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Original Research Paper

Transcriptome analysis and identification of leaf, tuberous root and fibrous root tissue-specific high temperature stress-responsive genes in sweet potato

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ABSTRACT

Sweet Potato is an important food crop, and its production is affected by environmental stresses, including high temperature. The gene expression patterns and molecular responses in different tissues of sweet potato under high temperature stress were studied using microarray data sets. Analysis revealed that modulation in the expression of key genes and pathways associated with various proteins including enzymes under high temperature stress in leaf, fibrous root and storage root tissues. Tissue-specific responses, with both common and unique cellular responses were observed among the tissues. Pathway analysis revealed the differential regulation of genes involved in DNA replication, metabolism, transport, signaling, and stress response during high temperature stress. Six genes viz., DnaJ-domain protein (IpDnaJ), nuclear protein (IpELF5), heat shock protein 90.1 (IpHsp90.1), ABC transporter (IpABC) hydrolase (IpNUDX1) and alternative oxidase 1a (IpAO1a), were up-regulated in the leaf, fibrous root and tuberous root tissues. These six genes might play an important role in imparting high temperature stress tolerance in the leaf, fibrous root and tuberous root tissues of sweet potato. The information generated provides valuable insights on leaf, tuberous root and fibrous root tissue-specific high temperature stress-responsive genes in sweet potato. These datasets will be helpful in selecting candidate genes and pathways for further functional and genomic analyses, facilitating the genetic improvement of sweet potato with enhanced stress tolerance.

Keywords : Fibrous root, high temperature stress, microarray, sweet potato, tuberous root

INTRODUCTION

Sweet potato [*Ipomoea batatas* (L.) Lam] holds immense importance as a staple crop in the tropical and sub-tropical regions across the globe. It is the only domesticated species in the *Ipomoea* genus (Ravi *et al*., 2014). Sweet potato is a highly nutritious crop due to its starch-rich storage root that provides a substantial amount of dietary energy and essential nutrients required to meet human nutritional requirements (van Jaarsveld and Faber, 2013). This versatile crop offers a range of benefits, including a high carbohydrate content, dietary fiber, bioactive compounds (such as proteins, vitamins, β-carotene, anthocyanins, and minerals), and nutritional composition comparable to cereals and pulses (van Jaarsveld and Faber, 2013; Ravi *et al*., 2014). Additionally, sweet potato has emerged as a "climate-resilient" and "famine-relief" crop, playing a crucial role in mitigating food shortages during natural calamities, saving numerous lives globally (Gurmu *et al.,* 2014; Ravi *et al*., 2014). Introduction of orange-fleshed sweet potatoes, rich in β-carotene, has effectively addressed vitamin A-related malnutrition disorders in pregnant women and young children in developing nations (van Jaarsveld and Faber, 2013; Gurmu *et al.,* 2014). Consequently, sweet potato holds immense potential as an essential dietary component for future human populations (Gurmu *et al.,* 2014).

High-temperature stress has detrimental effects on sweet potato growth, development, and overall yield. Studies have shown increased temperatures during early seasons result in fewer tubers and decreased yield, whereas, high temperatures during mid and late seasons promote shoot growth at the expense of root growth, ultimately affecting the final storage root yield (Gajanayake *et al.,* 2015). Moreover, elevated temperatures have been found to impact storage root growth and yield negatively, with potential reductions in sweet potato yield (Wijewardana *et al.,* 2018). It

has also been observed that high temperatures can depress photosynthetic rates, further affecting yield (Ravi *et al.,* 2014). To enhance the resilience of sweet potato crops against heat stress, it is crucial to identify and understand the genes involved explicitly in heat stress responses. By studying these genes, strategies can be developed to improve the crop's ability to with stand high temperatures and maintain optimal growth and yield.

