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Transcriptome Analysis of *Cocos nucifera* L. Seedlings Having Contrasting Water-Use Efficiency (WUE) under Water-Deficit Stress: Molecular Insights and Genetic Markers for Drought Tolerance

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Abstract: Perennials utilize complex adaptive strategies and molecular mechanisms to cope with water-deficit conditions. Two year old coconut seedlings of the varieties Kalpa Sree and Kalpatharu were subjected to soil water-deficit regimes (25% of available of soil moisture as in control). Biochemical, physiological and growth parameters underlying water-deficit stress revealed the differential enzymatic anti-oxidants, lipid peroxidation status and water use efficiency trait between the genotypes investigated. The whole plant water use efficiency at control condition was significantly low in Kalpatharu (4.06) compared to Kalpa Sree (4.74). Nevertheless, under severe stress [25% ASM] Kalpatharu exhibited highest WUE (5.68) as against dwarf variety Kalpa Sree (3.84). Furthermore, the leaf transcriptome profiles of the control and water-deficit stressed seedlings were examined utilizing paired-end RNA-Seq. In total, ~7300 differentially expressed genes have been identified between the seedlings under water-deficit stress and control. Analysis of control and stressed Kalpasree leaf transcriptome showed significant upregulation of PHLOEM PROTEIN 2-LIKE A1-like, WRKY transcription factor 40 isoform X1 and downregulation of glycerol-3-phosphate acyltransferase 3 transcripts. On the other hand, upregulation of transcripts encoding polyamine oxidase, arabinose 5-phosphate isomerase among others and downregulation of aquaporin PIP1-2 transcript was documented in Kalpatharu leaves. Besides, long non-coding RNA and genic SSRs were also identified from the transcriptome data to further enrich the genomic resources of coconut palm which would pave way for its utilization in developing climate-smart coconut crop. The implication of this study in molecular dissection of adaptive response of coconut to soil-water deficit is also discussed.

Keywords: coconut seedlings; climate-smart; drought adaptation; genic markers
RNA-seq

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Materials and Methods

Genotypes :

Two year old coconut seedlings of the varieties

a) **Kalpa Sree** (dwarf) b) **Kalpatharu** (tall)

Drought experiment

Seedlings were treated: 100% available soil moisture (ASM)-control; and 25% ASM-stressed

Biochemical characterization

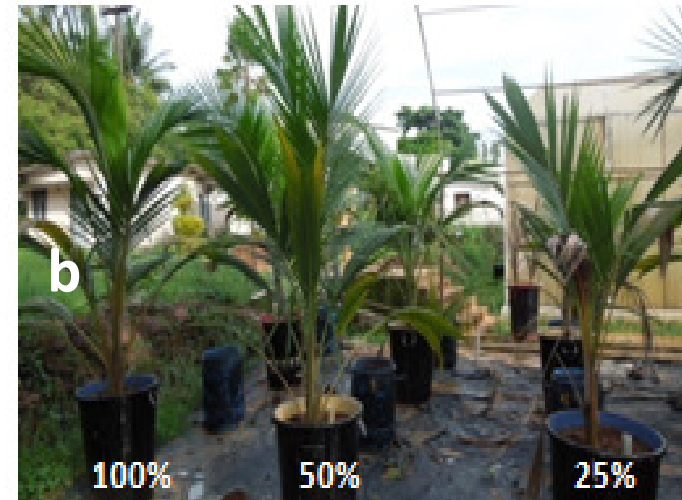
Epicuticular wax content, [MDA] and enzymatic antioxidants

RNA-Seq Analysis

Illumina TruSeq Stranded mRNA Library Preparation Kit, *de novo* assembly, unigenes prediction using CD-HIT package v 4.6.1, mapping of the unigene to the biological pathways performed using KAAS, Differential gene expression analysis, SSR and long ncRNA prediction

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Results and Discussion



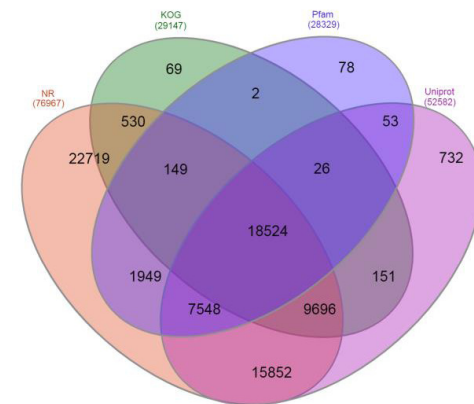
Parameters	Kalpatharu	Kalpasree
Epicuticular wax	88.59 $\mu\text{g cm}^2$	54.63 $\mu\text{g cm}^2$
SOD (sp.activity)	3.49 to 5.98	2.35 to 2.51
PPO (sp.activity)	0.16 to 1.37	0.14 to 1.18
Water use efficiency	5.68	3.84

Transcriptomic features and Unigene annotation

RNA-Seq Analysis

Four cDNA libraries yielded approximately 158.96 million bp reads with an average transcript length of 829bp and transcript N50 value of 1225.

