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Data Descriptor

Transcriptome Dataset of Strawberry (*Fragaria* × *ananassa* Duch.) Leaves Using Oxford Nanopore Sequencing under LED Irradiation and Application of Methyl Jasmonate and Methyl Salicylate Hormones Treatment

M Adrian [1], Roedhy Poerwanto [2], Eiichi Inoue [3] and Deden Derajat Matra [2,4,*]

[1] Agronomy and Horticulture Study Program, Graduate School, IPB University, Dramaga Bogor 16680, Indonesia
[2] Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Dramaga Bogor 16680, Indonesia
[3] College of Agriculture, Ibaraki University, Ami 300-0393, Japan
[4] Science Molecular Division, Advanced Research Laboratory (Ar-Lab), IPB University, Dramaga Bogor 16680, Indonesia
* Correspondence: dedenmatra@apps.ipb.ac.id

Abstract: This data descriptor introduces a transcriptome dataset of strawberry plant left exposed to an LED light treatment and plant hormones of Methyl Jasmonate (MeJA) and Methyl Salicylate (MeSA). These data consist of a transcriptome dataset (four libraries) obtained from the leaves of strawberry plants treated with LEDs of blue and red spectrums and the hormones of Methyl Jasmonate (MeJA) and Methyl Salicylate (MeSA), which allowed us to conduct a further analysis of the growth and development processes of strawberry plants. In addition, we describe detailed procedures on how the plants were prepared and treated and how the data were generated and processed beforehand. Further analysis of these data will significantly help to improve our understanding of the molecular mechanisms of LED light and MeJA-MeSA in strawberry plants.

Dataset: The data have been deposited in the DNA Data Bank of Japan (DDBJ) at Bioinformation and DDBJ Center under accession number DRA015235, https://ddbj.nig.ac.jp/resource/sra-submission/DRA015235 accessed on 9 December 2022.

Keywords: strawberry; long-read sequencing; lights; plant hormone; RNASeq

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1. Summary

Strawberries (*Fragaria* × *ananassa* Duch.) are members of the Rosaceae family. Strawberries are one of the most popular fruits worldwide due to their abundance of nutrients, vitamins, and minerals [1,2]. However, strawberry cultivation in the tropics could be successful if it was carried out in highland areas with an elevation above 1000 m above sea level with a temperature of 17–20 °C [3]. Based on this, it is necessary to make simple modifications in the development of strawberry cultivation techniques to increase fruit production and quality and make it easy to cultivate in all areas, especially lowland areas. In addition, the challenge of introducing highland plants to the lowlands is the frequent attacks of plant-disturbing organisms due to different environmental conditions.

Artificial light in cultivation techniques can shorten the annual cycle of crops such as barley, peas, and canola [4]. The Light Emitting Diode (LED) is an artificial light that is used in strawberry plants cultivation that can modify the plants' metabolic pathways by

accumulating photosynthate in the sinks and regulating the photosynthate flow. Carbohydrate translocation can also be optimized without interference from other sink competitors so that increased yield and fruit quality can be achieved [5].

Plant growth and development are regulated by hormones, including the induction and adaptation of plant defenses [6]. For example, the exogenous application of the active form of Jasmonate acid, Methyl Jasmonate (MeJA), is known to induce the plant's defense, thereby increasing the plant's resistance to herbivorous insect pests [7]. In addition, Jasmonate acid is generally associated with the activation of defenses against pests, necrotrophic pathogens, and nematodes [8,9], whereas salicylic acid is associated with the activation of defenses against biotrophic pathogens [10].

The transcriptomic approach has been widely used to study the genetic and molecular mechanisms underlying the metabolic processes and adaptations in plants. One of the transcriptomic methods utilized is the next-generation sequencing (NGS) method with MinION, which was developed by Oxford Nanopore Technologies. The results of NGS MinION in the form of long-read data are expected to produce complete information regarding the genes that play a role in and their expression in the adaptation mechanism of strawberry plants to artificial light with LEDs and hormones, especially in the lowlands. Thus, the interaction of the two factors can be a source of reference in strawberry cultivation techniques in the lowlands.

