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Molecular Analysis of Enzymes and Metabolites Regulated under Drought Stress in the Wild Plant Senna (*Cassia angustifolia*)

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This study aimed at studying differential presence of enzymes metabolites via KEGG analysis of trasncriptomes of the wild plant species senna (*Cassia angustifolia* Vahl.) due to watering. Senna is a shrub of the family Caesalpiniaceaewith important applications in pharmaceuticals. Firstly, RNA-Seq datasets were produced by next-generation sequencing (NGS) of Illumina Miseq of leaf (day 1) in order to detect the influence of wateringat day 2. Samples were harvested at three time points (e.g., dawn, midday and dusk) of the two days.*de novo* assembleddatasets and number ofannotated genes exceeded 2000 genes. As cluster analysis of gene expression almost showed no discrete differencesat the transcriptome level due to watering within time pointsof dawn and dusk, the study focused mainly on those of the midday across the two days. KEGG analysisfor genes whose differential expression between the two days wase"5 FC resulted in a number of enzymes that were found repressed due to watering, thus likely participate in the molecular mechanisms utilized by the organism to adapt to the long-lasting drought stress. The recovered regulated metabolites and enzymes included abscisic acid (ABA) receptor PYL4 and PYL9, auxin response factor (ARF) 5 and 15, ARF (or Aux/IAA) proteins IAA7 and IAA14, indole-3-pyruvate (or flavin) monooxygenase,phosphoinositide phosphatase SAC1 and SAC6, pre-mRNA splicing factors 8, 8A, 19, 40A and ISY1, andserine/arginine-rich splicing regultors SCL33, RS31 and RS34. The two pathways tryptophan metabolism and plant hormone signal transduction likely crosstalk in senna (*C. angustifolia*) towards the maintenance of normal growth under adverse condition.Many other regulated metabolites and proteins in senna (*C. angustifolia*) including brassinosteroid, heat shock protein 95s, ATPase, several protein kinases such as mitogen-activated protein kinase (MAPK) and cytochrome c oxidase. Other enzymes include phospholipase C2 and allene oxide cyclase as well as isochorismate pathway were also regulated in senna (*C. angustifolia*). In conclusion, we think that we have scoped the light on the possibleregulated metabolites under drought stress that might confer drought stress tolerance in the wild plant senna (*C. angustifolia*).

Caesalpiniaceae is a large family with several pharmaceutical applications as being mainly used as a laxative and to relieve constipation. *Cassia angustifolia*, formally *Sennaangustifolia* (2n = 28) is known for its important applications in pharmaceuticals. *C. angustifolia* is a wild medicinal

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drought-tolerant shrub (Ayoub 1977, Khalid *et al*. 2012) with many cathartic properties (Lemli 1986, Folkard 1995, Hammouda *et al*. 2005). Drought stress-related genes have been studied at the trancriptomic level in senna (*C. angustifolia*) (Mehta *et al*. 2017) as well as in many other plant species such as parsley (Li *et al*. 2014a,b), bean (Hiz *et al*. 2014), chrysanthemum (Xu *et al*. 2013), tall fescue (Hu *et al*. 2014), and grapevine (Rocheta *et al*. 2014). The lucine-rich repeat kinase family was recently found in senna (*C. angustifolia*) as the most abundant group of protein kinases under drought stress in addition to several families of transcription factors (e.g., bHLH, and bZIP, etc.) (Mehta *et al*. 2017). Previous studies on senna (*C. angustifolia*) deciphered some physiological, morphological and molecular mechanisms allowed the plant to tolerate drought stress (Ratnayaka and Kincaid 2005, Mehta *et al*. 2017).

Availability of water is a major obstacle for agricultural productivity. Wild plants growing in severe arid climates provide tools for studying plant response to extreme drought conditions. Generally speaking, drought, saltand heat stresses have large impacts on plant growthand productivity. Other abiotic deleterious stressesnowadays include increased chemicals and pollutants. Besides, it is likely that plants are exposed to more than one type of stress at a time. In particular, drought stress is amajor threat in at least 26 % of world's arable land (Blum1988). The effects of drought include delayedor stunted growth aswell as impaired physiological processes such as photosynthesis, respiration,and mineral exchange (Do *et al*. 2013). Therefore,it is crucial to get a better understanding the molecular and physiological impacts ofdrought stress inorder to find solutions to help the plant cope with or at least lower the influence of this stress for the sake ofmaintaining crop productivity and possibly cultivate more crops in arid lands to mitigate global food crisis.

Plants are adaptedto drought eitherby avoidance or tolerance,as the two main strategies, by which a crop plant can maintain yield components and minimize theloss due to the stress. Avoidance mechanisms include the occurrence of several morphological changes, such as stomatal closure and reduced leaf area to reduce respiration, as well asenlargingroot systems in order to gain more water with the same intensity of cultivation (Levitt 1980, Budak *et al*. 2013, Rama Reddy*et al*. 2014). Alternatively, drought tolerance is a subject of intense research as it mainly occurs due to several physiologicaland molecular mechanisms that help the plant to adapt with the osmotic pressure due to the shortage of water (Bartels and Sunkars2005). Tolerance mechanisms were proven to be genetically-dependent as different plant species have different strategies to cope with the problem. These strategies are supported by complex metabolicpathways that should link together and cross-talk in order to produce osmolytes and protein chaperons to secure the cell from the stress and avoid denaturation or damage of important compounds in the cell (Yamaguchi-Shinozaki andShinozaki 2006,Kantar *et al*. 2011,Shanker *et al*. 2014). There are several abiotic stress-related enzymes like glutathione reductase, catalase, superoxide dismutase considered as biomarkers for drought stress tolerance (Khammari *et al*. 2012).

The present study aim at studying drought-related dynamics of leaf transcriptome insenna (*C. angustifolia*)to detect the crosstalking pathways possibly associated withdrought stress tolerance to add to our understanding of the molecular mechanisms underlyingdrought stress tolerance in wild plants.

MATERIAL AND METHODS

Plant material sampling and watering regime

The field experiment of sample treatment and harvesting was conducted for senna (*C. angustifolia*) shrubs grown near Jeddah, Saudi Arabia. Three plants of equal size were selected in which leaf samples were harvested in two consecutive days at three time point of the day (1 h post-dawn, midday and 1 h post-dusk). At dawn of the day 2, plants were watered (25 liters dH_2O) and leaf samples were harvest at the same three time points.

RNA-Seq and KEGG analyses

Total RNA was extracted from three similar-sized (10 mm^2) leaf discs per plant of*Cassia angustifolia*, then shipped to Beijing Genome Institute (BGI), China, for next-generation sequencing. Recovered RNA-Seq datasets were *de novo* assembledusing the Trinity RNA-Seq Assembly package (r2013-02-25) with optimized parameters and K-mer size set to 25 (Zhang *et*

al. 2015). Differential expression and cluster analysis were done by EdgeR (version 3.0.0, R version 2.1.5). All predicted CDS were annotated against protein database in order to assign putative function of the transcriptome after translation into protein. To identify the biological pathways with enzymes that differentiate at midday samples, the detected genes were mapped to reference canonical pathways in the Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.ad.jp/kegg/).RNA-Seq datasets of *Cassia angustifolia* were validated via qRT-PCR (data provided upon request).

RESULTS AND DISCUSSION

Preliminary data analysis

Sequence assemblyresulted in a number of Ã5000 regulated genes due to watering and the number of annotated genes Ã2000 genes. The term enrichment in this study refers to the increase of a given enzymeor metabolitein the second day due to watering, while suppression indicates that theintensity of the enzyme or metabolitewas reduced due to watering. In other words, enzymeor metaboliteenrichment indicates thatthe encoding genes were highly expressed after watering, while repression indicates that the expression of the encoding drought-related genes isabolished as it is no longer required after watering. GO classification indicated that the subcategory "response to stimulus" is repressed as the stress in the second day is completely relieved.We expected that several biological processes of this subgroup can confer tolerance to drought stress.

KEGG analysis

In order to study the enzymes inselected biological pathways of *Cassia angustifolia*whose genes encoding them are highly (e"5 FC) regulated due to watering, we mapped the detected genes to reference canonical pathways in the Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.ad.jp/kegg/). Drought tolerance is a multigenic process with different metabolic pathways affecting plant growth (Mehta *et al*. 2017).Pathwaysthat were either enriched or represseddue to watering were examined (Table S1).Of which, we selected the pathways that might have emphasis on plant response to abiotic stress (Table 1). In addition, we have investigated few other pathways whose enzymes were suppressed due to watering, while showed no enzymes that were enriched due to the stress. When applying the criteria of e"5 FC gene regulation in KEGG analysis, a number of five groups of pathways including 14 subgroups with 22 pathways were selected. These groups are "metabolism", "Genetic Information Processing", "Environmental Information Processing", "Cellular Processes" and "Organismal Systems". Of which, 10 pathways showed no enrichment in their enzymes due to watering, rather they were repressed due to watering. This indicates that these pathways likely participate in the molecular mechanisms utilized by the organism to cope with the stress, whose enrichment is not required when water becomes available. These pathways are "Biosynthesis of siderophore group nonribosomal peptides", "Spliceosome", "VEGF signaling pathway", "Jak-STAT signaling pathway", "Autophagy - other", "Tight junction", "Longevity regulating pathway", "Longevity regulating pathway - multiple species", "Circadian rhythm" and "Thermogenesis" (Table 1). Enzymes or metabolites of the enriched and suppressed pathways are shown in Tables 2 and 3, respectively. The number of activated enzymes due to watering was 43 enzymes, while the number was 107 for the suppressed enzymes due to watering (Tables 2 and 3).

