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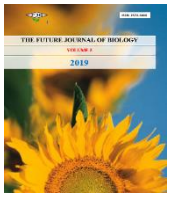

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# EXPRESSION PROFILE OF REDOX ACTIVE SECONDARY METABOLITES RELATED GENES OBTAINED BY TRANSCRIPTOME SEQUENCING UNDER SALT AND DROUGHT STRESS IN SWEET POTATO

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**ABSTRACT**: Under salt and drought stress conditions, sweet potato plants are producing secondary metabolites. Redox active secondary metabolites occasionally produced to accommodate changes arising in their fluctuating growth conditions, which in role enable functional flexibility. In the current study, transcriptome sequencing was applied for understanding the redox active secondary metabolites related genes and its pathway under stress conditions as compared to control plants. The obtained transcripts were annotated using BlastX search against NR_NCBI database to identify the sequence similarity with the available online sequence data. The detected up-regulated genes were involved in metabolic pathways as well as abiotic stress resistance. These up-regulated transcripts were aligned to 10 different known genes including 4-coumarate--CoA ligase-like 10 (4 CL), 9-cis-epoxycarotenoid dioxygenase chlorophyll(ide) b reductase NYC1 (chloroplastic), cytochrome P450 82C2-like , cytochrome P450 82C4-like, cytochrome P450 CYP72A219-like, cytochrome P450 CYP82D47-like isoform X1, Homogentisate 1,2-dioxygenase, methyltransferase DDB_G0268948 and Phosphoethanolamine N-methyltransferase 1-like. Detected down-regulated individual genes in the current experiment were involved in processes related to plant metabolic pathways. Some of these genes were significantly expressed in high levels at the beginning of stress then started to decrease with increasing the stress duration. This indicated that they are early responsive genes to stress, which contribute in determining plant resistance and growth at the second phase of stress. The down-regulated individual genes were aligned to 9 different known genes namely 24-methylenesterol C-methyltransferase 2, 3-oxoacyl-[acyl-carrier-protein] synthase I, cytochrome P450 71A3-like, cytochrome P450 76A1-like isoform X2, cytochrome P450 76A2-like, cytochrome P450 76C2-like, geraniol 10-hydroxylase-like protein, geraniol 8-hydroxylase-like and tocopherol O-methyltransferase, chloroplastic isoform X1. This study of hexaploid sweet potato redox active secondary metabolites related genes are providing valuable source for breeding applications. Additionally, it offers new candidate genes to breed salt and drought tolerant sweet potato cultivars.

**Keywords:** Ipomoea batatas; Sweet potato, Salt, drought, redox, secondary metabolites.

## INTRODUCTION

Sweet potato is a bio-efficient staple food crop, which serves for human and animal feed and used as a raw material for many industrial products. It is a low-input crop which require moderately warm climate with the ability to grow in lands ranging from the tropics to temperate zones **(Markos and Loha, 2016)**. In addition, sweet potato is considered an important source for starch, protein, dietary fibres, micronutrients, vitamins and bioactive compounds. It yields a high amount of dry matter and energy per unite area as compared to other economic crops. Therefore, it is expected that optimizing the annual yield of sweet potato will bridge the malnutrition and food shortage problems around the world, in general, and in the developing countries, in particular **(Motsa *et al.*, 2015)**.

Salinity and drought stresses are two factors mainly limit crop productivity worldwide. Moreover, salinity

and drought problems are expected to be worsen in the coming decade which will adversely affect crop production **(Raymundo *et al.*, 2014 and Gajanayake *et al.*, 2015)**. In fact, crops like sweet potato with its high nutritional contents and wide adaptability is a good choice to potentially address the food and nutrition security in the context with global climatic changes especially under salt and drought stress **(Devaux *et al.*, 2014)**.

Under salt and drought stress conditions, sweet potato plants have numerous mechanisms to accommodate changes arising in their fluctuating growth conditions which in role enable functional flexibility **(Bennett and Wallsgrove, 1994)**. This functional flexibility occurs without affecting cellular mechanisms or developmental processes by producing secondary metabolites, which contribute to stress response **(Wink, 1988)**.

Secondary metabolites are produced occasionally in the living plant cells as an adaptive capacity in coping with salt and drought as well as other stress conditions **(Alagna, 2013)**. Many of secondary metabolites behave as redox active compounds which are able to modulate the intercellular redox equilibrium in living cells **(Kapoor *et al.*, 2015)**. Some genes were found to control oxidation-reduction process *i.e.,* 9-cis-epoxycarotenoid dioxygenase 1 **(Anjum *et al.*, 2016)** and cytochrome P450 **(Gnanasekaran *et al.*, 2015),** which are up-regulated during stress time. On the other hand, some of these compounds cause oxidative modifications within the cell which ultimately lead to cell death **(Sha Valli Khan *et al.*, 2014)**.

Improving characteristics related to redox active secondary metabolites and its related genes becomes necessary to produce stress tolerance cultivars with strong defence mechanisms against salt and drought stress **(Wink, 1988 and Gruneberg *et al.*, 2015)**. Nowadays, the development of such resistant cultivars is considered as a main target for plant breeders and biotechnology industry **(Alagna, 2013)**. Despite the importance of secondary metabolites, still there is a wide gap on genes encoding for entire metabolic pathways and those involved in their regulation. Nerveless, a limited number of metabolic pathways in sweet potato have been completely clarified.