This study produced a set of long reads data from RNA sequencing results from four different treatment samples, namely, LED Red: Blue 2:1 + Methyl Salicylate (RMS), LED Red: Blue 2:1 + Methyl Jasmonate (RMJ), LED Red: Blue 1:2 + Methyl Salicylate (BMS), and RB Red: Blue 1:2 + Methyl Jasmonate (BMJ), and we produced 10,248 transcripts (Table 1), and we identified the differences in the abundance of genes affected by the LED and MeJA-MeSA hormones. This dataset will fundamentally contribute to determining the genes that play a role and are active in strawberry plants when they are exposed to LED irradiation and the application of hormones Methyl Jasmonate (MeJA) and Methyl Salicylate (MeSA).

Table 1. Summary of raw and clean reads and transcriptome assembly.

Sample	BMJ	BMS	RMJ	RMS
RAW READS				
Total Reads	634,035	697,857	633,288	144,667
Average (bp)	362.28	328.35	335.75	307.90
Largest (bp)	44,572	48,586	34,703	202,197
N50 (bp)	371, n = 195,583	321, n = 221,986	350, n = 206,574	297, n = 47,997
CLEANED READS				
Total Reads	523,511	564,539	475,749	104,408
Average (bp)	197.08	163.06	179.62	148.94
Largest (bp)	3638	5709	3585	3371
N50 (bp)	285, n = 100,363	225, n = 106,168	236, n = 99,241	195, n = 21,516

2. Data Description

A total of four RNA libraries (BMJ, BMS, RMJ, and RMS) were prepared and sequenced. The RNA-seq was performed using MinION ONT (oxford nanopore technologies). The transcriptome sequencing had an estimated number of reads of 634,035, 697,857, 633,288, and 144.667 for the BMJ, BMS, RMJ, and RMS conditions, respectively. The preprocessed analysis using phychopper [11] and cutadapt [12] showed approximately 72–82% high-quality reads. The de novo assembly was constructed using the RATTLE program and produced 10,248 transcripts. The results of the sequencing and pre-processing are summarized in Table 1. The open reading frames (ORFs) from the transcripts were determined using the TransDecoder program, and this produced 4053 Transcripts with 27.88% of them being 5′prime_partial, 3.73% of them being 3′prime_partial, 1.51% of them being Internal, and 66.89% of them being incomplete. The summary of the de novo transcriptome assembly

and open reading frames (ORFs) prediction characteristics is in Table 2. The gene abundance estimation was calculated using TPM, and it is presented in Figure 1. Figure 1 shows the differences in the abundance of genes affected by the LED and MeJA-MeSA hormones.

Table 2. Summary of de novo transcriptome assembly and open reading frames (ORFs) prediction characteristics.

Features	Numbers
Number and bases total (bp) of transcripts	10,248/8,461,077
Length range, average (bp), and N50 (bp) of transcripts	153–4190/825.63/955
- ORF transcripts.	4053
- ORFs Type:	
a. 5′prime_partial;	1130 (27.88%)
b. 3′prime_partial;	151 (3.73%)
c. Internal;	61 (1.51%)
d. Complete.	2711 (66.89%)