Drought stress triggers several plant responses at the gene expression levels, and likely result in the accumulation of secondary metabolites or osmolytes that help the plant stand the stress (Ramchandra Reddy *et al*. 2004, Ergen *et al*. 2009). In the present study, a large number of enzymes were found repressed due to watering, thus likely participate in the molecular mechanisms utilized to adapt to the long-lasting drought stress. Enrichment of these enzymes is not required when water becomes available, while re-enriched when land became dry again. KEGG analysis indicated that several gene families are involved as a safeguard against drought stress. Of these gene families, abscisic acid (ABA) receptor PYLseems to be required under drought stress for ABA-mediated responses such as stomatal closure (Hao *et al*. 2011). Suppression of two types of this receptor, namely PYL4 and PYL9 in senna (*C. angustifolia*)(Table 3, Figure 1), indicates that they participate in morphological changes as

Table 1. Selected pathways of *C.angustifolia*with enzymes encoded by genes highly (e”5 FC) regulated due to the watering.
Green box = pathway enriched due to watering, red box = pathway suppressed due to watering, blue box = regulated TOR-dependent pathway

Group	Subgroup	Pathway ID	Pathway	Enriched	suppressed
1. Metabolism	1.1 Carbohydrate metabolism	562	Inositol phosphate metabolism	Green box	red box
	1.3 Lipid metabolism	592	alpha-Linolenic acid metabolism	Green box	red box
	1.5 Amino acid metabolism	380	Tryptophan metabolism	Green box	red box
		400	Phenylalanine, tyrosine and tryptophan biosynthesis	Green box	red box
	1.9 Metabolism of terpenoids and polyketides	1053	Biosynthesis of siderophore group nonribosomal peptides		red box
2. Genetic information Processing	2.1 Transcription	3040	Spliceosome		red box
	2.3 Folding, sorting and degradation	4141	Protein processing in endoplasmic reticulum	Green box	red box
3. Environmental information processing	3.2 Signal transduction	4012	ErbB signaling pathway	Green box	red box
		4016	MAPK signaling pathway - plant	Green box	red box
		4072	Phospholipase D signaling pathway	Green box	red box
		4075	Plant hormone signal transduction	Green box	red box
		4150	mTOR signaling pathway	Green box	red box
		4151	PI3K-Akt signaling pathway	Green box	red box
		4370	VEGF signaling pathway		red box
		4630	Jak-STAT signaling pathway		red box
4. Cellular processes	4.1 Transport and catabolism	4136	Autophagy - other		red box
	4.2 Cell growth and death	4218	Cellular senescence	Green box	red box
	4.3 Cellular community - eukaryotes	4530	Tight junction		red box
5. Organismal systems	5.9 Aging	4211	Longevity regulating pathway		red box
		4213	Longevity regulating pathway - multiple species		red box
	5.10 Environmental adaptation	4710	Circadian rhythm		red box
		4714	Thermogenesis		red box

Table 2. Selected pathways of *C. angustifolia*with enzymes encoded by genes highly (≥5 FC) enriched at midday due to the watering. N = before watering, NR = after watering

No.	Pathway/Enzyme	Time point					
		Before watering			After watering		
0380	Tryptophan metabolism	N1	N2	N3	NR1	NR2	NR3
1	Probable acetyl-CoA acetyltransferase cytosolic 2	-0.24212	-0.24212	-0.24212	2.0615160	1.8123826	1.789422
2	Amidase 1	-0.64225	-0.66793	-0.67082	4.195597	2.802451	3.00827
3	Aldehyde dehydrogenase family 7 member A1	-0.50968	-0.64755	-0.38832	1.789422	2.655908	2.62422
0400	Phenylalanine, tyrosine and tryptophan biosynthesis (2)	N1	N2	N3	NR1	NR2	NR3
4	Arogenate dehydratase/prephenate dehydratase 2- chloroplastic	-0.48907	-0.48907	-0.48907	2.473292	2.290608	2.324863
0562	Inositol phosphate metabolism (3)	N1	N2	N3	NR1	NR2	NR3
5	Inositol oxygenase 4	-0.97716	-1.25336	-1.25336	2.448629	3.041257	2.848035
6	Inositol-tetrakisphosphate 1-kinase 3	-0.09205	0.007279	-0.27748	3.901911	3.545024	3.259165
7	Triosephosphate isomerase cytosolic	-0.50236	-0.14356	0.089004	3.068392	2.946959	3.529814
0592	alpha-Linolenic acid metabolism (1)	N1	N2	N3	NR1	NR2	NR3
8	Glyoxysomal fatty acid beta-oxidation multifunctional protein MFP-a	-0.08301	-0.29871	-0.4084	2.058619	2.266513	0.83222
4012	ErbB signaling pathway (2)	N1	N2	N3	NR1	NR2	NR3
9	Shaggy-related protein kinase kappa	-0.58381	-0.58381	-0.58236	2.858208	2.870764	3.025239
10	Serine/threonine-protein kinase TOR	-0.62635	-0.70496	-0.48959	3.435905	3.497071	3.2715
4016	MAPK signaling pathway - plant (2)	N1	N2	N3	NR1	NR2	NR3
11	Ethylene-insensitive protein 2	-0.43202	-0.43202	-0.43202	1.314289	2.52031	2.397217
12	Protein ETHYLENE INSENSITIVE 3	-0.60329	-1.40404	-1.40404	4.847172	4.881385	4.470712
4072	Phospholipase D signaling pathway (5)	N1	N2	N3	NR1	NR2	NR3
13	Phospholipase D zeta 1	-0.17576	-0.17576	-0.17576	1.624987	1.187133	1.17576
14	Dynamin-2B	-0.57567	-0.57279	-0.28525	1.046323	1.758612	2.629566
15	Serine/threonine-protein kinase TOR	-0.60295	-0.60295	-0.60295	3.150976	3.324852	2.568417
16	ADP-ribosylation factor 1	-0.65602	-0.65602	-0.65602	3.214932	2.680402	3.423353
17	ADP-ribosylation factor 2	-1.07784	-1.07784	-1.07784	4.767723	4.261117	3.38493
18	Ankyrin repeat- PH and SEC7 domain containing protein secG	-0.5132	-0.5132	-0.5132	1.569848	1.580703	0.971201

4075 Plant hormone signal transduction (5)	N1	N2	N3	NR1	NR2	NR3
19 Auxin response factor 1	-0.51286	-0.51286	-0.51286	3.266296	1.894222	2.251403
20 Histidine-containing phosphotransfer protein 1	-0.37534	-0.37534	-0.37534	-0.37534	2.328646	1.703269
21 Gibberellin receptor GID1B	-1.05549	-1.14079	-1.36132	3.321495	2.686399	2.662871
22 Ethylene-insensitive protein 2	-0.43202	-0.43202	-0.43202	1.314289	2.52031	2.397217
4141 Protein processing in endoplasmic reticulum (9)	N1	N2	N3	NR1	NR2	NR3
23 Cullin-1	-0.34984	-0.24015	-0.34984	0.731499	1.660223	1.86925
24 Heat shock cognate protein 80	-0.29447	-0.23504	-0.15431	2.723259	2.478121	1.853568
25 Dolichyl-diphosphooligosaccharide—protein glycosyltransferase subunit STT3A	-0.24265	-0.12895	-0.24265	1.843793	1.209944	1.24265
26 Calreticulin	-0.86438	-0.86438	-0.86438	3.981055	4.049367	3.634441
27 Heat shock protein 90-5- chloroplastic	-0.88796	-0.34992	-0.3892	4.341242	3.589975	2.973597
28 Alpha-mannosidase I MNS4	-0.54941	-0.54941	0.088435	1.76006	3.3388	2.170433
29 Protein transport protein Sec61 subunit alpha	-0.10453	-0.3406	-0.45163	1.002018	1.709612	2.032249
30 Derlin-1	-0.91968	-0.5126	0.469884	3.480923	3.095565	2.875045
31 Plant UBX domain-containing protein 4	-0.44073	-0.25043	-0.44073	1.155248	2.939165	2.130943
4150 mTOR signaling pathway (4)	N1	N2	N3	NR1	NR2	NR3
32 V-type proton ATPase catalytic subunit A	-0.33156	-0.33156	-0.33156	0.813791	2.564324	1.53317
33 V-type proton ATPase subunit F	-0.88151	-0.88151	-0.88151	4.827068	4.137752	4.25776
34 Shaggy-related protein kinase kappa	-0.76969	-0.76969	0.29795	0.655233	1.469709	0.984771
35 Serine/threonine-protein kinase TOR	-0.60295	-0.60295	-0.60295	3.150976	3.324852	2.568417
4151 PI3K-Akt signaling pathway (5)	N1	N2	N3	NR1	NR2	NR3
36 Shaggy-related protein kinase kappa	-0.76969	-0.76969	0.29795	0.655233	1.469709	0.984771
37 Serine/threonine-protein phosphatase regulatory subunit A	-0.59834	-0.63208	-0.97463	2.142898	2.687689	2.046494
38 Heat shock cognate protein 80	-0.29447	-0.23504	-0.15431	2.723259	2.478121	1.853568
39 Serine/threonine-protein kinase TOR	-0.60295	-0.60295	-0.60295	3.150976	3.324852	2.568417
40 Heat shock protein 90-5- chloroplastic	-0.88796	-0.34992	-0.3892	4.341242	3.589975	2.973597
4213 Longevity regulating pathway - multiple species (2)	N1	N2	N3	NR1	NR2	NR3
41 SNF1-related protein kinase regulatory subunit beta-2	-0.20111	-0.39645	-0.39645	-0.39645	2.435828	2.398065
42 Serine/threonine-protein kinase TOR	-0.60295	-0.60295	-0.60295	3.150976	3.324852	2.568417
4218 Cellular senescence (1)	N1	N2	N3	NR1	NR2	NR3
43 Serine/threonine-protein kinase TOR	-0.60295	-0.60295	-0.60295	3.150976	3.324852	2.568417

Table 3. Selected pathways of *C. angustifolia*with enzymes encoded by drought-related genes highly (≥ 5 FC) suppressed due to the watering. N = before watering, NR = after watering