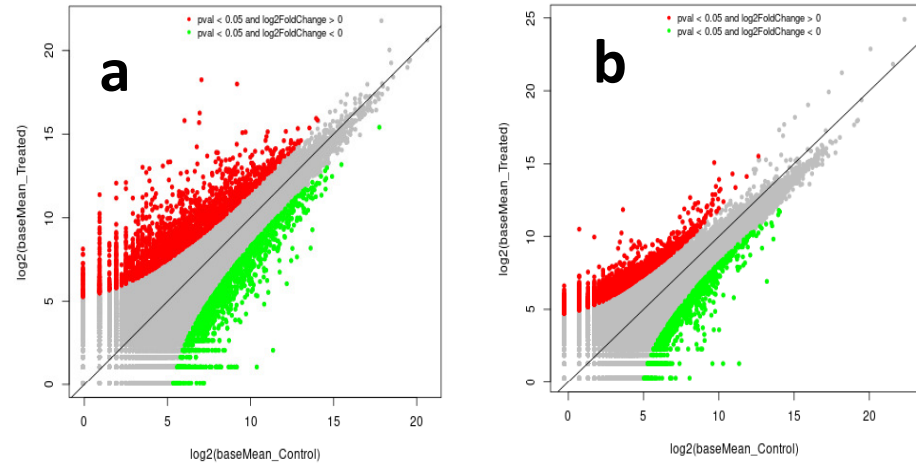
A maximum of 68482 transcripts belonged to class 300-400bp



Venn diagram representing share of various databases used for annotation of Unigenes

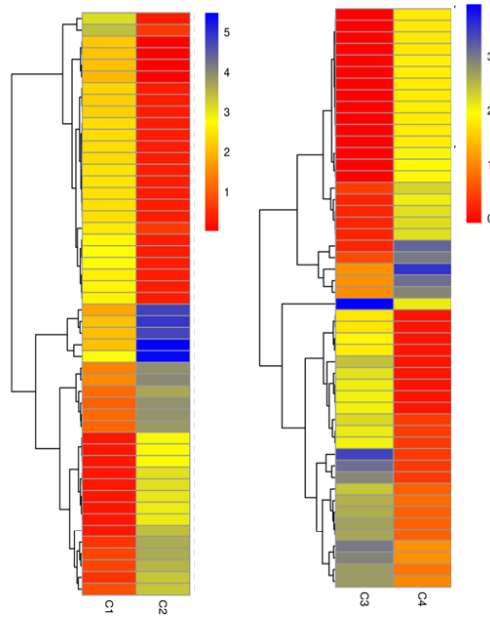
Transcriptomic response of the genotypes under drought

Analysis of transcripts of stressed and control Kalpa Sree seedlings revealed 2388 transcripts are significantly upregulated and 1278 are significantly downregulated whereas in Kalpatharu significant upregulation and downregulation of 2868 and 778 transcripts, respectively



Scatter plot representation of log₂ (base mean-treated) values for transcripts expressed in control and treated Kalpa Sree (a) and Kalpatharu (b)

Differential gene expression analysis



Heat map showing clusters of differentially expressed genes (a) Kalpa Sree(b) Kalpatharu

Kalpasree: Upregulation of PHLOEM 143 PROTEIN 2-LIKE A1-like (Log2 FC 11.19), WRKY TF-40 isoform X1 (Log2 FC 9.59)
Downregulation of glycerol-3-phosphate acyltransferase 3

Kalpatharu: Downregulation of Aquaporin PIP1-2 transcript (Log2 FC -1.8), ethylene responsive transcription factor ERF105 (Log2 FC -1.9)

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Genetic markers

17296 genic SSRs were identified from the transcriptome sequences

The most common SSRs were di-nucleotide repeats (10826) followed by tri-nucleotide repeats (5699) ; quad-, penta-, and hexa nucleotides are of 633, 89, and 49, respectively

Analysis for expression of long non-coding RNAs (lncRNAs) in transcriptome data set revealed a total of 63 Unigenes are potential lncRNAs

In Kalpa Sree 32 lncRNAs are differentially regulated whereas in Kalpatharu 59 are differentially regulated

Conclusions

Genotype-specific differential molecular responses of coconut cultivar to water stress

EST-derived SSRs uncovered would add to molecular markers in coconut

Underscores the importance of lncRNAs repertoire in shaping up the adaptive response of coconut cultivars to water deficit stress

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