Transcriptome analysis plays a crucial role in understanding the dynamic changes in gene expression under abiotic stress conditions (Katiyar *et al.,* 2015; Sun *et al.,* 2022). Comprehensive tissue-specific transcriptome analysis by employing techniques such as Microarray, RNA sequencing (RNA-Seq) etc., provides valuable insights into the regulatory programs that govern gene expression during organ development (Katiyar *et al.,* 2015; Ravi *et al.,* 2020). This approach is particularly relevant in the context of sweet potato, as it allows for the identification and characterization of genes specifically involved in heat stress responses, unveiling tissue-specific gene responses and regulatory networks that contribute to the crop's adaptation and resilience under high-temperature conditions (Sharma *et al.,* 2021). Tissue-specific transcriptome analysis has been employed in various plant species to uncover multiple responses to environmental stressors (Katiyar *et al.,* 2015; Tiwari *et al.,* 2023). Transcriptome analysis has provided valuable insights into tissue-specific gene expression profiles and regulatory networks involved in heat stress responses (Tao *et al.,* 2012; Sun *et al.,* 2022). These studies have revealed specific genes and pathways that are activated or suppressed in response to high temperatures in different plant tissues. Hence, in this study transcriptome analysis was performed to identify the high temperature-responsive genes in sweet potato tissues *viz*., leaf, fibrous root and tuberous root tissues using microarray. Analysis revealed that under high temperature stress, certain key genes and pathways associated with DNA replication, metabolism, transport, signaling, and stress response are modulated in leaf, fibrous root, and storage root tissues. Interestingly, tissue-specific responses were observed, with both common and unique cellular responses among the different types of tissues.

MATERIALS AND METHODS

Plant material and growth conditions

The sweet potato var. Sree Arun was grown in the earthen pots in the natural sunlight conditions with 12 hours sun light per day under 1700 µ mol m^2h^{-1}at $30^oC \pm 2^oC$ during day time and $23^oC \pm 1^oC$ during night time as described in Ravi *et al.* (2017). High temperature stress was imposed by exposing the plant to $40^oC \pm 2^oC$. High quality RNA was extracted from the leaf, storage root and fibrous root from 30 days old sweet potato plants (Ravi *et al.,* 2017).

RNA processing and cRNA synthesis

The RNA samples of leaf, fibrous root and tuberous root were labeled using Agilent Quick Amp Kit as per manufacturers protocol (Ravi *et al.,* 2017). 500 ng of RNA was reverse transcribed using oligodT primer tagged to T7 promoter sequence for synthesizing cDNA. The *in vitro* transcription step was performed to convert cDNA to cRNA using T7 RNA polymerase enzyme and Cy3 dye as per manufacturers protocol (Ravi *et al.,* 2017). The cRNA was further cleaned using Qiagen RNeasy columns (Qiagen, Cat No: 74106). The concentration and amount of dye incorporated was measured using Nano Drop Spectrophotometer (Thermo Scientific, USA).

Microarray and expression analysis

The Agilent 60-mer oligo microarray (Agilent control grid IS- 62976-8-V2-60K x 8-Gx-EQC-201000210) was used for studying the expression pattern of the genes of sweet potato (Ravi *et al.,* 2020). For these, 600 ng of labeled cRNA were hybridized on the array using the Gene Expression Hybridization kit following manufacturers instruction using Agilent Sure hybridization Chambers at 65º C for 16 hours. Hybridized slides were washed using Agilent Gene Expression wash buffers (Part No: 5188-5242). G2505C scanner (Agilent Technologies) was used to scan the slides. Sample preparation and microarray expression analysis was performed at the Genotypic Technology Pvt. Ltd., Bengaluru, India. The microarray datasets generated in this study was submitted to NCBI GEO: GSE51862. The raw data was processed and the expression of the probes was transformed into the $\log_2$ ratio. The gene expression with $\log_2$ FC >2 was considered as upregulated and gene expression with $\log_2$ FC < -2 was considered as downregulated. Differentially expressed genes (both upregulated and downregulated) were considered for further analysis. The sequence information of the sweet potato probes were used as a query and a blast search was performed against the *Arabidopsis* and rice genome database to identify the respective

Table 1 : Differentially expressed gene (DEGs) statistics under high temperature stress in sweet potato

Analysis group	Total DEGs (Log_2 FC>2 and Log_2 FC<-2)	Upregulated (Log_2 FC>2)	Downregulated (Log_2 FC<-2)
Leaf tissue	1871	967	904
Fibrous root tissue	2725	1461	1264
Tuberous root Tissue	2810	109	2701

orthologous. The gene annotated information and LOC details of *Arabidopsis* were used for predicting the gene ontology (Tian *et al.,* 2017).