No.	Pathway/Enzyme	Time point					
		Before watering			After watering		
00380	Tryptophan metabolism (2)	N1	N2	N3	NR1	NR2	NR3
1	Aldehyde dehydrogenase family 3 member F1	1.587468	1.287719	-0.50763	-1.456226318	-1.456226318	-1.456226318
2	Probable indole-3-pyruvate monooxygenase YUCCA10	2.227227	2.164615	1.962111	-4.308717014	-4.308717014	-4.308717014
00400	Phenylalanine, tyrosine and tryptophan biosynthesis (4)	N1	N2	N3	NR1	NR2	NR3
3	Probable aminotransferase TAT2	0.34544	0.16178	0.060001	-0.918195045	-0.918195045	-0.918195045
4	Anthranilate synthase alpha subunit 2- chloroplastic	0.685204	0.618131	0.077341	-1.623098806	-1.623098806	-1.623098806
5	3-dehydroquinate synthase	0.059773	0.131979	0.080449	-0.258688436	-0.258688436	-0.258688436
6	Bifunctional 3-dehydroquinate dehydratase/shikimate dehydrogenase- chloroplastic	0.339229	0.373643	-0.09642	-2.534977735	-2.534977735	-2.534977735
00562	Inositol phosphate metabolism (6)	N1	N2	N3	NR1	NR2	NR3
7	Putative 1-phosphatidylinositol-3-phosphate 5-kinase FAB1C	-0.18249	0.261804	-0.48236	-0.696482365	-0.696482365	-0.696482365
8	Inositol-3-phosphate synthase	-1.33722	-1.07768	-2.64815	-2.648146652	-2.648146652	-2.648146652
9	Phosphoinositide phospholipase C 2	0.044158	0.002828	-0.23448	-3.234123788	-3.234123788	-2.779947895
10	SAL1 phosphatase	-0.21719	0.201112	-0.62427	-0.624266235	-0.624266235	-0.624266235
11	Phosphoinositide phosphatase SAC6	-0.22023	0.207125	0.361932	-1.00264063	-1.00264063	-1.00264063
12	Phosphoinositide phosphatase SAC1	-0.28007	-0.00855	-0.60254	-0.689320217	-0.689320217	-0.689320217
00592	alpha-Linolenic acid metabolism (4)	N1	N2	N3	NR1	NR2	NR3
13	Peroxisomal acyl-coenzyme A oxidase 1	0.420476	0.332021	0.490144	-0.580245492	-0.580245492	-0.580245492
14	3-ketoacyl-CoA thiolase 2- peroxisomal	2.497536	0.943436	0.033103	-1.311157654	-1.223016057	-1.160597977
15	Allene oxide cyclase- chloroplastic	0.982792	0.939056	0.797635	-4.8274607	-4.747141535	-4.75890803
16	Fatty acid hydroperoxide lyase- chloroplastic	0.794242	0.693469	0.69914	-3.482149218	-4.223724066	-4.223724066
01053	Biosynthesis of siderophore group nonribosomal peptides (1)	N1	N2	N3	NR1	NR2	NR3
17	Isochorismate synthase- chloroplastic	0.246862	0.101321	0.281063	-0.416710774	-0.416710774	-0.416710774
03040	Spliceosome (21)	N1	N2	N3	NR1	NR2	NR3
18	Pre-mRNA-processing factor 19 homolog 2	0.60534	1.088448	1.057733	-1.29615449	-1.29615449	-1.29615449
19	Small nuclear ribonucleoprotein SmD1b	1.055236	0.646652	-0.28834	-1.809891101	-1.809891101	-1.809891101
20	U1 small nuclear ribonucleoprotein 70 kDa	0.810862	1.303077	-1.39203	-1.119405074	-1.392025529	-1.392025529

21	Probable small nuclear ribonucleoprotein F	1.16508	0.276073	-0.15363	-2.104102705	-2.104102705	-2.104102705
22	Sm-like protein LSM7	1.206399	0.436801	0.56956	-2.583434918	-2.583434918	-2.583434918
23	DEAD-box ATP-dependent RNA helicase 42	0.343033	0.826515	0.910273	-3.419209265	-3.419209265	-3.419209265
24	Pre-mRNA-splicing factor ATP-dependent RNA helicase DEAH1	0.070534	0.243749	-0.15351	-0.153506306	-0.153506306	-0.153506306
25	Pre-mRNA-processing protein 40A	0.82283	1.068422	0.835349	-2.682934189	-2.682934189	-2.682934189
26	RNA-binding protein 25	0.240599	0.642963	0.572124	-3.238113765	-3.238113765	-3.238113765
27	Splicing factor 3B subunit 2	0.270645	1.218281	-0.54197	-0.773399755	-0.657034999	-0.773399755
28	Splicing factor U2af small subunit B	0.454521	0.634509	-0.44233	-1.463101427	-1.463101427	-1.463101427
29	Splicing factor U2AF 65 kDa subunit	0.11815	0.929108	0.938241	-0.780631873	-0.79641487	-0.79641487
30	Protein RRC1	0.532613	0.082866	-0.39497	-1.366562985	-1.366562985	-1.366562985
31	Pre-mRNA-splicing factor 8 homolog	0.570287	0.259443	-0.02415	-3.086094827	-3.086094827	-3.086094827
32	Pre-mRNA-processing-splicing factor 8A	-0.11281	0.637654	0.642344	-2.907325618	-2.907325618	-2.907325618
33	Pre-mRNA-splicing factor ISY1 homolog	0.395342	0.524189	-0.37189	-0.371889402	-0.371889402	-0.371889402
34	THO complex subunit 1	0.093394	0.446651	-0.47707	-0.477068528	-0.477068528	-0.477068528
35	Serine/arginine-rich-splicing factor SR34	-0.2751	0.380628	0.087468	-1.088216087	-1.088216087	-1.088216087
36	Serine/arginine-rich splicing factor SC35	0.733706	0.937683	0.670312	-2.421557464	-2.421557464	-2.421557464
37	Serine/arginine-rich splicing factor RS31	0.58339	0.093735	0.63455	-0.452233488	-0.452233488	-0.452233488
38	Serine/arginine-rich SC35-like splicing factor SCL33	-0.1577	0.030388	0.045774	-0.454007978	-0.454007978	-0.454007978
04012 ErbB signaling pathway (1)		N1	N2	N3	NR1	NR2	NR3
39	Serine/threonine-protein kinase TOR	-0.13965	0.024261	0.222411	-0.467335376	-0.467335376	-0.467335376
04016 MAPK signaling pathway - plant (8)		N1	N2	N3	NR1	NR2	NR3
40	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1	-0.39322	0.158405	0.151183	-0.981064495	-0.981064495	-0.981064495
41	Transcription factor bHLH14	0.548965	0.884457	-0.22924	-0.229243769	-0.229243769	-0.229243769
42	Transcription factor MYC2	0.463226	0.388223	0.246732	-3.542892865	-3.542892865	-3.542892865
43	Abscisic acid receptor PYL4	1.852722	2.016651	1.774202	-2.549995805	-3.405985503	-2.273079691
44	Abscisic acid receptor PYL9	-0.04695	0.02713	0.14755	-0.851006972	-0.851006972	-0.851006972
45	Serine/threonine-protein kinase SAPK10	0.085518	0.408981	0.024526	-0.672358315	-0.672358315	-0.672358315
46	Serine/threonine-protein kinase SRK2I	0.352227	0.265327	0.300548	-0.464986643	-0.464986643	-0.464986643
47	Ethylene-insensitive protein 2	0.625007	0.49946	0.634267	-2.388455563	-2.388455563	-2.388455563
48	ETHYLENE INSENSITIVE 3-like 3 protein	0.006315	0.249024	0.482061	-0.302861221	-0.302861221	-0.302861221
49	Mitogen-activated protein kinase 3	-0.32703	-0.24247	-0.65804	-2.104820666	-2.104820666	-2.104820666
50	Mitogen-activated protein kinase kinase kinase NPK1	-0.10581	0.342507	-0.21778	-0.406812599	-0.406812599	-0.406812599

04072	Phospholipase D signaling pathway (3)	N1	N2	N3	NR1	NR2	NR3
51	Dynamin-2B	0.036532	0.427132	-0.81227	-0.812266541	-0.812266541	-0.812266541
52	Serine/threonine-protein kinase TOR	-0.13965	0.024261	0.222411	-0.467335376	-0.467335376	-0.467335376
53	Ankyrin repeat- PH and SEC7 domain containing protein secG	0.054366	0.039542	0.172577	-0.679422307	-0.679422307	-0.679422307
04075	Plant hormone signal transduction (12)	N1	N2	N3	NR1	NR2	NR3
54	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1	-0.39322	0.158405	0.151183	-0.981064495	-0.981064495	-0.981064495
55	Transcription factor bHLH14	0.548965	0.884457	-0.22924	-0.229243769	-0.229243769	-0.229243769
56	Transcription factor MYC2	0.463226	0.388223	0.246732	-3.542892865	-3.542892865	-3.542892865
57	ABSCISIC ACID-INSENSITIVE 5-like protein 2	0.147921	0.100752	0.205535	-0.336475436	-0.336475436	-0.336475436
58	Ubiquitin C-terminal hydrolase 22	0.85731	0.59537	-1.46606	-1.466060125	-1.466060125	-1.466060125
59	Auxin-responsive protein IAA14	1.958978	1.720019	1.690397	-2.897808345	-2.897808345	-1.762930291
60	Auxin-responsive protein IAA7	-1.12006	-1.0914	-1.24864	-2.252966449	-2.250083941	-2.251524475
61	Protein Transport Inhibitor Response 1 (TIR1)	1.184285	1.267837	1.534964	-2.01593676	-2.01593676	-2.01593676
62	Auxin response factor 5	-0.20576	0.035701	-0.49736	-0.497363523	-0.497363523	-0.497363523
63	Auxin response factor 15	-0.20652	-0.18762	-0.10993	-2.070066629	-2.070066629	-2.070066629
64	Two-component response regulator ARR3	0.66018	0.893755	0.665409	-0.801348332	-0.801348332	-0.801348332
65	Abscisic acid receptor PYL4	1.852722	2.016651	1.774202	-2.549995805	-3.405985503	-2.273079691
66	Abscisic acid receptor PYL9	-0.04695	0.02713	0.14755	-0.851006972	-0.851006972	-0.851006972
67	Serine/threonine-protein kinase SAPK10	0.182149	0.394438	0.649098	-0.879973008	-0.879973008	-0.879973008
68	Serine/threonine-protein kinase SRK2I	0.352227	0.265327	0.300548	-0.464986643	-0.464986643	-0.464986643
69	Methyltransferase-like protein 2	0.088466	0.244608	0.328877	-0.652245369	-0.652245369	-0.652245369
70	Ethylene-insensitive protein 2	0.625007	0.49946	0.634267	-2.388455563	-2.388455563	-2.388455563
71	ETHYLENE INSENSITIVE 3-like 3 protein	0.006315	0.249024	0.482061	-0.302861221	-0.302861221	-0.302861221
04136	Autophagy - other (3)	N1	N2	N3	NR1	NR2	NR3
72	Serine/threonine-protein kinase TOR	-0.13965	0.024261	0.222411	-0.467335376	-0.467335376	-0.467335376
73	Autophagy-related protein 8C	0.034356	0.272842	-1.83552	-1.835515633	-1.831194027	-1.58334222
04141	Protein processing in endoplasmic reticulum (9)	N1	N2	N3	NR1	NR2	NR3
74	Protein transport protein Sec61 subunit beta	-0.41036	-0.01659	-1.17928	-1.815265804	-1.815265804	-1.815265804
75	Heat shock protein 90-5- chloroplastic	1.960161	2.058363	1.856076	-4.675500173	-4.675500173	-4.675500173
76	Ubiquitin recognition factor in ER-associated degradation protein 1	0.284782	0.397391	-0.11243	-1.516790405	-1.516790405	-1.516790405
04150	mTOR signaling pathway (4)	N1	N2	N3	NR1	NR2	NR3
77	CBL-interacting protein kinase 1	0.411691	0.2668	0.148233	-1.62111525	-1.62111525	-1.62111525

