking of Regulated Transcription Factors Under Salt Stress in Wild Barley (*H. spontaneum*)

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Salt stress is among environmental conditions that severely retards plant growth. Scope of this work is the detection of transcription factors that might participate in regulating salt-stressed genesin wild barley (*Hordeumspontaneum*). Expression profiles of important types of transcription factors (TFs) were displayed. They includeWRKY and MYB, that were regulated under salt stress. WRKY19 and NAC96 are known to induce stress tolerance through activation of *DREB2A* (or *Ap2-ERF*). NAC96 concordantly upregulated with *DREB2A* gene under salt stress in *H. spontaneum*, a possible cross talking to compensate the negative performance of *WRKY19* gene. P5CS, for proline accumulation, is also known to be driven by ERF1 and genes encoding these proteins concordantly upregulated in *H. spontaneum* under salt stress supporting NAC96/ERF1/P5CS cross talking towards proline accumulation under stress. Genes encoding enzymes participating in the last steps of glucose, sucrose and maltose biosyntheses concordantly upregulated with WRKY11 that is also involved in driving genes encoding free proline. B-box zinc finger protein 21 (BZF21) concordantly expressed with genes encoding catalase and SAUR40 indicating that *BZF21* gene might drive expression of the two genes under salt stress. Upregulated WRKY41 and WRKY46 under salt stress in wild barley are known to exhibit enhanced stomatal closure, reactive oxygen species (ROS) scavenging, lateral roots development via regulation of ABA signaling and auxin homeostasis. The latter action is governed by *GH3.8* gene that was upregulated in wild barley. MYB30 is known for being SUMOylated by SIZ1. In the present study, *MYB30*, *MYB44* and *MYB3R-2* genes were concordantly expressed with *SIZ2* gene supporting their crosstalking under salt stress in *H. spontaneum*. Based on the regulation of *WRKY19* and *MYB30* genes under salt stress in *H. spontaneum*, we suggest that the first is a positive activator, while the second is a negative activator of *FT* gene that drives early flowing in plants. MYB44 that promotes stomatal closure under stress can also serve in conferring tolerance to abiotic stresses in wild barley. Several other downregulated genes under salt stress, e.g., *MYB1*, *MYB20* and *MYB73*, were previously reported to negatively regulate abiotic stress tolerance in plants. We suggest that WRKY gene family participates in salt stress responses in leaves of *H. spontaneum* following approaches different from those of other plants. Regulation of MYB gene family is almost similar to that of other plant species under salt stress. In conclusion, the present study addresses some of the regulatory frame works driving expression of salt-related genes in*H. spontaneum* that can be utilized in plant, e,g, cereals, breeding programsto improve their salt stress tolerance.

Keywords: WRKY, MYB, DREB, NAC, GH3, SAUR, SIZ, FT.

Salt stress is one of the most devastating environmental conditions that extremely restrict plant growth and yield. For a plant to survive such harsh condition, a series of tolerance mechanisms can occur to help plant adapt and respond properly to this condition[1]. Earlier reports indicate that

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expression levels of different stress-related genes are regulated by transcription factors (TFs) that work as stimulators of individual genes or act as master switches driving a battery of genes or a whole pathway such as stress signal transduction pathways[2-8]WRKYis among the largest families of TFs that play important roles in modulating physiological processes in plants under stress conditions[8-12]. Protein encoded by this TFgene family is characterized by a 60 amino acids domain of highly conserved WRKYGQKheptapeptide at the N-terminal and an atypical zinc finger-like motif at its C-terminal[13, 14]. This family contains over 70 members in Arabidopsis[13, 15]. 55 in cucumber[16], 119 in maize[17], 94 in barley[18], and 100 in rice[19]. Encoded proteins of this family are characterized by a 60-amino acids domain containing the WRKY amino acid sequence at its amino-terminal end and a putative zinc finger motif at its carboxy-terminal end. Based on number and diversity of WRKY domains, WRKY proteins are classified into three groups (I, II and III) of which category I proteins harbor two domains, while proteins of groups II and III harboronly one domain. Groups II and III proteins differ in zinc finger structures (C2H2 in group II, while C2HC in group III)[14, 20]. Previous study reported a number of 74 WRKY proteins in Arabidopsis, while over 100 in rice (*Oryza sativa*)[10]. WRKYTFs have specificity to bind W-box [TTGAC(C/T)] of promoters of their target genes, which subsequently wire genetic circuits towards downstream biological responses[20, 21].

WRKYTFs can either negatively or positively trigger a certain response under stress conditions[19]. These regulation patterns as well as members participating in a given condition can changes from a plant to the other. For example, *WRKY54* and *WRKY70* in Arabidopsis negatively regulate leaf senescence[22]. While, WRKY23 positively enhanced pathogen defense and over expression of maize WRKY58 in rice[23]. and wheat WRKY1 and *WRKY33* in Arabidopsis positively conferred drought and salt tolerance[24]. WRKYTFs also reported to be involved in abiotic stress by wiring ABA signaling pathway[25]. For example, Chrysanthemum *WRKY1* enhancedabiotic stress tolerance, whilecotton *WRKY17* overexpressed in tobacco reduced tolerance by regulating a number of genes in ABA signaling pathway and reactive oxygen species (ROS) production.

MYB is also a family of TFs involved in response to abiotic stresses in plants[26]. Of which, expression of MYB108 gene in Arabidopsis is induced in response to salt stress and participate in crosstalking between abiotic and biotic stresses via orchestration of signaling pathways of jasmonic acid (JA) and gibberellic acid (GA)[27, 28]. While, MYB65 participates in GA signaling in growth and flowering processes[29]. Our work also showed that the expression of this gene increased in roots in response to both stresses whereas, in leaves up-regulated only in response to salt stress. Expression of genes encoding MYB differs in different tissues in response to salt stress as MYB34 in Arabidopsis, for example, is normally upregulated in root tissue and its expression in leaves increases only in response to stress, whereas MYB47 and MYB32 were common in both tissues[28, 30].

In the present study, we have demonstrated important types of TFs including WRKY and MYB that were regulated under salt stress in wild barley *Hordeumspontaneum*. The information recovered from this work can be helpful in improving plant salt stress tolerance in the future.