A group of up-regulated genes, including 4-coumarate--CoA ligase-like 10, 9-cis-epoxycarotenoid dioxygenase 1，chlorophyll(ide) b reductase NYC1 (chloroplastic) cytochrome P450，Homogentisate 1,2-dioxygenase，methyltransferase DDB_G0268948 and Phosphoethanolamine N-methyltransferase 1-like play a critical role under salt and drought stress in sweetpotato leaves. These genes are involved in different biological processes including oxidation-reduction process, secondary metabolite biosynthetic process, lignin biosynthetic process and single-organism metabolic process **(Capriotti *et al.*, 2014)**. On the contrary, the down regulated genes are responsible for transferase activity, transferring acyl groups other than amino-acyl groups, oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, NAD (P) H, as one donor, and incorporation of one atom of oxygen and protochlorophyllide reductase activity **(Schaller, 2003)**.

The aim of the current study is to closely investigate the expression level of the redox active secondary metabolites related genes under drought and salt stress. In addition, we aim to study its behaviour during different time points. Precise identification of the most important redox-modulating metabolites will contribute to provide a promising new lead to target selectivity for tolerant cultivars.

## MATERIALS AND METHODS

### Experimental materials and stress treatments

Xuzi-8 sweet potato cultivar, used for this study, was obtained from Xuzhou sweetpotato research institute of Agriculture science in Jiangsu province, China. It have green leaves and purple flesh storage roots. It is a high yield, early mature, drought and salt tolerant sweet potato cultivar.

Xuzi-8 healthy plants grown in the field were used as a source for apical stem cuttings with an average length 15-20 cm. The stem cuttings grown hydroponically in Hoagland solution for 30 days till having suitable root system at 25±3 under 16/8 day/night prior for stress treatments **(Nedunchezhiyan *et al.*, 2012)**.

Seedlings were subjected to drought stress (PEG-6000, 30%) and salt stress (NaCL 200 mM) for 0, 1, 6, 12 and 48. Samples were collected from the third upper leaf at the different time points and placed in liquid nitrogen then stored at -80° C prior for RNA extraction.

### Total RNA extraction and Library Preparation

Total RNA extraction was performed on samples collected from drought, salt and control plants using TRIZOL reagent (Invitrogen) method. Concentration and purity of extracted RNA were determined using Nano Drop machine (Thermo Scientific Nano Drop 2000C Technologies). RNA integrity was determined using Nano Kit (RNA-6000) of the Agilent 2100 Bio-analyser (Agilent Technologies, CA, USA). cDNA first strand was synthesized according to manual instructions (PrimeScript™ kit, TAKARA) **(Kukurba and Montgomery, 2015)**. Subsequently, cDNA second strand synthesis was done using DNA polymerase I and RNase H. The library fragments were purified with the AMPure XP system (Beckman Coulter, Beverly, USA) to select fragments more than

250-200 bp. After that, 3 µL USER Enzyme (NEB, USA) was used with size-selected, adaptor-ligated cDNA at 37 °C for 15 min followed by 5 min at 95 °C before PCR. Universal PCR primers and Index (X) Primer were used. Finally, PCR products were purified and library quality was assessed on the Agilent Bioanalyzer 2100 system.

**Differential Expression Analysis**

Each library gene expression level was determined through RSEM **(Li and Dewey, 2011)**. Each library clean data were mapped into assembled transcriptome. Mapped results were used to form read counts for each gene.

Data analyses for Differentially Expressed Genes (DEGs) were conducted using DESeq2 and R software through the negative binomial distribution system. Benjamini and Hochberg method was used to adjust P-values for controlling the False Discovery Rate (FDR) **(Storey and Tibshirani, 2003)**. Differentially expressed genes were considered significant when FDR is more than 0.05 and log2 fold change (FC) is more than 1.

**Selecting redox active secondary metabolites related genes**

Depending on transcriptome sequencing data, clusters for orthologous groups (COG/ KOG) classifications were used to identify the transcripts belongs to the category "secondary metabolites biosynthesis, transport and catabolism". Significantly expressed unigenes were chosen according to the equation "Log2 FC more than 1 and P-value lower than 0.05". Determined significant transcripts were classified into two groups including up and down regulated unigenes as compared to the untreated plant (control).

**Transcriptome assembly and gene functional annotation**

Transcriptome sequencing was done using next and third generation sequencing analysis and Fragment per kilo base million (FPKM) was recorded for each library. Obtained sequences were aligned used for further analysis **(Grabherr *et al.*, 2011 and Haas *et al.*, 2013)**. Functional annotation was performed for the obtained sequence on different data base including NCBI (non-redundant protein sequence), KOG/ COG (clusters of orthologous group of proteins) and Swiss-pot database was used to classify the potential functions of unigenes **(Young *et al.* 2010)**. Blast2GO (v2.5) was used to determine Gene ontology (GO) for the annotation result of NR and Pfam (http://www.geneontlology.org).

**RESULTS**

**Transcripts identification and sequence alignment**

A number of 10 up-regulated and 9 down-regulated transcripts were identified with significant difference in stressed leaf tissues as compared to control plants **(Table 1)**. The obtained genes were annotated using BlastX search against NR_NCBI database to identify the sequence based on similarity with the available online sequence data. The up-regulated transcripts were aligned to 10 different genes including 4-coumarate--CoA ligase-like 10 (4 CL), 9-cis-epoxycarotenoid dioxygenase 1, chlorophyll(ide) b reductase NYC1 (chloroplastic), cytochrome P450 82C2-like, cytochrome P450 82C4-like, cytochrome P450 CYP72A219-like, cytochrome P450 CYP82D47-like isoform X1, Homogentisate 1,2-dioxygenase, methyltransferase DDB_G0268948 and Phosphoethanolamine N-methyltransferase 1-like. These up-regulated genes were obtained with average length ranged between 682 to 1899 base pairs (bp). While the down-regulated transcripts were aligned to 9 different genes namely 24-methylenesterol C-methyltransferase 2, 3-oxoacyl-[acyl-carrier-protein] synthase I, cytochrome P450 71A3-like, cytochrome P450 76A1-like isoform X2, cytochrome P450 76A2-like, cytochrome P450 76C2-like, geraniol 10-hydroxylase-like protein, geraniol 8-hydroxylase-like and tocopherol O-methyltransferase, chloroplastic isoform X1. Furthermore, the obtained down-regulated fragments length were ranged from 1115 to 1732 bp.