78	Serine/threonine-protein kinase TOR	-0.13965	0.024261	0.222411	-0.467335376	-0.467335376	-0.467335376
79	Serine/threonine-protein kinase ATG1c	-0.08368	-0.07452	0.311552	-0.581421762	-0.581421762	-0.581421762
80	Calcium-binding protein 39-like	0.015711	0.085252	0.051792	-1.643756869	-1.643756869	-1.520752915
04151 PI3K-Akt signaling pathway (5)		N1	N2	N3	NR1	NR2	NR3
81	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A beta isoform	0.810633	0.662534	-0.48803	-0.488025561	-0.488025561	-0.488025561
82	CBL-interacting protein kinase 1	0.411691	0.2668	0.148233	-1.62111525	-1.62111525	-1.62111525
83	Serine/threonine-protein kinase TOR	-0.13965	0.024261	0.222411	-0.467335376	-0.467335376	-0.467335376
84	Myb-related protein A	0.451647	0.311785	0.130007	-2.191054894	-2.191054894	-2.191054894
85	Heat shock protein 90-5- chloroplastic	1.960161	2.058363	1.856076	-4.675500173	-4.675500173	-4.675500173
04211 Longevity regulating pathway (2)		N1	N2	N3	NR1	NR2	NR3
86	CBL-interacting protein kinase 1	0.411691	0.2668	0.148233	-1.62111525	-1.62111525	-1.62111525
87	Serine/threonine-protein kinase TOR	-0.13965	0.024261	0.222411	-0.467335376	-0.467335376	-0.467335376
04213 Longevity regulating pathway - multiple species (2)		N1	N2	N3	NR1	NR2	NR3
88	CBL-interacting protein kinase 1	0.411691	0.2668	0.148233	-1.62111525	-1.62111525	-1.62111525
89	Serine/threonine-protein kinase TOR	-0.13965	0.024261	0.222411	-0.467335376	-0.467335376	-0.467335376
04218 Cellular senescence (3)		N1	N2	N3	NR1	NR2	NR3
90	Calcineurin B-like protein 3	1.070011	1.306436	1.147796	-1.734456047	-1.734456047	-1.734456047
91	Serine/threonine-protein kinase TOR	-0.13965	0.024261	0.222411	-0.467335376	-0.467335376	-0.467335376
92	Mitochondrial outer membrane protein porin 2	0.659578	0.668525	0.995054	-0.288275387	-0.288275387	-0.288275387
04370 VEGF signaling pathway (1)		N1	N2	N3	NR1	NR2	NR3
93	Calcineurin B-like protein 3	1.070011	1.306436	1.147796	-1.734456047	-1.734456047	-1.734456047
04530 Tight junction (3)		N1	N2	N3	NR1	NR2	NR3
94	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A beta isoform	0.810633	0.662534	-0.48803	-0.488025561	-0.488025561	-0.488025561
95	CBL-interacting protein kinase 1	0.411691	0.2668	0.148233	-1.62111525	-1.62111525	-1.62111525
96	Tubulin alpha-5 chain	0.591109	-0.00636	0.338417	-2.790866288	-2.790866288	-2.790866288
04630 JAK-STAT signaling pathway (1)		N1	N2	N3	NR1	NR2	NR3
97	Serine/threonine-protein kinase TOR	-0.13965	0.024261	0.222411	-0.467335376	-0.467335376	-0.467335376
04710 Circadian rhythm (1)		N1	N2	N3	NR1	NR2	NR3
98	CBL-interacting protein kinase 1	0.411691	0.2668	0.148233	-1.62111525	-1.62111525	-1.62111525
04714 Thermogenesis (9)		N1	N2	N3	NR1	NR2	NR3
99	Succinate dehydrogenase [ubiquinone] flavoprotein subunit 1- mitochondrial	1.353688	0.91732	1.373706	-1.736656898	-1.736656898	-1.736656898

100	Cytochrome c oxidase assembly protein COX11-mitochondrial	1.118139	0.44765	0.259696	-0.848660867	-0.848660867	-0.848660867	
101	Cytochrome c oxidase assembly protein COX15	1.006468	0.719522	0.640444	-0.994613871	-0.994613871	-0.994613871	
102	NADH dehydrogenase [ubiquinone] flavoprotein 1-mitochondrial	-0.18283	0.628526	-0.4885	-0.488504281	-0.488504281	-0.488504281	
103	CBL-interacting protein kinase 1	0.411691	0.2668	0.148233	-1.62111525	-1.62111525	-1.62111525	
104	Serine/threonine-protein kinase TOR	-0.13965	0.024261	0.222411	-0.467335376	-0.467335376	-0.467335376	
105	SWI/SNF complex subunit SWI3C	0.082451	0.391757	-0.14529	-0.145290342	-0.145290342	-0.145290342	
106	PsbP domain-containing protein 7- chloroplastic	0.099498	0.212171	0.147611	-0.243057157	-0.243057157	-0.243057157	
107	Protein arginine methyltransferase NDUFAF7 homolog- mitochondrial {ECO:0000250|UniProtKB:Q7L592}	0.684605	0.685377	0.493703	-2.538045118	-2.006975625	-2.538045118	

a mechanism of avoidance tolerance. PYL4 was reported to improved ABA-dependent inhibition of PP2CA and can modulate inhibition even in the absence of ABA (Pizzio *et al*. 2013). PP2CA plays a critical role to regulate both seed and vegetative responses toABAand regulates stomatal aperture through interaction with the anion channel SLOW ANION CHANNEL1 (SLAC1) and the kinase OPEN STOMATA1 (Kuhn *et al*. 2006,Yoshida *et al*. 2006,Lee *et al*. 2009). In addition, ABA receptor PYL9 was proven to promote drought resistance and leaf senescence in Arabidopsis and rice (Zhao *et al*. 2016).

Auxin response factor (ARF) 5 and 15 are among the mechanisms of senna (*C. angustifolia*) to tolerate drought stress(Table 3, Figure 2). ARF are potential mediators of biotic and abiotic stress responses in plant (Bouzroud *et al*. 2018). ARF5 gene was proven to increase the contents of carotenoids and enhance the tolerance to both salt and drought when expressed in Arabidopsis (Kang *et al*. 2018), while ARF15 regulates cell division activity during early tomato fruit development, thus, provides important influence on plant growth under drought stress (DeJong *et al*. 2015). ARF5 gene also act as an activator of auxin-responsive gene expression in *Arabidopsis* (Remington *et al*. 2004). This enzyme allows the activation of ARF, derepresses downstream auxin responsive pathways, thus mediates plant growth and development(Gray *et al*. 2001). Also, two ARF (or Aux/IAA) proteins, namely IAA7 and IAA14, exist in stressed plant only(Table 3, Figure 3). Aux/IAA proteins orchestrate several biological and physiological processes such as embryogenesis, leaf expansion and senescence, lateral root development and fruit development by regulating the expression of auxin response genes (Wilmoth *et al*. 2005, Sagar *et al*. 2013). IAA7 controls the morphological responses induced by light, e.g., promoting leaf development under adverse condition, while IAA14 acts in controlling lateral root formation by interacting with two ARF proteins (Luo *et al*. 2018). These two Aux/IAAs are produced due to the accumulated IAA generated is a result of tryptophan metabolism pathway and specifically oriented towards the induction of plant hormone signal transduction pathway to help the plant maintains normal performance inder drought stress. As the highly conserved domain II of the

other Aux/IAA proteins is a target for degradation process promoted by auxin,this action allows the participation of the auxin-induced Protein TRANSPORT INHIBITOR RESPONSE 1 (TIR1). Consequently, TIR1activates its target factors, e.g., ARFs, thus, allowing the auxin-responsive downstream genes, *AUX*/*LAX*, *LBD* and *SAUR*, to function and promote plant growth under adverse conditions. This Protein TRANSPORT INHIBITOR RESPONSE 1 (TIR1) also shown to be a major player under drought stress in senna as it is enriched only under the stress (Table 3, Figure 4). BRASSINOSTEROID INSENSITIVE 1(BRI1)-associated receptor kinase 1 (or BAK1) belongs to a large group of plant transmembrane proteins known as theleucine-rich repeatreceptor kinases (Belkhadir and Jaillais 2015). BAK1 has important role in brassinosteroidsignaling (Li *et al*.2002). Brassinosteroid is a plant hormone with important roles in cell proliferation and growth (Belkhadir and Jaillais 2015).BAK1 particularlyacts in repressing the development of unneeded stomatain plant