RESULTS AND DISCUSSION

The present study aimed to investigate the gene expression patterns in different tissues of sweet potato (leaf, fibrous root and tuberous root) in response to high temperature stress. Transcriptome analysis using microarray technology revealed the modulation of key genes and pathways associated with various proteins and enzymes in the different tissues of sweet potato under high temperature stress.

Differential expression analysis

Differential expression analysis revealed that 967, 1461 and 109 genes were upregulated in the leaf, fibrous root and tuberous root tissues, respectively, whereas, 904, 1264 and 2701 genes were down regulated in the leaf, fibrous root and tuberous root tissues, respectively during high temperature stress (Table 1 and supplementary Table 1). The details of the probes/genes, gene expression (fold change), description, etc., are presented in supplementary Table 1.

The present findings aligned with Ravi *et al*. (2017, 2020), who demonstrated the use of microarray analysis in understanding the molecular responses of tuber development in sweet potato. Differential expression analysis identified a substantial number of upregulated and downregulated genes in each tissue, highlighting the tissue-specific response to high temperature stress. Furthermore, the study identified common and unique cellular responses among the tissues, as supported by the differential regulation of pathway genes involved in DNA replication, metabolism, transport, signaling, and stress response (Tao *et al.,* 2012; Sharma *et al*., 2021; Sun *et al.,* 2022).

High temperature stress responsive genes in the leaf, fibrous root and tuberous root tissues of sweet potato

Molecular chaperones *viz*., heat shock proteins, DnaJ-domain, etc., protects the native proteins from the stress induced damages by retaining its native structures (Muthusamy *et al.,* 2016). The movement of these proteins across cell organelles was regulated with the help of coordinated function of various transporters such as *Ran GTPase* (Choudhury *et al.,* 2021). Alternative oxidase (AOX) protects the plant mitochondria under high temperature stress (Saha et al., 2016). In this study, sixty genes were differentially regulated in all three tissues viz., leaf, fibrous root and tuberous root tissues under high temperature stress (Fig. 1 and supplementary Table 2). Out of these sixty, six genes, DnaJ-domain protein (IpDnaJ), Nuclear protein (IpELF5), heat shock protein 90.1 (IpHsp90.1), Alternative oxidase 1a (IpAO1a), ABC transporter (IpABC) and hydrolase (IpNUDX1) were upregulated (Table 2), whereas, twenty-six genes were downregulated regulated in the leaf, fibrous root and tuberous root tissues respectively during high temperature stress (Fig. 1, supplementary Fig. 1 and supplementary Table 2). Thus, these six genes might play an important functional role in protecting the cellular proteins in leaf, fibrous root and tuberous root tissues under high temperature stress in sweet potato (Muthusamy *et al*., 2017; Saha *et al*., 2016; Choudhury *et al.,* 2021). In the present study, 376 were differentially regulated in both leaf and fibrous root tissues, whereas, 148 genes were differentially regulated in both leaf and tuberous root tissues during high temperature stress (Fig. 1 and supplementary Table 1). About 250 genes were differentially regulated in both fibrous root and tuberous root tissues under high temperature stress (Fig. 1 and supplementary Fig. 1). Several studies have shown the significant modulation in the expression of transcriptome involving important genes/

Table 2 : Details of the upregulated genes in leaf, fibrous root and tuberous root tissues of sweet potato under high temperature stress

Gene	Description/ function	Sweet potato Array Probe ID	Fold Change (log_2 FC)			*Arabidopsis* ortholog (LOC ID)
			Leaf	Fibrous root	Tuberous root	
IpDnaJ	DnaJ Chaperone	JP117116	3.16	2.23	2.26	AT5G03030.1
IpNUDX 1	cytosol-localized nudix hydrolase	JP135891	3.83	3.22	2.80	AT1G68760.1
IpABCC3	ABC transporter family protein	JP140208	4.09	2.75	2.31	AT3G13080.4
IpELF5	Nuclear Protein	JP144908	2.76	2.62	2.46	AT5G62640.2
IpHsp90.1	Heat shock protein 90 (HSP90)	JP146862	3.73	3.14	2.92	AT5G56010.1
IpAO1a	Alternative oxidase 1a	JP145717	5.45	4.26	3.90	AT3G22370.1