**Differentially expressed genes under salt and drought stress**

A number of 10 different genes were significantly up-regulated in Xuzi-8 leaf tissues under drought and salt stress among all time points as compared to control plants **(Figure 1)**. Among all genes the effect of drought stress on theses 10 genes was more pronounced than salt stress at all time points except for 4-coumarate--CoA ligase-like 10. The 4-coumarate--CoA ligase-like 10 gene expression gradually increased under salt and drought stress starting from 1 hour of stress till 6 hours then slightly decreased at 12 hours under both types of stress and finally increased again at 48 hours of stress to be at the same level of 1 and 6 hours. Furthermore, the 4-coumarate--CoA ligase-like 10 gene expression was highly affected with salt than drought stress as compared to control plants which stayed at the minimum level at all time points. 9-cis-epoxycarotenoid dioxygenase 1 (NCED1) gene was increased gradually by drought stress reaching to the peak at 12 hours and then decreased at 48 hours, while its level was slightly increased only at 6 hours of salt

stress and stayed at the same range of the untreated plant. Chlorophyll (ide) b reductase (NYC1) expression level under drought stress gradually increased reaching to the highest value (FPKM = 327) at 12 hours then decreased at 48 hours (FPKM = 223). Under salt stress conditions the NYC1 level increased gradually reaching to the maximum at 48 hours of NaCL-induced salt stress which was lower that the values of the same gene at 12 and 48 hours of PEG-induced drought stress. A number of five genes followed the same trend including cytochrome P450 82C2-like, cytochrome P450 CYP72A219-like, cytochrome P450 CYP82D47-like isoform X1, homogentisate 1,2-dioxygenase and methyltransferase DDB_G0268948. These five genes expression level only gradually increased under drought stress reaching to the highest level at 48 hours, while under salt stress the expression level was very low which was at the same level of control plants.

**Table 1. Detected transcripts identified as redox active secondary metabolites related genes under salt and drought stress in sweet potato leaf tissues.**

| No. | Unigene ID | Length (bp) | Gene Name (Nr annotation) | Regulation |
|---|---|---|---|---|
| 1 | g13642 | 1601 | 4-coumarate--CoA ligase-like 10 | up |
| 2 | g12468 | 1899 | 9-cis-epoxycarotenoid dioxygenase 1 | up |
| 3 | g13432 | 1693 | chlorophyll(ide) b reductase NYC1, chloroplastic | up |
| 4 | g14501 | 1490 | cytochrome P450 82C2-like | up |
| 5 | g14401 | 1485 | cytochrome P450 82C4-like | up |
| 6 | g17220 | 1146 | cytochrome P450 CYP72A219-like | up |
| 7 | g2162 | 1433 | cytochrome P450 CYP82D47-like isoform X1 | up |
| 8 | g15145 | 1394 | Homogentisate 1,2-dioxygenase | up |
| 9 | g5381 | 682 | methyltransferase DDB_G0268948 | up |
| 10 | g2045 | 1500 | Phosphoethanolamine N-methyltransferase 1-like | up |
| 11 | g3416 | 1115 | 24-methylenesterol C-methyltransferase 2 | Down |
| 12 | g1408 | 1732 | 3-oxoacyl-[acyl-carrier-protein] synthase I | Down |
| 13 | g15291 | 1344 | cytochrome P450 71A3-like | Down |
| 14 | g14010 | 1557 | cytochrome P450 76A1-like isoform X2 | Down |
| 15 | g2377 | 1379 | cytochrome P450 76A2-like | Down |
| 16 | g15242 | 1356 | cytochrome P450 76C2-like | Down |
| 17 | g14829 | 1433 | geraniol 10-hydroxylase-like protein | Down |
| 18 | g14985 | 1397 | geraniol 8-hydroxylase-like | Down |
| 19 | g17223 | 1121 | tocopherol O-methyltransferase, chloroplastic isoform X1 | Down |

Cytochrome P450 82C4-like gene gave expression level (FPKM = 20 – 60) at all time points under salt and drought stress as well as control plants, except for the samples taken after 48 hours of drought stress which gave FPKM value 75.88. The direction of Phosphoethanolamine N-methyltransferase 1-like (NMT2) expression take was opposite in the case of saline stress compared to water stress. Under salt stress the expression level of NMT2 started at 40 and increased reaching 120 at 6 hours the declined to be at the same level of untreated plants. On the other hand, under drought stress the expression level of NMT2 started from 20 and continued at the same level till 12 hours reaching 100 at 48 hours which was lower than the level of the same gene at 1 hour of salt stress.