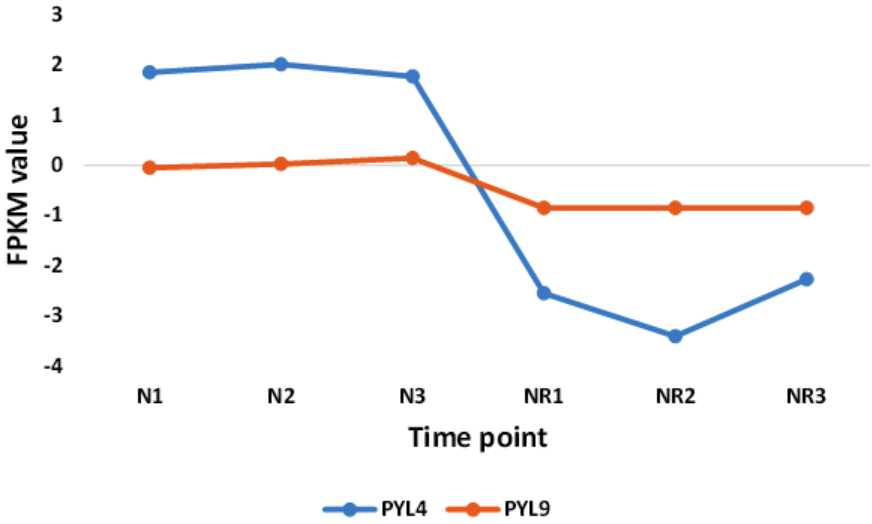

Fig. 1. Enrichment pattern of the abscisic acid (ABA) receptors PYL4 and PYL9 at midday before (N) and after (NR) watering

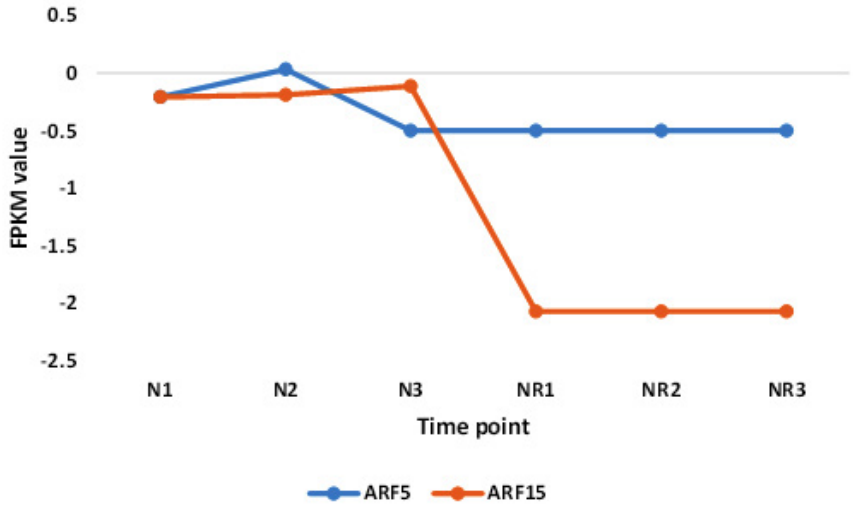

Fig. 2. Enrichment pattern of auxin response factor (ARF) 5 and 15 at midday before (N) and after (NR) watering

leaves, thus maintain water turgor under drought stress (Smakowska-Luzan *et al*. 2018). BAK1 also shown to be a major player under drought stress in senna as it is enriched only under the stress (Table 3, Figure 5). In senna, it is evident that PYL and BAK1 are induced by ABA and brassinosteroid towards the occurrence of stomatal closure and in cell elongation and division. These roles represent two bottle-necks in the plant hormone signal transduction pathway. Over and above, indole-3-pyruvate (or flavin) monooxygenase, encoded by *YUC2* gene, also participates in tryptophan metabolism pathway(Watanabe and Lam 2006) towards the biosynthesis of IAA that, as mentioned above, is required for triggering the plant hormone signal transduction pathway. Accordingly,this enzyme likely has a role in conferring drought stress tolerance in senna (*C. angustifolia*) (Table 3, Figure 6). The two pathways likely crosstalk in senna (*C. angustifolia*) towards the maintenance of normal growth under adverse condition (Figures 7 and 8).

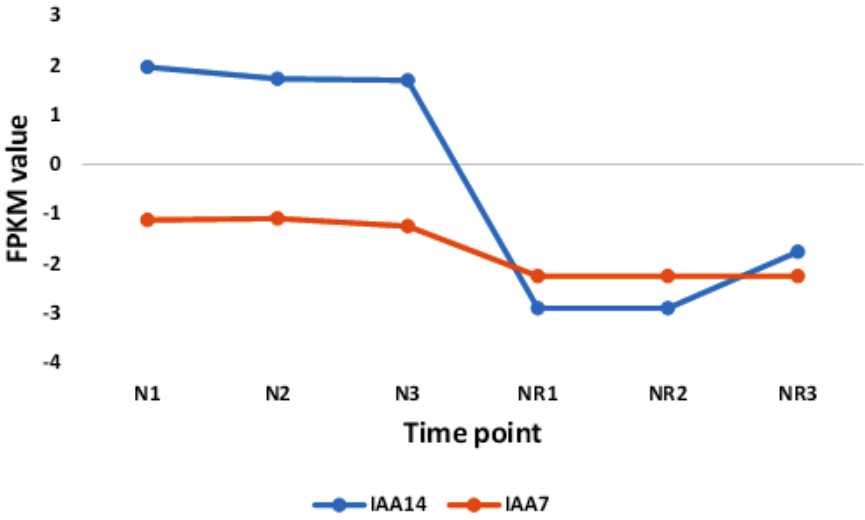

Fig. 3. Enrichment pattern of AUX/IAA factors IAA14 and IAA7 at midday before (N) and after (NR) watering

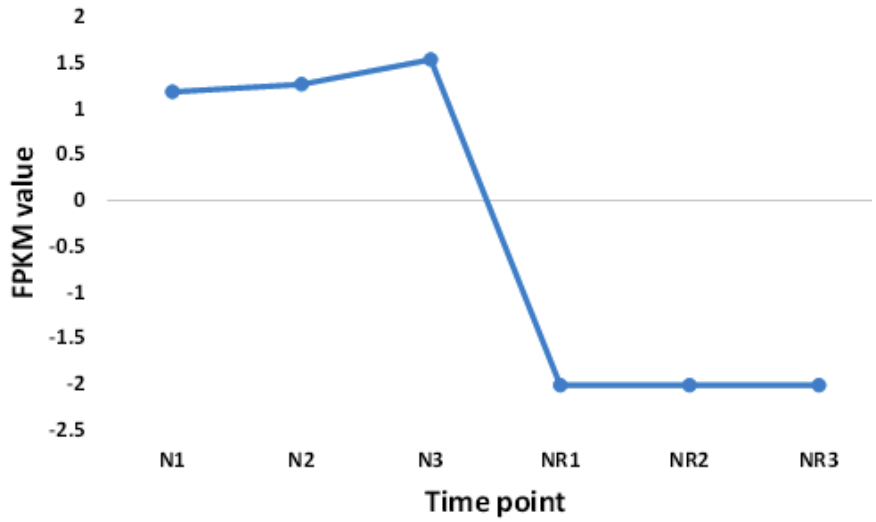

Fig. 4. Enrichment pattern of Protein TRANSPORT INHIBITOR RESPONSE 1 (TIR1) at midday before (N) and after (NR) watering.Details of enrichment pattern of this protein is shown in Table 3

Phosphoinositide phosphatase SAC1 and SAC6 can also be among the molecular mechanisms to cope with the stress in senna (*C. angustifolia*)(Table 3, Figure 9). The domains of SAC protein possess phosphoinositide phosphatase activities.SAC1 was previously shown to affect diverse cellular functions such as normal cell morphogenesis, Golgi function, and maintenance of vacuole morphology (Zhong *et al*. 2005), while SAC6 gene was predominantly expressed in flowers and proven to be highly induced by salinity in Arabidopsis (Zhong and Ye 2003).

A number of five pre-mRNA splicing factors as well as three serine/arginine-rich (SR) regulator factors were shown to participate in drought stress tolerance in senna (*C. angustifolia*) (Table 3, Figure 10). The pre-mRNA factors included splicing factors (SF) 8, 8A, 19, 40A and ISY1, while SRs included factors SCL33, RS31 and RS34. No particular function is assigned to

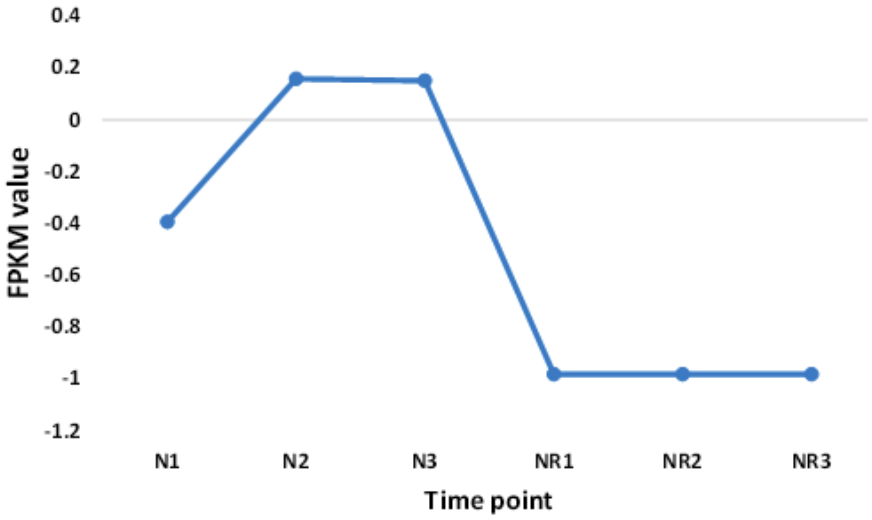

Fig. 5. Enrichment pattern of BRI1-associated receptor kinase 1(BAK1) at midday before (N) and after (NR) watering. Details of enrichment pattern of this enzyme is shown in Table 3