MATERIALS AND METHODS

Salt stress experiment was conducted on *H. spontaneum* as previously described[31]. Fourteen-day-old seedlings were treated with salt (500mMNaCl) and total RNAs were harvested in a replicated experiment from leaves at 0 (control), 2, 12 and 24 h time point using Trizol (Invitrogen, Life Tech, Grand Island, NY, USA). Then, RNAs were treated with RNase-free DNase (Promega Corporation, Madison, WI, USA) and 1 U/ul of RNasin® Plus RNase Inhibitor as described (Promega Corporation, Madison, WI, USA). Total RNA samples were, then, shipped to Beijing Genomics Institute (BGI), Shenzhen, China for deep sequencing using illuminaMiseq. Generated raw data were retrieved in FASTQ format and submitted to the NCBI and experiment received accession number of PRJNA227211 (https://www.ncbi.nlm.nih.gov/ bioproject?LinkName=sra_bioproject&from_uid=537429). Individual accession numbers of raw data of different samples are available in NCBI (https://www.ncbi.nlm.nih.gov/ sra?LinkName=bioproject_sra_all&from_uid=227211). Raw data was processed as

described[32] and clean data was subjected to genome-guided Trinity *de novo* transcriptome assembly (https://github.com/trinityrnaseq/trinityrnaseq/wiki/Genome-Guided-Trinity-Transcriptome-Assembly) with *Hordeumvulgare* genome (https://plants.ensembl.org/ Hordeum_vulgare/Info/ Index, Taxonomy ID 112509) used as the guide. Differential expression and cluster analysis were done by EdgeR (version 3.0.0, R version 2.1.5) with proper algorism and fold change values of e" 2 measured against *actin* house-keeping gene. Annotation of the recovered transcripts was done using Blast2GO (http://www.blast2go.org/). Subsequent bioinformatics approach was done as described[33]. Predicted CDSs were annotated against protein database in order to assign functions of transcripts. Protein domains common in TFswere identified using HMMER3 software[34].

Then, RNA-Seq datasets were validated via qRT-PCR of four randomly selected genes using the Agilent Mx3000P qPCR Systems (Agilent technology, USA) as previously described[31]. Transcripts selected from cluster analysis were upregulated at 2 and 12 h time points. Primer sequences are shown in Table S1. Calculations referring to expression levels of each transcript were done relative to that under control condition and barley actin gene was used as the house-keeping gene.

RESULTS AND DISCUSSION

For validating RNA-Seq datasets, qRT-PCR was done for four randomly selected transcripts encoding transcripts that were either upregulated at 2 and 12 h time points, upregulated at 2 h time point, or downregulated at 2 and 12 h time points of salt stress and results aligned with RNA-Seq datasets for transcripts used for validation (FigureS1).Cluster analysis resulted in the recovery of over 10000 differentially expressed (DE) transcripts with fold change of e" 2 under salt stress including over 600 TFs highlighted in Table S2that are separately shown in Table S3. Well-known TF families for their response to abiotic stresses include WRKY and MYB. Genes encoding WRKY activated at 2 and 12 h time points under salt stress include *WRKY2*, *WRKY11*, *WRKY41*, *WRKY46*, *WRKY50* and *WRKY71* (Figure 1a). Gene encoding *WRKY24* was upregulated at 2 h

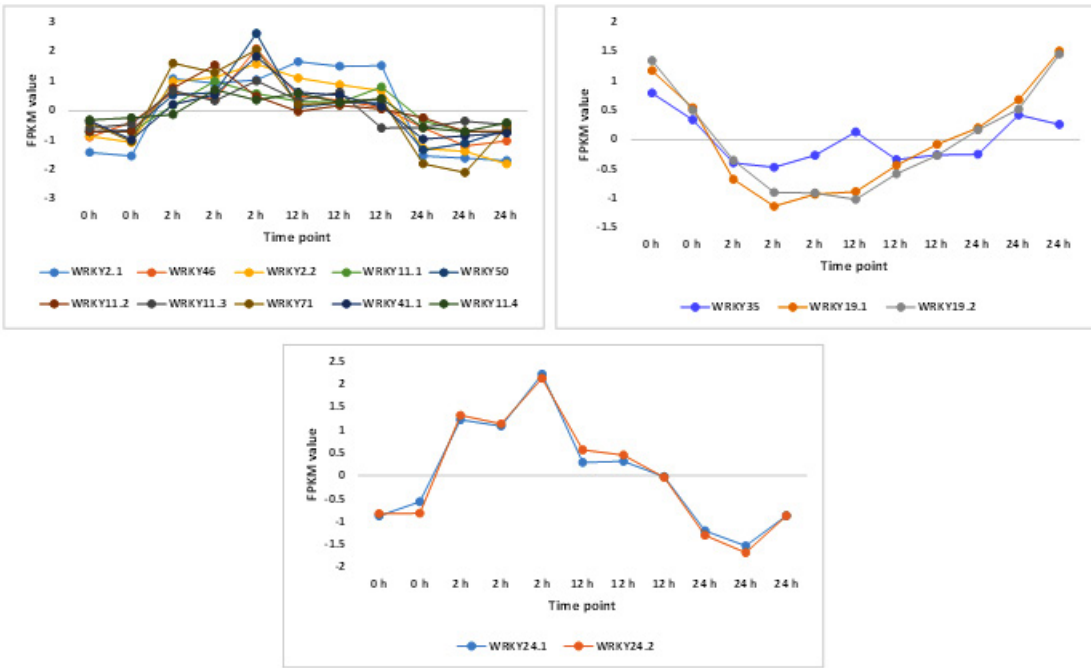

Fig. 1. Up- (a), downregulated (b) and up-/downregulated (c) transcripts of WRKY family under salt stress (1 M NaCl) across 0, 2, 12 and 24 h time points in leaves of H. spontaneum**.** Original RNA-Seq data is shown in Table S2

time point only, while downregulated at 24 h time point (Figure 1c). DownregulatedWRKYgenesin the present study include *WRKY19* and *WRKY35* (Figure 1b). Genes encoding MYB under salt stress include*MYB30*, *MYB44*,*MYB62*, *MYB3R-2* and *MYB3R-4*, while downregulatedMYBgenes include*MYB1*, *MYB20*,*MYB73* and *MYBS3* (Figure 2).