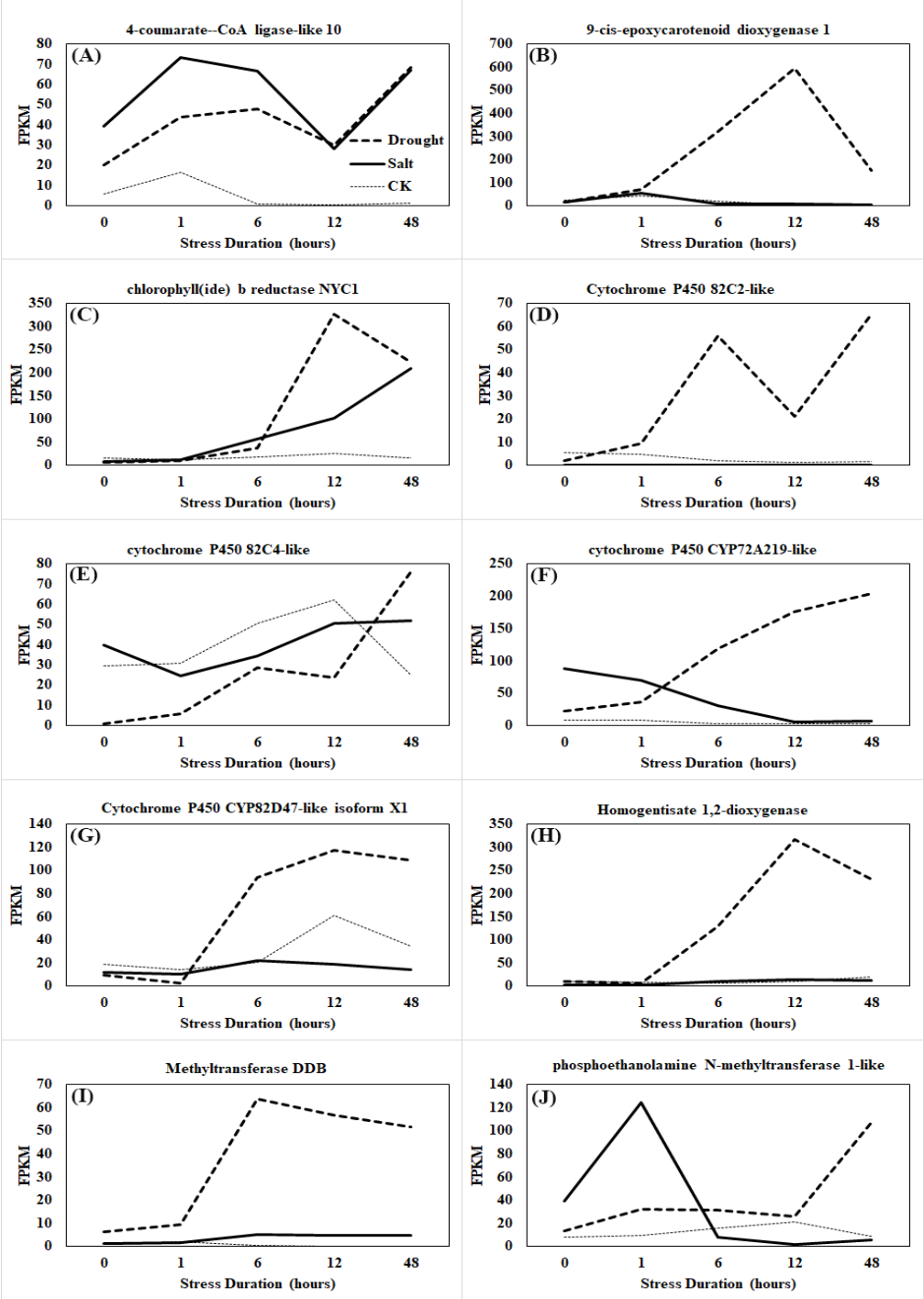


**Figure 1: Expression profile (Fragment per million kilo base) of detected up-regulated transcripts under salt and drought stress conditions as compared to control in sweet potato leaf tissues**

On the other direction, nine unigenes were down-regulated after the first phase of salt and drought stress conditions as compared to control **(Figure 2)**. The expression profile of genes 24-methylenesterol C-methyltransferase 2, cytochrome P450 76A1-like isoform X2 and cytochrome P450 76C2-like followed the same trend. All of these 3 genes significantly down regulated and stayed at the zero at the different durations of salt stress. While, under drought stress the expression level of the three genes started with its highest level at zero point then decreased reaching to the lowest level at 48 hours as compared to control plants which continued at high expression level. Another three genes expressed with a similar way during the different time points of stress including 3-oxoacyl-[acyl-carrier-protein] synthase I, geraniol 8-hydroxylase-like and tocopherol O-methyltransferase. These 3 unigenes give the highest

values of expression at the first time point of salt and drought stress, while the control treatment give lower expression level. Furthermore, the expression of the three genes gradually decreased under the different time points of drought and salt treatments but increased in the control treatment at 48 hours. The FPKM analysis of cytochrome P450 71A3-like, cytochrome P450 76A2-like and geraniol 10-hydroxylase-like protein indicated that the 3 genes expression level increased till 6 hours and then decreased gradually reaching to the minimum level at 48 hours of salt and drought stress. In control plants the expression level of both genes was decreasing following the same trend as treated plants then unlike stress-treated plants the expression level increased at 48 hours in cytochrome P450 71A3-like, cytochrome P450 76A2-like and at 12 hours for geraniol 10-hydroxylase-like protein.