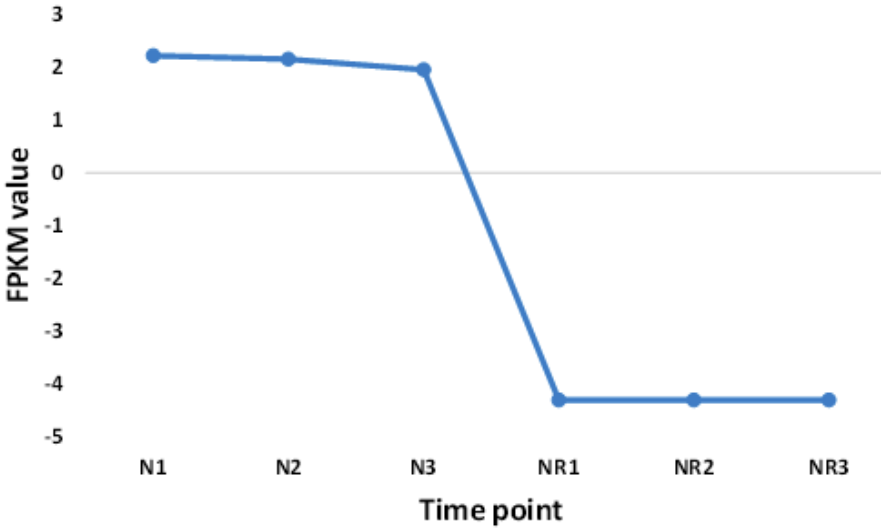

Fig. 6. Enrichment pattern of indole-3-pyruvate (or flavin) monooxygenase at midday before (N) and after (NR) watering. Details of enrichment pattern of this enzymeis shown in Table 3

any of these splicing factors and regulators except that each one might act on a certain protein or protein type. This conclusion warrant further experimentation and analysis. Pre-mRNAs usually contain introns that requires splicing or alternative splicing (AS) to produce structurally and functionally different proteins from the same gene (Palusa *et al*. 2007). Pre-mRNAs-related genes encode serine/arginine-rich (SR), which is a conserved protein family of splicing regulators in plant. Splicing of SR genes has proven to be developmental- and tissue-specific. Prior research indicated that abiotic stresses regulate the splicing of the pre-mRNAs of SR genes to produce different protein isoforms,thus different functions. We speculate that the altered splicing in senna seems like the action of the immune systems as to produce a specific antibodies for a specific antigen. Here, we think that alternative splicing of SR under stress produce the proper isoform of the proteins that can hold their structures and avoid denaturation or unfolding as a response to stimuli.

Many other regulated metabolites and proteins in senna (*C. angustifolia*)including protective proteins, such as heat shockprotein 95s were previously reportedto play functional roles in drought tolerancein plants (Hu and Xiong 2014, Umezawa *et al*. 2006). Senna might also

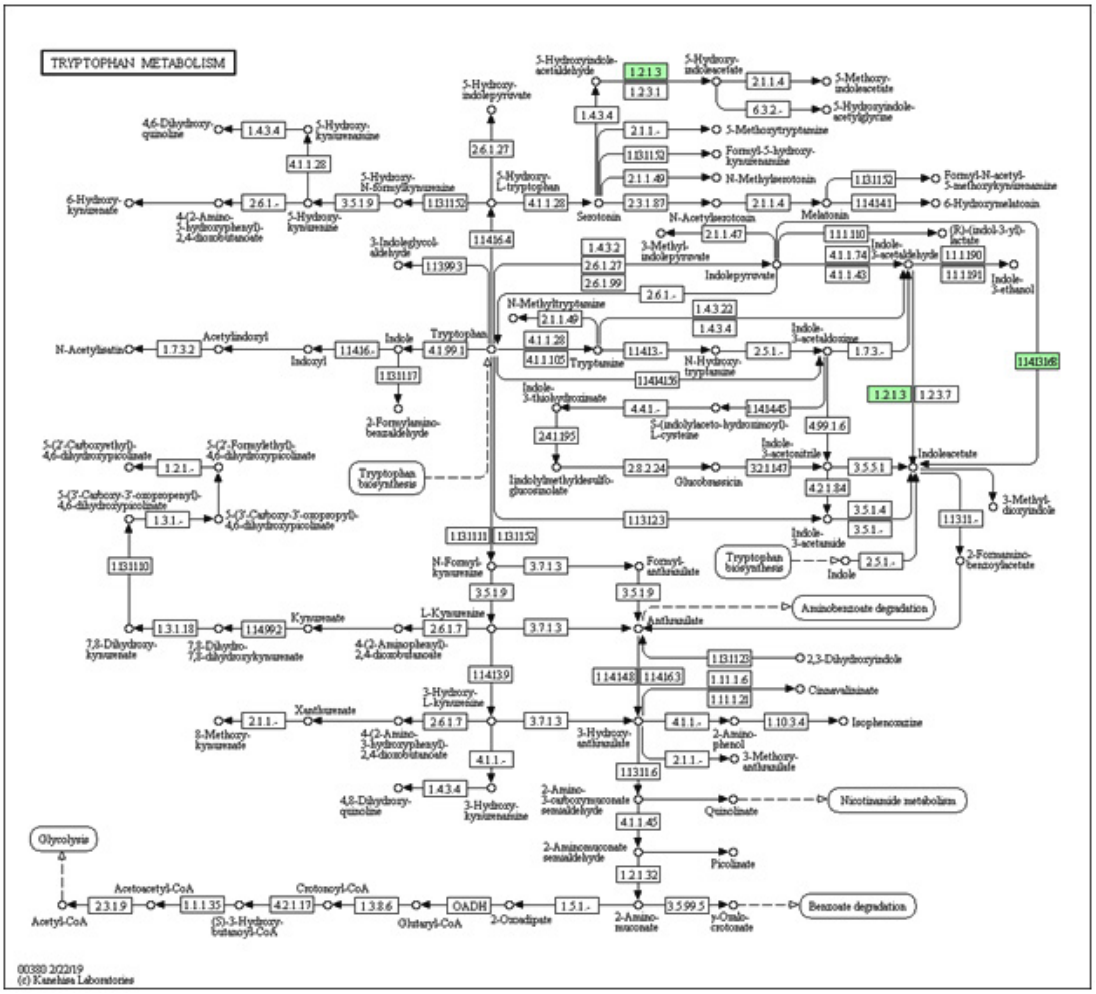

Fig. 7. Tryptophan metabolism pathway indicating two important enzymes Aldehyde dehydrogenase family 3 member F1 (EC: 1.2.1.3) and indole-3-pyruvate monooxygenase (EC: 11.41.31.68) that were enriched under drought stress, while suppressed due to watering. Details of enrichment pattern of these two enzymes are shown in Table 3

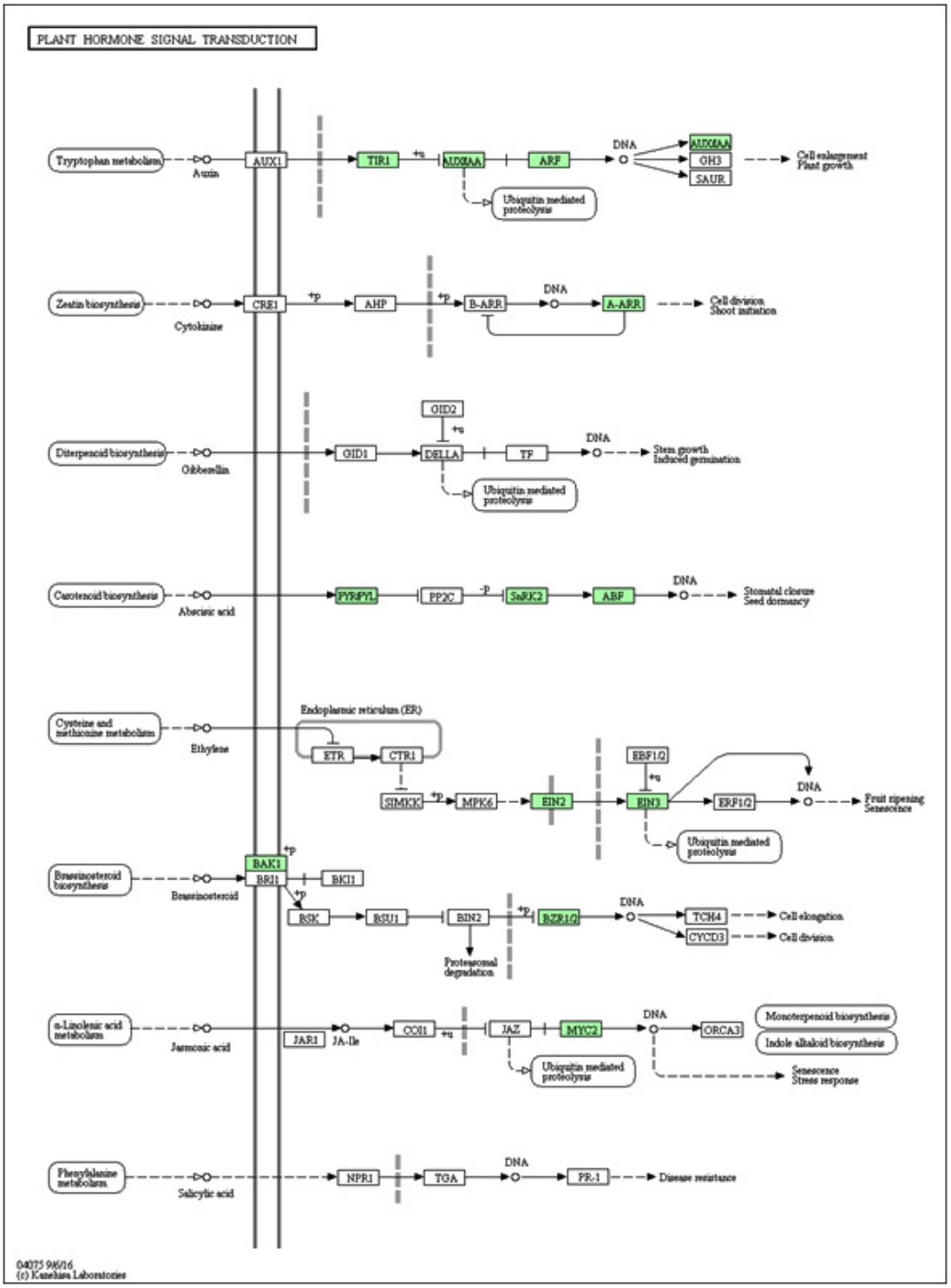

Fig. 8. Plant hormone signal transduction pathway indicating the auxin response factor (ARF) and the Aux/IAAthat were enriched under drought stress, while suppressed due to watering. Details of enrichment pattern of these two enzymes are shown in Table 3

developed efficient signal transductioncascades to cope with drought stress as it proved to regulate ATPase, which is a major signaling factor involved indrought stress signaling (Akpinar *et al*. 2012, 2013).Protein kinases participate in developmental and environmentalsignal transduction in plants (Rodriguez *et al*.2010, Liu *et al*. 2016) and play a key role in activating transcriptionfactors and drought-responsive proteins underdrought tolerance. Among them, mitogen-activated protein kinase(MAPK) and cytochrome c oxidase that were regulated in senna (*C. angustifolia*) (Table 3).