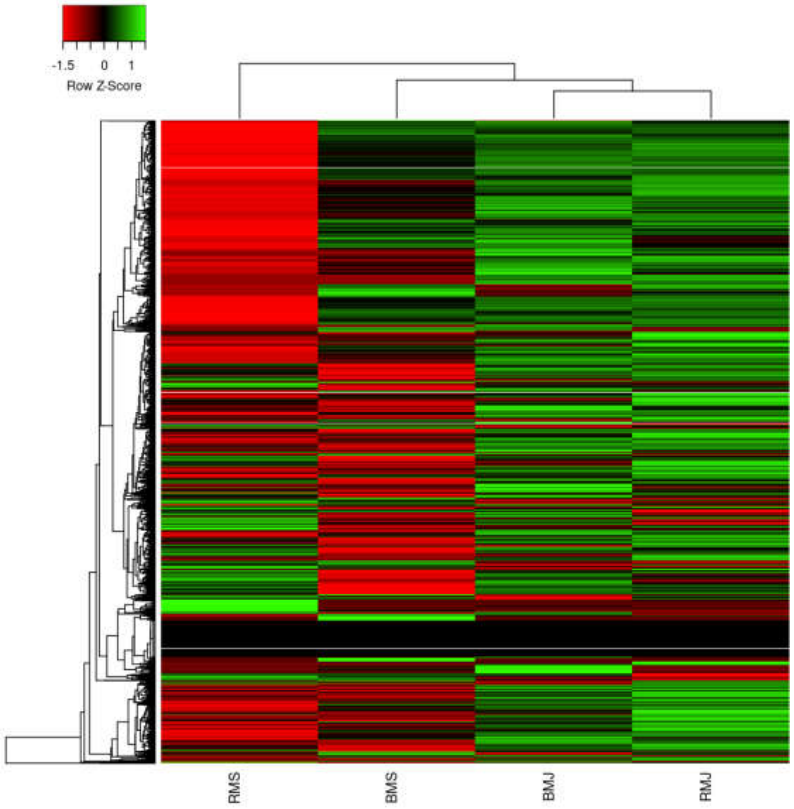

Figure 1. Clustering analysis of gene abundance estimation using heatmaps based on de novo assembled transcript.

3. Methods

3.1. Plant Treatment

The strawberry seedlings (*Fragaria* × *ananassa* Duch.) were collected from Lembang district, West Bandung Regency, West Java (1500 m above sea level). The seedlings were planted in the screenhouse with 60% shading net, Leuwikopo Experimental Field, Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University (210 masl). The strawberry plants were planted in pots with sizes of 38 × 31 × 12 cm. The Red: Blue 2:1 (R) and Red: Blue 1:2 (B) LED treatments lasted for one month. The experimental design used was a randomized block design with two factors. Biological replicates used were three plants with technical replicates of 3 samples. In addition, additional treatment was

given, the weekly application of MeSA (MS) and MeJA (MJ) hormones at a concentration of 15 mM each. The hormone application was achieved by spraying the leaves. The LED irradiation process was carried out with 21 h of irradiation time from 04.30–01.30 WIB. The dark phase occurred when the ambient temperature was at its lowest. The plant care regime consisted of watering them every two days, fertilizing them through the leaves, and pruning the old leaves. The sample used in this study was a leaf sample.

3.2. Isolation RNA and Sequencing

Leaf samples were collected after one month of treatment. First, the leaves were cleaned using alcohol by swiping the leaf surface, and then, the sample was put into liquid nitrogen. Next, the RNA extraction was performed with the RNeasy® Plant Mini Kit (QIAGEN). The quality and quantity of RNA were tested using a NanoPhotometer® NP80 (Implen) and a Qubit™ RNA Broad Range (BR) (Invitrogen) on a Qubit® Fluorometer. The total RNA was extracted from each biological replicate and pooled based on the treatment before the RNA library construction. The RNA sequencing process refers to the PCR-cDNA Barcoding protocol SQK-PCB109 (PCB_9092_v109_revB_ 10Oct2019). The RNA sequencing was run on MinION Mk1B using Flow Cell R9.4.1 (FLO-MIN106D).

3.3. Identification of Full-Length Transcripts

The raw fast5 files were base-called with default parameters using the guppy_basecaller v6.1.3+cc1d765d3. Next, the demultiplexing base-called reads were performed without trimming using guppy_barcoder. The data were pre-processed, and the mapping of the reads to the reference was achieved following the protocol at https://github.com/felixgrunberger/microbepore accessed on 1 November 2022. Next, the base-called reads were cleaned and trimmed using the phychopper [11] and using Cutadapt [12] to remove the remaining SSP (strand-switching primer) and VNP (oligo-dT30VN), and polyA-tails. De novo assembly was performed on all the clean, full-length reads using RATTLE v 1.0 [13]. The assembled transcripts were annotated using the BlastX program [14], with a cut-off of 10^{-5}, using the filtered UNIPROT database (Magnoliopsida (TaxID: 3398), downloaded on 19 October 2021). The gene ontology was performed using Blast2Go 6.0 software [15]. Finally, the open reading frames (ORFs) transcripts were predicted by TransDecoder (https://github.com/TransDecoder/TransDecoder, accessed on 1 November 2022) using default parameters [16].

3.4. Estimation Gene Abundance

The cleaned reads were mapped to the public strawberry genome of FAN_r1.1 (http://strawberry-garden.kazusa.or.jp, accessed on 1 November 2022) or the de novo assembled transcripts using Minimap2 [17]. To estimate them, gene abundances were calculated in an alignment-based mode using salmon v1.9.0 [18]. Finally, the transcripts per million (TPM) from each treatment were compared using a clustering analysis by using RStudio Software (4.1.2 version) with some packages such as gplots, cluster, and heatmap2 [19].

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