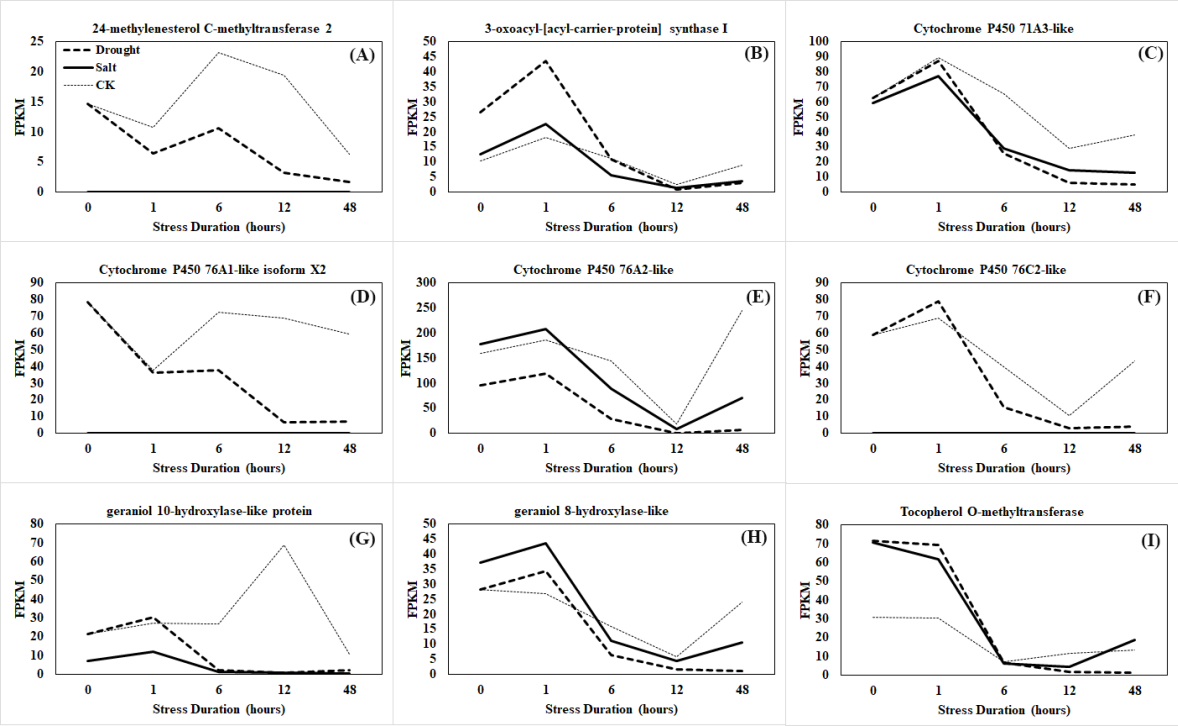


**Figure 2: Expression profile (Fragment per million kilo base) of detected down-regulated transcripts I leaf tissues under salt and drought stress conditions as compared to untreated plants of sweet potato**

**Function annotation of detected up and down regulated genes by Gene Ontology (GO)**

A BLAST2GO search against NR_NCBI protein database was used to annotate the obtained unigenes GO (http://www.geneontlology.org). In total, 10 up- and 9 down-regulated unigenes were detected under the category of secondary metabolites biosynthesis, transport and catabolism with high significant difference as compared to control plants.

According to GO terms, the functions of the obtained transcripts were divided into three different categories (cellular components, molecular function and biological process) as shown in **Table 2**. The obtained genes were aligned to different molecular functions including monooxygenase activity, 5-O-(4-coumaroyl)-D-quinate 3'-monooxygenase activity, dioxygenase activity, oxidoreductase activity, iron ion binding, metal ion binding, heme binding, binding, and trans-cinnamate 4-monooxygenase activity. Furthermore, the 10 detected genes were involved in different biological processes including oxidation-reduction process, secondary metabolite biosynthetic process, lignin biosynthetic process and single-organism metabolic process. For the cellular components category, the 10 unigenes were included under only two subcategories including integral component of membrane and membrane.

The nine down-regulated transcripts were involved in 27 different biological processes presented in **(Table 2).** Moreover, these nine genes were responsible for different molecular activities including oxidoreductase activity, transferase activity, transferring acyl groups other than amino-acyl groups, acting on paired donors and incorporation of one atom of oxygen and protochlorophyllide reductase activity. These nine genes were also present in five different cellular components including endoplasmic reticulum membrane, chloroplast, integral component of membrane and integral component of membrane.

**Table 2. Classification of up- and down-regulated proteins induced under salt and drought stress according to their protein sequence using gene ontology (GO) analysis.**