WRKY2 and WRKY19 were reportedby Niuet al. (35)to induce stress tolerance in wheat through activation of STZ (salt tolerance zinc finger) andDREB2A (dehydration-responsive element binding 2A) pathways, respectively. Although a large number of zinc finger genes[11] in the present study was regulated in leaves *H. spontaneum* under salt stress (Table S3), *STZ* gene was not regulated. Then, *STZ* gene cannot be used in tracing regulation of *WRKY2* gene. *WRKY19*gene was downregulated in leaves of *H. spontaneum*under salt stress (Figure 1b), thus, no activation of *DREB2A*gene is expected. *DREB2A* is among *AP2-ERF* (Apetala2/Ethylene responsive factor) gene family and a recent report indicated that *DREB2A* is also affected by other TFs, ex., NAC96(28). Interestingly, two *Ap2-ERF*gene isoforms and a gene encoding NAC96 were upregulated in cluster 1 under salt stress in leaves of *H. spontaneum*(Figure 3 and Table S2) indicating that upregulation of *Ap2-ERF* gene can compensate the negative regulation of *WRKY19*gene in *H. spontaneum*.

Overexpression of *WRKY2*gene in grapevine increased proline under salt stress[36]. Two other recent reports indicated that WRKY2 in wheat[37] and WRKY11 in soybean[38] also drive genes encoding free proline and soluble sugars

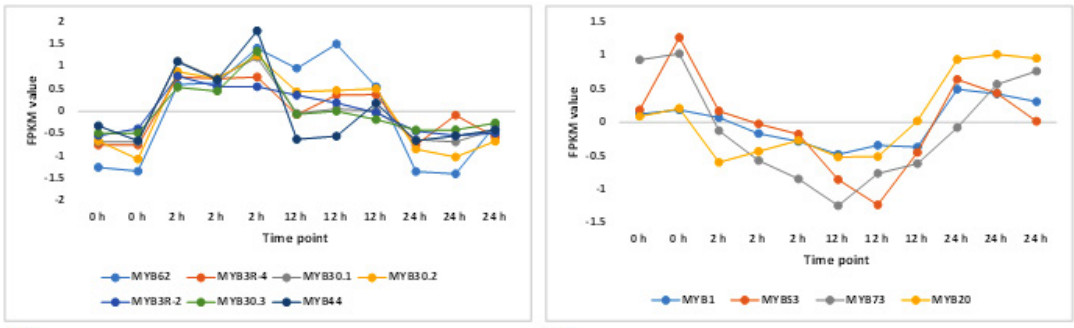

Fig. 2. Up- (a) and downregulated (b) transcripts of MYB family under salt stress (1 M NaCl) across 0, 2, 12 and 24 h time points in leaves of *H. spontaneum*. Original RNA-Seq data is shown in Table S2

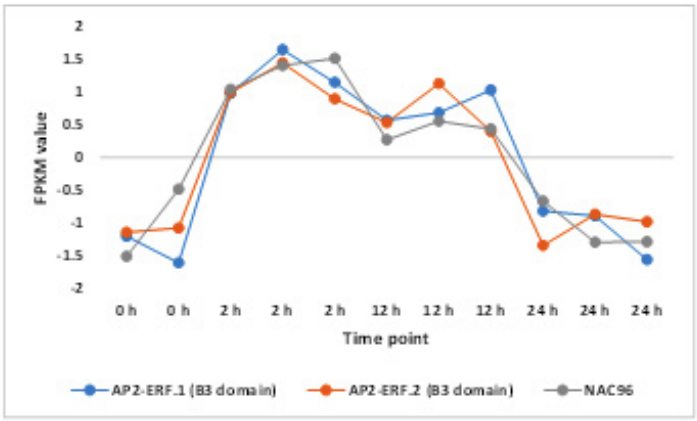

Fig. 3. Expression pattern of transcripts encoding two DREB2A (AP2-ERF) isoforms under salt stress (1 M NaCl) across 0, 2, 12 and 24 h time points in leaves of *H. spontaneum*. AP2-ERF = Apetala2/ethylene responsive factor. Original RNA-Seq data is shown in Table S2

under drought stress. Prolineand sugars are known as important osmolytesthat neutralize the effects of salt and alleviate stress[39, 40]. Interestingly, gene encoding Delta-1-pyrroline-5-carboxylate synthase (P5CS) for proline accumulation was concordantly upregulated under salt stress with that encoding *ERF1* (*ethylene responsive factor 1*)gene, other*DREB*gene derivative, in cluster 32 (Figure 4 and Table S2). Then, proline accumulation due to the function of *P5CS*gene in *H. spontaneum* under salt stress canalso be driven by *ERF1*gene that is likely controlled by NAC96, not by either WRKY2 or WRKY11. As per expected sugar levels under salt stressin *H. spontaneum*, results indicated that genes encoding enzymes participating in the last step of glucose (e.g., beta-glucosidase), sucrose (e.g., sucrose synthase 6) and maltose (e.g., beta-amylase 8) biosynthesis concordantly upregulated with WRKY2 in clusters1 and 5, whileWRKY11 and MYB3R-2 in cluster 6 (Table S2) under salt stress (Figure 5).Accordingly, we speculate that WRKY 2 and WRKY11 are involved

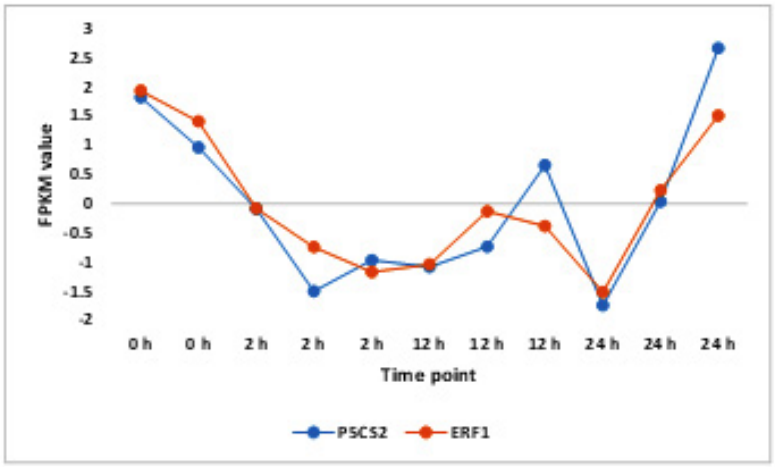

Fig. 4. Expression pattern of transcripts of DREB2A (ERF1) and P5CS concordantly upregulated under salt stress (1 M NaCl) across 0, 2, 12 and 24 h time points in leaves of *H. spontaneum*. P5CS2 = Delta-1-pyrroline-5-carboxylate synthase 2, ERF1 = ethylene responsive factor 1. Original RNA-Seq data is shown in Table S2.