Other enzymes includephospholipase C2 and allene oxide cyclase as well as isochorismate pathway were regulated in senna (*C. angustifolia*). The two enzymes play a role in JA pathway, while isochorismate pathway results in the production of salicylic acid (SA) (Kawano *et al*. 2004, Mustafa *et al*. 2009).The latter pathways have important applications in plant production.

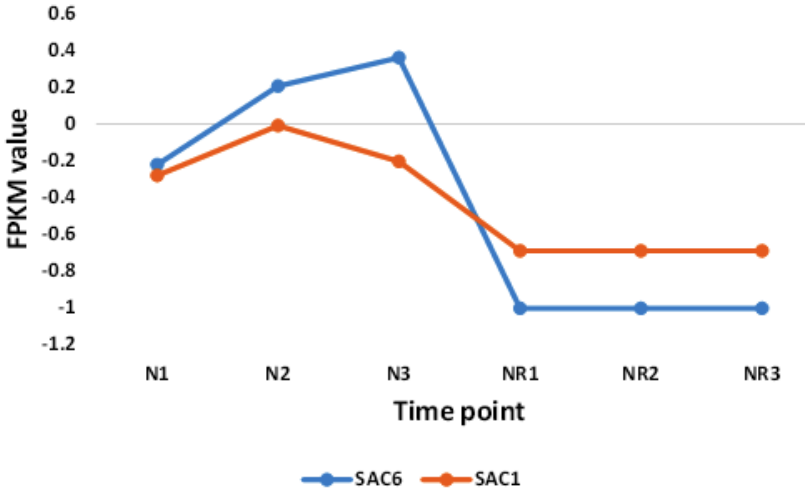

Fig. 9. Enrichment pattern of SAC1 and SAC6 at midday before (N) and after (NR) watering.Details of enrichment pattern of these proteins are shown in Table 3

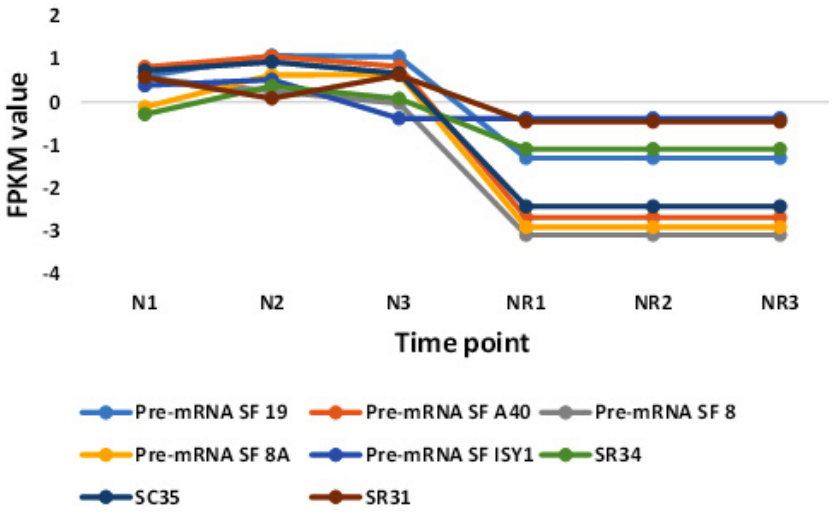

Fig. 10. Enrichment pattern of the five Pre-mRNA splicing factors SF19, SF A40, SF 8, SF 8A and SF ISY1 as well as the three serine/arginine-rich SR34, SC35 and SR31 regulator factors at midday before (N) and after (NR) watering.Details of enrichment pattern of these proteins are shown in Table 3

In conclusion, we speculate that we have expanded our understanding of the molecular mechanism underlyingdrought stress tolerance in senna (*C. angustifolia*). This information will be valuable resource for accelerated genomics-assisted genetic breeding programs targeting theimprovement of drought tolerancein economically important crop plants.

REFERENCES

1. Akpinar BA, Avsar B, Lucas SJ, Budak H. Plant abiotic stress signalling. *Plant Signal Behav,* 2012; **7**(11):1450–1455.
2. Akpinar BA, Lucas SJ, Budak H. Genomics approaches for crop improvement against abiotic stress. *Sci World J,* 2013; **15**:361921.
3. Ayoub AT. Some primary features of salt tolerance in senna (*Cassia angastifolia*). *J Exp Bot,* 1977; **28**: 484–492.
4. Bartels D, Sunkar R. Drought and salt tolerance in plants. *Cr Rev Plant Sci,* 2005; **24**(1):23–58.
5. Belkhadir Y, Jaillais Y. The molecular circuitry of brassinosteroid signaling". The New Phytologist, 2015; **206**: 522–40.
6. Blum A. Plant breeding for stress environments. CRC Press, Inc., 1988; Boca Raton.
7. Bolger AM, LohseM, Usadel B. Trimmomatic: a flexible trimmer for illumina sequence data. *Bioinformatics*, 2014; **30**: 2114–2120.
8. Bouzroud S, Gouiaa S, Hu N, Bernadac A, Mila I, Bendaou N, *et al*. Auxin Response Factors (ARFs) are potential mediators of auxin action in tomato response to biotic and abiotic stress (*Solanum lycopersicum*). *PLoS ONE*, 2018; **13**(2): e0193517.
9. Bowman MJ, Park W, Bauer PJ, Udall JA, Page JT, Raney J, Scheffler BE, Jones DC, Campbell BT. RNA-Seq transcriptome profiling of upland cotton (Gossypium hirsutum L.) root tissue under water-deficit stress. *PLoS One,* 2013; **8**(12):e82634.
10. Budak H, Kantar M, Kurtoglu KY. Drought tolerance in modern and wild wheat. *Sci World J,* 2013; **15**: 548246.
11. DeJong M, Wolters-Arts M, Schimmel BC, Stultiens CL., de Groot PF, *et al*. *Solanum lycopersicum* AUXIN RESPONSE FACTOR 9 regulates cell division activity during early tomato fruit development. *J. Exp. Bot.* 2015; **66**: 3405–3416.
12. Do PT, Degenkolbe T, Erban A, Heyer AG, Kopka J, Köhl KI, Hincha DK, Zuther E: Dissecting rice polyamine metabolism under controlled long-term drought stress. *PLoS One*, 2013; **8**(4):e60325.
13. Dong Y, Fan G, Deng M, Xu E, Zhao Z. Genome-wide expression profiling of the transcriptomes of four Paulownia tomentosa accessions in response to drought. *Genomics,* 2014; **104**(4):295–305.
14. Ergen NZ, Thimmapuram, Bohnert J, Hans J, Budak H. Transcriptome pathways unique to dehydration tolerant relatives of modern wheat. *Funct Integr Genomics,* 2009; **9**(3):377–396.
15. Folkard C. Encyclopedia of herbs and their uses. Herb Society of America, Dorling Kindersley Publishing Inc., New York 1995.
16. Gray WM, Kepinski S, Rouse D, Leyser O, Estelle M. Auxin regulates SCF^{TIR1}-dependent degradation of AUX/IAA proteins. *Nature,* 2001; **414**: 271-276.
17. Hammouda FM, Ismail SI, Abdel-Azim NS, Shams KA. A guide to medicinal plants in North Africa. In: Batanouny KH (eds) IUCN Centre for Mediterranean Cooperation, Malaga, Andalusia, Spain, 2005; pp 217–218.
18. Hao Q., Yin P., Li W., Wang L., Yan C., *et al*. The molecular basis of ABA-independent inhibition of PP2Cs by a subclass of PYL proteins. *Mol. Cell*, 2011; **42**: 662-672.
19. Hirayama T, Shinozaki K. Research on plant abiotic stress responses in the postgenome era: past, present and future. *Plant J,* 2010; **61**(6):1041–1052.
20. Hiz MC, Canher B, Niron H, Turet M. Transcriptome analysis of salt tolerant common bean (Phaseolus vulgaris L.) under saline conditions. *PLoS One*, 2014; **9**(3): e92598.
21. Hu H, Xiong L. Genetic engineering and breeding of droughtresistant crops. *Annu Rev Plant Biol,* 2014; **65**: 715–741.
22. Hu T, Sun X, Zhang X, Nevo E, Fu J. An RNA sequencing transcriptome analysis of the high-temperature stressed tall fescue reveals novel insights into plant thermotolerance. *BMC Genomics*, 2014; **15**: 1147.
23. Kang C, He S, Zhai H, Li R, Zhao N and Liu Q. A Sweetpotato Auxin Response Factor Gene (IbARF5) Is Involved in Carotenoid Biosynthesis and Salt and Drought Tolerance in Transgenic Arabidopsis. *Front. Plant Sci.,* 2018; **9**: 1307.
24. Kantar M, Lucas SJ, Budak H. Drought stress: molecular genetics and genomics approaches. *Adv Bot Res,* 2011; **57**: 445–493.
25. Kawano T, Furuichi T, Muso S. Controlled free salicylic acid levels and corresponding signaling mechanisms in plants. *Plant Biotechnol,* 2004; **21**: 319–335.
26. Khalid H, Abdalla WE, Abdelgadir H, Opatz T, Effert T. Gems from traditional north-African medicine: medicinal and aromatic plants from

Sudan. *Nat Prod Bioprospect,* 2012; **2**(3):92–103.