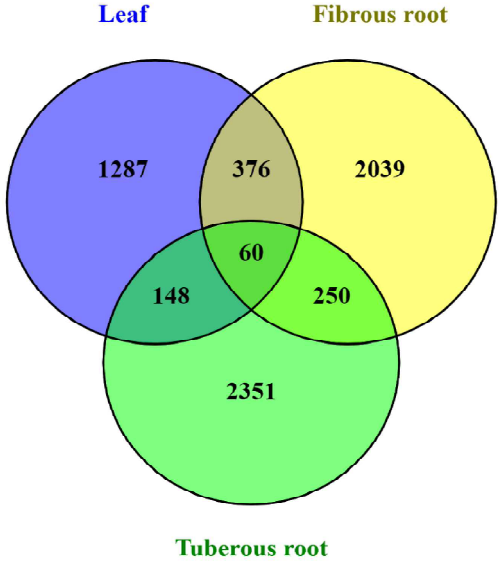
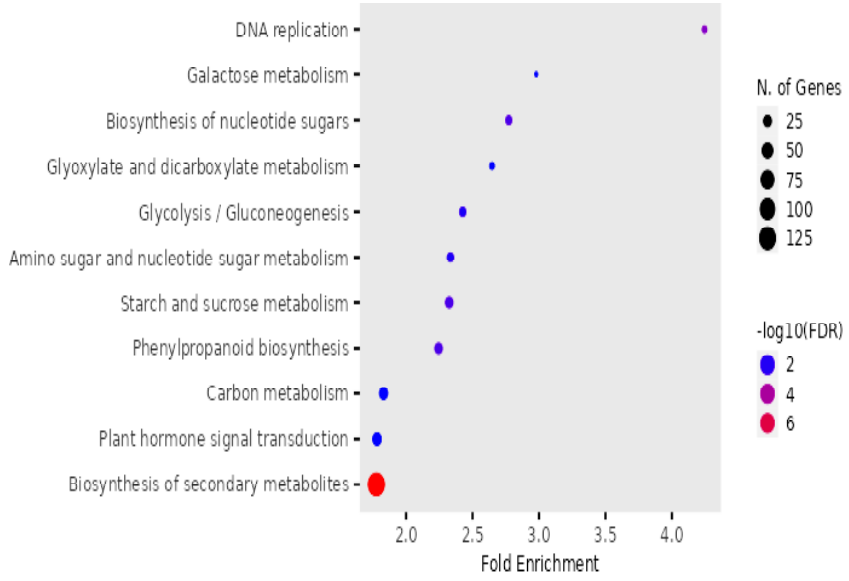


Fig. 1 : Venn diagram showing upregulated and downregulated genes in the leaf, fibrous root and tuberous root tissues of sweet potato under high temperature stress in comparison with control condition

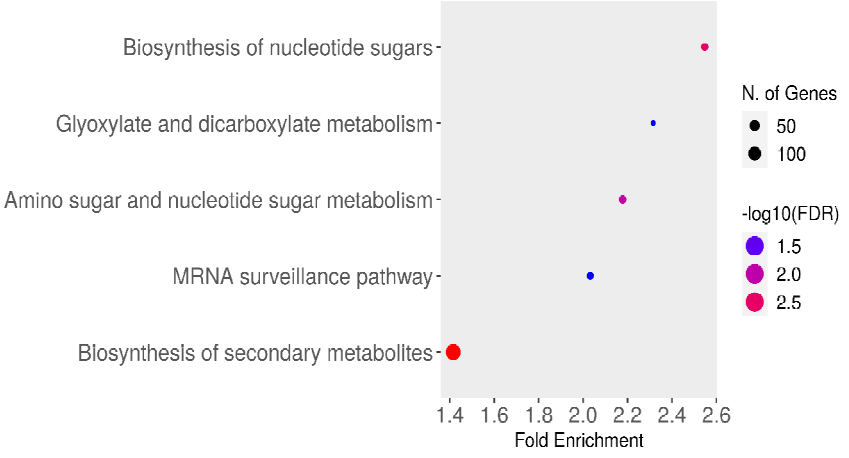
pathways during high temperature stress. The pathways/genes including phytohormones, molecular chaperones, signaling kinesis, ROS scavenging enzymes, Epigenetic modifications, transcription factors, etc., were differently expressed during high temperature stress (Sharma *et al*., 2021; Venkatesh *et al*., 2022).