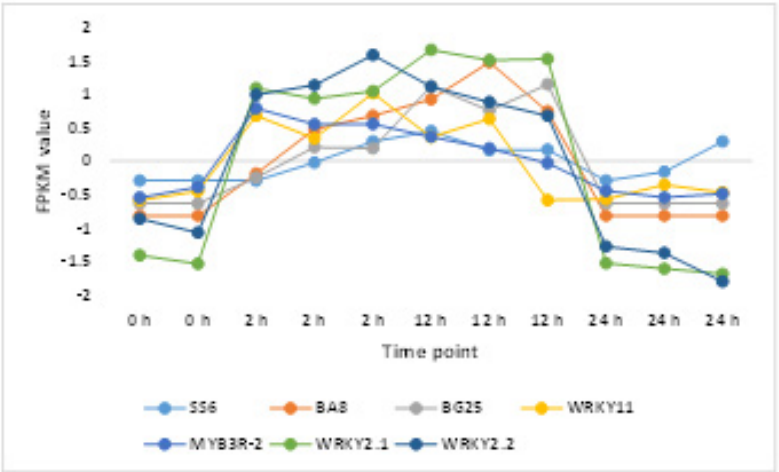

Fig. 5. Expression pattern of transcripts encoding SS6, BA8, BG25, WRKY2, WRKY11 and MYB3R-2 concordantly upregulated under salt stress (1 M NaCl) across 0, 2, 12 and 24 h time points in leaves of *H. spontaneum*. SS6 = Sucrose synthase 6, BA8 = Beta-amylase 8, BG25 = Beta-glucosidase 25, MYB3R-2 = myeloblastosis3R-2. Original RNA-Seq data is shown in Table S2

in driving genes encodingsoluble sugars as two different mechanisms of salt stress tolerance in *H. spontaneum*.

Recently, WRKY11 was also proven to induce elevated levels of superoxide dismutase (SOD) and catalase in soybean[38]. In the present study, upregulated *WRKY11*gene does not seem to concordantly express with *SOD* regulatedgene isoforms in *H. spontaneum*, where*SOD* gene isoforms were downregulated (Figure 6 and Table S2) as shown in clusters 2 and 27 and no other TFcan likely complementWRKY11 effect whose upregulation pattern of its three isoforms in*H. spontaneum* was different (cluster 1). Although both genes are upregulated, *WRKY11*gene does not either concordantly express with isoforms of gene encoding catalase(existing in cluster 23), but gene encoding another TF namely B-box zinc finger protein 21 (BZF21) concordantly expressed with the two isoforms of gene encoding catalase, thus, possibly drive expression of this gene in *H. spontaneum*instead of WRKY11 (Figure 7 and Table S2). B-box zinc finger proteins were reported to enhance salt and drought stresses tolerance in Arabidopsis (Liu et al., 2019). Interestingly, gene encoding SAUR40also concordantly expressedwith

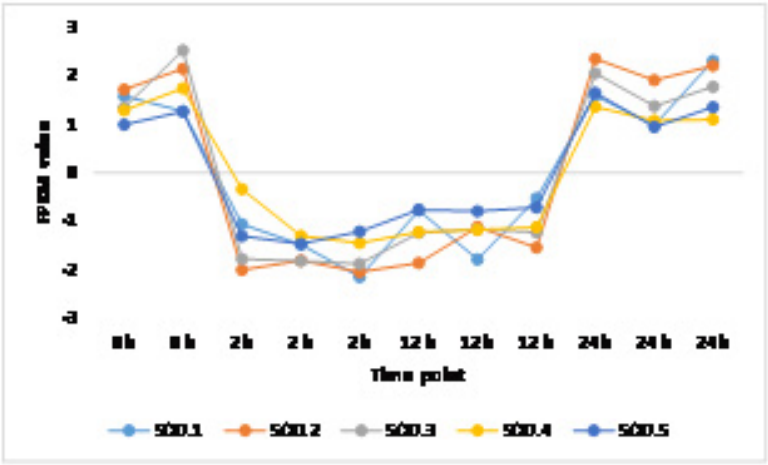

Fig. 6. Expression pattern of transcripts encoding SOD downregulated under salt stress (1 M NaCl) across 0, 2, 12 and 24 h time points in leaves of *H. spontaneum*. SOD = superoxide dismutase. Original RNA-Seq data is shown in Table S2

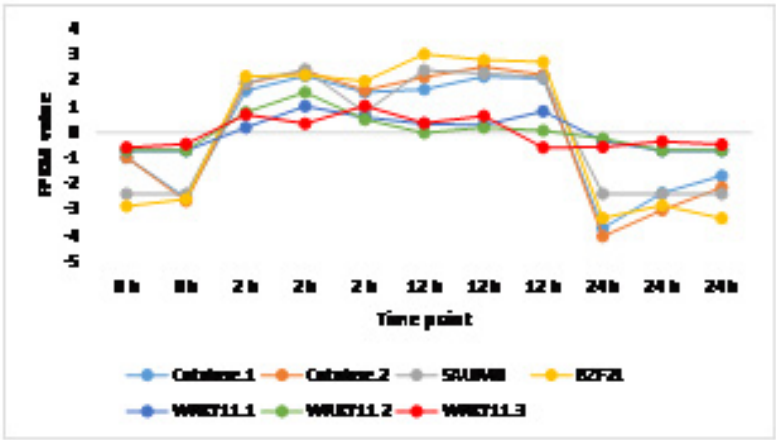

Fig. 7. Expression pattern of transcripts encoding isoforms of catalase concordantly upregulated with BZF21 and SAUR genes, while not with WRKY11 gene isoforms under salt stress (1 M NaCl) across 0, 2, 12 and 24 h time points in leaves of H. spontaneum. BZF21 = B-box zinc finger protein 21, SAUR40 = small a*uxin*-up RNA. Original RNA-Seq data is shown in Table S2

BZF21 and catalase genes in cluster 23 (Figure 7 and Table S2).*SAUR40*gene is among a family acting as a regulator of cell elongation and plant growth performance[41] and a stimulator of shoot elongation due to auxin signaling[42]. Thus, we speculate that BZF21 might drive expression of both *catalase* and *SAUR40* genes as genes encoding the three metabolites are concordantly expressed (Figure 7).