27. Khammari I, Galavi M, Ghanbari A, Solouki M, Poorchaman MRA. The effect of drought stress and nitrogen levels on antioxidant enzymes, proline and yield of Indian Senna (Cassia angustifolia L.). *J Med Plants Res,* 2012; **6**(11): 2125–2130.
28. Kuhn JM, Boisson-Dernier A, Dizon MB, Maktabi MH, Schroeder JI (2006). The protein phosphatase AtPP2CA negatively regulates abscisic acid signal transduction in Arabidopsis, and effects of abh1 on AtPP2CA mRNA. *Plant Physiol.* 2012; **140**(1):127-39.
29. Lee SC, Lan W, Buchanan BB, Luan S. A protein kinase-phosphatase pair interacts with an ion channel to regulate ABA signaling in plant guard cells. *Proc Natl Acad Sci USA*. 2009; **106**(50):21419-24.
30. Lemli J. The chemistry of senna. *Fitoterapia,* 1986; **57**: 33–40.
31. Levitt J. Responses of plants to environmental stress: chilling, freezing and high temperature stresses, vol 1, 2nd edn. Academic, New York 1980.
32. Li J, Wen J, Lease KA, Doke JT, Tax FE, *et al*. BAK1, an Arabidopsis LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. *Cell,* 2002; **110**: 213–22.
33. Li MY, Tan HW, Wang F, Jiang Q, Xu ZS, *et al*. De novo transcriptome sequence assembly and identification of AP2/ERF transcription factor related to abiotic stress in parsley (Petroselinum crispum). *PLoS One*, 2014a; **9**(9):e108977.
34. Li PS, Yu TF, He GH, Chen M, Zhou YB, *et al*. Genome-wide analysis of the Hsf family in soybean and functional identification of GmHsf-34 involvement in drought and heat stresses. *BMC Genom,* 2014b; **15**:1009.
35. Li WX, Oono Y, Zhu J, He XJ,Wu JM, *et al*. The Arabidopsis NFYA5 transcription factor is regulated transcriptionally and post-transcriptionally to promote drought resistance. *Plant Cell*, 2008; **20**(8):2238–2251.
36. Liu H, Che Z, Zeng X, Zhou X, Sitoe HM, *et al*. Genome-wide analysis of calcium-dependent protein kinases and their expression patterns in response to herbivore and wounding stresses in soybean. *Funct Integr Genom,* 2016; **16**(5):481–493.
37. Luo J, Jing-Jing Zhou J-J, Zhang J-Z. *Aux/IAA* Gene Family in Plants: Molecular Structure, Regulation, and Function. Int J Mol Sci. 2018; **19**(1): 259.
38. Mehta RH, Ponnuchamy M, Kumar J, *et al*. (2017). Exploring drought stress-regulated genes in senna (*Cassia angustifolia* Vahl.): a transcriptomic approach. *Funct Integr Genomics,* 2017; **17**:1–25.
39. Mustafa NR, Kim HK, Choi YH, Erkelens C, Lefeber AW, Spijksma G, van der Heijden R,Verpoorte R Biosynthesis of salicylic acid in fungus elicited Catharanthus roseus cells. *Phytochemistry,* 2009; **70**(4): 532–539.
40. Palusa SG, Ali GS, Reddy AS. Alternative splicing of pre-mRNAs of Arabidopsis serine/arginine-rich proteins: regulation by hormones and stresses. *Plant J*. 2007; **49**(6):1091-107.
41. Pizzio GA, Rodriguez L, Antoni R, Gonzalez-Guzman M, Yunta C, *et al*. The PYL4 A194T Mutant Uncovers a Key Role of PYR1-LIKE4/PROTEIN PHOSPHATASE 2CA Interaction for Abscisic Acid Signaling and Plant Drought Resistance. Plant *Physiol.* 2013; **163**(1): 441–455.
42. Rama Reddy NR, Ragimasalawada M, Sabbavarapu MM, Nadoor S, Patil JV. Detection and validation of stay-green QTL in post-rainy sorghum involving widely adapted cultivar, M35-1 and a popular stay-green genotype B35. *BMC Genom,* 2014; **18**(15):909.
43. Ramchandra Reddy A, Chaitanya KV, Vivekanandan M. Drought induced responses of photosynthesis and antioxidant metabolism in higher plants. *J Plant Physiol,* 2004; **161**(11):1189–1202.
44. Remington DL, Vision TJ, Guilfoyle TJ, Reed JW. Contrasting modes of diversification in the Aux/IAA and ARF gene families. *Plant Physiol,* 2004; 1**35**: 1738-1752
45. Ratnayaka HH, Kincaid D. Gas exchange and leaf ultrastructure of tinnevelly senna, cassia angustifolia, under drought and nitrogen stress. *Crop Sci,* 2005; **45**(3):840–847.
46. Rocheta M, Becker JD, Coito JL, Carvalho L, Amâncio S. Heat and water stress induce unique transcriptional signatures of heatshock proteins and transcription factors in grapevine. *Funct Integr Genomics,* 2014; **14**(1): 135–148.
47. Rodriguez MC, Petersen M, Mundy J. Mitogen-activated protein kinase signaling in plants. *Annu Rev Plant Biol,* 2010; **61**: 621–649.
48. Sagar M, Chervin C, Mila I, Hao Y, Roustan JP, *et al*. SlARF4, an auxin response factor involved in the control of sugar metabolism during tomato fruit development. *Plant Physiol.* 2013; **161**(3):1362-74.
49. Shanker AK, Maheswari M, Yadav SK, Desai S, Bhanu D, Attal NB, Venkateswarlu B. Drought stress responses in crops. *Funct Integr Genomics*, 2014; **14**(1):11–22.

50. Smakowska-Luzan E, Mott GA, Parys K, Stegmann M, Howton TC, *et al*. An extracellular network of Arabidopsis leucine-rich repeat receptor kinases. *Nature,* 2018; **553**: 342–346.
51. Thumma BR, Sharma N, Southerton SG. Transcriptome sequencing of Eucalyptus camaldulensis seedlings subjected to water stress reveals functional single nucleotide polymorphisms and genes under selection. *BMC Genomics,* 2012; **13**:364.
52. Umezawa T, Fujita M, Fujita Y, Yamaguchi-Shinozaki K, Shinozaki K. Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. *Curr Opin Biotechnol,* 2006; **17**(2): 113–122.
53. Watanabe N, Lam E. *Arabidopsis* Bax inhibitor-1 functions as an attenuator of biotic and abiotic types of cell death. *The Plant Journal.* 2006; **45**: 884-894.
54. Wilmoth JC, Wang S, Tiwari SB, Joshi AD, Hagen G, *et al*. NPH4/ARF7 and ARF19 promote leaf expansion and auxin-induced lateral root formation. *Plant J*., 2005; **43**(1):118-30.
55. Xu Y, Gao S, Yang Y, Huang M, Cheng L,Wei Q, Fei Z, Gao J, Hong B. Transcriptome sequencing and whole genome expression profiling of chrysanthemum under dehydration stress. *BMC Genomics,* 2013; **14**:662.
56. Yamaguchi-Shinozaki K, Shinozaki K. Transcriptional regulatory networks in cellular response and tolerance to dehydration and cold stresses. *Annu Rev Plant Biol,* 2006; **57** :781–803.
57. Yoshida T, Nishimura N, Kitahata N, Kuromori T, Ito T, *et al*. ABA-hypersensitive germination3 encodes a protein phosphatase 2C (AtPP2CA) that strongly regulates abscisic acid signaling during germination among Arabidopsis protein phosphatase 2Cs. *Plant Physiol*. 2006; **140**(1):115-26.
58. Zhang J, Ruhlman TA, Mower JP, Jansen RK. Comparative analyses of two Geraniaceae transcriptomes using next-generation sequencing. *BMC Plant Biology*, 2013b; **13**: 228.
59. Zhang X, Allan AC, Li C,Wang Y, Yao Q. De novo assembly and characterization of the transcriptome of the Chinese medicinal herb, Gentiana rigescens. *Int J Mol Sci,* 2015; **16**(5):11550–11573.
60. Zhao Y, Chan Z, Gao J, Xing L, Cao M, *et al*. ABA receptor PYL9 promotes drought resistance and leaf senescence. *PNAS*, 2016; **113**(7):1949-1954.
61. Zhong R, Burk DH, Nairn CJ, Wood-Jones A, Morrison WH, *et al*. Mutation of SAC1, an Arabidopsis SAC domain phosphoinositide phosphatase, causes alterations in cell morphogenesis, cell wall synthesis, and actin organization. *Plant Cell,* 2005; **17**:1449-1466.
62. Zhong R, Ye Z-H. The SAC Domain-Containing Protein Gene Family in Arabidopsis. *Plant Physiol*. 2003; **132**(2): 544–555.
63. Zhou X, Li L, Xiang J, Gao G, Xu F, Liu A, Zhang X, Peng Y, Chen X, Wan X. OsGL1-3 is involved in cuticular wax biosynthesis and tolerance to water deficit in rice. *PLoS One,* 2015; **10**(1):e116676.
64. Zhu H, Dardick CD, Beers EP, Callanhan AM, Xia R, Yuan R. Transcriptomics of shading-induced and NAA-induced abscission in apple (*Malus domestica*) reveals a shared pathway involving reduced photosynthesis, alterations in carbohydrate transport and signaling and hormone crosstalk. *BMC Plant Biol*, 2011; **11**:138.