| GO-category | Gene regulation | GO function annotation |
|---|---|---|
| Molecular functions | up | (GO: 0004497) monooxygenase activity<br>(GO: 0047083) 5-O-(4-coumaroyl)-D-quinate 3'-monooxygenase activity<br>(GO: 0051213) dioxygenase activity<br>(GO:0016705) oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen<br>(GO:0005506) iron ion binding<br>(GO: 0046872) metal ion binding<br>(GO: 0020037) heme binding<br>(GO:0005488) binding<br>(GO: 0016710) trans-cinnamate 4-monooxygenase activity |
| | Down | (GO:0016747) transferase activity, transferring acyl groups other than amino-acyl groups<br>(GO:0016709) oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, NAD(P)H, as one donor, and incorporation of one atom of oxygen<br>(GO:0016630) protochlorophyllide reductase activity |
| Biological Process | up | (GO: 0055114) oxidation-reduction<br>(GO: 0044550) secondary metabolite biosynthetic<br>(GO: 0009809) lignin biosynthetic<br>(GO: 0044710) single-organism metabolic |
| | Down | (GO:0000038) very long-chain fatty acid metabolic process<br>(GO:0000096) sulfur amino acid metabolic process<br>(GO:0006546) glycine catabolic process<br>(GO:0006636) unsaturated fatty acid biosynthetic process<br>(GO:0006733) oxidoreduction coenzyme metabolic process<br>(GO:0006766) vitamin metabolic process<br>(GO:0008652) cellular amino acid biosynthetic process<br>(GO:0009072) aromatic amino acid family metabolic process<br>(GO:0009106) lipoate metabolic process<br>(GO:0009108) coenzyme biosynthetic process<br>(GO:0009117) nucleotide metabolic process<br>(GO:0009416) response to light stimulus cold acclimation<br>(GO:0009695) jasmonic acid biosynthetic process<br>(GO:0009793) embryo development ending in seed dormancy<br>(GO:0009832) plant-type cell wall biogenesis<br>(GO:0015995) chlorophyll biosynthetic process<br>(GO:0016049) cell growth<br>(GO:0016117) carotenoid biosynthetic process<br>(GO:0019216) regulation of lipid metabolic process<br>(GO:0019288) isopentenyl diphosphate biosynthetic process, methylerythritol 4-phosphate pathway<br>(GO:0019684) photosynthesis, light reaction<br>(GO:0019748) secondary metabolic process<br>(GO:0030243) cellulose metabolic process<br>(GO:0031408) oxylipin biosynthetic process<br>(GO:0042335) cuticle development<br>(GO:0044272) sulfur compound biosynthetic process |
| Cellular components | up | (GO: 0016021) integral component of membrane<br>(GO: 0016020) membrane |
| | Down | (GO:0009507) chloroplast<br>(GO:0016021) integral component of membrane<br>(GO:0016020) membrane<br>(GO:0005789) endoplasmic reticulum membrane<br>(GO:0016021) integral component of membrane |

## DISCUSSION

To promote studies on transcriptomics and plant metabolic pathways, Illumina Hiseq platform has been used in a wide range of plant species **(Hirakawa *et al.*, 2014)**. In the current study, transcriptome sequencing was applied for more understanding redox active secondary metabolites related genes under stress conditions.

In the present study, the up-regulated genes were involved in metabolic pathways a well as abiotic stress resistance. In addition, 4CL stayed at a high expression level in leaf tissues under drought and salt stress. This gene considered as a key rate limiting enzyme in flavonoids and lignin metabolism. Furthermore, it is involved in production of resistance related substances in sweet potato under salt and drought stress **(Capriotti *et al.*, 2014)**. Our results indicate that 9-cis-epoxycarotenoid dioxygenase 1 is significantly up-regulated at 12 hours of drought stress.

It was previously reported that 9-cis-epoxycarotenoid dioxygenase-1 responsible for abscisic acid (ABA) biosynthesis and its increment accumulation in leaf tissues that's played a role in increased plant tolerance to several environmental conditions (**Kim *et al.*, 2012)**. In addition, it was found that 9-cis-epoxycarotenoid dioxygenase 1 contributed to increase ABA accumulation and enhance drought tolerance in transgenic sweet potato **(Zhai *et al.*, 2016)**. A chloroplast gene named chlorophyll (ide) b reductase (NYC1) was highly expressed in leaf tissues especially at 12 and 48 hours of drought and salt stress, respectively. It acts as a key regulator of chlorophyll degradation under salt and drought stress conditions. In addition, in tomato it was found to be involved in stress-induced leaf chlorosis **(Sakuraba *et al.*, 2014)**. Cytochrome P450s (CYPs) are the largest group belong to oxidoreductases class of enzymes. It is playing and diverse roles in sweet potato beyond xenobiotic metabolism including biosynthesis of hormones **(Mao *et al.*, 2013)**, fatty acids, and several defence compounds **(Tamiru *et al.*, 2015)**. Additionally, it is involved in protecting plant against adverse environmental conditions. In the current study CYPs were present in different forms including cytochrome P450 82C2-like, 82C4-like, CYP72A219-like and CYP82D47-like isoform X1 which were highly up-regulated in leaf tissues under drought stress and slightly increased under salt stress as compared to control.

Homogentisate 1,2-dioxygenase were expressed in the current experiment in leaf tissue of stress treated sweet potato plants which is previously defined to have a role in tocopherol biosynthesis under stress in leaves **(Falk and Munné-Bosch, 2010)**. Under salt and drought stress conditions methyltransferase genes contribute in DNA methylation that's in role enable modifying plant genomic DNA reversibly soon to void time consuming methods such as genetic recombination and diversity **(Ahmad *et al.*, 2014)**. That's ultimately results in adaptability of DNA under adverse environmental conditions. After exposure to stress conditions, sweet potato plants somatic cells can transfer the experience by epigenetic mechanisms which considered as a very important concern for plant breeding programs **(Francis, 2011)**. In agree with these results, the current results indicated that methyltransferase DDB gene highly expressed under drought stress in leaf tissue and also increased under salt stress conditions while it stayed at the low levels in the untreated plant leaves. We found that phosphoethanolamine N-methyltransferase was significantly up-regulated after one hour of salt stress then decreased from 6 hours till 48 hours of salt stress. On the contrary, under drought stress the expression level started with low level at 1 hour of drought stress then started to increase gradually reaching to thee peak at 48 hours of stress. In agree with our results, **Mou *et al.*, (2002)** induced mutant plants with less Phosphoethanolamine N-methyltransferase resulted into a hypersensitive Arabidopsis line to salinity. Furthermore, he demonstrated that this gene have role in plant growth and development. It was also found to be involved in abiotic stress tolerance, *i.e.*, salt and drought stress.

Down-regulated unigenes in the current experiment were involved in process related to plant metabolic pathways. Some of these genes were significantly expressed in high levels at the beginning of stress then started to decrease with increasing the stress duration. This indicated that those are early response genes to stress which determine the plant resistance and growth conditions in the second phase of stress.