Participation of the two TFs, namelyWRKY24 and WRKY71, as responsive elements under salt stress was argued in rice[43]. However, expression patterns of isoforms of these two TFs seem to be controversial (Figure 1) as gene encoding the first was upregulated at 2 h time point and downregulated at 24 h time point, while gene encoding the second was upregulated at 2 and 12 time points. Xie et al[19] indicated that

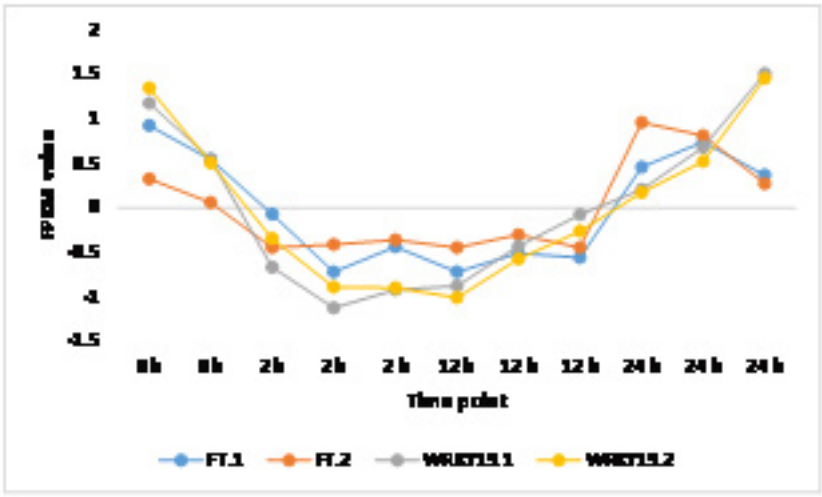

Fig. 8. Expression pattern of transcripts encoding isoforms of FT concordantly upregulated with two isoforms of WRKY19 genes under salt stress (1 M NaCl) across 0, 2, 12 and 24 h time points in leaves of *H. spontaneum*. FT = FLOWERING LOCUS T. Original RNA-Seq data is shown in Table S2

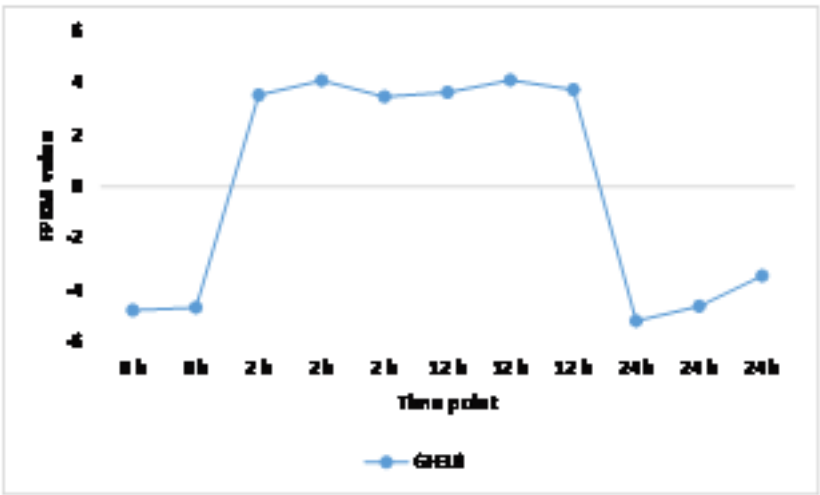

Fig. 9. Expression pattern of transcript encoding GH3.8 upregulated under salt stress (1 M NaCl) across 0, 2, 12 and 24 h time points in leaves of H. spontaneum. GH3.8 = GRETCHEN HAGEN 3.8. Original RNA-Seq data is shown in Table S2

*WRKY24*gene is induced by ABA signaling, while Basu and Roychoudhury[43] indicated that ABA signaling induces higher expression of *WRKY71*gene and many other TFs. Interestingly, the authors indicated that*WRKY24*gene showed expression even lower than that of the control untreated samples under salt stress. WRKY71 was recently reported to antagonistically act against both salt-delayed flowering and escaping salt stress in Arabidopsis through the induction of gene encoding FLOWERING LOCUS T (FT)[44]. Surprisingly, two isoforms of the latter gene in clusters 4 and 8 seem concordantly downregulated with gene encoding WRKY19of cluster 4 rather than with gene encoding WRKY71of cluster 21 (Figure 8 and Table S2). We cannot jump to conclusions on the relationship between *WRKY19* and *FT* genes unless an experiment to detect the consequences of *WRKY19* gene being knocked out in Arabidopsis model.

Aligning with the results of the present study, WRKY41and WRKY46 were reported to

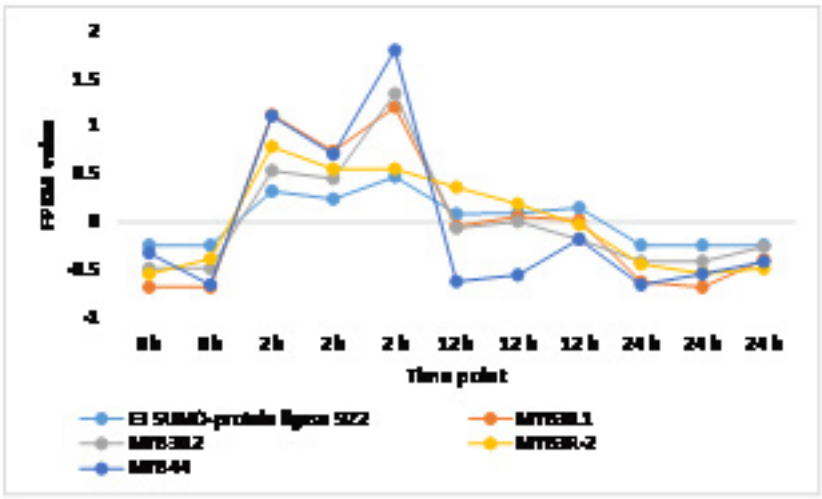

Fig. 10. Expression pattern of transcripts encoding SIZ2 concordantly upregulated with two isoforms of MYB30 gene as well as MYB3R-2 and MYB44 under salt stress (1 M NaCl) across 0, 2, 12 and 24 h time points in leaves of *H. spontaneum*. Original RNA-Seq data is shown in Table S2