Tissue specific molecular, biochemical and physiological response play important role in regulating high temperature response in plants (Muthusamy *et al.,* 2017; Sharma *et al.,* 2021; Xiang and Rathinasabapathi, 2022). Thus, in this study, under high temperature stress, the pathway genes *viz*., DNA replication, galactose metabolism, biosynthesis of nucleotide sugars, glyoxylate and dicarboxylate metabolism, glycolysis/gluconeogenesis, amino sugar and nucleotide sugar metabolism, starch and sucrose metabolism, phenylpropanoid biosynthesis, carbon metabolism, plant hormone signal transduction and biosynthesis of secondary metabolites were modulated in the leaf tissue, whereas, biosynthesis of nucleotide sugars, glyoxylate and dicarboxylate metabolism, amino sugar and nucleotide sugar metabolism, mRNA surveillance pathway and biosynthesis of secondary metabolites were modulated in the fibrous root tissues (Fig. 2).

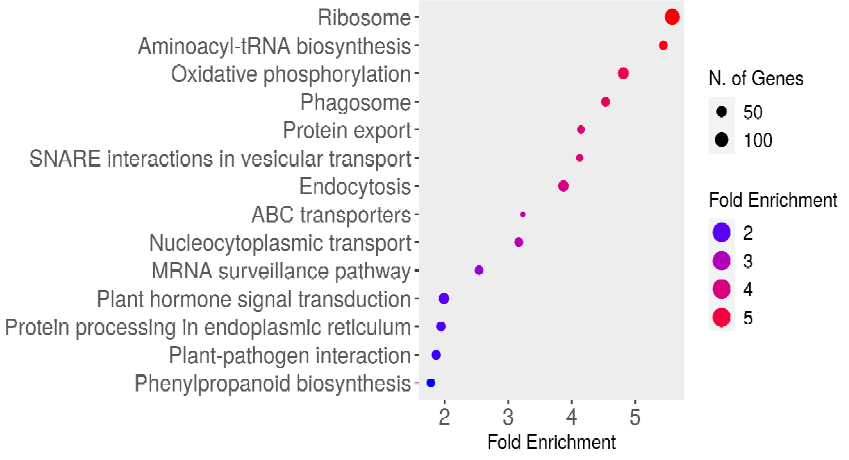
Similarly, in the tuberous root tissues, ribosome, aminoacyl-tRNA biosynthesis, oxidative phosphorylation, protein export, ABC transporters, nucleocytoplasmic transport, mRNA surveillance pathway, plant hormone signal transduction, protein processing in endoplasmic reticulum- plant-pathogen interaction and phenylpropanoid biosynthesis pathway genes were modulated. Additionally, previous research has elucidated the molecular responses of sweet potato to other stress conditions such as low temperature stress (Wijewardana *et al.,* 2018). These studies collectively contribute to understanding of the complex molecular mechanisms underlying stress responses in sweet potato (Wijewardana *et al.,* 2018; Ravi *et al.,* 2020;



A. leaf



B. fibrous root



C. tuberous root

Fig. 2 : GO categories of DEGs of sweet potato under high temperature stress.

Sun *et al.,* 2022; Xiang and Rathinasabapathi, 2022). Thus, present study demonstrates the presence of both collective and individual cellular responses to high temperature stress in the leaf, fibrous root, and tuberous root tissues of sweet potato.

CONCLUSION

The study sheds light on the effects of high temperature stress on the gene expression profiles and molecular responses in the leaf, fibrous root, and storage root tissues of sweet potato. The study identified both common and tissue-specific responses to high temperature stress among these tissues through comparative analysis. The findings provide valuable insights for identifying key genes and pathways involved in the response to high temperature stress, facilitating further functional and genomic studies aimed at enhancing the genetic improvement. By better understanding the molecular mechanisms underlying the response to high temperature stress, we can develop targeted strategies to enhance stress tolerance and improve the overall resilience of sweet potato.

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