In the current study, the 24-methylenesterol C-methyltransferase 2 gene downregulation led to reduce the plasma membrane fluidity and permeability under stress conditions which in role reduce water loss and increase the viscosity of fluids inside the plant resulting to enrich salt and drought resistance **(Schaller, 2003)**. According to the current results, the 3-oxoacyl-[acyl-carrier-protein] synthase I gene started with a high expression level in leaf tissue at the first hour of drought and salt stress then starting from 6 hours of stress the expression level started to decrease reaching to the minimum at 48 hours. In agree with our results **Leonhardt *et al.* (2005)** reported that 3-oxoacyl-[acyl-carrier-protein] catalyses the 3-oxoacyl-ACP reduction step in the fatty acid synthesis pathway and was repressed by abscisic acid in Arabidopsis leaves guard cells. Different cytochrome P450 forms including 71A3-like, 76A1-like isoform X2, 76A2-like and 76C2-like were also showed to have only an early response to drought and salt stress. These different forms of P450

protein was found to have high amino acid sequence similarity **(Mao *et al.*, 2013)**. The gene geraniol 10-hydroxylase-like protein (G10H) also belong to cytochrome P450 monooxygenase (P450). It was discovered that the overexpression of Arabidopsis PgG10H increase resistance to salt and drought stress **(Balusamy *et al.*, 2017)**. In another study, it was reported that G10H have a relation with binding energy and molecular dynamics as well as stability of protein-ligand complex **(He *et al.*, 2016)**. A chloroplastic gene named tocopherol O-methyltransferase was expressed during the first phase of salt and drought stress resulted in proliferation of the inner membrane envelope which decreases the susceptibility of salt stress by reducing the reactive oxygen species. These results are in agree with our data that explain the reason of salt and drought stress and its relationship with the expression level of tocopherol O-methyltransferase gene in leaf tissues.

Our findings are providing valuable sources for breeding applications of hexaploid sweet potato. In addition, the study of redox active secondary metabolites related genes will offer new candidate genes to breed salt and drought tolerant sweet potato cultivars.

## REFERENCES

**Ahmad, F.; Huang, X.; Lan, H.; Huma, T.; Bao, Y.; Huang, J. and Zhang, H. (2014).** Comprehensive gene expression analysis of the DNA (cytosine-5) methyltransferase family in rice (Oryza sativa L.). Genet. Mol. Res., 13(3): 5159-5172.

**Alagna, F. (2013).** Innovative Transcriptomics approaches for large scale identification of genes involved in plant secondary metabolism. Journal Plant Biochemistry Physiology, 1: e107.

**Anjum, N. A.; Khan, N. A.; Sofo, A.; Baier, M. and Kizek, R. (2016).** Redox homeostasis managers in plants under environmental stresses. Frontiers in Environmental Science, 4: 35.

**Balusamy, S. R. D.; Rahimi, S.; Cho, Y.-G.; Senthil, K. and Yang, D.-C. (2017).** Overexpression of geraniol 10-hydroxylase from Panax ginseng conferred enhanced resistance to Pseudomonas syringae in Arabidopsis. Plant growth regulation, 81(2): 305-316.

**Bennett, R. N. and Wallsgrove, R. M. (1994).** Secondary metabolites in plant defence mechanisms. New phytologist, 127(4): 617-633.

**Capriotti, A. L.; Borrelli, G. M.; Colapicchioni, V.; Papa, R.; Piovesana, S.; Samperi, R.; Stampachiacchiere, S. and Laganà, A. (2014).** Proteomic study of a tolerant genotype of durum wheat under salt-stress conditions. Analytical and bioanalytical chemistry, 406(5): 1423-1435.

**Devaux, A.; Kromann, P. and Ortiz, O. (2014).** Potatoes for sustainable global food security. Potato Research, 57(3-4): 185-199.

**Falk, J. and Munné-Bosch, S. (2010).** Tocochromanol functions in plants: antioxidation and beyond. Journal of experimental botany, 61(6): 1549-1566.

**Francis, R. C. (2011).** Epigenetics: How environment shapes our genes, WW Norton & Company.

**Gajanayake, B.; Raja Reddy, K. and Shankle, M. W. (2015).** Quantifying growth and developmental responses of sweetpotato to mid-and late-season temperature. Agronomy Journal, 107(5): 1854-1862.

**Gnanasekaran, T.; Vavitsas, K.; Andersen-Ranberg, J.; Nielsen, A. Z.; Olsen, C. E.; Hamberger, B. and Jensen, P. E. (2015).** Heterologous expression of the isopimaric acid pathway in Nicotiana benthamiana and the effect of N-terminal modifications of the involved cytochrome P450 enzyme. Journal of biological engineering, 9(1): 24.

**Grabherr, M. G.; Haas, B. J.; Yassour, M.; Levin, J. Z.; Thompson, D. A.; Amit, I.; Adiconis, X.; Fan, L.; Raychowdhury, R. and Zeng, Q. (2011).** Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nature biotechnology, 29(7): 644.

**Gruneberg, W.; Ma, D.; Mwanga, R.; Carey, E.; Huamani, K.; Diaz, F.; Eyzaguirre, R.; Guaf, E.; Jusuf, M. and Karuniawan, A. (2015)**. Advances in sweetpotato breeding from 1992 to 2012, CABI International.

**Haas, B. J.; Papanicolaou, A.; Yassour, M.; Grabherr, M.; Blood, P. D.; Bowden, J.; Couger, M. B.; Eccles, D.; Li, B. and Lieber, M. (2013).** De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nature protocols, 8(8): 1494.