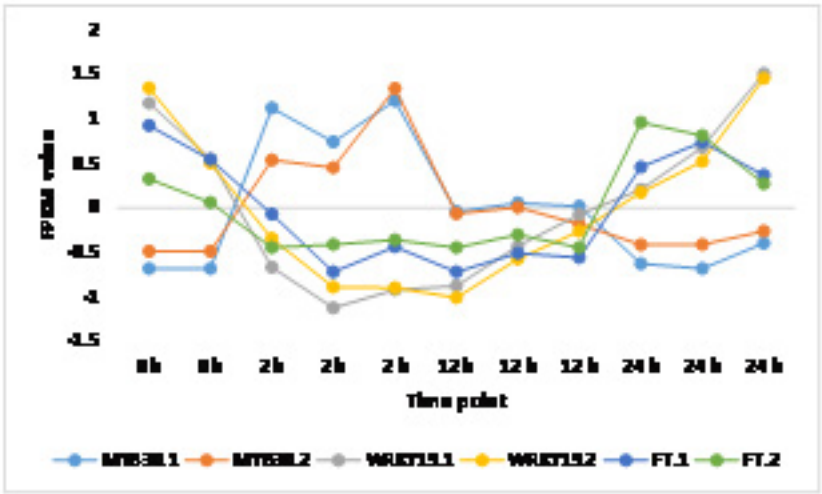

Fig. 11. Expression pattern of transcripts encoding MYB30 and the concordantly downregulated isoforms of WRKY19 and FT genes under salt stress (1 M NaCl) across 0, 2, 12 and 24 h time points in leaves of *H. spontaneum*. Original RNA-Seq data is shown in Table S2

positively relateto salt stress tolerance in tobacco[45]. Genes encoding the two TFs were upregulated under salt stress in *H. spontaneum* as shown in clusters 11 and 1, respectively (Figure 1 and Table S2). Overexpressing the cotton *WRKY41*gene in tobacco exhibited enhanced stomatal closure and reactive oxygen species (ROS) scavenging when plants were exposed to osmotic stress[45]. WRKY46acts in Arabidopsis in developinglateral roots under osmotic/salt stress via regulation of ABA signaling and auxin homeostasis[46]. Auxin homeostasis is known to be regulated by GRETCHEN HAGEN3 or *GH3* gene family in "Plant hormone signal transduction" pathway[42]. In the present study, *GH3.8*gene was upregulated in cluster 17 with no exact TF concordantly expressed with it (Figure 9 and Table S2). No conclusive information on the function of WRKY50 (cluster 1) is available except that it acts as a positive regulator in the salicylic acid (SA) signaling pathway and

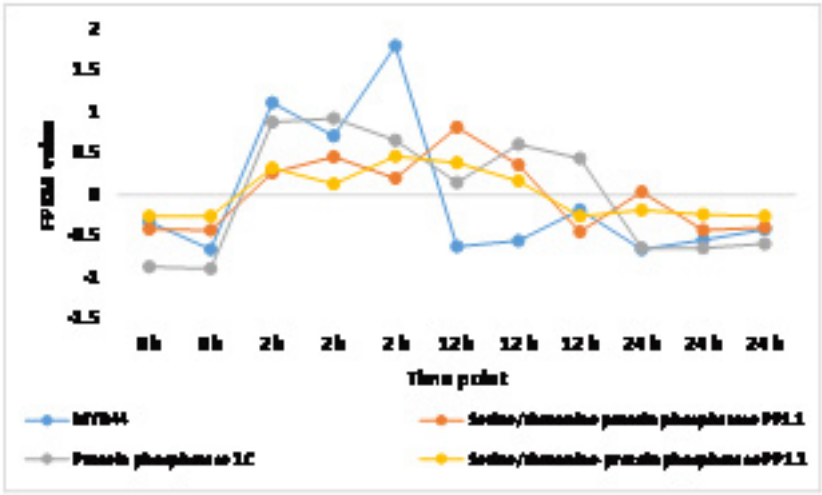

Fig. 12. Expression pattern of transcripts encoding MYB44 concordantly upregulated with genes encoding two isoforms of serine/threonine protein phosphatase PP1 gene as well as with gene encoding protein phosphatase 2C under salt stress (1 M NaCl) across 0, 2, 12 and 24 h time points in leaves of *H. spontaneum*. Original RNA-Seq data is shown in Table S2

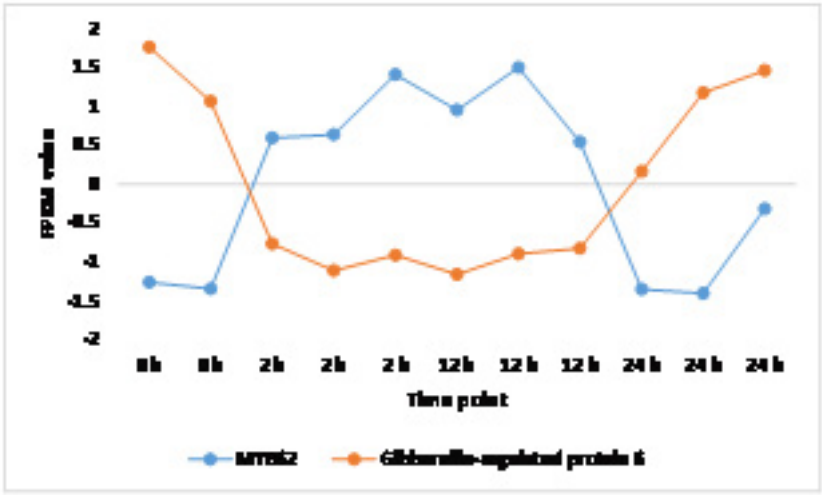

Fig. 13. Expression pattern of transcript encoding MYB62 that is expressed oppositely to gene encoding gibberellin-regulated protein under salt stress (1 M NaCl) across 0, 2, 12 and 24 h time points in leaves of *H. spontaneum*. Original RNA-Seq data is shown in Table S2

probably ABA signaling pathway in Arabidopsis, while a negative regulator in jasmonic acid (JA) signaling[47, 48].Very little is known about the mode of action of WRKY35 except that its expression participates in conferring salt stress tolerance in zoysia grass[49].We conclude that *WRKY* gene family participates in salt stress responses in leaves of *H. spontaneum* in ways different from those in other plant species.