**He, Y.; Yan, H.; Hua, W.; Huang, Y. and Wang, Z. (2016).** Selection and validation of reference genes for quantitative real-time PCR in Gentiana macrophylla. Frontiers in plant science, 7: 945.

**Hirakawa, H.; Shirasawa, K.; Miyatake, K.; Nunome, T.; Negoro, S.; Ohyama, A.; Yamaguchi, H.; Sato, S.; Isobe, S. and Tabata, S. (2014).** Draft genome sequence of eggplant (Solanum melongena L.): the representative solanum species indigenous to the old world. DNA research, 21(6): 649-660.

**Kapoor, D.; Sharma, R.; Handa, N.; Kaur, H.; Rattan, A.; Yadav, P.; Gautam, V.; Kaur, R. and Bhardwaj, R. (2015).** Redox homeostasis in plants under abiotic stress: role of electron carriers, energy

metabolism mediators and proteinaceous thiols. Frontiers in Environmental Science, 3(13).

**Kim, S. H.; Ahn, Y. O.; Ahn, M.-J.; Lee, H.-S. and Kwak, S.-S. (2012).** Down-regulation of β-carotene hydroxylase increases β-carotene and total carotenoids enhancing salt stress tolerance in transgenic cultured cells of sweetpotato. Phytochemistry, 74: 69-78.

**Kukurba, K. R. and Montgomery, S. B. (2015).** RNA sequencing and analysis. Cold Spring Harbor Protocols, (11): pdb. top084970.

**Leonhardt, N.; Kwak, J. M.; Robert, N.; Waner, D.; Leonhardt, G. and Schroeder, J. I. (2005).** Microarray Expression Analyses of Arabidopsis Guard Cells and Isolation of a Recessive Abscisic Acid Hypersensitive Protein Phosphatase 2C Mutant. Plant Cell 16, 596–615. Plant Cell, 17(4): 1330-1333.

**Li, B. and Dewey, C. N. (2011).** RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC bioinformatics, 12(1): 323.

**Mao, G.; Seebeck, T.; Schrenker, D. and Yu, O. (2013).** CYP709B3, a cytochrome P450 monooxygenase gene involved in salt tolerance in Arabidopsis thaliana. BMC plant biology, 13(1): 169.

**Markos, D. and Loha, G. (2016).** Sweet potato agronomy research in Ethiopia: Summary of past findings and future research directions. Agriculture and Food Sciences Research **3**(1): 1-11.

**Motsa, N. M.; Modi, A. T. and Mabhaudhi, T. (2015).** Sweet potato (Ipomoea batatas L.) as a drought tolerant and food security crop. South African Journal of Science, 111(11-12): 1-8.

**Mou, Z.; Wang, X.; Fu, Z.; Dai, Y.; Han, C.; Ouyang, J.; Bao, F.; Hu, Y. and Li, J. (2002).** Silencing of phosphoethanolamine N-methyltransferase results in temperature-sensitive male sterility and salt hypersensitivity in Arabidopsis. The Plant Cell, 14(9): 2031-2043.

**Nedunchezhiyan, M.; Byju, G. and Jata, S. K. (2012).** Sweet potato agronomy. Fruit, Veg. Cereal Sci. Biotech., 6(1): 1-10.

**Raymundo, R.; Asseng, S.; Cammarano, D. and Quiroz, R. (2014).** Potato, sweet potato, and yam models for climate change: A review. Field Crops Research, 166: 173-185.

**Sakuraba, Y.; Park, S.-Y.; Kim, Y.-S.; Wang, S.-H.; Yoo, S.-C.; Hörtensteiner, S. and Paek, N.-C. (2014).** Arabidopsis STAY-GREEN2 is a negative regulator of chlorophyll degradation during leaf senescence. Molecular plant, **7**(8): 1288-1302.

**Schaller, H. (2003).** The role of sterols in plant growth and development. Progress in lipid research, 42(3): 163-175.

**Sha Valli Khan, P.; Nagamallaiah, G.; Dhanunjay Rao, M.; Sergeant, K. and Hausman, J. (2014).** Chapter 2—Abiotic Stress Tolerance in Plants: Insights from Proteomics. Emerging Technologies and Management of Crop Stress Tolerance; Academic Press: Cambridge, MA, USA: 23-68.

**Storey, J. D. and Tibshirani, R. (2003).** Statistical significance for genomewide studies. Proceedings of the National Academy of Sciences, 100(16): 9440-9445.

**Tamiru, M.; Undan, J. R.; Takagi, H.; Abe, A.; Yoshida, K.; Undan, J. Q.; Natsume, S.; Uemura, A.; Saitoh, H. and Matsumura, H. (2015).** A cytochrome P450, OsDSS1, is involved in growth and drought stress responses in rice (Oryza sativa L.). Plant molecular biology, 88(1-2): 85-99.

**Wink, M. (1988).** Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivores. Theoretical and applied genetics, 75(2): 225-233.

**Young, M. D.; Wakefield, M. J.; Smyth, G. K. and Oshlack, A. (2010).** Gene ontology analysis for RNA-seq: accounting for selection bias. Genome biology, 11(2): R14.

**Zhai, H.; Wang, F.; Si, Z.; Huo, J.; Xing, L.; An, Y.; He, S. and Liu, Q. (2016).** A myo‐inositol‐1‐phosphate synthase gene, Ib MIPS 1, enhances salt and drought tolerance and stem nematode resistance in transgenic sweet potato. Plant biotechnology Journal, 14(2): 592-602.