Expression of five MYB genes,namely *MYB30*, *MYB44*, *MYB62*, *MYB3R-2* and *MYB3R-4*, was proven to be increased under salt stress in leaves of *H. spontaneum* (Figure 2a), while expression of four, namely *MYB1*, *MYB20*, *MYB73* and *MYBS3*, wasdecreased (Figure 2b). MYB30, anR2R3 MYBTF, was studied by Gong et al[50] and results indicated that expression in the perennial wall-rocket (*Diplotaxistenuifolia* L.) increased under salt stress up to 4 h time point, while gradually decreased up to 24 h time point in perfect alignment with results of the present study with regard to regulation of gene encoding this TF. MYB30 was proven to be SUMOylated by SIZ1(50, 51). SUMOylation represents a post-translational regulation involved in various cellular processes including response to stresses[52]. SmallUbiquitin-likeModifier (SUMO) proteins, like SIZ1 and SIZ2,represent a family of smallproteinscovalentlyattached to a certain protein, while detached from others to modify target protein's(e.g., MYB30) function. In the present study, two isoforms of *MYB30*gene as well as *MYB44* and *MYB3R-2* genes concordantly expressed with *SIZ2*gene in cluster 6 under salt stress in *H. spontaneum*(Figure 10and Table S2). MYB30also accelerates flowering both in long and short days. Early flowering is mediated by elevated expression of*FLOWERING LOCUS T*(*FT*)gene that is mainly activated byCONSTANS (CO). However, MYB30can also drive expression of *FT*gene[53], a phenomenon that we speculated for *WRKY19*gene under salt stress in *H. spontaneum*. The major difference between the possible regulation of *WRKY19* or*MYB30*gene is that the first is a positive activator, while the second is a negative activator of *FT* gene. This controversial speculated regulation of*WRKY19* and *MYB30*genes under salt stress in *H. spontaneum* is shown in Figure11.MYB30was alsoreported to participate in ABA signaling response[51], in accumulation of very-long-chain fatty acids such as waxes, phospholipids, and complex sphingolipids[54], and in promoting the expression of a subset of brassinosteroids (BRs) target genes[55, 56]. No results were detected on the regulation of genes encoding any of the above-mentioned compounds under salt stress in *H. spontaneum*.

Interestingly, MYB44 was proven to be a negative regulator of ABA signaling and abiotic stresses in Arabidopsis [57], while positively increased sensitivity of seed germination to ABA[58]. The latter authors indicated that phosphorylation of

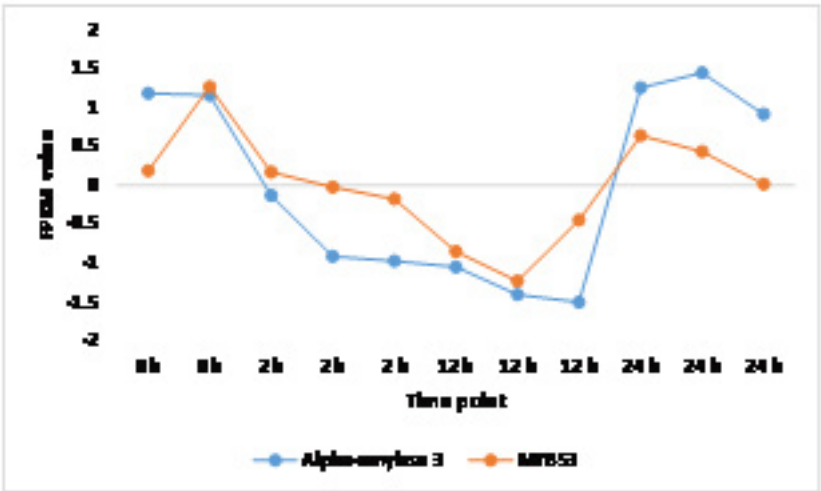

Fig. 14. Expression pattern of transcript encoding MYBS3 that concordantly downregulated with gene encoding alpha-amylase under salt stress (1 M NaCl) across 0, 2, 12 and 24 h time points in leaves of *H. spontaneum*. Original RNA-Seq data is shown in Table S2

MYB44 by MAPK is mandatory for its function. Nonetheless, Jung et al[59] indicated that MYB44 promotes stomatal closure, a characteristic shared with WRKY41 that consequently serves in conferring tolerance to abiotic stresses in Arabidopsis.Tolerance is conferred for plants overexpressing *MYB44* gene because they exhibit a reduced rate of water loss, reduced rate of genes encoding serine/threonine protein phosphatases 2C (PP2Cs), then enhanced tolerance to drought and salt stress. Nonetheless, genes encoding MYB44, protein phosphatase 2C and two isoforms of protein phosphatase 1 (PP1)in the present study are concordantly expressed in cluster 6 (Figure 12 and Table S2). Explanation of concordant expression of *MYB44* and genes encoding the two phosphatases might be that *MYB44* was upregulated only at 2 h time point only, while the other genes were upregulated also at 12 h time point. This indicates that negative regulation of *MYB44* might take place only at 12 h time point.

Devaiah et al[60] stated that MYB62is actingtowards suppression of several phosphate (Pi) starvation-induced genes and suppression of gibberellic acid (GA) biosynthesis under nutrient stress. Authors claimed that cross-talking between Pi homeostasis and GA is an adaptive mechanism underabiotic stresses. Therefore, it is logic that MYB62 negatively regulate expression of gibberellin-regulated protein under salt stress in *H. spontaneum* as previously described [60]. Inthe present study, MYB62 exists in cluster 1 whose

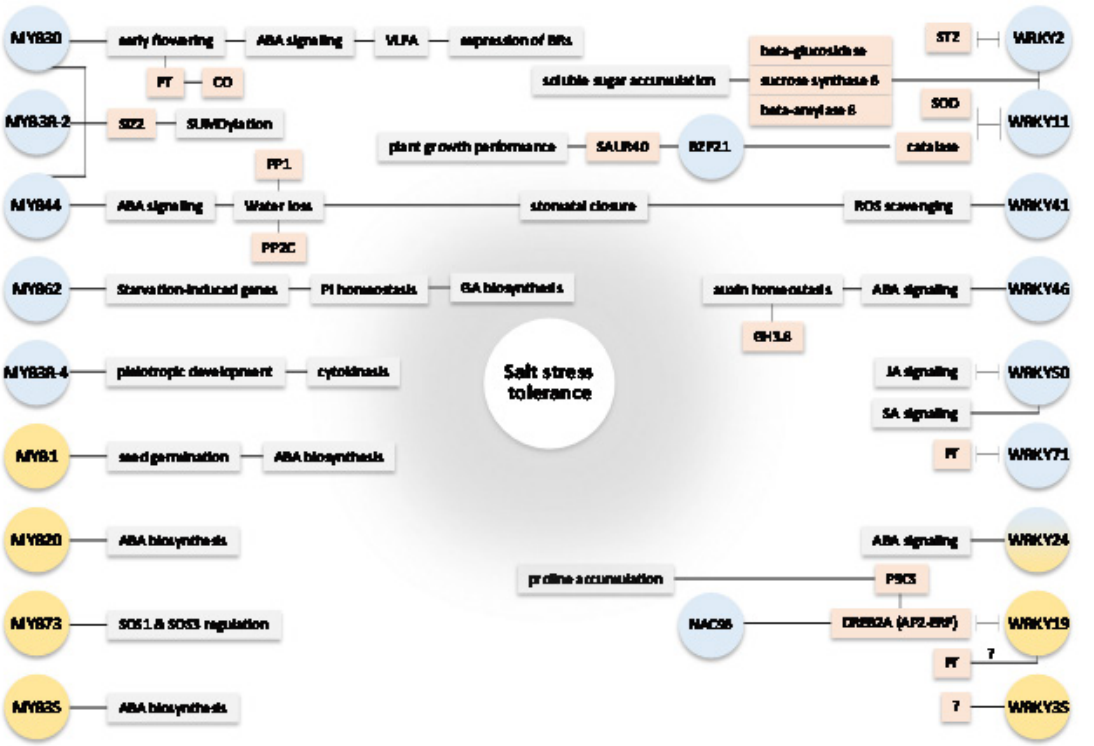

Fig. 15. Networking of transcription factor regulated in *H. spontaneum* under salt stress and their known contribution to salt stress tolerance in plants. Blue circles refer to transcription factor genes upregulated under salt stress, while orange circles refer to transcription factor genes downregulated under salt stress. WRKY24 is upregulated at 2 h time point, while downregulated at 24 h time point of salt stress. Pink boxes refer to regulated genes that are concordantly expressed with transcription factors, while grey boxes refer to biological processes that can lead to salt stress tolerance. STZ = salt tolerance zinc finger, FT = FLOWERING LOCUS T, CO = CONSTANS, SOD = superoxide dismutase, SIZ2 = SUMO E3 ligase (SAP and MIZ1 domain-containing ligase 2), SAUR40 = small a*uxin*-up RNA, PP1 = protein phosphatase 1, PP2C = serine/threonine protein phosphatases 2C, GH3.8 = GRETCHEN HAGEN3, P5CS = Delta-1-pyrroline-5-carboxylate synthase, DREB2A (AP2-ERF) = dehydration-responsive element binding 2A (Apetala2/Ethylene responsive factor)

expression of transcripts indicated upregulation at 2 and 12 h time points, while one gibberellin-regulated protein exists in cluster 4 whose expression of transcripts indicated downregulation at 2 and 12 h time points (Figure 13 and Table S2). As per MYB3R-4, Haga et al[61] indicated its participation in pleiotropic development and regulation of multiple G2/M-specific genes in Arabidopsis. None of the genes involved in the latter processes were regulated in *H. spontaneum* under salt stress.

MYB1, *MYB20*, *MYB73* and *MYBS3*genes were shown to be downregulated under salt stress in *H. spontaneum* (Figure 2b). These TFs were previously reported to negatively regulate abiotic stress tolerance in plants except forMYBS3 that was reported for its positive role in abiotic stresses, particularly cold stress tolerance in rice via mediation of *a-amylase* gene expression[62]. In *H. spontaneum*, MYBS3 seems concordantly expressed with *a-amylase* gene although the first exists in cluster 4, while the second exists in cluster 2 (Figure 14 and Table S2). Wang et al[63] claimed that MYB1 negatively regulates seed germination under saline conditions in Arabidopsis by regulating the levels of the stress hormone abscisic acid (ABA). Similar conclusions were reached by Gao et al[47] in their work on MYB20 in Arabidopsis with regard to the negative regulation of ABA under drought stress. Loss-of-function experiment of *MYB73*gene resulted in drought or/ and salt tolerance due to its negative regulation of *SOS1* (salt overly sensitive 1) and *SOS3* genes(64, 65). No *SOS* genes are regulated under salt stress in *H. spontaneum*.

Summary of the overall molecular networking involving transcription factors and their concordantly expressed genes along with downstream biological processes towards conferring salt stress tolerance in *H. spontaneum* under salt stress is shown in Figure 15.

CONCLUSION

In conclusion, we suggest that WRKY gene family participates in salt stress responses in leaves of *H. spontaneum* of which some of them follow different approaches, in terms of their regulation under salt stress as well as the downstream responsive genes, from those of other plant species. Regulation of MYB gene family in *H. spontaneum*seems similar, to a large extent, to that of other plant species under salt stress. The present study addressed some of the molecular mechanisms by which *H. spontaneum* follows under salt stress in order to stand severe salt stress. One of the important avenue towards improving salt stress tolerance is understanding the regulatory elements, e.g. transcription factors, that drive important salt-related genes. This information might be useful in subsequent breeding programs in cultivated barley and other cereal crops.

SUPPLEMENTARY INFORMATION

Supplementary Information accompanies this article at http://dx.doi.org/10.13005/bbra/2858

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